The Place of the Analytical Department in Modern Industry

WHAT is the place of the analyst in industry today? How does the analytical department fit into the modern "team approach" pattern we hear so much about in industry today, especially in the so-called chemical process fields? What do we mean when we talk about such subjects as research in analysis, quality control, adaptation of instrumental analysis methods in large scale manufacturing, and how are these and other factors related to other departments of a company?

In many respects, the analytical department can be said to be the nerve center in modern manufacturing operations. As a department, it has direct contact with manufacturing, purchasing, sales, research, market research and development, and top management. If it does not, something is vitally wrong in the organizational structure, a weakness that can be very detrimental to the success of a company where quality and new products are of prime importance.

Despite this generally close relationship with just about every other activity in a company setup, we do not recall ever having seen or read any comprehensive survey discussing these essential relationships.

When we mentioned this apparent absence in the literature at a meeting of the Advisory Board of ANALYTICAL CHEMISTRY, there was an immediate spontaneous enthusiasm for a series of articles that would discuss the relationship of the analytical department to all other phases of company management.

The first in this series appears in this issue, page 11 A. John E. McKeen, president and chairman of the board of Chas. Pfizer & Co., a nationally and internationally known chemical and pharmaceutical manufacturing concern, discusses how top management in his company views the analyst and the analytical department. In subsequent issues, widely known leaders in manufacturing, purchasing, sales, product development, engineering, and research will present their ideas of what constitutes an ideal relationship between their respective departments and the analytical department. Still other articles in the series will be contributed by an independent chemical consultant and an independent management consultant. The series will close with one or more articles written by prominent directors of a number of analytical departments.

We firmly believe that management today is conscious of the role of the analyst. We know from firsthand observation of many companies that the director of analytical research very frequently is made part of the over-all team assigned to the development of new processes, new products, and new large scale manufacturing operations. Thus the horizons of the analyst are constantly broadening from the classical concept that analysis has as its chief objective the maintenance of quality control. It is certainly true that the so-called "works chemist" or analyst of the 18th or 19th centuries no longer is looked upon by management as an expensive but, perhaps, necessary evil.

Very significantly the wheel of fortune for the analyst has come around a full 360 degrees. Historically analytical chemistry probably is the oldest field in the broad spectrum of chemistry. Obviously, the first challenge to the chemist was to determine the constituent parts of the physical things he could see or touch. Later emphasis shifted to organic chemistry, then to physical chemistry for the reason that the challenge then was to produce synthetically many natural substances or to produce products that did not exist in nature.

Ultimately, of course, the analyst reached the lowest ebb in stature when small chemical manufacturing plants sprang up. The analyst's main or sole duties consisted largely in performing routine tests to determine the quality of the raw materials coming in, so to speak, the back door, and the finished goods going out the front.

No longer is the analyst the "Cinderella" of the chemical profession. Nevertheless, despite the renaissance in his professional stature, the duties and contributions of the analytical chemist are still in many instances not too well defined nor too well understood.

The series which starts with Dr. McKeen's keen analysis of the role of the analytical department in Pfizer operations is specifically designed to develop discussion and controversy. Comments from readers will be most welcome.

Reaction Rates in Analytical Chemistry

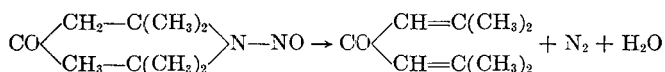## Rates, Mechanisms, and Solvent

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The application of kinetics to analytical problems is discussed with emphasis on the application of the theories of the influence of dielectric constant and ionic strength to the interpretation of rates and mechanisms for reactions in solution. The electrostatic effects of the ion atmosphere and the dielectric constant of the medium are treated with respect to ion dipolar molecule and ion-ion reactions. The electrostatic effect of the dielectric constant of the medium is discussed with respect to dipolar molecule–dipolar molecule reactions. Other factors, such as microscopic dielectric constant and selective solvation, are mentioned as perhaps being influential in rate processes.

ALTHOUGH this paper deals for the most part with fundamental theories of rates, mechanisms, and the effect of the solvent upon kinetic processes in solution, a brief mention of the historic and current applications of reaction kinetics to analytical problems is in order. The successful kineticist must have a fundamental grasp of analytical procedures, as his determinations of the rate of disappearance of reactants or appearance of products must in many cases depend upon chemical analysis. Kinetics, while not so essential to the analyst, nevertheless is a valuable tool for him to learn to use, because kinetic methods and theory throw much light upon the mechanisms and natures of chemical reactions.

Kinetic processes permit the determination of the actual concentrations of certain chemical species. Thus, the catalytic activities of hydrogen and hydroxyl ions in selected reactions are the historically oldest methods for the determination of the concentrations of these ions. Of course, titrimetric determinations of total acid and base concentrations preceded these catalytic methods for the determination of the concentrations of hydrogen and hydroxyl ions.

In 1912 Clibbens and Francis (*13*) found the decomposition of nitrosotriacetonamine into nitrogen and phorone to be a function of the catalytic activity of hydroxyl ion. The stoichiometric equation for the reaction is:

$$CO\left\langle{}^{CH_2—C(CH_3)_2}_{CH_3—C(CH_2)_2}\right\rangle N—NO \rightarrow CO\left\langle{}^{CH=C(CH_3)_2}_{CH=C(CH_3)_2}\right. + N_2 + H_2O$$

The original concentration of nitrosotriacetonamine was known and the rate of decomposition was found by measuring the volume of nitrogen produced as a function of time.

Francis, Geake, and Roche (*15*) found that at 30° C. the relationship between the apparent first-order velocity constant, k, for the reaction and the hydroxyl ion concentration [OH⁻] was given by the equation

$$k = 1.92 \ [OH^-] \tag{1}$$

The constant k has the units of sec. $^{-1}$ The constant 1.92 has the units of $l \times$ mole$^{-1} \times$ sec.$^{-1}$

Duboux (*14*) for acid-catalyzed inversion of sucrose found that the relation between the velocity constant, k, for the reaction and the concentration of hydrogen ion [H⁺] could be represented by the equation,

$$k = k_H \ [H^+] \tag{2}$$

when the acid was completely dissociated. In this equation k_H is a proportionality constant. The equation was used to calculate the concentration of hydrogen ion. Both k and k_H must have the same time units—e.g., sec.$^{-1}$ on min.$^{-1}$ The quantity k has the units of time^{-1}.

For acids which were not completely dissociated the formula for the dual catalysis by hydrogen ions and undissociated acid molecules was used. The formula is

$$k = k_H \ [H^+] + k_M \ (C - [H^+]) \tag{3}$$

The C term represents the total acid concentration both dissociated and undissociated, and k_M is another proportionality
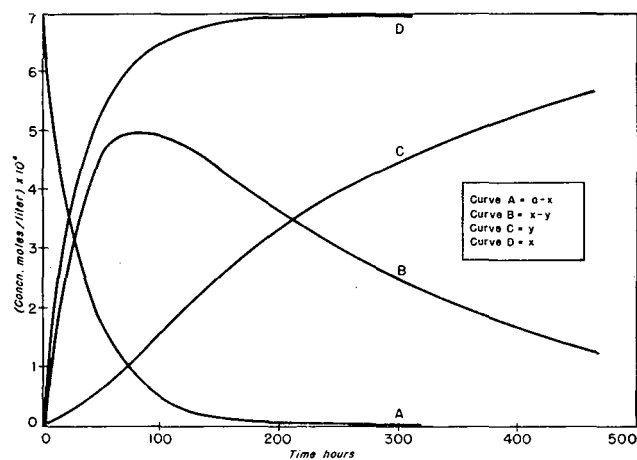

Figure 1. Plot of $(a - x)$, $(x - y)$, y and x in moles per liter *vs.* time in hours

$k_1 = 2.95 \times 10^{-2}$, $k_2 = 3.80 \times 10^{-3}$, temperature 25.1° C.

constant. The other terms have already been defined. The k_M term was calculated for any acid in any dilution with the aid of Taylor's formula by comparison with an acid for which the Taylor relation—namely,

$$K = (k_M/k_H)^2 \qquad (4)$$

is known. The accuracy is sufficient as k_M is very small compared with k_H. In this equation K is the dissociation constant of the acid.

A constant k_H, indispensable for the correct determination of hydrogen ion concentration, can be obtained only by preliminary measurements in completely dissociated acids for which

$$k_H = k/C \qquad (5)$$

Brønsted (10) suggested that the acid-catalyzed rate of addition of water to the nitratoaquotetramine cobaltic ion could be used to determine hydrogen ion concentrations.

The intention here is to point out the possibilities and not to give an exhaustive list of applications.

A second application of kinetics to analytical chemistry is to study the kinetic rate of decomposition of soluble complexes, or the reagents used in forming such, under experimental conditions and, thus, to ascertain the time limit during which a test is valid. Thus, palladous salt forms a soluble colored complex with acid p-fuchsin (29) which serves as a sensitive test for palladium. However, the color fades on standing. A series of kinetic measurements would establish the period of time in which the colored complex would maintain its capacity for light absorption sufficiently to give the correct concentration of palladium.

Carlton, Bradbury, and Kruh (12) found that dithizone in molten naphthalene gave colored complexes with bismuth, antimony, mercury, cadmium, and tin. The reaction of bismuth with dithizone in the molten solvent system was sensitive to 0.004 γ of bismuth and was reported as a spot test for bismuth ion by Carlton and Bradbury (11). The dithizone was found by Carlton, Bradbury, and Kruh to decompose in molten naphthalene with specific first-order rate constants of 0.07, 0.14, and 0.28 min.$^{-1}$, respectively, at 100°, 110°, and 137° C. The activation energy for the reaction at 100° C. was 18 kcal. per mole.

These data indicate that the dithizone reagent in molten naphthalene decomposes at the rates of 7, 14, and 28% per minute at the respective temperatures 100°, 110°, and 137° C., and that time is therefore an important consideration in using this reagent in this medium.

A third application of kinetics in analytical chemistry is the determination of the concentration of a particular reactant or product from known concentrations of reactants and the rates at which they decompose, or from the analytically determined concentration of a product.

Broach, Rowden, and Amis (9) studied the consecutive first-order reaction of tetrabromophenolsulfonphthalein with silver ion in aqueous nitric acid solution. If R represents the tetrabromophenolsulfonphthalein molecule except for the bromine atoms, the resulting reaction for the purpose of calculation can be represented by the following equations:

$$\overset{k_1}{RBr_4 \rightarrow} RBr_3(OH) \overset{k_2}{\rightarrow} RBr_2(OH)_2 \qquad (6)$$
$$(a) \qquad (x) \qquad (y)$$
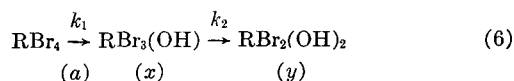
where a, x, and y represent the initial concentrations of the original dye, the concentration of the first substitution product, if no decomposition of this product occurred, and the concentration of the second substitution product, respectively. The

Table I. Ester Hydrolysis

Ester	Solvent	Temperature, ° C.	Type	Reference
Ethyl acetate	Water and water–ethyl alcohol	0.00, 9.80, 19.10	Alkaline	(26)
Ethyl acetate	Water and water–acetone	0.00, 15.87, 19.10	Alkaline	(6)
Methyl propionate	Water and water–acetone	25.13, 35.21, 45.48	Acid	(18)
Ethyl acetate	Water and water–dioxane and water and water–acetone	35.0, 45.0, 55.0	Acid	(23)
Ethyl formate	Water and water–acetone	35.01, 45.11, 55.02	Acid	(28)
Methyl propionate	Water and water–acetone	25.00, 35.03	Alkaline	(27)
Ethyl dichloroacetate	Water and water–acetone	25.00, 35.00, 45.00	Acid	(22)

quantity $(x - y)$ is the net concentration of the first substitution product.

The experimental determinations of the specific velocity constants, k_1 and k_2, for the first and second steps, respectively, from the rate of production of silver bromide were first carried out. Then the concentrations of the first and second substitution products as a function of time were calculated from the equations

$$x = a(1 - e^{-k_1 t}) \qquad (7)$$
and
$$y = \frac{ak_2(1 - e^{-k_1 t}) - ak_1(1 - e^{-k_2 t})}{k_2 - k_1} \qquad (8)$$

The remaining concentration $(a - x)$ of the reactant RBr$_4$ as a function of time was found by subtracting the calculated values of x at the different times from the known value of a. The net concentration, $(x - y)$ as a function of time of the intermediate product, was calculated from the corresponding concentrations with respect to time of x and y.

Plots of these concentrations as a function of time are shown in Figure 1.

Parsons, Seaman, and Woods (25) have reported the spectrophotometric determination of 1-naphthol in 2-naphthol, utilizing differences in reaction rates of the two naphthols with diazotized 2-naphthylamine-5,7-disulfonic acid at a controlled temperature and at a controlled acidity. The absorbancy of the reddish color formed was measured at 485 mμ. The standard deviation was ± 0.004% of 1-naphthol for samples containing 0.07 to 0.35% 1-naphthol. This is the direct application of a kinetic rate to an analytical determination of concentration.

Thus, kinetic procedures allow the determination of the concentrations of reyctants and products from a few data. Illustrations of these methods could be multiplied, but these examples suffice to illustrate the principle.

The remainder of this discussion shows how kinetic procedures permit the identification of possible intermediates in reaction processes. This is, in a sense, a qualitative analytical adaptation of rate studies.

The electrostatics of a rate process as influenced by the dielectric constant of the medium and by the ionic atmosphere in which reactant particles are located permit, under certain circumstances, an interpretation of the charge type of reactants involved in the rate controlling step of the reaction.

ION DIPOLAR MOLECULE REACTIONS

The author and his coworkers have studied both the acid and the basic hydrolysis of esters. Table I summarizes their work on ester hydrolysis.

The salt effect data in the case of alkaline hydrolysis were tested for obedience to theory using the Amis-Jaffé (4) equation which is

$$\ln k' = \ln k'_\kappa = 0 + \frac{Z_B \, \epsilon \cos \theta}{DkTr_0^2} [\mu_0^* - \mu^* e^{-\kappa r_0} (1 + \kappa r_0)] \qquad (9)$$

In this equation κ is the Debye kappa, $Z_B \epsilon$ is the charge on the ion, θ is the angle which the line drawn between the centers of

charge in the dipole makes with the line drawn from the ion to one of these centers of charge, μ^* is the Onsager enhanced moment, μ_0^* is the enhanced moment at zero ionic strength, r_0 is the distance of approach between the ion and the dipole necessary for reaction to take place, D is the dielectric constant of the medium, k is the Boltzmann gas constant, and T is the absolute temperature. The equation was transformed for the salt effect calculations by substituting in it the dimensionless variables

$$Z = \kappa a = \kappa r_0 \qquad (10)$$

and

$$W = \frac{(\ln k' - \ln k'_{\kappa=0})\, 2DkTr_0^2}{Z_B \epsilon \mu_0^* \cos \theta} \qquad (11)$$

and gave

$$W = \frac{Z^2}{(1 + Z + \frac{1}{2}Z^2) + \frac{N^2}{2D}(1 + Z)} \qquad (12)$$

A theoretical curve of W versus Z^2 was plotted and the data for the dependence of the velocity constant upon ionic strength were fitted to the curve using the constants of Table II.

Table II. Constants Used in Fitting Ionic Strength Data to Theoretical Curve

$r_0 = 7.5$ A.

Temp., °C. ± 0.02	$\mu_0^* \times 10^{18}$	N^2	Z^2 at Ionic Strength = 1	$k'_\kappa = 0$
0.00	13.2	12.7	7.95	0.530
9.80	7.66	6.51	7.72	1.46
19.10	11.7	11.0	7.48	3.07

The data fitted the theoretical curve well and the constants used compared favorably in magnitudes with those found in former applications of this theory.

The equation (1)

$$\ln k'_{D-D} = \ln k'_{D-\infty} + \frac{Z\epsilon\mu}{DkTr^2} \qquad (13)$$

has been developed for the limiting case of the head-on approach of an ion to a dipolar molecule from electrostatic considerations for the rate of reaction between ions and dipolar molecules. According to this equation if D is increased the rate of reaction should decrease for a positive ionic reactant and vice versa for a negative ionic reactant. If D is decreased the rate should increase for positive ions and vice versa for negative ions.

In deriving this equation, free energy and energy of activation were assumed to be equivalent. This assumption can be verified since, for reaction in solution, both $p\Delta V$ and the change of entropy of activation due to coulombic effects for rate processed carried out in solvents of constant dielectric constant (20) are each equal to zero.

The directions of change are in harmony with those predicted by the Amis-Jaffé equation. When Equation 13 is applied to the data of Potts and Amis and of Seigel and Amis the data fall on the theoretical plot of log k' versus $1/D$ and the slope, which is $\frac{Z\epsilon\mu}{2.303\ kTr^2}$, yields reasonable values of the parameter r. These values of r together with the values of r found by Quinlan and Amis (27), using the same approach for the alkaline hydrolysis of methyl propionate in water and water-acetone media, are recorded in Table III. These values of r, though somewhat small for the alkaline hydrolysis of ethyl acetate in water-ethyl alcohol media, are of the right order of magnitude for a molecular radius.

From these correlations of empirical data and theory, one would conclude that the rate-determining step in the hydrolysis

of these esters under the condition given is the reaction of a dipolar molecule with a negatively charged ion. The over-all mechanism may involve several steps.
These steps could be

$$(14)$$
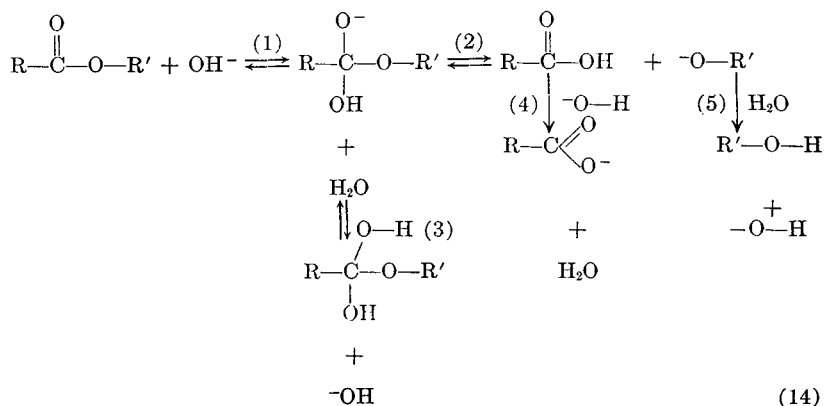
From the standpoint of the effect of the dielectric constant upon the rate, either Step 1 or some other step involving the reaction of a dipolar molecule with a negatively charged ion is rate determining. While Step 3 involves molecules and negatively charged ions, it involves water as one of the reactants and there is no kinetic evidence in these data that the concentration of water as such is involved in the rate process. Steps 4 and 5 are practically irreversible.

Hockersmith and Amis (18) and Nair and Amis (23) found that the hydrochloric acid hydrolysis of methyl propionate and of ethyl acetate, respectively, obeyed the predictions of Equation 13 with respect to the dielectric constant effect upon the rate of a reaction, the rate-controlling step of which involved a dipolar molecule and a positive ion, and when the specific velocity constant was also made specific with respect to water by dividing by the concentration of water. Only the hydrolysis of ethyl acetate in water and water-acetone at the highest temperature involved in these investigations—namely, at 55° C.—shows an unreasonable value of the r parameter. A summary of the r values for the various investigations is given in Table IV.
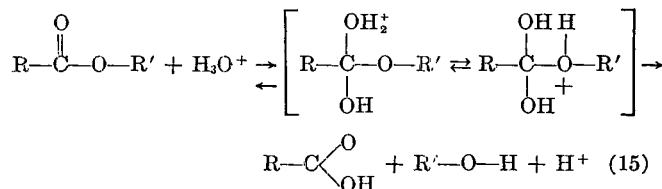
From the dielectric constant effect upon the rate, it appears that the mechanism for the acid hydrolysis of esters proposed by Bender (7)—namely,

Table III. Values of Parameter r for Alkaline Hydrolysis of Esters

Ester	Solvent	Temp., °C.	$\gamma \times 10^8$ Cm.
Ethyl acetate	Water and water-ethyl alcohol	0.00	0.94
		9.80	0.93
		19.10	0.98
Ethyl acetate	Water and water-acetone	0.00	2.00
		15.87	1.8
		25.10	1.5
Methyl propionate	Water and water-acetone	15.00	1.4
		25.00	1.4
		35.03	1.3

Table IV. Values of Parameter r for Acid Hydrolysis of Certain Esters

Ester	Solvent	Temp., °C.	$r \times 10^8$
Ethyl acetate	Water and water-dioxane	35.0	9.08
		45.0	9.18
		55.0	9.50
Ethyl acetate	Water and water-acetone	35.0	5.21
		45.0	6.19
		55.0	Unreasonably small
Methyl propionate	Water and water-acetone	25.13	3.43
		35.21	3.42
		45.48	3.01

$$R-\overset{\overset{\displaystyle O}{\|}}{C}-O-R' + H_3O^+ \underset{\leftarrow}{\rightarrow} \left[R-\overset{\overset{\displaystyle OH_2^+}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-O-R' \rightleftarrows R-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle +}{|}}{O}}-R' \right] \rightarrow$$

$$R-C\overset{\displaystyle O}{\underset{\displaystyle OH}{\diagdown}} + R'-O-H + H^+ \quad (15)$$

or some similar mechanism—is acceptable if the first step or a step involving the product of the concentration of a positive ion and the concentration of a dipolar molecule governs the rate.

It might be pointed out that the hydrolyses of esters are many times complex and are not amenable to like treatment in all cases (*22, 26, 28*).

Landskroener and Laidler (*21*) derive the general equation

$$\ln k' = \ln k_0' + \frac{\epsilon^2}{2kT}\left(\frac{1}{D} - 1\right) \times$$
$$\left(\frac{Z_A^2}{b_A} + \frac{Z_B^2}{b_B} - \frac{(Z_A + Z_B)^2}{b_\pm}\right) +$$
$$\frac{3\epsilon^2}{8kT}\left(\frac{2}{D} - 1\right)\left(\frac{G_A}{b_A^3} + \frac{G_B}{b_B^3} - \frac{G_\pm}{b_\pm^3}\right) \quad (16)$$

for the effect of the dielectric constant upon reaction velocities. In this equation k' is the observed specific velocity constant, k_0' is the specific velocity constant at the reference dielectric constant unity, Z_A is the valence of reactant A, Z_B is the valence of reactant B, $(Z_A + Z_B)$ is the valence of the intermediate complex, D is the dielectric constant, T the absolute temperature, k the Boltzmann gas constant, and ϵ the electric charge. G_A, G_B, and $G_\pm$ are complex functions of the charges and structures of the reactant A, reactant B, and the intermediate complex, respectively. Definite models of reactant molecules and of a complex have to be assumed in order to determine the charges, distances, and Legendre polynomials which make possible the determination of the G-factors.

According to Equation 16, a plot of log k' versus $1/D$ should yield a straight line, the slope, A, of which is given by

$$A = \frac{\epsilon^2}{2.303(2kt)}\left[\left(\frac{Z_A^2}{b_A} + \frac{Z_B^2}{b_B} - \frac{(Z_A + Z_B)^2}{b_\pm}\right) + \frac{3}{2}\left(\frac{G_A}{b_A^3} + \frac{G_B}{b_B^3} - \frac{G_\pm}{b_\pm^3}\right)\right] \quad (17)$$

For a univalent ion dipole reaction, $Z_A = \pm 1$ ($Z_A^2 = +1$), $Z_B = 0$, $G_A = 0$, and G_B is negligible. Substitution of these values into Equation 17 yields

$$A = \frac{\epsilon^2}{2.303(2kT)}\left(\frac{1}{b_A} - \frac{1}{b_\pm} - \frac{3}{2} - \frac{G_\pm}{b_\pm^3}\right) =$$
$$\frac{\epsilon^2}{(9.212)kTb_A}\left(\frac{2b_\pm^3 - 2b_\pm^2 b_A - 3G_\pm b_A}{b_\pm^3}\right) \quad (18)$$

A model had to be constructed for the activated complex in order to evaluate the $b_\pm$ and $G_\pm$. The models for the acid and base hydrolysis of esters are given as follows:
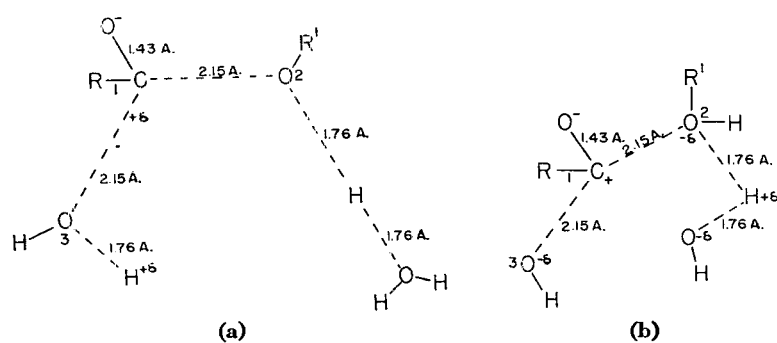
Using the model for the acid hydrolysis, $G_\pm$ is 9.1×10^{-16} sq. cm. and taking b_A as 1.7×10^{-8} cm., Equation 18 becomes

$$A = \frac{1.07 \times 10^4}{b_\pm^3 T}(2b_\pm^3 - 3.4b_\pm - 47.31) \quad (19)$$

Also from the model for the base hydrolysis $G_\pm$ becomes 5.4×10^{-16} sq. cm. and taking b_A as 1.4×10^{-8} cm. Equation 18 becomes

$$A = \frac{1.30 \times 10^4}{b_\pm^3 T}(2b_\pm^3 - 2.8b_\pm^2 - 22.68) \quad (20)$$

Thus to check the theory, plots of log k' versus $1/D$ were made. The slope of the straight line so obtained in the case of the acid hydrolysis was set equal to A in Equation 19 and $b_\pm$ calculated. Equation 20 was used in the case of basic hydrolysis for the calculation of $b_\pm$. The $b_\pm$ values were the effective radii of the solvated complex and, therefore, should have the magnitude of molecular dimensions—that is, they should be of the order of magnitude 10^{-8} cm. Calculated values of $b_\pm$ for both acid and basic hydrolysis of esters as given by Landskroener and Laidler are recorded in Table V.
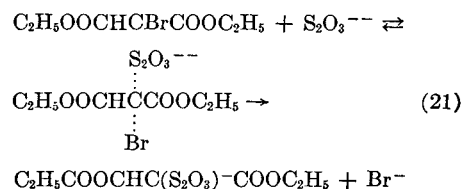
Table V. Values in Angstroms of $b_\pm$ Function in Both Acid and Base Hydrolysis of Esters

Ester	Solvent	Ionic Strength	Temp., ° C.	Slope	
Acid Hydrolysis					
Ethyl acetate	Dioxane-water	0.05	35.0	− 11	3.3
	Acetone-water	0.05	35.0	− 34	3.0
Methyl propionate	Acetone-water	0.02	35.3	− 31	3.0
Base Hydrolysis					
Ethyl acetate	Dioxane-water	0.05	25	− 24	2.5
	Acetone-water	0.05	25	− 32	2.4
	iso-PrOH-water	0.05	25	− 50	2.3
	tert-BuOH-water	0.05	25	− 61	2.2
	n-PrOH-water	0.05	25	− 74	2.1
	EtOH-water	0.05	25	− 96	2.0
	MeOH-water	0.05	25	−155	1.8

The $b_\pm$ values should be compared with the r values for ester hydrolysis obtained by use of Equation 13 and listed in Tables III and IV.

The kinetics of the reaction of ethyl bromomalonate with thiosulfate (*8*) obeyed Equation 9 with respect to the salt effect and Equation 13 with respect to the dielectric constant dependence of the rate. The r-values were assumed to be 2.5×10^{-8} cm. and the value of the moment, μ, calculated for the ethyl bromomalonate were 5.45×10^{-18} and 4.60×10^{-18} at $0.10°$ and $25.00°$ C., respectively, giving an average moment of 5.03×10^{-18} which is of the right order of magnitude.

From the electrostatics of the reaction the rate-governing step involved a dipolar molecule and a bivalent negatively charged ion. It was concluded that the reaction was of a substitution nucleophyllic reaction of the second order and that the mechanism was probably

$$C_2H_5OOCHCBrCOOC_2H_5 + S_2O_3^{--} \rightleftarrows$$

$$C_2H_5OOCH\overset{\overset{\displaystyle S_2O_3^{--}}{|}}{\underset{\underset{\displaystyle Br}{|}}{C}}COOC_2H_5 \rightarrow \quad (21)$$

$$C_2H_5COOCHC(S_2O_3)^-COOC_2H_5 + Br^-$$

The hydronium ion catalyzed inversion of sucrose (*1*) in dioxane-water solvents resulted in kinetics in obedience to Equation 13. The plot of log k' versus $1/D$ gave a straight line of positive slope. Using the moment, μ,

(a)

(b)

as 3.4 Debye units as found by Landt, the value of r from the slope was 5.5×10^{-8} cm. The mechanism then is one involving a positive ion and a dipolar molecule and can be written

glucose   fructose

The reaction rate of diacetone alcohol with hydroxide ion gave kinetics in obedience to the requirements of Equation 13. The plot of log k' versus $1/D$ is a straight line of negative slope, which, assuming a moment of 4.0 Debye units, gives an r value of 6.0 $\times 10^{-8}$ cm. The mechanism can be written as given below. The kinetics indicate the rate-controlling step to be a negative ion reacting with a dipolar molecule. Either, the first or second step seems the logical one, since these steps involve negative ions reacting with dipolar molecules and are independent of acetone concentration, and the reaction has been found not to depend on this quantity (17). Frost and Pearson (16) select step (2) in the forward reaction as being rate-determining.

Nelson and Butler's (24) mechanism—namely,

where B is a negative ion—would agree with the kinetics, but there are certain objections to this mechanism (16).

DIPOLAR MOLECULE–DIPOLAR MOLECULE REACTIONS

The equation (1)

$$\ln k'_{D-D} = \ln k'_{D-\infty} - \frac{2\mu_1\mu_2}{DkTr^3} \qquad (25)$$

has been derived, for the limiting case of zero angles of approach between two dipoles, to express the effect of the dielectric constant of the medium upon the rates of dipolar molecule—dipolar molecule reactions. This equation was tested by plotting the log k'_{D-D} versus $1/D$ for the reaction of tert-butyl chloride with water. The plot, as required by the equation, was a straight line of negative slope. Equating the slope of the line to ($-2 \, \mu_1 \, \mu_2/ kTr^3$), one obtains a value of r of 0.75×10^{-8} cm. if the moment of water and the moment of tert-butyl chloride (30) are taken as 1.84 and 2.10 Debye units, respectively. This is a reasonable, though somewhat small, value for r. The mechanism from these electrostatic considerations must show the slow step as a dipolar molecule reacting with a dipolar molecule (19) and could be step one as given in the following mechanism:

ION-ION REACTIONS

The well known Brønsted-Christiansen-Scatchard equation

$$\ln k = \ln k - \frac{Z_aZ_b\epsilon^2}{DkTr} + \frac{Z_aZ_b\epsilon^2}{DkT} \frac{\kappa}{1 + a\kappa} \qquad (27)$$
$$\kappa = 0$$
$$D = \infty$$

expresses the effect of both ionic strength and dielectric constant upon reaction rates when the reactants are both ions.

In the limiting form for dilute solutions when the dielectric constant of the medium is infinity and using decadic logarithms the equation becomes

$$\log k'_{D-\infty} = \log k'_{D-\infty} + Z_aZ_b A\sqrt{\mu} \qquad (28)$$
$$\kappa = 0$$

where A is 0.977, 1.014, and 1.060 at 5°, 25°, and 45° C., respectively, when water is the medium and a plot of log k' versus $\sqrt{\mu}$ should be a straight line of positive slope for ions of like charge sign and of negative slope for ions of unlike charge sign.

Also for ionic strength $\mu = 0$

$$\log k'_{\kappa=0} = \log k'_{D-\infty} - \frac{Z_aZ_b\epsilon^2}{2.303 \, DkTr} \qquad (29)$$
$$\kappa = 0$$

and a plot of log k' versus $1/D$ should yield a straight line of negative slope for ions of like charge sign and of positive slope for ionic reactants of unlike charge sign. In either case the slope of the line which is $\left(\frac{-Z_AZ_B\epsilon^2}{2.303 \, kTr}\right)$ should yield a reasonable value for r.

The alkaline fading of bromophenol blue (tetrabromophenolsulfonphthalein) is a reaction between the twice ionized negatively

charged quinoid dye ion and the negative hydroxide ion. Plots of the ionic strength data for water media at 5°, 35°, and 45° C. gave straight lines of the expected positive slopes and plots of the dielectric constant data in both water-methanol and water–ethyl alcohol solvents gave, at the temperatures mentiond above, straight lines of negative slopes. The values of r from the slopes at 25° C. were 1.49×10^{-8} cm. and 1.22×10^{-8} cm. in water–methanol and water–ethyl alcohol media, respectively (*2, 5*). These are reasonable values for r. The reaction, including Step 1 which is presumably the governing step, can be written as follows:

(c)
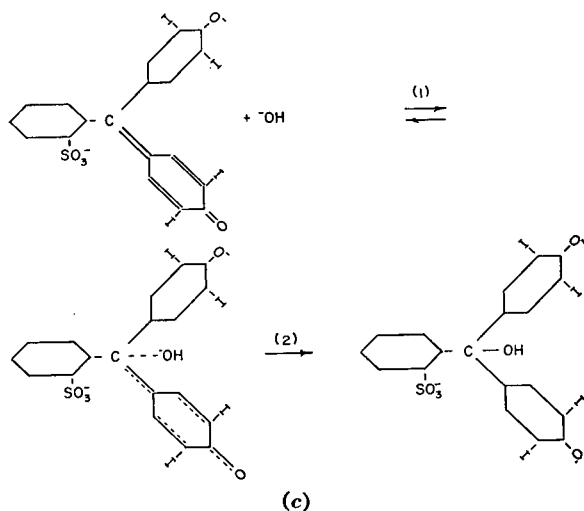
OTHER CONSIDERATIONS

Brief mention should be made of some of the factors not accounted for in the theories of the effect of the dielectric constant upon reaction rate. The macroscopic dielectric constant of the solvent is, in general, all that is available and this is used in calculations of the effect of dielectric constant upon reaction rates. Selective solvation and the greater electrostatic attraction of the solute for the more polar component of a mixed solvent may produce a region near a solute particle of dielectric constant entirely different from the average dielectric constant.

If the Walden equation for ionic solutes

$$\Lambda_0 \eta = \frac{\epsilon F}{1800\pi} \left(\frac{r_0^+ + r_0^-}{r_0^+ r_0^-} \right) \tag{30}$$

is used to calculate the term in parenthesis and its reciprocal—i.e., $\left(\dfrac{r_0^+ r_0^-}{r_0^+ + r_0^-} \right)$—is taken, some interesting conclusions can be drawn from the results. The reciprocal is termed the r-function, as it has the dimensions and the magnitude of an ionic radius.

A plot of the r-function versus the per cent of the organic component of the solvent is given in Figure 2 for potassium chloride in water-methanol and in water-acetone solvents at 25° C. The minimum at low percentages of organic component of the solvent may be significant. Landskroener and Laidler (*21*) discuss kinetic anomalies which occur in the region of 10% by weight of the organic component of the solvent.

It is apparent that the relatively slow increase of the r-function with the first additions of organic solvent can be interpreted as meaning that the potassium and chloride ions cling rather exclusively to the more polar water up to 30 or 40% by weight of the organic component. The ions hold relatively tightly to water until it is entirely replaced. This latter conclusion is justified by the sudden large increase of the r-function as the larger molar volume organic component replaces the last few per cent of water.

If the actual radius, r_s, of a solvated ion is assumed to be the radius, r_i, of the nonsolvated ion plus a term proportional to the electrostatic attractive force between the ion and the dipolar molecule of the solvent; if it is assumed that r_i is small compared to the distance at which electrostatic forces can be exerted; and if it is assumed that the Coulomb expression for head-on alignment between an ion and a dipolar molecule can be used to represent the electrostatic force between an ion and a dipole, the equation

$$r_s = \sqrt[3]{\frac{1800\pi K \eta \mu \Lambda_0}{DF}} \tag{31}$$

can, using Walden's equation, be derived for the effective distance, r_s for electrostatic force between an ion and a dipole (*3*). K is a constant of proportionality between the radius of the solvated ion and the electrostatic force exerted between the ion and the dipolar molecule, Λ_0 is the equivalent conductance of the electrolyte at infinite dilution, η is the viscosity of the solution, F is the faraday, and the other symbols have their usual significance. The equation was derived for univalent ions.
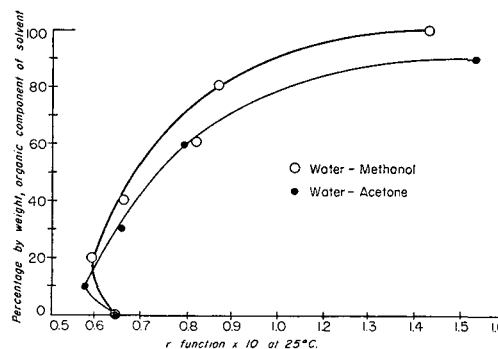
Figure 2.   Relation of r function to organic component of solvent

The r_s term has a much greater possibility of remaining constant than does the Walden r-function since, when the dielectric constant, D, which occurs in the denominator of the expression for r_s, increases the moment, μ, which occurs in the numerator, likewise increases and vice versa. The average deviation from the average of r_s is less than 3% when data for potassium chloride in water, methanol, and water-methanol solvents at three temperatures and that for tetramethylammonium chloride and for tetraethylammonium picrate at 25° C. in both methanol and ethyl alcohol are included. The average value of r_s found was 12.72 ± 0.32 A. when the constant K is taken as unity. K is perhaps not far from unity since the calculated values are reasonable values for r_s.

It is hoped that other methods of investigation will reveal just the role that selective solvation plays in the determination of the solvent sheath around a reactant particle and give insight as to the true nature of the dielectric constant in the vicinity of the solute particle.

LITERATURE CITED

(1) Amis, E. S., *J. Chem. Educ.*, **30**, 351 (1953).
(2) Amis, E. S., "Kinetics of Chemical Change in Solution," p. 80, Macmillan, New York, 1949.
(3) Amis, E. S., unpublished manuscript.
(4) Amis, E. S., and Jaffé, George, *J. Chem. Phys.*, **10**, 598 (1942).
(5) Amis, E. S., and LaMer, V. K., *J. Am. Chem. Soc.*, **61**, 905 (1939).
(6) Amis, E. S., and Siegal, Saul, *Ibid.*, **72**, 674 (1950).
(7) Bender, M. L., *Ibid.*, **73**, 1626 (1951).
(8) Broach, J., and Amis, E. S., *J. Chem. Phys.*, **22**, 39 (1953).

(9) Broach, W. J., Rowden, R. W., and Amis, E. S., *Ibid.*, 56, 189 (1952).
(10) Brønsted, J. N., *Z. physik. Chem.*, 122, 383 (1926).
(11) Carlton, J. K., and Bradbury, W. C., ANAL. CHEM., in press.
(12) Carlton, J. K., Bradbury, W. C., and Kruh, R., *Anal. Chim. Acta*, 12, 101 (1955).
(13) Clibbens, D. A., and Francis, F., *J. Chem. Soc.*, 1912, 2358.
(14) Duboux, Marcel, *Helv. Chim. Acta*, 7, 849 (1924).
(15) Francis, F., Geake, F. K., and Roche, J. W., *J. Chem. Soc.*, 1915, 1651.
(16) Frost, A. A., and Pearson, R. G., "Kinetics and Mechanism," pp. 283–96, Wiley, New York, 1953.
(17) Hammett, L. P., "Physical Organic Chemistry," p. 344, McGraw-Hill, New York, 1940.
(18) Hockersmith, J. L., and Amis, E. S., *Anal. Chim. Acta*, 9, 101 (1953).
(19) Laidler, K. J., and Eyring, H., *Ann. N. Y. Acad. Sci.*, 39, 303 (1940).
(20) LaMer, V. K., *J. Franklin Inst.*, 225, 709 (1938).
(21) Landskroener, P. A., and Laidler, K. J., "Effects of Solvation on Hydrolysis," Ph.D. dissertation, Catholic University of America, Catholic University of America Press, Washington, 1954. By permission.
(22) Nair, P. M., and Amis, E. S., *J. Am. Chem. Soc.*, 77, 3452 (1955).
(23) Nair, P. M., and Amis, E. S., *Anal. Chim. Acta*, 9, 111 (1953).
(24) Nelson, W. E., and Butler, J. A. V., *J. Chem. Soc.*, 1938, 957.
(25) Parsons, J. S., Seaman, W., and Woods, J. T., ANAL. CHEM., 27, 21 (1955).
(26) Potts, J. E., Jr., and Amis, E. S., *J. Am. Chem. Soc.*, 71, 2112 (1949).
(27) Quinlan, J. E., and Amis, E. S., *Ibid.*, 77, 4187, (1955).
(28) Shah, N. P., and Amis, E. S., *Anal. Chim. Acta*, 11, 401 (1954).
(29) West, P. W., and Amis, E. S., IND. ENG. CHEM., ANAL. ED., 18, 400 (1946).
(30) Wiswall, R. H., Jr., and Smyth, C. P., *J. Chem. Phys.*, 9, 356 (1941).

8th Annual Summer Symposium—Role of Reaction Rates

Induced Reactions in Analytical Chemistry

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Induced reactions can cause serious errors in both volumetric and colorimetric analytical procedures based on oxidation-reduction reactions. There are two types of induced reactions: coupled reactions and induced chain reactions. Both types involve the formation of an active intermediate in the primary reaction. In a coupled reaction, or over-all induced reaction of the first type, the actor reacts with both the acceptor and the inductor, in a fixed ratio of equivalents designated as the coupling index. The ratio of the equivalents of the coupled reaction to the equivalents of the primary reaction is designated as the coupling factor; this represents the relative extents of the competing reactions of the acceptor and the inductor with the active intermediate. In an induced chain reaction, the actor reacts only with the acceptor. The ratio of this reaction to the primary reaction, designated as the induction factor, likewise represents the relative extents of competing reactions. Methods of overcoming induced chain reactions are discussed.

INDUCED reactions have been recognized for 100 years (*9, 28*). From a classical, phenomenological standpoint, an induced reaction may be represented by the following scheme (*15*):

$$A + B \rightarrow \text{reaction}, \quad A + C \rightarrow \text{no reaction}$$

$$\begin{cases} A + B \rightarrow \text{reaction} \\ A + C \rightarrow \text{reaction} \end{cases}$$
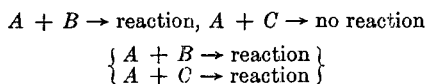
That is, the reaction between $A + C$, which ordinarily does not proceed (or proceeds very slowly), is brought about (or greatly accelerated) by the simultaneous occurrence of the reaction between A and B. In this scheme, A is called the actor, since it acts on both the other constituents; B is called the inductor; and C is called the acceptor. The reaction between A and B is the primary reaction, and that between A and C is the induced reaction. The extent of the induced reaction is conventionally expressed as the induction factor, F_i, defined as

$$F_i = \frac{\text{equivalents of induced reaction}}{\text{equivalents of primary reaction}}$$

Induced reactions are distinguished from catalyzed reactions, in which (phenomenologically) the mere presence of the catalyst brings about or accelerates the reaction in question. A catalyst is not altered by the occurrence of the catalyzed reaction, whereas an inductor is altered by its participation in the primary reaction.

Induced reactions must also be distinguished from side reactions. A side reaction is one which proceeds to an extent which is unaffected by the simultaneous occurrence of the principal reaction. For example, the air oxidation of iodide ions, which takes place spontaneously in acidic solutions, appears to constitute a side reaction in the iodometric determination of peroxides. Like other side reactions, this air oxidation of iodide is compensated for by carrying out a blank determination in the absence of the peroxide (*8, 35*). Possibly the magnitude of the air oxidation may depend on the extent of the primary reaction (*8*); in this case, as with other induced reactions, blank determinations would be of no value in compensating for the air oxidation. Fortunately the air oxidation of iodide can be kept comparatively small, and it is probably for this reason that this reaction has not yet been conclusively identified as an induced or side reaction.

The analytical chemist usually encounters induced reactions as a source of error in volumetric analyses. Thus, if it is attempted to determine the amount of a component B by titration with a reagent A, then the simultaneous occurrence of an induced reaction between A and C consumes an additional amount of the reagent A, and disrupts the stoichiometry of the primary titration reaction. The apparent result in this case is equal to the true result multiplied by a factor $(1 + F_i)$. In the case where the actor, A, is being titrated with the inductor, B, the apparent result is equal to the true result divided by the factor $(1 + F_i)$.

Induced reactions may also be encountered in colorimetric analyses where the substance to be determined first reacts with another substance, which is in turn measured colorimetrically (*14*).

The error resulting from an induced reaction cannot be compensated for by blank determinations, but may sometimes be estimated by means of control determinations—that is, by determinations on known materials in comparable concentrations, and under the same conditions as used in the determination of the unknown. However, the requirement that the control be closely similar to the unknown may involve stringent or obscure

specifications—for example, of the temperature, pH, agitation, etc. These specifications are best determined by study of the mechanism of the reaction. Elucidation of the mechanism may also lead to the development of procedures in which the induced reaction is either eliminated or else forced to assume a definite stoichiometric relationship to the primary reaction.

Studies of the mechanisms of various induced reactions have led to the general conclusion that induced reactions involve reactive intermediates formed in the primary reaction. These reactive intermediates may have the character of free radicals, or they may be metallic ions in abnormal valence states; they may arise from either the actor (23) or the inductor (24). In analytical chemistry, these intermediates are encountered almost exclusively in oxidation-reduction reactions.

COUPLED REACTIONS

Induced reactions fall into two categories. Typical of the first category is the arsenite-induced oxidation of manganous ions by sexivalent chromium. Thus, the reaction between arsenic(III) and chromium(VI) proceeds readily in strong acid, with the following stoichiometry, if no other ingredients are present:

$$2Cr(VI) + 3As(III) \longrightarrow 2Cr(III) + 3As(V) \qquad (A)$$
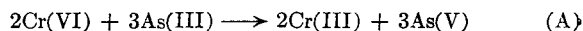
In the presence of manganese(II), additional chromium(VI) is consumed by a reaction which may be written with the following stoichiometry:

$$2Cr(VI) + 3Mn(II) \longrightarrow 2Cr(III) + 3Mn(IV) \qquad (B)$$
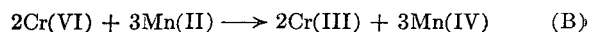
In the above scheme, chromium(VI) is the actor, arsenic(III) is the inductor, and manganese(II) is the acceptor. Reaction A is the primary reaction; and Reaction B, in the classical terminology, is called the induced reaction, but is here referred to as the apparent induced reaction. The induction factor, F_i, is the ratio of the equivalents of Reaction B actually taking place, to the equivalents of Reaction A.

Careful study of this reaction by Lang and Zwerina (17) has shown that the induction factor is 0.5, under all conditions under which reliable measurements could be made. [Experimentally observed induction factors between 0.4 and 0.5 were shown (17) to be due to a side reaction between manganese(III) and arsenic(III). Other experiments which gave induction factors of less than 0.4 were not actually valid, because the chromium(VI) was virtually all consumed during the course of the reaction.] The induction factor of 0.5 means that for every equivalent of chromium(VI) used in the oxidation of arsenic(III), a further 0.5 equivalent is also consumed in the oxidation of manganese(II). This is accounted for by the following mechanism, which is also in agreement with mechanisms for other reactions of these compounds:

$$Cr(VI) + As(III) \longrightarrow Cr(IV) + As(V) \qquad (1)$$

$$Cr(IV) + Cr(VI) \longrightarrow 2Cr(V) \qquad (2)$$

$$Cr(V) + As(III) \longrightarrow Cr(III) + As(V) \qquad (3)$$

$$Cr(IV) + Mn(II) \longrightarrow Cr(III) + Mn(III) \qquad (4)$$

$$2Mn(III) \rightleftharpoons Mn(IV) + Mn(II) \qquad (5)$$
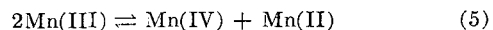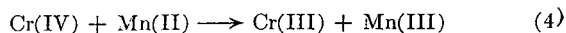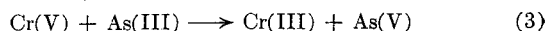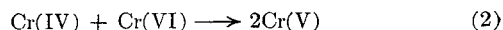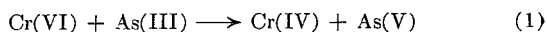
Here the reactants are represented only by their valence states, as the exact constitution of the chromium(V) and chromium(IV) is not actually known. It appears (34) that the chromium(VI) is reacting in the form of acid chromate, $HCrO_4^-$, which is in equilibrium with dichromate. The manganese goes from manganous to manganic, and these two are in equilibrium with manganese dioxide.

Step 1 represents the transfer of two electrons from arsenic(III) to chromium(VI). The arsenic(V) thus formed is stable, but the chromium(IV) is not. In order to arrive at stable valence states—namely arsenic(V) and chromium(III)—it would be necessary for the two reactants to undergo valence changes of

different magnitudes. Since the first step in the reaction sequence must be bimolecular, this step must lead to an unstable valence state of one of the reactants. This of course is an instance of a general rule. As another general rule, reactive intermediates can be expected whenever a reactant must undergo a valence change of more than two in order to arrive at a stable valence state, since it appears (34) that oxidation-reduction reactions may involve the transfer of either one or two electrons, but no more.

If a particular valence state of an element is unstable, this is because it is highly reactive—that is, it is a reactive intermediate, which can take part in induced reactions. However, it is also evident that there must be some path whereby the active intermediate can react further with the inductor and/or the actor and, thus, give an over-all reaction between actor and inductor with the formation of stable products; this is the primary reaction. In the present example, this path is represented by Steps 2 and 3, which together with Step 1, make up the primary Reaction A. [Although there is ample evidence for the existence of quinquevalent chromium, the nature of Step 2 is uncertain; it may, for example, be reversible, and it may conceivably lead to some complex between two chromium species of different valence states (34).]

If manganese(II) is present, the chromium(IV) formed in Step 1 reacts exclusively with manganese(II), as shown in Step 4. Thus, there is a stepwise reaction in which an acid chromate ion is first reduced by arsenious acid to form chromium(IV), which then oxidizes a manganous ion. The manganese(III) formed in Step 4 can disproportionate according to Step 5; and if this last step is considered as going to completion, then the sequence of Steps 1, 4, and 5 gives the over-all induced reaction, Reaction C:

$$2Cr(VI) + 2As(III) + Mn(II) \rightarrow$$
$$2Cr(III) + 2As(V) + Mn(IV) \qquad (C)$$
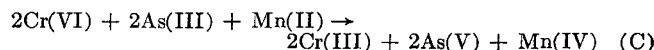
It appears preferable to think in terms of the over-all induced reaction rather than the apparent induced reaction. In terms of mechanism, the over-all induced reaction stands for an actual path for primary reactants to final products, whereas, there is no path whereby the apparent induced reaction can take place. In this particular case, the apparent induced reaction is thermodynamically impossible, and indeed in the absence of arsenite the reverse reaction proceeds slowly. Furthermore, induced reactions always involve some sort of competing reactions. The ratio between the rates of the two steps which are in actual competition is directly related to the relative extents of the primary reaction and of the over-all induced reaction; however, in the present instance, the competing reactions are not manifest, because in all experiments the competition between Steps 2 and 4 was entirely in favor of Step 4.

In the over-all induced reaction the inductor and acceptor, the arsenic(III) and the manganese(II), are coupled in their reaction with the actor, chromium(VI). In the classical terminology (23), the primary reaction and the apparent induced reaction are called "coupled reactions." It seems preferable to refer to the single over-all induced Reaction C as a coupled reaction, and to define a coupling factor, F_c:

$$F_c = \frac{\text{equivalents of coupled reaction taking place}}{\text{equivalents of primary reaction taking place}}$$

In the coupled reaction, itself, there is a definite ratio of equivalents of acceptor reacted to equivalents of inductor reacted; in this case, the ratio is 0.5, which is evidently the limiting induction factor in the classical terminology. However, in order to focus attention on the coupled reaction, this ratio is referred to as the coupling index, C.I.:

$$\text{C.I.} = \frac{\text{equivalents of acceptor reacting in coupled reaction}}{\text{equivalents of inductor reacting in coupled reaction}}$$

Sexivalent chromium is the actor in a number of induced reactions besides the one just considered; these induced reactions have been tabulated by Westheimer (34).

The reaction between chromium(VI) and vanadium(II) has been found to induce the oxidation of iodide ions by chromium(VI), with a coupling index of 2 (22). The data do not permit setting forth the mechanism in detail, but it may be presumed that chromium(V) is formed in the first step:

$$Cr(VI) + V(II) \longrightarrow Cr(V) + V(III) \qquad (6)$$
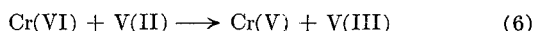
and that the chromium(V) can then react either with iodide ion, or with vanadium(II) by some as yet obscure mechanism. The coupling index of 2 arises from the fact that the active intermediate is chromium(V), so that the chromium undergoes a valence change of only 1 in the oxidation of the inductor, Cr(VI) $\longrightarrow$ Cr(V), and of 2 in the oxidation of the acceptor, Cr(V) $\longrightarrow$ Cr(III). This group of reactions is complicated by the further reaction of chromium(VI) with vanadium(III), which also induces the oxidation of iodide ions by chromium(VI), likewise with a coupling index of 2 (22). Owing to this complication, no exact kinetic treatment of the data of Luther and Rutter can be given, but their experiments, summarized in Table I, show clearly that the induction factor, F_i, approaches its limiting value of 2 as the concentration of iodide ion becomes very large relative to that of vanadium(II). Thus,

$$F_c = \frac{F_i + (F_i/\text{C.I.})}{1 - (F_i/\text{C.I.})}$$

Since the coupling index, C.I., is equal to the limiting value of F_i, F_c becomes infinite as F_i approaches its limiting value (Table I). As the ratio of iodide to vanadium(II) becomes very large, the relative extent of the two competing reactions involving these species becomes very large, and this is reflected in the very large values of F_c.

The reactions between dichromate and iodide or bromide, induced by the reaction between dichromate and ferrous iron, are particularly interesting inasmuch as dichromate is preferred to permanganate for the titration of ferrous iron because it does not attack chloride ions up to moderate concentrations. The induced reaction with iodide, studied in much greater detail than that with bromide, was first discovered in 1858 (29). It proceeds rapidly, but the possibility that the iron is acting as a simple catalyst is ruled out, because the direct reaction between the ferric and iodide ions proceeds rather slowly. The coupling index of the chromium(VI)–iron(II)–iodide ion system is 2 (25).

The mechanism of this coupled reaction involves first a reversible reaction between the actor, chromium(VI), and the inductor, iron(II) (3):

$$Cr(VI) + Fe(II) \rightleftharpoons Cr(V) + Fe(III) \qquad (7)$$
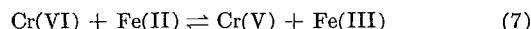
In the absence of iodide the chromium(V) is further reduced, probably by the following steps (22), of which the first is rate-controlling:

$$Cr(V) + Fe(II) \xrightarrow{k_{Fe}} Cr(IV) + Fe(III) \qquad (8)$$

$$Cr(IV) + Fe(II) \longrightarrow Cr(III) + Fe(III) \qquad (9)$$
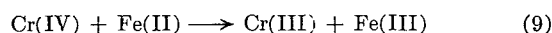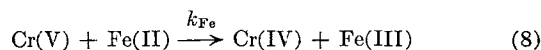
In the presence of the acceptor, iodide, the following steps can occur:

$$Cr(V) + I^- \xrightarrow{k_I} Cr(III) + IO^- \qquad (10)$$

$$IO^- + I^- + 2H^+ \longrightarrow I_2 + H_2O \qquad (11)$$
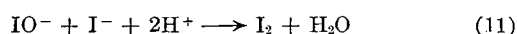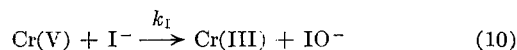
Here there is the possibility of direct competition between ferrous and iodide ions for reaction with chromium(V), according to Steps 8 and 10. This competition was investigated by Wagner and Preiss (32). These authors carried out the reaction in 1M

Table I. Coupled Reaction of Chromium(VI), Vanadium(II), and Iodide Ion

[Data of Luther and Rutter (22). Conditions, 0.009M K$_2$Cr$_2$O$_7$, approximately 0.00044M VSO$_4$, 0.007M H$_2$SO$_4$]

Concentration Ratio, I$^-$/V(II)	F_i	F_c
4.5	1.22	4.69
8.7	1.43	7.51
19.8	1.47	8.32
39.3	1.51	9.24
91.2	1.61	12.4
182.4	1.68	15.7
459	1.87	49.6
903[a]	1.96	147
1530[a]	1.99	600

[a] Concentration of VSO$_4$ lower than in the other experiments.

potassium chloride at 0° C., in order to retard the direct reaction between ferric and iodide ions. An amount of dichromate was added which was insufficient to oxidize all of the ferrous and iodide ions. After a reaction time of 1 minute, phosphoric acid was added and the iodine was titrated with thiosulfate. Wagner and Preiss calculated the ratio of the rate constants of Steps 8 and 10, taking into account the decrease in the concentrations of iron(II) and iodide ion as the reaction progressed. Typical data are given in Table II.

Table II. Coupled Reaction of Chromium(VI), Iron(II), and Iodide Ion

[Data of Wagner and Preiss (32). Conditions, 1.0M KCl, 0.04M HCl, 0.002M KI; 0.001N K$_2$Cr$_2$O$_7$]

Initial Ratio, I$^-$/Fe(II)	F_i	F_c	k_{Fe}/k_I
1.0	1.30	5.54	0.163
0.5	0.969	2.82	0.165
0.33	0.748	1.79	0.175
0.25	0.640	1.41	0.166
0.20	0.575	1.21	0.155
0.17	0.486	0.966	0.163
0.13	0.413	0.779	0.162
0.10	0.355	0.647	0.147

In the experiments of Table II, no attempt was made to reach the limiting conditions where $F_i = 2$ and $F_c = \infty$; rather, conditions were chosen so as to permit the determination of the ratio, k_{Fe}/k_I, with the greatest accuracy. This ratio is close to 0.16 in all experiments—that is, the chromium(V) reacts with the iodide ion about six times as readily as with the ferrous ion. The constancy of this ratio is good evidence that the extent of the induced reaction is controlled solely by the relative extent of the two competing steps.

In these experiments, the coupling factor, F_c, is almost directly proportional to the initial ratio of iodide to ferrous ions. This is because (a) the coupling factor, which is the relative extent of the coupled and primary reactions, must be equal to the relative extent of the actual competing steps; (b) the competing steps involve ferrous and iodide ions directly, without complications from equilibrium or side reactions or from pH; and (c) in these particular experiments the effective ratio of iodide to ferrous ions throughout each experiment was fairly close to the initial ratios. Under conditions where conditions (b) and (c) do not hold—for example, in the study of a more complex reaction, or with the actor taken in sufficient amount to react with all of the inductor—the coupling factor does not remain directly proportional to the initial ratio of acceptor to inductor. Nevertheless the coupling factor would still be equal to the relative extent to which the competing steps actually took place during the entire

course of the reaction. Thus, each time Step 10 proceeds, a chromium atom undergoes a valence change of 3 by the coupled reaction, which is the sum of Steps 7, 10, and 11, whereas for each occurrence of Step 8, a chromium atom undergoes the same valance change of 3 via the primary reaction, which is the sum of Steps 7, 8, and 9.

In studying a new induced reaction, the first goal is its characterization in terms of actor, inductor, and acceptor. The next goal is its classification as a coupled or chain reaction. The experiments required for this classification are generally sufficient to establish the coupling index, if the reaction is a coupled reaction. The experimental results can then be interpreted in terms of the coupling factor. This gives a first approach to the ratio of rate constants of the competing reactions; but further data are generally required in order to establish a kinetically valid ratio (19). The final goal, which is the complete elucidation of the mechanism, is to be sought after and approached in successive degrees, but perhaps never completely attained.

INDUCED CHAIN REACTIONS

Coupled reactions can give rise to errors of as much as two- or threefold in volumetric analyses. Much greater errors may result from the other recognized class of induced reactions. These reactions have been described empirically by Bray and Ramsey as "induced catalysis" (5); however, these authors recognized that the reactions of this type, which had been elucidated up to that time, actually proceeded by chain mechanisms. Study of other such reactions during the past 20 years permits the general conclusion that the primary reaction between the actor and inductor initiates a chain reaction between the actor and acceptor. Livingston has deplored the description of such reactions as catalyzed reactions and refers to them as induced chain reactions (21).

Induced chain reactions involving peroxides and ferrous salts are of analytical importance in view of the widely recommended use of ferrous salts as reagents for the determination of peroxides. No single method has found universal acceptance for the technically important determination of peroxides in natural products; the chemical methods in current use are based on reduction of the peroxide with ferrous salts (14, 16), iodides (8, 33, 35), arsenious oxide (30), or stannous chloride (1). Many of the early procedures for the ferrous methods give grossly erroneous results. These errors may be due to either of the following induced reactions, the one accounting for low analytical results in the absence of dissolved molecular oxygen, and the other accounting for high results in the presence of oxygen.
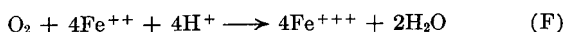
Stoichiometric Reaction
$$ROOH + 2Fe^{++} + 2H^+ \longrightarrow ROH + 2Fe^{+++} + H_2O \quad (D)$$
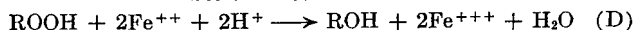
Induced Reduction of Peroxide
$$ROOH + S \longrightarrow ROH + S—O \quad (E)$$
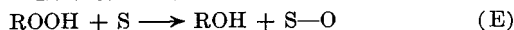
where S is the substrate and S—O is its oxidation product.
Induced Air Oxidation of Ferrous Ion

$$O_2 + 4Fe^{++} + 4H^+ \longrightarrow 4Fe^{+++} + 2H_2O \quad (F)$$

The peroxide has been represented as an organic hydroperoxide, ROOH, since peroxides of this type are those of the greatest technical importance. The course of the oxidation of the substrate—functioning as the acceptor in Reaction E—depends on the nature of the substrate, which may be the crude material (gasoline, lard, etc.) of which the peroxide content is being determined, or may be the solvent, or even the hydroperoxide molecule itself. In the scheme composed of Reactions D and E, the peroxide is the actor, and the ferrous ion is the inductor; while in the Scheme D and F, the ferrous ion is the actor, the peroxide is the inductor, and the molecular oxygen is the accep-

tor. Each of these over-all induced reactions, E and F, involves only the actor and the acceptor; this is a general distinction between induced chain reactions and coupled reactions, which involve both actor, acceptor, and inductor.

To illustrate the errors to which the ferrous method is subject, results of some peroxide determinations by various published procedures are presented in Table III. The results are expressed as per cent of the correct value, which was in each case determined iodometrically by procedures accurate to within a few per cent. The determinations which gave low results were carried out in solutions from which air had been removed; these results are expressed in terms of an induction factor which is the ratio of the extents of the over-all Reactions E and D. The determinations which gave high results were carried out in ordinary air-saturated solutions, and the induction factor used here is based on Reactions F and D. However, Reaction E may also take place to some extent even in the presence of air, so that the induction factor observed in these experiments is actually the resultant of the two over-all induced reactions. Similar considerations would apply to the reactions carried out in the nominal absence of air, if the removal of air was actually incomplete.

Table III. Peroxide Determinations by Ferrous Methods

Peroxide or Substrate	Result, %	Induction Factor B/A	Induction Factor C/A	References
Pure peroxides				
tert-Butyl hydroperoxide	690		5.9	(12),[a](16)[b]
Cumene hydroperoxide	56	0.79		(14)[a,b]
Peroxides formed in crude substrate				
Olive oil	366		2.66	(18)[a,b]
Diisobutylene	17	4.9		(33)[a], (4)[b]

[a] Reference to report of determination.
[b] Reference to procedure employed.

The large errors in the determinations of Table III are typical of procedures which are subject to induced chain reactions. For example, in the iodometric determination of vanadic acid, the primary reaction induces the air oxidation of iodide; induction factors as high as 12 have been reported (5), corresponding to an apparent result as high as 1300% of the true value. In the titration of stannous tin with dichromate, induced air oxidation of the stannous ions has been observed (20) to give induction factors of up to 57, or an apparent result equal to only 1.7% of the true value. The peroxide–ferrous ion reaction is unusual, however, in that two induced chain reactions can take place, in which either the peroxide or the ferrous ion can be the actor resulting in either low or high apparent results in the determination of the peroxide.

The mechanism proposed to account for these results in the peroxide–ferrous ion reaction is supported by the work of several groups during the past 10 years (2, 10, 13, 27, 31). The reaction of the simplest peroxide, hydrogen peroxide, with ferrous sulfate in pure aqueous sulfuric acid is considered first. The essential steps were proposed by Haber and Weiss in 1932 (7):

$$H_2O_2 + Fe^{++} \longrightarrow Fe^{+++} + OH^- + HO \cdot \quad (12)$$

$$HO \cdot + Fe^{++} \longrightarrow Fe^{+++} + OH^- \quad (13)$$
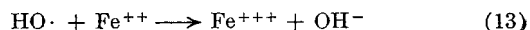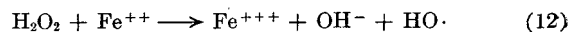
This reaction proceeds stepwise with the intermediate formation of the very reactive hydroxyl radical, HO·. Each step involves the transfer of a single electron. The sum of Steps 12 and 13 gives the stoichiometric Reaction D.

In the presence of various organic compounds, of which ethyl alcohol may be taken as typical, either high or low results are found in the peroxide determination, depending on whether oxygen is present or absent. The nature and magnitude of these

errors, as shown in Tables IV and V, are comparable to the nature and magnitude of the errors illustrated in Table III (13, 14). These errors are accounted for by induced chain reactions, which start with the reaction between the hydroxyl radical and the organic compound:

$$HO\cdot + CH_3CH_2OH \longrightarrow CH_3CHOH + H_2O \qquad (14)$$

(It is possible that the product may have the structure $CH_3CH_2O\cdot$ rather than CH_3CHOH)

In the absence of oxygen the organic radical reacts with a ferric ion:

$$CH_3CHOH + Fe^{+++} \longrightarrow CH_3CHO + H^+ + Fe^{++} \qquad (15)$$

Steps 14, 15, and 12 represent a chain mechanism whereby the over-all induced Reaction E takes place. Chain carriers are formed in each of these steps. Step 12, which is the first step of the primary reaction, forms a chain carrier (the hydroxyl radical), and hence is said to initiate the chain reaction.

It may be somewhat confusing to find that Step 12 is part of both the nonchain primary Reaction D and the induced chain Reaction E. A simpler mechanism can be written (27) in which Reaction E is composed of Steps 14 and 15′

$$CH_3CHOH + H_2O_2 \longrightarrow CH_3CHO + H_2O + HO\cdot \qquad (15')$$
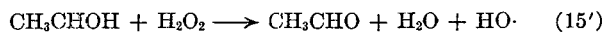
However, experiments carried out in the presence of oxygen have demonstrated that the reaction between hydrogen peroxide and the organic radical actually takes place through Steps 15 and 12, which add up to Step 15′, rather than through Step 15′ directly.

Table IV. Induced Reduction of Hydrogen Peroxide

[Reaction conditions, $[Fe^{++}]_0 = 2 \times 10^{-3}M$, $[H_2O_2]_0 = 5 \times 10^{-4}M$, in 1.5$N$ H_2SO_4 (13)]

Ethyl Alcohol, (M)	H$_2$O$_2$ Found (% of H$_2$O$_2$ Taken)	Induction Factor
10^{-4}	98	0.02
10^{-3}	82	0.22
10^{-2}	35	1.9
10^{-2}	11	8.5
10^{-1}	5.5	17

Table V. Induced Air Oxidation of Ferrous Iron

[Reaction Conditions, ethyl alcohol, 0.5M; H$_2$SO$_4$, 0.8N (26)]

Initial Concentrations ($M \times 10^5$)			H$_2$O$_2$ Found (% of H$_2$O$_2$ Taken)	Induction Factor
Fe^{++}	H$_2$O$_2$	Fe^{+++}		
2	0.036	..	1250	11.5
11	0.55	..	670	5.7
232	11.1	..	220	1.2
12	1.11	81	200	1.0
12	2.67	200	110	0.10

The chain reaction Steps 14, 15, and 12 can proceed indefinitely as long as there is any hydrogen peroxide and ethyl alcohol, until it is terminated by Step 13. The relative extent of the chain reaction—thus, the induction factor or the chain length—depends on the relative rates of the competing Steps 14 and 13, in which the hydroxyl radical reacts with either ethyl alcohol or ferrous iron, respectively. It has been established (26) in experiments carried out according to the technique of Merz and Waters (27), but in the absence of air, that the ratio of the rate constants for Steps 14 and 13 is 4; the same ratio was also found (26) for the analogous reaction with methanol in place of ethyl alcohol. Thus, in a particular experiment in which the concentration of ethyl alcohol is one half as great as that of ferrous iron, Step 14 occurs twice as often as Step 13, so that the induction factor (ratio of Reactions E and F) is equal to 2, as seen in the third experiment of Table IV. The induction factors observed in the last two experiments of Table IV are not as high as expected, probably owing to incomplete removal of oxygen.

Results in the presence of oxygen, shown in Table V, are accounted for by a mechanism in which the organic radical formed in Step 14 reacts with molecular oxygen:

$$CH_3CHOH + O_2 \longrightarrow CH_3\overset{|}{C}HOH \qquad (16)$$
$$\overset{|}{O}O\cdot$$

$$CH_3\overset{|}{C}HOH + Fe^{++} + H^+ \longrightarrow CH_3\overset{|}{C}HOH + Fe^{+++} \qquad (17)$$
$$\overset{|}{O}O\cdot \qquad\qquad\qquad\qquad \overset{|}{O}OH$$
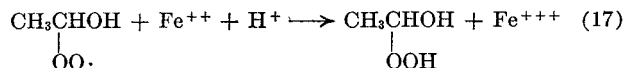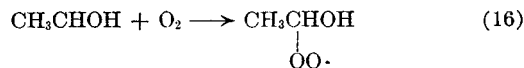
followed by various subsequent reactions of the hydroperoxide. Steps 15 and 16 are in competition, and the relative extent of these two steps—and thus of Reactions E and F—depends on the relative concentrations of ferric ions and oxygen molecules, as shown in Table V.

In the reaction between an organic hydroperoxide and ferrous iron, the first step is the formation of an alkoxy radical.

$$ROOH + Fe^{++} \longrightarrow RO\cdot + Fe^{+++} + OH^- \qquad (18)$$
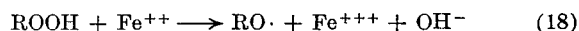
Some of the subsequent reactions are analogous to those presented above, with the alkoxy radical abstracting a hydrogen atom from the substrate or from another hydroperoxide molecule. Other reactions may involve the rearrangement of the various radicals (10).

From the standpoint of analytical chemistry, the detailed mechanisms of the induced chain reactions are of value chiefly in suggesting methods for the elimination of these reactions. Two approaches have been pursued to the point where this can be done in special cases, permitting the accurate determination of the peroxide in these cases.

The first approach makes use of chain-transfer reactions, by means of which the induced chain reactions can be suppressed. For example, in the presence of bromide ion, Step 12 is followed by Steps 19 and 29:

$$Br^- + HO\cdot \longrightarrow Br\cdot + OH^- \qquad (19)$$

$$Br\cdot + Fe^{++} \longrightarrow Br^- + Fe^{+++} \qquad (20)$$

The sum of Steps 19 and 20 is equivalent to Step 13, so that the bromide ion is in effect a catalyst for Step 13. Step 19 is referred to as a chain-transfer step, inasmuch as it involves transfer of the chain-carrying function from the hydroxyl radical to the bromide ion. If the concentration of bromide is sufficiently high relative to that of ethyl alcohol, then virtually all the hydroxyl radicals react according to Step 19 rather than according to the competing Step 14. The bromide atom formed in Step 19 reacts exclusively according to the chain-terminating Step 20, rather than entering into any chain-propagating steps analogous to 14 and 15. Thus the net reaction becomes the sum of Steps 12, 19, and 20, which is the stoichiometric Reaction D.

The suppressing action of the bromide ion has been put to practical use in the determination of persulfate by reduction with ferrous iron. Here a sulfate radical ion, $SO_4\cdot^-$, is formed in the first step:

$$S_2O_8^{--} + Fe^{++} \longrightarrow Fe^{+++} + SO_4^{--} + SO_4\cdot^- \qquad (21)$$
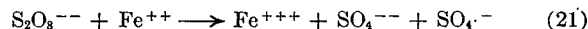
This sulfate radical ion can undergo subsequent reactions analogous to those of the hydroxyl radical. Thus, erroneous results are observed in the presence of ethyl alcohol; but by the addition of bromide in a concentration of 1M, persulfate can be determined in the presence of as much as 0.25M ethyl alcohol, with an accuracy of 5 parts per thousand (11). This procedure is stated (11) to offer certain advantages over alternative procedures—e.g., iodometric—for the determination of persulfate in the presence of ethyl alcohol.
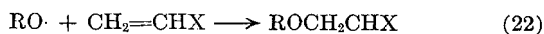
Other substances which act as suppressors include chloride ion, acetone, and acetic acid. The last two are effective only in the absence of oxygen, whereas chloride and bromide are

effective in either the presence or absence of oxygen. By using acetone as a solvent and suppressor (in the absence of oxygen), cumene hydroperoxide can be determined satisfactorily in a colorimetric procedure based on the oxidation of ferrous iron (14). However, this procedure does not give accurate results with peroxides in fats and oils.
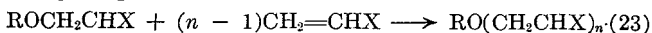
The addition of a suppressor can force the reaction between a peroxide and ferrous iron to proceed in the molar ratio of 1 to 2, as expressed in Equation D. In another approach, the addition of a monomer has been used to force the reaction between a peroxide and ferrous iron to proceed in the molar ratio of 1 to 1, as expressed in Equation G:

$$2ROOH + 2Fe^{++} + 2nCH_2{=}CHX \longrightarrow$$
$$2Fe^{+++} + 2OH^- + RO[CH_2CHX]_{2n}OR \quad (G)$$
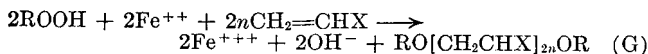
The mechanism of the over-all Reaction G is of course the familiar free radical chain polymerization, with initiation by Step 18 followed by:

$$RO{\cdot} + CH_2{=}CHX \longrightarrow ROCH_2CHX \quad (22)$$

then propagation:

$$ROCH_2CHX + (n-1)CH_2{=}CHX \longrightarrow RO(CH_2CHX)_n{\cdot}(23)$$

and termination:

$$2RO(CH_2CHX)_n{\cdot} \longrightarrow RO(CH_2CHX)_{2n}OR \quad (24)$$
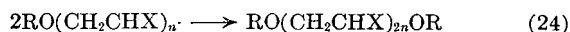
Thus, the reaction between cumene hydroperoxide and ferrous iron has been made to proceed quantitatively according to Equation G by the use of acrylonitrile as the monomer (6) in the absence of oxygen.

From the standpoint of the acrylonitrile, Equation G represents an induced chain reaction, since the polymerization of acrylonitrile is a chain reaction which is induced by the peroxide-iron reaction. However, this chain reaction (Steps 23 and 24) does not involve either of the primary reactants (the peroxide and the ferrous iron). From the standpoint of the primary reactants, the over-all induced Reaction G is simply a coupled reaction, with a coupling index of unity.

From the practical analytical standpoint, this illustrates an important difference between coupled reactions and induced chain reactions. In the absence of acrylonitrile, the over-all reaction consists of the primary Reaction D and the induced chain Reaction E, which, because they proceed in an indefinite ratio, give an indefinite stoichiometry. In the presence of sufficient acrylonitrile, the primary Reaction D is completely displaced by the coupled Reaction G, which has a definite stoichiometry with regard to the primary reactants and therefore

can serve for the determination of one of these reactants by means of the other.

LITERATURE CITED

(1) Barnard, D., and Hargrave, K. R., *Anal. Chim. Acta*, 5, 476 (1951).
(2) Baxendale, J. H., Evans, M. G., and Park, G. S., *Trans. Faraday Soc.*, 42, 155 (1946).
(3) Benson, Clara, *J. Phys. Chem.*, 7, 1, 356 (1903); 8, 116 (1904).
(4) Bolland, J. L., Sundralingam, A., Sutton, D. A., and Tristram, G. R., *Trans. Inst. Rubber Ind.*, 17, 29 (1941).
(5) Bray, W. C., and Ramsey, J. B., *J. Am. Chem. Soc.*, 55, 2279 (1933).
(6) Fordham, J. W. L., and Williams, H. L., *J. Am. Chem. Soc.*, 73, 1634 (1951).
(7) Haber, F., and Weiss, J., *Naturwissenschaften*, 20, 948 (1932); *Proc. Roy. Soc. (London)*, A147, 332 (1934).
(8) Hartman, L., and White, M. D. L., ANAL. CHEM., 24, 527 (1952).
(9) Kessler, F., *Pogg. Ann.*, 119, 218 (1863); 95, 224 (1855).
(10) Kharasch, M. S., Fono, A., and Nudenberg, W., *J. Org. Chem.*, 15, 763 (1950).
(11) Kolthoff, I. M., and Carr, E. M., ANAL. CHEM., 25, 298 (1953).
(12) Kolthoff, I. M., and Laitinen, H. A., private communication, cited in (14).
(13) Kolthoff, I. M., and Medalia, A. I., *J. Am. Chem. Soc.*, 71, 3777, 3784, 3789 (1949).
(14) Kolthoff, I. M., and Medalia, A. I., ANAL. CHEM., 23, 59t (1951).
(15) Kolthoff, I. M., and Stenger, V. A., "Volumetric Analysis," vol. I, 2nd ed., p. 168, Interscience, New York, 1942.
(16) Laitinen, H. A., and Nelson, J. S., IND. ENG. CHEM., ANAL..ED., 18, 422 (1946).
(17) Lang, R., and Zwerina, J., *Z. anorg. u. allgem. Chem.*, 170, 389 (1928).
(18) Lea, C. H., *J. Soc. Chem. Ind.*, 64, 106 (1945).
(19) Lee, T. S., in Friess, S. L., and Weissberger, A., eds., "Technique of Organic Chemistry," vol. VIII, pp. 100–30, Interscience, New York, 1953.
(20) Lensson, E., and Löwenthal, J., *J. prakt. Chem.*, 87, 193 (1862).
(21) Livingston, Robert, in Friess, S. L., and Weissberger, A., eds., "Technique of Organic Chemistry," vol. VIII, pp. 219, 224, Interscience, New York, 1953.
(22) Luther, R., and Rutter, T. F., *Z. anorg. Chem.*, 54, 1 (1907).
(23) Luther, R., and Schilow, N., *Z. physik. Chem.*, 46, 777 (1903).
(24) Manchot, W., *Ann. Chem. Justus Liebig*, 325, 93 (1902).
(25) Manchot, W., and Wilhelms, O., *Ibid.*, 325, 105 (1902).
(26) Medalia, A. I., unpublished experiments.
(27) Merz, J. H., and Waters, W. A., *J. Chem. Soc.*, 1949, S15.
(28) Schönbein, C. F., *J. prakt. Chem.*, 75, 108 (1858).
(29) Schönbein, C. F., *Pogg. Ann.*, 100, 34 (1857).
(30) Siggia, S., ANAL. CHEM., 19, 827 (1947).
(31) Stein, G., and Weiss, J., *J. Chem. Soc.*, 1951, 3265.
(32) Wagner, C., and Preiss, W., *Z. anorg. u. allgem. Chem.*, 168, 265 (1928).
(33) Wagner, C. D., Smith, R. H., and Peters, E. D., ANAL. CHEM., 19, 976, 982 (1947).
(34) Westheimer, F., *Chem. Rev.*, 45, 419 (1949).
(35) Wheeler, D. H., *Oil & Soap*, 9, 89 (1932).

8th Annual Summer Symposium—Role of Reaction Rates

Kinetic Behavior of Halide Complexes

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The stepwise hydrolysis of halide complexes is discussed from the kinetic point of view covering the following subjects: experimental methods for studying the kinetics, structural factors affecting the rate, and mechanistic features of the reaction. Examples are also given of the utilization of kinetic data in identifying ionic species in solution.

WHEN metal halides are dissolved in the corresponding halogen acid it is a common reaction for the metal to become part of a complex halo anion. Isolation of salts containing the halo anion has been performed in a sufficient number of cases to leave little doubt as to the great prevalence of this type of reaction. Evidence that such ions also exist in solution has been obtained by several different physical methods. It is only

the most electropositive elements that do not seem to form complexes of this type.

It is also a well-known fact that if such solutions are diluted the halo complexes generally decompose, and in many cases a precipitate forms. The nature of the precipitate depends on the metallic element; it may be a basic salt, hydroxide, or hydrous oxide, and often its actual nature is difficult to determine. In accordance with the modern concepts of complex ions it is believed that the transition from the halo complex to the final product must proceed in several discrete steps, the initial steps of which are successive replacements of the halogen atoms in the complex. In a few favorable cases the stepwise hydrolysis products have been isolated. For example, in the case of chromium(III), salts containing the anions, $CrCl_6^{---}$ and $Cr(H_2O)Cl_5^{--}$, have been isolated. With platinum(IV), salts containing the ions, $PtCl_6^{--}$, $Pt(OH)Cl_5^{--}$, $Pt(OH)_2Cl_4^{--}$, etc., are known.

These two examples illustrate that the hydrolytic species, whether present in solution or isolated as salts, will be of a form where halide ions have been replaced by either a water molecule or hydroxide ion. Which of these forms appears depends merely upon whether the ion $M(H_2O)X_y^{-z}$ is a strong acid or weak acid under the conditions of the experiment. Presumably the case might also arise where the acid strength could be such that comparable amounts of $M(H_2O)X_y^{-z}$ and $M(OH)X_y^{-(z+1)}$ would be present under the experimental conditions.

Although only a few good examples of this stepwise hydrolysis can be demonstrated by chemical isolation, it is assumed that this is a general type of behavior. In some favorable cases, like that of the complexes of antimony(V) in hydrochloric acid (7), it is possible to demonstrate this type of behavior from physical evidence even when isolation is not possible.

From the kinetic point of view each step in the hydrolysis is merely a particular substitution reaction; hence some of the general features of substitution reactions are worth recalling. Taube (13), in reviewing these reactions, has divided complexes into two classes on the basis of their electronic structure. This classification is made by considering that there is some covalent character to the metal-halide bond, and that this covalent character can be described in terms of the occupancy of certain orbitals of the metal ion by electrons from the ligand. For coordination number six, one S, three P, and two D orbitals are involved. If the orbitals are $(n - 1) D^2 nS nP^3$, the complex is called an "inner orbital" complex; if the orbitals are $nS nP^3 nD^2$, the complex is called an "outer orbital" complex.

Reactions of inner orbital complexes, in general, are rapid if at least one of the three remaining $(n - 1) D$ orbitals is not occupied by electrons from the metal. If these $(n - 1) D$ orbitals are occupied, the complex gives slow reactions in general. Several halo complexes of this last type, giving slow rates of hydrolysis and slow rates of substitution, are known. Probably the most familiar hydrolysis reaction falling into this category is the slow conversion of chromium(III) from the green form in concentrated hydrochloric acid to the violet form in very dilute acid.

Most complexes of the outer orbital type exhibit rapid reactions, but if the central atom of the complex is sufficiently electronegative, resulting in considerable covalent character to the metal-ligand bond, the substitution reactions become slow enough to be measurable. Most of the kinetic data to be discussed are for an ion of this last type, $SbCl_6^-$. It is well to bear in mind that the mechanistic features displayed in the hydrolysis of $SbCl_6^-$ may not be characteristic of all halo complexes, and possibly may not even be characteristic of all outer orbital halo complexes.

METHODS FOR MEASURMENT OF KINETICS

A spectrophotometric method of following the kinetic has proved most successful in the case of $SbCl_6^-$. Since all halo complexes exhibit a "charge-transfer" absorption in the ultraviolet region, this method should be of general utility in studying the hydrolysis of these ions. Because these complexes are generally studied in the presence of the corresponding halogen acid the wave length region, which can be observed, is primarily determined by the absorption of the acid itself. For chloro complexes in hydrochloric acid the lower wave length limit imposed by the solvent is about 210 mμ, varying somewhat with the acid concentration and the instrument. In general the molar absorbance index at an absorption maximum is the order of 10,000, so that solutions as dilute as $10^{-4} M$ in the complex can be used satisfactorily.

The general characteristics of this absorption in the ultraviolet region can be seen from the several examples in the papers by Rabinowitch (10) and by Rogers and coworkers (2, 5). The spectral characteristics of antimony(V) and its dependence on time are indicated in a previous article (7). In actual rate studies the change in absorbance after dilution can be observed at several selected wave lengths. The results of several experiments (9) of this type are discussed in the next section in connection with the mechanism of the hydrolysis. The methods used to obtain the desired rate constants from the experimental data will now be indicated without going into detail. If the reaction goes to completion—i.e., essentially all of the $SbCl_6^-$ is converted to one or another of the hydrolytic species—a plot of log $(D - D_\infty)$ vs. time gives a straight line from whose slope the rate constant, k_h, for hydrolysis can be obtained. If the reaction goes only to an equilibrium [where an appreciable fraction of antimony(V) still remains as $SbCl_6^-$], the log $(D - D_\infty)$ vs. time plot gives $(k_h + k_f)$, where k_f is the pseudo-first-order rate constant for the formation of $SbCl_6^-$ from the first hydrolytic species, $Sb(OH)Cl_5^-$. One can obtain k_h from the same experimental data by plotting log $(D - D_P)$ vs. time, where D_P is the absorbance the system would have if all the $SbCl_6^-$ had been converted to the first hydrolysis product. This plot is not a straight line, but from its initial slope one can obtain k_h. In such a reaction it is thus possible to obtain both k_h and k_f.

Two other methods (11) have been used for studying this reaction, but they are not as convenient or satisfactory as the spectrophotometric method. The concentration of $SbCl_6^-$ during the hydrolysis reaction can be determined by a polarographic method, because the $SbCl_6^-$ ion is reversibly reduced, giving a well-defined diffusion current. The hydrolytic species do not interfere since they are reduced irreversibly at more negative potentials. The second method utilizes the fact that $SbCl_6^-$ can be extracted (with an appropriate cation to form an ion pair) into organic solvents, whereas the hydrolytic forms are not extracted appreciably. The choice of cation and solvent is, of course, important; the protonated form of Rhodamine B as cation and benzene as solvent have been used for quantitative determination of $SbCl_6^-$ (11).

Whether the last two methods could be used for other hydrolysis reactions would require investigation of the properties of the halo ions and their hydrolysis products. These methods would not seem to have the general utility of the spectrophotometric method.

MECHANISTIC FEATURES OF HYDROLYSIS OF SbCl$_6$−

The reaction is, as expected, first order in $SbCl_6^-$. In any given experiment the concentration of all other reactants was far in excess of that of $SbCl_6^-$, hence each reaction appeared pseudo-first-order. The effect of other reactants was determined by observing the change in the pseudo-first-order rate constant, k_h, resulting from varying their concentrations from one experiment to another. Because the dependence on water remains undetermined, it is impossible to say whether the water molecule enters the complex simultaneously with or subsequent to the loss of the chloride ion. Or, in other words, it is impossible to say whether the reaction is of the SN-1 type or of the SN-2 type.

There is a statement in the earlier literature (14), based on the observation of formation of precipitate, that the rate of hydrolysis of $SbCl_6^-$ is decreased by increasing acidity. From equilibrium data (7) it is apparent that formation of a precipitate is not a valid measurement of the hydrolysis of $SbCl_6^-$; in $6M$ hydrochloric acid $SbCl_6^-$ is about 99% hydrolyzed, but there is no formation of precipitate. And even at acidities where all of the antimony(V) precipitates the rate of hydrolysis of $SbCl_6^-$ may not be the rate-determining step in the formation of the precipitate.

Cheek (1) has shown that increasing acidity actually increases the rate of hydrolysis. His experiments were performed in hydrochloric acid of varying concentrations, so that $[H^+]$, $[Cl^-]$, and ionic strength were all varying. To observe the exact nature of this hydrogen ion dependence, experiments have now been performed (9) in solutions of constant $[Cl^-]$, but varying $[H^+]$, by use of hydrochloric acid–lithium chloride mixtures. At a total chloride concentration of $6M$ the rate constant observed is given by the expression $k_h = (3.9 + 0.8\ [H^+]) \times 10^{-3}$ min.$^{-1}$. At a total chloride concentration of $9M$ the expression $k_h = (5.3 + 1.6\ [H^+]) \times 10^{-3}$ min.$^{-1}$ was found to hold.
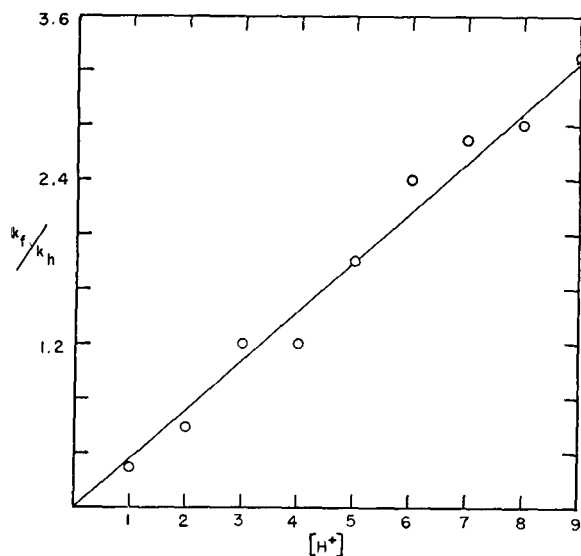
Figure 1. k_f/k_h as a function of $[H^+]$ for solutions $9M$ in chloride ion

There are then two mechanisms operative under these conditions. The first is independent of hydrogen ion, and is the reaction of $SbCl_6^-$ by either an SN-1 or SN-2 mechanism. The role of the hydrogen ion in the second, and hydrogen ion dependent, mechanism is undoubtedly attack on the chloride. The data thus point to the existence of a species $HSbCl_6$, but whether this is a true chloro-acid of reasonable lifetime, or is better described as merely a transition state, cannot be decided.

It was also observed that the presence of antimony(III) accelerated the hydrolysis of $SbCl_6^-$. The most reasonable explanation of this effect, and one that is consistent with the fact that the acceleration is greater at lower acidities, is that antimony trichloride is the effective form of antimony(III). Its action, like that of hydrogen ion, would be in abstracting a chloride ion from the complex. From the magnitude of the effects observed antimony trichloride is several orders of magnitude more effective than hydrogen ion in this action.

These results suggest that any Lewis acid should be effective in the same way. The difficulty with aqueous systems is, of course, the fact that few Lewis acids exist as such in water, and

the stronger the acid is the less likely it is to exist. Experiments were tried with tin(IV), iron(III), and aluminum(III), but in none of these was there any effect on the hydrolysis that could be attributed to the corresponding chlorides.

USE OF KINETIC DATA TO IDENTIFY SPECIES IN SOLUTION

Occasionally kinetic data give useful evidence about the ionic species in solution, although it rarely is conclusive unless supported by other evidence. Two examples of such a utilization of kinetic data are given.

The first example comes again from the antimony(V) system, and concerns the identity of the first hydrolysis product. It has been stated that this product is $Sb(OH)Cl_5^-$, without indicating any of the evidence. The kinetic evidence comes from measurements of the rates of hydrolysis and formation of $SbCl_6^-$ in solutions $9M$ in chloride ion, and of varying hydrogen ion concentration. In solutions of such high chloride concentration the hydrolysis, from the point of view of equilibrium, does not go beyond the first step.

On the basis of the discussion of the types of hydrolytic species to be expected, it is reasonable to assume that the first hydrolysis product is either $Sb(OH)Cl_5^-$ or $Sb(H_2O)Cl_5$. If the product is $Sb(OH)Cl_5^-$ then the rate of formation of $SbCl_6^-$ is equal to $k_f[Sb(OH)Cl_5^-]$, and the rate of its hydrolysis is equal to $k_h[SbCl_6^-]$. At equilibrium the two rates are equal, and $[SbCl_6^-]/[Sb(OH)Cl_5^-] = k_f/k_h$. Also at equilibrium

$K = \dfrac{a_{H_2O}a_{SbCl_6^-}}{a_{Sb(OH)Cl_5^-}a_{H^+}a_{Cl^-}}$. Although it is not completely certain that the use of lithium chloride–hydrochloric acid mixtures of constant chloride concentration guarantee a constancy of the activity coefficients, it is certainly the best that can be done experimentally in this regard. Assuming that the coefficients are reasonably constant

$K' = \dfrac{[SbCl_6^-]}{[Sb(OH)Cl_5^-][H^+]}$. The ratio $\dfrac{[SbCl_6^-]}{[Sb(OH)Cl_5^-]} =$

$$K'[H^+] = \frac{k_f}{k_h}$$

A similar analysis for the assumption that the first hydrolysis product is $Sb(H_2O)Cl_5$ gives

$$\frac{[SbCl_6^-]}{[Sb(H_2O)Cl_5]} = \frac{k_f}{k_h},$$

$$K = \frac{a_{H_2O}a_{SbCl_6^-}}{a_{Sb(H_2O)Cl_5}a_{Cl^-}}, \text{ and } K' = \frac{[SbCl_6^-]}{[Sb(H_2O)Cl_5]}$$

Figure 1 shows the experimentally observed dependence of k_f/k_h on $[H^+]$. The data can be seen to satisfy well the relationship $k_f/k_h = K'[H^+]$. The kinetic evidence then supports the position that $Sb(OH)Cl_5^-$ is the first hydrolytic species. All the equilibrium evidence (7) also supports this position.

The second example of the use of kinetic evidence for identification purposes arises in regard to the chemistry of the synthetic element astatine (8). The half life of this element is such that the chemistry of the element can only be studied on the tracer scale, and hence conclusive evidence in regard to oxidation state and ionic form is difficult to obtain. If a solution of astatine in concentrated hydrochloric acid is treated with chlorine, the astatine is converted to a form that can be extracted into ethers. This behavior suggests that the astatine is present as either $AtCl_6^-$ or $AtCl_4^-$. (The ether extractability of both of these would be expected because of their similarity to $SbCl_6^-$ and and ICl_4^-.) If the ion is $AtCl_6^-$ it should display a slower rate of hydrolysis than $SbCl_6^-$, because of the greater electronegativity of astatine(V) compared to antimony(V), and the rate could be measured by extraction experiments. Actually the extraction experiments indicate a rapid reaction. On this basis it is unlikely that the astatine exists as $AtCl_6^-$, but rather it appears to be

AtCl$_4$$^-$. In this regard the behavior of astatine is identical to that of iodine, which forms ICl$_4$$^-$ under the same conditions.

GENERALIZATIONS AND CORRELATIONS

For complexes of the outer orbital type, and which differ only in the central atom, slower rates correspond to increased covalent character in the metal-halide bond. There is ample evidence of this from substitution reactions in general (13), and this generalization has been used in the argument concerning the form of astatine in the last section.

If complexes of a given metal differing only in the halide ligand are considered, one might be inclined from the statement of the previous paragraph to feel that chloro complexes hydrolyze more rapidly than bromo complexes, because the latter are more covalent. It is more likely, however, that the rates of halide replacement in these inorganic systems parallel those in organic halides—that is, iodo complexes hydrolyze more rapidly than bromo complexes, which in turn hydrolyze more rapidly than chloro complexes, etc. In these comparisons extent of covalent character is probably not the determining factor; polarizability and ion size are probably more important.

It had been reported (7) that the hydrolysis of Sb(OH)Cl$_5$$^-$ is more rapid than that of SbCl$_6$$^-$. Unfortunately no comparative rate data are available for Sb(OH)Cl$_5$$^-$ and Sb(OH)$_2$Cl$_4$$^-$. If the trend continues in the direction indicated it would suggest that the remaining metal-halide bonds become more ionic as successive halide ions are removed, leading to the faster rates of hydrolysis.

Although the metal-oxygen bond is different from the metal-halide bond, similarities may be expected between the reactions of inorganic oxy anions and the halo anions. The reaction in the oxygen system corresponding to the hydrolysis of the halide system is the oxygen exchange between water and the oxy anion. Acceleration of the oxygen exchange by hydrogen ion has been observed for many oxy anions (3, 6), and mechanistically the effect of hydrogen ion can be visualized to be the same in the two cases.

There are several examples of halide substitution being accelerated by Lewis acids, similar to the influence of antimony trichloride on the hydrolysis of SbCl$_6$$^-$. In the organic field Lewis acids, most commonly Ag$^+$ and mercuric chloride, are often used to accelerate the hydrolysis of alkyl halides (4). An example in inorganic systems is the catalytic effect of platinum(III) on the radioactive exchange between Cl$^-$ and PtCl$_6$$^{--}$ (12).

There is still much to be learned about these reactions.

LITERATURE CITED

(1) Cheek, C. H., Ph.D. thesis, Washington University, St. Louis, Mo., January 1953.
(2) DeSesa, M. A., and Rogers, L. B., Anal. Chim. Acta, 6, 534 (1952).
(3) Hall, N. F., and Alexander, O. R., J. Am. Chem. Soc., 62, 3455 (1940).
(4) Hughes, E. D., Quart. Revs. (London), V, 245 (1951).
(5) Merritt, C., Hershenson, H. M., and Rogers, L. B., ANAL. CHEM., 25, 572 (1953).
(6) Mills, G. A., J. Am. Chem. Soc., 62, 2833 (1940).
(7) Neumann, H. M., Ibid., 76, 2611 (1954).
(8) Neumann, H. M., unpublished data.
(9) Neumann, H. M., and Ramette, R. W., unpublished data.
(10) Rabinowitch, E., Revs. Mod. Phys., 14, 112 (1942).
(11) Ramette, R. W., Ph.D. thesis, University of Minnesota, Minneapolis, Minn., June 1954.
(12) Rich, R. L., and Taube, H., J. Am. Chem. Soc., 76, 2608 (1954).
(13) Taube, Henry, Chem. Revs., 50, 69 (1952).
(14) Weinland, R. F., and Schmid, H., Z. anorg. Chem., 44, 37 (1903).

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8th Annual Summer Symposium—Role of Reaction Rates

Competing Rates of Oxime Formation

Determination of Aromatic Aldehydes in Presence of Aromatic Ketones

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In the analysis of mixtures of organic compounds containing a common functional group, methods based on differences in rates of reaction with a common reagent are particularly useful. The example of the rates of oxime formation with mixed aromatic aldehydes and ketones, specifically vanillin and acetovanillone, illustrates the general technique. Calibration curves relate the concentration at a chosen time with the original concentration. The calculation of the extent of reaction with time is illustrated. The selection of the optimum conditions of reaction is described.

THE usual methods of analysis applied to mixtures of two constituents which differ only slightly in chemical properties often lead to unsatisfactory results. This situation occurs frequently in organic syntheses where two homologous products having the same functional group are obtained. It is not always practicable to utilize a physical method for quantitative analysis of such mixtures.

A technique which can sometimes solve these problems depends on the fact that minor differences in substituents or structure frequently cause pronounced changes in the relative rates of reaction with a given reagent. Kolthoff and Lee (5–7) cite examples of the use of this technique for the analysis of mixtures of esters, aldehydes, ethylenic compounds, etc. Hass and Weber (4) analyzed mixtures of primary isoamyl chlorides by the rate of reaction with potassium iodide.

A method based on the application of rates of oxime formation enables vanillin to be determined simply in the presence of acetovanillone (4'-hydroxy-3'-methoxyacetophenone) with excellent precision and accuracy (2). p-Hydroxybenzaldehyde and syringaldehyde behave quantitatively like vanillin, and acetosyringone (4'-hydroxy-3',5'-dimethoxyacetophenone) behaves like acetovanillone. Trials of the method (without attempting to determine optimum conditions) were made on several aromatic aldehyde-ketone pairs with favorable results (Table I). The principles underlying this example illustrate the general criteria for using competitive reaction rates as an analytical tool.

The reaction of carbonyl compounds with hydroxylamine to form oxime is second order (3). There is general acid catalysis, but the use of half-neutralized hydroxylamine hydrochloride in excess buffers the system sufficiently to make this effect negligible (10). The relative rates for a number of aromatic aldehydes and ketones have been compared by Vavon and Monthéard

(11). Lester *(8)* has studied the effects of substituents on the ketones. Mitchell *(9)* and Bryant and Smith *(1)* have shown that in general the reaction with aldehydes goes to analytical completion much faster than with ketones.
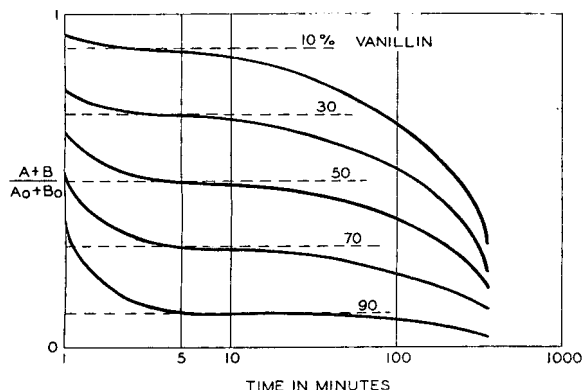

Figure 1. Calculated carbonyl concentration as function of reaction time for various starting concentrations of vanillin

Where a mixture of an aldehyde and a ketone is reacted with hydroxylamine, the rate equations are:

$$-d(A)/dt = k'(A)(R) \tag{1}$$

$$-d(B)/dt = k''(B)(R) \tag{2}$$

$$-d(R)/dt = [k'(A) + k''(B)](R) \tag{3}$$

where (A) is the concentration of the aldehyde, such as vanillin, (B) is the concentration of the ketone, acetovanillone, and (R) is the concentration of the reagent, hydroxylamine. The general solution of Equation 3, so that a measurement of R, or of $A + B$, at any time enables the original ratio $A_o/(A_o + B_o)$ to be determined, is too complex to be of much use. A special solution, where $R_o = A_o + B_o$, is given by Kolthoff and Lee *(5)*, but it is not always convenient to arrange the reaction conditions in this way. These authors have also considered the case where reactions are first order, in which case the integrated equation is

$$A + B = A_o e^{-k't} + B_o e^{-k''t} \tag{4}$$

Where the reactants are in very large excess over the reagent, second-order reactions become pseudo-unimolecular and Equation 4 can be used. Where the reagent is in very large excess over the reactants, the reaction is again pseudo-unimolecular, but the integrated equation is

$$A + B = A_o e^{-R_o k't} + B_o e^{-R_o k''t} \tag{5}$$

In these latter three cases a knowledge of k', k'', R_o and $A_o + B_o$ (found by running the reaction to completion, for instance) enables a calibration plot of $(A + B)/(A_o + B_o)$ vs. $A_o/(A_o + B_o)$ for any chosen time of reaction to be calculated. A basic re-

quirement is that k' shall not equal k''. The larger the ratio k'/k'' is, the more suitable the technique and the better the accuracy that can be obtained.

In the example under consideration, the operating conditions are such that the rate constant, k', for the aldehyde is several hundred times as large as that for the ketone, k''. Exact details of the procedure are given elsewhere *(2)*, but essentially an $0.1M$ solution of aldehyde plus ketone in alcohol solution is reacted for a chosen time and temperature with $0.3M$ half-neutralized hydroxylamine hydrochloride. The excess of hydroxylamine is then determined by titration with alcoholic hydrochloric acid and the carbonyl content calculated. Experiments made at various concentrations and at temperatures of 5° and 25° C. gave values for the specific rate constants of: $k' = 5 \times 10^{10}$ $e^{-13,000/RT}$ and $k'' = 10^9 e^{-14,000/RT}$, for vanillin and acetovanillone, respectively. No great precision can be claimed for these figures, but they are adequate for the purpose and are in agreement with data for similar compounds *(8, 10, 11)*. At 25° C., the specific rate constants are $k' = 23$ and $k'' = 0.06$ liter per mole minute.
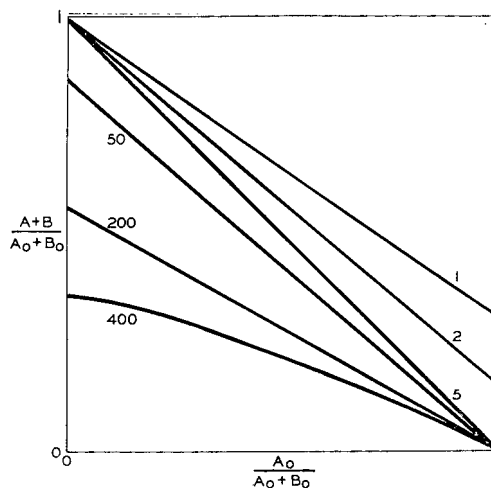

Figure 2. Calculated carbonyl concentration as function of initial concentration of vanillin for reaction times from 1 to 400 minutes

At 5° C., $k' = 4.5$ and $k'' = 0.010$. The ratio k'/k'' thus is about 400. Ratios of this magnitude apparently are general for aromatic aldehyde-ketone systems.

When the ratio of the rate constants is greater than 25, and particularly when it exceeds 100, a simplifying assumption can be made to eliminate the integration of Equation 3. It may be assumed that the reaction of A (the aldehyde) is substantially complete before any appreciable amount of B (the ketone) is reacted. Thus, Equation 1 can be integrated with concentrations $A = A_o$ and $R = R_o$ at $t = 0$ and Equation 2 with concentrations $B = B_o$ and $R = (R_o - A_o)$ at $t = 0$. The resultant equations are:

$$k't = \frac{2.303}{(R_o - A_o)} \log \frac{A_0}{R_0} \frac{(R_0 - A_0 + A)}{A} \tag{6}$$

$$k''t = \frac{2.303}{(R_o - A_o - B_o)} \log \frac{A_o}{(R_o - A_o)} \frac{(R_o - A_o - B_o + B)}{B} \tag{7}$$

If substitutions are made in these equations of various $A_o/(A_o + B_o)$ ratios, $(A + B)/(A_o + B_o)$ as a function of the time of reaction can be calculated. Figure 1 shows the results of such a series of calculations, using the rate constants for 5° C.

Table I. Aldehyde Content of Aldehyde-Ketone Mixtures

Aldehyde	Ketone	% Aldehyde[a] Taken	% Aldehyde[a] Found
Salicylaldehyde	Acetophenone	88.5	87.3
Benzaldehyde	Acetophenone	86.3	87.9
	o-Hydroxyacetophenone	85.0	84.7
Ethylvanillin	o-Hydroxyacetophenone	88.8	87.9
	m-Nitroacetophenone	89.9	90.2
p-Nitrobenzaldehyde	m-Nitroacetophenone	90.0	84.4

[a] Av. of duplicates.   Std. dev. 0.4%.

An examination of Figure 1 shows that the assumption of independence of reaction is well justified in the region of 90% aldehyde, (as vanillin), and that it should be possible, using a time of 5 to 10 minutes, to determine the vanillin concentration as if it were the only carbonyl present. That is, where $A_o/(A_o + B_o)$ is near 0.9, it is not necessary to determine $A_o + B_o$ in this case.

Figure 2 shows plots of $(A + B)/(A_o + B_o)$ as a function of $A_o/(A_o + B_o)$ for various times of reaction. For any given time, the plot constitutes a calibration curve by which the original $A_o/(A_o + B_o)$ could be obtained from the determination of the remaining $(A + B)/(A_o + B_o)$. The slope of the calibration curve is a measure of the precision obtainable by the method. The error of measurement in $(A + B)/(A_o + B_o)$ will be propagated into the desired answer, $A_o/(A_o + B_o)$, approximately as dictated by the right triangle for which the calibration curve determines the hypotenuse and the error in $(A + B)/(A_o + B_o)$, the ordinate. In instances where the calculations are not as simple as here, an experimental curve relating the variables can be constructed.

An approximate check of the optimum time of reaction is afforded by the equation offered by Kolthoff and Lee for the situation $R_o >> (A_o + B_o)$:

$$\text{Optimum time} = \frac{\ln(k'/k'')}{R_o(k' - k'')} \qquad (8)$$

Substitution of the values for 5° C. gives 4.5 minutes as the time giving the maximum slope to the calibration curve, which is confirmed by the reported (2) experimental finding that at less than 3 minutes or more than 9 the results diverged from known values by more than the experimental error, with 5 minutes as a good working choice. If the data for 25° C. are substituted in

Equation 8, the calculated optimum time turns out to be about 30 seconds. This is confirmed by the experimental finding (2) that the results are unreliable at room temperature. Although the gain in k'/k'' (450 vs. 400) obtained by lowering the temperature in this case is unimportant, the gain in operating convenience is marked.

LITERATURE CITED

(1) Bryant, W. M. D., and Smith, D. M., J. Am. Chem. Soc., 57, 57 (1935).
(2) Fowler, L., Kline, H. R., and Mitchell, R. S., ANAL. CHEM., 27, 1688 (1955).
(3) Hammett, L. P., "Physical Organic Chemistry," McGraw-Hill, New York, 1940.
(4) Hass, H. B., and Weber, P., IND. ENG. CHEM., ANAL. ED., 7, 231 (1935).
(5) Kolthoff, I. M., and Lee, T. S., Ann. N. Y. Acad. Sci., 53, 1093 (1951).
(6) Lee, T. S., "Organic Analysis," vol. 2, Mitchell, J., Kolthoff, I. M., Proskauer, E. S., and Weissberger, A., eds., Interscience, New York, 1954.
(7) Lee, T. S., "Technique of Organic Chemistry," vol. 8, Friess, S. L., and Weissberger, A., eds., Interscience, New York, 1953.
(8) Lester, C. T., Craft, M. J., Landrum, B. F., and Surett, E. C., J. Am. Chem. Soc., 73, 4462 (1951).
(9) Mitchell, J., "Organic Analysis," vol. 1, Mitchell, J., Kolthoff, I. M., Proskauer, E. S., Weissberger, A., eds., Interscience, New York, 1953.
(10) Stempel, G. H., Jr., and Schaffel, G. S., J. Am. Chem. Soc., 66, 1158 (1944).
(11) Vavon, G., and Monthéard, P., Bull. soc. chim., 7, 551 (1940).

8th Annual Summer Symposium—Role of Reaction Rates

(COMPETING RATES OF OXIME FORMATION)

Determination of Vanillin in Presence of Acetovanillone

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A volumetric method using oxime formation has been developed for the determination of the vanillin content of crude concentrates from the oxidation of alkaline lignin sulfonates in which the major impurity is acetovanillone. By control of the temperature (0° to 5° C.) and the time of reaction (3 to 9 minutes), vanillin can be estimated in the presence of up to 30% acetovanillone and of other impurities. The precision of the method is within 0.3%; the accuracy, 0.5%. It is rapid and convenient. Other aldehydes would interfere.

IN THE manufacture of vanillin by the oxidation of alkaline lignin sulfonates from gymnosperms, such as spruce and hemlock, the major impurity found in concentrates of the crude vanillin is acetovanillone (4'-hydroxy-3'-methoxyacetophenone) to the extent of 5 to 10% (1, 5, 15). Minor impurities are phenolics, such as guaiacol, and other degradation products. A rapid method for the quantitative assay of the vanillin content of such concentrates is desirable.

Peniston, Agar, and McCarthy (8) describe an ionophoretic technique for resolving such materials. Leger and Hibbert (5) use m-nitrobenzoylhydrazide similarly. Weinberger (14) describes the use of dimethylcyclohexanedione as a precipitating

agent for vanillin in the presence of ketones. Mitchell and Smith (7) describe the use of silver oxide oxidation for this purpose. Various chromatographic approaches have been applied (9, 12). In general these methods do not permit as much speed and/or accuracy as could be desired.

Summaries of sundry quantitative methods applicable to aldehydes such as vanillin are available (4, 6, 10). Of these the volumetric oxime formation seemed most promising. Although this reaction can be used for both aldehydes and ketones, the rates of reaction are markedly different, aldehydes reacting much more rapidly than the corresponding ketones (3, 6, 13). Exploration showed that, with proper control of temperature and time, vanillin can be determined quantitatively in the presence of acetovanillone and other impurities.

REAGENTS

The hydroxylamine reagent used is similar to that of Stillman and Reed (11). Forty grams of reagent grade potassium hydroxide are dissolved in 20 ml. of water and diluted to 1 liter with formula 30 alcohol. Five grams of barium chloride are added, the mixture shaken well and allowed to stand 24 hours before use. Hydroxylamine hydrochloride, 21.5 grams of reagent grade, is dissolved in 40 ml. of water and diluted to 400 ml. with formula 30 alcohol. Bromophenol blue indicator is prepared by triturating 0.1 gram of the dye with 1.5 ml. of 0.1N sodium hydroxide solution and diluting to 25 ml. with water. Into 400 ml. of the

hydroxylamine hydrochloride solution is decanted slowly, with stirring, 300 ml. of the alcoholic potassium hydroxide, followed by 5 ml. of the bromophenol blue solution. The mixture is allowed to stand 1 hour and then filtered with suction through a Whatman No. 42 paper. This reagent is not useful more than about 10 days after making.

Alcoholic hydrochloric acid solution, 0.5N, is prepared by making up 43 ml. of reagent grade hydrochloric acid per liter with formula 30 alcohol and standardizing against any suitable material, such as sodium carbonate. Aqueous 0.5N acid can be used, though the end point is not as sharp. Other materials used were:

VANILLIN, U. S. Pharmacopoeia, melting point 81.5° C. or above.

ACETOVANILLONE, as supplied by the Marathon Corp., Rothschild, Wis., melting range 113.2° to 114.2° C.

ETHYL VANILLIN, melting point 76.5° C. or above.

GUAIACOL, technical grade, melting point 27.8° C. or above.

SKELLYSOLVE-B, Skelly Oil Co., Kansas City, Mo.

Other aldehydes and ketones were Eastman White Label grade.

PROCEDURE

The general procedure followed was to weigh accurately about 1.0 gram of sample into one of a pair of 250-ml. Erlenmeyer flasks. Into each flask 20 ml. of formula 30 alcohol (neutralized to bromophenol blue) was added, and the sample flask shaken to complete solution. Both flasks were then cooled in an ice-water bath for 5 minutes. Then 50 ml. of the hydroxylamine reagent solution was precisely pipetted into each of the flasks, which were swirled to mix and were retained for a chosen time in the ice-water bath until titrated with the 0.5N alcoholic hydrochloric acid.

Table I. Effect of Time on Recovery of Vanillin

Time, Min.	% Vanillin Added	Found
1	94.9	91.4
3	95.3	95.3
5	See Tables II, III, IV	
7	96.2	96.8
9	95.2	95.6
15	96.7	97.3

Table II. Effect of Acetovanillone Content

Added % Acetovanillone	% Vanillin[a] Taken	Found
2.9	97.1	97.2
4.8	95.2	95.3
10.3	89.7	89.9
15.0	85.0	84.8
20.0	80.0	80.1
25.0	75.0	75.0
30.0	70.0	70.3
35.0	65.0	65.5
40.0	60.0	60.7
45.0	55.0	56.1
50.0	50.0	51.6

[a] Av. of two or more samples. Std. dev. = 0.2%.

The blank flask was titrated first to a yellowish green end point, the volume of titrant noted, and the flask retained to serve as a standard for the end-point color match with the sample solution. The sample solution was titrated as rapidly as possible, being kept in the ice-water bath until just before the end point, at which time it was removed if necessary and the last few drops of titrant added quickly. The vanillin content was calculated as:

% vanillin = (ml. HCl for blank − ml. HCl for sample) × normality × 15.21/sample weight

TIME AND TEMPERATURE EFFECTS

Preliminary experiments at room temperature indicated that, although vanillin reacts very rapidly, acetovanillone reacts rapidly enough to make it difficult to get a clean separation. With the ice-water bath the time of reaction prior to titration can safely fall between 3 and 9 minutes (Table I). For all further work 5 minutes was used as the hold time between addition of the reagent and titration of the sample. The time required for the

titration itself is a minute or less. The actual temperature of the reaction mixture during the hold time is about 5° C. The reagent can be cooled to this point before addition without affecting the results, but it becomes cloudy because of the decreased solubility of the salts present.

EFFECT OF ACETOVANILLONE CONTENT

To evaluate the range of applicability of the method as a function of the acetovanillone content, a series of samples of various concentrations was evaluated (Table II). It appears that serious error does not begin to occur until above 30% acetovanillone.

EFFECT OF OTHER IMPURITIES

To simulate the effect of other materials present in crude concentrates, guaiacol and an inert diluent, Skellysolve, as well as acetovanillone, were added in various concentrations. The results are given in Table III. Although the accuracy is not as good as with acetovanillone alone, no specific trend is shown.

PRECISION AND ACCURACY

To establish the reproducibility of the method, further replications on pure and crude vanillin samples were made. The results are summarized in Table IV. The over-all standard deviation of precision is 0.33%. Comparison with another laboratory (2) showed a standard deviation of reproducibility of 0.5%. Taking all the available data into account the standard deviation of accuracy is about 0.5%. Routine use of the method in production control for over 2 years has shown this to be a reasonable estimate.

DISCUSSION

Once the reagent and titrant have been prepared, the total elapsed time per sample is no more than 15 minutes. Some familiarity with the end point of the titration and the technique of operations is required, but the method is suitable for routine use by technicians with no unusual or expensive equipment required. It is good practice to make determinations in duplicate.

Table III. Effect of Various Impurities

Aceto-vanillone	Guaiacol	Inert	% Vanillin Taken	Found
4.9	..	5.3	89.8	89.1
4.8	8.0	..	87.2	87.0
..	8.1	3.4	88.5	88.7
..	8.0	3.0	89.0	89.1
2.9	4.3	4.3	88.5	89.5
3.0	2.5	5.6	88.9	89.5
3.8	2.1	4.1	90.0	90.5
4.6	3.6	6.4	85.4	86.2

Table IV. Precision of Determinations on Actual Samples

% Vanillin Found	No. of Replications	Std. Dev.
100.0	5	0.35
95.5	6	0.31
91.3	7	0.30
86.2	4	0.33

The authors have been advised (2) that a 30% methanol-70% isopropyl alcohol solvent substitutes adequately for the formula 30 alcohol used.

It is possible to substitute a potentiometer or pH meter for the bromophenol blue indicator. Calomel and glass electrodes can be used. The end-point "break" is about 50 mv. and occurs

at about 150 ml. below the initial value. Some dexterity of operation is required to avoid taking too much time during the titration. Some samples may be colored enough to require this technique.

The stability of the hydroxylamine reagent with time is somewhat variable. A simple means of verifying its stability is to analyze a sample of pure vanillin. The indicated purity should fall between 99.7 and 100.3%.

ESTIMATION OF ACETOVANILLONE CONTENT

A simple modification of the procedure enables the total carbonyl content of samples to be determined. A similar sample size and volume of reagent are taken and the mixture is refluxed for about 1 hour. After cooling to room temperature, the reaction mixture is titrated with the 0.5N alcoholic hydrochloric acid to the same end point. After correcting for a reagent blank carried through the same procedure, the amount of hydroxylamine required for the total carbonyl less that required for the vanillin is calculated as acetovanillone. This reaction time probably could be shortened, but no demonstration has been made.

OTHER ALDEHYDES AND KETONES

If the crude vanillin has been derived from angiosperms such as birch, p-hydroxybenzaldehyde and syringaldehyde may be present. Limited tests showed that these will be titrated quantitatively as vanillin. Acetosyringone (4'-hydroxy-3'5'-dimethoxyacetophenone) apparently behaves like acetovanillone.

LITERATURE CITED

(1) Brauns, F. E., "Chemistry of Lignin," Academic Press, New York, 1952.
(2) Bryan, C. C., private communication.
(3) Bryant, W. M. D., and Smith, D. M., J. Am. Chem. Soc., 57, 57 (1935).
(4) Guenther, E., and Langenau, E. E., ANAL. CHEM., 25, 12 (1953).
(5) Leger, F., and Hibbert, H., J. Am. Chem. Soc., 60, 565 (1938).
(6) Mitchell, J., "Organic Analysis," vol. 1, Interscience, New York, 1953.
(7) Mitchell, J., and Smith, D. M., ANAL. CHEM., 22, 746 (1950).
(8) Peniston, Q., Agar, H., and McCarthy, J., Ibid., 23, 994 (1951).
(9) Reaville, E. T., and Shreve, G. W., Ibid., 27, 565 (1955).
(10) Smith, W. T., Jr., Wagner, E. F., and Patterson, J. M., Ibid., 26, 155 (1954).
(11) Stillman, R. C., and Reed, R. M., Perfumery Essent. Oil Record, 23, 278 (1932).
(12) Stone, J. E., and Blundell, M. J., ANAL. CHEM., 23, 771 (1951).
(13) Vavon, G., and Monthéard, P., Bull. soc. chim., 7, 551 (1940).
(14) Weinberger, W., IND. ENG. CHEM., ANAL. ED., 3, 365 (1931).
(15) Wise, L. E., and Jahn, E. C., "Wood Chemistry," 2nd ed., vol. 1, Reinhold, New York, 1952.

8th Annual Summer Symposium—Role of Reaction Rates

Use of Microorganisms as Analytical Tools

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Microorganisms occupy a definite place in the field of analytical analysis. They can be used successfully to assay various antibiotics, vitamins, and amino acids with an acceptable degree of accuracy even when crude and unknown types of materials are involved. In general, they are inexpensive to run, are not difficult to handle, and can be adapted for large-scale operations. They have shortcomings, particularly with interfering substances causing assay drifts with the vitamin and amino acid assays. In many cases they represent the only satisfactory analytical tool available for use in specific chemical, stability, biological standardization, nutritional, and microbiological studies.

THE literature is full of references on the use of microorganisms in assaying antibiotics, vitamins, and amino acids. Although various microorganisms, including bacteria, fungi, yeasts, and protozoa have been studied, the bacteria have demonstrated the widest degree of usefulness This paper is confined to their use in the various microbiological analytical techniques. Bacteria are extremely small, unicellular plantlike, microscopic organisms which differ from true plants in that, with one known exception, they lack chlorophyll (9). They reproduce by binary fission and their size generally varies from 0.2 to 5 microns. Cell growth, or the measurement of some manifestation thereof, is the basis for all microbiological assays. In the growth of a bacterial culture, a series of phases, characterized principally by variations in the growth rate, may be demonstrated. In general,

the following definitions (23) illustrate the sequence of events found in a typical bacterial sigmoid growth curve.

LAG PHASE. The growth rate is essentially null.

ACCELERATION PHASE. The growth rate is slow but increasing.

EXPONENTIAL PHASE. The growth rate is constant. The cells are dividing regularly and at maximum speed in a geometric progression.

RETARDATION PHASE. The growth rate is decreasing. Here the bacterial cells cease to multiply at maximum rates and cell divisions become less and less.

STATIONARY PHASE. The growth is essentially null again. Actually the bacterial population remains almost constant, but theoretically there is an equilibrium between dying and newly formed cells.

PHASE OF DECLINE. The growth rate is negative. Here the cells are dying at a constant rate or nearly so.

The first two phases may be shortened by the use of young, actively growing cells, a principle frequently used in microbiological assays. Many physical and chemical factors may also alter one or more of the above phases, sometimes eliminating them. The bacteria commonly used for microbiological assay work have a generation time—i.e., the time required for one cell to divide into two cells—of usually less than 1 hour (26).

Bacteria are adaptable to the assay of a number of vitamins and amino acids necessary in animal nutrition because, in general, all forms of life share certain common metabolic processes in their basal cellular biochemistry (16). However, bacteria may also be used for the assay of antibiotics which do not, in general, exert cellular metabolic effects in higher animals. The apparent discrepancy between the response of the cells of higher animals and of certain microorganisms appears to be due to the cellular organization in the two classes of organisms (16).

Of the many practical advantages in the use of microbiological assays (16) the rapidity with which the test bacteria react is certainly impressive. This, together with the fact that only small quantities of the test substances are needed to obtain a specific and measurable response, makes possible a relatively small scale field of operations for performing a reasonably large number of assays.

ANTIBIOTIC ASSAYS

Microbiological assays for the quantitative estimation of antibiotics have had widespread use (10, 13, 19, 25, 28). They have served as efficient guides in chemical isolation and purification studies, in pharmacological studies, in routine production and control activities, and in microbiological research and development work. There are certain advantages for these bioassays. Apart from the sensitivity factor, the bioassay may often be applied as well to an unknown as to a known antibiotic (10). Also, although the antibiotic material to be investigated is often chemically heterogeneous and of variable composition, a microbiological method can usually be applied without preliminary fractionation. This generally holds true for the assay of materials in body fluids as well. On the debit side, however, is the fact that a microbiological method does not necessarily differentiate between two or more antibiotics.

There are certain general methods which have been shown to be applicable to fermentation broths as well as chemically isolated preparations of varying degrees of purity. These include the dilution, diffusion and turbidimetric techniques. The dilution method represents the all or none end-point type of response whereas the diffusion and turbidimetric methods represent the graded response.

The principle on which the bioassay of antibiotics is based is that equivalent doses of a given antibiotic exert equal inhibitory effects on similar populations of a given species of bacterium under established standardized conditions (28). Thus, the measurable response can be correlated with the dosage of test material used by a mathematical relationship which can be represented graphically by a dosage response curve.

SERIAL DILUTION METHODS

In the standard serial dilution method (10, 28), a number of tubes, each containing initially the same number of bacterial cells and the same volume of liquid nutrient medium, are dosed with varying amounts of antibiotic. The tubes are then incubated until sufficient growth of the uninhibited bacteria has occurred. Depending on the assay conditions, this incubation period may vary from a few hours to several days. The end-point or threshold concentration is taken as the lowest concentration of antibiotic that prevents growth of the test bacterium as evidenced by a lack of turbidity in the broth. A similar set of tubes containing known amounts of the test material is run as a control, and for tests requiring more than 3 to 4 hours, sterile tests preparations are required. Because an all or none response is being dealt with, a definite value for the end point cannot be given, only a range. The range can be narrowed by more intermediate tubes, but the degree of sensitivity desired must be equated against the increased time, effort, and cost. This bioassay technique can be made accurate and figures such as the following have been reported (30) with a penicillin assay where the concentration of test material decreased by 10% increments:

Assays	
1	± 8.9% std. dev.
3	± 6.3% std. error
5	± 4.5% std. error
8	± 3.4% std. error
16	± 2.3% std. error

Serial dilution assays may not only be carried out in liquid nutrient medium but also in solid nutrient medium (32). Whereas the results obtained are less accurate than in broth and the procedure is somewhat more cumbersome, they do have the advantages that sterile preparations are generally not required and that the threshold concentrations may be obtained simultaneously against several different bacteria.

DIFFUSION METHODS

By far the most widely used bioassay technique for antibiotics is the diffusion or cylinder-plate method. In this procedure a solid nutrient medium is seeded with a test bacterium. The solution of the antibiotic to be assayed is brought into contact with the solid seeded medium either by the means of metal cylinders or paper disks placed on the surface or in holes cut in the solid medium itself. After an incubation period of usually 18 to 24 hours, the diameters of the inhibition zones are measured. The resulting zone value is essentially a measure of the equilibrium condition between two factors—namely, outward diffusion of antibiotic and growth of the test bacterium which tends to cover the nutrient surface. If a standard is set up at the same time using known amounts of the same antibiotic, when possible, a dosage response curve can be plotted from which the strength of the unknowns can be determined.

The paper disk technique is capable of high accuracy although some workers feel it is not as sensitive to such low dilutions as the other plate diffusion methods (10). Volumes as small as 0.02 ml. of liquid can be used per disk, however. With the cylinder plate technique, the concentration of the substance in the solution to be assayed determines the inhibition zone whereas with the paper disk technique it is the amount of the substance deposited on the disk which is the controlling factor, provided it is freely soluble.

There are many more or less controllable factors which are capable of affecting the inhibition zone sizes obtained in the diffusion methods. These include the following as outlined by Lees and Tootill (19):

Choice of test organism
Condition of test organism
Density of seeding of test organism on assay plates
Formulation and condition of assay medium
Depth of seeded agar in assay plate
Potency of test solution
Volume of test solution applied to the plate
Area of seeded agar to which test solution is initially applied
Time of application of potent solution
Temperature of incubation of assay plate
Length of incubation cycle

While all of these factors are extremely important, certain of them are of particular interest. The basic assumption one makes in an antibiotic assay is that the zone size produced by the antibiotic solution under test is determined solely by the antibiotic itself. If, however, the solution is a mixture of two or more antibiotics, then the response of the bacterium varies and, thus, does not result in a valid response. Where these other "contaminating" factors are known or suspected efforts should be made to incorporate them into the assay medium.

The size of the inhibition zone is inversely related to the seeding density of the test bacterium. Thus, a compromise must be worked out between zone size and clarity and a suitable linear-dose response curve with a satisfactory slope. The effect of the pH of the medium must be taken into account. Various test bacteria differ in their response to antibiotics depending on the pH of the medium and, thus, it must be buffered accordingly. Likewise the oxidation-reduction potential may be of importance. The lack of sufficient poising capacity may result in a gradient in O/R potential between the agar surface exposed to the atmospheric oxygen and the solid medium in contact with the glass bottom of the culture dish receiving oxygen solely by the slow process of diffusion (19). If this factor is critical enough, the test bacterium may show a differential response through the solid nutrient medium, producing, in some cases, a conical rather than

a cylindrical zone. This can be prevented by having a non-seeded base layer of nutrient medium overlaid with a thin seeded layer.

It is also known that the margins of the inhibition zones depend on the diffusion rate of the particular substance under assay, and this diffusion rate is controlled to some degree by the water content of the solid nutrient medium. Thus, variations in the moisture content throughout the assay medium may lead to local increased absorption of the moisture from the test solution with the resulting effect of increasing the extent of its diffusion. This can be a problem with large glass plates. The inhibition zone of an antibiotic varies inversely with the depth of the solid nutrient medium in the test dish or plate. Thus, differences in medium depth may result in the test solutions diffusing at different rates. At the same time variations in the area covered by the test solution can affect slightly the zone size obtained. Although this is generally not a problem with cylinders or paper disks, it can be a problem with holes cut in the medium or with beads, as diffusion of the test solution from the area of deposition is not at once wholly horizontal, but also vertical (19). It can be demonstrated that the application time of two solutions of equal potency to the assay plates is extremely important.

The size of the inhibition zone is also a function of the growth rate of the test bacterium. Thus, the more rapidly the test organism grows during the incubation period, the smaller the resulting inhibition zones will be. In the event the plate is allowed to remain at room temperature for any period of time after the test solutions are applied before incubating, the diffusion begins immediately, whereas growth of the test bacterium is essentially nil. Relatively larger zones result. This technique can be applied for low potency samples.

The size of the inhibition zone depends to a large extent on the diffusion rate of the test substance, the duration of the lag phase of the test bacterium, and its subsequent growth rate in the exponential phase.

Several interesting techniques have been developed by Goyan, Dufrenoy, Strait, and Pratt (12) and Pratt and Dufrenoy (27) to shorten the incubation period in the pencillin assay. They were able to produce visible latent inhibition zones after 3 hours of incubation by treating the plates with silver salts, exposing to light, and then submitting them to photographic development. The resulting dose-response curve was linear for penicillin concentrations between 1 and 8 microns per ml. More recently (27) it was reported that these latent zones could be revealed by treating the plates with dyes such as bromocresol purple, phenolsulfone phthalein, Nile blue, Janus green, methyl green, and safranine O, or reagents such as cadmium acetate, cobalt nitrate, Schiff's reagent azo-coupling reagent, and ferricyanide followed by ferric sulfate. The curves obtained relating concentration of penicillin to zone diameter were flatter than with the more usual methods having a longer incubation period. This resulted in a wider range suitable for assay purposes, although accuracy probably was somewhat less.

TURBIDIMETRIC METHODS

In these techniques, the amount of growth of a test bacterium in a liquid nutrient medium, dosed with various levels of known antibiotic solution, is measured turbidimetrically and plotted in graph form. Several levels of test solution run simultaneously in the same fashion are calculated against the standard. Generally, turbidity or light absorption is measured photoelectrically and the resulting dose-response curve is established by plotting concentration directly against instrument reading. Because the period of incubation is frequently short with this technique, the concentration of inoculum and time and temperature of incubation are extremely important to ensure constant growth rates and, thus, reproducible assays. While this technique is capable of high accuracy, and requires only a short operation time, there are certain disadvantages (10). The procedure

is more sensitive to impurities than most other procedures which may stimulate or inhibit the test bacterium. Colored or turbid samples obviously interfere and introduce errors as do any non-specific changes, such as pH, color, or turbidity, produced in the liquid medium during bacterial growth.

With assays of the graded response type (turbidimetric, diffusion) the probable error can be calculated easily and it is not too difficult to increase the accuracy to any desired scientific and economic degree by sufficient replication. In the case of the dilution, or all or none type procedure, the answer is given only as range. Increasing the number of intermediate dilutions can only narrow this range.

In the early days of antibiotic assay development, the standard error associated with the assay ranged about ±10 to 15% or less with sufficient replication. More recently Lees and Tootill (19, 20) have shown that with large plates and routine care a standard error of ±5% could be obtained, whereas with more precise precautions this could be lowered to ±1%.

The following antibiotics can be assayed by the indicated test organisms:

Antibiotic	Method	Test Organism
Bacitracin	Cylinder-plate	*Micrococcus flavus* ATCC10240
	Turbidimetric	*Staphylococcus aureus* ATCC10537
Chloramphenicol	Cylinder-plate	*Sarcina lutea* P.C.I. 1001
Chlortetracycline	Cylinder-plate	*Sarcina lutea* P.C.I. 1001
	Turbidimetric	*Micrococcus pyogenes* var. *aureus* ATCC6538-P
Dehydrostreptomycin sulfate	Cylinder-plate	*Bacillus subtilis* 6633
	Turbidimetric	*Klebsiella pneumoniae* ATCC10031
Erythromycin	Cylinder-plate	*Sarcina lutea* P.C.I. 1001
Neomycin sulfate	Cylinder-plate	*Micrococcus pyogenes* var. *aureus* ATCC6538P
	Turbidimetric	*Klebsiella pneumoniae* ATCC10031
Penicillin	Cylinder-plate	*Micrococcus pyogenes* var. *aureus* ATCC6538P
Polymyxin B sulfate	Cylinder-plate	*Micrococcus pyogenes* var. *aureus* ATCC4617
Streptomycin sulfate	Cylinder-plate	*Bacillus subtilis* ATCC6633
	Turbidimetric	*Klebsiella pneumoniae* ATCC10031
Tyrothricin	Turbidimetric	*Streptococcus hemolyticus* Lancefield Group D, ATCC9854

Specific details of the above assay procedures are given in the United States Pharmacopeia (25).

VITAMIN ASSAYS

The knowledge gained of the nutritional requirements of various microorganisms has proved to be a very useful approach to the study of animal nutrition. The essential nature of inositol, pantothenic acid, p-aminobenzoic acid, pyridoxal, pyridoxamine, biotin, and nicotinic acid was discovered for the nutrition of microorganisms before their essentiality in animal nutrition was established (4, 22, 31). Likewise the studies on folic acid and vitamin B_{12} were facilitated by the use of microbial assays (31).

As in the case with the antibiotic assays, there are advantages to the microbiological vitamin assays (1). They have the advantages of speed and small requirements of space, labor, and materials when compared to other biological methods using animals or humans. Secondly, they can be used, unlike most chemical methods, before the chemical nature of the vitamin being assayed has been determined. Lastly, they share with other biological assays the property of biological specificity.

Microorganisms, which can be used to assay various members of the known vitamin B complex (1, 4, 14, 22, 31) are now known. This is illustrated below.

Vitamin	Method	Test Organism
p-Aminobenzoic acid	Gravimetric	*Neurospora crassa* (mutant)
Biotin	Acidimetric	*Lactobacillus arabinosus*
Choline	Gravimetric	*Neurospora crassa* (mutant)
Folic acid	Acidimetric	*Streptococcus faecalis*
	Acidimetric	*Lactobacillus casei*
Inositol	Gravimetric	*Neurospora crassa* (mutant)
	Turbidimetric	*Saccharomyces carlsbergensis*
Nicotinic acid	Acidimetric	*Lactobacillus arabinosus*
Pantothenic acid	Acidimetric	*Lactobacillus arabinosus*
Riboflavin	Acidimetric	*Lactobacillus helveticus*
	Acidimetric or turbidimetric	*Lactobacillus casei*

Thiamine	Turbidimetric	*Lactobacillus fermenti*
Vitamin B₆		
Total	Gravimetric	*Neurospora sitophila* (mutant)
	Turbidimetric	*Saccharomyces carlsbergensis*
Pyridoxamine +		
pyridoxal	Turbidimetric	*Streptococcus faecalis*
Pyridoxal	Turbidimetric	*Lactobacillus casei*
Vitamin B₁₂	Turbidimetric	*Lactobacillus leichmannii*

Specific details of the above assay procedures may be found in the excellent texts of Barton-Wright (*4*,) Snell (*31*), and others (*1*, *25*).

Although a number of x-ray mutants of the mold *Neurospora* and certain strains of yeasts can be used, the lactic acid bacteria are undoubtedly the most useful and most important. It is an established fact that when the lactic acid bacteria are used for microbiological assays, the measurement of their response is simple and direct since, with the exception of vitamin B₁, the lactic acid produced by the growth and metabolic activities of these bacteria is directly proportional over a certain range to the vitamin concentration in the liquid nutrient medium (*4*). All that is necessary is to titrate this acid produced with sodium hydroxide and prepare a standard curve. From this standard curve, the amount of vitamin in the various levels of test solution can be determined by interpolation. This type of assay is thus based on a total response. In certain cases, the growth of the test organism after a short incubation period is measured directly by turbidimetric measurement. In this case, one is measuring the comparative rates of response (*31*). Generally speaking, assays based on a total response are likely to be more reliable than those based on rate of response, because much information remains to be learned about factors present in crude preparations which affect response rates, either to slow them down or to accelerate them, but which generally have little or no effect on total response.

The assay medium generally includes some natural material such as casein hydrolyzate from which contaminating vitamins have been removed, plus pure vitamins, purines, salts, cystine, tryptophan, fermentable sugar (usually glucose), and a suitable buffer such as sodium acetate. The latter has a stimulating effect on growth apart from its effect on pH. Below is listed the composition of a typical assay medium (*1*):

Acid-hydrolyzed casein (vitamin-free)
Cystine-tryptophan
Adenine-guanine-uracil
B_1, B_2, *p*-aminobenzoic acid, B_6
Calcium pantothenate
Biotin
Niacin
$K_2HPO_4 - KH_2PO_4$
$MgSO_4.7H_2O$, $NaCl$, $FeSO_4.7H_2O$, $MnSO_4.4H_2O$
Glucose anhydrous
Sodium acetate anhydrous

While the composition of the above basal medium may vary with the different assays, it is necessary to remember only that it be nutritionally complete for the test organism except for the one vitamin being measured.

The medium is prepared in tubes which are then dosed with the vitamin preparation to be tested. Following sterilization by autoclaving, the tubes are cooled in a water bath or at room temperature until the temperature within the tubes is absolutely uniform. This precaution is particularly important when turbidimetric measurements of growth are to be made after 16 to 18 hours. Even slight differences in initial temperature can influence the early growth rate much more markedly than they would the total growth over a 72-hour period. The constancy of the incubation temperature throughout the incubator or bath is even more important in the short term incubations, because of its resultant affect on growth rates although it is still important with the longer assays.

In vitamin assays of natural or crude preparations, the sample treatment frequently becomes of prime importance because the vitamin or unknown growth factor must be in a state readily utilizable by the test organism. Where stability is of no concern, treatment with acid or alkali at high temperatures is most useful. In other cases, enzymatic digestion must be used when the vitamins or unknown growth factors are destroyed by one of the above treatments. Care must be taken that the enzymes themselves, which are generally crude preparations, do not contain growth factors which interfere in the assay.

The basic assumption in microbiological assays is that growth and metabolism of the test bacteria are influenced in the same way by the growth factor being assayed, whether it is in the standard solution or in the unknown solution, and by no other factor in the unknown solution (*1*). Under these conditions, there is no tendency for the assay values obtained on the test solution to increase or decrease with the dilution level used. This latter condition is known as assay drift and may lead to nonvalid assay results. It can be caused by different factors—for example, in the case of upward drift substances closely related to vitamins (*24*), growth factors, or complexes of the vitamin may stimulate the growth of the test bacterium, but to different proportionate degrees from that of the standard vitamin at the various dilution levels. In addition, chemically unrelated, nonvitamin substances may alter the response to a vitamin (*31*). For example, fatty acids may affect the response of *Lactobacillus casei* to riboflavin resulting in inhibition or stimulation depending on the concentration. Likewise high levels of thymine and thymidine may replace folic acid and vitamin B₁₂, respectively. Where the drift is due to a known medium inadequacy, it can frequently be averted by adding the interfering substances to the medium. Where there is a downward drift, it generally indicates the presence in the sample of some toxic or inhibitory substance (*1*). A high salt concentration, resulting from medium neutralization, may also inhibit the test bacterium.

Various other factors have been investigated for their effect on the dose response curve in the vitamin assays. The critical nature of temperature, both on the amount of growth and, even more important in some cases, on the growth rate, has been emphasized. Furthermore, the requirements of certain bacteria for riboflavin (*29*) and phenylalanine (*7*) may even be altered by temperature differences. It has been mentioned that the nutrient medium must be buffered, because the pH optima for the lactic acid bacteria can be altered during growth thus making conditions unsatisfactory for further growth. Varying the amount of carbon dioxide in the surrounding atmosphere, while generally producing no effect, can in certain instances affect certain bacterial requirements for phenylalanine, histidine, aspartic acid (*21*), and biotin (*14*). The oxidation-reduction potential of the medium may likewise affect certain nutritional requirements. Although the bacteria used in these assays are either microaerophilic (the rod forms, Genus *Lactobacillus*) or facultative aerobes (the coccoid forms, Genera *Streptococcus* and *Leuconostoc*), they generally are able to grow under the described conditions which promote a rather favorable oxidation-reduction potential. Reports have appeared pointing out that the microbial requirements for pyridoxine (*6*) and vitamin B₁₂ (*15, 17, 18*), may be altered significantly by various oxygen tensions.

In recent years several new techniques have been introduced for microbiological vitamin assays (*14*). One involves the use of cylinder plate or diffusion assays similar to those described for the antibiotic assays (*2, 3, 11*). Here one obtains zones of exhibition or growth, and the usual dosage response curves can be plotted. The procedure is faster and lends itself readily to large-scale routine handling. However, the sensitivity is considerably reduced, and 10 to 2000 times as much vitamin is required (*14*). Closely allied to the diffusion plate technique is the use of bacteria in bioautography. Here a tool is provided for the location of vitamins and their closely related analogs, which have been separated by paper chromatographic techniques. Removal of the indicated active locations by leaching and testing by the usual tube assays has led to quantitative estimation of B₁₂, B₁₂ₐ, and B₆ mixtures (*33, 34*).

The accuracy claimed for microbiological vitamin assays is generally ±10 to 15% of the mean. It has been shown by

Bessey and Hull (5), however, that much greater accuracy can be obtained with careful control of all the variables and with adequate media. They report a probable error for the mean of ±0.7 to 1.2% with pure materials and ±3% with crudes.

AMINO ACID ASSAYS

The microbiological assay of amino acids was the sequel to, and the logical outcome of, the earlier assay studies on vitamins. Basically the same fundamental principles apply. The general assay media and test bacteria are the same as those described for the vitamin assays. The following amino acids can be assayed by the indicated test organism.

Amino Acid	Method	Test Organism
α-Alanine	Acidimetric	*Lactobacillus citrovorum* 8081
Arginine	Acidimetric	*Streptococcus faecalis*
Aspartic acid	Acidimetric	*Leuconostoc mesenteroides* P. 60
l-Cystine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Glutamic acid	Acidimetric	*Lactobacillus arabinosus*
Glycine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Histidine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Isoleucine	Acidimetric	*Lactobacillus arabinosus*
Leucine	Acidimetric	*Lactobacillus arabinosus*
Lysine	Acidimetric	*Leuconostoc mesenteroides* P. 60
DL-Methionine	Acidimetric	*Lactobacillus fermenti* 36
L-Methionine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Phenylalanine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Proline	Acidimetric	*Leuconostoc mesenteroides* P. 60
Serine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Threonine	Acidimetric	*Streptococcus faecalis*
Tryptophan	Acidimetric	*Lactobacillus arabinosus*
L-Tyrosine	Acidimetric	*Leuconostoc mesenteroides* P. 60
Valine	Acidimetric	*Lactobacillus arabinosus*

Specific details of the above assay procedure may be found in the excellent text of Barton-Wright (4).

The general methods used for measuring growth—i.e., lactic acid production or turbidity—are the same as those used in the vitiamin assays. All the precautions described apply here with equal importance for the performance of these assays and the preparation of the samples.

If the basal medium is composed only of amino acids essential for the growth of the test bacteria one does not get maximum growth. Thus, the stimulatory amino acids must also be added to give growth comparable to that obtained with acid hydrolyzed casein plus tryptophan and cystine. The latter two amino acids must be added, since acid hydrolysis of the casein destroys tryptophan and very little cystine is normally present.

Preparation of the sample is frequently a serious problem, particularly with foodstuffs. Many interfering substances are frequently present resulting in either abnormal stimulation or inhibition. As such, they affect the rates of growth of the test organism.

It has been noted by various workers (8, 14) that the relationship of the concentrations of the various amino acids in the medium can be critical. In some cases it may determine the essentiality of a given acid or it may cause the inhibition of the test bacterium. Thus, the role of amino acid antagonisms can be critical and the assayer must establish a medium with sufficiently high levels of amino acids to offset any possible distortion by the addition in the sample of other amino acids.

By controlling all the factors discussed, it has been found that amino acid assays can be run with an error of ±5%.

LITERATURE CITED

(1) Association of Vitamin Chemists, Inc., "Method of Vitamin Assay," 2nd ed., p. 129, Interscience, New York, 1951.
(2) Bacharach, A. L., *Nature*, 160, 640 (1947).
(3) Bacharach, A. L., and Cuthbertson, W. F. J., *Analyst*, 73, 334 (1948).
(4) Barton-Wright, E. C., "Microbiological Assay of Vitamin B-Complex and Amino Acids," p. 3, Pitman, New York, 1952.
(5) Bessey, O. A., and Hull, T. Z., *J. Biol. Chem.*, 155, 71 (1944).
(6) Bohonos, N., Hutchings, B. L., and Peterson, W. H., *J. Bacteriol.*, 44, 479 (1942).
(7) Borek, E., and Waelsch, H., *J. Biol. Chem.*, 190, 191 (1951).
(8) Brickson, W. L., Henderson, L. M., Solhjell, I., and Elvehjem, C. A., *Ibid.*, 176, 517 (1948).
(9) Bryan, A. H., and Bryan, C. G., "Principles and Practice of Bacteriology," 3rd ed., p. 13, Barnes & Noble, New York, 1945.
(10) Florey, H. W. F., Chain, E., Heatley, N. G., Jennings, M. A., Sanders, A. G., Abraham, E. P., and Florey, N. E., "Antibiotics," vol. 1, p. 110, Oxford Univ. Press, New York, 1949.
(11) Foster, J. C., Lally, J. A., and Woodruff, H. B., *Science*, 110, 507 (1949).
(12) Goyan, F. M., Dufrenoy, J., Strait, L. A., and Pratt, R., *J. Am. Pharm. Assoc., Sci. Ed.*, 36, 65 (1947).
(13) Heatley, N. G., *Biochem J. (London)*, 41, XIII (1947).
(14) Hendlin, D., *Bacteriol. Revs.*, 16, 241 (1952).
(15) Hendlin, D., and Soars, M. H., *J. Biol. Chem.*, 188, 603 (1951).
(16) Knight, B. C. J. G., *Biochem. J. (London)*, 41, i (1947).
(17) Kocher, V., *Intern. Z. Vitaminforsch.*, 20, 369 (1949).
(18) Koditschek, L. K., Hendlin, D., and Woodruff, H. B., *J. Biol. Chem.*, 179, 1093 (1949).
(19) Lees, K. A., and Tootill, J. P. R., *Analyst*, 80, 95 (1955).
(20) *Ibid.*, p. 110.
(21) Lyman, C. M., Moseley, O., Wood, S., Butler, B., and Hale, F., *J. Biol. Chem.*, 167, 177 (1947).
(22) Macrae, T. F., *Biochem. J. (London)*, 41, x (1947).
(23) Monod, J., "Annual Review of Microbiology," 3, p. 373, Annual Reviews, Stanford, 1949.
(24) Pfifftner, J. J., Calkins, D. G., Bloom, E. S., and O'Dell, B. L., *J. Am. Chem. Soc.*, 68, 1392 (1946).
(25) "Pharmacopeia of the United States of America," Committee of Revision, pp. 848–61, 885–89, Mack Publ. Co., Easton, Pa., 1955.
(26) Porter, J. R., "Bacterial Chemistry and Physiology," p. 129, Wiley, New York, 1946.
(27) Pratt, R., and Dufrenoy, J., *Nature*, 159, 576 (1947).
(28) Pratt, R., and Dufrenoy, J., "Antibiotics," 2nd ed., p. 72, Lippincott, Philadelphia, 1953.
(29) Price, S. A., and Graves, H. C. H., *Nature*, 153, 461 (1944).
(30) Rake, G., and Richardson, A. P., *Ann. N. Y. Acad. Sci.*, 48, 143 (1946).
(31) Snell, E. E., "Vitamin Methods," vol. 1, p. 327, Academic Press, New York, 1950.
(32) Waksman, S. A., and Reilly, H. C., IND. ENG. CHEM., ANAL. ED., 17, 556 (1945).
(33) Winsten, W. A., and Eigen, E., *Proc. Soc. Exptl. Biol. Med.*, 67, 513 (1948).
(34) Woodruff, H. B., and Foster, J. C., *J. Biol. Chem.*, 183, 569 (1950).
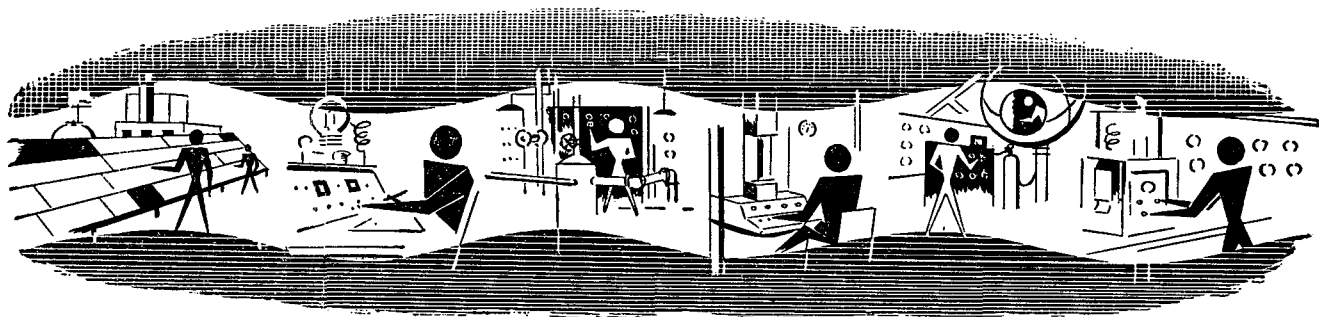
Identification of Short-Lived Species in Chemical Reactions

Use of Flash Photolysis and Rigid Glassed Solvents in Identification and Rate Studies of Reaction Intermediates

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There are three classes of intermediate short-lived species which participate in chemical reactions: molecular (or ionic) complexes, atoms and free radicals, and atoms and molecules in excited electronic states. Intermediates of the first class are best studied in flow systems. The identification and subsequent study of the other two classes require their spontaneous generation in high concentration. This has been solved in large measure by the "flash photolysis" technique. When flow methods and flash photolysis are coupled with rapid spectroscopic methods it is possible to measure their absorption spectra and life times. High concentrations of short-lived species can also be achieved by irradiation of reactants dissolved in rigid glassy solvents. In this state atoms and radicals produced on irradiation remain stabilized and their spectra can be measured in a conventional manner. Radiative processes of excited molecules are readily studied in the glassy state.

THE field of reaction kinetics as it grows in scope and complexity offers an increasing challenge to the analyst. With this growth the emphasis has shifted to physical rather than chemical methods of analysis not only because physical methods are more rapid and versatile, but also because a physical change can generally be converted to a corresponding electrical change and, thus, be continuously monitored with suitable recording devices.

Recently, considerable effort has been directed toward the development of physical techniques for studying "fast reactions," reactions whose lifetimes vary from several seconds to several microseconds. The impetus for this effort arises from the results of classical kinetic studies which have indicated that a large body of chemical reactions involves short-lived intermediate species. These intermediates disappear by very rapid intra- or intermolecular processes and, hence, the study of these processes requires fast reaction techniques.

In order to identify these short-lived intermediates some property uniquely characteristic of the individual species must be measured; ideally this property is the absorption spectrum. Rapid spectrographic and spectrophotometric techniques have been developed recently which, when coupled with appropriate methods of producing these intermediate species, make it possible both to identify them and to study their reactions.

For the present purpose it is convenient to classify these short-lived species into three classes: molecular (or ionic) complexes, atoms and free radicals, and excited states of atoms and molecules.

Species of the first class, molecular complexes, are formed by the direct union of reactants. Many reactions involve such species, but the number of cases where the intermediates have actually been identified and studied are relatively few. Most prominent of these cases are the enzymatic reactions of biochemistry.

Early kinetic studies on enzyme reactions (*10, 15*) indicated that intermediate enzyme-substrate complexes were involved, and these in turn determined the course and rate of the over-all reaction. Mathematical equations were derived by Michaelis and Menten (*34*) for general enzyme reactions based on the scheme,

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \tag{1}$$

$$ES \overset{k_3}{\rightarrow} E + P \tag{2}$$

where an enzyme, E, combines with substrate, S, to form the enzyme-substrate complex, ES, which in turn decomposes, regenerating the free enzyme together with the products, P, of the reaction.

The first qualitative evidence for the existence of an enzyme-substrate complex was demonstrated by Keilen and Mann (*18*), who showed that the addition of hydrogen peroxide to a solution of the enzyme, horse-radish peroxidase, produced a red color which disappeared upon the addition of the reducing substrate, leucomalachite green. In this case the reaction sequence is represented by Equations 1 and 3.

$$ES + AH_2 \rightarrow E + SH_2 + A \tag{3}$$
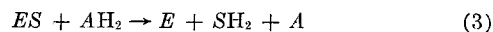
where S and AH_2 are hydrogen peroxide and leucomalachite green, respectively, and ES is the peroxidase-peroxide complex. Several years later quantitative measurements on this reaction were made by Chance (*6, 7, 9*) providing, for the first time, direct substantiation of the mathematical equations of the Michaelis theory.

Rapid reactions involving molecular complexes are best studied by the flow technique of Hartridge and Roughton (*16*) where the reactants are rapidly mixed and forced under pressure through an observation tube. At convenient positions along the tube (their distances from the site of mixing being a measure of the time of the reaction) some physical property characteristic of the complex is measured.

Significant improvements in the Hartridge-Roughton technique, particularly in speed range and fluid economy, have been made by Chance (*8, 11*) who developed the "accelerated" and "stopped" flow methods. In this modification the reactant fluids (ca. 0.1 ml.) are introduced into the mixing chamber through syringes, whose plungers are driven manually by a short, sharp blow. Several milliseconds after the plunger stroke is completed, the flow in the observation tube stops, and the course of the reaction is recorded by a rapid and very sensitive spectrophotometric technique. Using this system Chance determined absorption spectra and studied the kinetics of the reactions of a number of enzyme-substrate complexes.

Comprehensive discussions of the flow techniques recently have been published by Chance (*10, 11*) and Roughton (*42*).

The second class of short-lived species, atoms, and free radicals (ionic fragments might also be included here) participate in a variety of reactions, but are usually associated with chain reactions. These reactions may vary in rate from explosions to very

slow oxidations (formation of peroxides in hydrocarbons). Most gas reactions and a large number of reactions in solution, such as polymerization, halogenation, and photochemical decomposition of organic molecules, occur by free radical mechanisms.

Of all classes of reactions free radical reactions have been studied the most intensively, but, on the whole, the least is known about them. Even though their over-all rates may be slow enough to measure by classical techniques, their detailed mechanisms can only be arrived at by inference. To prove the mechanisms it is necessary to establish the identity of the participating radicals together with the specific reactions which they undergo and the rate constants for these reactions. Up until very recently, attempts to find out this information have met with limited success.
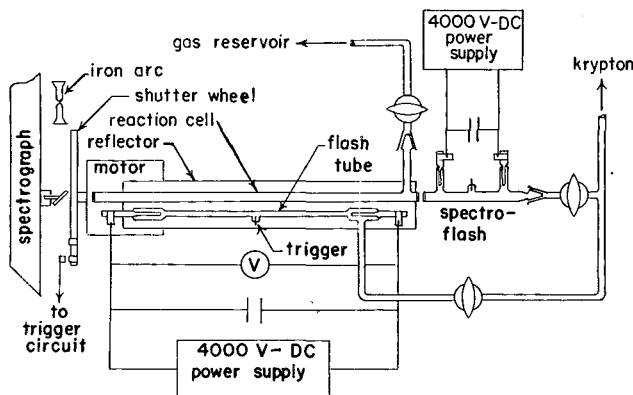
Figure 1. Flash photolysis apparatus

The third class of short-lived species, atoms, and molecules in excited electronic states, though generally associated with free radical reactions, differ from radicals in that they may lose their stored energy by processes other than reaction—e.g., fluorescence, phosphorescence, and radiationless transitions. Species of this type participate in photosensitized reactions. A typical example is the primary process in photosynthesis—the transfer of energy from the excited chlorophyll molecule (in green plants) to substrates resulting in the scission of the water molecule. The important characteristics of these species are their absorption spectra, emission (fluorescence or phosphorescence) spectra, lifetime, and the manner in which they transfer energy to substrates.

These short-lived species, whether they be atoms, radicals, or molecules in excited states, are usually present in minute concentration. Thus the problem of their identification and subsequent study of their reactions is their generation (or isolation) in sufficiently high concentration so that physical properties such as absorption spectra can be measured.

In principle the generation of these high concentrations can be accomplished thermally, electrically, or photochemically. The difficulty, however, is that the energy must be introduced into static systems in large amounts and in times short compared to the life times of the reactive species formed. Furthermore, it is desirable that the energy be introduced homogeneously (35) into the system if the concentrations and properties of these short-lived species are to be measured accurately. In addition, it is also desirable that this energy be specific in its action—i.e., that only one type of specie be produced.

Energy may be rapidly introduced into a system thermally by means of a shock wave, but this technique though useful in some cases (3, 4) is limited to gases. Furthermore, the mode of energy introduction is not homogeneous and its action may be nonspecific. The same objections may be raised against energy introduction by means of an electrical discharge.

For a number of reasons it would appear that the ideal method of introducing energy into a system is photochemically. These reasons are:

The mode of introduction is essentially homogeneous; hence no large thermal or concentration gradients exist, making the determination of the concentration of short-lived species and their reaction rates a relatively simple problem.

Absorbed light energy is specific in its action—i.e., as to what bonds are broken and hence what radical species are produced or what particular molecule is excited.

Up until recently the chief difficulty of introducing energy rapidly into a system photochemically has been in obtaining a sufficiently intense source of radiation. The most powerful steady sources of light in the near ultraviolet seldom exceed 10^{-6} einstein per second, which means that, at best, about 10^{-4} mole of reactive species can be produced in a millisecond.

FLASH PHOTOLYSIS

Five years ago Porter (38) introduced the flash photolysis technique, a powerful and general method of introducing large amounts of light energy into a system rapidly. Essentially the technique involves the triggered discharge of energy stored in a capacitor across a large spark gap, which is simply a pair of tungsten electrodes enclosed in a quartz tube filled with inert gas. The energy of the discharge appears primarily as light whose spectrum is a continuum of roughly constant intensity in the region 2000 to 4500 A., gradually decreasing towards the red. This technique has the advantage in that light energy up to several thousand joules can be introduced into a system in times of the order of 100 microseconds, and if the energy of the flash is reduced the time of the flash can be cut to approximately 10 microseconds. Thus, it is possible to produce as much as 10^{-4} mole of intermediates with a single flash, a quantity sufficient for measuring absorption spectra.

In Figure 1 is shown the flash photolysis apparatus essentially as developed by Porter (38) for studying gas reactions. At one focus of an elliptical reflector is mounted a cell, 1 meter long and 1 cm. in diameter, filled with reactant gases from a reservoir. At the other focus is a quartz flash tube filled with krypton. Focused along the axis of the reaction cell and onto the slit of a large aperture spectrograph is a second flash tube whose flash intensity is about one fiftieth of the main flash. A shutter wheel rotates in front of the slit, and on its periphery are mounted two platinum contacts which serve to trigger first the main flash, and then, the spectroflash. The slit is open to the system only when the spectroflash is triggered in order to eliminate scattered light from the main flash. By adjusting the distance between the contacts on the wheel, the time between the main flash and the spectroflash may be varied. In this manner a photographic record of the absorption spectrum may be taken at any arbitrary time after the main flash.

One of the systems studied by Norrish and Porter (37) was the explosion of hydrogen and oxygen sensitized by nitrogen dioxide. They carried out a series of duplicate flash photolysis experiments on the system; 10 mm. of hydrogen + 5 mm. of oxygen + 0.75 mm. of nitrogen dioxide, varying the time interval between the photolytic and spectroflashes. The series of spectrograms obtained showed that the 1,0 and 2,0 bands of the hydroxyl radical appeared immediately after the photolytic flash, grew in to maximum intensity in approximately 1.2 milliseconds, and then gradually disappeared. Further experiments on mixtures of nitrogen dioxide and hydrogen alone showed that the rate of fall off in intensity of the 1,0 bands of the hydroxyl radical increased with hydrogen pressure.
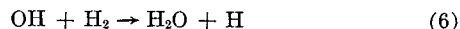
Since photolysis of the light-absorbing species occurs by the reaction

$$NO_2 + h\nu \rightarrow NO + O \qquad (4)$$
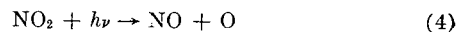
The fact that the spectrum of hydroxyl radicals appeared immediately after the flash indicates that the reaction

$$H_2 + O \rightarrow H + OH \qquad (5)$$
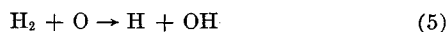
starts the chain. The increased rate of disappearance of the hydroxyl radical with hydrogen pressure shows that the reaction

$$OH + H_2 \rightarrow H_2O + H \qquad (6)$$

participates in the chain. In this way direct evidence for two of the postulated elementary reactions in the explosion of hydrogen and oxygen was furnished for the first time.

If precise rate data on the appearance or disappearance of a particular intermediate formed in flash photolysis are desired, then the spectrograph is replaced by a monochromator or suitable filters and a steady source of radiation is used in place of the spectroflash. Rapid changes in transmitted light intensity at wave lengths corresponding to characteristic absorption peaks of the intermediate may be measured with a multiplier phototube and oscilloscope. Satisfactory results have been obtained (1) with this technique when precautions were taken to minimize the flash duration and scattered light, and when the multiplier phototube has a very high signal to noise ratio.

The versatility of the flash photolysis technique may be shown by reference to Figure 2, which illustrates some of the possible processes that occur when a molecule, AB, absorbs ultraviolet or visible light. The initial absorption of a photon may raise the molecule to an excited singlet state. In this state it may lose energy by several processes: fluorescence radiation, radiationless transition to the ground state with the energy appearing as heat, or radiationless transition to a long-lived excited state, generally assumed to be a triplet (23). The molecule may remain in this state for a considerable length of time (ca. 10^{-3} second in fluid solvents) gradually losing its energy by phosphorescence emission and radiationless processes, or it may also undergo a variety of processes such as scission into radicals, ions, loss of an electron, or reaction with substrates. However, these latter processes need not occur exclusively from the lowest triplet state. Which of these processes actually does occur depends on the nature of the molecule and its surroundings, as well as its physical condition—i.e., whether it is in fluid or rigid media.

Flash photolysis may be used to produce any of the above intermediates and to study their various reactions and decay processes. The technique does, however, have limitations (36)—the chief ones being that the intermediates studied must have spectra in quartz-ultraviolet or visible regions, and that the rate processes studied must necesarily be longer than the duration of the flash.

The absorption spectra (17, 38), as well as the reactions (27, 35, 41) of a number of radicals, have been determined by flash photolysis. One process, the rate of recombination of iodine atoms, has received considerable study (12, 33, 43) by this technique. It has also been applied to the study of triplet states of a number of aromatic molecules (40).

A unique application of flash photolysis is the very rapid and homogeneous introduction of large quantities of heat into a system. This is accomplished by using a sensitizer molecule which has a discrete absorption spectrum in the spectral range of the flash tube. In this case all the energy absorbed by the sensitizer is rapidly converted to heat, generating temperatures as high as 1000° C. (35). This technique has been used by Knox, Norrish, and Porter (19) to study the mechanism of carbon formation at high temperatures.

An excellent discussion of the utility of the flash photolysis technique has recently been published by Norrish and Porter (35).

SPECTROSCOPY IN RIGID SOLVENTS

There is another method by which high concentration of short-lived species may be produced, and that is by use of glass forming

solvents. If a molecule is dissolved in an appropriate transparent solvent, such as an 8 to 3 to 5 mixture of ether, isopentane, and alcohol (EPA), frozen to liquid nitrogen temperatures where the solution sets to a rigid optically clear glass, and the solution irradiated by light absorbed only by the solute molecule, the molecule may split in radical or ionic fragments. These fragments remain stabilized since their bimolecular recombination reaction is prevented in this rigid glassy state. The initial splitting occurs because a certain fraction of the excitation energy is converted into heat which melts the solvent in the close vicinity of the excited molecule, allowing the fragments to move apart from each other by several molecular diameters. This local heating is quickly dissipated throughout the solvent and freezing sets in immediately, preventing recombination of the fragments. As the fragments produced remain stabilized, conventional spectroscopic method may be used to study their absorption spectra.

Excited states of molecules may be produced in rigid glassed solvents and, though they are not stabilized as in the case of radicals and ions, bimolecular quenching processes, as well as "internal conversion" to the ground state, are impeded (23) making it possible to study their natural radiative processes—i.e., fluorescence and phosphorescence. Triplet-singlet transitions (phosphorescence) under these conditions often have lifetimes of the order of several seconds. Thus, by using intense cross illumination to populate the triplet, it is possible in some cases to observe its absorption spectrum (32).

G. N. Lewis and his school introduced this technique and have applied it to the study of a number of photoprocesses (20–23). The spectra of a number of complex radicals such as diphenyl nitrogen, phenyl sulfur, and triphenylmethyl were measured (22). More recently Porter and Norman (39) have observed the spectra of CS, ClO, and benzyl radicals.
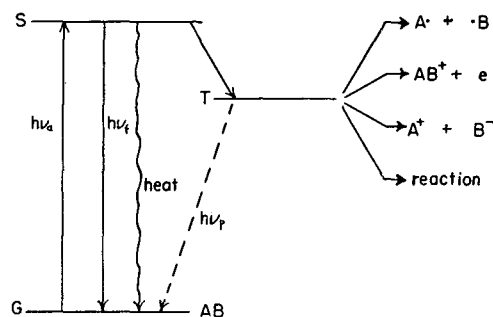

Figure 2. Possible processes occurring when molecule, AB, absorbs ultraviolet or visible light

The process of photoionization or electron ejection is particularly favored in the rigid glassed state, especially for complex molecules such as phenols and amines, and anions such as the triphenyl methide ion.

Linschitz, Rennert, and Korn (26) have observed an interesting case where the same semiquinone is formed in a mixture of ether, isopentane, and alcohol glass by two reciprocal processes; in the first instance by photooxidation of diphenyl-p-phenylenediamine and, in the second, by photoreduction of diphenyl-p-phenylenediamine.

Abnormal luminescence effects (20) may often be associated with certain processes in rigid glasses. Linschitz, Berry, and Schweitzer (24) studied the system, lithium diphenylamide in the glassed solvent; 2 to 3 to 3 to 1 ether, isopentane, triethylamine, and trimethylamine (EPTM). On irradiation of this anion a new peak developed at 7500 A., characteristic of the

neutral diphenyl nitrogen radical. Concurrently a broad absorption in the far red appeared apparently because of the ejected electrons which had been trapped in the solvent. When the irriadated glass was allowed to melt slowly a brilliant long-lived luminescence lasting several minutes was observed. When the solution was quickly refrozen, the luminescence was quenched. Measurement of the absorption spectrum at this point showed a drop in both the radical and solvated electron absorption peaks which indicated that the luminescence arises from recombination of the radical and electron.

A further significant feature of the delayed luminescence was that its emission spectrum matched closely the phosphorescent spectrum of the parent amine indicating that radical-electron recombinations resulted in the formation of triplet states. In no case studied was fluorescence emission observed on melting the glass. Hence, it appears that photoionization and the recombination process in molecules of this type occur through triplet states (cf. Figure 2).

LONG-LIVED EXCITED STATE OF CHLOROPHYLL MOLECULE

The kinetics of a number of chlorophyll sensitized reactions in vitro (14) indicate that energy is stored in the molecule in some long-lived excited state, presumably a triplet (5). Similar considerations (13) indicate that such a state is also involved in the in vivo process, photosynthesis. For this reason, there has been considerable interest in establishing the existence and properties of this state. Studies in rigid glassed solvents and by flash photolysis have been instrumental in this regard.

Calvin and Dorough (5) demonstrated the existence of a triplet state in chlorophyll by observation of its phosphorescence in a rigid glassed solvent of ether, isopentane, and alcohol. Livingston (29) disputed this observation, but recently Becker and Kasha (2) have established its existence conclusively.

Linschitz and Rennert (25) made a qualitative study of the absorption spectrum of the long-lived state of chlorophyll by observing changes in transmittance of chlorophyll (in the rigid glassed solvent ether, isopentane, and alcohol) when it was intensely cross-illuminated. They observed partial "bleaching" of the characteristic blue (4720 A.) and red (6550 A.) absorption peaks of chlorophyll and enhanced absorption in the green and far red (7000 A.) regions of the spectrum where chlorophyll is transparent.

Livingston (28), Livingston, Porter, and Windsor, (30), and Livingston and Ryan (31) have made preliminary studies on the reversible bleaching of chlorophyll solutions at room temperature using the flash photolysis technique. They observed, on flashing, a bleaching of the blue absorption peak and a growing in of a new peak in the green characteristic of the long-lived state. Their results (31) also indicated that two long-lived states were involved in the bleaching reaction.

More recently, a systematic study of the bleached state of chlorophyll has been undertaken using improved flash photolysis techniques, and the complete absorption spectrum of this state has been obtained (1). In addition to the absorption peaks in the green observed by Livingston, a characteristic absorption peak was found in the red (7000 A.) confirming the observations made by Linschitz and Rennert (25). The apparent existence of two long-lived states (31) was also confirmed.

SUMMARY

Classical kinetic studies have shown that intermediate short-lived species participate in the majority of chemical reactions. Today considerable effort is directed toward developing methods of identifying these intermediates and studying their reactions. Flow methods and flash photolysis have been particularly useful in this respect. Rigid glassy solvents have also been used to advantage in both isolating and studying the decay processes of many of these short-lived species.

LITERATURE CITED

(1) Abrahamson, E. W., and Linschitz, H., J. Chem. Phys., in press.
(2) Becker, R. S., and Kasha, M., J. Am. Chem. Soc., 77, 3669 (1955).
(3) Britton, D., Davidson, N., and Schott, G., Discussions Faraday Soc., 17, 58 (1954).
(4) Carrington, T., and Davidson, N., J. Phys. Chem., 57, 418 (1953).
(5) Calvin, M., and Dorough, G., Science, 105, 433 (1947).
(6) Chance, B., Arch. Biochem., 22, 224 (1949).
(7) Chance, B., J. Biol. Chem., 151, 553 (1943).
(8) Chance, B., J. Franklin Inst., 228, 459 (1940).
(9) Chance, B., Science, 92, 445 (1940).
(10) Chance, B., in "Techniques of Organic Chemistry," Weissburger, A., editor, vol. 8, chap. IX, Interscience, New York, 1953.
(11) Ibid., chap. X, part 2.
(12) Christie, M., Norrish, R., and Porter, G., Nature, 174, 785 (1954).
(13) Franck, J., Ann. Rev. Plant Physiol., 2, 57 (1951).
(14) Gaffron, H., Biochem. Z., 264, 251 (1933).
(15) Haldane, J. B. S., "Enzymes," Longmans, Green, London, 1937.
(16) Hartridge, H., and Roughton, F., Proc. Roy. Soc. (London), A104, 376 (1923).
(17) Herzberg, G., and Ramsay, D. A., Discussions Faraday Soc., 14, 11 (1953).
(18) Keilen, D., and Mann, T., Proc. Roy. Soc. (London), B122, 119 (1937).
(19) Knox, K., Norrish, R., and Porter, G., J. Chem. Soc. 210, 439 (1952).
(20) Lewis, G. N., and Bigeleisen, J., J. Am. Chem. Soc., 65, 2419 (1943).
(21) Lewis, G. N., and Kasha, M., Ibid., 66, 2100 (1944).
(22) Lewis, G. N., and Lipkin, D., Ibid., 64, 2801 (1942).
(23) Lewis, G. N., Lipkin, D., and Magel, T., Ibid., 63, 3005 (1941).
(24) Linschitz, H., Berry, M., and Schweitzer, D., Ibid., 76, 5833 (1954).
(25) Linschitz, H., and Rennert, J., Nature, 169, 193 (1952).
(26) Linschitz, H., Rennert, J., and Korn, M., Ibid., 76, 5839 (1954).
(27) Lipscomb, F., Norrish, R., and Porter, G., Ibid., 174, 785 (1954).
(28) Livingston, R., J. Am. Chem. Soc., 77, 2179 (1955).
(29) Livingston, R., Officer of Naval Research, First Annual Report, Research Project 059,028 (1948).
(30) Livingston, R., Porter, G., and Windsor, M., Nature, 173, 485 (1954).
(31) Livingston, R., and Ryan, V., J. Am. Chem. Soc., 75, 2176 (1953).
(32) McClure, D., J. Chem. Phys., 19, 670 (1951).
(33) Marshall, Royal, and Davidson, N. R., Ibid., 21, 659 (1953).
(34) Michaelis, L., and Menten, M., Biochem. Z., 49, 333 (1913).
(35) Norrish, R., and Porter, G., Discussions Faraday Soc., 17, 40 (1954).
(36) Norrish, R., and Porter, G., Nature, 164, 658 (1949).
(37) Norrish, R., and Porter, G., Proc. Roy. Soc. (London), A210, 439 (1952).
(38) Porter, G., Proc. Roy. Soc. (London), A200, 284 (1950).
(39) Porter, G., and Norman, I., Nature, 174, 508 (1954).
(40) Porter, G., and Windsor, M., Ibid., 17, 178 (1954).
(41) Porter, G., and Wright, F., Discussions Faraday Soc., 14, 23 (1953).
(42) Roughton, F., "Techniques of Organic Chemistry," Weissburger, A., editor, vol. 8, chap. X, Interscience, New York, 1953.
(43) Russell, K. E., and Simons, J., Proc. Roy. Soc. (London), A217, 271 (1953).

Kinetics and Mechanism in Formation of Slightly Soluble Ionic Precipitates

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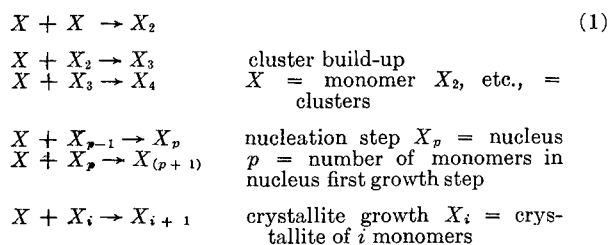*Department of Chemistry, University of Michigan, Ann Arbor, Mich.*

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It is believed that precipitation occurs in two stages: the first involves nucleation and growth, the second involves only the growth process. The formation of primary particles is effectively terminated early in the precipitation process when the existing particles have reached a size such that their surface contribution to the growth rate in combination with the higher solubility of small particles reduces the effective rate of nucleation to a relatively small value. Chronometric integrals derived on the basis of the above concepts are shown to describe the precipitation process using barium sulfate as an experimental model.

THE kinetics of the precipitation process occupies an important place in the fundamentals upon which precipitation technology rests. For analytical chemistry, this technology has special importance. Within recent years, new concepts have been developed concerning the mechanism and kinetics of formation of slightly soluble ionic precipitates. Because the literature in this area is somewhat abstruse and scattered, an attempt is made here to present a simple interpretation of its content and an explanation of its main ideas.

From the standpoint of mechanism, precipitation is regarded as a series of stepwise reactions proceeding as follows:

$$X + X \rightarrow X_2 \tag{1}$$

$$X + X_2 \rightarrow X_3$$
$$X + X_3 \rightarrow X_4$$

cluster build-up
X = monomer X_2, etc., = clusters

$$X + X_{p-1} \rightarrow X_p$$
$$X + X_p \rightarrow X_{(p+1)}$$

nucleation step X_p = nucleus
p = number of monomers in nucleus first growth step

$$X + X_i \rightarrow X_{i+1}$$

crystallite growth X_i = crystallite of i monomers

All polymers smaller than the nucleus are called clusters. They are a part of the mother phase and are in a quasi-equilibrium state with each other. With increasing size, the chemical potential of clusters presumably increases, and their equilibrium concentration decreases. Polymers exceeding the nucleus size are termed crystallites, or particles. They are in a separate phase and their chemical potentials, or solubilities, decrease with increasing size in a manner generally indicated by the Thomson equation. Standing between the clusters and the crystallites in size and at a maximum with respect to chemical potential is the nucleus. Addition of a monomer to the nucleus initiates a series of spontaneous further monomer additions, which is the growth reaction. The reactions within the cluster equilibrium leading to replacement of the growth-removed nuclei constitute the nucleation reaction.

Experimentally, the systems most useful to nucleation and growth studies are those in which the contributions of the sepa-

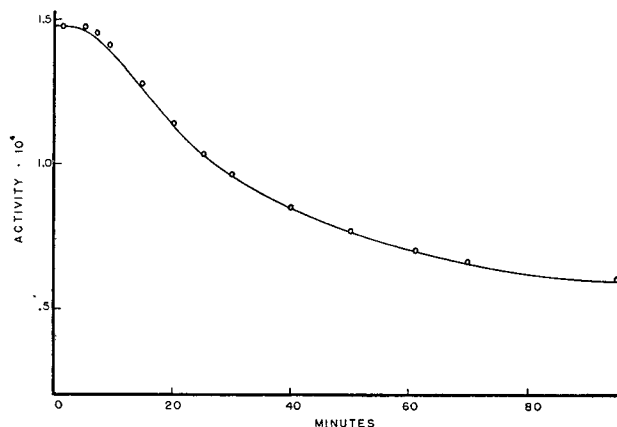[1] Present address, Shell Development Co., Emeryville, Calif.

Figure 1. Variation of $[Ba^{++}] = [SO_4^{--}]$ with time

Experimental points, measured conductometrically *(14)*. Curve calculated from empirical growth rate expression, Equation 6
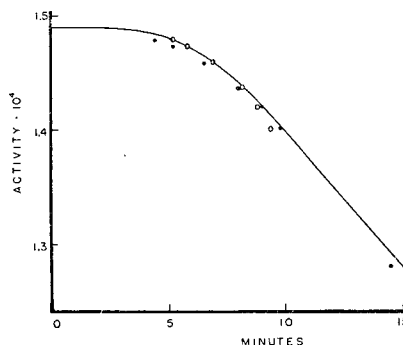

Figure 2. Induction period and growth surge of barium sulfate
○ $\sigma = 2/3$
● $\sigma = 1/2$

rate reactions are well separated with respect to the timing and the magnitude of their contribution. For these conditions a prototype system is one in which a long induction period is possible at high supersaturations. Certain supersaturations of barium sulfate admirably fit this prototype, and consequently this salt has been the subject of considerably more kinetic investigation than any other salt of its type. A typical precipitation curve is shown in Figure 1 and a close-up of the induction period is given in Figure 2.

It is profitable to begin our study by examining separately the natures of the nucleation and growth reactions and proceed then to consideration of the interplay of the two processes in the course of an actual precipitation.

One of the objectives of the studies is to find a chronometric integral (chronomal) which describes the experimental precipitation curve in terms of the nucleation and growth processes and which corresponds to a reasonable physical picture and reaction mechanism.

NUCLEATION

If the intermediates leading to nucleation (Equation 1) are in the same phase and in a quasi-equilibrium state, the nucleation reaction may be represented as $pX \to Xp$ and the nucleation rate as

$$\frac{d\nu}{dt} = KC^p \qquad (2)$$

where ν is the number of nuclei, K is the rate constant, and C is the concentration of monomers, or, for a salt of 1 to 1 configuration, $C = \sqrt{[A][B]}$.

It is assumed that the supersaturation is large enough that the dissociation of the nucleus does not take place and that monomer addition to the nucleus is a relatively rapid reaction. From a steady state treatment of the above nucleation process, Christiansen (4) found an indication in available experimental data that the steady state for nucleation is reached instantaneously. He derived the above rate expression with the aid of steady state assumptions.

The incipient crystallites are relatively soluble. Correspondingly, the back reaction for nucleation is undoubtedly greater than that for growth. As a consequence, as precipitation progressively depletes the supersaturation, the nucleation rate must be curtailed more sharply than the growth rate.

The magnitude of p is the index of rate of change of nucleation rate with change in monomer concentration. A comparison of the nucleation of water droplets and of barium sulfate serves to illustrate this point. Water nuclei are estimated to consist of approximately 100 molecules (19). Correspondingly, the appearance of nuclei is found experimentally to be critically dependent upon supersaturation. The range between the supersaturation above which nucleation occurs instantaneously and below which nucleation does not occur is a narrow one.

In contrast, for barium sulfate, the supersaturation limit at which turbidity appears immediately (17) is four or five times greater than the limit below which autonucleation is practically nonexistent (18). This comparison between water and barium sulfate indicates a lower dependence of nucleation rate on concentration for the latter. Kinetic treatments by Christiansen (5) and by Johnson and O'Rourke (14) of experimental data bear out this indication.

The probability of a low order for the nucleation of crystalline ionic substances has fundamental implications to problems related to the methods of bringing about the initial supersaturation. If the reactants are brought together by direct mixing, there arises the question of the number of nuclei generated from momentary higher supersaturations occurring in the mixing process in comparison to those formed after homogenization. Because the differential of nucleation rate with respect to concentration is of a low order, the extra amount of nuclei due to mixing fluctuations is not expected to be great relative to those arising after homogenization.

This expectation is confirmed for the barium sulfate system as shown by the data in Table I, which reveal that the number of particles formed is nearly independent of concentration and, thus, rejects the mixing fluctuation hypothesis for this system. This conclusion is further confirmed by the experiments and theory of Duke and Brown (9), discussed in a section below, which clearly relates the number of particles to kinetic properties of the precipitation reaction. Accordingly, the hypothesis of homogeneous nucleation taking place throughout the induction period and for a period thereafter is expected to prevail for the slightly soluble salts in concentrations presenting an induction period. In some experiments (13, 17) the mixing fluctuations are avoided

by chemical generation of the precipitating solute or one of the reactants—i.e., homogeneous precipitation. If the nucleation reaction is higher order, as is probably the case for sulfur hydrosol formation (24), it may be expected that a myriad of nuclei are formed almost instantaneously upon attainment of a certain critical supersaturation and that this reaction easily reduces the supersaturation below the limits in which nucleation occurs. As a consequence, further nucleation does not occur and a monodisperse sol is formed. In contrast, for a low order nucleation the gradually increasing supersaturation brings about, over a considerable concentration range, a steadily increasing flow of nuclei into the growth reaction. The complication of the variable nucleation rate probably affords more difficulties in interpretation than the uncertainties due to mixing fluctuations. Direct mixing is accordingly preferred for studies on crystalline precipitates.

Table I. Particle Count and Initial Concentration of Barium Sulfate

Solutions used. Barium chloride, aged 1250 hours, and sodium sulfate, aged 1400 hours

Procedure. Equal volumes of reagents were rapidly mixed to give the concentration indicated. Particles were counted microscopically when precipitation was complete.

$[Ba^{++}] = [SO_4^{--}]$ (Moles/Liter) $\times 10^4$	Particles/Liter $\times 10^{-9}$
2.5	1.2
5.0	1.1
10.0	1.2
15.0	1.2
20.0	1.0
25.0	1.8[a]

[a] Precipitation began immediately on mixing.

The possibility of induced nucleation is present in any nucleating system. Probably seeding is the most important and also the best understood of the induced nucleations. The effectiveness of seeds in inducing precipitation is closely related to the similarity between the lattices of the seed and of the precipitating substance (22). This limitation makes accidental seeding on foreign nuclei a much smaller consideration for nucleation of crystals than for nucleation of liquid droplets. With regard to the induction of nucleation by stirring, little evidence is available from systematic experimentation to indicate the effectiveness of this mechanism.

A special effect may be mentioned as induced nucleation. Fischer (11) has shown that, if freshly prepared solutions of barium chloride are used for the formation of barium sulfate, the particle size (and thereby the number of particles formed) is a function of the age of the solution. The number of particles decreases with increasing age of the reagent solution and the variability tends toward a constant. Tests carried out in these laboratories are summarized in Table II to illustrate the effect. The effect has not been satisfactorily explained and may account for certain contradictory results in the literature of barium sulfate formation.

Table II. Variation of Particle Count with Age of Barium Chloride Solution

(Equal volumes of $2 \times 10^{-3}M$ reagents, barium chloride and sodium sulfate, were rapidly mixed. Particles were counted microscopically when precipitation was complete)

Age of BaCl₂ Solution, Hours	Particles/Liter $\times 10^9$
0	16.4
3.0	4.0
4.5	3.5
10	1.9
22	1.3
34	1.2
72	1.1
166	0.7
1248	1.2

In general, the great number of nuclei formed and the reproducibility of their concentration in experiments with barium sulfate and other ionic precipitates indicate that processes bringing about nucleation in an accidental or random way are probably of minor importance for this type of system. From this and other considerations summarized, the following picture emerges for the nucleation process in slightly soluble ionic salt systems in which an induction period occurs. After the reagents are mixed (homogenization can be assumed to be instantaneous) nucleation takes place at a uniform rate. With the depletion of supersaturation at the end of the induction period, the nucleation rate, because of the relatively low stability of nuclei and incipient crystallites, decreases rapidly and its effective contribution to the precipitation rate is eliminated at a concentration substantially greater than the macro solubility. Accordingly, growth is the sole precipitation reaction beyond a certain intermediate stage in the process.

GROWTH

In general, the rate of crystal growth is a function of two variables, the surface available for growth and the concentration of the precipitating solute; hence,

$$dC/dt = - k \times f(S) \times f(C) \qquad (3)$$

where $f(C)$ is a function of the concentration of the precipitating solute and $f(S)$ is a function of the available surface. (Note that $C = [AB] = \sqrt{[A^+][B^-]}$ or $C = [A^+] = [B^-]$.)

DEPENDENCE OF GROWTH RATE ON CONCENTRATION OF PRECIPITATING SOLUTE

In a numerical integration of the portions of the barium sulfate precipitation curves attributable solely to growth, Johnson and O'Rourke (14) found the function of concentration, $f(C)$, to be $(C - C_s)^4$. Davies and Jones (6, 7) and Kobayashi (15) found the growth rate of silver chloride upon seed crystals to be dependent upon $(C - C_s)^2$.

Because diffusion control predicts that the concentration function be first order, this mechanism is ruled out for these systems. Also it has been shown by Turnbull (21) that, for very small particles, surface control rather than diffusion control is indicated for the growth process.

Growth by random accretion of ions, or ion pairs, on plane crystal surfaces is not generally held to be a probable mechanism for crystal growth because of the low energy release involved and the corresponding low stability of the added ions.

A more probably hypothesis has been developed, notably by Volmer (23) and by Becker and Doering (1) which involves the formation of "two-dimensional growth nuclei" followed by two-dimensional growth over the respective planes. Growth in the third dimension takes place by successive repetitions of two-dimensional nucleations followed by respective spreading reactions. The mechanism and kinetics of two-dimensional nucleation are expected to follow along lines discussed in the preceding section. The reaction may be expressed as follows:

$$qX \to Xq \qquad (4)$$

in which q represents the number of monomers in the growth nucleus. If growth over the plane spreads rapidly after each nucleation, the growth rate assumes the dependence on precipitant concentration characteristic of the nucleation reaction—i.e., the growth rate is expected to be q-order. Thus, the results cited indicate a growth nucleus of two ion pairs for barium sulfate and of a single ion pair for silver chloride.

A hypothesis for growth which does not require the postulation of growth nuclei has arisen from consideration of imperfections, or dislocations, in the lattice of the developing crystallite (12). Dislocations in the form of molecular terraces serve to stabilize entering growth units. Certain dislocations perpetuate themselves throughout the crystallite's development, thus permitting

continuous growth without the high energy requirement imposed by the formation of two-dimensional nuclei. The prevalence of dislocations in crystal lattices is well established and surface structures have been microscopically observed which lend support to this hypothesis. Addition to the dislocations is expected to take place in the smallest neutral units. Accordingly, the growth rate is predicted to be second order for silver chloride or barium sulfate. Although the experimental growth order for silver chloride, second order, meets this prediction, that for barium sulfate, fourth order, is not in agreement with the dislocation theory.

The solubility term, C_s, enters the experimental rate expressions cited above by modifying the precipitant concentration term to be a supersaturation term—i.e., the rate is dependent upon $(C - C_s)^q$, not C^q. Davies and Jones (7) explain this for silver chloride by assuming that growth occurs only from a surface film in which an equilibrium concentration of silver ions and of chloride ions, C_s, is maintained in an intact absorbed monolayer. Only concentrations in excess of C_s,—i.e., $(C - C_s)$—are then available for growth.

SURFACE FUNCTION AND GROWTH RATE

The surface function is probably the resultant of several effects taking place during growth. Although it is not possible to resolve these effects experimentally especially for rapidly growing, incipient particles, the contributions of certain of the effects can be predicted. Perhaps then a first approximation of the mechanisms in play can be made.

For ionic crystals with the sodium chloride structure, Stranski (20) has calculated the energy involved in adding an ion to various sites on a crystal surface. The calculations indicate that the inception of new layers is more likely to occur at edges or corners than in the face. Observations of growing crystals made under the microscope by Bunn and Emmett (3) revealed layers growing as a general rule from points on faces. The growth nucleation rate is independent of particle size if confined to corners. If nucleation takes place on edges only, the rate is proportional to $i^{1/3}$; for surface nucleation the corresponding exponential is $i^{2/3}$ (i is the number of monomers in the growing particle). These considerations are valid if the crystal form does not change in the process. However as high index faces are eliminated in the particle's development, simpler forms evolve with relatively fewer corners and edge and surface development. These changes, if averaged into the growth nucleation rate, have the effect of adding a negative component to the exponentials described—e.g., for surface nucleation, $i^{1/2}$ might represent the nucleation function averaged over a series of form simplifications. Upon conversion of the nucleation rate to growth rate, a positive component is added. If growth spreads immediately over the face, or contiguous faces, from the nucleation site, the growth interval resulting from each nucleus expands with increasing particle size; thus,

$$dC = - i^{2/3}dn \qquad (5)$$

in which n refers to growth nuclei. The corresponding surface functions of growth rate, dC/dt, for corner, edge, and surface nucleation are then, respectively, $i^{2/3}$, i, and $i^{4/3}$, other effects, such as form simplifications, being neglected.

The foregoing are primary considerations arising from the surface nucleation theory of growth. Similar considerations are derivable for the dislocation theory. Although they can suggest mechanisms, their present status does not permit conclusions to be drawn from existing experimental evidence.

Experimentally, the surface function for barium sulfate is $i^{2/3}$. This was determined by Johnson and O'Rourke (14) in two ways: by numerical integration of the last part of the precipitation curve (Figure 1), assuming a homodisperse set of particles and by analysis of the curvature at the termination of the induction

period as described in the next section. For silver chloride, Kobayashi (15) has found the surface function to be $i^{1/3}$ from growth measurements made on seeded systems.

The surface function brings to the growth rate its autoinduction character. The abrupt termination of the induction period is largely dependent upon this function. The curvature following this terminating surge is controlled largely by the order of the growth reaction, q.

A summary of the cited experimental evidence yields for a homodisperse set of growing barium sulfate particles

$$-dC/dt = k\nu^{1/3}(C_0 - C)^{2/3}(C - C_s)^4 \qquad (6)$$

The corresponding equation for silver chloride is

$$-dC/dt = k\nu^{2/3}(C_0 - C)^{1/3}(C - C_s)^2 \qquad (7)$$

INDUCTION PERIOD AND GROWTH SURGE TERMINATING IT

Phenomenologically, the striking features of the induction period are the absence of any detectable change throughout its lifetime and its abrupt termination as evidenced by the appearance of turbidity and decrease in reagent concentration. The two empirical relationships pertinent to these features are, respectively, the reciprocity relationship of the induction period

$$C_0^x t_i = \text{constant} \qquad (8)$$

in which t_i is the length of the induction period, and x is a constant, and the chronomal for the growth surge terminating the induction period is

$$(C_0 - C) = bt^y \qquad (9)$$

b and y being constants. The activity of the precipitating solute is included in the constant b term, thus limiting the extent to which the relationship is valid. However, the order of time dependence, y, determined at the outset of the growth surge appears to be decisive in establishing the kinetics and mechanism during the induction period. Mechanisms and mathematical treatments to explain this behavior have been proposed following either of two premises.

Nucleation occurs as an instantaneous burst of reaction [Frisch and Collins (13) and Turnbull (21)]. The explanation of the induction period and the observable precipitation process is based on growth of the nuclei thus formed.

Nuclei formed at a uniform rate after the moment of mixture steadily expand the available growth sites. This premise has served as the basis for two independent approaches to the kinetics of precipitation: (a) the "dynamic" approach of Johnson and O'Rourke (14) [cf., (2) and (10)] and (b) the steady state, or "static," approach of Christiansen (5). After following the above treatments to their respective chronomals examination of their agreement with data obtained is made, in particular from experiments on barium sulfate, and also the plausibility of their physical bases.

Instantaneous nucleation is considered unlikely. It is instructive, however, to derive the chronomal for barium sulfate corresponding to this hypothesis. If ν particles are formed and develop according to the growth rate of Equation 6, the chronomal during the induction period and immediately upon its termination (where C^q is constant) is

$$C_0 - C = k^3\nu C^{3q}t^3 \qquad (10)$$

Third-order time dependence for the growth surge chronomal—y = 3—is thus indicated.

A fourth-order time dependence is obtained for the barium sulfate system if nucleation is assumed to make a considerable contribution parallel to the growth reaction during the induction period. Within any period t after mixing the reactants, there are infinitesimal intervals, $d\tau$, $(0<\tau<t)$ in each of which an infinitesimal number of nuclei, $d\nu$, is created and the nuclei proceed to grow according to the experimental growth law. The amount of

precipitate formed by each group of particles from the time of their inception, τ, to time t is given by Equation 10 with $d\nu$ replacing ν and $(t - \tau)$ replacing t. Upon combining this relationship with the nucleation rate equation (Equation 2) and integrating from the moment of mixing to time t with C^{3q+p} held constant, an expression indicating fourth-order time dependence is obtained for the over-all reaction:

$$C_0 - C = \frac{Kk^3C^{3q+p}}{27}\int_0^t (t - \tau)^3 d\tau \qquad (11)$$

$$= \frac{Kk^3C^{3q+p}}{108}t^4$$

Bransom and Dunning (2) generalized the concentration functions of the nucleation and growth rates to be $F(S_o)$ and $f(S_o)$, respectively, and following considerations similar to those of the previous paragraph, also predicted fourth-order dependence of time on the amount of precipitate.

The curtailment of nucleation, or its effective contribution to the over-all reaction, was used by Duke and Brown (9) as the basis for predicting the dependence of the number of particles on the initial concentration as a function of the differential between nucleation and growth orders $(p - q)$. Combination of Equations 2 and 11 with the assumption that the growth reaction effectively stops nucleation when the particles have attained a certain average size, containing λ moles per particle gives

$$\nu = \frac{K}{k}(108\lambda)^{1/3}C^{p-q} \qquad (12)$$

Hence, the particle count data in Table I, obtained using a hemocytometer, indicate that p and q are equal for barium sulfate. This conclusion is in agreement with the evaluation obtained by another route described below.

Christiansen (4, 5) has approached the kinetics of precipitate formation via steady state assumptions and treatment. _ _tations below follow the notations already established in thi _er.)

The growth process is represented by a flow sheet in wl _u polymers made up of successively greater numbers of monomers are represented as follows.

$$X_1 \xrightarrow{s} X_2 \xrightarrow{s} X_: \xrightarrow{s} . \xrightarrow{s} X_p \to \ldots \to X_i \xrightarrow{s} \ldots \xrightarrow{s} X_n$$
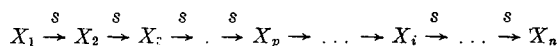
At the steady state, the rates of monomer addition within respective classes of polymers are all the same—i.e., s. At any time t in the process, the concentrations of polymers of n or fewer monomers are steady, whereas the concentration of the polymer X_{n+1} and the polymers containing more than $n+1$ monomers do not exist. The steady state rate, s, is subject to change with advancement of the value of n.

The concentration of each i-mer, c_i, is shown to be

$$c_i = \frac{s}{ki^\sigma C^q} \qquad (13)$$

in which i is analogous to the surface function discussed in the preceding section. The "concentration" of monomers bound in each i-mer, $(C_o - C)_i$ is

$$(C_o - C)_i = \frac{si^{1-\sigma}}{kC^q} \qquad (14)$$

When the particles in the advancing front contain n monomers, the total depletion in precipitating solute concentration is found to be

$$(C_o - C) = \frac{sn^{2-\sigma}}{kC^q(2 - \sigma)} \qquad (15)$$

Reference to the steady state flow diagram recalls that at the steady state the rate of disappearance of polymers within each class is equal to the nucleation rate. The nucleation rate ex-

pression derived from Christiansen's steady state treatment is the same as that assumed in the preceding treatment, Equation 2. Also the over-all rate of reaction at the point at which the particles in the advancing front contains n monomers is $s(n+1) \sim sn$; hence,

$$dC/dt = -sn = -kC^p n \qquad (16)$$

Combining Equations 14 and 15 and symbolizing $\dfrac{1}{2-\sigma}$ by m produces an expression in differential form

$$-dC = [(C_o - C)(2 - \sigma)(kC^q)(KC^p)^{1-\sigma}]^m dt \qquad (17)$$

If C^q and C^p are held constant, integration yields

$$(C_o - C)^{1-m} = (1 - m)(K^{1-\sigma}k/m)^m C^{m[q + (1-\sigma)p]} \times t \qquad (18)$$

Equation 17 is a general chronomal for the induction period and the region of its immediate termination. It is important that an expression in complete agreement with this chronomal is obtained by the dynamic derivation, which led to Equation 11, if the general surface function, $(C_o - C)^\sigma$, is used instead of the specific term for barium sulfate $(C_o - C)^{2/3}$.

From the induction period chronomal can be derived the two empirical expressions of the induction period, Equations 8 and 9, and thereby the surface and concentration functions of the former can be related to the experimental constants of the latter.

By raising Equation 18 to the $\left(\dfrac{1}{1-m}\right)$ power, the form describing the termination of the induction period, Equation 9, is obtained —i.e., $C_o - C = \text{Const} \times t^{\frac{1}{1-m}}$, and the order of time dependence is found:

$$y = \frac{1}{1-m} = \frac{2-\sigma}{1-\sigma} \qquad (19)$$

The result indicates that the curvature in this region is determined solely by the surface function. In the log $(C_o - C)$ vs. log t plot, Figure 3, the solid straight line represents a slope of 4 and the dashed line a slope of 3. The line of slope 4 gives a much better representation of the limiting slope of the experimental curve; and it is concluded that the value of y is 4. The solution of Equation 19 yields $\sigma = 2/3$ in agreement with the value used in the numerical integration of the growth portion of the curve, Equation 6. Data obtained by Turnbull [Figures 6, 7, and 8 of reference (21)] also reveal a limiting value near 4 if the first points are used. If the time order, y, is in fact 3, then as Christiansen has indicated, $\sigma = 1/2$.

From Kobayashi's expression for the growth rate of silver chloride, Equation 7, $\sigma = 1/3$; hence, from Equation 19, $y = 5/2$ is predicted.

The reciprocity relationship of the induction period, Equation 8, is implicit in Equation 18 if it be assumed that the end of the induction period can be experimentally observed when a certain amount of precipitate has been formed—i.e., the term $(C_o - C)$ is a constant.

$$C^{mq - (1-m)p} t_i = \text{constant} \qquad (20)$$

With respect to the empirical Equation 8

$$X = mq + (1 - m)p \qquad (21)$$

For barium sulfate, the plot of log C_o vs. t_i is linear. Estimation of the slope yields $x = 4$. With $\sigma = 2/3$ and $q = 4$ the nucleation order, p, is calculated to be 4. Similar plots of data by Kobayashi on the silver chloride system yield $x = 4$, (15). When this value is combined with $q = 2$ and $\sigma = 1/3$ experimental growth constants for this system, the nucleation order found is 7.

Phenomenologically, the scheme which appears to fit the process during the growth surge is the following. The particles nucleated in the first moments of the induction period reach a stage of surface development at which their growth becomes rela-

tively very rapid. Higher order Tyndall effect is observable in the growth surge and confirms that the particles appearing at the end of the induction period are in a narrow age group (16). The effect of the rapid depletion of precipitable material is to choke off nucleation and maintain the already established particles as the prime contributors to further growth. On the basis of this picture, an approximate model is assumed for the last part of the precipitation—namely, beyond a given point, an invariant number of homodisperse crystallites grows until equilibrium is reached. Thereby, Johnson and O'Rourke found by numerical integration the growth rate expression given in Equation 6. From the particle counts given in Table I, the constant k for barium sulfate is estimated at 2.2×10^8 moles liters^{-1} seconds^{-1}. From this value of k, the nucleation constant, K, can be found with the aid of the growth surge data and Equation 11; the value thus obtained is 1.4×10^{22} particles liters^{-1} seconds^{-1}.

EFFECT OF TEMPERATURE

The absence of an effect of temperature on growth of silver chloride from seeded suspensions is reported by Davies and Nancollas (8). This effect is in contrast to results from the same study indicating an activation energy of 15.4 kcal. for dissolution of silver chloride. This is in good agreement with the heat of solution, 15.6 kcal., calculated from the solubility of silver chloride at various temperatures.
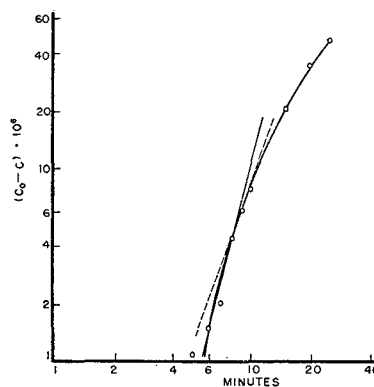

Figure 3.   Growth surge of barium sulfate

For barium sulfate, the effect of temperature on the concentration-induction period relationship, Equations 8 and 20, was studied by O'Rourke (18). The concentration exponent, $x = \dfrac{3q + p}{4}$ was found to be invariant, indicating that the precipitation mechanism is not affected by temperature. The constant term showed a slight decrease with increasing temperature. Since the rate constants appear in the denominator of the constant— viz., as Kk^3—the slight positive temperature dependence is indicated. However, the effect of temperature on the rate constants is small in approximate agreement with the results of Davies and Nancollas.

CONCLUSIONS

For the analysis of the kinetics and mechanism of precipitation, systems presenting an induction period terminated by appearance of precipitate and a surge of reaction are particularly useful.

Nucleation takes place at a regular rate throughout the induction period, probably according to $dv/dt = KC^p$. The rapid depletion of supersaturation after the induction period sharply curtails nucleation; hence, beyond a certain point precipitation occurs solely through growth.

Growth is a function of the available precipitate surface and of the supersaturation—viz., $dC/dt = k(C_o - C)^\sigma(C - C_0)^q$ the surface exponent, σ, represents, approximately, a complicated set of surface functions and is expected to be a simple fraction. The supersaturation exponent indicates the size of the two dimensional growth nuclei (*1*, *23*). For $q = 2$, the dislocation theory of growth is also possible.

A general chronometric integral (chronomal), derived by two independent methods on the basis of simultaneous nucleation and growth reactions, relates the experimental concentration to the constants and functions of those reactions. The chronomal is limited to the induction period and the growth surge which terminates it.

The curvature of the concentration-time curve at the termination of the induction period is determined solely by the surface function of the growth reaction and is useful for determining this function.

The effect of initial concentration on the length of the induction period is given by $C_o^x t_i = $ constant, in which $x = mq + (1 - m)p$, m being $\dfrac{1}{2 - \sigma}$. After the growth terms have been determined, the nucleation rate terms can be estimated from this relationship.

The variation of particle count with initial supersaturation is proportional to the difference between the nucleation and growth order—i.e., $(p - q)$. Since $p = q$ for barium sulfate the number of particles formed is independent of the initial concentration, 10^9 particles per liter are formed if an induction period precedes appearance of precipitation.

LITERATURE CITED

(1) Becker, R., and Doering, W., *Ann. Physik*, 24, 719 (1935).

(2) Bransom, S. H., and Dunning, W. J., *Discussions Faraday Soc.*, 5, 96 (1949).
(3) Bunn, C. W., and Emmett, H., *Ibid.*, 5, 119 (1949).
(4) Christiansen, J. A., *Acta Chem. Scand.*, 8, 909 (1954).
(5) *Ibid.*, p. 1665.
(6) Davies, C. W., and Jones, A. L., *Discussions Faraday Soc.*, 5, 103 (1949).
(7) Davies, C. W., and Jones, A. L., *Trans. Faraday Soc.*, 51, 812 (1955).
(8) Davies, C. W., and Nancollas, G. H., *Ibid.*, 51, 823 (1955).
(9) Duke, F. R., and Brown, L. M., *J. Am. Chem. Soc.*, 76, 1443 (1954).
(10) Dunning, W. J., *Discussions Faraday Soc.*, 5, 195 (1949).
(11) Fischer, R. B., and Rhinehammer, T. B., ANAL. CHEM., 25, 1544 (1953).
(12) Frank, F. C., *Discussions Faraday Soc.*, 5, 48 (1949).
(13) Frisch, H. L., and Collins, F. C., *J. Chem. Phys.*, 21, 2158 (1953).
(14) Johnson, R. A., and O'Rourke, J. D., *J. Am. Chem. Soc.*, 76, 2124 (1954).
(15) Kobayashi, K., *J. Chem. Soc. Japan*, 70, 125 (1949); *Science Repts. Tôhoku Univ.*, 37, 125 (1953).
(16) LaMer, V. K., and Barnes, M. D., *J. Colloid Sci.*, 1, 71 (1946).
(17) LaMer, V. K., and Dinegar, R. H., *J. Am. Chem. Soc.*, 73, 380 (1951).
(18) O'Rourke, J. D., Ph.D. thesis, University of Illinois, Urbana, Ill., 1953.
(19) Rodebush, W H., *Proc. Natl. Acad. Sci., U. S.*, 40, 789 (1954); *Ind. Eng. Chem.*, 44, 1289 (1952).
(20) Stranski, I. N., *J. physik Chem. A.*, 155, 466 (1928).
(21) Turnbull, D., *Acta Met.*, 1, 684 (1953).
(22) Turnbull, D., and Vonnegut, B., *Ind. Eng. Chem.*, 44, 1292 (1952).
(23) Volmer, M., "Kinetik der Phasenbildung," T. Steinkopf, Dresden and Leipzig, 1939.
(24) Zaiser, E. M., and LaMer, V. K., *J. Colloid Sci.*, 3, 371 (1948).

8th Annual Summer Symposium—Role of Reaction Rates

Slow Precipitation Processes

Application of Precipitation from Homogeneous Solution to Liquid-Solid Distribution Studies

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The direct addition of a precipitant to a solution results temporarily in a heterogeneity of conditions. In the vicinity where the precipitant has been introduced the formation of the solid phase takes place under conditions such that the solution concentrations vary between very wide limits. Therefore, results of coprecipitation studies obtained with conventional precipitation procedures may also vary markedly. Precipitation from homogeneous solution offers an ideal technique for controlling the rate and mode of addition of a precipitant. It permits a slow precipitation process, which allows near equilibrium to be established between the surface of the solid and the solution. It is thereby possible to determine the nature and extent of coprecipitation. Applications of this technique are described in which Doerner-Hoskins' distribution coefficients have been obtained for systems containing barium-radium mixtures. Other coprecipitation studies are also described, particularly some which have revealed that the extent of coprecipitation is negligible except during the initial and final stages of the precipitation process.

IN 1937, Willard (*45*, *46*) published the results of an investigation illustrating the use of urea in a slow precipitation process. This study, in which the aluminum ion was slowly precipitated as a basic salt, served as the stimulus for many subsequent papers describing applications of the technique now referred to as precipitation from homogeneous solution. The virtue of precipitation from homogeneous solution lies in the production of a very dense precipitate which minimizes coprecipitation. The principles of this technique and its merits have been reviewed in two papers (*9*, *38*).

Either an anion or a cation can be generated homogeneously within a solution to serve as a precipitant. Urea (*39*, *41*, *42*, *44–46*), hexamethylenetetramine (*25*), and acetamide (*10*, *38*) have been used to release hydroxyl ion. The sulfate ion can be produced from sulfamic acid (*8*, *34*) and either dimethyl (*5*, *6*) or diethyl sulfate (*38*). Oxalate from dimethyl (*11*, *20*, *41*) or diethyl oxalate (*1*, *4*, *12*); phosphate from metaphosphoric acid (*43*) or trimethyl or triethyl phosphate (*40*); carbonate from the trichloroacetate ion (*30*); sulfide from thioacetamide (*7*); iodate from periodate (*18*); periodate from iodate (*33*); and chromate from dichromate (*13*) are other examples.

The generation of a cation in a solution may be effected by one

or two methods. The cation may be released from a complex by removal of the ligand. This can be done either by a change in pH, use of an oxidizing agent to destroy the complexing reagent, or by temperature. Ethylenediaminetetraacetic acid [(ethylenedinitrilo)tetraacetic acid] complexes have been thus utilized (17, 24, 28). The silver-ammonia complex has been dissociated with hydrogen ion resulting from the slow hydrolysis of an ester (14).

By the other method, a cation may be converted from one oxidation state to the desired one. Cerium(III) iodate is soluble, whereas cerium(IV) iodate is not. Thus, cerium(III) may be oxidized in the presence of iodate to produce cerium(IV) iodate (47).

Precipitation from homogeneous solution has been used primarily in the development of improved gravimetric methods. However, it has also been used as a method for the removal of a major constituent—e.g., iron (44) from minor constituents—and in the separation by fractional precipitation of chemically similar pairs such as zirconium-hafnium (40) and the rare earths (11, 17).

Recent studies (14, 15, 19, 26, 32) have been concerned with the nature and extent of coprecipitation. Precipitation from homogeneous solution provides an excellent tool with which to study coprecipitation. For example, a precipitation rate can be slowed down, so that it will require 8 days to precipitate 150 mg. of silver chloride. Thus, a precipitation process can be examined at various stages and a study made of the distribution of trace materials in solid-liquid systems analogous to those of liquid-liquid distribution investigations. This paper describes the utilization of the technique of precipitation from homogeneous solution in such studies. The latter have been divided into two sections. One considers coprecipitation due to isomorphous mixed crystal formation and the other to adsorption.

COPRECIPITATION BY ISOMORPHOUS MIXED CRYSTAL FORMATION

Barium-Radium. Systems consisting of radium and barium salts have been studied extensively; an excellent review may be found in the text by Wahl and Bonner (35). The results have usually been interpreted in terms of two distribution laws.

Consider a system in which barium is precipitated as the sulfate in the presence of a minute quantity of radium. Thus

$$Ba^{++} (aq.) + SO_4^{--} (aq.) = BaSO_4(s)$$

$$K_1 = \frac{N_{Ba}}{(Ba^{+2})(SO_4^{-2})} \quad (1)$$
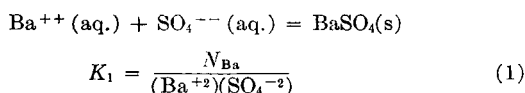
$$Ra^{++} (aq.) + SO_4^{--} (aq.) = (RaSO_4) \text{ dissolved in barium sulfate}$$

$$K_2 = \frac{N_{Ra}}{(Ra^{++})(SO_4^{--})} \quad (2)$$

Since the mole fraction of barium, N_{Ba}, is virtually 1, and since N_{Ra} essentially equals $(Ra^{++}/Ba^{++})_{crystal}$, then:

$$\frac{K_1}{K_2} = \left(\frac{Ra^{++}}{Ba^{++}}\right)_{solution} \times \left(\frac{Ba^{++}}{Ra^{++}}\right)_{crystal} \quad (3)$$

Rearrangement, and substitution of D for K_1/K_2, gives:

$$\left(\frac{Ra^{++}}{Ba^{++}}\right)_{crystal} = D \left(\frac{Ra^{++}}{Ba^{++}}\right)_{solution} \quad (4)$$

Equation 4 is known as the homogeneous distribution law. The equation describes a system in which, at equilibrium, the microcomponent is homogeneously distributed throughout the host crystal. Obviously, it may take considerable time for the system to reach this equilibrium state.

Doerner and Hoskins (3) considered that a more realistic approach assumed that only the crystal surface would be in equilibrium with the solution. Thus, Equation 4 becomes

$$\left(\frac{Ra^{++}}{Ba^{++}}\right)_{surface\ of\ crystal} = D \left(\frac{Ra^{++}}{Ba^{++}}\right)_{solution} \quad (5)$$

From this equation, Doerner and Hoskins obtained:

$$\ln \frac{Ra_{initial}}{Ra_{final}} = \ln \frac{Ba_{initial}}{Ba_{final}} \quad (6)$$

The subscripts in Equation 6 refer to initial and final solution concentrations.

Equation 6 is known as the logarithmic (or heterogeneous) distribution law and prescribes a nonuniform distribution of the microcomponent within the host crystal. The amount of microcomponent increases or decreases logarithmically from the center of the crystal outward depending on whether the value of the distribution coefficient is less than or greater than unity.

The determination of the distribution coefficient is difficult when a conventional precipitation process is employed. The addition of one solution to another results temporarily in a heterogeneous mixture in which the solution concentrations of the ions may vary over a very wide range. For example, the radium and barium in the vicinity of a drop of added precipitant may be depleted in such a manner that the radium-barium ratio of the right-hand member of Equation 5 could assume almost any instantaneous value.

In 1927, Henderson and Kracek (22) studied the radium-barium chromate system and concluded that the system obeyed the homogeneous distribution law. Their results were obtained by precipitation of barium chromate in the presence of radium by a conventional precipitation procedure.

Salutsky, Stites, and Martin (32) in 1953 used the technique of precipitation from homogeneous solution to re-examine the radium-barium chromate system and conclude that the system obeyed the logarithmic distribution law.

The values of λ, which Salutsky obtained, were not constant with "fraction of barium precipitated" as Equation 6 predicts. These investigators proposed, as a result of their work and their own survey of studies by others, that the values of the distribution coefficient be extrapolated to zero per cent precipitated and that λ be reported as a limiting distribution coefficient. The question arises as to why λ should vary. Salutsky ascribed this to recrystallization; the conditions under which Equation 6 is obeyed would, given sufficient time, revert to those under which Equation 6 would be obeyed.

The radium-barium sulfate system has been studied by Doerner and Hoskins (3), Marques (29), and Gordon and Rowley (10, 16). Wherever conventional precipitation techniques were used, the values obtained for the logarithmic distribution coefficient have been somewhat erratic. However, where conditions conforming to or approaching precipitation from homogeneous solution were employed, the results have indicated conformity to the logarithmic distribution law.

When Doerner and Hoskins added sulfuric acid to "radium-barium chloride solutions without regard to temperature or agitation," their values for λ varied from 1.003 to 1.314. When "dilute sulfuric acid was added in very small portions to hot, agitated radium-barium chloride solution and the crystals were digested between each addition of acid" these authors obtained 1.568 to 1.686. When these authors obtained mixed crystals by evaporation and cooling of a solution containing radium, barium, and sulfate, they obtained 1.713 to 1.893.

Marques slowly added 0.01N sulfuric acid to radium-barium mixtures, at 20° C., and obtained values of λ from 1.54 to 1.71 in the range of 5 to 96% barium precipitated. By isothermal evaporation at 20° C. of a dilute solution of the sulfates, she obtained 1.84 to 2.01 in the range 67 to 91% barium precipitated.

Gordon and Rowley hydrolyzed sulfamic acid at 90° C. to slowly precipitated barium sulfate in the presence of trace radium. In the range of 3 to 96% of barium precipitated, λ was virtually constant; the average value was 1.21. The calculated values of D varied continuously from 1.18 to 1.92. Because the system obeys the Doerner-Hoskins equation, it seems reasonable to conclude that the radium must be logarithmically distributed

within the carrier substance. However, in a recent investigation by Jucker and Treadwell (26) in which sulfamic acid was also used to precipitate mixed crystals of barium and radium sulfates, it was concluded on the basis of radio-autographs that the radium is uniformly distributed. These conflicting views will have to be eventually resolved.

The distribution coefficient (cf. previous equations) is the ratio of the solubility products if the two compounds constituting the mixing crystal form an ideal solid solution or nearly so. Thus, λ is the ratio of the solubility products of barium sulfate and radium sulfate. A distribution experiment might then provide a potential means for determining the solubility of a trace substance since the experiment would require only that concentration of trace ions required for radiochemical analysis. However, Hahn (21) has indicated that a simple relationship between solubility and distribution coefficient does not exist.

Barium-Strontium. The barium-strontium sulfate system has been studied by Gordon, Reimer, and Burtt (15). Barium and strontium were slowly precipitated with sulfate produced as a result of the hydrolysis of dimethyl sulfate (5, 6). The system was studied in the range where 58 to 95% of the barium was precipitated. The system apparently conforms to the Doerner-Hoskins equation.

Rare Earths. Weaver (36) precipitated pairs of rare earths with oxalate obtained by the hydrolysis of dimethyl oxalate. He concluded that these systems obeyed the homogeneous distribution law; the logarithmic distribution law was apparently not considered. The conditions employed by Weaver in his investigation were those under which it might be expected that the systems would have more closely obeyed the logarithmic distribution law. Weaver's conclusions have been discussed by Callow (2) and by Salutsky and Gordon (31).

Hermann (23) investigated a system similar in many respects to those studied by Weaver. Lanthanum was precipitated in the presence of actinium using dimethyl oxalate. The results indicated adherence to the logarithmic distribution law.

COPRECIPITATION BY ADSORPTION

Basic Stannic Sulfate. Precipitation from homogeneous solution has been used to determine the extent and nature of the coprecipitation of manganese(II) with basic stannic sulfate (19). The latter was precipitated by the urea method (42).

In experiments in which the tin and manganese concentrations were approximately 10^{-2} and $10^{-3}M$, respectively, the results indicated that the coprecipitation of manganese occurs primarily in the initial and final stages of precipitation of the carrier. Relatively little occlusion, defined as adsorption followed by covering over with subsequent layers, occurs during the intermediate precipitation stages.

The coprecipitation occurring during the initial stages of carrier formation is apparently linked with the supersaturation effect existing during nucleation (27). That this may be the case was demonstrated in other experiments in which pure preformed basic stannic sulfate was initially present. In these experiments, coprecipitation did not occur in the initial stage but only in the final stages.

In the final stages of precipitation, when the solution has been virtually depleted of carrier ions, the precipitate behaves as an adsorbent for manganous ions. However, during the intermediate stages of precipitation, it is much more selective in its choice of cations as may be expected from the Paneth-Fajans-Hahn rule (37).

By using a slower precipitation rate, accomplished by lowering temperature which reduces the rate of urea hydrolysis, it was possible to minimize considerably the supersaturation effect and consequently the initial extent of coprecipitation.

When the manganous ion concentration was reduced to $10^{-6}M$ (and finally to $10^{-11}M$) somewhat similar coprecipitation phenomena were observed. The fraction of manganese coprecipi-

tated increased slightly, but definitely, during the intermediate precipitation stages. For example, the manganese coprecipitated amounted to 1 and 5% respectively, at 20 and 70% of tin precipitated. In the described experiments in which the initial concentration of manganese was $10^{-3}M$, this increase in occlusion was not evident, because the fraction of manganese absorbed at any stage was so very small in terms of the amount initially added.

An important consideration is that occlusion can be reduced to a minimal effect. This is accomplished by utilizing the technique of precipitation from homogeneous solution in order to prevent the depletion of carrier ions in any portion of the solution. Such depletion allows the precipitate to adsorb other ions in its vicinity. This can occur repeatedly in a conventional precipitation process upon each addition of precipitant. If, on the other hand, the precipitant is generated under controlled conditions, depletion of the carrier ions occurs only at the conclusion of the precipitation process; thus, adsorption—i.e., occlusion—during the intermediate stages of precipitation is minimized. However, adsorption by the quantitatively precipitated carrier poses a difficult problem with which to cope.

Willard and Sheldon (44) have proposed a simple but unique solution to this problem. They utilize a two-stage process in which they initially remove the carrier by filtration when about 95% has been precipitated. They then continue the precipitation process and finally remove the residual carrier. The two-stage process never permits more than a small fraction of the carrier to coexist in solution in the presence of contaminant only. The results obtained by Willard and Sheldon bear out the efficacy of the procedure.

Ferric Periodate. Acetamide was hydrolyzed at 80° C. in a slightly acid solution containing iron(III) and periodate (10). As the pH increased, iron periodate slowly precipitated over time intervals comprising many hours. Coprecipitation studies, utilizing aluminum and yttrium as contaminants, have confirmed the general conclusions reached in the described studies with basic stannic sulfate.

Silver Chloride. Silver ions were slowly released, in the presence of chloride ions, from the silver-ammonia complex which was dissociated with hydrogen ions generated by the hydrolysis of β-hydroxyethyl acetate (14). This process produced large crystals of silver chloride.

Because the cation in this case is derived from a complex ion and because its concentration is determined by the amount of chloride, the concentration of free silver ion can actually increase during the precipitation process. This is in contrast to the radium-barium sulfate case—for example, where the barium concentration is steadily decreased with the formation of barium sulfate.

The investigation with silver chloride as carrier utilized thallium(I) at approximately $10^{-6}M$ in the coprecipitation study. The free silver ion concentration was smaller than this in many instances, although the total silver concentration was initially $10^{-2}M$. The initial concentration of chloride was varied from 0.01 to 1.00M.

Although thallium(I) chloride does not mix isomorphously with silver chloride, the coprecipitation of thallium could have conceivably obeyed one of the two distribution laws. Cases of anomalous mixed crystal formation have been noted by Hahn (21) who refers to these as "isodimorphism."

In the application of either Equation 4 or 5 to the thallium-silver system, account must be taken of the fact that it is the silver ion which is involved. Thus, for example, integration of Equation 5 results in a different equation (14) from Equation 6. The data did not fit either Equation 4 or 5, as modified, under the experimental conditions which were employed. Thus, the system under the conditions investigated is not a case of Hahn's isodimorphism.

The thallium-silver ratio in the crystal, under any given set of conditions, remained constant throughout the precipitation

process; the mole ratio, thallium to silver in the precipitate, was of the order of 10^{-7}. So little of the initial thallium added was coprecipitated, about 1 part per 1000, that the solution concentration of thallium remained essentially unchanged during the precipitation process. Other experiments indicated that the thallium-silver ratio in the crystal was dependent on the solution concentration of thallium. Thus, the system was one in which

$$\left(\frac{Tl}{Ag}\right)_{precipitate} = K$$

for a given initial concentration of thallium. Apparently, what happens is that each layer of silver chloride adsorbs essentially the same small amount of thallium from the solution which contains an essentially constant thallium concentration. Thus, an apparently homogeneous distribution results even though the data do not fit the homogeneous (or logarithmic) distribution law.

SUMMARY

In 1950 Willard (38) wrote as follows: "The work on precipitation from homogeneous solution was begun at this university (Michigan) over 20 years ago and is still continuing. The advantages of this method are beginning to be realized, as evidenced by the work of other authors. There are many different ways of applying this principle. Some have been described and others are being investigated."

Today, five years later, precipitation from homogeneous solution is widely recognized as an important technique which can be used to develop new methods of analysis and to improve existing ones. It can also be used to increase the efficiency of separation methods which utilize fractional precipitation. Some of its most useful applications are in coprecipitation studies where it can be used under near equilibrium conditions to determine the true nature and extent of coprecipitation. In particular it facilitates the measurements of distribution coefficients in heterogeneous systems in which a solid carrier phase is precipitated.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Caley, E. R., Gordon, L., and Simmons, G. A., Jr., ANAL. CHEM., **22**, 1060 (1950).
(2) Callow, R. J., *Research*, **7**, No. 9, 549 (1954).
(3) Doerner, H., and Hoskins, W., *J. Am. Chem. Soc.*, **47**, 662 (1925).
(4) Elving, P. J., and Chao, P. C., ANAL. CHEM., **21**, 507 (1949).
(5) Elving, P. J., and VanAtta, R. E., *Ibid.*, **22**, 1375 (1950).
(6) Elving, P. J., and Zook, W. C., *Ibid.*, **25**, 502 (1953).
(7) Flaschka, H., *Chemist Analyst*, **44**, 2 (1955).
(8) Freund, H., dissertation, University of Michigan, Ann Arbor, Mich., 1945.
(9) Gordon, L., ANAL. CHEM., **24**, 459 (1952).
(10) Gordon, L., "Annual Progress Report," U. S. Atomic Energy Commission, NYO-3558 (1955).
(11) Gordon, L., Brandt, R. H., Quill, L. L., and Salutsky, M. L., ANAL. CHEM., **23**, 1811 (1951).
(12) Gordon, L., and Caley, C. R., *Ibid.*, **20**, 560 (1948).
(13) Gordon, L., and Firsching, F. H., *Ibid.*, **26**, 759 (1954).
(14) Gordon, L., Peterson, J. I., and Burtt, B. P., *Ibid.*, in press.
(15) Gordon, L., Reimer, C. C., and Burtt, B. P., *Ibid.*, **26**, 842 (1954).
(16) Gordon, L., and Rowley, K., unpublished research.
(17) Gordon, L., and Shaver, K. J., ANAL. CHEM., **25**, 784 (1953).
(18) Gordon, L., and Stine, C. R., *Ibid.*, **25**, 1519 (1953).
(19) Gordon, L., Teicher, H., and Burtt, B. P., *Ibid.*, **26**, 992 (1954).
(20) Gordon, L., and Wroczynski, A. F., *Ibid.*, **24**, 896 (1952).
(21) Hahn, O., "Applied Radiochemistry," Cornell University Press, Ithaca, N. Y., 1936.
(22) Henderson, L., and Kracek, F., *J. Am. Chem. Soc.*, **49**, 738 (1927).
(23) Hermann, J. A., "Separation of Americium from Lanthanum by Fractional Oxalate Precipitation from Homogeneous Solution," U. S. Atomic Energy Commission, AECD-3637 (1953).
(24) Heyn, A. H. A., and Schupak, E., ANAL. CHEM., **26**, 1243 (1954).
(25) Ismail, A. M., and Harwood, H. F., *Analyst*, **62**, 185 (1939).
(26) Jucker, H., and Treadwell, W. D., *Helv. Chim. Acta*, **37**, 113 (1954).
(27) LaMer, V. K., and Dinegar, R. H., *J. Am. Chem. Soc.*, **72**, 4847 (1950).
(28) MacNevin, W. M., and Dunton, M. L., ANAL. CHEM., **26**, 1246 (1954).
(29) Marques, B. E., *Compt. rend.*, **198**, 1765 (1934).
(30) Quill, L. L., and Salutsky, M. L., *J. Am. Chem. Soc.*, **72**, 3306 (1950).
(31) Salutsky, M. L., and Gordon, L., communication submitted to ANAL. CHEM.
(32) Salutsky, M. L., Stites, J. G., and Martin, A. W., *Ibid.*, **25**, 1677 (1953).
(33) Vanselow, C. H., M.S. thesis, Syracuse University, Syracuse, N. Y., 1951.
(34) Wagner, W. F., and Wuellner, J. A., ANAL. CHEM., **24**, 1031 (1952).
(35) Wahl, A. C., and Bonner, N. A., "Radioactivity Applied to Chemistry," Wiley, New York, 1951.
(36) Weaver, B., ANAL. CHEM., **26**, 479 (1954).
(37) Weiser, H. B., "Colloid Chemistry," 2nd ed., p. 112, Wiley, New York, 1949.
(38) Willard, H. H., ANAL. CHEM., **22**, 1372 (1950).
(39) Willard, H. H., and Fogg, H. C., *J. Am. Chem. Soc.*, **59**, 1197 (1937).
(40) Willard, H. H., and Freund, H., IND. ENG. CHEM., ANAL. ED., **18**, 195 (1946).
(41) Willard, H. H., and Gordon, L., ANAL. CHEM., **20**, 165 (1948).
(42) *Ibid.*, **25**, 170 (1953).
(43) Willard, H. H., and Hahn, R. B., *Ibid.*, **21**, 293 (1949).
(44) Willard, H. H., and Sheldon, J. L., *Ibid.*, **22**, 1162 (1950).
(45) Willard, H. H., and Tang, N. K., IND. ENG. CHEM., ANAL. ED., **9**, 357 (1937).
(46) Willard, H. H., and Tang, N. K., *J. Am. Chem. Soc.*, **59**, 1190 (1937).
(47) Willard, H. H., and Yu, S. T., ANAL. CHEM., **25**, 1754 (1953).

Significance of Rates and Equilibria in Electroanalytical Chemistry

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On the basis of Nernstian equilibria and kinetics, reversibility and irreversibility of simple electrode reactions are interpreted in terms of two competing rate processes—viz., mass transfer and electron transfer. The corresponding relationships are illustrated by experiments of hydrodynamic voltammetry where current-voltage curves are determined in solutions which are allowed to stream with varying flow velocities past a stationary indicator electrode. By adjusting the rate of flow, the shape of the current-voltage wave of ferricyanide can be changed from reversible to irreversible over an appreciable range of potentials. A generalized wave equation is derived for current voltage curves obtained by hydrodynamic voltammetry, which can be made use of for the determination of the rate of electron transfer. Because the latter is a monotonic exponential function of the potential, control by mass transfer prevails whenever a true limiting current is attained, irrespective of the apparent reversibility or irreversibility of the ascending portion of the wave. Since the rate of diffusive and/or convective transport is in all known instances proportional to the bulk concentration of the electroactive species, limiting currents of this type may always be applied with confidence to quantitative analysis.

I N THIS day and age, a ghost called irreversibility is haunting electroanalytical chemists. Irreversibility appears to be responsible for a good deal of lack of confidence in the analytical utilization of certain electrode processes. The two methods primarily affected are potentiometry and voltammetry. Notwithstanding the very successful application of some procedures based on admittedly irreversible electrode reactions—e.g., potentiometric titrations with permanganate—the loose common usage of the adjective "irreversible" implies "not well understood."

CRITERIA OF REVERSIBILITY

The criterion by which electrode processes are classified into reversible and irreversible is the Nernst equation. In potentiometry, the zero current potential is compared with the theoretical equilibrium potential. Thus, in an "electrode equilibrium" of the type

$$Ox + ne \rightleftharpoons Red \qquad (1)$$
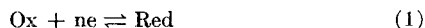
where both the oxidized and the reduced form of the electroactive species are soluble, reversibility is considered to prevail whenever the zero current potential, $E_{i=0}$, varies in accordance with the equation:

$$E_{i=0} = E^0 + (RT/nF) \ln (a_{Ox}/a_{Red}) \qquad (2)$$

a_{Ox} and a_{Red} denote the activity of the oxidized and of the reduced form, respectively.

In voltammetry, reversibility or irreversibility is established by considering the entire current-voltage curve and taking into account concentration polarization. The equation of a reversible current-voltage wave corresponding to Reaction 1 has the form:

$$E = E_{1/2} + (RT/nF) \ln \frac{i_c - i}{i - i_a} \qquad (3)$$

where E denotes the potential of the indicator electrode, $E_{1/2}$ the half-current (or half-wave) potential, i the current at potential E, and i_a and i_c the anodic and cathodic limiting currents, respectively.
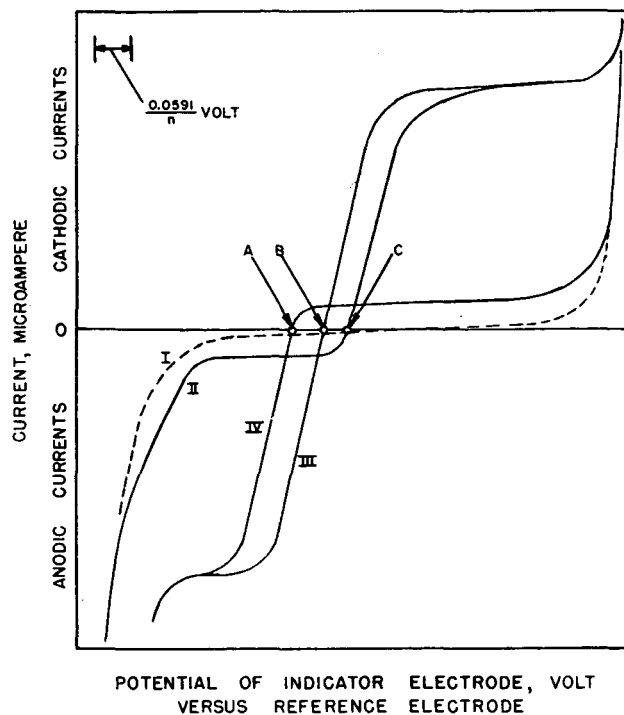

POTENTIAL OF INDICATOR ELECTRODE, VOLT VERSUS REFERENCE ELECTRODE

Figure 1. Idealized composite current-voltage curves

(Illustrating criteria of reversibility for electrode reaction: Ox + ne $\rightleftharpoons$ Red)

I. Residual current
II. Ox = 10 Red
III. Ox = Red
IV. 10 Ox = Red
A, B, C. Potentiometric zero current potentials

The correlation between the potentiometric and voltammetric tests for reversibility is illustrated in Figure 1. In the figure, the potentiometric criterion represents a single point in the current-voltage wave. The potentiometric zero-current value is rigorously significant only when the corresponding residual current is negligibly small, as was first pointed out by Kolthoff and Orlemann (7). Consequently, a voltammetric wave-equation represents generally a more reliable criterion of reversibility than the classical potentiometric test.

PHENOMENOLOGICAL INTERPRETATION OF REVERSIBILITY

For an insight into the physical significance of reversibility, it is necessary to consider that even the simplest electrode reaction involves two kinds of competing rate processes:

I. Mass transfer processes—i.e., the transport of one form of the electroactive species from the bulk of the solution to the sur-

face of the indicator electrode (and the analogous transport of the other form in the opposite direction).

II. Electron transfer between the electrode on the one hand, and the oxidized and reduced forms of the electroactive species on the other.

If electron transfer is rapid compared to mass transfer, the concentrations at the electrode surface of the oxidized and reduced forms of the electroactive species at any given potential adjust instantaneously to the ratio of values corresponding to Nernstian equilibrium. This yields a current-voltage wave of the shape shown in Figure 2, curve 1, which has been calculated on the basis of Equation 3, assuming that the bulk concentration of the oxidized species is finite and that of the reduced form equal to zero. If the rate of electron transfer is comparable to (or slower than) the rate of mass transfer, the adjustment to the equilibrium ratio of the electrode surface concentrations (and the corresponding current) on the ascending portion of the wave lags behind the change in potential and a drawn out, irreversible wave (Figure 2, curve II) is obtained, everything else being equal.

In the case of multistage electron transfer processes, and in situations complicated by chemical (nonelectrode) reactions, other factors must be taken in regard besides I and II. The treatment in this paper, however, is confined to the type of reaction which consists of a single electron transfer process and does not pertain to catalytic waves, etc.

QUANTITATIVE ASPECTS OF ELECTRON TRANSFER AND MASS TRANSFER IN VOLTAMMETRY

A unified theory of current-voltage waves at the dropping mercury electrode, covering currents controlled by diffusion and by the rate of electron transfer, was developed by Delahay (1). A generalized equation for current-voltage curves obtained in streaming solutions at indicator electrodes of constant area is now presented. Its derivation was inspired by unpublished work carried out in 1954 at the University of Minnesota (6).

Consider Reaction 1, involving one oxidized and one reduced form of the electroactive species, both soluble. Let C_{Ox} and C_{Red} be the corresponding bulk concentrations expressed in moles per cubic centimeter and assume:

$$C_{Ox} > 0; \quad C_{Red} = 0 \qquad (4)$$

Under these initial conditions, cathodic current-voltage waves of the type shown in Figure 2 are obtained. The current density (corrected for residual current) at a given potential represents a measure of the rate, R_Σ, of the over-all net electrode process:

$$R_\Sigma = \frac{i}{nFA} \qquad (5)$$

where i is expressed in amperes and A denotes the area of the indicator electrode expressed in square centimeters.

The net electron transfer involves a forward (reduction) and a backward (oxidation) process. The corresponding rates are expressed in accordance with Kimball's treatment of the Eyring theory (3, 4) applied to electrode reactions, for which experimental verification is available in the literature (2, 15).

Forward. Ox + ne → Red; $R_{Red} = k_{Red}a^0_{Ox}$; where $k_{Red} = k^0 \exp[\alpha(E^0 - E)nF/RT]$ (6)

Backward. Red → Ox + ne; $-R_{Ox} = k_{Ox}a^0_{Red}$; where $k_{Ox} = k^0 \exp[(1 - \alpha)(E - E^0)nF/RT]$ (7)

Net electron transfer. $R_{Red} - R_{Ox} = k_{Red}a^0_{Ox} - k_{Ox}a^0_{Red} = k_{Red}f_{Ox}C^0_{Ox} - k_{Ox}f_{Red}C^0_{Red}$ (8)

The symbols k_{Red} and k_{Ox} denote first-order rate constants referred to unit electrode area and expressed in centimeters per second; k^0 is called the specific rate constant at the standard potential where $k_{Ox} = k_{Red} = k^0$. α is a transfer coefficient defined by Kimball (3, 4); a^0 and C^0 indicate activities and concentrations at the electrode surface, respectively, and f denotes activity coefficients.

Mass transfer affects the oxidized species, which is consumed at the electrode interface, as well as reduced species which is formed concomitantly. The rate of mass transfer depends on its mechanism, which may be diffusion (9), forced convection (5), or both (2, 10, 16). In all known instances, however, the rate of mass transfer is proportional to the difference between the bulk concentration and the concentration at the electrode surface. Generally, the rate of mass transfer can be expressed as follows:

For the oxidized species. $R_{M,Ox} = m_{Ox}(C_{Ox} - C^0_{Ox})$ ...   (9)

For the reduced species. $-R_{M,Red} = m_{Red}(C^0_{Red} - C_{Red}) = m_{Red}C^0_{Red}$ (10)

m denotes mass transport coefficients expressed in centimeters per second.

In streaming solutions a steady state is assumed to prevail (2). Consequently:

$$R_\Sigma = R_{Red} - R_{Ox} = R_{M,Ox} = -R_{M,Red} \qquad (11)$$

Substituting in Equation 11 values from Equations 5, 8, 9, and 10 and eliminating the $C^0 - s$, the following expression is obtained:

$$\frac{i}{nFA} = \frac{C_{Ox}}{\dfrac{1}{k_{Red}f_{Ox}} + \dfrac{1}{m_{Ox}} + \dfrac{1}{m_{Red}} \cdot \dfrac{f_{Red}}{f_{Ox}} K}$$

$$\text{where } K = \frac{k_{Ox}}{k_{Red}} = \exp[(E - E^0)nF/RT] \quad (12)$$

In the limiting current (i_1) region $C_{Ox} \gg C^0_{Ox}$; and, therefore, it follows from Equations 5, 9 and 11 that:

$$C_{Ox} = \frac{i_1}{nFAm_{Ox}} \qquad (13)$$

which substituted in Equation 11 yields:

$$i = \frac{i_1}{\dfrac{m_{Ox}}{k_{Ox}f_{Red}} + \dfrac{m_{Ox}}{m_{Red}} \times \dfrac{f_{Red}}{f_{Ox}} K + 1} \qquad (14)$$

Expression 14 represents a generalized wave equation which takes into account control by both mass transfer and electron transfer
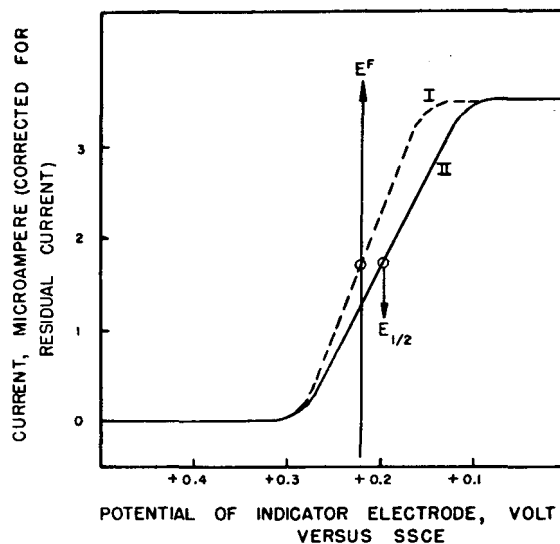

Figure 2. Current-voltage curves of 10^{-4} M ferricyanide in 1M potassium chloride

I.   Calculated reversible wave, assuming $A = 1.25 \times 10^{-1}$ sq. cm., $m_{Ox} = 2.89 \times 10^{-2}$
II. Irreversible wave obtained in hydrodynamic voltammetry cell at flow velocity at 136 cm. per sec.
E_F. Formal potential of ferricyanide–ferrocyanide couple in 1M KCl

and is, as such, analogous to expressions derived by various authors, pertaining to somewhat different experimental situations (2).

The significance of Equation 14 becomes apparent if we assume that $m_{Ox} = m_{Red}$ [a reasonable assumption when convection controlled mass transfer prevails (5), or when the diffusion coefficients of the oxidized and reduced form are equal] and that $f_{Red} = f_{Ox} = 1$. Considering, under these simplifying assumptions, the point on the wave which corresponds to the standard potential where $K = 1$ and $k_{Red} = k^0$, the following expression is obtained:

$$i = \frac{i_1}{\frac{m_{Ox}}{k^0} + 2} \tag{15}$$

Obviously the current is controlled by the rate of mass transfer when $\frac{m_{Ox}}{k^0} \ll 2$, or $k^0 \gg m_{Ox}/2$. If this condition is satisfied, the current-voltage wave is reversible. Conversely, if $k^0 \ll m_{Ox}/2$ the current is controlled by the rate of electron transfer and the wave is irreversible. Similar considerations hold for the entire ascending portion of the wave. On the limiting current region, however, control by mass transfer prevails in all instances. Control by electron transfer could not account for a limiting current region, because both k_{Red} and k_{Ox} are monotonic exponential functions of the applied potential (Equations 6 and 7). Equation 13 is valid whenever a true limiting current is attained. The latter is always proportional to the bulk concentration of the electroactive species. Therefore, voltammetric limiting currents may be used with confidence for quantitative determinations, even when the ascending part of the wave is irreversible.

HYDRODYNAMIC VOLTAMMETRY

On the basis of the preceding theoretical considerations it was anticipated that it might be possible to alter experimentally the reversible or irreversible character of a current-voltage wave, by varying the conditions of mass transfer in an electrolysis cell. A suitable rotated cell and a cylindrical stationary platinum microelectrode were designed. By maintaining judiciously controlled hydrodynamic conditions and a carefully selected geometry, it was found possible to vary the rate of flow of the solution past the indicator electrode by a factor of about 100.

Experimental. Details of the experimental setup are sketched in Figure 3. With the aid of a suitable motor, a variable transmission and gears, the polyethylene electrolysis cell was rotated counterclockwise at speeds between about 0.02 and 2 revolutions per second. These were timed by connecting in series an electric stopwatch and an electric counter (productimer counter supplied by Durant Mfg. Co., Milwaukee, Wis.). The latter was actuated by a microswitch which was adjusted to close after each completed revolution of the electrolysis cell upon contact with a wooden protuberance attached to the upper rim of the cell. In each experiment, 1.70 liters of solution was used and a temperature of 25.0° ± 0.1° C. was maintained with the aid of an external infrared heating lamp, a mercury thermoregulator immersed into the solution, and an electronic relay.

The indicator electrode consisted of the surface of a platinum wire 0.210 cm. in length and 0.019 cm. in diameter, extended tightly between two ends of a fork made of Geon, a saturated chloroethylene plastic, (manufactured by the B. F. Goodrich Co.) as illustrated in Figure 4. The fork was attached to a rod made of Micarda, a laminated phenolic resin manufactured by
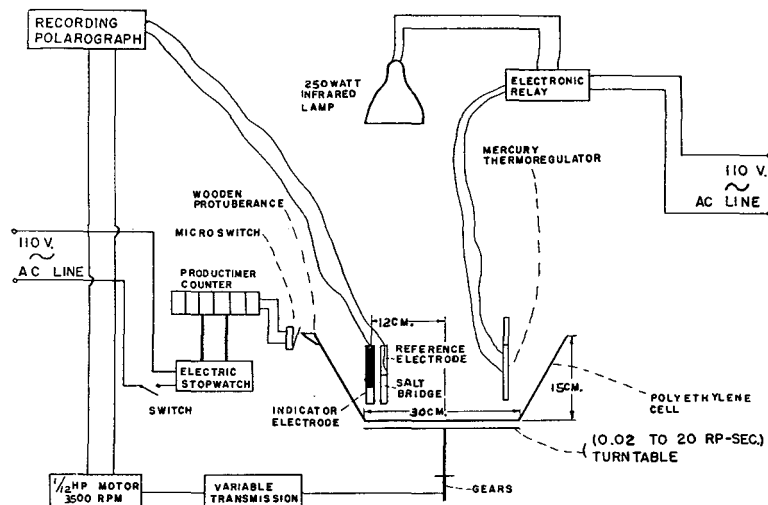

Figure 3. Diagram of experimental setup of hydrodynamic voltammetry cell

Westinghouse Electric. The indicator electrode was held stationary at a distance of 12.0 cm. from the center of rotation, in a horizontal position at an angle of 90° to the direction of flow. The velocity of flow of the solution past the indicator electrode was computed from the rate of rotation assuming that the liquid was quasi rigidly attached to the cell. Under these experimental conditions, the mass transport coefficient is equal to:

$$m = 0.55\, D^{2/3} v^{1/2} d^{-1/2} \eta^{-1/6} \tag{16}$$

where D denotes the diffusion coefficient expressed in square centimeters per second, v the rate of flow of the solution expressed in centimeters per second, d the diameter (in centimeters) of the indicator electrode, and η the kinematic viscosity of the solution expressed in square centimeters per second. Equation 16 is based on rigorous theoretical hydrodynamic considerations and takes into account the empirically established distribution of lines of flow around the indicator electrode. It was derived by Ranz (14) by adapting heat transfer relationships (12) to mass transfer and is similar, except for the numerical constant, to equations reported in the literature in slightly modified forms (2, 10, 16). In accordance with Equation 16 it was verified experimentally that the mass transport coefficients (calculated for our limiting currents using Equation 13) for ferricyanide and ferrocyanide in $1M$ potassium chloride were proportional to $v^{1/2}$ in a range of velocities of flow between 1.5 and 150 cm. per second.

A tubular silver–silver chloride electrode (SSCE), saturated in potassium chloride, served as reference electrode. It was constructed in conjunction with an agar plug saturated in potassium chloride, which provides a convenient low resistance salt bridge as described by Lingane (11). The resistance of the entire electrolysis cell in $1M$ potassium chloride was only 150 ohms, obviating corrections for IR drops.

Current-voltage curves were recorded with a Leeds & Northrup Model E Electrochemograph.

The hydrodynamic voltammetry of ferricyanide provided a striking illustration of the relativity of reversibility and irreversibility in electrode processes. At velocities of flow up to 2.1 cm. per second, ferricyanide in $1M$ potassium chloride yielded a reversible reduction wave with a half-wave potential of +0.277 volt vs. SSCE. When v was equal to 2.06 cm. per second the limiting current in $10^{-4}M$ solution was 0.430_μ A. which corresponds to a value of 3.56×10^{-3} cm. per second for m_{Ox}.

As the velocity of flow was increased to between 40 and 150 cm. per second the ferricyanide wave became increasingly irre-

versible, everything else being equal. A typical example is shown in Figure 2, curve II.

At all flow velocities investigated, the limiting current of ferricyanide was proportional to concentration in a range between 10^{-5} and $10^{-3}M$.

The anodic limiting current of ferrocyanide in $1M$ potassium chloride was also determined, the indicator electrode being anodically preconditioned, according to Laitinen and Kolthoff (9). The mass transport coefficient for ferrocyanide, m_{Red}, was evaluated and it was found that the ratio m_{Ox}/m_{Red} was equal to 1.12 at all velocities of flow (cf. Equation 16).
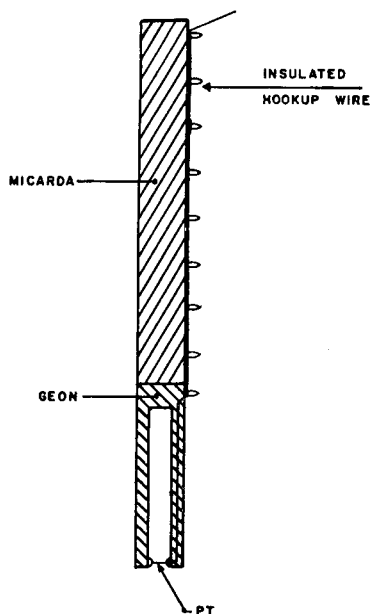
Figure 4. Indicator electrode

The formal potential in $1M$ potassium chloride of the ferricyanide-ferrocyanide couple was determined potentiometrically using precautions recommended by Kolthoff and Tomsicek (8). A value of $+0.275$ volt vs. SSCE [$+0.472$ volt vs. normal hydrogen electrode (NHE)] was obtained.

EVALUATION OF RATE OF ELECTRON TRANSFER IN ELECTRODE PROCESSES

From Equation 14 the value at the formal potential of the product $k_{Red}f_{Ox}$ can readily be explicited in terms of experimentally accessible quantities:

$$k_{Red}f_{Ox} = \frac{m_{Red}i}{m_{Red}i_l - (m_{Ox} + m_{Red})i} \cdot m_{Ox} \qquad (17)$$

Substituting in Equation 17 the corresponding data, a numerical value of

$$k_{Red}f_{Ox} = (8 \pm 1) \times 10^{-2} \text{ cm. per sec. at } 25°$$

is obtained for the electroreduction of ferricyanide at $+0.472$ volt vs. NHE in $1M$ potassium chloride. This is in satisfactory agreement with a value at 20° of 9×10^{-2} cm. per second determined by Randles and Somerton (13) with the aid of an alternating current relaxation method. (Randles and Somerton set their values equal to the rate constant k_{Red} itself rather than to the product $k_{Red}f_{Ox}$. This implies the assumption by Randles and Somerton that the activity of ferricyanide may within experimental error be considered equal to concentration. The accuracy of the hydrodynamic voltammetry data appears to warrant the taking into account of the activity coefficient, which, while not actually known, is probably of the order of 0.1.)

It appears that hydrodynamic voltammetry is ideally suited for accurately determining the rates of electron transfer processes involving interactions between soluble electroactive species and the electrode. Work is in progress in this laboratory with a view to extending the applicability of the method to flow velocities up to 2 kilometers per second. It is hoped that the knowledge of the rates of the electrode reactions might eventually help to elucidate their detailed mechanism, which represents a challenge to fundamental research in analytical chemistry.

LITERATURE CITED

(1) Delahay, P., J. Am. Chem. Soc., 75, 1430 (1953).
(2) Delahay, P., "New Instrumental Methods in Electrochemistry," pp. 72–86, 227–39, Interscience, New York, 1954.
(3) Glasstone, S., Laidler, K. J., and Eyring, H., "The Theory of Rate Processes," p. 575, McGraw-Hill, New York, 1941.
(4) Kimball, G. E., J. Chem. Phys., 8, 199 (1940).
(5) Kolthoff, I. M., and Jordan, J., J. Am. Chem. Soc., 76, 3843 (1954).
(6) Kolthoff, I. M., Jordan, J., and Prager, S., unpublished results.
(7) Kolthoff, I. M., and Orlemann, E. F., J. Am. Chem. Soc., 63, 644 (1941).
(8) Kolthoff, I. M., and Tomsicek, W. J., J. Phys. Chem., 39, 945 (1935).
(9) Laitinen, H. A., and Kolthoff, I. M., Ibid., 45, 1061, 1079 (1941).
(10) Levich, B., Discussions Faraday Soc., 1, 37 (1947).
(11) Lingane, J. J., "Electroanalytical Chemistry," p. 263, Interscience, New York, 1953.
(12) McAdams, W. H., "Heat Transmission," 3rd ed., McGraw-Hill, New York, 1954.
(13) Randles, J. E. B., and Somerton, K. W., Trans. Faraday Soc., 48, 937 (1952).
(14) Ranz, W. E., personal communication.
(15) Rubin, H., and Collins, F. C., J. Phys. Chem., 58, 958 (1954).
(16) Tobias, C. W., Eisenberg, M., and Wilke, C. R., J. Electrochem. Soc., 99, 360 (1952).

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Concept of Polarographic Currents Limited by Rate of a Chemical Reaction and Some of Its Applications

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The concept of rate-controlled polarographic currents is discussed and illustrated by several examples. Both rate constants and equilibrium constants, which are not accessible to measurement by other methods, may be calculated in a simple manner from rate-controlled polarographic waves. The simple, but essentially correct theory, derived from the concept of reaction volume, is used and the statistical meaning of reaction volume is explained. Only examples which can be regarded as fully rate-controlled have been chosen to achieve greatest possible simplicity.

THE purpose of this article is to show the applicability of rate-controlled polarographic waves to the determination of extremely rapid reaction rates and also to the determination of equilibrium concentrations, which are not accessible to direct measurement. The simplest form of the theory (*11*) of fully rate-controlled currents, later found essentially correct by rigorous calculations (*6, 7*), is used, because it makes possible an easy understanding of the processes involved.

Consider the following system:

$$A \underset{k'}{\overset{k}{\rightleftharpoons}} B \qquad \frac{A}{B} = \frac{k'}{k} = K$$
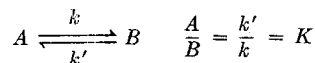
Assume that B is reducible at the dropping mercury electrode and A is either not reducible or reducible at a potential more negative than B. Obviously the height of a polarographic reduction wave of B will be in the most general case determined by the rate of diffusion of B from the bulk of the solution to the electrode (i_{d_B}) and by the rate of transformation of A into B in the electrode environment (i_{k_B}). Now further limit the case by assuming that the equilibrium concentration of B is so small that its diffusion current, i_{d_B}, is negligible as compared with the kinetic current, i_{k_B}; and the kinetic current i_{k_B} is in turn much smaller than the diffusion current of A (i_{d_A}) or, in the case that A is not reducible, than the hypothetical diffusion current of A calculated from the diffusion coefficient of A by means of the Ilkovič equation. This case is extremely simple to deal with and has considerable practical importance, because it is possible to find conditions in which the above limitations are valid for many systems. Further it is possible by adjustment of concentrations, pH, or other variables, to bring the rate-controlled current i_{k_B} into the optimum range for measurement, whereas i_{d_B} is completely negligible and i_{d_A}, which does not have to be measured, is several hundred or more times larger than a measurable polarographic wave.

Under conditions defined in this manner the concentrations of A in the bulk of the solution and in the vicinity of the electrode are the same, as the extent to which A is exhausted in the interface is negligibly small. Roughly the ratio of the concentration of A in the bulk of the solution and in the vicinity of the electrode is given by the expression

$$\frac{i_{d_A}}{i_{d_A} - i_{k_B}}$$

In the specific situation being considered i_{k_B} is negligibly small with respect to i_{d_A}.

Consequently an expression for i_{k_B} may be very simply formulated

$$i_{k_B} = nF \times 10^{-3}\, \bar{q} . \mu . k\; [A] \qquad (1)$$

where k = rate constant for the conversion of A into B
n = number of electrons required for the reduction
F = 96,500 coulombs
$\bar{q}$ = average surface area of the electrode
$\mu = \dfrac{3}{5} 0.85\,(mt)^{2/3}$
m = outflow velocity of mercury in grams per second
t = time of one drop in seconds

The effective thickness of the reaction layer, μ, is a statistical quantity and can be derived in the following manner (*11*).

The condition for reduction of each molecule of B, formed at a distance X from the electrode, is that it must reach the surface of the electrode (the potential of which, of course, corresponds to the limiting current) within its lifetime—that is, it must reach the electrode before it is reconverted into A. Clearly X must be a distance that the molecule can travel in its lifetime.

The average displacement of a molecule in the two directions perpendicular to the electrode surface is given by the Einstein formula, in which D is the diffusion constant of the molecule considered:

$$\Delta = \sqrt{2D\tau}$$

Consequently molecules of B with lifetime, τ, are, on the average, reduced only if they have been formed at a distance from the electrode

$$X \lessgtr \frac{1}{2} \Delta = \frac{1}{2}\sqrt{2\,D\tau}$$

The factor $\dfrac{1}{2}$ results from the fact that only one half of the molecules move toward the electrode.

If now the individual lifetimes of the molecules are replaced by their mean lifetime $\dfrac{1}{k'}$ (k' is the rate constant for the reaction $B \to A$), an average of the average displacements $\bar{\Delta} = \sqrt{\dfrac{2D}{k'}}$ and a condition valid for the reduction of all molecules of B are obtained—viz.,

$$X \lessgtr \frac{1}{2}\bar{\Delta} = \frac{1}{2}\sqrt{\frac{2\,D}{k'}} = \sqrt{\frac{1}{2}}\sqrt{\frac{D}{Kk}} = \mu \qquad (2)$$

A rigorous treatment of the problem has later given (*7*) $\mu = \sqrt{\dfrac{D}{K.k}}$, which differs only by the constant factor, $\sqrt{\dfrac{1}{2}}$, from the statistical derivation.

The introduction of this value for μ into Equation 1 gives Equation 3, which constitutes a complete solution of the problem under consideration.

$$i_{k_B} = nF \times 10^{-3} \, \bar{q} \, \sqrt{\frac{D}{K}} \sqrt{k} \, [A] \qquad (3)$$

This result was corroborated 5 years later in another manner by Delahay (5).

The Equation 1 for i_{k_B} further reveals an interesting property which has been pointed out in the first clearly recognized case of a rate-controlled current (13). Because i_{k_B} is proportional to the average surface of the electrode, $\frac{3}{5} \times 0.85(mt)^{2/3}$, it is independent of the height of the mercury reservoir. This is due to the fact that (mt) is the weight of one drop and is consequently a constant independent of mercury pressure. It is well known that a diffusion-controlled current is proportional to the square root of the height of the mercury column. In this respect a rate-controlled current differs significantly from a diffusion current and can be easily distinguished from it.
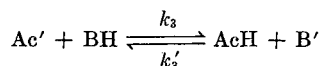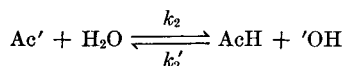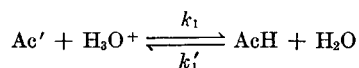
As an example of the determination of an extremely rapid reaction rate from the fully rate-controlled current let us consider the case of dissociation of reducible acids, which has been treated approximately and rigorously in the region of joint diffusion and rate control (1-4, 6, 7, 11).

In many cases a polarographic wave of an undissociated acid (phenylglyoxylic, pyruvic, and many others) is located at a more positive potential than the wave of the corresponding anion. Since this separation into two waves occurs in a pH region where the undissociated acid is present in negligible concentration, the height of the more positive wave must be determined by the rate of the reaction

$$Ac' + H^+ \underset{k'}{\overset{k}{\rightleftharpoons}} AcH$$
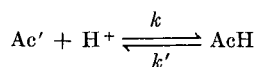
Phenylglyoxylic acid has been recently studied (14) under conditions where the limitations specified in this article are strictly valid. In the pH region between 8 and 10 the more positive wave is at least 200 times smaller than the wave of the anion. In order to make it precisely measurable one can increase the concentration of the acid to $0.02M$, which of course brings the diffusion current of the anion out of range.

In the presence of buffer (borate in this case) proton transfer is mediated by the following reactions:

$$Ac' + H_3O^+ \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} AcH + H_2O$$

$$Ac' + H_2O \underset{k_2'}{\overset{k_2}{\rightleftharpoons}} AcH + {}'OH$$

$$Ac' + BH \underset{k_3'}{\overset{k_3}{\rightleftharpoons}} AcH + B'$$

AcH is here the reducible acid; BH the buffer acid.

By using Equation 3 for these three reactions the Equation 4 may be easily derived:

$$i_{k_{AcH}} = 2 \, F\bar{q} \times 10^{-3} \, [Ac'] \cdot \sqrt{\frac{D}{K_{Ac}}} \left[k_1 + \frac{k_2[H_2O]}{[H_3O^+]} + \frac{k_3 C_B}{[H_3O^+] + K_B} \right]^{1/2} [H_3O^+] \qquad (4)$$

K_{Ac} = dissociation constant of reducible acid
K_B = dissociation constant of buffer acid
C_B = buffer concentration

By fitting the experimental data obtained at various buffer concentrations and various hydrogen ion concentrations to Equation 4, it has been possible to elucidate the three rate constants in question:

$k_1 = 5.73 \times 10^{10}$ liter moles^{-1} sec.$^{-1}$
$k_2 \times (H_2O) = 22$ sec.$^{-1}$
$k_3 = 6.38 \times 10^2$ liter moles^{-1} sec.$^{-1}$

The precision of k_1 is much better than that of k_2 and k_3, these having the nature of second-order corrections. This result at the same time shows that in the more acidic range studied earlier the recombination with hydronium ions is the only important mechanism of proton transfer.

Koutecký (6) recently achieved a rigorous way of dealing with cases in which diffusion limits the current jointly with reaction rate. This solution has been amply verified experimentally, since Koutecký's asymptotic solution is again identical in form (except for a constant factor) with the solution arrived at very early (11) by substitution of Equation 2 for μ in an approximate equation (2) for the case in which the current is rate and diffusion controlled. This treatment in turn has been known for a long time to agree precisely with the experimental data. Thus the complete clarification of the problem of recombination of acid anions with hydronium ions has culminated in Koutecký's brilliant mathematical treatment, and in spite of the difficulties of this treatment has resulted in very simple equations that can be used easily by chemists. The way is now open to a systematic study of recombination rates of various substituted pyruvic (3, 4), phenylglyoxylic (10), and other acids with hydronium ions and also to an interesting study of the same reactions in heavy water (10). These experiments are under way at the University of New Brunswick.

To illustrate further the possibilities of polarographic rate-controlled currents, the case of glucose in which it has been possible to derive the value of the concentration of the open chain glucose tautomer by analysis of the rate-controlled current of glucose may be mentioned. It was shown some time ago that the polarographic current of glucose is controlled completely by the rate of formation of the aldehyde tautomer from α- and β-glucose (12). It is now possible to solve the system of glucose in the following manner (9).

The mechanism of mutarotation of glucose may be represented by:

$$\alpha \underset{k_1'}{\overset{k_1}{\rightleftharpoons}} [ald] \underset{k_2}{\overset{k_2'}{\rightleftharpoons}} \beta$$
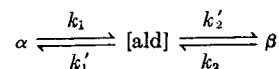
Since it is possible to determine the rate-controlled current due to the formation of the aldehyde glucose from α and β glucose separately, there are two independent equations involving the unknowns k_1, k_1' and k_2, k_2'. The remaining equations required to calculate all four unknowns are available from well known data.

The mutarotation velocity expressed by the four unknowns is:

$$k_{mut} = (k_1 k_2' + k_1' k_2)/(k_1' + k_2')$$

The over-all equilibrium constant of α- and β-glucose is

$$\frac{k_1 \times k_2'}{k_1' \times k_2} = 1.740$$

Consequently there are four independent equations, and the four unknown rate constants can be calculated. From these in turn the unknown equilibrium concentration of aldehyde glucose can be calculated. This comes out to 0.0027% of the glucose present.

This procedure is an approximation, because in reality the glucose system should be represented by the following scheme.

$$\alpha \rightleftharpoons [ald] \rightleftharpoons \beta$$
$$\updownarrow$$
$$[hydrate]$$
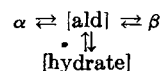
The correction for the influence of hydration of the aldehyde group is, however, not a serious one and experiments designed to elucidate it have been under way for some time at the University of New Brunswick (8).

LITERATURE CITED

(1) Brdička, R., Chem. Listy, 40, 232 (1946); Collection Czechoslov. Chem. Communs., 12, 212 (1947).
(2) Brdička, R., and Wiesner, K., Ibid., p. 138; Chem. Listy, 40, 66 (1946).
(3) Clair, E., M.Sc. thesis, University of New Brunswick, New Brunswick, Canada, May 1950.
(4) Clair, E., and Wiesner, K., Nature, 165, 202 (1950).
(5) Delahay, P., J. Am. Chem. Soc., 74, 3506 (1952).
(6) Koutecký, J., Collection Czechoslov. Chem. Communs., 18, 597 (1953).
(7) Koutecký, J., and Brdička, R., Ibid., 12, 337 (1947).
(8) Los, J. M., and Simpson, B., unpublished results, University of New Brunswick, New Brunswick, Canada, 1955.
(9) Los, J. M., and Wiesner, K., J. Am. Chem. Soc., 75, 6346 (1953).
(10) Wheatley, Mary, unpublished results, University of New Brunswick, New Brunswick, Canada, 1955.
(11) Wiesner, K., Chem. Listy, 41, 6 (1947).
(12) Wiesner, K., Collection Czechoslov. Chem. Communs., 12, 64 (1947).
(13) Wiesner, K., Z. Elektrochem., 49, 164 (1943).
(14) Wiesner, K., Wheatley, Mary, and Los, J. M., J. A. Chem. Soc., 76, 4858 (1954).

[END OF EIGHTH ANNUAL SUMMER SYMPOSIUM]

Spectrochemical Determination of Boron in Carbon and Graphite

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Moving plate studies showed that the available material which best matched the volatility behavior of boron in carbon and graphite in the direct current arc was finely powdered iridium. When the discharge was burned in a 76 to 24 (by volume) argon-oxygen mixture, the line pair B 2497/Ir 2543 gave intensity ratios having a relative standard deviation of 1.8% or less between 0.5 and 4 p.p.m. boron. By use of a powder spark method, the sensitivity was extended to 0.25 p.p.m. boron. In this technique, a "sifter" electrode (a porous cup electrode with a perforated floor) was filled with powdered sample and used as the upper electrode in a high voltage spark discharge. The opening at the top of the electrode was closed with a small cork. During the discharge, the sample material sifted into the discharge area and was excited. When this discharge was conducted in argon, the line pair B 2497/Cu 2492 gave a relative standard deviation of 1.8% or less between 0.25 and 7 p.p.m. boron.

CHEMICAL determination of small amounts of boron in carbon or graphite is commonly accomplished by igniting the sample in the presence of calcium oxide or fusing it with an alkaline oxidizing mixture, and determining the resultant increase in total borate by colorimetry or titration. Because of the large blanks and long processing involved, however, such methods begin to lose accuracy and precision as the boron concentration decreases to the parts per million range.

Several attempts have been made to perform this analysis spectrochemically, but the results achieved appear to have been semiquantitative. In 1936, Gatterer (4) suggested that boron, as well as other impurities in spectrographic graphite, might be determined by evaporating appropriate amounts of solutions containing the salts of interest on flat-topped pure graphite electrodes, and burning to completion. Concentrations were estimated by comparing the times required for the untreated graphite and the standards to produce spectral lines of equal density. This procedure ignored the possibility of the premature escape of boron oxides from the standard electrode, however, and at best yielded semiquantitative results. No data on sensitivity, accuracy, or precision were given by the author.

Shugar (23) recently published a procedure for determining boron in carbon or graphite used in nuclear piles. He mixed the carbon or graphite with a calcium hydroxide slurry, dried the mixture, ignited it 1.5 hours at 850° to 900° C., and exposed it spectrographically, using beryllium as internal standard. The author stated that 0.1 p.p.m. of boron could be "detected" and 0.2 p.p.m. "estimated" by this method. He regarded the method as semiquantitative, however, and "not as precise as colorimetry."

Mitrovic (13) gave an absolute sensitivity limit of 0.01 to 0.03 γ for the detection of boron in carbon or graphite.

In view of the semiquantitative nature of the procedures available, and the ubiquity of boron as a contaminant of spectrographic and other carbon, it was considered advisable to examine the factors which complicate this analysis, and if possible, to devise a quantitative procedure.

DIRECT CURRENT ARC PROCEDURE

PHYSICOCHEMICAL PROPERTIES OF BORON CARBIDE AND THEIR ROLE IN SPECTROCHEMICAL ANALYSIS

The spectrochemical determination of traces (0.2 to 5 p.p.m.) of boron in carbon and graphite, or in their presence, is considerably complicated by the behavior of the boron-carbon system at high temperatures. At 2500° to 2600° C., boron, boric oxide (20), borates, and even boron nitride (24) react with carbon to give boron carbide (B_4C). This compound is extremely stable and unreactive; it can safely be assumed (see below) that all elemental or combined boron which has not volatilized before the sample reaches the above temperature will react with any carbon present to give boron carbide. Any attempt to devise a spectrochemical procedure for determining trace concentrations of boron in carbon or graphite must therefore provide for the volatilization of this compound.

The literature does not appear to contain any quantitative information on the vapor pressure of boron carbide; however, its volatility is known to be very low. Ridgway (20), who was the first to isolate pure boron carbide, described its vapor pressure as "inappreciable" at the melting point [2450° C. (5)]; Rusanov (21) confirmed this in stating that traces of boron are still present in graphite which has been heated at 3000° C. for 30 seconds. Steinle (25) observed that when a direct current arc was struck to an anode containing a mixture of less than 1% boric oxide in carbon, the surface of the anode was immediately covered with tiny globules of a molten substance, later shown by x-ray diffraction to be boron carbide contaminated with carbon. As the burning proceeded, these globules coalesced into large drops. The fact that the boron carbide failed to volatilize completely when in a fine state of subdivision is further evidence that its vapor pressure

is extremely low, even at the surface temperature of a carbon anode (3300° to 3700° C.) (*3, 9*).

The low volatility of this compound introduces two separate difficulties into the present determination: It makes the contamination of electrode and dilution stock by boron carbide almost inevitable, and creates considerable uncertainty as to the mechanism by which boron is transported into the arc column.

Contamination of Electrode and Dilution Stock by Boron Carbide. Boron carbide cannot be completely eliminated from carbon or graphite by either high temperatures or chemical purification (*26*), but boron-free electrode stock can be prepared from boron-free raw materials. Such stock is occasionally found in the output of commercial suppliers, and can be used to circumvent this difficulty.

Uncertainty as to Mechanism of Volatilization. The appearance of atomic boron lines in carbon arc spectra indicates that boron originally existing in the electrode as the carbide is found later in the analytical gap in the atomic state. Any effort to devise a procedure which will combine the best attainable sensitivity, accuracy, and precision must be based on some theory as to the manner in which boron is transported from the electrode into the arc column. Present knowledge of the characteristics of boron carbide suggests the following possibilities.

ENTRAINMENT OF GLOBULES. Microscopic molten globules of boron carbide might be swept up into the arc column by the stream of gases coming from the anode; the volatilization and dissociation processes would then be completed in the arc column. [According to Huldt (*7*) and Lochte-Holtgreven and Maecker (*11*), temperatures as high as 7300° C. exist in carbon arcs.] This mechanism could be effective only for boron carbide existing on the exposed surface of the anode.

VOLATILIZATION. Boron carbide might be volatilized in molecular form and dissociated in the arc column. The observations of Steinle do not preclude this possibility in the present case, since even a very low vapor pressure might suffice for volatilization of the globules formed by the small quantities of boron carbide contemplated. Since the temperature of the anode decreases very rapidly with depth below the surface on which the arc impinges, this mechanism, even if effective, could volatilize boron carbide only from this surface or the region immediately below.

DISSOCIATION. Boron carbide might dissociate on, or immediately below, the anode surface, giving elemental boron (boiling point 2550° C.), which would volatilize readily at anode surface temperatures. However slight the degree of dissociation, continuous removal of the boron might drive this reaction to completion.

CHEMICAL REACTIONS AT ANODE SURFACE. These can be involved in several ways in the transportation of boron into the arc column.

Figure 1. Electrode arrangement for arc method

Boron Present as Carbide. It is very probable that boron originally present as the carbide cannot be volatilized into the arc column from the interior of the anode—i.e., that such boron can be removed from the anode only by direct exposure of each boron carbide particle in place to the action of the arc itself. This implies that progressive and complete consumption of the anode is necessary for quantitative volatilization of the boron. Steinle has shown (*25*) that at low amperages, the consumption of a carbon anode is due almost entirely to chemical reaction with the ambient gases, rather than to volatilization of carbon. The present analysis therefore must be carried out in an atmosphere capable of reacting with carbon to give gaseous products.

Oxidation of Exposed Boron Carbide Particles. Oxidation of the exposed particles must also be examined as a possible volatilization mechanism. Boron present as the carbide in graphite is known to be partially volatilized on ignition in air or oxygen unless appropriate chemical measures are taken to retain it. It is doubtful, however, that such boron is volatilized as an oxide from the interior of a carbon anode. Although the temperature there is sufficiently high to volatilize boron as boric oxide, there is no oxygen available at that point for the formation of this oxide. Conditions appear no more favorable for the oxidation of boron carbide at the anode surface. The temperature at this point is probably high enough for the formation and volatilization of boron monoxide (BO), which is stable at arc temperatures (*16*). However, any oxygen existing at the anode surface is probably present as carbon monoxide, so that oxidation of the boron carbide is unlikely in this case also.

While Steinle's observation of the coalescence of small globules of boron carbide on the face of a carbon anode does not entirely preclude the volatilization, dissociation, and chemical reaction mechanisms, it counts rather heavily against them. The entrainment of microgram quantities of the carbide in the anode vapors, on the other hand, appeared to be a plausible mechanism and was\adopted as a working hypothesis. Information subsequently obtained from moving plate exposures provided circumstantial evidence supporting this assumption (see discussion of selection of internal standard).

REQUIREMENTS FOR ANALYTICAL PROCEDURE

Highest Possible Anode Surface Temperature. This is required in order to ensure liquefaction of boron carbide, and to promote its entrainment by causing the most vigorous evolution of vapors possible from the anode. The temperature of a carbon anode surface in air can be raised to 3300° to 3700° C. by increasing the arc current density (amperes per square centimeter of anode end-surface area). Once the surface has attained this temperature, further increases in the electrical or chemical energy liberated at the anode serve only to increase the area over which this temperature exists and thus to accelerate consumption of the anode. This maximum surface temperature can be maintained with a moderate amperage if the rate of heat loss from the anode is kept to a minimum.

The anode loses heat principally by conduction through the electrode and by conduction and convection to the atmosphere. The first loss can be minimized by choosing a poorly conducting electrode material—e.g., carbon as opposed to graphite—and by reducing the cross-sectional area of the electrode. Losses to the atmosphere can be minimized by using an atmosphere showing poor thermal conductivity at anode temperatures and by reducing the surface area of the electrode.

Complete Consumption of Anode. This is required by all of the above vaporization mechanisms, since all require that each boron carbide particle be exposed to the direct action of the arc. Steinle (*25*) found that a carbon anode lost only 1 mg. per minute in argon, but lost 115, 70, and 156 mg. per minute in air, nitrogen, and carbon dioxide, respectively. Consumption of the anode in a reasonable length of time thus requires that the ambient at-
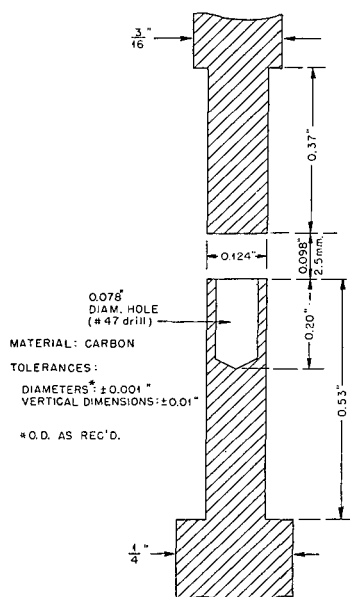
mosphere contain a component capable of reacting with carbon at arc temperatures to give a gaseous product.

Immobility of Arc Column. The smooth entrainment and vaporization of boron carbide requires uniform current density (temperature) over the face of the anode and the absence of turbulence in the arc column. Ordinary arc wandering, such as is encountered with carbon electrodes 0.25 inch in diameter at currents of 3 to 10 amperes, can be avoided by reducing the diameter of the electrode. This step is desirable in any case as a means of attaining high anode surface temperatures. Unfortunately, however, reducing the anode diameter, which is equivalent to increasing the current density, also leads to an extremely undesirable change in the behavior of a pure carbon arc.

As the current density is increased beyond a fairly well-defined value characteristic of the electrode gap geometry, the arc begins to hiss, and an anode spot (much brighter than the rest of the anode surface) forms. Under the current and gap geometry conditions used in spectrochemistry, the anode spot wanders erratically over the face of the anode, changing direction thousands of times per second. This motion causes uneven heating of the anode surface, and creates turbulence in the arc column. The anode spot in turn contains a microspot 0.3 to 0.5 mm. in diameter. According to Finkelnburg (*3*), the microspot "apparently represents the point of entry of the arc current into the anode at a given instant." If this is true, the current density in the anode spot is of the order of 50,000 amperes per sq. cm. This high current density causes the localized emission of a jet of carbon vapor. The microspot moves within the anode spot some 50,000 to 80,000 times per second, or oftener; this motion is sometimes a regular oscillation. There appeared to be little prospect of effecting a smooth entrainment or volatilization of boron carbide in the presence of this turbulence; it was therefore necessary to find a way to avoid it.

DERIVATION OF OPTIMUM EXPOSURE CONDITIONS

Electrode Geometry, Amperage, and Atmosphere. Preliminary experiments guided by requirements for highest possible anode surface temperature and complete consumption of the anode, as well as by restrictions imposed by the external illumination system, led to the use of carbon electrodes arranged as shown in Figure 1. The arc was operated at 9.0 amperes. This arc was found to be on the verge of the hissing region, and gave analytical results whose accuracy and precision fluctuated considerably. Some change in burning conditions was obviously necessary if satisfactory results were to be obtained.

List and Jones (*10*) have shown that the critical amperage for the onset of hissing in a carbon arc varies linearly with anode area and arc length. An increase in anode area, or a corresponding decrease in current, was considered undesirable because of the requirements for the analytical procedure. Lengthening of the arc gap sufficiently to stop hissing was found to lead to poor precision at low boron levels. It was therefore decided to determine whether the above described operating conditions could be retained, and hissing avoided, by using some atmosphere other than air which met the requirements for the highest possible anode surface temperature. Nitrogen, carbon dioxide, Freon 12 (CCl_2F_2), oxygen, and mixtures of oxygen in various proportions with helium and argon, were investigated; in some cases the gases were saturated with water vapor. All experiments were carried out in an Owen fused quartz gas chamber (*17*), as described below.

The smoothest burning and best analytical precision were obtained by using a 76% argon–24% oxygen mixture (3.2 and 1.0 liters per minute of argon and oxygen, respectively). The gas pressure within the chamber was maintained 5 cm. of water higher than that of the atmosphere.

Internal Standard. DISCUSSION OF CRITERIA. Volatility. In carbon arc exposures of metal, salt, or oxide samples, the temperature of the arc column tends to rise during the exposure. If good analytical precision is to be obtained in such cases, the

volatilization *vs.* time curves of internal standard and subject elements must either resemble each other closely or be extremely reproducible. In the present case, however, the sample consisted of essentially pure carbon. The composition, and hence the temperature, of the arc column remained almost constant throughout the exposure. The requirements as to similarity in volatilization behavior between subject element and internal standard were therefore less strict; particles encountered the same chemical species and temperature in the arc column, regardless of when they entered it.

Excitation Energy and State of Ionization. Similarity in excitation energy between subject element line and internal standard line helps the observed energy ratio, or intensity ratio, to be invariant to changes in arc column temperature, provided that these changes are not accompanied by nonreproducible changes in the relative abundance of the two species in the arc column. In the present case, the temperature of the column varied little as a function of time. The use of matched excitation potentials was still desirable, however, to compensate for changes in temperature as a function of radial distance from the axis of the arc column.

The only boron lines available for consideration were the atomic lines B I 2496.778 and B I 2497.733; both have excitation potentials of 4.94 e.v. (*15*). In order to avoid complications due to possible variation in degree of ionization of the internal standard, only atomic lines of the latter were examined.

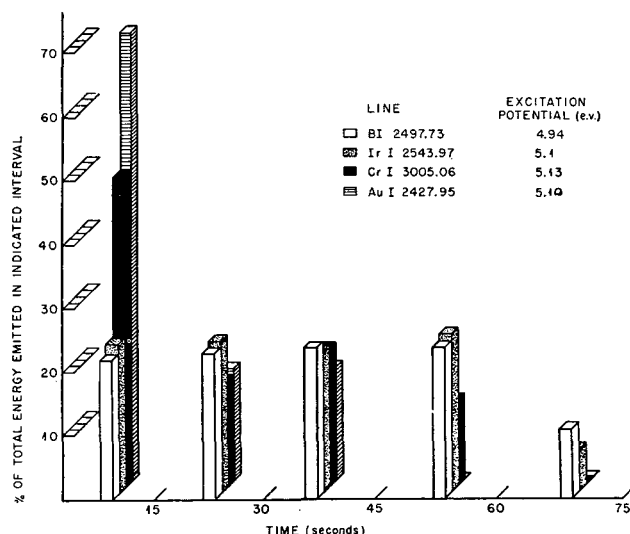Freedom from interference is discussed later.

Figure 2. **Volatilization curves**

SELECTION OF INTERNAL STANDARD. In view of the above discussion, resemblance in volatilization behavior between the internal standard and boron carbide was considered highly desirable, but not essential. The substances selected for testing were auric chloride ($AuCl_3$), chromic oxide (Cr_2O_3), and metallic iridium. All three metals possess suitably located lines having acceptable excitation potentials. As can be seen from moving plate exposures, however, the volatilization behavior of these substances varies widely (Figure 2).

Auric chloride decomposes at 254° C. Gold therefore is present in the electrode in the metallic state almost from the beginning of the exposure. The metal melts at 1063° C., boils at 2600° C., and does not react with carbon. Thus, it volatilizes readily under the experimental conditions employed.

Chromic oxide, like boric oxide, reacts with carbon at high temperatures to give carbides. Since a large excess of carbon is

present in the anode, the species formed in this case can be assumed to be chromium carbide, Cr_3C_2, [melting point 1890° C.; boiling point 3800° C. (5)]. The shape of the volatilization curve indicates that some chromium may be volatilized as an oxide or the metal before carburization is complete.

Iridium [melting point 2454° C. (2), boiling point 4800° C. (2)], although in no way chemically resembling boron, duplicates the volatilization behavior of boron carbide almost exactly. It does not react with carbon.

The data relevant to the use of these substances as internal standards are summarized in Table I.

Table I. Characteristics of Tentative Internal Standards

Substance	M. P.[a], ° C.	B. P.[a], ° C.	Spectrum Line of Interest	
			Wave length, A.[b]	Excitation potential, e.v.
Au	1063	2600	Au I 2427.95	5.10 (19)
Cr	1890	2200	Cr I 3005.06	5.13 (15)
Cr_2O_3	1990	...		
Cr_3C_2	1890	3800		
Ir	2454	4800	Ir I 2543.97	5.1 (14)

[a] Data on Ir taken from (2); other data from (5).
[b] Wave lengths taken from (6).

These data, taken in conjunction with the moving plate exposures shown in Figure 2, furnish support for the hypothesis that boron carbide is transported into the arc column by entrainment. Figure 2 shows that the elimination of chromium carbide was completed before that of boron carbide—i.e., before the anode was consumed. This indicates that volatilization of the chromium carbide must have been due, at least in part, to molecular distillation. Since the highest temperature attained on the anode, ~3700° C., (3, 9) is close to the boiling point of chromium carbide (3800° C.), this appears possible. Boron carbide is distinctly less volatile than chromium carbide, however, and since no temperatures higher than ~3700° C. are available on the anode, the volatilization of boron carbide must be due to some mechanism other than molecular distillation.

This is even more evident from comparison of the vaporization curves of boron carbide and iridium. Since the boiling point of iridium (4800° C.) is far higher than the highest temperature attainable on a carbon anode, entrainment probably plays an important role in transporting iridium into the arc column. The exact agreement between its volatilization vs. time curve and that of boron carbide strongly suggests that the two materials were transported by similar mechanisms.

The choice of internal standard was based on the above considerations and on the results of further analysis of results of conventional and moving plate exposures. The reproducibility of intensity ratio exhibited by the best line pair for each internal standard is shown in Table II. Each precision figure represents at least six replicate exposures. The evidence available thus clearly dictates the choice of iridium as the internal standard for the determination of boron present as boron carbide.

External Illumination System. The spectrograph used was a Jarrell-Ash 3.4-meter Wadsworth grating instrument. The following criteria were set up for its external illumination system.

Light from only the center 50% of the image was to be used.
Scattered light within the instrument was to be kept to a minimum.
Maximum optical speed was desired—i.e., the aperture of the system was to be completely filled.
Spectral lines were to show the greatest possible vertical uniformity.

These requirements were met by using a 10-cm. spherical quartz lens to form a stigmatic intermediate image of the arc gap on a slotted diaphragm located on the optical bench. An 8-cm. spherical quartz lens located at the slit formed an image of the above-mentioned slot on the collimating mirror. The size, shape,

and location of this slot were first chosen so that its image exactly filled the available area of the collimating mirror. This satisfied the second and third requirements. The arc gap and the 10-cm. spherical lens were next positioned so that the image of the 2.5-mm. gap thrown upon the diaphragm was twice as high as the slot, and suitably centered. This satisfied the first requirement. The only existing stigmatic images of the arc (those on the diaphragm and collimating mirror) were sufficiently far removed from the slit to ensure satisfaction of the last requirement (Figure 3).

ANALYTICAL TECHNIQUE

Materials. CARBON ELECTRODE STOCK AND POWDER. National Special Spectroscopic Electrodes, 0.25 inch in diameter by 12 inches (Carbon grade L113SP), were obtained from the National Carbon Co., Cleveland, Ohio, and were tested for boron content as follows: Electrodes shaped as shown in Figure 1, but having no cavity, were burned until the portion 0.124 inch in diameter was completely consumed. If five electrodes from a given lot, burned in this way, showed no evidence of B I 2497.73 A., the lot was used for electrode preparation. Carbon powder for the dilution of standards was prepared by machining electrode stock selected as above. This powder was checked after preparation for contamination.

BORON CARBIDE. Pure boron carbide was obtained from the Norton Co., Chippawa, Ont., Canada. A wet chemical analysis performed by John M. Chilton showed the material to conform to within 0.2% to the formula B_4C.

AURIC CHLORIDE AND CHROMIC OXIDE. Analytical reagent grade reagents were used.

IRIDIUM. Finely powdered iridium was obtained from the American Platinum Works, Newark 5, N. J. Its metallic state was verified by x-ray diffraction exposures made by Gilbert E. Klein. Electron microscope measurements made by T. E. Willmarth and F. E. Toomer showed that 99.3% of the particles of this material were smaller than 0.1 micron in diameter.

Table II. Reproducibility of Intensity Ratios for Boron-Internal Standard Line Pairs

Line Pair	Relative Std. Dev. of Observed Intensity Ratio, %
$\dfrac{\text{B I 2497.73}}{\text{Au I 2427.95}}$	6.1
$\dfrac{\text{B I 2497.73}}{\text{Cr I 3005.06}}$	8.4
$\dfrac{\text{B I 2497.73}}{\text{Ir I 2543.97}}$	1.5

Preparation of Standards. Since the amount of sample to be burned was only 10 mg., it was essential that the standards and samples be perfectly homogeneous with regard to the distribution of boron and iridium. Adherence to the grinding procedures was necessary in order to assure this homogeneity. All dilutions of standards and samples were made with carbon powder, because it was easier to grind than graphite. Test showed that analytical curves obtained in carbon and graphite bases were identical in both the arc and spark techniques.

BORON STOCK. Amounts of finely ground boron carbide and boron-free carbon powder calculated to give a mixture containing 1.0% boron by weight were transferred to an agate mortar and ground for 4 hours.

IRIDIUM STOCK. A mixture containing 1.00% iridium in carbon was prepared by grinding appropriate amounts of iridium and boron-free carbon as above.

ANALYTICAL STANDARDS. Amounts of the above stock mixtures calculated to give a mixture containing 35 p.p.m. of iridium and the desired concentration of boron were ground together for 15 minutes in an agate mortar. No standard or sample was ever diluted more than tenfold in a single grinding.

Preparation of Sample. The greatest care was observed to ensure representative sampling of the original material. As large a sample as could conveniently be handled was subdivided by mixing and quartering, or by riffling. The aliquot used for analysis was next ground for 30 to 60 minutes, depending on its apparent original homogeneity, and passed through a 360-mesh

silk screen sieve. A mixture of 90 mg. of this sample material and 10 mg. of iridium stock was then ground in an agate mortar for 15 minutes. Carbon electrodes shaped as in Figure 1 (anode) were then filled with sample mixture and tamped; approximately 10 mg. of the mixture was required.

Electrodes. These carbon electrodes (see Figure 1) were prepared with tools having tungsten carbide cutting edges.
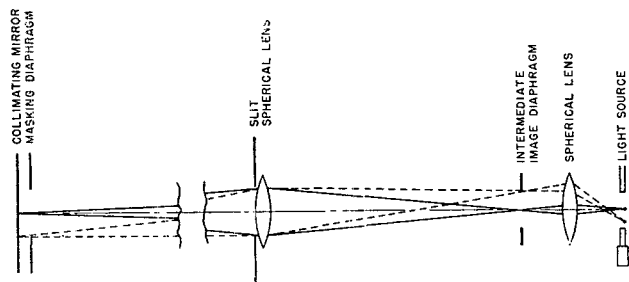

Figure 3. Illumination system (side view)

Gas Chamber. An Owen fused quartz gas chamber (17), provided with detachable front and rear windows to facilitate cleaning, was used for all exposures. The essential dimensions of this chamber are given in Figure 4. The chamber was supported, independently of the arc stand, by a V-block which was mounted on an optical bench rider and provided with the degrees of freedom necessary for positioning the chamber.

Gas Supply. In the gas chamber 3.2 liters per minute of argon and 1.0 liter per minute of oxygen were mixed and passed from the front to the rear. A positive pressure of 5 cm. of water was maintained in the chamber.

External Illumination System. The surface of the diaphragm contained a scale calibrated in terms of actual millimeters of electrode gap (see Figure 3 and discussion on external illumination system). The slot in the diaphragm had a height equivalent to 1.25 mm. of gap width.

Slit, 25 microns (fixed) $\times$ 2 mm.

Reciprocal linear dispersion, 5 A. per mm.

Emulsion, Eastman Kodak Spectrum Analysis No. 1 35-mm. roll film.

Power supply, Motor-generator set, supplying 230 volts direct current; continuously variable series resistance.

Exposure Technique. The electrodes were inserted in the clamps so that the lower electrode, containing the sample, was the anode. The electrodes were then advanced into the gas chamber; the analytical gap was adjusted to 2.5 mm. and centered over the diaphragm slot by projection of a shadow image of the electrodes on the diaphragm. The chamber was swept with the argon-oxygen mixture for 15 seconds, and the cathode brought into contact with the anode. The current was then adjusted to 9.0 amperes, the shutter opened, and the cathode retracted to its former position. The exposure was continued for 90 seconds.

Photographic Processing. Films were developed for 3 minutes at 20° C. in Eastman Kodak D-19 developer, washed for 15 seconds in 4% acetic acid solution, fixed for 90 seconds in fresh Eastman Kodak acid fixing solution, rinsed in running water for 5 minutes, immersed for 30 seconds in water containing Eastman Kodak Photo-Flo, and hung in an air-conditioned room (50% relative humidity) until dry.

Densitometry. The emulsion was calibrated by the two-step method (Seidel function, 1). All spectra had a clearly visible background, which was subtracted from observed line plus background readings. All densitometric measurements were made on a National Spectrographic Laboratories Spec-Reader recording densitometer.

Although interference of Fe II 2497.72 with B I 2497.73 and Fe 2543.92 with Ir I 2543.97 was expected, it was found that no such interference was encountered until the iron reached a concentration of 1000 p.p.m. in the first case, and 500 p.p.m. in the second. None of the samples analyzed so far have been contaminated with iron to this extent. However, the intensity of the interfering iron line can safely be calculated as a constant multiple of the intensity of another iron line originating from the same, or a closely related, term level (see section on iron interference).

Since the need to use monitor lines has not arisen in use of the arc procedure, no such lines have been selected.

RESULTS

The working curve obtained is shown in Figure 5. The fact that the lowest experimental point lay on the same straight line as the remaining points indicates that no significant amount of residual boron was present in either the electrodes or the dilution stock.

The standard deviation of the intensity ratio was not significantly greater for the lowest standard concentration (0.50 p.p.m. of boron) than for the others. Although the standard mixtures gave a standard deviation of 1.1% for quadruplicates, the corresponding mean figure for all sample material to date has been approximately 1.8%.
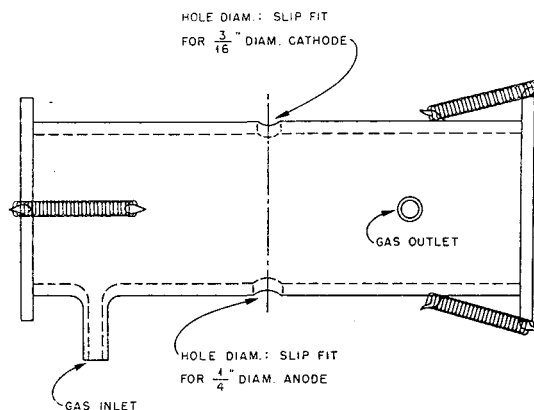

Figure 4. Owen fused quartz arc chamber (modified)

The limit of sensitivity was set by the presence of lines believed due to C_2 bands. No attempt was made to lower this limit, since another technique, developed concurrently with the present procedure, was available for lower concentrations of boron.

HIGH VOLTAGE SPARK (SIFTER ELECTRODE) PROCEDURE

The principal difficulties encountered in the development of the direct current arc procedure were concerned with volatilizing boron carbide—i.e., transporting it into the analytical gap. It was felt that some of these difficulties might be circumvented by feeding a stream of finely divided sample particles into the analytical gap through a hollow electrode and using spark excitation. Although good absolute sensitivity was not to be expected from random encounters between the spark and the suspended sample particles, this approach appeared to offer the possibility of achieving satisfactory concentrational sensitivity through the use of large samples.

DERIVATION OF OPTIMUM CONDITIONS

Electrode Geometry. Techniques of this type which have been suggested have employed gravity, gas currents, and electrostatic repulsion for propelling sample material into the analytical gap.

In 1940, Safonov (22) suggested that powdered ore samples might be fed from a reservoir into a funnel by means of a vibrator. The funnel emptied into a tubular upper electrode. A

thin stream of particles was thus fed into an arc or spark discharge through an opening 2.5 mm. in. diameter.

In 1951, Owen (18) suggested that a stream of air or gas containing sample particles in suspension be sent into the analytical gap through holes bored axially through both electrodes.

Johnson and Norman (8), in 1943, and Milbourn and Hartley (12), in 1948, placed powdered nonconducting samples in an open cup-shaped (lower) electrode. They then passed a high voltage spark across the analytical gap; electrostatic repulsion caused the sample particles to be projected into the analytical gap, where they were vaporized and excited by the discharge.

The first two methods had the disadvantage of requiring auxiliary feeding apparatus. Preliminary experiments with electrostatic ejection gave relatively poor sensitivity for boron in carbon, probably because the spark necessarily struck to one side of the emerging cloud of particles.

Experimentation with a number of electrode shapes and perforation patterns led to the development of an electrode which combined the gravity feed and electrostatic ejection principles. This electrode, which was called the sifter electrode, is shown in Figure 6.
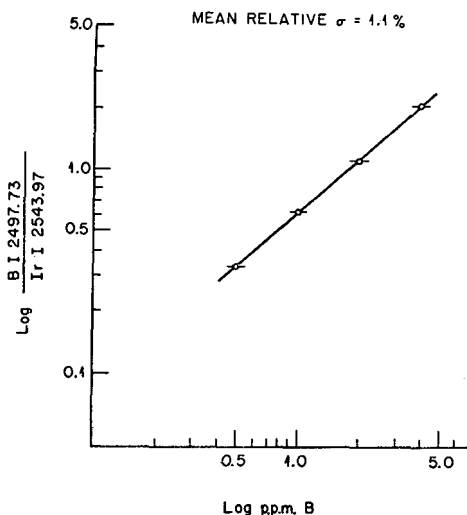
Figure 5. Analytical curve for arc method

This design forced intimate contact between sample particles and discharge channel by requiring the spark to strike between two or more closely neighboring orifices.

The electrode was filled almost to the top with dry powdered sample material. The opening was then closed with a cork to prevent the escape of this material from the top of the electrode during the exposure.

The passage of a high voltage spark across the analytical gap was found to propel sample particles through the holes at a steady rate without the use of auxiliary feeding devices. (A carbon or graphite powder, which had been exposed to an exceedingly dry or an exceedingly humid atmosphere, was occasionally found to flow irregularly through the holes. When this difficulty occurred, it was remedied by storing the material overnight in a 50% humidity chamber.)

In the analysis of carbon or graphite powder, approximately 90 seconds were required for the consumption of a 110-mg. charge. Concentrational sensitivity appeared promising. The gap width and counter electrode shape were selected on the basis of sensitivity tests. Since the sifter electrode appeared to offer a satisfactory method of transporting the boron carbide into the analytical gap, attention was turned to the remaining problems: exci-

tation conditions, atmosphere, selection of an internal standard, and interferences.

Electrical Parameters. No theoretical criteria were available for predicting the optimum electrical conditions for the present technique. The only parameters which were variable on the spark source used were the primary voltage and the secondary series inductance. Optimum values were selected on the basis of the sensitivity and precision shown (see analytical curve).
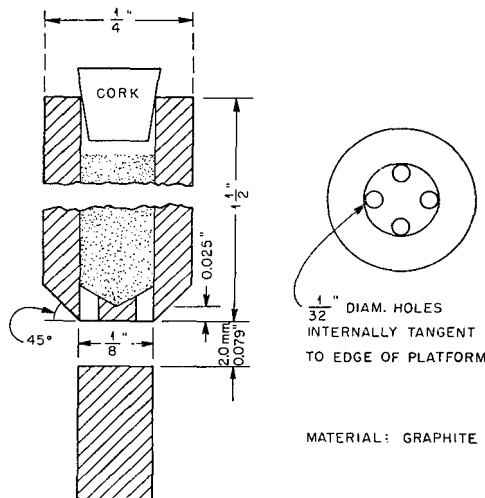
Figure 6. Filled sifter electrode and counter, in position for exposure, and bottom view of sifter electrode

Atmosphere. Neither hissing nor electrode consumption was a problem in the present technique. However, the nature of the atmosphere was thought to be capable of influencing results, since the atoms and molecules of this atmosphere served as the medium of conveying energy to and from the sample and internal standard atoms. Test exposures were run in all of the gases listed in the discussion on electrode geometry, amperage and atmosphere under direct current arc procedure. The best sensitivity obtained in air was 1.25 p.p.m. of boron. The best sensitivity obtained in any other atmosphere was 0.25 p.p.m., which was found in commercial argon.

Selection of Internal Standard. DISCUSSION OF CRITERIA. Volatility. With this type of sample feeding, the relative volatility of boron carbide and internal standard could not affect the relative abundance of these two materials in the analytical gap. Nor was this factor expected to seriously affect the vaporization of sample particles by the spark; the temperatures existing in a spark channel are several thousand degrees higher than those encountered on a direct current arc anode, and complete volatilization of particles was expected except in cases where the particle was found in the periphery of the spark channel. To guard against any effect which the latter factor might have on precision, the substances tested included both volatile and nonvolatile materials. The present technique offered an additional advantage in the selection of an internal standard, which was not available in the direct current arc technique. Minor lines of the internal standard element could not be used in the arc technique, because this would have required the addition of large proportions of this element to the sample. The presence of this added material would have caused the temperature of the arc column to vary during the exposure (see section on volatility under direct current arc procedure). The same situation did not exist in the sifter technique, so that additional elements were available for testing.

EXCITATION ENERGY AND STATE OF IONIZATION. Since excitation conditions in this case fluctuated rapidly in both space and time, particular stress was laid on the matching of excitation potentials.

CHOICE OF INTERNAL STANDARD. The substances listed in selection of internal standard under direct current arc procedures were tested as internal standards; Ir I 2543.97 again gave the best precision. In testing additional materials, however, it was found that Cu I 2492.15 also gave excellent precision when the copper was used in the form of thoroughly ignited cupric oxide (CuO). (It was essential to prepare this compound from metallic copper powder. Cupric oxide prepared by igniting the nitrate was always somewhat hygroscopic, and clogged the electrode.) Since cupric oxide was more readily available than iridium, it was used as the internal standard. Cu I 2492.15 is a weak line, so that a relatively large amount of cupric oxide was needed.

External Illumination. The general aims in setting up the external illumination system were the same as in the direct current arc procedure, and were achieved in the same way. In the present case, however, a 2.0-mm. analytical gap was used, and light from only the center 0.4 mm. was desired. The lens producing the intermediate image was therefore chosen and positioned in such a way as to permit this region to pass through the slot in the diaphragm.

Correction for Iron Interference. Both the boron and the copper lines were found to have Fe II interferences when the concentration of iron exceeded 200 p.p.m. Independent measurements showed, however, that an accurate and precise estimate of the intensities of the interfering iron lines could be made by multiplying the observed intensity of Fe II 2502.39 by an empirically determined intensity ratio. The excitation potentials of all of these iron lines (15) agreed to within 0.03 e.v. The value of this ratio was, of course, characteristic of the exposure conditions and power source used, and would have to be determined anew if any of these were changed. A summary of the lines used and intensity ratios observed is given in Table III.

In order to test the accuracy of this correction, synthetic mixtures were prepared and exposed by the technique described below. Results are shown in Table IV.

Table III. Correction of Iron Interference in Sifter Procedure

Line Interfered with	Interfering Line	Monitor Line	Observed Intensity Ratio: Interfering Line / Monitor Line In air	In argon
Cu I 2492.15	Fe II 2492.34	Fe II 2502.39	0.148 ± 0.004	0.252 ± 0.001
B I 2497.73	Fe II 2497.82	Fe II 2502.39	0.0835 ± 0.005	0.0881 ± 0.006

Table IV. Accuracy of Correction for Iron Interference

Fe Present, P.P.M.	Boron Added, P.P.M.	Boron Recovered, P.P.M.
500	4.00	4.03 ± 0.08
500	3.00	2.97 ± 0.05

In view of the quantitative recoveries obtained, it was felt that this correction would not introduce any abnormal variation in precision, although both accuracy and precision might be affected when extremely low boron concentrations occurred simultaneously with extremely high iron concentrations.

ANALYTICAL PROCEDURE

Materials. ELECTRODE STOCK. Samples of counterelectrode stock were run through the analytical exposure procedure. If five electrodes from a lot failed to show B I 2497.73, the lot was accepted for use.
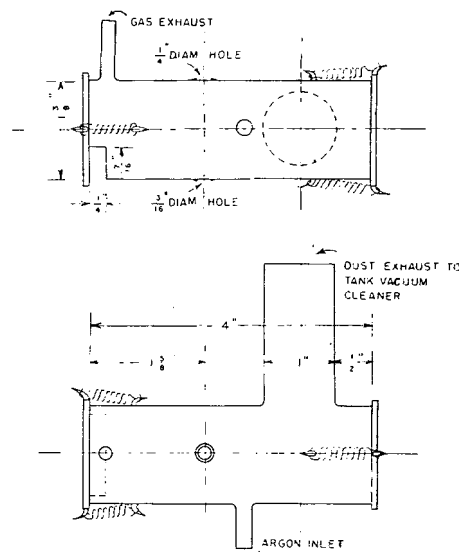
DILUTION STOCK, prepared from boron-free electrode stock.

Figure 7. Chamber used with sifter electrode

BORON CARBIDE. See Materials under direct current arc procedure.

CUPRIC OXIDE, prepared by igniting metallic copper powder in air for 1 hour at 800° C. (Ignition at lower temperatures may give a hygroscopic product.) If powdered copper is not available, prepare it by reducing any pure cupric oxide with hydrogen.

Preparation of Standards. BORON CARBIDE STOCK. See preparation of standards under direct current arc procedure.

CUPRIC OXIDE STOCK. Boron-free carbon was combined with sufficient cupric oxide to make the mixture 10% in copper by weight, and thoroughly ground in an agate mortar.

STANDARDS. If exposures were to be made in argon, boron carbide stock and cupric oxide stock were combined in amounts to give a mixture containing 0.400% copper. If exposures were to be made in air, this mixture was made 0.500% in copper. Standards were kept in a 50% humidity chamber until used.

Preparation of Samples. Samples were crushed and ground if necessary, and sieved through 360-mesh silk screening. For a 600-mg. sample, 24 mg. of cupric oxide stock was mixed thoroughly with 576 mg. of sample material, and transferred to a 50% humidity chamber until used. The sifter electrode was filled by means of a small spatula, with occasional gentle tapping. Tight packing was avoided. The electrode was corked.

Electrodes. Electrodes were prepared by drilling four $1/32$-inch (0.0792-mm.) holes in the end of boron-free graphite porous cup electrodes (United Carbon Co. Style 201 or similar), which have the form shown in Figure 6, except for the 45° bevel. (In this investigation, the drills were held in a pin vise, and the drilling done by hand. A jig was used to ensure proper placement and alignment of the holes.) A flat-topped $1/8$-inch counterelectrode was used, with an analytical gap of 2.0 mm.

Gas Chamber. In order to minimize contamination of the laboratory, the chamber shown in Figure 7 was employed, regardless of whether or not an argon atmosphere was used. The exhaust served both to remove argon and to draw a current of air past the inner surface of the front window. This prevented the accumulation of sample material on this window. The tank vacuum cleaner was left connected to the side arm during exposures, but was operated only between exposures. The chamber was made of borosilicate glass, and was independently supported on the optical bench.

Gas Supply. When argon was used, it was supplied to the chamber at 1.8 liters per minute.

Exhaust. A house exhaust line giving 35 cm. of vacuum was used.

External Illumination. The center 0.4 mm. of the gap was projected on the diaphragm. Other conditions were as discussed in external illumination under direct current arc procedure.

Power Source. Excitation was provided by the high voltage spark section of a 1943 model Baird Associates power source. This unit has a 110/25,000-volt transformer.

The primary of this transformer is fed by an autotransformer through a fixed 18.3-ohm (660-volt) resistor. During operation, the autotransformer was adjusted so that the voltmeter placed

directly across the primary windings read 90 volts. A synchronous interrupter had been added to the original circuit. This interrupter had hemispherical tungsten tips with a radius of $^3/_{16}$ inch (4.76 mm.); the gap spacing in the interrupter was 0.020 inch (0.508 mm.). The interrupter was set to discharge at the peak of the voltage wave. In view of the above circuit characteristics, the condenser may be assumed to have been charged to approximately 20,500 volts at the instant of discharge.

The capacitance was 0.005 μfd.; the series inductance was 0.037 mh. No ohmic resistance was added to the secondary circuit.

The RF ammeter (hot wire-thermocouple type) in the secondary circuit read 4.8 amperes.

Other instrumental characteristics were as described under direct current arc procedure.

Exposure Technique. The electrodes were positioned, and the chamber swept with argon for 15 seconds. The exposure was continued until the charge was consumed (approximately 90 seconds). If greater sensitivity was desired, an additional exposure was superimposed on the first. After exposures of a given sample were completed, the dust was swept from the chamber by means of a tank vacuum cleaner (see Figure 7).
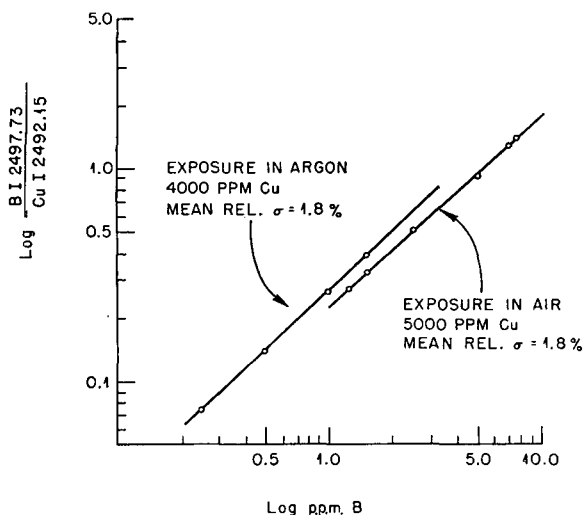
Figure 8. Analytical curves for spark (sifter electrode) method in argon and air

Photographic Processing. Processing was carried out as in photographic processing under direct current arc procedure, except that film was subjected to a controlled intentional fogging in the darkroom to bring the spectral background within the measurable range (Section 14, *1*).

Densitometry. See discussion of densitometry under direct current arc procedure. If the iron concentration appeared to exceed 200 p.p.m., the intensities of Fe II 2497.82 and Fe II 2492.34 were determined as indicated in correction for iron interference above. These intensities were subtracted from the net (background-corrected) intensities of BI 2497.73 and CuI 2492.34.

RESULTS

Spectral Background. The spectral background in sifter electrode exposures was very smooth, with no evidence of the band structure found in arc exposures. This greatly facilitated densitometry, and lowered the practical sensitivity limit. It also made accurate corrections for iron interference possible at boron levels as low as 0.3 p.p.m.

Analytical Curve. The analytical curves obtained in air and argon are shown in Figure 8. The sensitivity limits given are for double exposures in both cases. The relative standard deviations obtained in quadruplicate exposures of samples and standards have ranged from 0.4 to 3.6%; the mean of the relative standard deviation values obtained was 1.8%. The curves obtained with carbon and graphite dilution stock were superimposable.

COMPARISON OF METHODS

The arc and spark methods are supplemental in applicability. Although either method may be used in some cases, various characteristics of the sample, or the availability of equipment, may decide in favor of one procedure or the other. Table V presents information to guide in this choice.

Where there are no advance contraindications, the spark-in-air method is usually used first. If this exposure fails to give a measurable boron line, one of the other techniques is employed.

DETERMINATION OF OTHER IMPURITIES IN CARBON AND GRAPHITE

The present procedures were designed principally to deal with the peculiar problems presented by the determination of boron in carbon and graphite. Either approach could be used for the determination of other elements. It would seem that the sifter technique, in particular, might form the basis of a general procedure for the determination of impurities in these materials.

LITERATURE CITED

(1) Am. Soc. for Testing Materials, Philadelphia, Pa., "Methods for Emission Spectrochemical Analysis," pp. 22–5, 1953.
(2) Dushman, S., "Scientific Foundations of Vacuum Technique," Wiley, New York, 1949.
(3) Finkelnburg, W., "Hochstromkohlebogen," Springer, Berlin, 1948.
(4) Gatterer, A., Z. Ver. deut. Ing., 80, 129 (1936).
(5) Handbook of Chemistry and Physics, 35th ed., (C. D. Hodgman, editor), Chemical Rubber Publ., Cleveland, Ohio, 1954.
(6) Harrison, G. R., "M.I.T. Wave Length Tables," Wiley, New York, 1939.
(7) Huldt, L., thesis, U. of Uppsala, 1948.
(8) Johnson, W. W. A., and Norman, D. P., IND. ENG. CHEM., ANAL. ED., 15, 119 (1943).
(9) Kohn, H., and Guckel, M., Z. Physik, 27, 305 (1924).
(10) List, B. H., and Jones, T. B., Armed Forces Technical Information Agency, Dayton, Ohio, Report AD-1765.
(11) Lochte-Holtgreven, W., and Maecker, H., Z. Physik, 105, 1 (1937).
(12) Milbourn, M., and Hartley, H. E. R., Spectrochim. Acta, 3, 320 (1948).
(13) Mitrović, M., Rec. trav. inst. recherches Structure Matière (Belgrade), 2, 23–6 (1953) (in English).
(14) Moore, C. E., private communication.
(15) Moore, C. E., "Ultraviolet Multiplet Table," Natl. Bureau of Standards Circ. 488, Section I (1950); Section II (1952).
(16) Mulliken, R. S., Phys. Rev., 25, 259 (1925).
(17) Owen, L. E., J. Opt. Soc. Amer., 41, 139 (1951).
(18) Ibid., p. 709.
(19) Platt, J. R., and Sawyer, R. A., Phys. Rev., (2) 60, 866 (1941).
(20) Ridgway, R. R., Trans. Electrochem. Soc., 66, 117 (1934).
(21) Rusanov, A. K., "Spectral Analysis of Ores and Minerals," p. 24, Gosgeolizdat, Moscow-Leningrad, 1948.
(22) Safonov, S. I., Zavodskaya Lab., 9, 187 (1940).
(23) Shugar, D., Bull. centre phys. nucléaire univ. libre Bruxelles, No. 34 (1952).
(24) Stähler, E., Ber. deut. chem. Ges., 46, 2067 (1913).
(25) Steinle, H., Z. angew. Mineral., 2, 28 (1939).
(26) Zeeman, P. B., Spectrochim. Acta, 6, 337 (1954).

Ultraviolet Spectrophotometric Determination of Bismuth by Iodide and Thiourea Methods

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A spectrophotometric study was undertaken to investigate the feasibility of increasing the sensitivity of iodide and thiourea methods for the determination of bismuth by making spectrophotometric measurements in the ultraviolet region of the spectrum. The absorption spectra of both complexes have characteristic ultraviolet absorbance maxima. By using ultraviolet absorbance maxima instead of the absorbance maxima found in the visible region, a large increase in sensitivity is achieved for both methods. The effect of diverse ions and the optimum conditions for the formation of each complex were studied. The optimum concentration range for both ultraviolet spectrophotometric methods is 0.6 to 6 p.p.m. of bismuth when 1.000-cm. absorption cells are used.

ONE of the classical colorimetric methods for the determination of bismuth is based on the yellow color of the tetraiodobismuthate(III) complex (*5, 7, 10, 11*). A colorimetric method for determining antimony is based on the tetraiodoantimonate(III) complex. Elkind, Gayer, and Boltz (*2*) showed that the tetraiodoantimonate(III) complex has a characteristic ultraviolet absorbance maximum, which could be used to increase the sensitivity of the iodide method by approximately 350%, and that bismuth(III) interfered in the ultraviolet spectrophotometric determination of antimony. This spectrophotometric investigation was made to ascertain whether increased sensitivity could be obtained for bismuth(III) complexes by performing spectrophotometric measurements in the ultraviolet region of the spectrum.

A survey of the literature revealed that Merritt, Hershenson, and Rogers (*8*) recommended the use of hydrochloric acid to form the tetrachlorobismuthate(III) complex. This complex exhibited maximum absorbance at 327 mμ and served as a suitable basis for the ultraviolet spectrophotometric determination of bismuth. They also showed the ultraviolet spectrophotometric curves for the tetraiodo- and tetrabromobismuthate(III) complexes, which have characteristic absorbance maxima at approximately 340 and 375 mμ, respectively. Kingerly and Hume (*4*) showed that the hexathiocyanobismuthate(III) complex has a characteristic absorbance maximum at 330 mμ. In addition to the tetraiodobismuthate(III) complex it was decided to study the bismuth(III)–thiourea complex, which has been used in colorimetric analysis although its sensitivity is much less than the dithizone method and slightly less sensitive than the iodide method (*6*). Recently, Nielsch and Böltz (*9*) investigated the use of thiourea as a reagent in the colorimetric determination of bismuth in nitric, hydrochloric, and hydrobromic acid solutions.

GENERAL EXPERIMENTAL WORK

Apparatus and Solutions. A Warren Spectracord and a Beckman DU spectrophotometer were used for spectrophotometric measurements. All measurements were made in 1.000-cm. silica

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cells with a reagent blank solution in reference cell, unless otherwise stated.

A standard bismuth solution was prepared by dissolving 0.2321 gram of hydrated bismuth nitrate, $Bi(NO_3)_3.5H_2O$, in 5 ml. of perchloric acid, or in 5 ml. of concentrated nitric acid, and diluting to 1 liter in a volumetric flask. The use of perchloric acid is preferable for ultraviolet measurements. The standard solution has a titer of 0.100 mg. of bismuth per ml., this value being confirmed by a gravimetric determination as bismuthyl chloride.

The potassium iodide–ascorbic acid reagent was prepared by dissolving 140 grams of reagent grade potassium iodide and 10 grams of ascorbic acid in distilled water and diluting to 1 liter.

A 10N sulfuric solution was prepared by adding carefully 280 ml. of concentrated reagent grade sulfuric acid to distilled water and diluting to 1 liter.

The thiourea solution was prepared by dissolving 60 grams of reagent grade thiourea in approximately 500 ml. of distilled water and warming to effect dissolution. The solution was filtered through a sintered-glass filter crucible of medium porosity and diluted to 500 ml. Each milliliter of the thiourea solution contained 0.12 gram of thiourea.

Reagent grade (70%) perchloric acid was used.

The solutions used in studying the effect of diverse ions were prepared using reagent grade chemicals.

General Procedure. The required volume of the standard bismuth(III) solution is transferred to a 50-ml. volumetric flask. After the desired volume of acid and any diverse ion solution is added, the color-forming reagent is added. The solution is then diluted to the graduation mark with distilled water. The reference absorption cell contains a solution, which has the same concentration of acid and color-forming reagent.

IODIDE METHOD

Effect of Solution Variables. BISMUTH CONCENTRATION. Figure 1 shows the absorption spectrum for the tetraiodobismuthate(III) complex, which exhibits absorbance maxima at 337 and 465 mμ. This figure also indicates the increase in sensitivity obtainable by making the spectrophotometric measurements in the ultraviolet region at 337 mμ. The ultraviolet spectrophotometric curves obtained with 0.5, 1, 2, 4, and 10 p.p.m. of bismuth are shown in Figure 1. Conformity to Beer's law was found at 337 mμ. The optimum concentration is 0.6 to 6 p.p.m. of bismuth for spectrophotometric measurements at 337 mμ, based on the virtual linear portion of a Ringbom plot.
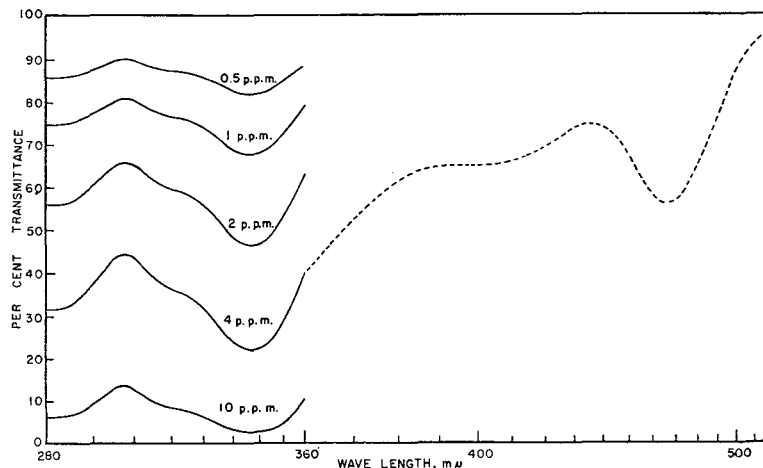

Figure 1. Absorption spectra of tetraiodobismuthate(III) complex

IODIDE CONCENTRATION. The effect of iodide concentration was determined, using 4 p.p.m. of bismuth, 10 ml. of 10N sulfuric acid, and amounts of potassium iodide varying from 0.25 to 3.5 grams per 50 ml. It was found that 2.5 to 3.5 grams of potassium iodide per 50 ml. was sufficient to assure attainment of maximum absorptivity. Hence, in subsequent work, 20 ml. of the potassium iodide–ascorbic acid reagent were used giving a final concentration of 5.6% in respect to potassium iodide. This concentration is much higher than that usually used in the colorimetric determination of bismuth.
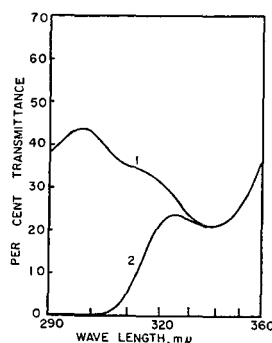
ACID CONCENTRATION. The use of sulfuric acid is recommended, because hydrochloric acid tends to decrease the absorbance through formation of tetrachlorobismuthate(III) ion, nitric acid has a characteristic absorbance in the ultraviolet region, and perchloric acid precipitates potassium perchlorate. The concentration of sulfuric acid was varied from 1.5 to 2.5N with no appreciable effect on absorptivity.

TIME. The tetraiodobismuthate(III) complex is stable for at least 24 hours, using the original reference blank solution in the reference cell. The absorbances of the blank and the sample change slowly, but at the same rate; thus, for maximum precision and accuracy, both should be the same age. A difference of 5% has been observed on substituting a fresh blank for an aged blank when measuring a solution that has been standing for 24 hours.

DIVERSE ION CONCENTRATION. The effect of certain diverse ions was determined using 4 p.p.m. of bismuth, 20 ml. of the potassium iodide–ascorbic acid reagent per 50 ml., and a final acidity of 2N in respect to sulfuric acid. The spectrophotometric measurements were made at 337 mμ. An error of less than 2.5% was obtained with 1000 p.p.m. of the following ions: acetate, ammonium, bromide, cadmium, calcium, chloride, ferrous, fluoride, manganous, magnesium, nitrate, perchlorate, potassium, sodium, sulfate, Versenate [(ethylenedinitrilo)tetraacetate], and zinc. Table I lists those ions which were found to interfere.

Although a preliminary separation of bismuth(III) from copper(II) is possible by extracting with dithizone (3), the effect of cupric ion was investigated inasmuch as it was expected that iodine would be liberated by cupric ions. Figure 2 shows that 50 p.p.m. of cupric ion can be tolerated, as the iodine liberated does not affect the spectrophotometric measurement at 337 mμ. Larger amounts of cupric ion interfere because of the development of a turbidity due to cuprous iodide. Ferric ions which were expected to interfere seriously in the iodide method did not liberate sufficient iodine to introduce a serious interference. The ascorbic acid in reagent is presumably effective in reducing the oxidation potential of the ferric ion below the value necessary for oxidation of iodide ions to iodine. The fact that ferric fluoride complex does not liberate iodine from an iodide solution

and the fact that fluoride ions do not interfere in the determination of bismuth, also, suggests another method of increasing the tolerance to ferric ions. An attempt to eliminate the interference due to antimony(III) by complexing the antimony(III) with fluoride ions was not very successful. Using 3 p.p.m. of bismuth and 0.8 p.p.m. of antimony an error of 45% (+1.35 p.p.m. bismuth) was obtained. A solution of the same concentration in respect to bismuth and antimony, but also 500 p.p.m. in fluoride ions, gave an error of 33%, (+1 p.p.m. bismuth). With the iodide concentration, which was adopted for the ultraviolet spectrophotometric method, a tolerance larger than 0.1 p.p.m. of antimony cannot be achieved by utilizing a fluoride concentration of 500 to 1000 p.p.m. A preliminary separation of antimony from bismuth by distillation of antimony(III) halides was not investigated. Likewise, a preliminary separation of bismuth from lead by electrolysis was not investigated as a means of circumventing the interference due to the plumbous ion (1).

Figure 2. Effect of cupric ion

1. 4 p.p.m. Bi
2. 4 p.p.m. Bi + 50 p.p.m. Cu

Recommended General Procedure. PREPARATION OF SAMPLE. Weigh, or measure by volume, a sample containing sufficient bismuth, so that the resulting solution contains 0.3 to 3 mg. of bismuth per 100 ml. of prepared solution. The solution should be 1 to 2N in sulfuric acid.

MEASUREMENT OF DESIRED CONSTITUENT. Transfer a 10-ml. aliquot of the prepared solution to a 50-ml. volumetric flask. Add 10 ml. of the 10N sulfuric acid and 20 ml. of the potassium iodide–ascorbic acid reagent. Dilute to the mark with distilled water and mix thoroughly. Measure the transmittance, or absorbance at 337 mμ, using 1.000-cm. silica cells. A reagent blank solution is used in the reference absorption cell.

THIOUREA METHOD

Effect of Solution Variables. BISMUTH CONCENTRATION. Figure 3 shows the characteristic absorption spectrum for the bismuth(III) thiourea complex, which has absorbance maxima at 322 and 470 mμ. The effect of 0.5, 1, 2, 4, and 6 p.p.m. of bismuth on the ultraviolet absorption spectrum are shown in Figure 3. Conformity to Beer's law was found at 322 mμ. The optimum concentration range is 0.6 to 6 p.p.m. of bismuth when measurements are made in the ultraviolet region on the basis of a Ringbom plot.

ACID CONCENTRATION. The effect of perchloric acid concentration was studied using 5 p.p.m. of bismuth. The final acidity was varied from 0.1 to 2N using variable amounts of 6N perchloric acid. It was found that with final acid concentrations below 1N the maximum absorptivity was not obtained. No change in absorptivity was noted in solutions which were 1 to 2N in perchloric acid. A final acidity of 1N was used in subsequent testing for the effect of diverse ions.

THIOUREA CONCENTRATION. The effect of thiourea concentration was studied using 5 p.p.m. of bismuth. The thiourea concentration was varied from 0.6

Table I.   Interfering Diverse Ions

		Iodide Method			Thiourea Method		
Ion	Added As	Amount added, p.p.m.	Error, in p.p.m. Bi	Tolerance^a, p.p.m.	Amount added, p.p.m.	Error, in p.p.m. Bi	Tolerance^a, p.p.m.
Antimony	KSbC$_4$H$_4$O$_7$	0.8	1.35	0	0.8	0.4	0
Lead(II)	Pb(NO$_3$)$_2$	5	0.6	0	2	0.18	1
Mercury(II)	Hg(C$_2$H$_3$O$_2$)$_2$	−1	0.3	0	5	0.17	3
Arsenic(III)	Na$_3$AsO$_3$	50	0	50	30	0.35	10
Tin(II)	SnCl$_2$	10	0^b	10	...	...	...
Silver	AgClO$_4$	10	0^b	10	...	...	...
Copper(II)	Cu(ClO$_4$)$_2$	50	0^b	50	5	0.23	1
Cobalt(II)	Co(NH$_4$)$_2$(SO$_4$)$_2$	50	−0.2	25	...	...	...
Iron(III)	Fe(ClO$_4$)$_3$	1000	0.25	500	40	0.25	10
Nickel(II)	NiCl$_2$	150	0^b	150	1000	0.2	500
Vanadate	Na$_3$VO$_4$	10	−0.2	5	300	0.18	200
Tungstate	Na$_2$WO$_4$	10	0.2	5	10	0.24	5
Iron(II)	FeSO$_4$	...	...	...	1000	0.3	300
Chloride	KCl	...	...	...	500	−0.35	100
Bromide	KBr	...	...	...	500	−0.25	200
Fluoride	KF	...	...	...	500	−0.10	200

^{a} As p.p.m., causing less than 2.5% error using 4 p.p.m. of bismuth.
^{b} Larger amounts caused interference.

to 4.8 grams of thiourea per 50 ml. The absorptivity increases rapidly until a concentration of approximately 2.5 grams of thiourea per 50 ml. is reached with a very slight increase in absorptivity being detected after the concentration exceeds 3 grams of thiourea per 50 ml. A final concentration of 3 grams of thiourea per 50 ml. is recommended.

TIME. Solutions of the bismuth(III) thiourea complex were found to be stable for at least 24 hours.

DIVERSE ION CONCENTRATION. The effect of diverse ions was studied using 5 p.p.m. of bismuth, 25 ml. of the thiourea reagent, and 10 ml. of 1 to 1 perchloric acid solution. An error of less than 2.5% was considered negligible. It was found that 1000 p.p.m. of the following ions did not cause interference: acetate, ammonium, cadmium, cobalt(II), manganese(II), magnesium, nitrate, phosphate, potassium, silver, sodium, sulfate, Versenate, and zinc. Those ions causing interference are listed in Table I.

Recommended General Procedure. PREPARATION OF SAMPLE. Weigh, or measure by volume, a sample containing sufficient bismuth, so that the resulting solution contains 0.3 to 3 mg. of bismuth per 100 ml. of prepared solution. The solution should be 1 to $2N$ in perchloric acid.

MEASUREMENT OF DESIRED CONSTITUENT. Transfer a 10-ml. aliquot to a 50-ml. volumetric flask. Add 10 ml. of a 1 to 1 perchloric acid and 25 ml. of the thiourea reagent. Dilute to the mark with distilled water and mix thoroughly. Use a reagent blank solution in the reference absorption cell, 1.000-cm. silica cells, and measure the transmittance, or absorbance, at 322 mμ.

DISCUSSION

An ultraviolet spectrophotometric study has been made of the iodide method for the spectrophotometric determination of bismuth, and it has been found that greatly increased sensitivity can be obtained by making spectrophotometric measurement at the ultraviolet absorbance maximum, 337 mμ, instead of at the absorbance maximum in the visible region, 465 mμ. The recommended general procedure for the iodide method, using 4 p.p.m. of bismuth, gave a standard deviation of 0.004 absorbance unit, 0.6%, for eight samples.

It was found that the bismuth(III) thiourea complex has an absorbance maximum at 322 mμ in addition to its known absorbance maximum at 470 mμ. Using the recommended general procedure developed, the sensitivity of the thiourea method was increased almost fourfold by making spectrophotometric measurements at the ultraviolet absorbance maximum. The molar absorptivities are 3.6×10^4 and 9.3×10^3 at 322 mμ and 470 mμ, respectively. Thus, whereas the iodide method is more sensitive than the thiourea method for spectrophotometric measurements made in the visible region, the thiourea
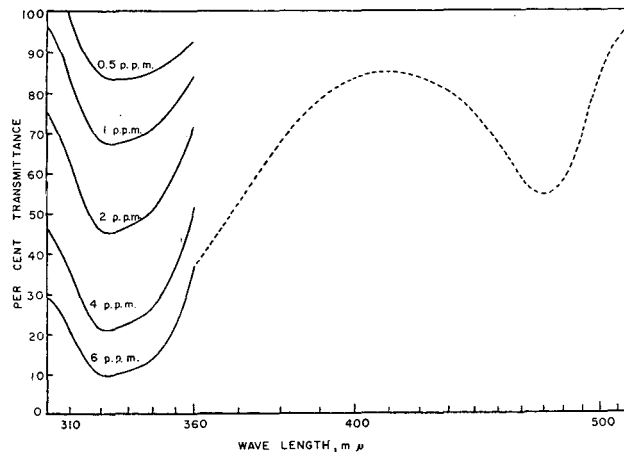

Figure 3. Absorption spectra of bismuth thiourea complex

method is slightly more sensitive when spectrophotometric measurements are made in the ultraviolet region. An indication of the precision of the ultraviolet spectrophotometric determination of bismuth by the thiourea method was ascertained from the results of eight samples, each containing 4 p.p.m. of bismuth. These samples gave a mean absorbance value of 0.684 with a standard deviation of 0.009, or 1.3%.

LITERATURE CITED

(1) Clarke, B. L., Wooten, L. A., and Luke, C. L., *Ind. Eng. Chem.,* 8, 411 (1935).
(2) Elkind, A., Gayer, K. H., and Boltz, D. F., ANAL. CHEM., 25, 1744 (1953).
(3) Haddock, L. A., *Analyst,* 59, 163 (1934).
(4) Kingerly, W. D., and Hume, D. N., *J. Am. Chem. Soc.,* 71, 2393 (1949).
(5) Leonard, C. S., *J. Pharmacol.,* 28, 81 (1926).
(6) Mahr, C., *Z. anal. Chem.,* 94, 161 (1933); 97, 96 (1934).
(7) McChesney, E. W., IND. ENG. CHEM., ANAL. ED., 18, 146 (1946).
(8) Merritt, C., Jr., Hershenson, H. M., and Rogers, L. B., ANAL. CHEM., 25, 572 (1953).
(9) Nielsch, W., and Böltz, G., *Z. anal. Chem.,* 142, 321 (1954); 143, 13, 168 (1954).
(10) Sproull, R. C., and Gettler, A. O., IND. ENG. CHEM., ANAL. ED., 13, 462 (1941).
(11) Wiegand, C. S. W., Lann, G. H., and Kalich, F. V., *Ibid.,* 13, 912 (1941).
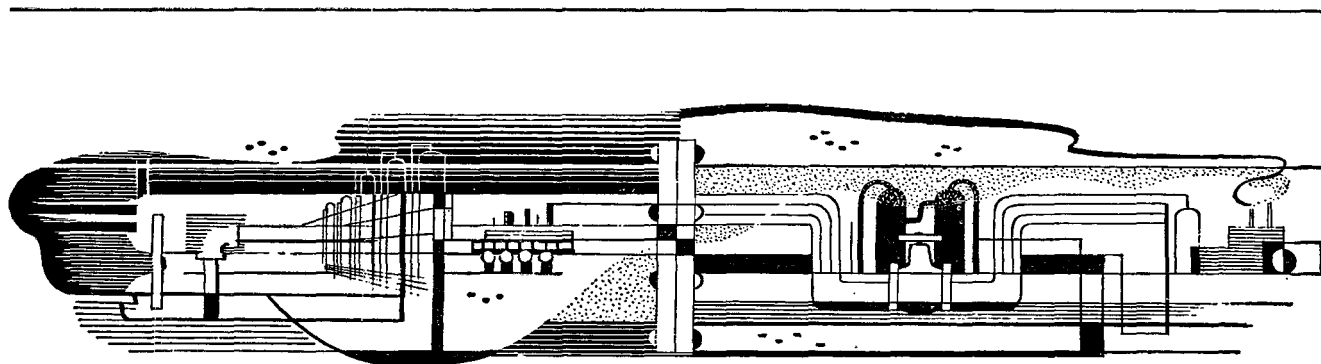
Separation and Determination of Millimicrogram Amounts of Cobalt

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A method is described for the separation of millimicrogram amounts of cobalt from biological materials, such as blood. The sample is dry-ashed, excess iron and alkali chlorides are removed, and the cobalt is separated on an anion exchange resin, Dowex 1. It is eluted from the resin substantially free from other elements, with recoveries of over 90%. This technique has been combined with spectrochemical analysis to determine the amount of cobalt in human blood, where its concentration is found to be below 1 part per billion. A spectrochemical method of determining cobalt is described which is sensitive to 1 mγ (1 $\times$ 10^{-9} gram). The direct current arc is used with the sample in the cathode. The conditions under which this sensitivity is achieved are discussed. A standard deviation of about 10% of the result may be expected when between 1 and 500 mγ of cobalt are present.

QUANTITATIVE analysis, since the time of Berzelius, has progressed through the gram and milligram stages, and with present instrumentation, the measurement of microgram quantities is routine. The next step, measurement of millimicrograms, is accompanied by special difficulties. The quantification of millimicrogram amounts of metals often requires the complete separation of the desired constituent from the original matrix material. The excess of mass of extraneous elements in the sample may prevent the determination of the trace constituent by diluting it beyond the limit of concentrational sensitivity. Chemical interferences not detectable at equimolar ratios arise when one constituent exceeds another by a wide margin, and the elements which ordinarily do interfere with a given procedure are certain to present significant problems when millimicrogram amounts of an element are to be determined (7). While a particular analytical technique may be capable of directly determining very small amounts of an ion when present alone in a solution, its sensitivity is generally greatly decreased when other ions or organic materials are present.

The determination of cobalt in human blood is chosen as an example of such a problem. It is known that cobalt occurs in human blood since vitamin B$_{12}$, a normal blood constituent, contains this element (10, 15). Vitamin B$_{12}$ is the cyano-complex of the cobalt-containing organic compound, cobalamin, C$_{63}$H$_{90}$O$_{14}$N$_{14}$PCo, and hydroxo-, nitrito-, chloro-, bromo-, sulfato-, cyanato-, thiocyanato-, and other complexes of the cobalt of cobalamin exist and produce varying degrees of vitamin activity. The concentration of vitamin B$_{12}$ in human blood has been measured by means of microbiological assays, and found to be in the parts per billion range (Table VI). Although data have been reported on the concentration of cobalt in human blood and serum (17, 18), no method of determining this concentration has been given in detail and the present communication supports the view that cobalt cannot have been detected as described. The concentrational levels reported here are much lower than those indicated elsewhere and are below the sensitivity limits for previous methods. As vitamin B$_{12}$ has been shown to play a role in protein metabolism, blood formation, etc., and is at present the subject of a great deal of research, the determination of cobalt in blood is of real interest even though its concentration is extremely low.

This paper describes a technique which can quantitatively separate as little as 2 mγ of cobalt from the other elements present in blood, and a new sensitive spectrochemical method of determining it. The cobalt can be separated so completely that spectrochemical analysis can detect no iron, nickel, or alkali metals accompanying it. The separation method has been shown to be applicable to much larger amounts of cobalt (6) and should provide a general method of separating it as preparation for any analytical procedure where a pure cobalt solution is required. The spectrochemical method can determine from 1 to 500 mγ (grams $\times$ 10^{-9}) of cobalt with a standard deviation about 10% of the result, and can be used whenever iron, nickel, and the alkali metals are absent.

QUANTITATIVE SEPARATION OF MILLIMICROGRAM AMOUNTS OF COBALT FROM BLOOD

A sample of blood which contains at least 2 mγ of cobalt is taken for analysis. It is dry-ashed, excess iron and alkali chlorides are removed, and the cobalt is separated on an anion exchange resin, Dowex 1-X8, according to the method of Kraus (6, 9). The effluent containing the cobalt is then evaporated to a small volume in hydrochloric acid, and analyzed by spectrochemistry.

REAGENTS

Hydrochloric Acid. Anhydrous hydrogen chloride gas (Matheson Co., Inc.) is scrubbed in a bubble tower with concentrated sulfuric acid, filtered through packed borosilicate glass wool, scrubbed again in a bubble tower by distilled water which has been saturated with the gas, and then dissolved in triply distilled water, or its equivalent, at 0° C. to give an acid concentration greater than 9M. The acid strength is determined and 9.0M, 4.0M, and 0.01M solutions of hydrochloric acid are obtained by proper dilution of this concentrated acid with triply distilled water. Acid thus produced is free of the traces of alkali and alkaline earth metals found in the reagent grade.

Figure 1. Ion exchange column

Isopropyl Ether. Alcohol-free ether (Eastman Organic Chemical) is redistilled from an acid cleaned still.

Ion Exchange Resin. Dow Chemical, Dowex 1-X8 resin, 50 to 100 mesh, is used in the absorption column. It is a strongly basic, quaternary amine type, styrene-divinylbenzene polymer containing approximately 7.5% of cross-linking divinylbenzene (6, 9).

Cobalt-60 Chloride Solution. This reagent was used only in checking the method. A cobaltous chloride solution having a specific activity of 4831 mc. per gram and a radiochemical purity of 99.9% was obtained from the Oak Ridge National Laboratory. The cobalt-60 was taken up into approximately 60 ml. of twice distilled hydrochloric acid. The solution was analyzed colorimetrically by the nitroso-R salt procedure described by Sandell (12) and was diluted one thousandfold, giving a final concentration of 13.3 mγ of cobalt per milliliter.

Tagged Vitamin B$_{12}$. This reagent was used only in checking the method. One-half milliliter of a solution containing 13 γ of vitamin B$_{12}$ tagged with cobalt-60 was diluted to 10.0 ml. with triply distilled water. Based on the value 4.5% as the amount
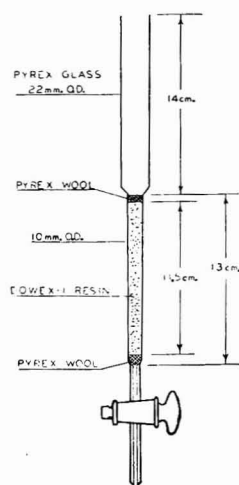
of cobalt in vitamin B_{12} (2), the metal concentration was calculated to be 29 mγ per ml. This material was provided through the courtesy of Merck & Co., Inc.

Sodium Heparin, Solid Salt (USP, Organon Inc., Orange, N. J.). Qualitative spectrochemical analysis of an ashed sample showed this material to be free of cobalt.

Triply Distilled Water. Laboratory distilled water was distilled twice more. The final condenser was made of fused silica, and the water was stored in polyethylene bottles.

APPARATUS

Ion Exchange Column. The column shown in Figure 1 was constructed of glass. A glass wool plug was saturated with water to eliminate air and placed in the bottom of the column, after which the Dowex 1 was added as a slurry in triply distilled water to give a resin bed 11.5 cm. long and 8.0 mm. in diameter. A second plug of glass wool saturated with water was placed on top of the resin bed.

Muffle Furnace. Electric furnaces operated by automatic controllers (Wheelco Model 241P) were used in ashing the samples. The temperature settings could be maintained within 20° C.
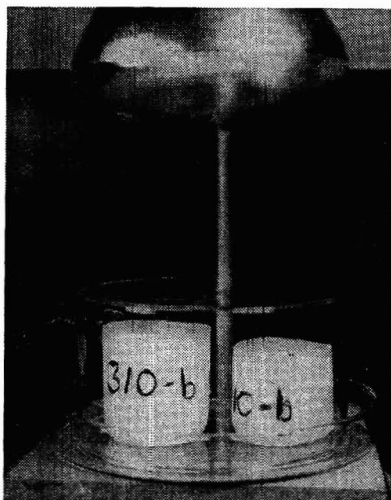

Figure 2. Evaporation cover

Geiger Counter. This apparatus was used only in checking the method. A Tracerlab Model SC-2A scaler with a TGC-4 counting tube was used in the tracer studies to determine the gamma activity of cobalt-60. The counter was dipped into the sample solution (10 ml. in volume) contained in a 1-inch-diameter test tube. A National Bureau of Standards cobalt-60 solution was used as activity standard.

Evaporation Cover. In order to protect the samples from air-borne contamination, apparatus like those shown in Figure 2 were fabricated from crystallizing dishes. Dried, filtered air was introduced through the side arm of the cover to remove vapor, thus preventing condensation inside the apparatus, and speeding the evaporation. This method of protecting the sample during evaporation proved necessary in order to avoid high and erratic results.

METHOD

Sampling. It is necessary to draw the blood in a careful manner in order to avoid contamination. One of two sampling techniques is used, depending on the quantity of blood being taken. One technique utilizes standard transfusion equipment, the other, 50-ml. syringes.

If a desired sample is 100 ml. or less in volume, it is drawn with 50-ml. hypodermic syringes fitted with special platinum–ruthenium alloy needles (J. Bishop and Co., Platinum Works, Malvern, Pa.). These needles are size No. 19, and their collars and joints are heavily plated with gold. Prior to use, they are cleaned by the technique used for cleaning glassware with the exception that they are immersed in the cleaning solution for only 15 or 20 seconds. The clean syringes and needles are placed in glass jackets, which have also been cleaned, and are sterilized by heating them in an oven at 160° C. overnight. One and one-

half milligrams of solid sodium heparin are added to the chamber of the syringe to prevent coagulation of the sample during drawing. A volume of blood slightly greater than that desired for the sample is drawn into the syringe. Then the volume is adjusted to that desired by using the syringe graduations and discharging the slight excess along with any bubbles produced during drawing. Previously calibrated syringes are therefore required. The samples are then transferred directly to the silica dish for ashing, or to polyethylene bottles if the sample is to be separated into blood fractions.

Samples larger than 100 ml. are drawn into soft-glass bottles regularly used for the collection of transfusion blood. The bottles (Abbott Laboratories, North Chicago, Ill.) are emptied of their anticoagulant solution, thoroughly cleaned, and after being charged with the proper amount of sodium heparin (1.5 mg. per 50 ml. of sample desired) and a few drops of triply distilled water, the bottles are re-evacuated to the vapor pressure of water. Abbott Laboratories blood collection sets were used in drawing the sample. They employ stainless steel needles connected with polyethylene tubing.

The first few milliliters of blood were discarded on the theory that it cleaned out the collection set. No actual contamination from the blood flushed through a new collection set was detected, however, when this was tried. In both methods of drawing blood it is important that the blood be shaken gently while the sample is being taken, as well as afterwards to ensure mixing of the anticoagulant or else difficulties may arise because of clotting. The samples are left in the transfusion bottles and are stored in a refrigerator until analyzed. Aliquots of the samples are measured in appropriately sized graduated cylinders and are transferred to silica dishes for ashing. Where separation of cells and serum is desired, this is done by centrifuging the fresh blood samples in a small polyethylene bottle and taking off the fractions with a syringe of the same type as was used for drawing small samples.

Table I. Recovery of Cobalt-60 in Blood Samples Dry Ashed at 450° C.

| Compound Added | Co^{60} Added, mγ | Activity of Co^{60} | | Recovery, % | Probable Error, % |
		Counts added	Counts recovered		
$Co^{60}Cl_2$	6.7	4,370	4,720	108[a]	...
	6.7	4,150	4,148	100	±2.0
	6.7	2,608	2,610	100	±3.0
	13.0	10,080	10,192	101	±1.0
	13.0	9,736	9,800	101	±1.0
	13.0	9,744	9,616	99	±1.0
Vitamin B_{12}	29.0	21,568	21,424	99.2	±0.7
	29.0	21,648	21,688	100.1	±0.7

[a] High result attributed to error in standardization of Geiger counter.

Ashing the Sample. The sample of whole blood, plasma, or cells, is placed in a 100-ml. fused silica dish, which is in turn placed in the evaporation cover shown in Figure 2. A 250-watt infrared lamp placed above the covered dish is used to dry the sample in a current of filtered air, slowly at first, but with increasing intensity after the water has evaporated and the sample appears dry and brittle. The heat of the infrared lamp is continued and is carefully augmented by the heat from a hot plate under the apparatus until the sample assumes a charred appearance. This preliminary charring is necessary to prevent the samples from foaming and spewing out of the dish when heated in the muffle furnace, and is particularly helpful when ashing volumes of sample larger than 50 ml.

The silica dish containing the charred sample is covered with a watch glass and placed in a cool muffle furnace. The temperature is slowly brought up to 450° C. and is maintained there until the sample has ashed completely, which usually takes about 18 hours.

If upon dissolving the ash in the next step appreciable amounts of carbon remain, a filtration is performed through a clean, sintered-glass filter crucible, and the crucible, with the carbon, is returned to the furnace to complete the ashing. The ash remaining on the crucible is then dissolved as part of the next step, by using this filter as the one which removes insoluble chlorides.

Solution of the Ash. The sample ash is treated in the silica dish with 5 ml. of 4M hydrochloric acid. The dish is heated to aid and ensure complete solution of the ash. The volume of

the solution is then reduced to approximately 1 ml. by evaporation under the cover. At this point, 2 ml. of $9M$ hydrochloric acid is added and the resulting solution is allowed to cool. Certain insoluble chloride salts precipitate on cooling (but they proved in tracer studies to contain no detectable amount of the cobalt). The cool supernatant liquid is then decanted through a sintered-glass filter of medium porosity. The silica dish and salt precipitate are rinsed with 1-ml. portions of $9M$ hydrochloric acid, each portion being decanted into, and sucked through the filter before the addition of the next. A total of 7 ml. of the $9M$ hydrochloric acid is used in the rinsing. Usually the salt is free of its original yellow ferric iron color by the end of five rinsings.

Extraction of Excess Iron. Prior to separation of the cobalt on the anion exchange resin, part of the large excess of iron which results when whole blood is ashed must be removed. The $9M$ hydrochloric acid solution is transferred to a 30-ml. separatory funnel, and is extracted with 10-ml. portions of isopropyl ether. One extraction is sufficient for plasma samples; two are necessary for samples of whole blood or cells. The ether phase is most easily removed by suction through a clean capillary tube. The separated acid phase is returned to a beaker and is boiled for 1 or 2 minutes until the solution is clear to remove dissolved ether. The solution is allowed to cool before beginning the next step.

Column Separation. The Dowex 1 resin column is washed with 30 ml. of $0.01M$ hydrochloric acid to remove elements remaining from the previous separation. Then it is washed with 5 ml. of $9M$ hydrochloric acid to condition it for the subsequent sample. The sample solution is transferred to the column reservoir and is allowed to flow through the column at a rate regulated within the limits of 0.75 to 1 ml. per minute. The flow is stopped when the surface of the liquid reaches the glass wool plug. Using the same rate of flow, the column is next washed with 20 ml. of $9M$ hydrochloric acid. This frees the column of the unabsorbed ash constituents, including the alkali metals, alkaline earth metals, nickel, manganese, and other ions. Thirty milliliters of $4M$ hydrochloric acid is added to the reservoir and is allowed to flow through the column at the same rate. The cobalt is eluted by this acid, which is collected in a clean polyethylene cup. The copper, iron, and zinc of the sample remain on the column, and are removed before running the next sample.

Concentration of Cobalt Solution. For spectrochemical analysis nickel is added in the same amount to each sample as an internal standard (1). By measuring the ratio of cobalt to nickel rather than the absolute amount, sample losses and spectrochemical variations are balanced out, and much better precision is obtained. It is at this point that the internal standard

is best added. The polyethylene cup containing the solution of cobalt (plus internal standard) is placed in the cover apparatus and evaporated by heating with an infrared lamp. Precautions must be taken to see that the temperature is not elevated so far as to soften the polyethylene.

When the volume has been reduced to less than 1 ml. the cup is tilted, and a watch is maintained to see that the solution collects into a single drop and that it does not evaporate completely. Since the solution does not wet the surface of the polyethylene, collection of all the dissolved material in the last drop of the evaporating solution is automatic unless the liquid separates in two portions and dries to form more than one final drop. When the volume of the final drop reaches approximately 0.05 ml., the heat is removed and the cup is allowed to cool. The sample is then ready for transfer to the spectrographic electrode. The transfer should not be delayed too long, lest evaporation upon standing carry the sample to dryness. To prevent this, it is suggested that evaporation of the final few milliliters of sample not be made until time permits immediate transfer to the electrode.

RESULTS

The completeness of the removal of nickel from the column was checked by spectrochemical analysis of the eluates when 2.5 γ of nickel and 5.0 mγ of cobalt were separated. More than 10, but not more than 20 ml. of $9M$ acid, were required to free the column from nickel.

In order to determine whether or not losses of cobalt resulted from any of the steps in the method, radioactive cobalt-60 was utilized. It was added as either cobalt chloride or as vitamin B_{12}, of which the cobalt atom was cobalt-60. By adding a known count, passing it through whatever step was being checked, and counting the sample afterwards, the percentage recovery of the cobalt and the losses during that particular step could be quantified. This was done for the ashing procedure, the precipitation of the chlorides and their removal, the evaporation step, the extraction of excess iron, the separation of cobalt on the column, and, finally, the placing of the drop containing the cobalt on the electrode for spectrochemical analysis. The results of these checks showed that 100% recovery was the rule. An example of the data obtained is given by Table I where the recovery in the ashing procedure is listed. The only step in the method where the recovery differed from 100% by more than the probable error of the method was that involving the ion exchange column, as shown by Table II. The loss at this point was about 5% of the total, which had little effect on the over-all method.

In several cases, the method as a whole was checked by adding to blood itself known amounts of radioactive cobalt-60 and measuring the radioactivity of the sample after it had passed through the complete procedure. The results for these analyses are included in Table II. They show the loss due to the ion exchange step. In order to test the possibility of contamination in the method, spectrochemical analyses were carried out qualitatively on large samples of each reagent with negative results, and quantitatively on simulated blood samples to which a known amount of cobalt had been added, with good recoveries. In addition to this, analyzed blood samples were "salted" with known amounts of cobalt, both as cobalt chloride and as vitamin B_{12}, and the recovery of the known amount of cobalt was measured. The data on the salted samples are given in Table III.

The results of the analyses to date of whole human blood and plasma and cell fractions of human blood are summarized in Tables IV and V. The values for cobalt found in the aliquot were corrected both for the amount of cobalt in the reagent blank and for the loss during the Dowex 1 resin column separation. The latter correction was made by dividing the blank corrected value by 0.94, the average efficiency found for the recovery of cobalt-60 by the Dowex 1 column. The concentration of cobalt

Table II. Separation of Cobalt by Dowex 1 as Determined with Cobalt-60

Sample Composition	Co^{60} Added, mγ	Activity of Co^{60}		Recovery, %	Probable Error, %
		Counts added	Counts recovered		
Cobalt alone	6.7	2,208	2,080	94	±3
	13.0	4,960	4,792	97	±2
Blood ash	6.7	2,536	2,560	101	±3
	6.7	2,568	2,512	98	±3
	6.7	2,592	2,472	95	±3
	6.7	2,512	2,272	91	±3
Blood ash^a	6.7	2,608	2,440	94	±3
	13.0	10,024	9,528	95	±1
	29.0	10,824	10,056	93	±1

^{a} Samples were passed through complete procedure before counting and therefore check method as a whole. Other samples check only separation step.

Table III. Recovery of Cobalt Added to Blood Aliquots as Vitamin B_{12} Determined Spectrochemically

Sample	Volume of Aliquot, Ml.	Millimicrograms of Cobalt			
		In aliquot	Added^a	Total	Found
310-C	50	14.5	9.0	23.5	24
	50	14.5	9.0	23.5	22
413	42	8.4	9.0	17.4	18
	42	8.4	18.0	26.4	26
431	50	20.0	27.0	47.0	39
523	50	4.4	9.0	13.4	11

^{a} Added as a solution of crystalline vitamin B_{12} which had been analyzed colorimetrically for cobalt (12).

in parts per billion of the sample was calculated from the average value of cobalt found in the sample aliquots using the values 1.06, 1.02, and 1.09 for the specific gravity of the whole blood, plasma, and cells respectively. These values are too few in number to give an estimate of the average concentrational levels of cobalt in blood. Although extremely low, they are more than enough to account for the vitamin B_{12}–bound cobalt. This is shown by Table VI, which gives estimates of the concentration of the vitamin B_{12}–bound cobalt, based on reported microbiological assays.

DISCUSSION

Solvent extraction has often been applied to separate cobalt from biological material, using dithizone, 1-nitroso-2-naphthol, or other reagents (12). However, the quantities of cobalt which have been successfully extracted are in the microgram rather than the millimicrogram range. In extensive work with dithizone the authors found that at the millimicrogram level major losses invariably occur, making the technique unusable.

SPECTROCHEMICAL ANALYSIS OF MILLIMICRO-GRAM AMOUNTS OF COBALT

Among the methods of determination available for the element cobalt, only spectrochemical analysis proved sensitive enough for the small absolute amount of the metal found in blood samples of routine size and then only after conditions had been found which substantially improved sensitivity. This paper reports the details of the new method which is capable of determining cobalt

Table IV.   Results of Analysis of Whole Human Blood

Sample Number	Volume of Aliquot, Ml.	Cobalt Found on Replicate Analyses, mγ	Average, mγ	Concentration of Cobalt[a], P.P.B.
310-B[b]	75	26, 15, 28	23	0.29
310-C[b]	50	15, 11, 14, 17	14.5	0.27
413	75	17, 15, 13	15	0.19
431	50	17, 16, 27	20	0.38
470	50	5.5	5.5	0.10
471	50	5.7, 8.1	6.9	0.13
482	50	6.6	6.6	0.12
485	50	3.7, 7.3	5.5	0.10
523	50	3.8, 4.9, 4.6	4.4	0.083

[a] Based on average and a specific gravity of 1.06 for whole blood.
[b] Samples from same person; 2 day interval between samples.

Table V.   Results of Analyses of Human Blood Fractions

Sample Number	Blood Fraction	Volume of Aliquot, Ml.	Cobalt Found, mγ	Concentration of Cobalt, P.P.B.
516	Plasma	30	1.9	0.062
	Cells	50	12	0.22
(Total = whole blood)		(80)	(13.9)	(0.16)
523	Plasma	40	3.2	0.083
		40	3.7	
	Cells	59	5.9	0.11
		59	7.7	
(Total = whole blood)		(198)	(20.5)	(0.098)[a]

[a] Compare with sample 523 of Table IV.

Table VI.   Estimates of Cobalt Concentration in Human Blood from Results of Vitamin B_{12} Assays

Blood Fraction	Reference	Test Organism	No. of Detns.	Vitamin B_{12} Reported, P.P.B.		Estimated Cobalt Concentration[a], P.P.B.
				Range	Average	
Whole Blood	(3)	Lactobacillus leichmanii	10	0.6 to 1.4	0.8	0.03 to 0.06
Serum	(11)	Lactobacillus leichmanii	24	0.08 to 0.42	0.2	0.004 to 0.019
Serum	(8)	Euglena gracilis	65	0.100 to 0.700	0.358	0.004 to 0.031

[a] Based on 4.5% cobalt in vitamin B_{12} (2).

from 1 to 500 mγ in amount, using nickel as an internal standard and the direct current arc cathode-layer method of excitation.

APPARATUS AND REAGENTS

Spectrograph. Applied Research Laboratories 2-meter grating spectrograph, modified with exterior optics and ignitor (16).
Excitation Source. Applied Research Laboratories rectifier unit, 0–15 amperes direct current.
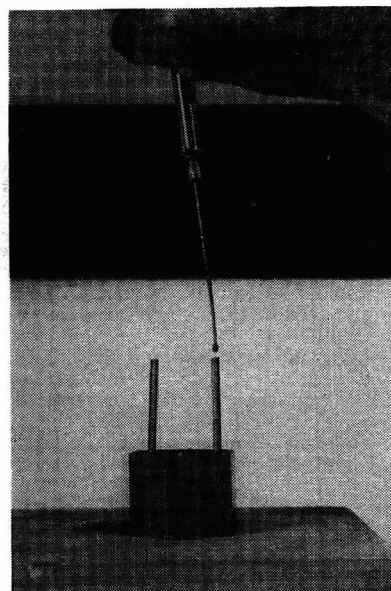**Densitometer.** Applied Research Laboratories film projection comparator-densitometer.

Figure 3.   Placing sample on electrode

Electrodes. National Carbon special spectrographic graphite, $^1/_8$-inch-diameter rod, cut to 1.5-inch lengths. These were used both as the sample holding electrode and the counter electrode. The former were prepared by drilling the $^1/_8$-inch rod to a depth of 3.0 mm. with a $^3/_{32}$-inch drill. The cup thus formed was filled with a mixture of alumina and graphite as a spectrographic base material. The filling was accomplished by pressing the electrode into the mixture to pick some up, then tamping in the mixture with the flat end of a small glass rod to ensure complete filling of the cup.
Graphite-Alumina Base Mixture. Alumina in a high state of purity was prepared by dissolving reagent grade aluminum nitrate (hydrated) in twice distilled constant boiling hydrochloric acid, then passing this solution through a column of Dowex 1 anion exchange resin, and precipitating aluminum hydroxide from the effluent by adding redistilled ammonium hydroxide. The aluminum hydroxide was washed several times with hot triply distilled water and ignited to the oxide in a muffle furnace. Equal weights of this alumina and the SP-2 grade of National Carbon special spectrographic graphite powder were mixed thoroughly by grinding in a mullite mortar.
Standard Cobalt Solution. A 0.500-gram sample of high purity cobalt metal (Johnson Matthey and Co.) was dissolved in 20 ml. of 6M hydrochloric acid and was diluted to 100 ml. with triply distilled water.

PROCEDURE

The cobalt to be determined (along with 0.25 γ of nickel, as nickel chloride which is the internal standard) is concentrated into a small volume of constant boiling hydrochloric acid solution, approximately

Table VII. Determination of Intensity Ratios for Cobalt Standards

Amount of Cobalt, mγ	1	2	3	4	5	Average Value	Standard Deviation
0	0.030	0.030	0.028	(0.090)[a]	0.030	0.029	0.002
0.50	0.13	0.11	0.12	0.08	0.09	0.11	0.021
1.50	0.12	0.09	0.16	0.18	0.13	0.14	0.035
5.00	0.38	0.37	0.39	0.38	0.40	0.38	0.012
15.0	1.33	1.25	1.22	1.10	1.12	1.20	0.095
50.0	4.45	4.02	4.44	4.98 ·	4.80	4.54	0.37
150.	13.5	12.2	12.2	11.9	10.1	12.0	1.2
500.	27.4	28.0	21.5	24.8	(40.0)[a]	25.4	3.0

[a] Rejected with 90% surety (4).

Table VIII. Effect of Graphite–Alumina Base on Precision of Intensity Ratios for Two Line-Pairs

	Line-Pairs	
	Co 3453.5 / Ni 3452.9	Co 3453.5 / Ni 3446.9
	St. Dev., Expressed as % of Results	
With base	5	6
Without base	15	13
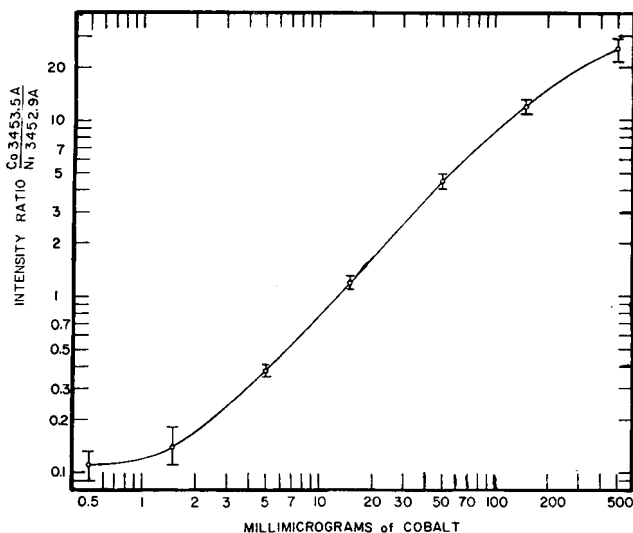
Figure 4. Working curve

0.05 ml. This small volume is transferred to, and evaporated upon, the end of an electrode. To facilitate the evaporation, the electrode is supported in a soapstone block which is in turn heated by a hot plate. Figure 3 shows this arrangement as well as that of a 1-ml. tuberculin syringe, fitted with a micropipet, which is used in effecting the transfer. Micropipets made from polyethylene tubing resist wetting by the solution and permit much more efficient transfer of the small volume. After loading, the electrodes are dried for at least 1 hour at 110° C.

The dry sample electrode is placed in the lower electrode holder of the arc stand and is made cathode. It is positioned so that the light from 1 mm. of the electrode tip and 3 mm. of the adjacent arc column falls upon the grating of the spectrograph. The counter electrode is adjusted to give a 10-mm. gap, and the slit is opened to 60 microns. After opening the camera shutter, the arc, preset for 14-ampere current and 3-second operation, is started by means of the ignitor. Exposures are for the full period of the arcing.

The region of the spectrum including the area from 6800 to 7000 A. (3400 to 3500 A. second order) is photographed on Spectrum Analysis No. 2 film. The exposed film is then processed.

Developer. Eastman Kodak D 19. Five-minute development, at 70° F.

Short Stop. Four and one half milliliters of glacial acetic acid in 350 ml. of water. Fifteen-second duration.

Fixer. Eastman Kodak rapid liquid fixer. Five-minute duration.

Water Wash. Fifteen-minute duration.

Drying. In air.

The processed film is placed in the densitometer and the per cent transmittance, based on a clear film reading of 100%, is obtained for the cobalt line at 3453.5 A., the nickel line 3452.9 A., and the weakest background between these two lines. The nickel line at 3446.9 A. is also read if the 3452.9 A. line is weak.

The intensity ratio of the cobalt and nickel lines (background corrected) is next obtained by applying the per cent transmittance values to a film calibration curve (5). If the intensity ratio thus obtained is for a sample of unknown cobalt content, it is applied to the working curve to find the amount of cobalt in millimicrograms in the sample.
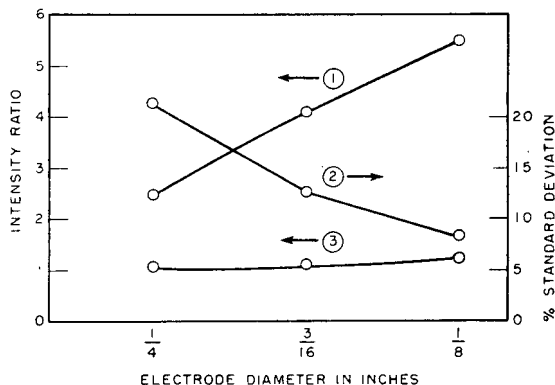
STANDARDS

Suitable dilutions were made of the standard cobalt and nickel solutions to give solutions with cobalt concentrations of 0.01, 0.03, 1.0, 3.0, and 10.0 p.p.m. and a constant nickel concentration of 5.0 p.p.m. These dilutions were made in 4M hydrochloric acid. By evaporating 0.05 ml. of these solutions upon the electrodes, replicate electrodes were obtained which held from 0.5 to 500 mγ of cobalt. By treating these according to the spectrochemical procedure intensity ratios were obtained for cobalt 3453.5 A. to nickel 3452.9 A. with backgrounds subtracted.

The results of five such replicates are shown in Table VII along with the average value and an estimate of the standard deviation for each concentration level. The average value of the intensity ratio for each of the standards was plotted against the amount of cobalt on the electrode, using logarithmic coordinates. This plot constitutes the working curve, shown in Figure 4. The standard deviation of the values obtained at each concentration is shown in the working curve as a vertical line above and below the average value.

DISCUSSION

The method proved to be sensitive enough to determine as little as 1 mγ and to have an over-all precision (expressed as a percentage standard deviation) of less than 10% in the usual working range. In order to achieve this sensitivity, which surpasses that of any other available method by a factor of at least 30 (13), it was necessary to establish the optimum conditions with respect to every possible variable.

Figure 5. Effect of electrode diameter on

1. Sensitivity
2. Precision
3. Element to internal standard ratio

Matrix. It was found by a trial of various matrices including pure graphite, and calcium, lithium, and sodium carbonates, that alumina, Al_2O_3, when mixed with graphite, provided a base which gave the highest sensitivity for cobalt of any applicable materials. This is in agreement with the work of Scott (14). Because of the work of Wolff (18), a matrix of sodium chloride was investigated. It proved to be inferior to alumina, because, under conditions where precision was adequate, the presence of sodium decreased rather than increased the sensitivity of the method.

It is common practice to use a pure salt in the electrode as a buffer to increase the precision of spectrochemical methods. Experiments were therefore performed using replicate determinations of 50-mγ samples of cobalt to compare the precision obtained with the alumina–graphite base to that obtained when the graphite electrode alone was used. Table VIII gives the percentage standard deviation of the results and shows that the base acted as a buffer to provide substantially better precision.

Electrode Diameter and Cup Depth. A two-factor experiment was performed to test the effect of electrode diameter and cup depth on the sensitivity of the determination and also on its precision. The experiment involved replicate determinations of 50-mγ samples on electrodes $1/8$, $3/16$, and $1/4$ inch in diameter with 1-, 4-, 7-, and 10-mm. depths of alumina-graphite packing. The former proved to be a very significant factor and the data are shown in Figure 5. The best precision, as well as the best sensitivity, can be achieved by using electrodes $1/8$ inch in diameter. Attempts to use smaller electrodes failed because of practical difficulties in handling them. Cup depth proved not to be significant, and a value of 3.0 mm. was chosen arbitrarily.
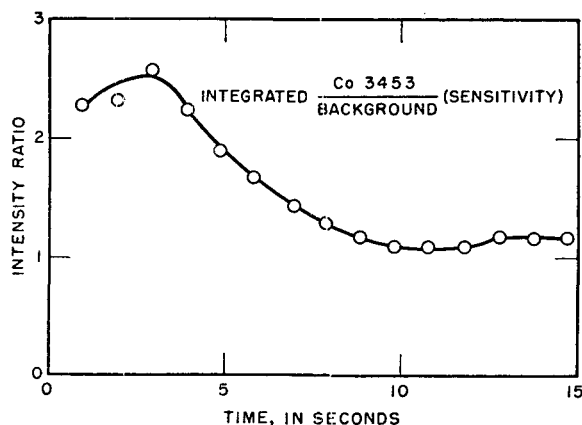

Figure 6.   Effect of exposure time on sensitivity

The experiment also indicated that nickel is a good internal standard for cobalt, because the variation of the intensity ratio of the cobalt to nickel lines is very small, whereas the individual lines vary markedly with electrode diameter. This is also shown in Figure 5.

Exposure Time and Amperage. The optimum amperage and exposure time for maximum sensitivity were sought. Replicate determinations on 50-mγ samples were made and the ratio of the cobalt line intensity to the intensity of the adjacent background was taken as a measure of relative sensitivity.

By "moving-film" studies of volatilization rates, it was found that the two factors in question were so related that at any selected amperage, maximum sensitivity could be achieved when the exposure time multiplied by the current was equal to 40-ampere seconds. Figure 6 illustrates this for 14-ampere current. It shows, as ordinates, the line to background ratio that would be obtained by terminating the exposure at the times given in the abscissas. Utilizing this relation, the data of Figure 7 were obtained, which show that sensitivity increases with amperage, but that the ratio of line to internal standard is relatively unaffected. Although Figure 7 makes information on higher currents appear desirable, it could not be obtained with the available apparatus. The conditions were therefore set at 14 amperes and 3.0 seconds.

These exposure conditions do not necessarily volatilize all of the cobalt from the electrode. (In fact, if more than 50 mγ

of cobalt are present on the electrode a duplicate spectrum can be taken on the same electrodes with good results.) They were set up as described to obtain maximum sensitivity. The ratio of the cobalt to nickel lines was not affected by variations in exposure time up to about 10 seconds, but the sensitivity was, as is shown by Figure 6.
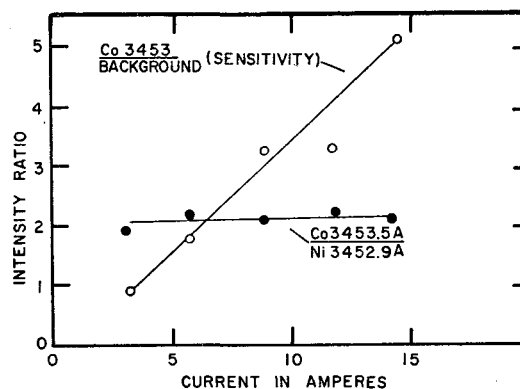

Figure 7.   Effect of current on sensitivity and intensity ratio at constant ampere seconds

Internal Standard. Nickel was chosen as the internal standard for the following reasons. The nickel line at 3452.9 A. is suitably close to the cobalt line at 3453.5 A. and in addition another nickel line at 3446.9 is available. The latter nickel line is somewhat stronger than the former one and acts similarly with respect to variations in the source conditions. The two lines were therefore used interchangeably (with appropriate working curves) depending on the spectrum strength. The precision was the same in both cases. The nickel and cobalt presumably exist on the electrode as dichlorides, and the boiling points of the two compounds are very similar, that of nickel dichloride being 973°C. and of cobalt dichloride being 1049°C. The excitation potentials of the three lines in question are cobalt 3453.5, 4.0 e.v., nickel 3446.9, 3.7 e.v., and nickel 3452.9, 3.7 e.v. Finally, chemical changes occurring in the arc should be similar for the two elements since they are similar in chemical properties.

TESTS OF RECOVERIES

The cobalt was concentrated from samples of human blood and determined by the method described. To known aliquots of the blood thus analyzed, known amounts of cobalt were added as a solution of vitamin B_{12}, the cobalt content of which had been determined colorimetrically. These salted samples were then carried through the analytical procedure. The results of these determinations are given in Table III. Their consistency is evidence for the validity of the method.

INTERFERENCES

The method requires that the cobalt be separated completely from nickel, the internal standard element, and as completely as possible from other elements. Complete separation from nickel means that the nickel remaining must be less than 10% of the amount to be added as internal standard, or 25 mγ. Complete separation from sodium or iron, the two elements from biological samples most likely to cause trouble, means that less than about 25 γ of either must be present. While this is a very small absolute amount, the specificity of the spectrographic method is illustrated by the fact that this represents up to 25,000 times as much sodium or iron as the cobalt being determined.

LITERATURE CITED

(1) Ahrens, L. H., "Spectrochemical Analysis," p. 81, Addison-Wesley, Cambridge, Mass., 1950.
(2) Brink, N. G., Wolf, D. E., Kaczka, E., Rickes, R. L., Koniuszy, F. R.; Wood, T. R., and Folkers, K., J. Am. Chem. Soc., 71, 1854–6 (1949).
(3) Couch, J. R., Witten, P. W., and Colby, R. W., Am. J. Physiol., 163, 77 (1950).
(4) Dean, R. B., and Dixon, W. J., ANAL. CHEM., 23, 636–8 (1951).
(5) Harvey, C. E., "Spectrochemical Methods," pp. 238, 274 ff., Applied Research Laboratories, Glendale, Calif., 1950.
(6) Kraus, K. A., and Moore, G. E., J. Am. Chem. Soc., 75, 1460–2 (1953).
(7) Mitchell, R. L., Commonwealth Bureau of Soil Science, Harpenden, England, Technical Communication No. 44, 121–7 (1948).
(8) Mollin, D. L., and Ross, O. I. M., J. Clin. Pathol., 5, 129 (1952).
(9) Moore, G. E., and Kraus, K. A., J. Am. Chem. Soc., 74, 843 (1952).
(10) Rickes, R. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., and Folkers, K., Science, 108, 134 (1948).
(11) Rosenthal, N. L., and Sarrett, H. P., J. Biol. Chem., 199, 433 (1952).
(12) Sandell, E. B., "Colorimetric Determination of Traces of Metals," 2nd ed., p. 271 ff, Interscience, New York, 1950.
(13) Scott, R. O., J. Soc. Chem. Ind. (London), 64, 189–94 (1945).
(14) Scott, R. O., and Mitchell, R. L., Ibid., 62, 4–8 (1943).
(15) Smith, E. L., Nature, 162, 144–5 (1948).
(16) Thiers, R. E., and Yoe, J. H., Soc. Appl. Spectroscopy Bull., 5, 8–12 (1951).
(17) Weissbecker, L., Klin. Wochschr., 25, 855 (1947).
(18) Wolff, H., Ibid., 28, 280 (1950).

Ultraviolet Spectrophotometric Determination of Cobalt

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An ultraviolet spectrophotometric study has been made of two classical methods for determining cobalt. An investigation of the thiocyanate and 1-nitroso-2-naphthol methods has shown that increased sensitivity can be obtained for both methods, provided that absorbance measurements are made in the ultraviolet region.

THE only ultraviolet spectrophotometric method for determining cobalt reported thus far in the chemical literature involves the use of hydrogen peroxide and sodium bicarbonate to give a cobalt complex exhibiting an absorbance maximum at 260 mμ (3). This method has serious limitations because so many diverse ions precipitate in the basic solution used.

Therefore, an ultraviolet spectrophotometric study has been made of the thiocyanate and 1-nitroso-2-naphthol methods for determining cobalt. This study has shown that increased sensitivity can be obtained for both of these classical methods for determining cobalt if the absorbance measurements are made in the ultraviolet region.

APPARATUS AND SOLUTIONS

Apparatus. The absorbance measurements were made with a Beckman Model DU spectrophotometer equipped with an ultraviolet accessory set and 1.000-cm. silica absorption cells. The reference cells contained a reagent blank solution unless otherwise stated. The hydrogen discharge lamp was used for measurements from 220 to 400 mμ and the tungsten lamp for the 400- to 700-mμ region. The pH measurements were made with a Beckman Model H pH meter equipped with a glass electrode.

Solutions. THIOCYANATE METHOD. Standard Cobaltous Sulfate. Dissolve 0.3350 gram of cobaltous ammonium sulfate hexahydrate in redistilled water and dilute to 500 ml. One milliliter of this solution contains 0.100 mg. of cobalt.

Ammonium Thiocyanate (33%). Dissolve 125 grams of reagent grade ammonium thiocyanate in 250 ml. of redistilled water.

Isoamyl Alcohol (ACS specifications). Saturate with ammonium thiocyanate and remove excess reagent by filtering through a fritted-glass funnel.

Diverse ions. Prepared from reagent grade chemicals.

1-NITROSO-2-NAPHTHOL METHOD. Ammonium citrate. Dissolve 250 grams of citric acid in 60 ml. of water and neutralize with 250 ml. of ammonium hydroxide (specific gravity 0.9).

1-Nitroso-2-naphthol (Eastman, practical grade). Prepare by boiling 1 gram of the reagent in 200 ml. of water containing 10 ml. of 5N sodium hydroxide. Cool solution, filter, and dilute to 1 liter.

Hydrochloric Acid, 5N. Prepare using redistilled water and reagent grade hydrochloric acid.

THIOCYANATE METHOD

One of the classical colorimetric methods for determining cobalt has been the thiocyanate method. Although this color reaction of cobalt with thiocyanate was known before Vogel's time, he is generally given credit for its discovery. Since 1900 there have been numerous papers related to the use of this reaction in analytical chemistry. There are two common variations in the procedure used for determining cobalt colorimetrically by the thiocyanate method. According to one procedure acetone is added to prevent dissociation of the cobalt thiocyanate complex, thus serving to intensify the color normally obtained in aqueous solution. The other procedure involves extraction of the cobalt thiocyanate complex with isoamyl alcohol. The blue color of the cobalt thiocyanate complex has been measured spectrophotometrically at its absorbance maximum at about 620 mμ.

This part of the investigation was concerned with studying the ultraviolet absorption spectrum of the cobalt thiocyanate complex and the effect of certain solution variables upon the absorbing system.

General Experimental Procedure. A definite amount of the standard cobaltous sulfate solution was transferred to a 50-ml. volumetric flask. After the desired amount of 33% ammonium thiocyanate solution was added, the solution was diluted to 50 ml. with redistilled water. The complexed cobalt was extracted from the water solution with an isoamyl alcohol solution which had been previously saturated with ammonium thiocyanate. The extracts were added to a 50-ml. volumetric flask. The extract was diluted to the graduation mark with isoamyl alcohol–thiocyanate reagent and the contents of the flask were thoroughly mixed. The absorbance measurements were made immediately using the extractant in the reference cell. The solutions were sufficiently stable to permit the necessary absorbance measurements to be made. During the study of the effect of diverse ions, all ions were added before complexation.

Effect of Cobalt Concentration. The absorption spectrum of the cobalt thiocyanate complex for various concentrations of

cobalt was studied. Figure 1 shows the comparison of the ultraviolet absorbance maximum at 312 mµ with the small absorbance maximum at 620 mµ. The concentration of cobalt was 6 p.p.m. Conformity to Beer's law was found at 312 mµ for concentrations from 0.2 to 10 p.p.m. of cobalt.

Effect of Thiocyanate Concentration. The effect of variable amounts of ammonium thiocyanate was determined using 6 p.p.m. of cobalt. The absorbance of each solution was measured at 312 mµ after extraction. Any amount of thiocyanate solution added exceeding 40 ml. did not appreciably change the absorbance. In order to eliminate adding such a large volume of reagent it is recommended that the concentration of ammonium thiocyanate be increased to 44%. It was found that 25 ml. of a 44% ammonium thiocyanate reagent gave maximum absorbance measurements.

Effect of Acidity. The effect of acidity using 6 p.p.m. of cobalt was studied by varying the pH of the final aqueous solution from 1.6 to 7.0. A dilute solution of perchloric acid was used to decrease the pH and a dilute solution of ammonium hydroxide was used to increase the pH. The pH was read before the extraction with isoamyl alcohol. Maximum absorbance was obtained for solutions having a pH of 1.6 to 1.9. Because absorbance readings at this pH were not very reproducible and because the absorbance decreases such a small amount from the range 1.6 to 1.9 to the range 3.0 to 5.3, this latter pH range is recommended. The use of hydrochloric acid and sulfuric acid does not give as reproducible results, possibly due to traces of iron(III), and is not recommended.

Effect of Diverse Ions. The effect of diverse ions was studied using 1000 p.p.m. of the ion and 6 p.p.m. of cobalt, complexing, adjusting the pH to 5.0, extracting, and reading the absorbance at a wave length of 312 mµ. Successively smaller amounts of the diverse ion were added until a deviation of 3% or less was recorded. Ions which were expected to interfere were added in lesser amounts for the original reading. The reference cell contained the extractant. A negligible error was obtained with 1000 p.p.m. of acetate, arsenate, ammonium, cadmium, chloride, calcium, magnesium, nitrate, potassium, sodium, and sulfate. Table I list those ions which were found to interfere.

Extraction. The cobalt thiocyanate complex was extracted with isoamyl alcohol saturated with ammonium thiocyanate. Without extraction the cobalt complex ion does not exhibit an absorbance maximum in the ultraviolet region of the spectrum. The isoamyl alcohol was saturated to ensure complete color formation and to increase the stability of the extracted complex. This effect was studied by comparing the absorbance measurements of solutions extracted with isoamyl alcohol and with isoamyl alcohol saturated with ammonium thiocyanate. In order to ensure constant absorbance for the extractant, it is necessary to filter the excess ammonium thiocyanate from the isoamyl alcohol. The effect of the number of extractions was
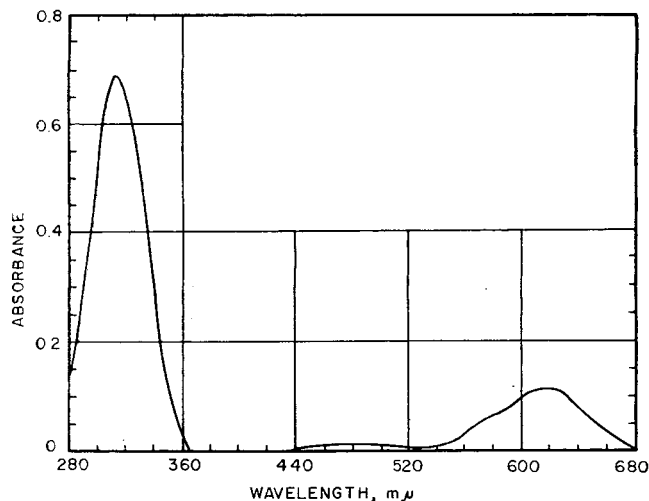

Figure 1.   Absorption spectrum of cobalt thiocyanate complex

6 p.p.m. of cobalt

studied and it was found that two 20-ml. extractions were just as satisfactory as were four 10-ml. extractions, or two 10-ml. and one 20-ml. extractions. It is recommended that all isoamyl alcohol used for extracting purposes, regardless of manufacturer or purity, be tested for 100% transmittance at 312 mµ with water in the reference cell.

Stability. The stability of the extracted solutions was studied by treating a solution containing 4 p.p.m. of cobalt in the usual manner. An absorbance reading at 312 mµ was taken immediately after extraction. Other readings were taken after increasing intervals of time. The last measurement was read after 24 hours and this absorbance value agreed with the original reading within the range of experimental error. It was concluded that the extracted solution was stable long enough for ordinary absorbance measurements.

1-NITROSO-2-NAPHTHOL METHOD

Cobaltous ions in a slightly acidic citrate-buffered solution when treated with 1-nitroso-2-naphthol solution give a precipitate of cobalt-1-nitroso-2-naphtholate. This complex is soluble in chloroform, producing a yellow orange solution (2). A procedure for determining cobalt photometrically using the colored extract of a filtered and dried cobalt-1-nitroso-2-naphtholate precipitate was developed by Waldbauer and Ward (4). A study of the ultraviolet spectrum of the extracted compound revealed an absorbance maximum in the ultraviolet region. The possibility of extracting the precipitate from the solution without filtering and the elimination of the interference of iron was also investigated.

General Experimental Procedure. A definite amount of the standard cobalt solution was added to 10 ml. of the ammonium citrate reagent and 10 ml. of water in a conical flask. The mixture was heated to boiling and 10 ml. of the 1-nitroso-2-naphthol reagent were added. The solution was cooled and allowed to stand for 2 hours. The precipitate was extracted three times with 30 ml. of chloroform. The final volume was adjusted with chloroform to 100 ml. and the absorbance measurements were made on the chloroform extract in the ultraviolet region.

Effect of Cobalt Concentration. The absorption spectra for various concentrations of cobalt were determined and conformity to Beer's law was found at 317 mµ in concentrations from 0.2 to 2 p.p.m. of cobalt. Figure 2 shows the characteristic absorbance maxima obtained. The discontinuity of the curve in the 365 to 400 mµ region is due to the high absorbance of the blank and the limited sensitivity of the spectrophotometer.

Table I.   Effect of Diverse Ions

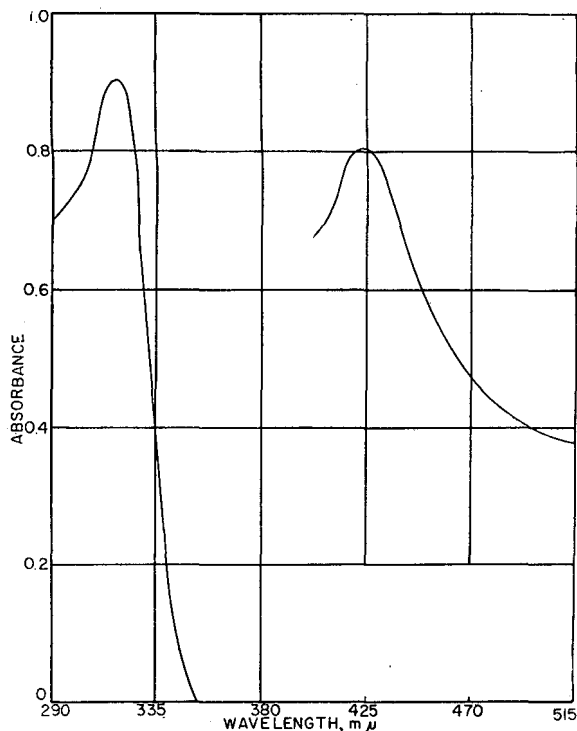| Ion	Added As	Added, P.P.M.	Error, %	Permissible Amount, P.P.M.
		(Thiocyanate method)		
VO3-	NH4VO3	10	1.7	10
MoO4--	Na2MoO4	1000	- 3.8	100
Fe+++	Fe(NO3)3	10	34.0	0
UO2++	UO2(C2H3O2)2	10	23.5	0
Cr+++	CrCl3	10	65.3	0
Cu-+	CuCl2	10	1.6	10
Al-++.	Al(NO3)3	1000	9.3	100
Mn++	MnCl2	100	3.5	75
Pb++	Pb(NO3)2	100	8.9	25
Fe(CN)6-4	K4Fe(CN)6	10	34.5	0
Ni++	NiCl2	10	4.4	5
Cr2O7--	K2Cr2O7	10	39.7	0
IO3-	KIO3	50	1.2	50
SnO3--	Na2SnO2	100	-16.6	0
Zn++	Zn(ClO4)2	100	1.9	100
HC6H6O7--	(NH4)2HC6H5O7	100	-14.6	0
HC4H4O6-	NH4HC4H4O6	100	2.9	100
Ti+4	Ti(SO4)2	20	- 3.2	0

Figure 2. Absorption spectrum of tris-(-nitroso-naphtholo)-cobalt(III)

2 p.p.m. of cobalt

Effect of Acid Concentration. The effect of various concentrations of hydrochloric acid was determined using 2 p.p.m. of cobalt, 10 ml. of ammonium citrate, and 10 ml. of water with 0, 1, 2, and 5 ml. of 5N hydrochloric acid and 10 ml. of precipitant. It was found that the absorbance of the extracted complex decreased rapidly with increased acidity. A maximum in absorbance was obtained when no acid was added, or the solution had a pH of about 5.1. The pH should be adjusted within the range 4.0 to 5.5.

Effect of Digestion Time on Precipitate Formation. After the precipitate was formed it was allowed to stand for 30 minutes, 1 hour, 2 hours, and 3 hours, respectively, before extraction with chloroform. It was found that the absorbance increased with longer periods of digestion before extraction until the digestion time of 2 hours was used. Increasing the time of digestion from 2 to 3 hours increased the absorbance inappreciably. Therefore, the 2-hour standing period was adopted as a satisfactory period for digestion of the precipitate.

Stability of Complex. The absorbance was measured over a 24-hour period and was found to be constant.

Effect of Diverse Ions. The effect of diverse ions was studied using 1 p.p.m. of cobalt. Absorbance readings were taken at 317 mμ. A change of 3% or less was considered negligible. A negligible error was obtained with 500 p.p.m. of chloride, nitrate, perchlorate, sulfate, and tungsten. A negligible error was also obtained with 200 p.p.m. of aluminum, cadmium, chromium, molybdenum, and zinc. Table II lists the interfering ions.

Table II. Interfering Ions

(1-nitroso-2-naphthol method) *

Element	Added as	Amount Added, P.P.M.	Error, %	Permissible Amount, P.P.M.
Cu	CuSO₄	20	52	0
Fe	Fe₂(SO₄)₃(NH₄)₂SO₄	5	45	0
Mn	MnSO₄	200	3	150
Ni	Ni(NO₃)₂	50	0	50
Pb	Pb(NO₃)₂	200	10	50
Sn	SnCl₂	50	7	10
Ti	K₂TiO(C₂O₄)₂	20	6	5
V	Na₃VO₄	200	18	20

Removal of Ferric Ions Prior to Spectrophotometric Determination of Cobalt. Inasmuch as 5 p.p.m. of ferric ions produced an error of 45% in the ultraviolet spectrophotometric determination of 1 p.p.m. of cobalt an effort was made to circumvent this interference. Lundell and Hoffman have shown that ferric ions can be removed by extraction with ether (1). It was found that appreciable amounts of iron could be removed using the following procedure.

Place the sample containing cobaltous and ferric ions in a separatory funnel. Add an equal volume of an ether solution which was prepared by shaking 100 ml. of diethyl ether twice with 100 ml. of 6N hydrochloric acid. After shaking, the aqueous layer is allowed to separate. The aqueous layer is extracted again with the ether solution. The aqueous layer is boiled to remove traces of ether. The precipitation of the tris-(-1-nitroso-2-naphtholo)cobalt(III) is performed using the general experimental procedure.

One part per million of cobalt was determined in samples containing 5, 10, and 20 p.p.m. of ferric ions with a negligible error (less than 2.5%). Thus, a preliminary extraction with ether is effective in removing the iron from the cobalt.

LITERATURE CITED

(1) Hoffman, J. I., and Lundell, G. E. F., Bur. Standards J. Research, 8, 659 (1932).
(2) Sandell, E. B., "Colorimetric Determination of Traces of Metals," 2nd. ed., p. 273, Interscience, New York, 1950.
(3) Telep, G., and Boltz, D. F., Anal. Chem., 24, 945 (1952).
(4) Waldbauer, L., and Ward, N. M., Ind. Eng. Chem., Anal. Ed., 14, 727 (1942).
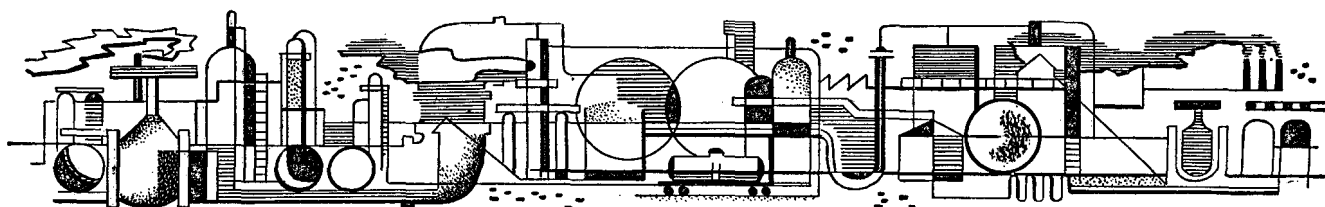
Infrared Spectra of 3-Phenyl-2-thiohydantoins of Amino Acids and Their Application to Identification of N-Terminal Groups in Peptides

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Infrared spectra of the phenylthiohydantoins of 22 amino acids are recorded. A modified method has been used for preparing the phenylthiohydantoins of asparagine and glutamine. Experiments with five peptides demonstrate the usefulness of infrared spectra for positive identification of N-terminal amino acid residues.

THE thiohydantoins and phenylthiohydantoins of amino acids recently have assumed importance in amino acid sequence studies on peptides and proteins. Schlack and Kumpf (14) identify C-terminal amino acids in proteins as the 2-thiohydantoins. The determination of amino acid sequence in peptides and proteins by the method of Edman (3) involves characterization of the residues through the 3-phenyl-2-thiohydantoins. Methods have been developed for their chromatographic separation and identification, on specially treated papers (10, 15). The molecular extinction coefficients for the thiohydantoins in the region of 270 mμ are of considerable value in their quantitative determination, but the spectra of these compounds in the ultraviolet region are of little use for their identification (5).

This communication presents the infrared spectra of the 3-phenyl-2-thiohydantoins of 22 amino acids. A characteristic spectrum was obtained for each compound tested. The amino acids from which these compounds arose, therefore, could be identified positively. The N-terminal residues in five synthetic peptides were identified correctly by use of the above spectra.

EXPERIMENTAL METHODS AND RESULTS

Materials. PREPARATION OF 3-PHENYL-2-THIOHYDANTOINS OF AMINO ACIDS. The 3-phenyl-2-thiohydantoins of glycine, alanine, leucine, isoleucine, valine, methionine, proline, hydroxyproline, tryptophan, tyrosine, phenylalanine, 3,4-dihydroxyphenylalanine (not previously described), aspartic acid, glutamic acid, lysine, arginine, and histidine were prepared according to the method of Edman (2). Each compound was crystalline, and had a melting point in satisfactory agreement with that reported by Edman (2). It was not possible, however, to remove the yellow color associated with the derivative of tryptophan by repeated recrystallization from glacial acetic acid and water. The use of Edman's method for preparing the phenylthiohydantoin of asparagine yielded only the derivative of aspartic acid.

The identity of the compound was proved by melting point (1, 2), mixed melting point, and comparison of infrared absorption spectra. This indicated that the amide group of the phenylthiocarbamyl asparagine had been hydrolyzed during the conversion to the phenylthiohydantoin. By using milder conditions for this reaction, the phenylthiohydantoin of asparagine and also of glutamine was obtained easily. To assure preservation of the amide group, the procedure was modified as follows:
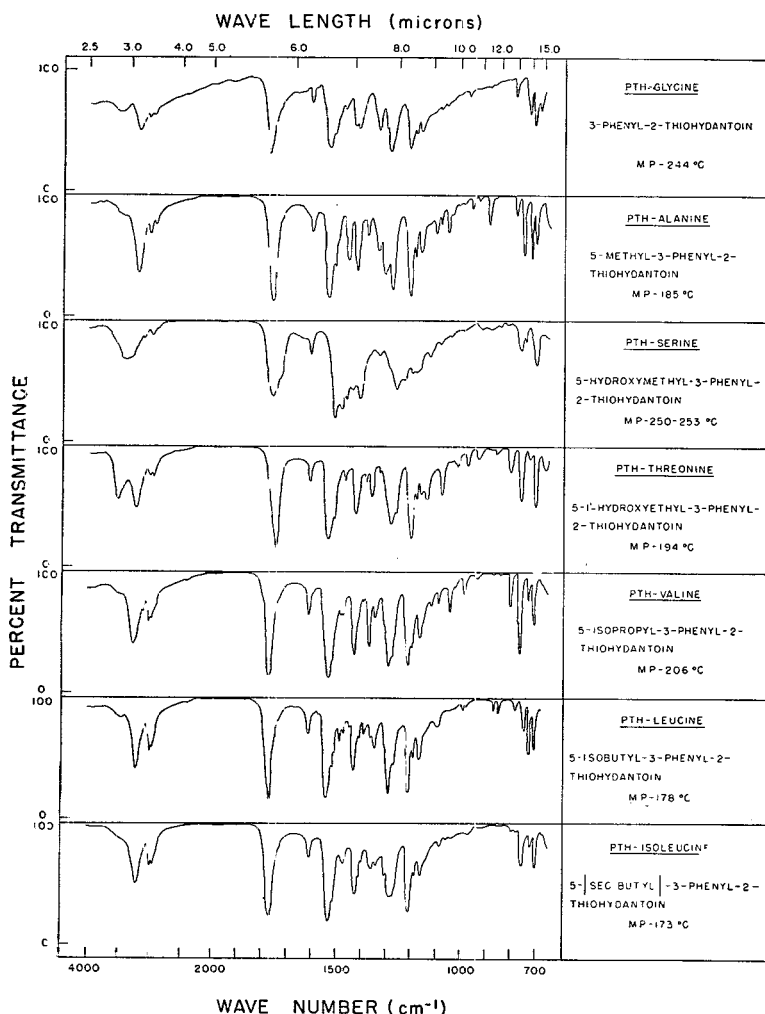

WAVE LENGTH (microns)

WAVE NUMBER (cm⁻¹)

Figure 1. Infrared spectra of phenylthiohydantoins

Crystalline state

The reaction mixture containing the crude N-phenylthiocarbamyl derivative of asparagine was extracted repeatedly with benzene. It was then adjusted to pH 1 with 2N hydrochloric acid and allowed to stand at room temperature for 2 days. The crystalline material which had settled out was collected by filtration, after chilling, washed with water, and recrystallized from ethyl alcohol and water. The phenylthiohydantoin of asparagine so obtained had a melting point of 239° C. (found: carbon, 52.87; hydrogen, 4.448; nitrogen, 16.79; calculated: carbon, 53.01; hydrogen, 4.450; nitrogen, 16.87), and displayed the infrared absorption bands characteristic of the —CO—NH₂ grouping (two bands in the 3200- to 3400-cm.⁻¹ region and one other near 1660 cm.⁻¹). The phenylthiohydantoin of glutamine had a melting point of 218° C. (found: carbon, 54.88; hydrogen, 4.980; nitrogen, 15.85; calculated: carbon, 54.75; hydrogen, 4.978; nitrogen, 15.97).

Since the completion of this work the authors' attention has been drawn to a recent review (6) in which a general method using milder conditions for ring closure is advocated for the preparation of the phenylthiohydantoins of amino acids. The melting points of the derivatives of asparagine and glutamine were reported as 234° and 193° C., respectively. No elementary analyses of the compounds were given, and it is not clear whether the melting point tabulated for the phenylthiohydantoin of asparagine was obtained on a preparation by the method suggested, or was that found by Edman (2).

Phenylthiohydantoins of the hydroxyamino acids were prepared according to the method of Ingram (8). The phenylthiohydantoin of threonine (2-amino-3-hydroxybutyric acid) obtained had the reported physical constants. Difficulty was encountered in preparing the pure phenylthiohydantoin of serine (2-amino-3-hydroxy propionic acid). Different samples possessed widely different melting points. Some insoluble amorphous material separated during attempts to recrystallize the derivative from ethyl alcohol–water mixtures (5).

The phenylthiohydantoin of hydroxylysine, 5-[3 - hydroxy - 4 - (β - phenylthioureido)butyl] - 3-phenyl-2-thiohydantoin—not previously described—was prepared from a racemic sample of hydroxylysine isolated from gelatin (11). The derivative melted at 119° C. (found: carbon, 57.93%; hydrogen, 5.35%; calculated: carbon, 57.60%; hydrogen, 5.38%).

Instrumental. MEASUREMENT OF INFRARED SPECTRA. The infrared spectra were recorded with a Perkin-Elmer, Model 21 infrared spec-

trophotometer equipped with a rock salt prism. The compounds were mounted in potassium bromide (13, 17). Two methods of sample preparation were applied: The pure crystalline compound (1 mg.) was thoroughly ground in a mortar with 500 mg. of powdered potassium bromide. The compound was first dissolved in a minimum amount of ethyl alcohol (about 0.5 ml.) and then mixed with potassium bromide. The mixture was dried in a vacuum desiccator over phosphorus pentoxide for 2 to 3 hours. Both samples were pressed into windows for infrared recording.

The infrared spectra of the phenylthiohydantoins of the 22 amino acids are given in Figures 1 to 3.

EDMAN DEGRADATION OF SIMPLE PEPTIDES FOR IDENTIFICATION OF N-TERMINAL RESIDUES. Experiments were done to determine whether infrared spectra could be used to identify the N-terminal residues of some peptides through the phenylthiohydantoins. The following peptides were used: DL-alanylglycylglycine, L-leucyl-glycine, DL-phenylalanylglycine, DL-histidylhistidine, and γ-glutamylcysteinylglycine (glutathione). Reaction with phenyl isothiocyanate was carried out in 50% pyridine at pH 8.6. Ring closure and cleavage of the N-terminal residue as the phenylthiohydantoin was effected by anhydrous nitromethane–hydrogen chloride (3); the extract containing the phenylthiohydantoin derivative of the N-terminal amino acid was evaporated to dryness and the material recrystallized for identification. The procedure was applied to 10- to 20-mg. samples of the peptides, alanylglycylglycine, leucylglycine, and phenylalanylglycine. With all three the N-terminal residues were correctly identified, by comparison with the infrared spectra of phenylthiohydantoins of known amino acids. With 2.7 mg.
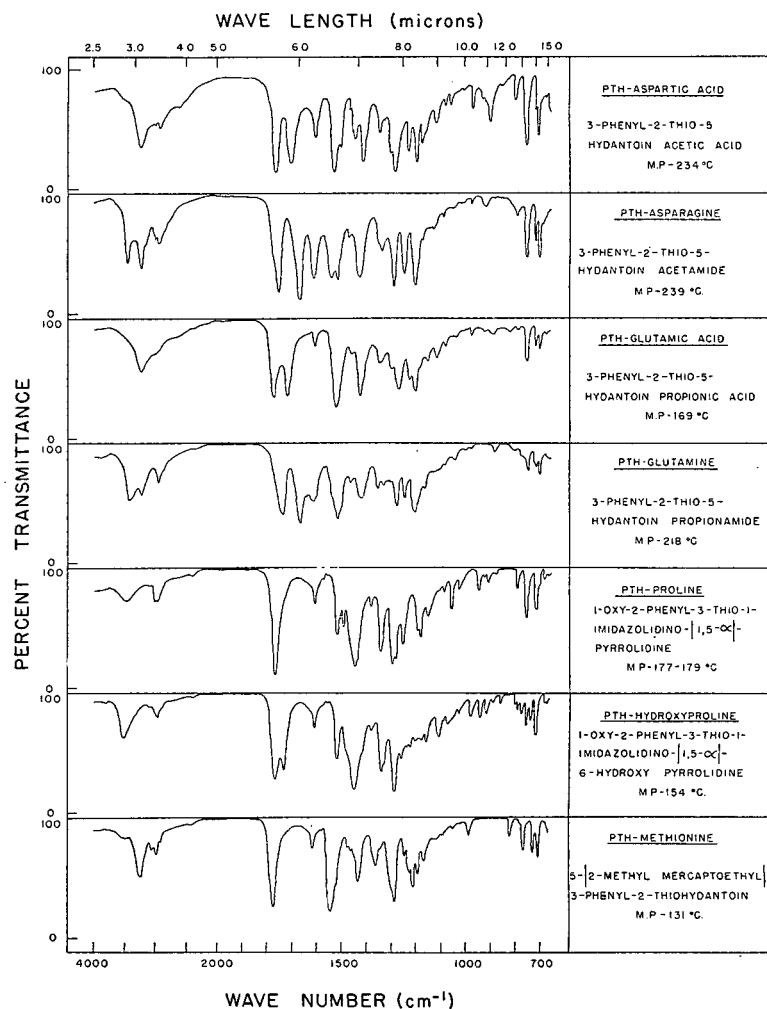
Figure 2. Infrared spectra phenylthiohydantoins

Crystalline state

WAVE LENGTH (microns)

PERCENT TRANSMITTANCE

PTH-ASPARTIC ACID
3-PHENYL-2-THIO-5 HYDANTOIN ACETIC ACID
M.P-234 °C

PTH-ASPARAGINE
3-PHENYL-2-THIO-5- HYDANTOIN ACETAMIDE
M.P-239 °C

PTH-GLUTAMIC ACID
3-PHENYL-2-THIO-5- HYDANTOIN PROPIONIC ACID
M.P-169 °C

PTH-GLUTAMINE
3-PHENYL-2-THIO-5- HYDANTOIN PROPIONAMIDE
M.P-218 °C

PTH-PROLINE
1-OXY-2-PHENYL-3-THIO-1- IMIDAZOLIDINO-|1,5-∝|- PYRROLIDINE
M.P-177-179 °C

PTH-HYDROXYPROLINE
1-OXY-2-PHENYL-3-THIO-1- IMIDAZOLIDINO-|1,5-∝|- 6-HYDROXY PYRROLIDINE
M.P-154 °C.

PTH-METHIONINE
5-|2-METHYL MERCAPTOETHYL|- 3-PHENYL-2-THIOHYDANTOIN
M.P-131 °C.

WAVE NUMBER (cm⁻¹)

of histidylhistidine, the phenylthiohydantoin derivative could not be crystallized to yield a preparation devoid of yellow coloring matter. When 10-mg. amounts of histidylhistidine were processed, crystalline phenylthiohydantoin of histidine identical with a standard preparation in melting point and spectral characteristics was obtained.

Reaction of 10 mg. of glutathione with phenylisothiocyanate was 93% complete, as indicated by alkali consumption. With this peptide, however, the insoluble product obtained by treating the reaction mixture with nitromethane–hydrogen chloride was not the free phenylthiohydantoin of glutamic acid. Although ring closure had occurred the reagent did not hydrolyze the γ-glutamyl peptide bond. The product was therefore refluxed for 4 hours in 4N hydrochloric acid, and then extracted with ether. The residue from the ether extract after recrystallization possessed the same spectral characteristics as those of glutamic acid phenylthiohydantoin.

DISCUSSION

For many of the amino acid derivatives, the spectrum obtained by the second sampling technique was the same as obtained by the first method, where the sample was maintained in the crystalline state. For identification through the infrared spectra, it, therefore, is advisable to treat the crystalline derivatives, to be identified, by the first sampling technique. If, however, the derivative is obtainable only in solution, then the spectra of the known derivatives for reference should be obtained using the same sampling method as used for the unknown. Thus, when the second sampling technique was used, the phenylthiohydantoins of aspartic acid, glutamic acid, tryptophan, tyrosine, and lysine showed marked changes in their infrared absorption properties (Figure 4). The absorption bands were generally broader, slightly shifted in wave number, and weaker. In the case of aspartic and glutamic acids the two carbonyl bands (near 1775 and near 1700 cm.$^{-1}$), which are well resolved for the crystalline state, added up to form one broad band with a maximum near 1750 cm.$^{-1}$ Changes in infrared absorption properties, similar to the ones mentioned, are known to be caused by a change in the physical state of the substance (7, 9, 12). It appeared that the mentioned phenylthiohydantoins did not crystallize during the drying operation and remained in the liquid state. The same "liquid" spectra can be obtained after heating the potassium bromide window prepared by the first sampling technique to the melting point of the corresponding compound.

All spectra exhibited characteristic bands, which made it possible to recognize the phenylthiohydantoin ring (I) in the compounds.

R—CH——NH
| |
O=C   C=S   R = H or side chains of the
   \   /   corresponding α - amino
    N   acids
    |
   C₆H₅
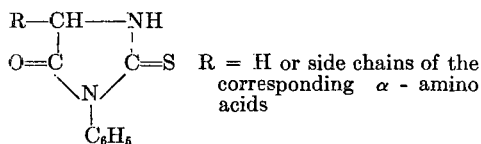
These bands appeared in the following regions of the infrared spectrum:

1770 to 1740 cm.$^{-1}$ assigned to ring C=O vibration.
1600 cm.$^{-1}$ assigned to phenyl group vibration.
1425 to 1400 cm.$^{-1}$ assigned to C=S vibration.
3300 to 3150 cm.$^{-1}$ } assigned to NH vibration (the N—H bands
1530 to 1500 cm.$^{-1}$ } are not shown by the proline and hydroxyproline phenyl-
thiohydantoins).

In the arginine derivative, the phenyl band at 1600 cm.$^{-1}$ is overlapped by the strong C=NH absorption. The N—H stretching vibration in the 3300 to 3150 cm.$^{-1}$ region is often modified by hydroxyl stretching vibrations of the derivatives of the hydroxy amino acids.

The identification of the phenylthiohydantoin derivative of an amino acid by infrared spectroscopy requires 1 mg. of the derivative, thus imposing a lower limit on the amount of peptide that may be degraded. In general, samples containing at least 1 mg. of a single N-terminal amino acid are required, and for convenience in handling, peptide samples of 10 to 20 mg. are desirable. For analysis of microquantities, the application of paper chromatography, on specially treated papers, is necessary. The latter method can be used to detect as little as 2 to 3 γ of phenylthiohydantoins of amino acids (12).

The identification of N-terminal amino acids in peptides or

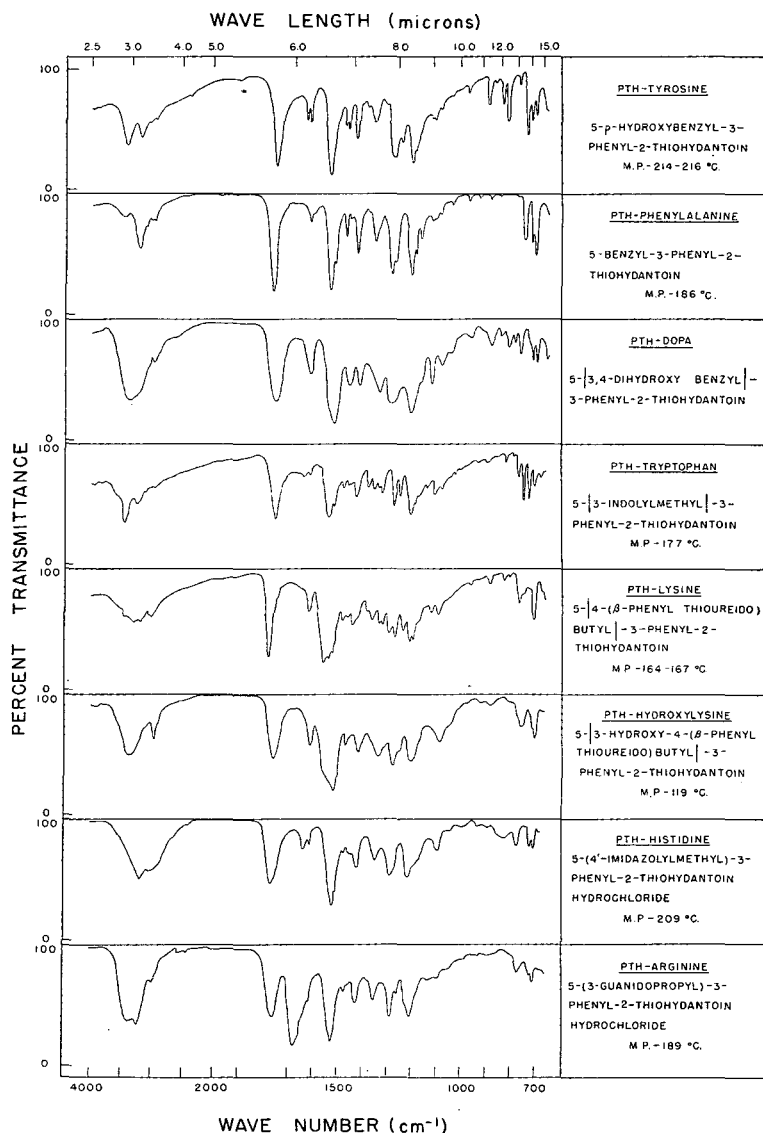**Figure 3. Infrared spectra of phenylthiohydantoins**

Crystalline state

WAVE LENGTH (microns)

PTH-ASPARTIC ACID

3-PHENYL-2-THIO-5-
HYDANTOIN ACETIC ACID
M P - 234 °C

PTH-GLUTAMIC ACID

3-PHENYL-2-THIO-5-
HYDANTOIN PROPIONIC ACID
M P -169 °C

PTH-LYSINE

5-|4-(δ-PHENYL THIOUREIDO)
BUTYL|-3-PHENYL-2-
THIOHYDANTOIN
M P -164-167 °C

PTH-TRYPTOPHAN

5-|3-INDOLYLMETHYL|-3-
PHENYL-2-THIOHYDANTOIN
M P -177 °C

PTH-TYROSINE

5-p-HYDROXYBENZYL-3-
PHENYL-2-THIOHYDANTOIN
M P-214-216 °C
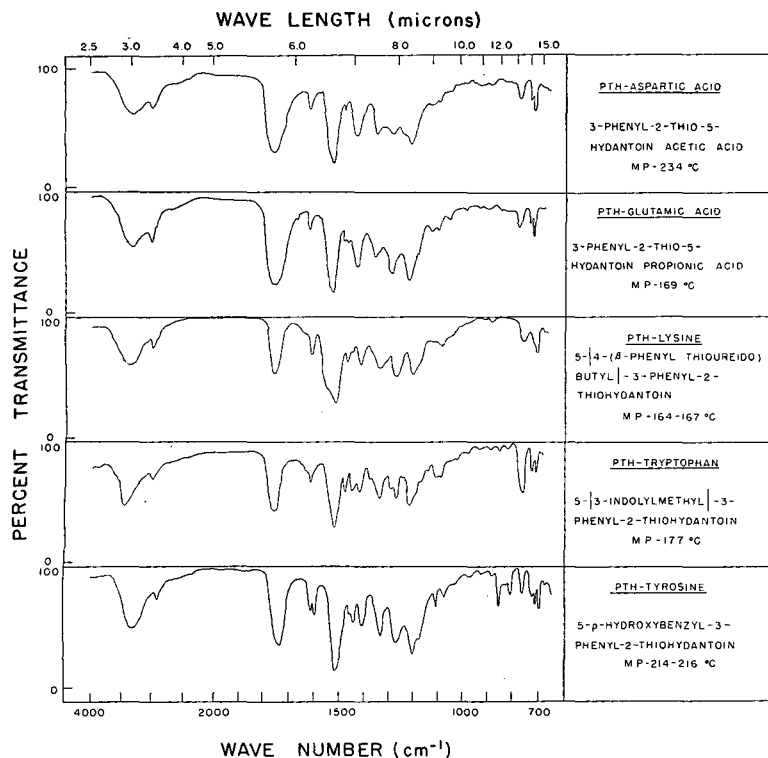
WAVE NUMBER (cm⁻¹)

Figure 4. Infrared spectra of phenylthiohydantoins

Liquid state

proteins by the infrared spectra of their phenylthiohydantoin derivatives is also limited to samples containing a single peptide species that does not possess branched chains. Satisfactory methods for separation and purification of the phenylthiohydantoins of amino acids (16) and for peptides increase the usefulness of the method by materially extending the cases for which it is applicable. Where sufficient quantities of pure

materials are available, the infrared method, as used herein, provides a simple and powerful means for the unambiguous identification of 3-phenyl-2-thiohydantoins of amino acids.

REFERENCES

(1) Brautlecht, C. A., J. Biol. Chem., 10, 139 (1911).
(2) Edman, P., Acta Chem. Scand., 4, 277 (1950).
(3) Ibid., p. 283.
(4) Edman, P., "The Chemical Structure of Proteins" (G. E. W. Wolstenholme and M. P. Cameron, ed.), p. 98, J. & A. Churchill, London, 1954.
(5) Fraenkel-Conrat, H., and Harris, J. I., J Am. Chem. Soc., 76, 6058 (1954).
(6) Fraenkel-Conrat, H., Harris, J. I., and Levy, A. L., "Methods of Biochemical Analyses," D. Glick, ed., vol. II, p. 391, Interscience, New York, 1955.
(7) Halford, R. S., and Schaeffer, O. A., J. Chem. Phys., 14, 141 (1946).
(8) Ingram, V. M., J. Chem. Soc., 1953, 3717.
(9) Kletz, T. A., and Price, W. C., Ibid., 1947, 644.
(10) Landmann, W. A., Drake, M. P., and Dillaha, J., J. Am. Chem. Soc., 75, 3638 (1953).
(11) Ramachandran, L. K., and Sarma, P. S., J. Sci. Ind. Research (India), 12B, 4 (1953).
(12) Richards, R. E., and Thompson, H. W., Proc. Roy. Soc. (London), A195, 1 (1948).
(13) Schiedt, U., and Reinwein, H., Z. Naturforsch., 7b, 270 (1952).
(14) Schlack, P., and Kumpf, W., Z. physiol. Chem., 154, 125 (1926).
(15) Sjöquist, J., Acta Chem. Scand., 7, 447 (1953).
(16) Sjöquist, J., Biochim. et Biophys. Acta, 16, 283 (1955).
(17) Stimson, M. M., and O'Donnell, M. J., J. Am. Chem. Soc., 74, 1805 (1952).

High Precision Spectrophotometric Microanalysis with Application to Vanadium-Aluminum Alloys

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Optically matched cuvettes are necessary for existing high precision spectrophotometric methods. The maintenance of optical matching is not practical for routine analysis at high precision. Procedures are presented utilizing a modification of the Beer's law equation which permit the use of unmatched cuvettes for high precision spectrophotometric analysis. The errors brought about by the use of unmatched cuvettes are calculated and they indicate the optimum conditions, relative to mismatching, for the use of Hiskey's method. These procedures were applied to the analysis for vanadium in two vanadium-aluminum alloys. The accuracy obtained on synthetic samples averaged within 0.1%. The reproducibility on the actual samples averaged within 0.2%.

THE most economical analyses are those which are shortest and simplest in their execution. From this standpoint spectrophotometric analysis is very attractive. It is one of the leading methods for trace analysis but, because of its lack of reproducibility, has been little used for the determination of major components.

Ringbom (10), Hiskey (5–7, 13), and Bastian (1, 2) have shown that very good reproducibility may be obtained in spectrophotometric analysis if a solution of high absorbance is used for the blank. Hiskey (5) has shown that the error in comparing two solutions of similar absorbance may be as low as 0.04% based on concentration. This increased precision results from the use of the spectrophotometer at scale values allowing maximum

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precision, even though the absolute absorbance of the solutions exceeds the maximum scale value of the instrument. By this method, colorimetric analysis can compete with other types of analyses in the accurate determination of major components. The precise determination of manganese in pyrolusite ores by conversion to permanganate was carried out by Young and Hiskey (13). Bastian (1) determined copper in bronzes. Both used a blank of high absorbance in their determination. The amount of sample used in each case would classify them as macro-methods, but Bastian (1) recognized the possible use of this method for microchemical analysis.

These methods require the use of carefully matched cuvettes. If the use of the high absorbance blank is to be extended to routine analysis, however, it is difficult to obtain four cuvettes which are closely matched. It is also likely that the cuvettes may become unmatched with use. For these reasons it is desirable to include the transparency ratio in the basic equation and also in the analytical procedure.

In the present report, the Beer-Lambert law is modified to correct for differences in the thickness and transparency of the cuvettes used. The thickness ratio, β', does not change and is determined only once for each pair of cuvettes. The transparency ratio, R, is determined at the slit width used in the analysis. The value of the extinction coefficient, K', changes only with the thickness of the blank cuvette, so it is determined only once for each No. 1 cuvette. This method gives precise results and permits the use of ordinary borosilicate cuvettes. It is assumed that the colored system obeys Beer's law.

CUVETTE CHARACTERISTICS

The work of Bastian (2) and Hiskey (5) was based on the following statement of the Beer-Lambert law:

$$\frac{P_2}{P_1} = 10^{-ab_1(\beta c_2 - c_1)} \tag{1}$$

Inverting and taking the logarithm of both sides, this becomes,

$$\text{Log} \frac{P_1}{P_2} = ab_1 (\beta c_2 - c_1) = K(\beta c_2 - c_1) \tag{2}$$

in which β is the ratio b_2/b_1 of the lengths of the two cuvettes and a is the absorptivity. There is the implicit assumption in this equation that the two cuvettes, in which solutions of concentration c_2 and c_1 are compared, are equally transparent at all slit widths used.

In practical spectrophotometric determinations, the time required for analysis is decreased if more than two cuvettes can be used. It is shown below that the blank cuvette must be matched to better than 1% transmittance with each of the cuvettes used for the above equation to give an accuracy of 1 part per thousand. If a cuvette transparency term is introduced in the above equation, the cuvettes need not be matched so exactly and the extension of this method to more than one cuvette is simplified. It is recommended in the Beckman DU operation manual that the absorbance difference of the two cuvettes filled with distilled water be subtracted from the absorbance reading of the absorbing sample. The equation justifying this correction may be derived from the Beer-Lambert law and a consideration of the changes taking place in the intensity of a light beam passing through a cuvette. Figure 1,A, illustrates the changes which take place in the intensity P_0 of an incident light beam while passing through a colorless blank, and Figure 1,B, represents these changes for a colored solution in the same cuvette. The symbols x and y represent the transmittances of the cuvette walls indicated. Figure 1,B, illustrates the decrease of light intensity from P_0 to P_0x before entering the solution and the decrease from P to Py after leaving the solution. Equation 3 is a simple mathematical statement of the Beer-Lambert law applied to the solution only, in cuvette 1B:

$$\text{Log} \frac{P_0x}{P} = abc \tag{3}$$

If P_0x/P is multiplied by y/y and the subscripts are applied which indicate that this is cuvette No. 1,

$$\text{Log} \frac{P_0x_1y_1}{Py_1} = ab_1c_1 \tag{4}$$

The same treatment may be used for a second cuvette containing a solution of concentration c_2:

$$\text{Log} \frac{P_0x_2y_2}{Py_2} = ab_2c_2 \tag{5}$$

Subtracting Equation 4 from 5 and rearranging:

$$\text{Log} \frac{P_0x_2y_2}{P_0x_1y_1} \times \frac{Py_1}{Py_2} = a(b_2c_2 - b_1c_1) = ab_1(\beta'c_2 - c_1) \tag{6}$$

Simplifying the symbols:

$$\text{Log} R + \log \frac{P_1}{P_2} = K'(\beta'c_2 - c_1) \tag{7}$$

This equation represents the comparison of solutions of concentrations c_1 and c_2 in two different cuvettes which have wall transmittances x_1, y_1 and x_2, y_2.

In this equation, R is the ratio of transparencies of the two cuvettes. As β and K no longer contain the effect of cell transparency, they are marked with a prime.

The equivalent equation using Hiskey's symbolism is:

$$P_1 = \frac{P_2}{R}10^{A_1(\beta'\alpha - 1)} \tag{8}$$

in which $\alpha = c_2/c_1$

MEASUREMENT OF β'

For that comparison in which cuvettes 1 and 2 both contain a solution of concentration c, Equation 7 may be written as follows:

$$\text{Log} \frac{P_1}{P_2} = K'c(\beta' - 1) - \log R \tag{9}$$

If this comparison is carried out for each of a series of concentrations, a plot of $\log P_1/P_2$ against concentration should yield a straight line of slope $K'(\beta' - 1)$ and of intercept $\log R$. An example of this type of plot is given in Figure 2. The data for this plot were obtained from the peroxy-vanadium complex. An approximate value of K' is sufficient to calculate the value of β'. The usual Beer's law plot gives a sufficiently accurate value for this calculation. The adherence of the experimental points to the straight line is a measure of the constancy of the transparency ratio with varying slit width. If this variation is excessive for any cuvette, it should be replaced. If a pattern of excessive variation is evident in the experimental points for all three cuvettes, the No. 1 cuvette should be replaced and the measurements repeated. In the accompanying plot the experimental error illustrated is $\pm$ 0.1% transmittance, which is
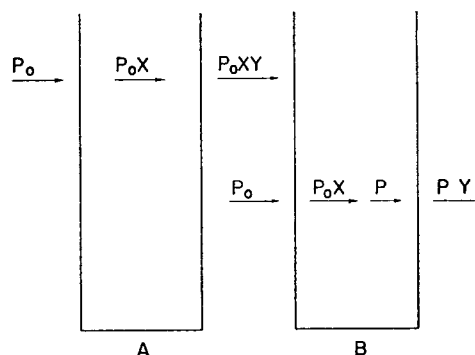

Figure 1. Illustration of change in light intensity in its passage through cuvette

$$\beta' = \frac{SLOPE}{K} + 1 = \frac{SLOPE}{.212} + 1$$

$$\Delta - \beta' = \frac{.000984}{.214} + 1 = 1.0046$$

$$\square - \beta' = \frac{.001649}{.214} + 1 = 1.0078$$

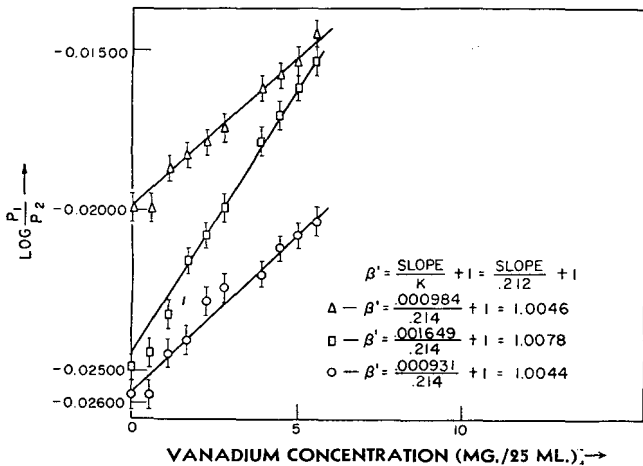$$\circ - \beta' = \frac{.000931}{.214} + 1 = 1.0044$$

Figure 2. Plot to determine β'

probably good for 9 of 10 readings. Therefore, these cuvettes are satisfactory, although there is a slight defect in the No. 1 cuvette which can be seen at a concentration of 0.55 mg. per 25 ml. The procedure given below is for the vanadium-peroxy complex; however, it is readily adaptable to other problems.

DETERMINATION OF K'

The value of K' is given by rearranging Equation 7:

$$K' = \frac{\log R + \log \frac{P_1}{P_2}}{\beta'c_2 - c_1} \tag{10}$$

In this equation $\log (P_1/P_2)$ can be measured for two solutions of known concentrations c_1 and c_2. Log R for cuvettes 2, 3, and 4 can be determined by using only one solution of concentration c_1 in all four cuvettes. Log R is then given by:

$$\text{Log } R = K'c_1 (\beta' - 1) - \log (P_1/P_2') \tag{11}$$

However, as K' is sensitive to the value of log R, the two equations should be solved simultaneously to give:

$$K' = \frac{\log \frac{P_1}{P_2} \times \frac{P_2'}{P_1}}{\beta'(c_2 - c_1)} = \frac{\log \frac{P_2'}{P_2}}{\beta'(c_2 - c_1)} \tag{12}$$

It is convenient to determine the value of K' at the same time as the first analysis. The standard solution used as blank in the analysis may also be used as the blank in the determination of K'. The other three standard solutions are made to have concentrations 1.3, 1.4, and 1.5 mg. (per 25 ml.) greater than the standard solution used as a blank.

DETERMINATION OF LOG R

The values of K' and of β' are constants for the cuvette pairs used. However, log R will vary with the preparation of the cuvette and perhaps with the slit width used. The value of log R is calculated from Equation 11 using the value of P_2 measured when both cuvettes are filled with solution of concentration c_1. This measurement is made with the slit width unchanged.

COMPARISON OF METHODS

The method of Hiskey (5, 13), based on Equation 2, will give exact results in two cases: (a) with matched cuvettes $(R = 1)$ or (b) if the value of K is calculated from measurements on a standard solution of the same concentration as the unknown sample. Since (b) is impractical, both Hiskey and Bastian used condition (a).
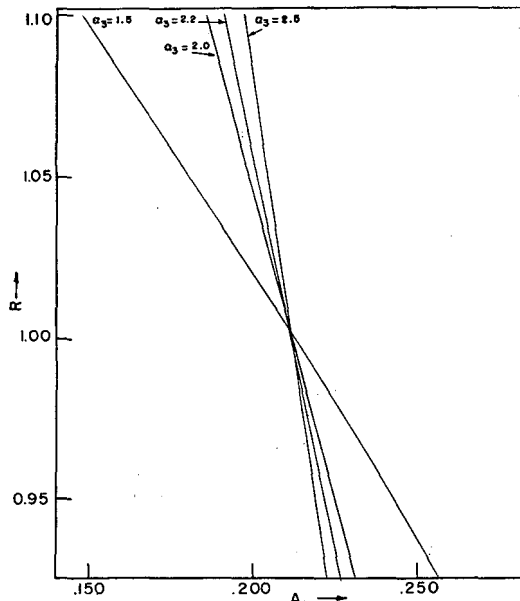
Figure 3. Calculated variation of absorbance constant with cuvette transparency ratio for selected values of a_3 (Equation 13)
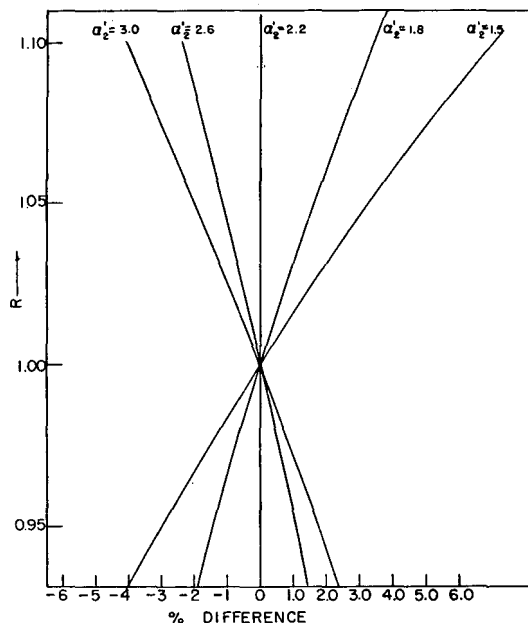
Figure 4. Calculated error in analytical results with cuvette transparency ratio for selected values of a_2' (Equation 17)

The comparison of the two equations is important for two reasons. First, the importance of using the log R term in the equation for very accurate work should be emphasized. Second, this comparison points out the best conditions for the use of Hiskey's method in so far as the cuvette transparencies affect the analytical results. In order to evaluate the expected error using Hiskey's method with unmatched cuvettes, the equation was written for each measurement of the two methods. The equations for the measurement of β and β' are:

$$\text{Log } \frac{P_1}{P_2} = A(\beta - 1) = ab_1c(\beta - 1) \tag{13}$$

and

$$\text{Log } \frac{P_1}{P_2} = A'(\beta' - 1) - \log R \tag{14}$$

Since the measured value of $\log \frac{P_1}{P_2}$ is the same for the two methods at the concentration c, $1 \rightarrow$ and $A \cong A'$ is a reasonable assumption in this case:

$$\beta' - \beta = \frac{\log R}{A} \qquad (15)$$

By defining the value of R and of A, the deviation of β from the true value β' can be calculated.

Although the methods are identical for the measurement of A and A', the value of β is different from that of β'. Also the measured value of $\log R$ affects the actual value obtained for A_1'. Equations similar to 13 and 14 were written relating A_1 and A_1'. In Figure 3 the variation of the A_1 value obtained by Hiskey's method with the value of R is graphed for some values of α_3.

The methods are also identical for the analysis. However, the calculation of the unknown concentration involves a measured value of $\log R$ and the values A_1' and β' in the modified case and the values A_1 and β in the unmodified case. The appropriate equations were written, the dependence of c_2 on c_2' was solved for, and the value of c_2 was calculated for various values of R and α_2'. The values of $c_2' - c_2$ were converted to per cent of c_2' and are graphed in Figure 4.

The values assigned for the above calculation were:

A_1' = 0.212 (true absorbance)
C_1 = 1.000 mg./25 ml. (standard blank)
β' = 1.000 (true length ratio)
C_2' = concentration of unknown solution
C_3 = concentration of standard used to obtain A_1 and A_1' (assigned value of 2.2 for calculation of c_2)
$\alpha_3 = \dfrac{C_3}{C_1}$, $\alpha_2' = \dfrac{C_2'}{C_1}$
A_1, β, C_2 = values of absorbance, length ratio, and unknown concentration calculated with unmodified method.

These calculations indicate that the following conditions will give the best values for Hiskey's method: (1) Cuvettes should be as closely matched as possible for all operations, (2) the best value of β should be obtained at a high absorbancy, (3) the best value of A_1 should be obtained at a large value of α_3, and (4) the unknown concentration should be as close as possible to the concentration of the standard used to measure A_1.

Conditions 1 and 2 are followed in existing procedures. It is interesting that 3 and 4 indicate a best analysis at large values of α_3 whereas Hiskey's consideration of the instrumental error predicted the most accurate analysis at values of α_3 near 1. It seems that the practical value will lie somewhere between 1 and $\dfrac{0.4343}{A} + 1$.

DETERMINATION OF VANADIUM IN VANADIUM-ALUMINUM ALLOYS

Four methods have been published for the microchemical determination of vanadium. In the sole microgravimetric method (8) vanadium is precipitated and weighed as silver vanadate. The three volumetric methods differ in the mode of reagent addition and of end-point detection, but all depend on the reduction of vanadium(V) to vanadium(IV) with ferrous ion. The coulometric-amperometric method of Furman, Reilley, and Cooke (3) seems to be the most accurate of the three. The method of Gale and Mosher (4) is a dead-stop method in which standard ferrous solution is added from a weight microburet. The method of Parks and Agazzi (9) is a standard amperometric titration. All three of these methods were extended into the microgram range.

The method proposed in this paper is comparable to those mentioned above in simplicity and equals the accuracy of the method of Furman, Reilley, and Cooke. As it is based on a different reaction [hydrogen peroxide with vanadium(V)] it may be considered as a complementary method to those outlined above. It can readily be adapted to large numbers of samples and it represents a type of analysis which should be readily applicable to other stable, well-behaved colorimetric systems.

This method is based on the standard peroxy-vanadium method investigated by Wright and Mellon (12) and also by Telep and Boltz (11). It contains features from both papers. No prior publications have been found in which a blank of high absorbance was used in microanalysis.

ANALYSIS OF VANADIUM-ALUMINUM ALLOYS

The analysis procedure follows this sequence:
Preparation.
    Reagent solutions are prepared.
    β' values are obtained for cuvettes 2, 3, and 4.
    K' value is measured.
Analysis.
    Sample is dissolved, perchloric acid and hydrogen peroxide are added, and it is diluted to 25 ml.
    Sample is compared against distilled water blank to obtain approximate concentration.
    Standard solution is prepared for blank.
    Samples are compared to blank at 460 mμ.
    Log R is measured and calculated.
    Sample concentrations are calculated.
Reagents and Solutions. Standard Vanadium Solution. Vanadium pentoxide (1.8 grams) of known purity is accurately weighed and dissolved in 800 ml. of 25% perchloric acid. After being cooled to room temperature it is diluted to 1 liter. The standard vanadium solution used in this work contained 1.0842 mg. per ml. at 31° C. and had a measured density of 1.1074 grams per ml. at 28.5° C.

Aluminum perchlorate (2 mg. per ml.). Reagent grade aluminum perchlorate nonahydrate (9.0 grams) is dissolved in about 250 ml. of distilled water.

Perchloric acid, reagent grade.

Hydrogen peroxide. Reagent grade 30% hydrogen peroxide is diluted to 5% in a mixing graduate.

Apparatus. The Beckman Model DU spectrophotometer with four 1-cm. cuvettes. This spectrophotometer should be voltage-stabilized.

Microbalance.

Standard Solution Preparation. Weigh the necessary amount of standard vanadium solution into a 25-ml. volumetric flask. Add sufficient concentrated perchloric acid to bring the total amount of concentrated perchloric acid to 5 ml. (The standard vanadium contains 0.2 ml. of concentrated perchloric acid per ml. of solution.) Add the approximate amount of aluminum perchlorate to equal the amount of aluminum in the sample. Dilute this solution to about 20 ml., mix by swirling, and cool to room temperature. Add 1 ml. of 5% hydrogen peroxide and dilute to the mark.

General Spectrophotometric Procedure. HANDLING CUVETTES. Mark the cuvettes so that they may always be replaced in the cuvette holder in the same slot and with the same side facing the photocell. Clean the cuvettes thoroughly, fill them with distilled water, and dress the outside of the optical surfaces with Desicote using a cotton swab. Wipe excess Desicote from them with benzene on a tissue. Allow the cuvettes to age 3 hours, then place in the cuvette holder. The cuvette in slot 1 is the blank cuvette. Empty, rinse, and refill these cuvettes with two long-barreled reagent droppers or with pipets. Leave the cuvettes in the holder during these operations.

ADJUSTMENT OF SLIT WIDTH. Adjust the dark current. Set the sensitivity knob 2.5 turns from its counterclockwise limit, the wave length at 460 mμ, and the diaphragm open to the blank cuvette. Adjust the slit width until the galvanometer is approximately in balance with the selection switch on check. The dark current and 100% adjustments are now made in the standard manner for the instrument. Make these adjustments as exact as possible then measure the per cent transmittance of the comparison cuvette, estimating the reading to 0.1% transmittance unit. Repeat the dark current and 100% adjustments and read again. Repeat until two check readings are obtained.

Determination of β'. Prepare six standard peroxy-vanadium solutions containing 0, 1, 2, 3, 4, and 5 ml. of the standard vanadium solution by the procedure given above, except that the aluminum perchlorate may be omitted. Rinse and fill all four cuvettes with the solution containing no vanadium. Compare

each with the No. 1 cuvette by the spectrophotometric procedure given above. Repeat the filling and measuring procedure with each standard solution. Plot the log $100.0/P$ against concentration in milligrams per 25 ml. Measure the slope and calculate the value of β' for cuvettes 2, 3, and 4 from the equation:

$$\beta' = \frac{slope}{0.214} + 1 \qquad (16)$$

As the value of β' is not particularly sensitive to concentration, volume measurements may be used in this procedure.

Determination of K'. Prepare four standard peroxy-vanadium solutions according to the procedure given above. Use 3.5, 4.8, 4.9, and 5.0 ml. of the standard vanadium solution per 25 ml. Fill cuvette 1 with the 3.5-mg. standard and cuvettes 2, 3, and 4 with the 4.8-, 4.9-, 5.0-mg. standards. Compare each of these with the No. 1 cuvette. Empty cuvettes 2, 3, and 4, rinse, and refill them with the 3.5-mg. standard. Compare each of these with the No. 1 cuvette.

Sample Preparation. Weigh out a sample of alloy containing 2 to 5 mg. of vanadium on the microbalance. It is convenient to do this in a micro combustion boat. Slide this boat into a 16-mm. test tube supported in a 125-ml. Erlenmeyer flask. Add 1 ml. of distilled water and then 2 ml. of concentrated perchloric acid. Mix them by swirling and place the mixture on the hot place at high heat. Heat this mixture until the fumes of perchloric acid appear, and then cool. If the sample has not entirely dissolved, add 1 ml. of distilled water and concentrate the sample again. Cool the dissolved sample and transfer it quantitatively to a 25-ml. volumetric flask. Add 3 ml. of concentrated perchloric acid, dilute to about 20 ml. with distilled water, swirl to mix, and cool to room temperature. Add 1 ml. of 5% hydrogen peroxide and dilute to the mark. Mix well and compare by the procedure for the analysis.

Analysis. Prepare the samples for analysis as described above. If it is not already known, determine the approximate per cent of vanadium by spectrophotometric comparison with a distilled water blank at 460 mμ using 1-cm. borosilicate glass cells. Calculate the approximate concentration in milligrams per 25 ml. by dividing the absorbance by 0.214.

Prepare a standard peroxy-vanadium solution to be used as blank. The concentration of this standard should be 0.5 to 1.5 mg. less than any sample. Fill cuvette 1 with standard solution and the remaining cuvettes with sample solutions. Compare these solutions according to the spectrophotometric procedure, then empty, rinse, and refill cuvettes 2, 3, and 4 with other samples, leaving the standard solution unchanged. Repeat until all samples have been compared. Lastly, fill all cuvettes with the standard solution and compare to obtain the data necessary to calculate log R.

CALCULATIONS

β' is calculated graphically as demonstrated in the section on the determination of β'. K' is calculated from Equation 12. Log R is calculated from Equation 11. The vanadium content is calculated from the following equation:

$$\% \text{ vanadium} = \frac{100}{S} \frac{(\log R + \log P_1/P_2 + K'c_1)}{K'\beta'} \qquad (17)$$

in which P_1 equals 100.0%, P_2 is the per cent transmittance read, c_1 is the concentration of the standard solution used as blank, and S is the sample weight in milligrams.

SAMPLE CALCULATIONS

For the calculation of β' see Figure 2.

$$K' = \frac{\log (99.8/58.0)}{1.0078 (4.9579 - 3.8605)} = 0.2131$$

$$\text{Log } R = (0.2131)(3.4699)(1.0046 - 1) - \log \frac{100.0}{104.4} = 0.02210$$

% vanadium =

$$\frac{100}{12.036} \frac{[0.02210 + \log (100.0/70.4) + (0.2131)(2.1736)]}{(0.2131) \ (1.0046)}$$

$$= 24.75\%$$

NOTE. The measurement for the calculation of K' was made with a different cuvette ($\beta' = 1.0078$) than the one used for log R and % vanadium ($\beta' = 1.0046$).

Table I. Effect of Aluminum

0.1M Al(ClO$_4$)$_3$, Ml.	Vanadium, %	Sample Size	K
0	100	4.9	0.2155
1	64	7.6	0.2148
2	47	10.3	0.2138
5	27	18.4	0.2124
10	15	31.9	0.2128

Av.　0.2139 ± 0.0010

Table II. Results on Synthetic Samples

Aliquot	Actual Concentration, Mg./25 Ml.	Measured Concentration, Mg./25 Ml.	Error, %
1	2.098	2.105	0.3
2	1.998	2.003	0.3
3	2.198	2.198	0.0
4	3.0434	3.0453	0.06
5	3.1532	3.1531	0.00
6	3.2588	3.2585	0.01
7	3.3629	3.3621	0.02
8	3.4699	3.4756	0.16

Av. 0.11

Table III. Alloys Analyzed

Alloy	Measured Concentration, Mg./25 Ml.	Vanadium, %	Av. Reproducibility, %
1	2.094	20.91	0.3
	2.079	20.96	
2	2.984	24.70	0.1
	3.133	24.72	

EFFECT OF ALUMINUM

The effect of aluminum is slight, as may be seen in Table I. Therefore, it is not necessary to add an accurate amount of aluminum to the comparison solutions. Columns 2 and 3 of Table I are approximate calculations to illustrate the range covered.

ANALYTICAL DATA AND RESULTS

The analytical data are summarized in Tables II and III. Aliquots 1, 2, and 3 in Table II and alloy 1 were analyzed without weighing any solutions. The remainder of the analyses were made by the full weighing procedure.

FURTHER APPLICATIONS

This analysis should be applicable to any mixture containing vanadium in which the other constituents do not absorb at 460 mμ. Wright and Mellon's (12) work may be consulted for a listing of 23 ions which do not interfere in this method.

LITERATURE CITED

(1) Bastian, R., ANAL. CHEM., 21, 972 (1949).
(2) Bastian, Robert, Weberling, Richard, and Pallila, Frank, Ibid., 22, 160 (1950).
(3) Furman, N. H., Reilley, C. N., and Cooke, W. D., Ibid., 23, 1665 (1951).
(4) Gale, R. H., and Mosher, Eve, Ibid., 22, 942 (1950).
(5) Hiskey, C. F., Ibid., 21, 1440 (1949).
(6) Hiskey, C. F., Rabinowitz, Jacob, and Young, I. G., Ibid., 22, 1464 (1950).
(7) Hiskey, C. F., and Young, I. G., Ibid., 23, 1196 (1951).
(8) Kroupa, Edith, Mikrochemie ver. Mikrochim. Acta, 32, 245 (1944).
(9) Parks, T. D., and Agazzi, E. J., ANAL. CHEM., 22, 1179 (1950).
(10) Ringbom, A., Z. anal. Chem., 115, 332 (1938).
(11) Telep, G., and Boltz, D. F., ANAL. CHEM., 23, 901 (1951).
(12) Wright, E. R., and Mellon, M. G., IND. ENG. CHEM., ANAL. ED., 9, 375 (1937).
(13) Young, I. G., and Hiskey, C. F., ANAL. CHEM., 23, 506 (1951).

Spectrophotometric Determination of Rhodium with Tin(II) Chloride

Simultaneous Determination of Rhodium and Platinum

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A method is given for the spectrophotometric determination of rhodium, based upon the pink to red color, absorbance maximum at 475 mμ, formed by addition of tin(II) chloride in hydrochloric acid solution. The color develops slowly at room temperature, but forms rapidly at the boiling point. The color is reproducible and stable. Wide variations in the amount of tin(II) chloride or in the amount of hydrochloric acid are without effect on the absorbance. The optimum concentration range for measurement in 1.00-cm. cells is about 4 to 20 p.p.m. of rhodium. Ruthenium, osmium, palladium, gold, and chromium, which interfere with the determination of rhodium, are easily removed. A method is given for the simultaneous determination of rhodium and platinum from measurements of the absorbance at two wave lengths; the relative error of the simultaneous determination is approximately 1% for each element.

FEW methods for the colorimetric determination of rhodium have appeared in the literature. Ayres and Young (*3*) reported a spectrophotometric method based on the blue color (absorbance maximum at 665 mμ) formed by treating rhodium-(III) solutions with excess hypochlorite; the system required about 1 hour for complete color development and also required rather close pH control.

Ivanov (*6*) observed that rhodium(III) salts in hydrochloric acid solution slowly developed a red color when treated with tin(II) chloride. The reaction has been used as a qualitative test for rhodium (*10, 11*), and also for its estimation (*4*), but appears not to have been applied for the spectrophotometric determination of rhodium. The composition of the colored solute is not known (*9*).

The present investigation was undertaken to study the color system produced by reaction of rhodium(III) with tin(II) chloride; to determine the optimum conditions for color development; to establish the range and reliability of the method; to determine the nature and extent of interferences and methods for their removal; and to test the method for the determination of rhodium, especially in samples containing other platinum metals and gold.

APPARATUS

Absorbance measurements were made with a Beckman Model DU quartz spectrophotometer, using matched 1.00-cm. cells. The instrument was operated at constant sensitivity. Calibrated weights and calibrated volumetric ware were used.

REAGENTS

Spectrographically pure rhodium metal powder, obtained from A. D. Mackay, Inc., was used for preparation of the standard rhodium solution. Standard platinum solution was prepared from Grade 1 platinum thermocouple wire, using the procedure described by Ayres and Meyer (*1*). Solutions of the other platinum elements were prepared from the metals or their compounds, obtained from A. D. Mackay, Inc.; all these materials were checked spectrographically for purity with respect to foreign platinum elements. All other chemicals were analytical reagent grade.

Tin(II) chloride solution, prepared from the dihydrate, was 1M in tin(II) chloride and 2.5M in hydrochloric acid.

EXPERIMENTAL

Preparation of Standard Rhodium Solution. Rhodium metal powder, mixed with excess potassium chloride, was converted to the hexachlororhodate(III) by high temperature treatment with chlorine, as described by Ayres and Young (*3*).

Spectral Characteristics. Figure 1 shows spectral curves for rhodium(III) solutions color-developed with tin(II) chloride by the standardized procedure described below. In addition to the absorption band at 475 mμ, there is strong absorption at about 330 mμ. Near 330 mμ, absorbance is sensitive to small variations in the concentration of tin(II) chloride, whereas this is not the case at 475 mμ; for this reason, absorbance measurements were made at 475 mμ in studying the influence of various factors on the color development. A region on either side of 475 mμ was scanned in order to detect any possible shift in the position of maximum absorbance; no shift was observed in any case.
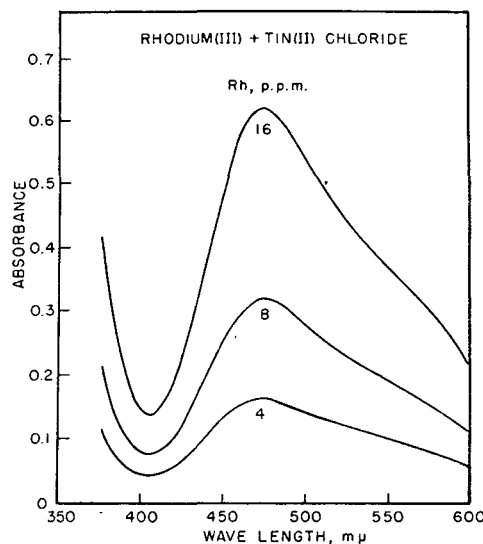
Figure 1. Spectral curves for rhodium(III) solutions color-developed with tin(II) chloride

A constant amount of rhodium, 10.0 p.p.m., was used in testing the effect of different variables on the color intensity.

Rate of Color Development. At room temperature, about 12 hours were required for complete color development; at temperatures near the boiling point, the full color intensity developed in 3 to 5 minutes; additional heating for 30 minutes had no effect on the absorbance.

Effect of Acid Concentration. The amount of hydrochloric acid (or perchloric acid) was varied from 1 to 40 ml. of concentrated acid per 100 ml. of final solution. Absorbance readings were constant within the limit of accuracy of making the measurement.

Effect of Tin(II) Chloride Concentration. Using a constant amount of rhodium and of hydrochloric acid (10 ml. of concentrated acid per 100 ml. of final solution), the amount of tin(II) chloride reagent was varied from 5 to 50 ml. per 100 ml. of final volume. Absorbance readings at 475 mμ were constant.

Stability and Reproducibility. Solutions measured after 2 weeks had the same absorbance as when freshly prepared. Throughout the entire investigation, the measurements at 475 mμ for all solutions containing a constant amount of rhodium did not vary by more than 0.2% (absolute) transmittance.

Table I. Effect of Diverse Ions

Ion	Tolerance, P.P.M.
Platinum(IV)	1
Ruthenium(III)	1
Palladium(II)	2
Osmium(IV)	8
Iridium(IV)	450
Gold(III)	5
Chromium(VI)	5
Iron(III)[a]	50
Cobalt(II)	50
Copper(II)	50
Nickel(II)	2500
Iodide	75
Bromide	1.5×10^4
Nitrate[a]	200
Sulfate[a]	1×10^4

[a] These substances produced a decrease in absorbance, relative to rhodium alone; all others produced an increase in absorbance.

Standardized Procedure. An aliquot of the working standard solution (or sample for analysis) to give the desired final concentration of rhodium was added to 10 ml. of concentrated hydrochloric acid. After addition of 10 ml. of $1M$ tin(II) chloride, the solution was diluted to about 30 ml. with water, and boiled gently for about 10 minutes. The cooled solution was treated with an additional 10 ml. of tin(II) chloride solution, to replace any tin(II) that might have been air-oxidized, and finally diluted to 100 ml. The absorbance was measured at 475 mμ, using a reagent blank for comparison. In the concentration range studied, up to 24 p.p.m., the system conforms to Beer's law. A Ringbom plot ($100 - \%T$ vs. log concentration) shows the optimum range, for measurement in 1.00-cm. cells, to be about 4 to 20 p.p.m. The range can be extended by any of the customary methods.

Effect of Diverse Ions. Platinum is known to interfere by forming an amber to red solution with tin(II) chloride (1). Interference might be expected also from the other platinum elements, certain colored metal ions, and ions—e.g., gold—which form colored reduction products with tin(II). For studying interference effects, the various elements were used in the oxidation states that would normally be present in the solution after the usual dissolution procedures. The tin(II) reduced chromium(VI) to chromium(III), iron(III) to iron(II), gold(III) to blue colloidal gold, and palladium(II) apparently to black colloidal metal.

In order to establish the tolerance limit of the rhodium system for another element, solutions containing 10.0 p.p.m. of rhodium and varying amounts of the foreign element (chloride solution for the metal ions, and alkali salts for the anions) were developed and measured in the usual way. The tolerance was taken as the largest amount of foreign substance that would give a per cent transmittance within 0.4 of that of the rhodium solution alone; for 10.0 p.p.m. of rhodium, this corresponded to an absorbance difference of about 0.005. The tolerances are given in Table I.

Removal of Interfering Ions. Table I shows the principal interfering substances to be platinum, ruthenium, palladium, osmium, gold, and chromium; all of these except platinum are easily removed. Fuming down with perchloric acid, in the presence of excess chloride, volatilizes chromium as chromyl chloride, and osmium and ruthenium as their tetroxides; anions of volatile acids are also removed. Gold can be removed by extraction with amyl acetate from hydrochloric acid solution (7). Palladium can be removed either by precipitation with dimethylglyoxime (5), or by extraction of palladium-phenylthiourea with amyl acetate (2). In the latter process, rhodium is not extracted, but platinum is extracted to the extent of about 40%; a solution containing 10 p.p.m. of platinum would require several successive extractions with amyl acetate to lower the platinum concentration in the aqueous solution to its tolerance limit for the determination of rhodium with tin(II) chloride. Platinum carried with the palladium into the organic solvent does not interfere with subsequent determination of palladium unless present in the extract in an amount about three times as much as the palladium (2).

Analysis of Mixtures. Sample 1 contained 10.0 mg. each of rhodium, palladium, gold, and iron. The solution, acidic with hydrochloric acid, was extracted with amyl acetate to remove the gold. The aqueous layer was treated with phenylthiourea solution and again extracted with amyl acetate; after separation, the organic layer was used for the spectrophotometric determination of palladium with bromide (2); the aqueous layer was treated with nitric acid then with perchloric acid to remove organic matter, and the rhodium was determined with tin(II) chloride. The following results, on triplicate samples, are typical: palladium found, 10.0, 10.1, and 9.8 mg.; rhodium found, 10.1, 10.1, and 10.3 mg.

Sample 2 contained 10.0 mg. each of rhodium, palladium, cobalt, nickel, and copper in solution. Palladium was removed by precipitation, from hydrochloric acid solution, with dimethylglyoxime; the palladium dimethylglyoximate was decomposed with nitric and hydrochloric acids, and the palladium was determined spectrophotometrically with bromide. The filtrate from the palladium separation was evaporated with nitric acid then fumed with perchloric acid, and the rhodium was determined with tin(II) chloride. In typical triplicate samples, the palladium found was 10.1, 9.9, and 9.9 mg.; rhodium found was 10.2, 10.1, and 9.9 mg.
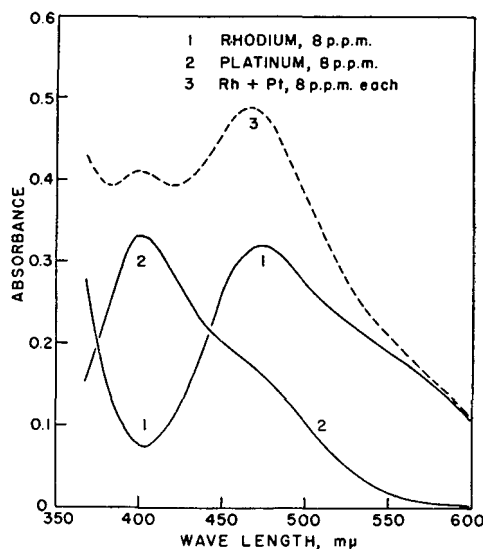
Figure 2. Spectral curves for rhodium, platinum, and mixture

When platinum is present along with palladium, and the latter is separated from rhodium by treatment with phenylthiourea and extraction with amyl acetate, platinum is also extracted to a moderate extent. Use of this method for the removal of platinum would require several successive extractions, and subsequent separation of palladium and platinum if these elements are to be determined. Removal of platinum is unnecessary, and both rhodium and platinum can be determined by making measurements of the absorbance at two different wave lengths, appropriately chosen.

SIMULTANEOUS DETERMINATION OF RHODIUM AND PLATINUM

Solutions of platinum(IV, II) react with tin(II) chloride in hydrochloric acid to form orange to red solutions having maximum absorbance at 403 mμ (1, 9). The colored product formed by reaction of rhodium(III) with tin(II) has an absorbance minimum at 403 mμ and a maximum at 475 mμ. The spectral

curves for rhodium, platinum, and a mixture of the two are shown in Figure 2.

Simultaneous determination of two components from the absorbances at two different wave lengths is based upon the additivity of absorbances of the components. Application of the spectrophotometric law to the mixture of rhodium and platinum, color-developed with tin(II) and measured at 403 and at 475 mμ in cells of the same optical path, gives

$$A_{475} = a_1 c_{Rh} + a_2 c_{Pt} \tag{1}$$

and

$$A_{403} = a_3 c_{Rh} + a_4 c_{Pt} \tag{2}$$

where a_1 and a_2 are the absorptivities of rhodium and platinum, respectively, at 475 mμ, and a_3 and a_4 are the absorptivities of rhodium and platinum at 403 mμ. Solving Equations 1 and 2 gives

$$c_{Rh} = \frac{a_4 A_{475} - a_2 A_{403}}{a_1 a_4 - a_2 a_3} \tag{3}$$

and

$$c_{Pt} = \frac{a_1 A_{403} - a_3 A_{475}}{a_1 a_4 - a_2 a_3} \tag{4}$$

Proof of Additivity of Absorbances. Before applying the method, additivity of absorbances of the two components was proved by measuring the absorbance of solutions of rhodium, platinum, and mixtures of rhodium and platinum, color-developed with tin(II) by the standardized procedure given previously. Many mixtures were measured, from 2 to 16 p.p.m. of each element, present in equal amounts and in widely different amounts. The absorbances were additive over the entire region from 375 to 600 mμ, within the limits of error of the absorbance measurements (about 0.001 to 0.005 absorbance, depending on the magnitude of the absorbance measured).

The absorptivities, a_1, a_2, a_3, and a_4, were determined from the measured absorbances, at 403 and at 475 mμ, of color-developed solutions of each element; each absorptivity used in Equations 3 and 4 was the average from several concentrations of each element at each wave length, and was equal to the slope of the plot of absorbance against concentration.

Analysis of Mixtures. The method was applied to synthetic mixtures of rhodium and platinum, varying widely in amounts of these elements and in their ratio. Typical results are shown in Table II. In the analysis of 20 samples (including the 10 samples given in Table II), the average relative error in the estimation of rhodium was 1.1%, and in the estimation of platinum, 1.0%.

The selection of the wave lengths to be used in the analysis of a mixture is based mainly on two considerations: the spectral position of the peak of the absorption band of the principal absorber and/or the position at which only one of the components shows appreciable absorption (8). The wave lengths 403 and

Table II. Simultaneous Determination of Rhodium and Platinum

Taken, P.P.M.		Found, P.P.M.		Difference, %	
Rh	Pt	Rh	Pt	Rh	Pt
2.0	2.0	2.0	2.0	0	0
4.0	4.0	4.0	4.0	0	0
8.0	8.0	7.7	8.0	4	0
10.0	10.0	9.9	9.9	1	1
16.0	16.0	16.0	16.2	0	1.2
2.0	16.0	2.0	16.1	0	0.6
4.0	16.0	4.0	16.1	0	0.6
2.0	20.0	2.1	19.7	5	1.5
20.0	2.0	19.9	1.9	0.5	5
			Av.	1.1	1.0

475 mμ were chosen on the basis of the first consideration. At 475 mμ, however, the platinum contributes an appreciable fraction of the total absorbance, especially in mixtures containing a high ratio of platinum to rhodium. At wave lengths in the region 560 to 600 mμ the platinum system shows very little absorption, but the absorption due to rhodium is only about one third of its value at 475 mμ. Several mixtures were analyzed from the absorbances measured at 403 and 560 mμ; the accuracy of the determinations at these wave lengths was the same as that obtained from measurements at 403 and 475 mμ. Either pair of wave lengths is equally satisfactory, provided that the absorptivities for use in Equations 3 and 4 are determined accurately.

LITERATURE CITED

(1) Ayres, G. H., and Meyer, A. S., Jr., ANAL. CHEM., 23, 299 (1951).
(2) Ayres, G. H., and Tuffly, B. L., *Ibid.*, 24, 949 (1952).
(3) Ayres, G. H., and Young, H. F., *Ibid.*, 22, 1403 (1950).
(4) Bouvet, Pierre, *Ann. pharm. franç.*, 5, 293 (1947).
(5) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 281, Wiley, New York, 1929.
(6) Ivanov, V. N., *J. Russ. Phys.-Chem. Soc.*, 50, 460 (1913).
(7) Lehner, V., and Kao, C. H., *J. Phys. Chem.*, 30, 126 (1926).
(8) Mellon, M. G., ed., "Analytical Absorption Spectroscopy," p. 493, Wiley, New York, 1950.
(9) Meyer, A. S., Jr., and Ayres, G. H., *J. Am. Chem. Soc.*, 77, 2671 (1955).
(10) Thompson, S. O., Beamish, F. E., and Scott, M., IND. ENG. CHEM., ANAL. ED., 9, 420 (1937).
(11) Wölbling, H., *Ber.*, 67, 773 (1934).

Potentiometric Titrations Involving Chelating Agents, Metal Ions, and Metal Chelates

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This study was initiated to find a suitable method for analyzing chelating agents in general and also metal chelates. The method can be used for determining mixtures of chelating agents and, in reverse, for determining metals and mixtures of certain metals. The general approach is the potentiometric titration of the chelating agent with a metal ion (or vice versa) using an electrode system that indicates any excess of metal ion in the system. Conditions, metal ion, and electrode systems can be varied so that mixtures of chelating agents can be determined. The chelating agent, conditions, and electrode system can be varied so that mixtures of metal ions can be determined. Metal chelates can also be analyzed for unchelated metal ion present, excess chelating agent, and the amount of metal chelate present. The method is simple to apply, and the precision and accuracy are generally better than ±1% for the systems where good titration curves are obtained.

CHELATING agents have found their way into many diverse applications. The number of chelating agents in use in industry has increased beyond the well-known ethylenediaminetetraacetic acid [EDTA; also known as (ethylenedinitrilo)-tetraacetic acid]. Industry now employs mixtures of chelating agents to achieve certain desirable characteristics. Metal chelates have become industrially important in recent years, especially in the agricultural field. This diversification of applications and the increase in the scope of chelation chemistry have necessitated the development of analytical methods to follow the processes and investigations being carried out.

Chelating agents have, in recent years, become of great importance from the standpoint of analysis. Biedermann and Schwarzenbech (1) devised a method for determining various metallic ions with EDTA, involving the use of a special indicator. The procedure can be reversed to determine EDTA by titrating with magnesium ion. The procedure cannot be applied to some of the other chelating agents which are produced today.

Furness, Crawshaw, and Davies (6) determined EDTA polarographically. Blaedel, Knight, and Malmstadt (2–4) used high frequency titration of metal ions with EDTA. Laitinen and Sympson (9) employed amperometric titrations, and Hall and others (7) used conductometric titrations.

Přibil, Koudela, and Matyska (10) used potentiometric titrations for determining iron by direct titration with EDTA solution. Platinum was used as the indicating electrode in an aqueous medium. Iron was the only metallic ion that could be determined directly. Aluminum, copper, cadmium, zinc, nickel, lead, and bismuth were also determined, but indirectly, by addition of excess EDTA and back-titration of the excess with a standard solution of ferric chloride.

It was found that by varying the solvents and especially the electrodes employed, the potentiometric approach described by Přibil could be extended not only to the direct titration of a number of metals other than iron but also to the titration of chelating agents other than EDTA. The electrode systems given in the tables were the best of the several tried for detecting the metal in question. The platinum electrode worked in only a few cases. Pyridine intensified the magnitude of the titration breaks and also made possible the solution of the acid forms of the chelating

agents and of many different types of sample. Mixtures of chelating agents also could be determined if the proper electrodes solvent systems, and pH were used. This approach was found to be suitable for the analysis of metal chelates, making possible the determination of any excess chelating agent or metal ion that may be present and also the amount of metal chelate present.

Potentiometric titration, in addition to being a versatile approach in chelating agent–metal analysis, is a simple tool to use. A pH meter may be used for the titrations, and the electrodes used are easily made. This puts the approach at the disposal of laboratories and plants with limited equipment. The accuracy and precision of the method vary with the chelating agent, metal ion, conditions, and electrodes used; however, accuracy and precision within ±1% are often obtainable.

DETERMINATION OF CHELATING AGENTS AND METAL IONS

The same procedures are involved in the determination of chelating agents and metal ions, except that for determining chelating agents a standard solution of the metallic ion is used, and for determining the metallic ion a standard solution of chelating agent is used. The electrode and solvent systems are the same in whichever direction the titration is performed. Typical titration curves are shown in Figure 1, and results are shown in Table I.

Table I shows that EDTA chelates well with all the metals tried. A sharp titration break appears to indicate a small dissociation constant for the metal chelates formed on titration.

Ammonia–triacetic acid gives sharp titration breaks with copper, zinc, and lead. Iron gives sharp breaks only under certain conditions. Mercury chelates only weakly, and under some conditions no break at all is visible. Calcium and magnesium do not noticeably chelate with this agent under the conditions employed.

N,N-di(β-hydroxyethyl)glycine exhibits relatively poor chelating properties under the conditions tried. Only copper and lead could be used to titrate this agent, and the precision was not too good, as only fair titration curves were obtained.

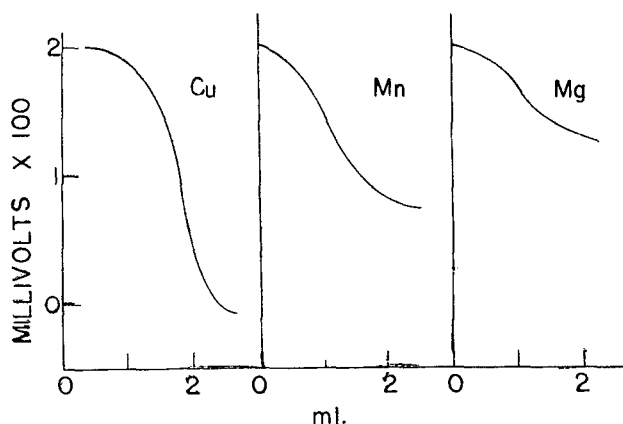Table I shows that chelation varies depending on three fac-

Figure 1. Curves of ethylenediaminetetraacetic acid titrated with copper, manganese, and magnesium

Mercury on platinum *vs.* calomel electrode system and 1 to 1 pyridine-water solvent system

Table I. Titration Results with Various Metal Ions[a]

Metal Used	Titrant, 0.1N	Solvent System	Found, %[b]	pH Range During Titration	Electrodes	Remarks
		Titration of Ethylenediaminetetraacetic Acid $\begin{pmatrix} HOOCCH_2 \\ \qquad\qquad NCH_2\text{—}CH_2N \\ HOOCCH_2 \end{pmatrix}\begin{matrix} CH_2COOH \\ \\ CH_2COOH \end{matrix}$				
Fe	FeCl₃	H₂O	101.0	4.70- 1.78	Pt vs. cal	Good break
		H₂O + 1 g. Na₂CO₃	No break	10.17-10.00	Pt vs. cal	Fe will not chelate when system is this alkaline
		H₂O + 5 ml. 0.5N NaOH	No break	12.03- 7.00	Pt vs. cal	
		H₂O	99.8	4.7 - 1.8	Pt vs. cal	Good break
		H₂O	98.5	4.7 - 1.8	Pt vs. cal	Good break
		H₂O	99.7	4.5 - 1.7	Pt vs. cal	Fair break[c]
		H₂O + 4 ml. of 1N NaOH	99.7	8.2 - 2.1	Pt vs. cal	Good break[c]
		H₂O + 1 ml. 1N NaOH + 1 g. NaAc	99.9	5.9 - 4.2	Pt vs. cal	Good break[c]
Cu	Cu(NO₃)₂	H₂O + pyridine (50–50)	99.95	7.61- 7.20	Pt vs.	Very good break
		H₂O + 1 g. Na₂CO₃	100.08	10.2 - 9.9	Pt vs. cal	Small but sharp break
		H₂O + pyridine (50–50)	100.0	7.1 - 6.7	Pt vs. cal	Fair break[c]
			100.0	7.1 - 6.7	Pt vs. cal	Fair break[c]
			99.4	7.1 - 6.7	Hg on Pt vs. ca	Good break[c]
Hg	Hg(Ac)₂	H₂O + pyridine (50–50)	100.9[d]	7.2 - 6.5	Ag vs. cal[e]	Good break
			100.1	7.2 - 6.5	Ag vs. cal[e]	Good break
			100.2[f]	8.9 - 7.2	Ag vs. cal[e]	Good break
			99.1[f]	8.9 - 7.2	Ag vs. cal[e]	Good break
		H₂O + pyridine + 0.1 ml. 0.5N NaOH	100.2[f]	9.2 - 7.3	Ag vs. cal[e]	Good break
		H₂O + pyridine + 1.0 ml. 0.5N NaOH	99.5[f]	9.6 - 7.4	Ag vs. cal[e]	Good break
		H₂O + pyridine + 4.0 ml. 1.0N NaOH	99.2	8.3 - 7.1	Ag vs. cal	Good break[c]
		H₂O + pyridine + 5.0 ml. 1.0N NaOH	99.8	9.3 - 7.4	Ag vs. cal	Good break[c]
		H₂O + pyridine + 3.0 ml. 1.0N NaOH	100.4	7.8 - 7.1	Ag vs. cal	Good break[c]
Zn	Zn(NO₃)₂	H₂O + pyridine (50–50)	99.0	7.65- 6.75	Hg on Pt vs. cal	Very good break
			100.3	7.65- 6.75	Hg on Pt vs. cal	Very good break
			100.0	8.01- 7.72	Hg on Pt vs. cal	Very good break
			99.03	6.68- 6.63	Hg on Pt vs. cal	Very good break[c]
			99.01	6.7 - 6.6	Hg on Pt vs. cal	Very good break[c]
			99.05	6.7 - 6.6	Hg on Pt vs. cal	Very good break[c]
Pb	Pb(NO₃)₂	H₂O + pyridine (50–50)	99.5	7.7 - 6.8	Hg on Pt vs. cal	Very good break
			99.95	7.7 - 6.8	Hg on Pt vs. cal	Very good break
			99.83	7.7 - 6.8	Hg on Pt vs. cal	Very good break
			99.51	7.03- 6.50	Hg on Pt vs. cal	Good break[c]
			99.66	7.03 -6.50	Hg on Pt vs. cal	Good break[c]
			99.60	7.03- 6.50	Hg on Pt vs. cal	Good break[c]
Mn	MnCl₂	H₂O + pyridine (50–50)	100.0	7.7 - 6.6	Hg on Pt vs. cal	Good break
			100.0	7.7 - 6.6	Hg on Pt vs. cal	Good break
			98.9	7.7 - 6.6	Hg on Pt vs. cal	Good break
	Mn(Ac)₂	H₂O + pyridine + 2 ml. 1N NaOH	99.3	9.0 - 7.4	Hg on Pt vs. cal	Good break[c]
			99.3	9.0 - 7.5	Hg on Pt vs. cal	Good break[c]
Ca	CaCO₃ + HCl	H₂O + pyridine (50–50)	100.23	7.42- 7.31	Hg on Ag vs. cal	Good break
			100.45	7.42- 7.31	Hg on Ag vs. cal	Good break
			99.84	7.42- 7.31	Hg on Ag vs. cal	Good break
			99.55	7.15- 6.83	Hg on Pt vs. cal	Good break
			99.96	7.15- 6.83	Hg on Pt vs. cal	Good break
			98.95	7.15- 6.83	Hg on Pt vs. cal	Good break
Mg	MgCl₂	H₂O + pyridine + 2 ml. of 1N NaOH	100.00	9.2 - 7.4	Hg on Pt vs. cal	Good break
			100.0	9.2 - 7.4	Hg on Pt vs. cal	Good break
Ni	Ni(NO₃)₂	H₂O + pyridine (50–50)	99.71[d]	.....	Hg on Pt vs. cal	Very good break
			99.54[d]	.....	Hg on Pt vs. cal	Very good break
			99.72[d]	.....	Hg on Pt vs. cal	Very good break
Co	Co(NO₃)₂	H₂O + pyridine (50–50)	99.16	.....	Hg on Pt vs. cal	Small break
			100.21	.....	Ag vs. cal	Small break
			100.04	.....	Pt vs. cal	Small break
		Titration of N,N-Di(β-hydroxyethyl)glycine $\begin{pmatrix} HOCH_2CH_2 \\ \qquad\qquad N\text{—}CH_2COOH \\ HOCH_2CH_2 \end{pmatrix}$				
Fe	FeCl₃	H₂O	...[d]	.....	Pt vs. cal	No break[c]
		H₂O + Na₂CO₃	...[d]	.....	Pt vs. cal	No break[c]
		H₂O + NaAc	...[d]	.....	Pt vs. cal	No break[c]
Cu	Cu(NO₃)₂	H₂O + pyridine + 5 ml. of 1.0N NaOH	98.6[d]	11.9 - 8.1	Hg on Pt vs. cal	Good break[c]
		H₂O + pyridine + 4 ml. 1.0N NaOH	96.2[d]	12.1 - 7.7	Hg on Pt vs. cal	Fair break[c]
Hg	Hg(Ac)₂	H₂O + pyridine (50–50)	...[d]	.....	Pt vs. cal	No break[c]
			...[e]	.....	Ag vs. cal	No break[c]
		H₂O + pyridine + 2 ml. 1.0N NaOH	...[d]	.....	Ag vs. cal	No break[c]

tors—the agent used, the metallic ion used, and the conditions, usually pH of the titration. [Nullapons (Antara Chemicals, 435 Hudson St., New York 14, N.Y.) indicates the relative chelating power of various metals with EDTA at various pH's.] Using a certain metallic ion and certain titration conditions, one chelating agent can be determined in the presence of another. An example is shown in Table II. In this case there is a mixture of EDTA and ammonia–triacetic acid which is a mixture sometimes obtained in the production of EDTA. The EDTA in the mixture can be titrated with mercuric ion under conditions which exclude the titration of ammonia–triacetic acid. Then the total of EDTA and ammonia–triacetic acid can be determined by using an ion, such as copper, which titrates both chelating agents The content of ammonia–triacetic acid is then obtained by difference.

It was ascertained later in this work that a zinc titration on such a mixture yields two breaks in the curve (see Figure 2); the first break represents the titration of the EDTA, and the second break represents the titration of the ammonia–triacetic acid. Values shown for the analysis of mixtures using zinc are given in Table II.

Conversely, chelating agent and conditions can be varied so that mixtures of metals can be determined. For instance, mixtures involving iron can be titrated at alkaline pH's where iron

Table I. Titration Results with Various Metal Ions[a] (continued)

Titration of N,N-Di(β-hydroxyethyl)glycine $\left(\begin{array}{c}HOCH_2CH_2 \\ HOCH_2CH_2\end{array}N-CH_2COOA\right)$

Metal Used	Titrant, 0.1N	Solvent System	Found, %[b]	pH Range During Titration	Electrodes	Remarks
Pb	Pb(NO₃)₂	25% H₂O + 25% pyridine + 50% acetone	99.89[d]	7.7 – 6.8	Hg on Pt vs. cal	Fair break
		50% pyridine + 50% acetone	100.76[d]	7.7 – 6.8	Hg on Pt vs. cal	Fair break
Ca	CaCO₃ + HCl	H₂O + pyridine (50–50)	...	.....	Hg on Pt vs. cal	No break[c]

Titration of Ammonia Triacetic Acid $\left(\begin{array}{c}HOOCCH_2 \\ HOOCCH_2\end{array}N-CH_2COOH\right)$

Metal Used	Titrant, 0.1N	Solvent System	Found, %[b]	pH Range During Titration	Electrodes	Remarks
Fe	FeCl₃	H₂O	99.91	7.63– 1.73	Pt vs. cal	Good break
		H₂O + 2 ml. 1.0N NaOH	99.4	9.1 – 2.4	Pt vs. cal	Good break[c]
		H₂O	99.8	3.6 – 2.1	Pt vs. cal	Poor break[c]
		H₂O + 2 ml. 1.0N NaOH	99.4	9.1 – 2.4	Pt vs. cal	Good break[c]
Cu	Cu(NO₃)₂	H₂O + pyridine (50–50)	99.71[d]	7.00– 6.88	Pt vs. cal	Very sharp break
		H₂O + pyridine + 1 ml. 1.0N NaOH	99.0	8.8 – 6.8	Pt vs. cal	Fair break[c]
			99.0	9.1 – 7.0	Hg on Pt vs. ca	Good break[c]
Hg	Hg(Ac)₂	H₂O + pyridine + 4 ml. 1.0N NaOH	99.8	11.6 – 7.8	Ag vs. cal	Poor break[c]
			99.8	11.6 – 7.9	Ag vs. cal	Poor break[c]
			99.8	11.8 – 8.1	Ag vs. cal	Poor break[c]
Zn	Zn(NO₃)₂	H₂O + pyridine (50–50)	99.0	7.63– 7.35	Hg on Pt vs. cal	Good break
			98.6	7.63– 7.35	Hg on Pt vs. cal	Good break
			99.0	7.60– 7.30	Hg on Pt vs. cal	Good break
			99.44	7.90– 7.30	Hg on Pt vs. cal	Good break[c]
			99.40	7.90– 7.30	Hg on Pt vs. cal	Good break[c]
			99.44	7.90– 7.30	Hg on Pt vs. cal	Good break[c]
Pb	Pb(NO₃)₂	H₂O + pyridine (50–50)	98.82	7.42– 6.88	Hg on Pt vs. cal	Good break[c]
			99.01	7.42– 6.88	Hg on Pt vs. cal	Good break[c]
			98.82	7.42– 6.88	Hg on Pt vs. cal	Good break[c]
Mn	Mn(Ac)₂	H₂O + pyridine + 2 ml. 1.0N NaOH	97.5	10.5 – 7.9	Hg on Pt vs. cal	Fair break[c]
Ca	CaCO₃ + HCl	H₂O + pyridine (50–50)	...	.....	Hg on Pt vs. cal	No break[c]
Mg	MgCl₂	H₂O + pyridine + 2 ml. 1.0N NaOH	...	.....	Hg on Pt vs. cal	No break[c]
Ni	Ni(NO₃)₂	H₂O	...	.....	Hg on Pt vs. cal	No break[c]
		H₂O + pyridine (50–50)	99.41	.....	Hg on Pt vs. cal	Good break
			99.13	.....	Hg on Pt vs. cal	Good break
			99.25	.....	Hg on Pt vs. cal	Good break
Co	Co(NO₃)₂	H₂O + pyridine (50–50)	99.72	.....	Hg on Pt vs. cal	Good break
			99.72	.....	Hg on Pt vs. cal	Good break
			99.66	.....	Hg on Pt vs. cal	Good break

[a] All chelating agents were prepared and recrystallized in the free acid form. Their purity was checked by C, H, and N analysis and by titration with standard alkali.
[b] All chelating agents used were in form of disodium salt. except where designated otherwise. All salt prepared from pure free acids.
[c] Run on macro scale. All other determinations run on semimicro scale.
[d] Free acid.
[e] Calomel electrode with KNO₃ bridge (5).
[f] Tetrasodium salt.

Table II. Titration of Ethylenediaminetetraacetic Acid–Ammonia Triacetic Acid Mixture with Various Metal Ions[a]

Metal Used	Titrant, 0.1N	Compn. of Mix, Wt. %		Found, %		pH Range During Titration	Electrodes	Remarks
		EDTA	ATA	EDTA	ATA			
Hg	Hg(Ac)₂	66.9	33.1	66.3	...	7.3–6.4	Ag vs. cal[b]	1 good break
		83.49	16.51	84.10	...	7.2–6.5	Ag vs. cal[b]	1 good break
		95.29	4.71	95.99	...	7.2–6.5	Ag vs. cal[b]	1 good break
		Mole		Mole				
Cu	Cu(Ac)₂	0.0003428	0.0005000	0.0008419[c]		...	Pt vs. cal	Very good break
		0.0001714	0.0003000	0.0004705[c]		...	Pt vs. cal	Very good break
		0.0001028	0.0003000	0.0004005[c]		...	Pt vs. cal	Very good break
		%		%				
Zn	Zn(NO₃)₂	79.54	20.46	79.86	20.46	7.2–6.5	Hg on Pt vs. cal	2 very good breaks
		84.74	15.26	85.25	15.21	7.2–6.5	Hg on Pt vs. cal	2 very good breaks
		91.81	8.19	92.18	8.24	7.2–6.5	Hg on Pt vs. cal	2 very good breaks

[a] Solvent system used in this series, 1 to 1 pyridine-H₂O. [b] Calomel electrode using KNO₃ bridge (5). [c] Total moles EDTA and ATA.

does not chelate. If the mixtures involve mercury, calcium, or magnesium, the titrations can be run using either ammonia–triacetic acid or N,N-di(β-hydroxyethyl)glycine, which show no chelation for these ions under certain conditions. Table III shows results of analysis of mixtures of copper-calcium or copper-iron using these described approaches. It is possible to choose electrode systems which are specific for certain metals (8) in a mixture, although this was not tried in this series of experiments. A mixture of calcium, lead, and zinc ions was titrated with EDTA to see if a differential titration could be obtained. In pyridine-water all three were titrated with only one break in the curve. It may well be possible, however, to obtain a differential titration

of mixtures of ions by selecting the proper titrant, solvent, electrodes, and conditions.

METAL CHELATES

The approach described makes it possible to analyze a metal chelate sample to determine the amount of any free chelating agent or any free metal ion that may be in the sample. In titrating any free chelating agent in a metal chelate, the metal ion used as titrant should be the same metal used in the chelate. This is necessary because if a metal ion used as titrant forms a more stable chelate with the agent used in the metal chelate, the metal used as titrant will replace the metal in the chelate, and the final result of the titration will not represent the free chelating agent

but the total chelating agent (free plus combined) in the sample. The same is true in titrating free metal ion in a metal chelate; the chelating agent used as titrant should be the same as that used in the metal chelate.

EDTA-metal complexes may also contain the ammonia–triacetic acid–metal complex, for most commercially available EDTA contains some ammonia–triacetic acid. If an EDTA solution is used to titrate the excess metal ion in an EDTA-metal complex containing ammonia–triacetic acid–metal complex, then the EDTA added will not only complex the free metal ion but also remove the metal from the ammonia–triacetic acid causing the value for free metal to be high. This is due to the fact that EDTA is a much stronger complexing agent than ammonia–triacetic acid.

In the case, then, of EDTA–metal complexes it would be advisable to titrate the excess metal with a standard solution of ammonia–triacetic acid, as this chelates only the free metal and cannot break up any of the complexes in the system. However, titrating a metal with ammonia–triacetic acid results in poorer titration breaks than titrating this chelating agent with a metal. For best results, a known amount of ammonia–triacetic acid is added to the sample, and the excess is titrated with the same metal used in the complex. The amount of ammonia–triacetic acid consumed is a measure of the free metal in the sample. Metals such as calcium, magnesium, or mercury, which show little or no chelation with ammonia–triacetic acid, cannot be handled in this manner.
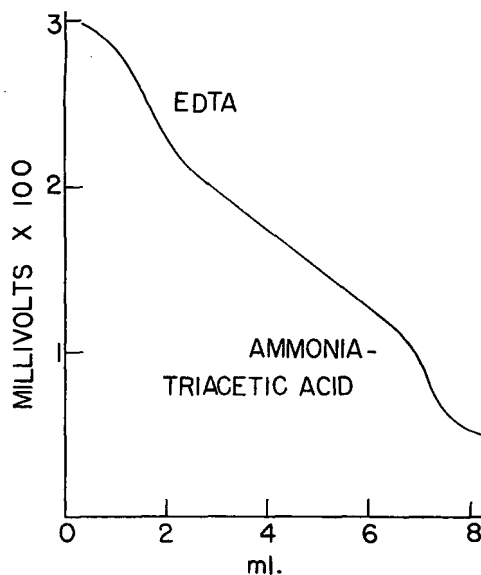

Figure 2. Titration of ethylenediamine-tetraacetic acid–ammonia–triacetic acid mixtures with zinc

Mercury on platinum vs. calomel electrode system and 1 to 1 pyridine-water solvent system

Table III. Analysis of Copper-Calcium and Copper-Iron Mixtures

Titrant, 0.1N	Metal Used, Millimole			Metal Found, Millimole	Remarks
	Cu	Ca	Fe		
	Copper-Calcium Mixtures[a]				
EDTA	0.3018	0.3096	....	0.6088 total	Good break
	0.3018	0.2064	....	0.5081 total	Good break
	0.3018	0.1032	....	0.4059 total	Good break
ATA	0.3018	0.3096	....	0.2966 Cu	Poor break
	0.3018	0.2064	....	0.3001 Cu	Poor break
	0.3018	0.1032	....	0.2984 Cu	Poor break
	Copper-Iron Mixtures				
EDTA[b]	0.3018	....	0.3162	0.3018 Cu	Good break
	0.3018	....	0.6324	0.3056 Cu	Good break
	0.3018	....	0.1054	0.3013 Cu	Good break
ATA[c]	0.3027	....	0.1956	0.4930 total	Good break
	0.3066	....	0.3162	0.6211 total	Good break
	0.3027	....	0.0978	0.3988 total	Good break

[a] Ca content can be obtained by difference. Pyridine-water, 1 to 1, was used as solvent, and Hg on Pt vs. calomel electrodes were used.
[b] Pyridine, 1 to 1, was used as solvent during tiration with EDTA. This permits titration of copper alone. Water alone was used as solvent during ATA titration. Ag vs. calomel electrodes were used in both titrations. Iron content can be obtained from difference between ATA and EDTA titrations.
[c] Excess ATA added and excess back-titrated with 0.1N cupric acetate.

The metal chelate content of a sample can be determined in one of several ways. The free metal ion in a sample of metal chelate is first determined as just outlined. Then a separate sample is digested by an acid (sulfuric acid or a mixture of sulfuric and nitric acids) digestion to destroy the organic portion of the sample, and the total metal is determined by standard inorganic methods or by titration with EDTA. The difference between free metal ion and total metal present is taken as the metal chelate. Another approach is the determination of free chelating agent as outlined, then titration of a separate sample with a metal that forms a more stable complex with the chelating agent than the metal already on the chelate. For example, the calcium–EDTA complex can be titrated with iron at somewhat acid pH's, and the iron replaces the calcium on the chelate (see Figure 3). Some results are shown in Table IV.

A modification of this approach is to vary the titration conditions to destroy the metal chelate and then titrate the freed chelating agent with a metal ion that will complex under these conditions. For example, iron-EDTA under certain alkaline conditions is not stable, and the iron will form the hydroxide. The freed EDTA can be titrated under alkaline conditions using copper (see Table IV). Mercuric ion can also be used instead of copper.

When the metal chelate contains a mixture of chelating agents such as EDTA–ammonia triacetic acid, care should be exercised in choosing conditions and titrants. In the case of an iron chelate made from EDTA containing ammonia–triacetic acid, the amount of each chelate can be determined as follows. Any free iron or excess chelating agent is determined as described above. A separate

Table IV. Titration of Ethylenediaminetetraacetic Acid Metal Chelates

Metal Used	Titrant, 0.1N	Solvent System			Used, Mole	Found, %	Electrodes	Remarks
		H₂O, ml.	Pyr., ml.	TSP[a] g.				
		Ethylenediaminetetraacetic Acid–Iron Chelate (NaFe-EDTA)						
Hg	Hg(Ac)₂	35	15	1.0	..	98.56	Ag vs. cal[b]	Very good break
		35	0	2.0	..	100.30	Ag vs. cal[b]	Poor break
		35	15	1.5	..	100.25	Ag vs. cal[b]	Very good break
		35	15	2.0	..	99.25	Ag vs. cal[b]	Very good break
Cu	Cu(Ac)₂	35	15	1.0	..	100.02	Pt vs. cal	Very sharp break
		35	15	1.5	..	99.83	Pt vs. cal	Very sharp break
		35	15	2.0	..	99.54	Pt vs. cal	Very sharp break
		Ethylenediaminetetraacetic Acid–Calcium Chelate (EDTA-CaNa₂)						
Fe	FeCl₃	H₂O			..	98.70	Pt vs. cal	Very good break
					..	98.81	Pt vs. cal	Very good break
		Mixture of Ethylenediaminetetraacetic Acid–Iron Chelate and Ammonia–Triacetic Acid–Iron Chelate						
					EDTA-NaFe	ATA-Fe	Mole	
Hg	Hg(Ac)₂	35	15	2.0	0.000247	0.000128	0.000244[c]	Ag vs. cal	Very good break
Cu	Cu(Ac)₂	35	15	2.0	0.000247	0.000128	0.000378[d]	Ag vs. cal	Poor break

[a] Trisodum phosphate.
[b] Calomel electrode, using KNO₃ bridge (5).
[c] EDTA complex alone.
[d] Total of EDTA and ATA complexes.

sample is treated with trisodium phosphate in a pyridine-water system. The alkalinity of this system breaks the iron chelate. The liberated chelating agents can be titrated with mercury which yields only the EDTA content. A separate, similarly treated sample can be titrated with copper to yield the total of EDTA and ammonia triacetic acid. The various components of the sample can then be obtained from these data. Table IV indicates some results obtained on synthetic mixtures of an iron chelate composed of a mixture of the EDTA and ammonia-triacetic acid complexes.

Preparations of Titrants

1.	$0.1N$ FeCl$_3$	27 grams ferric chloride hexahydrate dissolved in water.
2.	$0.1N$ Cu(NO$_3$)$_2$	6 grams of copper dissolved in concentrated nitric acid. If pure copper is used for this solution, the strength of the solution can be determined from the weight of copper used.
3.	$0.1N$ Hg(CH$_3$COO)$_2$	31 grams mercuric acetate dissolved in water and a few milliliters of acetic acid to clear solution.
4.	Zn(NO$_3$)$_2$	30 grams zinc nitrate hexahydrate dissolved in water.
5.	$0.1N$ Pb(NO$_3$)$_2$	33 grams lead nitrate dissolved in water.
6.	$0.1N$ MnCl$_2$	20 grams manganese dichloride tetrahydrate dissolved in water.
7.	$0.1N$ Mn(CH$_3$COO)$_2$	24.5 grams manganous acetate tetrahydrate dissolved in water.
8.	$0.1N$ CaCO$_3$	10 grams calcium carbonate dissolved in concentrated hydrochloric acid.
9.	$0.1N$ MgSO$_4$	25 grams magnesium sulfate heptahydrate dissolved in water.
10.	$0.1N$ Co(NO$_3$)$_2$	29.1 grams cobaltous nitrate hexahydrate dissolved in water.
11.	$0.1N$ Ni(NO$_3$)$_2$	29.1 grams nickel nitrate hexahydrate dissolved in water.

In each case the titrant was diluted to 1 liter with water.

STANDARDIZATION OF TITRANTS

All the titrants used can be standardized by inorganic means. A more convenient and much more rapid method of standardization is to titrate a known amount of disodium EDTA dihydrate potentiometrically, using the electrodes and solvent system for each metal from Table I. This EDTA salt can be used as a primary standard (2, 3).

PREPARATION OF ELECTRODES

Mercury on Platinum Electrode. A 1.5 × 1.5 cm. piece of platinum foil is welded to a piece of platinum wire about 10 to 12 cm. long. Two of these electrodes are placed in a 1 to 3% mercuric acetate solution containing 3 to 5 ml. of concentrated nitric acid per 100 ml. The electrodes are then connected to a 4- to 6-volt direct current supply and allowed to plate from 15 to 20 minutes. The electrode connected to the negative terminal will then be covered with a smooth coating of mercury.

The platinum should be cleaned thoroughly before attempting to plate with mercury. This can be done by dipping the electrode into concentrated nitric acid and burning in a Bunsen burner flame. Frequent replating is advisable to keep the breaks as large and sharp as possible.

Platinum Electrode. The platinum electrode connected to the positive terminal when preparing the mercury-platinum electrode is cleaned by dipping in concentrated nitric acid and burning in a Bunsen burner flame. This is then used for all the titrations involving a platinum electrode.

Silver Electrode. A piece of silver wire 10 to 12 cm. long is used for all titrations involving the silver electrode.

Calomel Electrode. For all titrations, except the semimicro mercury titrations, a Beckman No. 1170 saturated calomel electrode is used.

Calomel Electrode with Potassium Nitrate Bridge. This electrode (5) is used to keep the solution free of chloride ion contamination, which could give erroneous results in the micro scale method when mercury(II) ion is used as titrant. On the macro scale, the calomel electrode described above was used for mercury titrations and operated well.

PROCEDURE

For semimicroanalyses, a 0.1-gram sample is used; for macro-analyses, a 1.0-gram sample is taken. If the agent is in the free

acid form, it can be converted to the disodium salt by adding 2 moles of sodium hydroxide per mole of agent. The sample is dissolved in the sodium hydroxide solution (heating will help in most cases). Converting to the disodium salt is done for solubility purposes. When pyridine plus water is used as the solvent for the titration, converting to the disodium salt is not necessary, although in most cases it improves the breaks obtained.

For determining the amount of chelating agent in EDTA-iron chelate the procedure is varied somewhat:

One-tenth gram of iron chelate is dissolved in 35 ml. of water. From 1 to 2 grams of trisodium phosphate is added. After the chelate and the trisodium phosphate are dissolved, 15 ml. of pyridine is added and the solution heated at 60° to 70° C. for 5 minutes. After cooling to room temperature, the solution is titrated with mercury or copper ion using the electrodes obtained from Table I. Further details and results can be seen in Table IV.
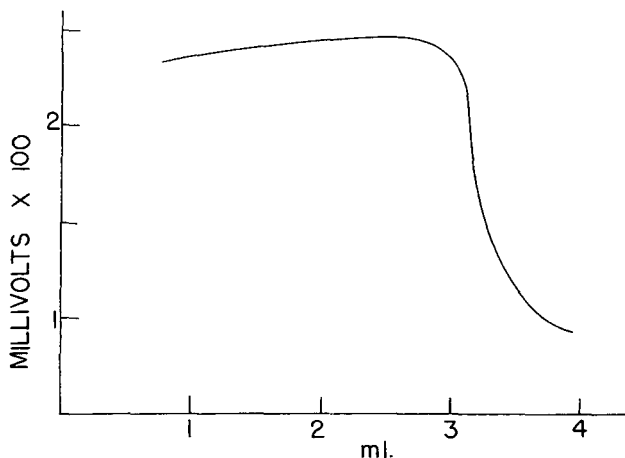
Figure 3. Ethylenediaminetetraacetic acid–calcium chelate titrated with iron(III) chloride

For semimicroanalyses the sample solution should consist of 30 to 40 ml. in a 150-ml. beaker. The electrode systems used can be obtained from Table I. The potentiometric titration is carried out using a semimicroburet.

For macrodeterminations, 60 to 80 ml. of solution is used. A Model H2 Beckman pH meter is used for the titrations.

LITERATURE CITED

(1) Biedermann, W., and Schwarzenbach, G., *Chimia (Switz.)*, 2, 56 (1948).
(2) Blaedel, W. J., and Knight, H. T., ANAL. CHEM., 26, 741 (1954).
(3) *Ibid.*, p. 743.
(4) Blaedel, W. J., and Malmstadt, H. V., *Ibid.*, 24, 455 (1952).
(5) Edsberg, R. L., Eichlin, D., and Garis, J. J., *Ibid.*, 25, 798 (1953).
(6) Furness, W., Crawshaw, P., and Davies, W. C., *Analyst*, 74, 629 (1949).
(7) Hall, L. H., Gibson, J. A., Jr., Wilkinson, R. P., and Phillips, H. O., ANAL. CHEM., 26, 1484 (1954).
(8) Kolthoff, I. M., and Furman, N. H., "Potentiometric Titrations," 2nd ed., Wiley, New York, 1931.
(9) Laitinen, H. A., and Sympson, R. F., ANAL. CHEM., 26, 556 (1954).
(10) Přibil, R., Koudela, Z., and Matyska, B., *Collection Czechoslov. Chem. Communs.*, 16, 80 (1951).

Coulometric Titrations with Electrolytically Generated Uranous Ion

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Both dilute ceric sulfate and dilute potassium dichromate solutions were titrated with uranium(IV). The uranium(IV) was generated electrolytically with constant current at a platinum cathode, which was immersed directly in the test solution. The end point was detected by means of a platinum wire–saturated calomel electrode system, and the electrolysis time was measured automatically.

THE titration results show that cerium(IV) and chromium(VI) ions can be reduced with 100% current efficiencies by use of uranium(IV) as a coulometric intermediate.

Belcher, Gibbons, and West (1) have reported the use of uranium(IV) as a volumetric reducing agent. They concluded that uranium(IV) in acid solution is a moderately strong reductant, which can be used for direct titration of the stronger oxidants, but which in practice is less convenient to use than are other existing reagents. This inconvenience is due to the manner in which uranium(IV) solutions must be prepared and stored and to their instability. The present work was undertaken in order to determine the feasibility of using electrolytically generated uranium(IV) as an intermediate in coulometric reductions, thus eliminating most of the disadvantages associated with volumetric solutions of this ion. Such a coulometric titration method should be especially useful in the determination of strong oxidants which may be present as minor constituents or impurities in uranium solutions; the uranium in the sample itself could then serve as the source of uranium(IV).

The determination of uranium coulometrically has been studied by Carson (2) and by Furman, Bricker, and Dilts (3).

APPARATUS AND REAGENTS

Electrical Apparatus. The automatic coulometric titrator (ACT) used in this work was developed and built by Stelzner, Fisher, and Kelley of the Instrumentation Group of the Analytical Chemistry Division at this laboratory and is to be described fully in a forthcoming paper (5). It electronically provides and maintains a constant—i.e., to better than $\pm 0.1\%$—generating current, which can be adjusted continuously to any value between zero and 10 ma. An ammeter is included in the circuitry and indicates approximately the current flowing. The exact value of the generating current is computed from Ohm's law by use of the measured potential drop across a standard 100-ohm ($\pm 0.5\%$) resistor that is incorporated in the generating circuit; the potential drop is measured by means of a Rubicon potentiometer (Rubicon Co., Catalog No. 2731).

The automatic coulometric titrator, in conjunction with a Leeds & Northrup pH meter (Catalog No. 7664), also provides for automatic stoppage of the generating current at any preselected end-point potential between -1.4 and $+1.4$ volts. The potential of the solution is constantly monitored during the titration. When the potential, as indicated on the pH meter, reaches the value that has been preselected as the end-point potential, the automatic coulometric titrator stops the flow of generating current. Should the potential fall below the cutoff value, the flow of current begins and continues until the cutoff potential is again attained. An electric timer (Standard Electric Time Co., Type S-10) calibrated in 0.1-second intervals is incorporated; it measures the time of current flow through the generating circuit.

This instrument was designed for coulometric titrations of 0.5 to 20 μeq. of substances. In practice, a portion of the substance being determined is first titrated automatically to the end point; the sample to be analyzed is then added and automatically titrated back to the end point. The same constant generating current is used in each of these titrations so that any errors caused of over- or undertitration are cancelled. At constant current, the time required to titrate the sample back to the end point indicates

the amount of unknown substance titrated. The operation of the automatic coulometric titrator has been very satisfactory in the coulometric generation of several intermediates by this technique.

Mechanical Apparatus. Titrations were made in a titration cell that was similar to the cell used by Furman, Cooke, and Reilley (4). A 15-ml. weighing bottle was fitted tightly with a rubber stopper that supported two salt bridges, two electrodes, and a carbon dioxide inlet tube. The generating electrode system consisted of a 1×2 cm. platinum flag (cathode) mounted vertically inside the cell with its "mast" protruding through the rubber stopper and a platinum gage cylinder (anode) 1 cm. high and 1 cm. in diameter immersed in a 3% ammonium sulfate–3% sulfuric acid solution that was connected to the test solution by means of one of the 3% ammonium sulfate–4% agar salt bridges. The indicating electrode system consisted of a 6-inch length of 16-gage platinum wire electrode (indicator), mounted vertically through the rubber stopper, and a saturated calomel electrode (reference) that was connected to the test solution through the second salt bridge. The solution was stirred with a magnetic stirrer.

Reagents. A $0.1000N$ potassium dichromate stock solution was prepared from National Bureau of Standards reagent No. 136a by weighing. Solutions of the desired chromium(VI) concentration were prepared from this stock solution by dilution.

A ceric sulfate stock solution was prepared from reagent grade ceric ammonium sulfate to be $0.1N$. This solution was standardized against National Bureau of Standards arsenious oxide reagent No. 83a with osmium tetroxide as the catalyst. The average normality, calculated from the results of five determinations, was 0.1015. Solutions of the desired cerium(IV) concentration were made from this stock solution by dilution.

A $0.2N$ uranyl sulfate solution, which was $0.5N$ in sulfuric acid, was used to supply the uranium(VI) to be reduced. This solution was prepared by heating approximately 13 grams of pure uranium trioxide in 50 ml. of water that contained 9 ml. of concentrated sulfuric acid until dissolution was complete, and then diluting the solution to a final volume of 500 ml. The final solution was shown by gravimetric analysis to contain 21.9 mg. of uranium per milliliter. Calibrated volumetric glassware was used when standardizing the stock solutions.

PROCEDURE AND RESULTS

Before automatic titrations can be made with the Stelzner titrator, the end point or cutoff potential must be established. This was done by coulometrically titrating a known quantity of the substance to be determined and plotting the indicated potential of the solution vs. current generation time on rectilinear graph paper. The cutoff potential was then taken as that potential at which the rate of change in potential per unit time was greatest. In the present work, the cutoff potential was taken as 0.880 volt vs. the S. C. E. for titrations of cerium(IV), whereas 0.180 volt vs. the S. C. E. was used as the cutoff potential for titrations of chromium(VI). Although this cutoff potential for chromium(VI) differs from the indicated calculated equivalence point of 0.68 volt by approximately 0.5 volt, 0.18 volt was found to be the point of maximum potential inflection. The choice of the potential at which cutoff occurred was not very critical, because large (300 to 500 mv.) and rapid potential changes occurred at the end points of these titrations, and the electrolyte in which a sample was to be titrated was first brought to the cutoff potential before the sample was titrated.

In order to make an automatic titration, the instruments were turned on and allowed to warm up for 10 minutes. During this time, the cutoff control was adjusted so that cutoff occurred at the desired potential, the generating current was adjusted to a value such that about 200 seconds would be required for titration of the sample, and the gas flow was adjusted to provide an adequate carbon dioxide blanket for the solution. Three milliliters of the uranium solution and 1 ml. of the sample were then placed in the weighing bottle together with a small glass-covered stirring

bar. After the vessel was positioned so that contact was made between the solution and the various electrodes and after the stirring motor was adjusted to give rapid mixing, the generating current was turned on and the titration was allowed to proceed automatically to cutoff. During this preliminary titration, the generating current was adjusted to the exact value desired as indicated by the measured internal-resistance drop across a standard 100-ohm resistor. When the flow of generating current stopped, a 1-ml. portion of the sample was accurately pipetted in the solution in the cell. The clock was then set at zero and the electrolysis was begun again and allowed to continue automatically until cutoff was again attained. The time indicated on the clock and the known generating current were then used to calculate via Faraday's law the amount of substance titrated as follows:

$$\text{Equivalents electrolyzed} = \frac{I(\text{amp.}) \times t(\text{sec.})}{96{,}493}$$

$$\text{Micrograms of chromium(VI)} = 0.1796 \times I(\text{ma.}) \times t(\text{sec.})$$

$$\text{Milligrams of cerium(IV)} = 1.452 \times I(\text{ma.}) \times t(\text{sec.})$$

A second 1-ml. portion of the sample could then be added to the solution, and the titration of it performed as above. Consecutive titration of more than three 1-ml. portions of the sample in one pretitrated uranyl sulfate solution was not satisfactory because the total solution volume became too large for adequate mixing. The electrodes and vessel were rinsed thoroughly with distilled water between each series of titrations, and the electrodes were left immersed in distilled water when they were not in use.

The results of titrations of known quantities of chromium(VI) and of cerium(IV) by the above procedure are given in Tables I and II.

Table I. Results of Titrations of Potassium Dichromate

(Three 1-ml. aliquots of four solutions containing varying amounts of potassium dichromate stock solution were titrated consecutively in $0.2N$ uranyl sulfate. Total volume was 5 to 7 ml. Cutoff potential was 0.180 volt vs. the S.C.E.)

No. of Aliquots	Generating Current, Ma.	Chromium(VI), γ Taken	Found (av.)	Relative Std. Dev. γ	%	Max. Dev. from Av. γ	%
12	7.000	260.1	261.1	0.48	0.18	−0.9 +0.8	−0.3 +0.3
12	5.000	173.4	173.6	0.64	0.37	−1.4 +0.7	−0.8 +0.4
13	2.000	69.3	69.3	0.28	0.41	−0.4 +0.4	−0.6 +0.6
12	0.500	17.3	17.5	0.21	1.22	−0.5 +0.2	−2.8 +1.2

The data were analyzed statistically to determine whether a bias existed in the method.

The bias B was calculated by the formula $B = E \pm \dfrac{ts}{\sqrt{N}}$

when

$$E = \frac{(\overline{X} - Y)}{Y} \, 100$$

$\overline{X}$ = av. value
Y = known value

$\dfrac{ts}{\sqrt{N}}$ = relative standard error

Because E ($= -0.3\%$) was less than $\dfrac{ts}{\sqrt{N}}$ ($= \pm 0.5\%$) no bias was considered to exist between the amount of chromium and cerium found and the known amount that was titrated.

DISCUSSION

The concentration of uranium in the uranyl sulfate supporting electrolyte solution was varied from 0.1 to $1.0N$. An increase in

uranium concentration resulted in somewhat smaller potential changes at the end point, but the cutoff potential remained essentially constant. The titration was therefore not adversely affected. The free acid concentration in the uranyl sulfate solution may vary from 0.2 to $1.0N$, the optimum being $0.5N$. Hydrolysis sometimes occurred at acidities less than $0.2N$, whereas acidities greater than $1.0N$ resulted in a slight loss in precision. Therefore, the acidity of the sample should be such that no large change in acidity occurs upon the addition of the sample to the pretitrated uranyl sulfate solution.

Table II. Results of Titrations of Ceric Sulfate

(Three 1-ml. aliquots of four solutions containing varying amounts of ceric sulfate stock solution were titrated consecutively in $0.2N$ uranyl sulfate. Total volume was 5 to 7 ml. Cutoff potential was 0.880 volt vs. the S.C.E.)

No. of Aliquots	Generating Current, Ma.	Cerium(VI), Mg. Taken	Found (Av.)	Relative Std. Dev. Mg.	%	Max. Dev. from Av. Mg.	%
12	7.500	2.277	2.276	0.006	0.27	−0.011 +0.008	−0.5 +0.4
10	5.000	1.423	1.421	0.005	0.37	−0.008 +0.009	−0.6 +0.6
11	2.500	0.712	0.716	0.003	0.46	−0.006 +0.005	−0.8 +0.7
15	0.500	0.142	0.146	0.002	1.42	−0.004 +0.002	−2.5 +1.6

Some work on the determination of iron(III) was done. Because of the sluggishness of the reaction between iron(III) and uranium(IV), it was necessary to carry out this titration at an elevated temperature. At 60° C. the speed of the reaction was increased to the extent that titrations could be made, but only with poor precision. With the setup described herein, elevation of the temperature above 60° C. caused bubble formation and volume losses by evaporation. This titration should prove feasible if a setup of larger scale than that of the present one could be used; it would then be possible to use larger volumes of solution, and the temperature could be elevated to 80° to 90° C. without difficulty. Although the work reported here was done entirely with apparatus designed for the titration of small quantities of substances in small volumes of solution, there is no apparent reason why this method could not be used on a larger scale.

There is evidence that some uranium(III) is formed during the electrolysis—e.g., upon titration past the end point, the potential of the solution falls to a much lower value than would be expected if only uranium(IV) were generated. A uranous sulfate solution was prepared by passing the uranyl sulfate solution through a Jones reductor and then aerating it to give a uranium(IV) solution. When this solution was electrolyzed the potential dropped to −0.150 volt vs. the S. C. E., indicating that some uranium(III) was produced. Further study of the reduction of uranium(IV) is warranted.

LITERATURE CITED

(1) Belcher, R., Gibbons, D., and West, T. S., ANAL. CHEM., 26, 1025 (1954).
(2) Carson, W. N., Jr., Ibid., 25, 466 (1953).
(3) Furman, N. H., Bricker, C. E., and Dilts, R. V., Ibid., 25, 482 (1953).
(4) Furman, N. H., Cooke, W. D., and Reilley, C. N., Ibid., 23, 945 (1951).
(5) Stelzner, R. W., Fisher, D. J., and Kelley, M. T., unpublished report.

Precision Coulometric Titrator

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An instrument for carrying out coulometric titrations in an industrial laboratory within 0.1% precision and accuracy gives constant currents up to 450 ma. The electric time clock is operated by a frequency standard, so that the instrument can be used on industrial power systems where the frequency is not rigidly controlled.

A N INSTRUMENT was needed for performing coulometric titrations at constant currents of several hundred milliamperes so that as much as 1 meq. of material could be titrated in a reasonably short time. The constant current supply of Reilley, Adams, and Furman (6) was modified to obtain currents up to 450 ma. It was necessary to employ a tuning fork source as a frequency standard for operation of the electric time clock, as available plant power frequency variations caused errors in time measurements of ±0.5 to 1% relative. Fry and Baldeschwieler (3) indicate that utility system power may vary by ±0.2 cycle, resulting in ±0.3% error in time. Craig, Satterthwaite, and Wallace (2) show that frequency errors in time are less than 0.2% for the Pittsburgh area.

INSTRUMENTATION

The circuit for the constant current supply is shown in Figure 1. Both the power transformer and choke in the rectifier section are rated at 500 ma. A pair of 866/866A rectifier tubes was chosen, because of their high current capacity and their inherently better regulation characteristics as compared with vacuum rectifiers. The single L section choke input filter yields a satisfactory ripple value. The thermal delay relay (30 seconds) is used in the interest of rectifier life. The current control section is an exact duplication of Reilley's circuit with the addition of two series regulator tubes and additional lamp bulbs (series load resistors) to handle the higher currents encountered. Physically, the rectifier section was constructed on a separate chassis and connected to the control section by a four-conductor cable. All output connections and controls with the exception of the main power switch are located on the controller chassis. A 500-ma. meter was provided for rough indication of the current level.

A Riverbank standard tuning fork (Cenco, 60 cycles, 5 p.p.m

precision, rated at 40-volt, 2-watt output) was employed for precise frequency control in operating the time clock. The power amplifier of the Cenco Riverbank source was redesigned for 115-volt output. A separate power supply for the above power amplifier was constructed as a separate unit together with the direct current source for operation of the clock clutch. The schematics of the revisions are not given, since a commercial frequency unit (Type 2005, American Time Products, Inc.) is available in a convenient package for direct operation of the clock. A 65-ma. selenium rectifier, a 10-henry choke and a 10 × 10 μfd condenser were used in the construction of the direct current source for the time clock clutch.

The electric time clock operated by the above frequency standard (Model S-1, Standard Electric Time Co.) has a capacity of 60 seconds with 0.01-second divisions and is provided with a direct current clutch having a start-and-stop operation error of ±0.002 second. Another clock (Standard Electric Time Co., SM-60), which was operated by plant power, was used to indicate minutes. The accuracy of the timer was checked by comparing with signals sent out by the Bureau of Standards Station WWV.

The circuit for the various component parts of the titrator is shown by the block diagram in Figure 2. These components were assembled in a standard 19-inch rack cabinet. Figure 3 is a photograph of the front panel showing the various controls. The equipment was provided with a connector for using the Beckman autotitrator as an end-point detector for automatic coulometric titrations as suggested by Lingane (4). The current was determined accurately by measuring the IR drop across a standard resistor in series with the electrolysis cell.

PERFORMANCE OF INSTRUMENT

The data in Table I show errors in time to be less than 0.1% (relative) in all cases where the timer was operated with a standard tuning fork. Most of this error for the short time periods is probably due to human error in starting and stopping the clock at the beginning and end of the Bureau of Standards time signal. The constant current supply maintains a current constant to considerably less than 0.1% for all current levels. At the 263-ma. setting, current measurements made during actual coulometric titrations over a 4- to 6-hour time period were all much less than 0.1% (maximum spread). Table II indicates the accuracy of milliequivalents determined from the current and time measurements as compared with the milliequivalents of silver deposited by passing the current through a silver coulometer.

The performance of the titrator for the coulometric titration of Bureau of Standards potassium dichromate with electrolytically generated ferrous ion (1) is shown by the results of Table III. The end point for the first five titrations shown in Table III was

Table I. Accuracy of Time

Time Interval (Between Station WWV Signals), Minutes	Electric Time Clock (Model S-1) (Operated by Standard Tuning Fork), Error in Seconds
5	−0.21
5	−0.05
5	−0.27
5	−0.02
5	−0.04
5	−0.03
4	−0.14
10	−0.07
15	−0.08
20	−0.10
25	+0.08
60	−0.46
60	−0.79

Table II. Comparison of Current × Time Relationship with Silver Coulometer Data

Current, Ma.	No. of Measurements	Maximum Spread, % Relative	Time, Seconds	Meq., $\frac{i \times t}{96.5}$	Meq., Silver Coulometer	Difference, % Relative
157.1	30	0.09	1805.0	2.938	2.937	+0.04
265.8	25	0.04	1500.0	4.132	4.132	0.00
296.3	14	0.04	918.3	2.819	2.822	−0.09
434.0	12	0.05	720.1	3.238	3.234	+0.12

Table III. Titration with Electrolytically Generated Ferrous Ion

Potassium Dichromate Taken, Meq.	Current, Ma.	Time, Seconds	Potassium Dichromate Found, Meq.	Error, %
0.6261	149.7	403.0	0.6251	−0.16
0.6261	149.7	403.2	0.6254	−0.11
0.6261	149.7	404.2	0.6269	+0.13
0.6261	149.7	404.3	0.6271	+0.16
0.6261	148.6	406.2	0.6253	−0.13
0.6261[a]	149.7	404.2	0.6269	+0.13
0.6261[a]	149.7	405.3	0.6278	+0.27

[a] 100 starts and stops.
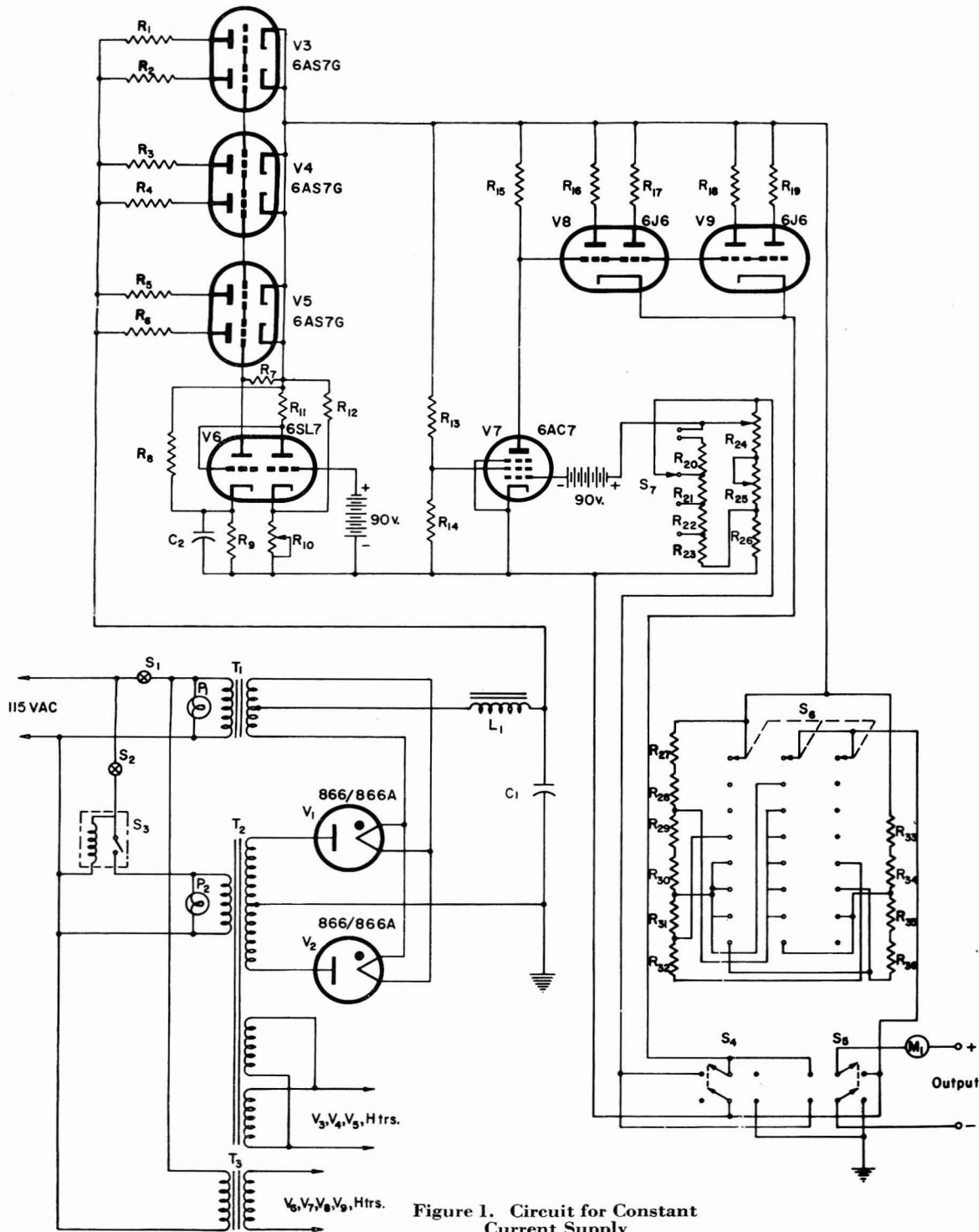
Figure 1. Circuit for Constant Current Supply

R_1 to R_6.	100-ohm, 1-watt carbon resistors
R_7, R_{11}, R_{15}.	470,000-ohm, 2-watt carbon resistors
R_8, R_{12}.	12,000-ohm, 25-watt wire-wound resistors
R_9.	15,000-ohm, 25-watt wire-wound resistor
R_{10}.	10,000-ohm, 25-watt wire-wound variable resistor
R_{13}.	100,000-ohm, 20-watt wire-wound resistor
R_{14}.	50,000-ohm, 20-watt wire-wound resistor
R_{16} to R_{19}.	2700-ohm, 1-watt carbon resistors
R_{20}.	7500-ohm; two 15,000-ohm, 20-watt wire-wound resistors in parallel
R_{21}.	4000-ohm; four 4000-ohm, 20-watt wire-wound resistors in series-parallel
R_{22}.	2000-ohm; four 2000-ohm, 20-watt wire-wound resistors in series-parallel
R_{23}.	1000-ohm; four 1000-ohm, 20-watt wire-wound resistors in series-parallel
R_{24}.	70,000-ohm, 7-watt wire-wound potentiometer
R_{25}.	10,000-ohm, 7-watt wire-wound potentiometer
R_{26}.	1750-ohm; eight 3500-ohm, 20-watt wire-wound resistors, four paralleled in series with four paralleled
R_{27} to R_{32}.	6-watt, 115-volt tungsten lamps
R_{33} to R_{36}.	25-watt, 115-volt tungsten lamps

C_1.	10-mfd., 1000-volt d.c. oil capacitor
C_2.	2-mfd., 600-volt d.c. capacitor
L_1.	10-henry, 500-ma. choke, UTC-CG 108 or equivalent
T_1.	Filament transformer, 2.5-volt at 10A, UTC-CG 34 or equivalent
T_2.	Power transformer, 500-0-500-volt at 500 ma., 6.3-volt at 5A, 6.3-volt at 3A, UTC-CG 431 or equivalent
T_3.	Filament transformer, 6.3-volt at 4A, UTC-CG 33 or equivalent
S_1, S_2.	S.P.S.T. toggle switch
S_3.	30-second thermal delay relay
S_4.	D.P.D.T. toggle switch
S_5.	D.P.D.T. center off toggle switch
S_6.	3-circuit, 8-position nonshorting switch
S_7.	Single circuit, 5-position, nonshorting switch
M_1.	0 to 500 d.c. millimeter
V_1, V_2	866/866A, half-wave rectifier tubes
V_3 to V_5.	6AS7G, series regulator tubes
V_6.	6SL7, d.c. amplifier tube
V_7.	6AC7, shunt control tube
V_8.	V_9, 6J6, series regulator tubes
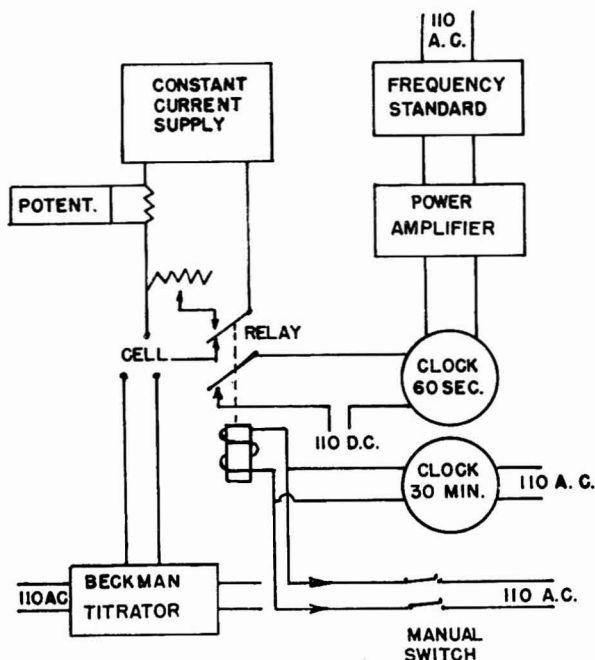
Figure 2. Block circuit diagram for coulometric titrator

determined potentiometrically with stopping and starting the current about ten times in the vicinity of the end point. For the last two titrations in Table III the current was interrupted 100 times. These two values indicate that this large number of starts and stops do not cause appreciable error.

The instrument was found to perform very satisfactorily for a large number of coulometric titrations with electrolytically generated titanous ion (5).

LITERATURE CITED

(1) Cooke, W. D., and Furman, N. H., ANAL. CHEM., **22**, 896 (1950).
(2) Craig, R. S., Satterthwaite, C. B., and Wallace, W. E., *Ibid.*, **20**, 555 (1948).

Figure 3. Coulometric titrator

(3) Fry, E. M., and Baldeschwieler, E. L., IND. ENG. CHEM., ANAL. ED., **12**, 472 (1940).
(4) Lingane, J. J., ANAL. CHEM., **26**, 622 (1954).
(5) Parsons, J. S., and Seaman, W., *Ibid.*, **27**, 210 (1955).
(6) Reilley, C. N., Adams, R. N., and Furman, N. H., *Ibid.*, **24**, 1044 (1952)

Coulometric Titrations with Low-Inertia Integrating Motor

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A small electromechanical motor for integrating current and time in a coulometric titration has given satisfactory performance in the titration of dichromate, the automatic titration of macro quantities of chloride, and the automatic titration of 0.1-meq. quantities of acid, with electrolytically generated ferrous ion, silver ion, and hydroxide ion.

COULOMETRIC titration equipment reported in the literature (4) has consisted of various regulator circuits for maintaining current constant and a good electric timer as the main

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components in the circuitry. A small motor, which integrates current and time, makes it possible to perform coulometric titrations with an unstabilized current. Coulometric titration equipment could be simplified and made less expensive by eliminating the need for a precisely controlled constant current supply, an accurate timer, and a frequency standard to drive the timer where frequency fluctuates as it may with industrial plant power (3).

Recently, Bett, Morris, and Nock (1) reported the use of a low-inertia integrating motor for coulometric titrations at high current levels. Meites (5) has used a relay for integrating current at low levels. This requires calibration for each current level and has a blank count. More recently, Meites (6) reported

a more accurate, but also more elaborate, instrument for integrating current in controlled potential electrolysis. Chemical integrators or coulometers such as used by Szebelledy and Somogyi (8) in their original work on coulometric titrations, although accurate, are inconvenient.

Wheatley (9) described low-inertia motors for use as electromechanical integrators. These are permanent magnet direct current motors with negligible friction, low brush contact resistance, and low iron losses, so that the speed of the motor is a linear function of the driving voltage. Furthermore, the time constant is small (about 10 milliseconds), so that little error is caused by starting and stopping.
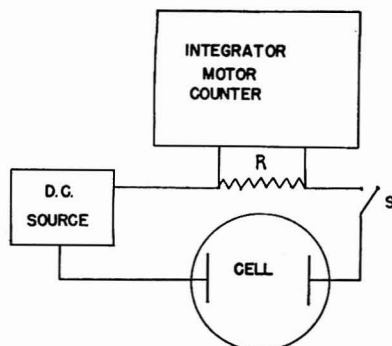
Figure 1. Block diagram of circuit

Integration of current and time is accomplished by allowing the *IR* drop or voltage across a fixed resistor in series with the electrolysis cell to supply power to the motor-counter unit. By keeping the resistance of the series resistor constant, the speed of the motor, or counts per second, is a linear function of the current flowing through the series resistor. The motor may be calibrated to read quantity of electricity (coulombs per count).

CALIBRATION OF INTEGRATOR

A block diagram of the manual coulometric titration circuit is shown in Figure 1. The integrating motor (Electro Methods, Ltd., Stevenage, Herts, England, type 913, 24 volts with mechanical counter) was connected across an approximately 70-ohm fixed setting of a 100-ohm, 100-watt potentiometer resistor. A photograph of the motor-counter unit is shown in Figure 2. The resistor was kept immersed in a reservoir of transformer oil, to prevent it from being heated excessively. A constant-current supply and a timer operated by a standard frequency tuning fork (3, 7) were used to calibrate the integrator. An accurately measured constant current was passed through the 70-ohm resistance for an accurately measured time, and the corresponding count on the integrating motor was read. The calibration factor was expressed by the following equation:

$$\frac{i(\text{amperes}) \times t(\text{seconds})}{96.5 \times \text{counts}} = \text{milliequivalents per count}$$

Calibration data obtained are presented in Table I.

Data in Table I indicate that variations in the calibration factor of the motor lead to an uncertainty of ±0.13% (standard deviation) for integration of current and time. The difference of 0.15% in the average value for the factor at the 260- to 270- and 138- to 154-ma. level is due to a slight deviation from linearity of the motor speed-voltage curve. Deviations from linearity become greater than 0.5% when the motor is operated at an input voltage below 5 volts. Data in Table I also indicate that the reproducibility of the factor is better than 0.1% for a series of determinations on a given day. The variation on different days may be due to an effect of temperature. The

manufacturers indicate that the speed of the motor with no load varies approximately 0.35% per 10° C. Another possible source of error is the load on the motor which is due to the mechanical counter. A load on the motor reduces the speed range within which the speed is linear with respect to voltage input. A load also increases the current necessary to operate the motor. According to the manufacturers, a current of 0.225 ma. is required to operate the motor at its nominal 24-volt speed with no load.

For current levels lower than 75 ma. the series resistor (*R*) in Figure 1 should be greater than 70 ohms, so that the motor will be operating on the linear portion of its speed-voltage curve. Data obtained at current levels of 12.5 to 13.7 ma. over a period of several months with a 1000-ohm series resistor gave a calibration factor with a standard deviation of ±0.18% (calculated from 26 determinations). Lower currents were not studied.

The motor-counter unit should not be mounted near iron, or other magnetic material. By supporting the unit on the base of an iron ring stand, the calibration factor was decreased 1.5%.

The unstabilized direct current sources, employed for the coulometric titrations described in this paper, were used because of their availability at the time this work was done. A selenium rectifier bridge with choke and condenser for filtering has since been found to be equally satisfactory. For high currents it is

Figure 2. Integrating motor-counter unit

Table I. Calibration Factor

Date	Current, Ma.	Time, Seconds	Count	Factor, Meq./Count
8/5/54	261.2	300.0	40.39	0.02010
	261.3	300.0	40.39	0.02011
	261.4	300.0	40.40	0.02012
	261.4	300.0	40.38	0.02013
	261.3	300.0	40.39	0.02011
8/17/54	272.6	300.0	42.14	0.02011
	272.6	330.1	46.37	0.02011
	273.1	2400.0	337.91	0.02010
9/21/54	262.6	300.0	40.51	0.02016
	262.7	600.0	81.05	0.02015
	262.8	180.0	24.32	0.02016
	262.8	240.0	32.42	0.02016
10/11/54	268.6	300.0	41.48	0.02013
	268.6	360.0	49.78	0.02013
				0.02013

Std. dev. = ±0.11%

Date	Current, Ma.	Time, Seconds	Count	Factor, Meq./Count
9/23/54	138.8	300.1	21.36	0.02021
	138.9	230.1	16.40	0.02020
	139.5	360.1	25.78	0.02019
	139.7	270.0	19.36	0.02019
10/11/54	153.9	360.1	28.52	0.02014
	153.9	600.0	47.53	0.02013
	153.9	300.1	23.77	0.02013
	153.9	420.1	33.27	0.02014
10/12/54	153.6	360.1	28.43	0.02016
	153.7	360.1	28.44	0.02017
	153.7	420.0	33.20	0.02015
				0.02016

Std. dev. = ±0.14%

recommended that the output voltage of the source be 100 to 200 volts, so that a fairly high resistance may be used in series with the electrolysis cell in order to prevent excessive fluctuations of the current.

PERFORMANCE OF INTEGRATION MOTOR IN COULOMETRIC TITRATIONS

Titration of Dichromate. The coulometric titration of National Bureau of Standards potassium dichromate with electrolytically generated ferrous ion (2) was used to test the motor. An unstabilized source of direct current (about 40 volts) obtained from the rectifier section of a potentiostat was used. A series resistor was adjusted to give 140 to 150 ma. through the electrolysis cell. The titration procedure and cell design of Cooke and Furman (2) were employed with the exception that the total volume of the titration solution was increased to about 140 ml., and a perforated cylindrical platinum electrode cathode (2 inches high by 1 inch in diameter) was used. The titration was carried out in an atmosphere of nitrogen and the end point was determined with a platinum–tungsten electrode pair. The results of titrations with 25 ml. of $0.025N$ potassium dichromate (based on weight of Bureau of Standards $K_2Cr_2O_7$ diluted to volume in calibrated glassware) are shown in Table II. The data were corrected for ferrous ion in the ferric ion reagent.

Data given by titrations 6 and 7 where the current was switched "off" and "on" 400 to 500 times indicate that this factor causes little, if any, error.

Automatic Macrotitration of Chloride. The integrating motor was used in conjunction with the Beckman automatic titrator for the automatic coulometric titration of chloride with electrolytically generated silver ion. The basic circuit shown in Figure 1 was used except for a double pole–double throw relay and the Beckman titrator in place of toggle switch (S) to terminate the end point. Lingane (4) has described the method of connecting the relay to the Beckman titrator. Uncontrolled direct current power (about 350 volts) was obtained from the rectifier section of a constant current supply (3). A large series resistor was adjusted to give a current of about 260 ma. through the electrolysis cell.

The electrolysis cell design was similar to that recommended by Lingane (4) except that the large stirrer which is provided with the Beckman titrator and a 600-ml. beaker were employed.

Titration was carried out in the solution obtained from a 15-gram sodium peroxide Parr bomb combustion after neutralization with a slight excess of nitric acid, dilution to 150 ml., and addition of 300 ml. of alcohol (formula 3a, denatured). The isolated cathode compartment was kept filled with a similar chloride-free solution. For the titrations described in this paper, the chloride was added to the solution after the combustion so as to eliminate any possible effect of incomplete combustion on these studies. The Beckman was set at $+0.240$ volt (standard calomel electrode). The anticipation was set to provide for the addition of several hundred increments in the vicinity of the end point. With integration of current and time, the large number of "starts" and "stops" did not cause significant error. The large number of increments had further advantages in preventing overstepping of the end point, allowing time for equilibrium to be established and requiring less care in the positioning of the electrodes in the cell. Results of automatic titrations are presented in Table III.

Two manual titrations with less than 10 starts and stops and with 1 meq. of chloride present gave 99.4 and 99.6% recovery. The slope of the titration curve in the vicinity of the end point, when 1 meq. of chloride is titrated, leads to an error of about 0.4% chloride for 10 mv. At a current level of 260 ma. 1 meq. of chloride requires about 370 seconds of generation time or 49.50 counts as read on the integrator. The end point used to obtain the values for chloride in Table III may be slightly in advance of the true end point as the titrator was set to stop at $+0.240$ volt (S.C.E.), whereas the end point as determined from a manual curve was $+0.253$ volt (S.C.E.).

Automatic Semimicro Titration of Acid. The automatic titration of acid with electrolytically generated hydroxide was also studied. At a current level of 13 ma., it was necessary to replace R in Figure 1 with a 1000-ohm resistor so that the IR drop would be sufficient to operate the motor at a favorable speed.

The constant-current supply and accurate timing equipment (3) and the Beckman titrator were used. The cell, a 100-ml. weighing bottle, contained a magnetic stirrer and a rubber stopper. The latter held a cylindrical cathode of platinum gauze (52-mesh, 1.8 cm. in diameter by 1.5 cm. high), glass and calomel electrodes, tubes for keeping the titration solution under an atmosphere of nitrogen and one leg of a U-tube (25 cm. by 8 mm. in diameter), which was filled with 3% agar in $1M$ sodium chloride. The other leg of the U-tube dipped into a separate vessel containing $1M$ sodium chloride and a platinum anode.

Titrations were carried out on 10-ml. portions of standard $0.01N$ hydrochloric acid in about 50 ml. of $0.1N$ sodium chloride. Reagents were prepared with boiled distilled water and the titration vessel was kept under an atmosphere of nitrogen. The Beckman titrator was set at pH 7.0.

Results in Table IV indicate excellent agreement between values determined from current and time measurements and by means of the integrating motor.

For the titration in Table IV the anticipation was set so that 132 to 240 increments were obtained. The pH of the solution after the completion of the titration was 7.0 to 7.1. Current was passed for about 730 seconds at about 13.2 ma. Approximately 75 counts were obtained. The calibration factor of the motor was 0.001325 meq. per count.

LITERATURE CITED

(1) Bett, N., Morris, G., and Nock, W., ANAL. CHEM., **26**, 784 (1954); *Analyst*, **79**, 607 (1954).
(2) Cooke, W. D., and Furman, N. H., ANAL. CHEM., **22**, 896 (1950).
(3) Gerhardt, G. E., Lawrence, H. C., and Parsons, J. S., *Ibid.*, **27**, 1752 (1955).
(4) Lingane, J. J., *Ibid.*, **26**, 622 (1954); "Electro-analytical Chemistry," Interscience, New York, 1953.
(5) Meites, L., ANAL. CHEM., **24**, 1057 (1952).
(6) *Ibid.*, **27**, 1116 (1955).
(7) Parsons, J. S., and Seaman, W., *Ibid.*, **27**, 210 (1955).
(8) Szebelledy, L., and Somogyi, Z., *Z. anal. Chem.*, **112**, 313, 323, 332, 385, 391, 395, 400 (1938).
(9) Wheatley, B. M., *Brit. J. Radiol.*, **26**, 382 (1953).

Table II. Manual Titration of Dichromate with Electrolytically Generated Ferrous Ion

Titration	% Recovery
1	100.04
2	100.06
3	99.99
4	100.03
5	100.09
6 (500 starts and stops)	100.09
7 (400 starts and stops)	100.12
Av.	100.06
Std. dev.	= ±0.05

Table III. Automatic Titration of Chloride

Chloride Added, Gram	Number of Determinations	% Recovery	Std. Dev.
0.0355	15	99.6	0.3
0.0532	1	99.9	..
0.0709	1	99.7	..

Table IV. Titration of Acid

Titration	% Recovery Time and current	% Recovery Integration
1	100.2	100.2
2	99.9	99.8
3	99.7	99.8
4	99.8	99.7
5	99.8	99.8
6	99.7	99.8
7	99.8	99.8
Av.	99.84	Av. 99.85
Std. dev.	= ±0.18	Std. dev. = ±0.14

Automatic Differential Potentiometric Titrations

Electrode Systems for Aqueous and Nonaqueous Titrations

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The initial success and interest in the automatic differential potentiometric titrator have led to an investigation of its characteristics and applicability in all general types of titrations. Examples of precipitation, complex formation, aqueous and nonaqueous acid-base and oxidation-reduction titrations illustrate the important features of the automatic differential technique. The potential response characteristics of various indicator electrodes for the different titration types are demonstrated with both recorded second derivative titration curves and automatically obtained titration data. Indicator and reference electrodes that shift or do not establish a reproducible potential are nevertheless applicable for automatic differential titrations. The effectiveness of an electrode system of platinum–rhodium alloy indicator electrode vs. graphite reference electrode in nonaqueous titrations is demonstrated. The titrator yields automatic titration results of excellent precision, even with titrant delivery rates greater than 10 ml. per minute in some cases.

A NEW type of automatic potentiometric titrator was recently described (24) whereby the inflection point of a potentiometric curve was automatically detected and used to turn the buret off. This was accomplished by electronically producing a voltage which was proportional to the second derivative of the ordinary potentiometric titration curve. This voltage function was ideally suited to trigger a relay system which turned the buret off at the inflection point (end point) of the titration.

Several advantages (24) characterize the automatic differential titrator. The equipment is simple, compact, and inexpensive, and there are no end-point potentials to set or other instrument adjustments to make. Various types of reference and indicator electrodes can be used, even though their absolute potentials shift from one titration to the next, or the electrodes undergo a drift in potential during a titration.

The automatic differential titrator is not suited for titrations where the solution or electrodes reach equilibria very slowly (24). However, for most titrations the solution equilibria are attained sufficiently rapidly, even for rather rapid titration rates. Electrodes are available which respond very rapidly to potential changes at the end point for precipitation, soluble complex, oxidation-reduction, aqueous acid-base, and nonaqueous acid-base titrations. Although certain electrodes have slow rates of potential response, these rates are relatively constant, and the precision obtained with such electrodes is good.

The glass electrode cannot be used with the titrator as originally described because of its high resistance, but this can be remedied by preceding the first amplifier stage with a high input impedance stage. However, a noble metal indicator electrode can be used in the titration cases where a glass electrode is usually used thus eliminating the necessity of a high input impedance stage, unless the solution resistance across the electrode pair is extremely high, as in the case of certain nonaqueous titrations. If it is necessary or desirable to use the glass electrode or if the resistance of the electrode pair is very large, a commercial direct-reading pH meter can be used by connecting directly from the feed-back resistor of the pH meter to the input of the differentiator.

The data presented for the examples of precipitation, soluble complex, aqueous acid-base, and nonaqueous acid-base titrations indicate that a wire of platinum–10% rhodium alloy serves as an excellent indicator electrode. The platinum–rhodium wire might prove to be an extremely versatile indicator electrode for automatic differential potentiometric titrations; however, it will have to be tried in many more titration systems to establish its versatility.
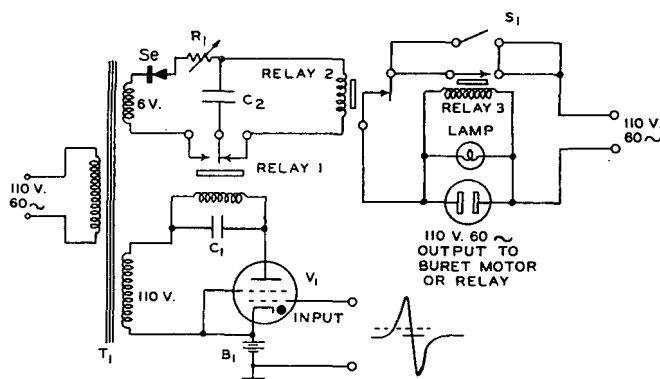
Figure 1. Relay system for automatic differential titrator

R_1.   200-ohm wire-wound potentiometer
C_1.   8 μfd., 150-volt electrolytic condenser
C_2.   500 μfd., 20 WVDC electrolytic condenser
S_1.   Push button (spring return) SPST switch
Relay 1.   110-volt a.c. SPDT relay
Relay 2.   6-volt d.c., SPST, normally closed relay
Relay 3.   110-volt a.c., SPST, normally open relay
B_1.   3-volt bias battery
Lamp.   110-volt a.c. indicator lamp
V_1.   2D21 thyratron tube
T_1.   Transformer (isolation); primary 110-volt, 60 cycles: secondary 110-volt, 60 cycles, 100 ma., and 6-volt, 60 cycles, 1 ampere
Se.   Selenium half-wave rectifier

The relay system as originally designed prevents false end points from various types of electrode noise which sometimes occur in certain titration procedures. This is illustrated with recorded curves for specific systems. Titrant flow rates up to 10 ml. per minute are possible in some cases without appreciable overshoot of the equivalence point. Examples are also given where flow rates should not exceed about 2 ml. per minute so that solution equilibria are maintained. Many of the experimental data were obtained at relatively fast flow rates, which are especially desirable for routine titrations.

EQUIPMENT AND GENERAL PROCEDURE

All experimental results were obtained using the automatic titrator (24). A uniform flow buret (4) in conjunction with a relay-operated pinch-clamp device was used for delivery of the titrant. It was found desirable to supplement the sintered-glass tubes for varied lengths of capillary tubing for use with alkali titrants, and to replace daily the rubber tubing running through the pinch clamp when used with the benzene–sodium methoxide solutions.

In most cases the relay system was satisfactory (24). This system, however, required that the electrode potential swing negative in the end-point region, because the relay operated on the negative pulse of the second derivative curve. In the case

of the glass electrode it was impossible to reverse the electrode connections on the pH meter input. When the glass electrode was used and the potential break was initially a positive voltage swing at the end point, use of a redesigned relay circuit was necessary to operate on the positive pulse of the second derivative curve. This was accomplished by connecting the relay circuit as shown in Figure 1.

The operation of the relay circuit is essentially the same as described (24), but the thyratron is biased negatively by a 3-volt bias cell so that the thyratron does not conduct until a positive voltage is applied to the grid. Relay 1 in the plate circuit of the thyratron must be connected so that the contacts are as shown in Figure 1 when not energized (no signal on grid of thyratron). An isolation transformer for the 60-cycle, 110-volt plate supply of the thyratron also is provided to eliminate the necessity of a polarized plug. The 6-volt winding on the isolation transformer is used in conjunction with a half-wave selenium rectifier to provide the charging voltage for condenser C_2. A variable time delay is provided by the potentiometer, R_1.

A Beckman Model H-2 pH meter was employed for use with the high resistance glass electrode. In this case, the electrodes were connected to the pH meter in the usual manner, and the voltage across the feed-back resistor was fed to the input of the amplifier-differentiator circuit. No connections in the pH meter need to be changed, as the leads across the feed-back resistor can be attached with clips.

In a nitrogen atmosphere the titration vessel was closed with a large rubber stopper equipped with holes for the electrodes, stirrer, buret tip, and nitrogen inlet and outlet. The buret was filled by nitrogen pressure by employing a wide-top buret, which had a rubber stopper with holes for delivery and an air outlet equipped with an Ascarite drying tube.

The recording of the second derivative titration curves served primarily as a permanent record for each titration, and, also, made it simple to observe electrode behavior while trying different electrode systems for various types of titrations. The two types of recorders employed were a full scale Brown recording potentiometer, 50 mv., and an Esterline-Angus recording milliammeter with an amplifier to convert it to a recording millivoltmeter. The curves discussed in the following sections are faithful reproductions of the titration curves obtained with one of the above recorders in the circuit.

The electrode tip must be below the surface of the solution and the stirring must be efficient. Motor-driven paddle stirrers provide good stirring, but magnetic stirrers do not generally stir effectively as they usually result in streaming of titrant from the buret tip. This often causes electrode noise.

REAGENTS AND ELECTRODES

HYDRAZINE SULFATE, commercially pure.

POTASSIUM IODATE SOLUTION. A 5.350-gram sample of potassium iodate was dissolved and diluted to 1.000 liter with freshly boiled distilled water to make a 0.02500M solution.

ACETIC ACID SOLUTION. Prepared to approximately 0.1N by diluting reagent grade glacial acetic acid.

HYDROCHLORIC ACID SOLUTION. Prepared approximately to 0.1N by diluting concentrated reagent grade hydrochloric acid.

SODIUM HYDROXIDE SOLUTION. Six milliliters of saturated sodium hydroxide was diluted with freshly boiled distilled water to make 1 liter of approximately 0.1N solution.

SODIUM CHLORIDE SOLUTION. Six grams of reagent grade sodium chloride was dissolved and diluted with water to make 1 liter of approximately 0.1N solution.

SILVER NITRATE SOLUTION. A 17-gram sample of reagent grade silver nitrate was dissolved and diluted to make 1 liter of approximately 0.1N solution.

POTASSIUM CYANIDE SOLUTION. Seven grams of reagent grade potassium cyanide was dissolved and diluted with distilled water to make 1 liter of approximately 0.1N solution.

MERCURIC NITRATE SOLUTION. A 25-gram sample of reagent grade mercuric nitrate was dissolved and diluted with distilled water to make 1 liter of approximately 0.1N solution.

GLACIAL ACETIC ACID, reagent grade.

ANILINE SOLUTION. Approximately 9 ml. of redistilled aniline was dissolved and diluted with glacial acetic acid to make 1 liter of approximately 0.1N solution.

PERCHLORIC ACID SOLUTION. With glacial acetic acid 9.8 ml. of 72% perchloric acid was diluted to 1 liter. Then 18.5 ml. of acetic anhydride was added and the solution allowed to stand for 24 hours. This makes an approximately 0.1N solution.

BENZENE, purified grade.

BENZOIC ACID, primary standard grade.

METHANOL, absolute.

BENZENE–METHANOL SOLUTION. Mix three volumes of benzene with one of methanol.

SODIUM METHOXIDE SOLUTION. Six grams of commercially available sodium methylate was added to 150 ml. of methanol and then diluted with 900 ml. of benzene.

GLASS ELECTRODE, Beckman 4990–80.

CALOMEL ELECTRODES, Beckman fiber capillary type and Leeds & Northrup sleeve type.

ANTIMONY ELECTRODE. Spectrographically pure antimony was sealed in glass tubing with mercury contact to lead-out wire.

GRAPHITE ELECTRODE, six-inch lengths of $^1/_4$-inch spectrographic graphite electrodes.

WIRE ELECTRODES. Platinum, platinum–10% rhodium, platinum–40% rhodium, rhodium, palladium, and silver wires of about 1-inch lengths and 0.5 mm. in diameter were sealed into 6-ml. glass tubing with about 0.5 inch of wire protruding. Mercury was used inside the glass tube for contact between the electrode wire and lead-out wire.

QUINHYDRONE ELECTRODE, Leeds & Northrup quinhydrone with platinum wire.

AQUEOUS ACID-BASE TITRATIONS

There are more than 100 literature references to various indicator electrodes which have been used for pH measurements and acid-base titrations (13–17). However, the glass electrode has become the favorite electrode in most laboratories for both pH measurements and acid-base titrations. The other pH-sensitive electrodes have been primarily of academic interest in recent years. It would seem logical, then, also to employ the glass electrode as the indicator electrode for acid-base titrations when using the automatic differential titrator, but there are two reasons why other electrodes are usually preferable with this instrument. The primary reason is that the glass electrode does not respond instantaneously to changes of pH in the solution; therefore, the titrant flow rate must be relatively slow to prevent overshooting the equivalence point, or a blank correction must be applied to correct for the delay in electrode response. Another reason is that its very high resistance necessitates a high input impedance stage to precede the differentiator circuit, and this unnecessarily complicates the equipment if a suitable low resistance electrode is available.

The antimony, platinum, platinum–10% rhodium alloy, platinum–40% rhodium alloy, rhodium, palladium, and graphite electrodes were used to compare the response of the glass electrode to other pH-sensitive electrodes for the titration of 10 ml. of approximately 0.1N acetic acid with 0.1N sodium hydroxide. The important point of the comparison is the relationship between the inflection point of the electrode potential curve to the equivalence point, as illustrated in Figure 2.

Indicator and Reference Electrodes. The curves in Figure 2 are the ordinary and second derivative potentiometric titration curves for each electrode system as recorded on a Brown recording potentiometer for the titration of acetic acid with sodium hydroxide. All curves were obtained with a titrant flow rate of 2 ml. per minute. The broken lines represent the phenolphthalein end point. The displacement of the inflection point of the potential curves from the broken line roughly illustrates the delay of electrode response to changes of pH in the solution.

Curve A in Figure 2 shows the ordinary and second derivative curves for a glass–calomel electrode system. The inflection point of the titration curves lags the phenolphthalein end point by a small amount when the titrant flow rate is 2 ml. per minute. A greater lag of inflection point is illustrated in curve B for the antimony indicator electrode with all other conditions the same. The lag in the antimony electrode is not too surprising in view of its complicated electrode reaction (6). Quantitative data are presented to illustrate that the lag is reproducible. Curve C illustrates that an untreated platinum wire electrode also responds to changes of pH, but its response is not reproducible and the inflection point lags the phenolphthalein point by a rather large amount. Attempts to use a platinum electrode for acid-base titrations have been numerous (13, 17, 20), but it was found
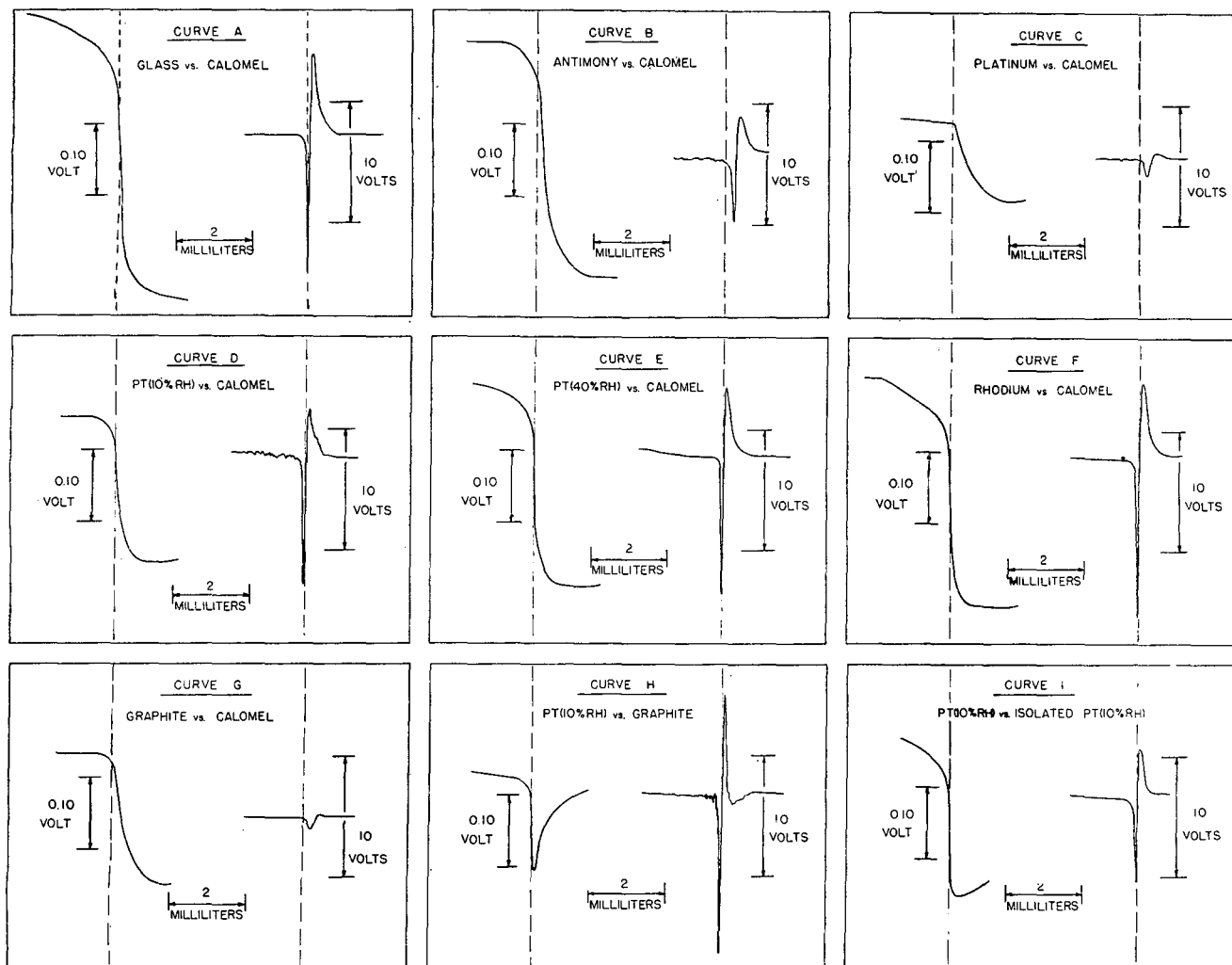
Figure 2.   Recorded ordinary and second derivative potentiometric titration curves

For various electrode systems in aqueous acid-base titrations

(7, 32) to give a poor change of potential at the equivalence point, and this was substantiated by curve C.

The potential curves for the same acid-base titration with identical conditions, but with platinum-rhodium alloys and pure rhodium indicator electrodes, are given in Figure 2, curves D, E, and F.   The inflection point of these curves nearly coincides with the phenolphthalein point, and the magnitude of the potential increases.   This phenomenon is not considered in the present discussion, but should prove interesting for future investigations. All three of these electrodes appear ideal for acid-base automatic differential potentiometric titrations, although they would not be too suitable for conventional potentiometric titration methods, because their absolute potentials often shift from one titration to the next and drift during the titration.   Most of the quantitative data presented were obtained with the platinum-10% rhodium indicator electrode, because the rhodium and platinum-40% rhodium electrodes were not available.   The rapid response of the platinum-10% rhodium electrode to pH changes precludes much improvement with the use of other electrodes.   The use of platinum-tantalum (5), indium, rhodium, osmium, and ruthenium (30) as electrodes for acid-base systems has been reported.

The response of a graphite rod electrode to pH changes in the solution is shown in curve G, resembling somewhat the response of a platinum wire electrode.   There is a rather large lag in elec-

trode response, which makes it undesirable as an indicator electrode for automatic differential titrations, but it does suggest its use as a reference electrode (7, 19, 32).   Curve H shows the ordinary and second derivative curves with a platinum-10% rhodium indicator electrode and a graphite reference electrode. The second derivative curve is obtained before the graphite starts to change potential, and the inflection point nearly coincides with the equivalence point.   About 0.5 minute after the equivalence point is passed the potential difference across the electrode pair is again very small, and such a pair is useless for this titration by the conventional titration procedure.   Because of the slow response of the platinum wire electrode as shown in curve C, it also serves as a reference electrode for acid-base automatic differential titrations.

Another possible reference electrode is an isolated indicator electrode.   The electrode can be isolated by several procedures (18, 27, 34).   Titration curves for the electrode system of a platinum-10% rhodium indicator electrode and a platinum-10% rhodium reference electrode isolated in the tip of the buret are shown in curve I, Figure 2.

The titration curves with palladium as indicator electrode are not given in Figure 2.   They are similar in shape to those for platinum-40% rhodium, curve E, but the magnitude of the potential change is less.

Quantitative Results at Various Titrant Flow Rates. Table I summarizes the quantitative results obtained at various flow rates for the titration of acetic acid with sodium hydroxide and with electrode systems of platinum–10% rhodium *vs.* calomel or graphite, glass *vs.* calomel, and antimony *vs.* graphite. After the titration was terminated by the automatic differential titrator, the pH of the solution was measured on a calibrated pH meter and the values were recorded. The reproducibility of successive titrations is given as standard deviation expressed both in terms of pH and milliliters of 0.1N sodium hydroxide. At fast flow rates the over-shoot of the equivalence point becomes significant especially with the glass and antimony electrodes. Even with these electrodes the precision is good, and it is possible to make a blank correction for a given flow rate, but it would generally be best to select an indicator electrode and flow rate where the blank is negligible or very small.

Figure 3 shows a manual titration curve using a pH meter and glass–calomel electrode system for the titration of 10 ml. of approximately 0.1N acetic acid in 50 ml. of solution with 0.1N sodium hydroxide. Titrant was delivered dropwise from a buret, and the solution was allowed to reach equilibrium before the pH values were measured. The average end point pH values for this same titration with the different indicator electrodes and various flow rates are indicated on the titration curve in Figure 3.
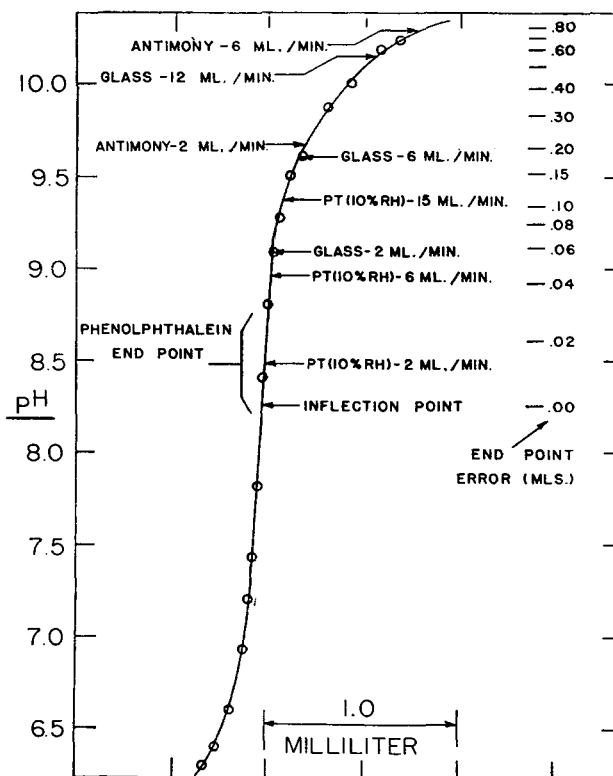
Figure 3. Potentiometric curve of end-point region for titration of acetic acid with sodium hydroxide

Table I. Automatic Differential Titrations of Acetic Acid with Sodium Hydroxide

(10-ml. aliquots of approximately 0.1N acetic acid in 50 ml. of solution were titrated with approximately 0.1N sodium hydroxide at various flow rates)

Conditions	End Point pH	Std. Dev. pH	Std. Dev. Ml.
Pt (10% Rh) vs. calomel, 2 ml./minute	8.50	0.07	0.005
	8.42		
	8.51		
	8.49		
	8.59		
	Av. 8.48		
Pt. (10% Rh) vs. calomel, 15 ml./minute	9.30	0.07	0.013
	9.38		
	9.43		
	Av. 9.37		
Pt (10% Rh) vs. graphite, 6 ml./minute	8.93	0.06	0.006
	8.88		
	9.02		
	8.97		
	Av. 8.95		
Pt. (10% Rh) vs. calomel, 6 ml./minute	8.91	0.08	0.009
	8.95		
	9.08		
	8.88		
	8.80		
	9.02		
	9.00		
	8.90		
	8.95		
	9.02		
	Av. 8.96		
Glass vs. calomel, 2 ml./minute	9.13	0.04	0.005
	9.08		
	9.11		
	9.01		
	9.07		
	9.08		
	9.12		
	9.08		
	9.14		
	9.06		
	Av. 9.09		
Glass vs. calomel, 6 ml./minute	9.64	0.07	0.08
	9.57		
	9.68		
	9.51		
	9.60		
	Av. 9.60		
Glass vs. calomel, 12 ml./minute	10.20	0.05	0.05
	10.13		
	10.10		
	10.10		
	10.14		
	Av. 10.14		
Antimony vs. graphite, 2 ml./minute	9.60	0.04	0.05
	9.68		
	9.64		
	9.61		
	9.69		
	Av. 9.64		
Antimony vs. graphite, 6 ml./minute	10.30	0.06	
	10.20		
	10.36		
	10.27		
	10.31		
	Av. 10.29		
Antimony vs. graphite, 10 ml./minute	10.52	0.04	
	10.60		
	10.50		
	10.57		
	10.51		
	Av. 10.54		

The end-point error—i.e., the difference in milliliters between equilibrium inflection point and automatically terminated end point—is given in the vertical column to the right of the curve.

Strong Acid–Strong Base Titrations. Ten-milliliter aliquots of 0.1N hydrochloric acid in 50 ml. of solution were titrated with carbonate-free 0.1N sodium hydroxide. The electrode system was platinum–10% rhodium *vs.* either calomel or graphite and the flow rate was 2 ml. per minute. Five aliquots of hydrochloric acid were automatically titrated in the presence of bromocresol green indicator, and five in the presence of phenolphthalein indicator. The titrations were all automatically terminated on the basic color of bromocresol green (blue) and the acid side of phenolphthalein (colorless). No attempts were made to measure the pH accurately after the titrations were automatically terminated, but the above color tests indicate that they were all terminated between about pH 6 and 8. For a strong acid–strong base titration this represents a very small milliliter error. At flow rates greater than 5 ml. per minute the pink color of phenolphthalein was just perceptible after automatic termination of the titration.

The hydrochloric acid–sodium hydroxide titrations were also performed with a quinhydrone indicator electrode with very good results. Ten samples were automatically titrated and the

average of these results was 10.59 ml. with a standard deviation of 0.03 ml. (about reading error of the buret), which compares with the average value of 10.58 ml. obtained manually using bromocresol green indicator.

NONAQUEOUS ACID-BASE TITRATIONS

Acid-base titrations in nonaqueous solvents have been rapidly gaining popularity in recent years. A serious drawback in the performance of these titrations has been the difficulty in the detection of the end point. Internal indicators, because of lack of a firm theoretical basis, have been chosen largely on a hit and miss basis. Likewise, lack of fundamental information on electrode systems has hindered the use of potentiometric methods. Many of the electrodes now used give an indication of the proper end point, but the potentials obtained are not reproducible (10).

With the automatic differential titrator it is not necessary to reproduce the electrode potentials from one titration to the next, and therefore the differential procedure should be used to advantage in nonaqueous titrations if electrodes respond rapidly at the equivalence point.

One nonaqueous system investigated was the titration of aniline with perchloric acid using glacial acetic acid as solvent. Among the electrode systems suggested for this solvent have been the chloranil-calomel (8), glass-calomel (25), glass-silver, silver chloride (11). Even with the glass-calomel system the absolute potential curve was not reproducible from day to day, but varied as much as 0.1 volt. The other nonaqueous system was the titration of benzoic acid with sodium methoxide using benzene-methanol as solvent. The glass electrode cannot be used in this solvent (12), but such systems as antimony-calomel, hydrogen-calomel, and antimony–isolated antimony, where the reference antimony electrode is placed in the buret tip (27) have been used.
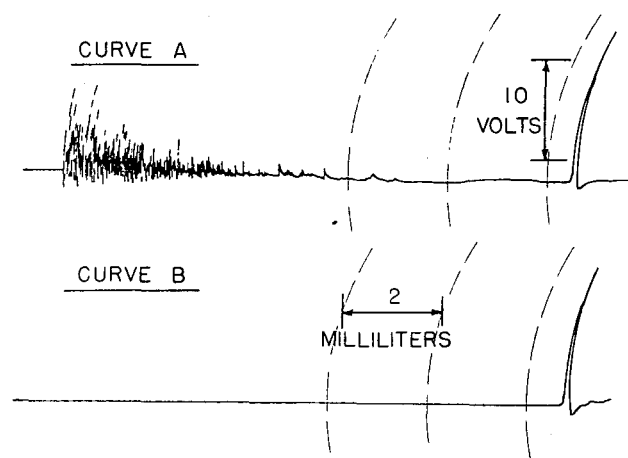

Figure 4. Recorded second derivative curves showing effect of lithium chloride in benzene-methanol solvent

The automatic differential titrations of aniline with perchloric acid and benzoic acid with sodium methoxide were performed with platinum–10% rhodium alloy, glass and antimony indicator electrodes, and a graphite reference electrode. The graphite reference electrode eliminates the use of salt bridges or special equipment, and is applicable to both types of solvents.

In acetic acid and benzene-methanol, as well as in most other nonaqueous solvents, there is a very high resistance between the indicator and reference electrodes, which generally requires a high input impedance circuit to the titrator. This high input impedance stage can be readily provided in most laboratories by a Beckman Model H-2 pH meter or other suitable pH meter or electrometer amplifier. For titrations in benzene-methanol

solvent about 0.1 gram of lithium chloride reduced the resistance at the start of the titration (Figure 4).

The curves were recorded on the Esterline-Angus recorder and curve A was for the titration of benzoic acid with sodium methoxide in benzene-methanol solvent without addition and curve B with addition of lithium chloride. The titration curves were obtained with the relay system in the circuit and one half of the second derivative curve at the end point accordingly is cut off. Figure 4, A, illustrates that the rapid oscillatory electrode noise does not turn the buret off. The rapidly oscillating voltage fed to the relay circuit causes the contacts of relay 1, Figure 1, to open and close rapidly, but the capacitance, C_2, does not obtain a sufficient charge to open relay 2 until the end point is reached. The "noise" level of the derivative curve decreases as the titration proceeds, because of the salt formation, but the initial chatter of relay, R_1, Figure 1, is annoying, and it is best to prevent this by adding some salt at the start of the titration.

Table II. Titration of Benzoic Acid with Sodium Methoxide in Benzene-Methanol Solvent

(Weighed samples of benzoic acid were dissolved in about 50 ml. of 3 to 1 benzene-methanol and titrated with approximately $0.15N$ sodium methoxide in 6 to 1 benzene-methanol solution)

Method	Wt. of Sample	Ml. of Sodium Methoxide	Equivalents per Liter of Sodium Methoxide
Manual	0.1246	6.98	0.1463
Using thymol blue as	0.1084	6.07	0.1464
indicator	0.1130	6.32	0.1466
Automatic	0.1152	6.46	0.1465
Pt (10% Rh) indicator	0.2198	12.28	0.1467
electrode,	0.0648	3.63	0.1463
2 ml./minute	0.2345	13.13	0.1464
Automatic	0.1094	(6.26–0.14) 6.12	0.1465
Pt (10% Rh) electrode,	1835	(10.41–0.14) 10.27	0.1465
6 ml./minute	0.0583	(3.40–0.14) 3.26	0.1466
	0.1913	(10.86–0.14) 10.72	0.1463
Automatic	0.1360	(7.96–0.34) 7.62	0.1463
Sb electrode,	0.1806	(10.41–0.34) 10.07	0.1462
2.4 ml./minute	0.2228	(12.83–0.34) 12.49	0.1462
	0.1200	(7.15–0.34) 6.71	0.1466

Titration of Benzoic Acid with Sodium Methoxide in Benzene-Methanol Solvent. Samples of reagent grade benzoic acid were weighed and dissolved in about 50 ml. of benzene-methanol solvent and titrated with approximately $0.15N$ sodium methoxide using the automatic differential titrator to terminate the titrations and electrode systems of platinum–10% rhodium vs. graphite or antimony vs. graphite. Data were obtained also by the manual titration procedure with thymol blue as indicator (Table II). There is no apparent overshoot of the equivalence point at a titrant flow rate of 2 ml. per minute with the platinum–10% rhodium indicator electrode, but at 6 ml. per minute there is a significant time lag of electrode response. The lag is reproducible, however, and can be corrected by a blank correction. The blank at a given titrant flow rate appears to be independent of concentration and temperature, and also to remain constant from day to day.

The antimony indicator electrode was found to have a large lag in potential response, similar to its action in aqueous acid-base titrations. With the antimony indicator electrode and a flow rate of 0.040 ml. per second, it was repeatedly observed that there was an 8-second time lag between the thymol blue color change and the automatic termination of the titration. This represents about a 0.32-ml. blank correction, which is in good agreement with the calculated value of 0.34 ml. The data in Table II illustrate the excellent results that can be automatically obtained, even with the antimony electrode when the blank correction is applied.

Titration of Aniline with Perchloric Acid in Glacial Acetic Acid. Each aliquot of approximately $0.1N$ aniline was diluted in the titration beaker to about 50 ml. with glacial acetic acid

Table III. Titration of Aniline with Perchloric Acid in Glacial Acetic Acid

(Aliquots of approximately 0.1N aniline were added to about 50 ml. of glacial acetic acid and titrated with 0.1N perchloric acid in glacial acetic acid)

Conditions	Ml. of Aniline	HClO₄-Blank
Pt (10% Rh) vs. graphite, 4 ml./minute	10.00	9.03 − 0.11 = 8.92
	10.00	9.09 − 0.11 = 8.98
	10.00	9.05 − 0.11 = 8.94
		Av. 8.95
	20.00	17.97 − 0.11 = 17.86
	20.00	18.01 − 0.11 = 17.90
	20.00	17.96 − 0.11 = 17.85
		Av. 17.87
Pt. (10% Rh) vs. graphite, 10 ml./minute	10.00	9.24 − 0.24 = 9.00
	10.00	9.20 − 0.24 = 8.96
	10.00	9.20 − 0.24 = 8.96
		Av. 8.97
	20.00	18.21 − 0.24 = 17.97
	20.00	18.17 − 0.24 = 17.93
	20.00	18.20 − 0.24 = 17.96
		Av. 17.94
	30.00	27.17 − 0.24 = 26.93
	30.00	27.13 − 0.24 = 26.89
	30.00	27.15 − 0.24 = 26.91
		Av. 26.91
Glass vs. graphite, 4 ml./minute	10.00	9.10 − 0.16 = 8.94
	10.00	9.14 − 0.16 = 8.98
	10.00	9.11 − 0.16 = 8.95
		Av. 8.96
	20.00	18.09 − 0.16 = 17.93
	20.00	18.06 − 0.16 = 17.90
	20.00	18.10 − 0.16 = 17.94
		Av. 17.92
Glass vs. graphite, 10 ml./minute	10.00	9.21 − 0.29 = 8.92
	10.00	9.24 − 0.29 = 8.95
	10.00	9.29 − 0.29 = 9.00
		Av. 8.96
	20.00	18.20 − 0.29 = 17.91
	20.00	18.20 − 0.29 = 17.91
	20.00	18.22 − 0.29 = 17.93
		Av. 17.92

and titrated with 0.1N perchloric acid. An electrode pair of either platinum–10% rhodium vs. graphite or glass vs. graphite was used in conjunction with the automatic differential titrator. The results of these titrations are summarized in Table III.

The potential break in this titration is not very sharp and the magnitude of the second derivative curve is accordingly small. Therefore, the titrant flow rate was maintained at about 4 ml. per minute or greater to ensure a derivative curve of sufficient magnitude to operate the relay system. Slower flow rates would be possible by modifications of either the amplifier-differentiator or relay circuit. At the relatively fast flow rates used for this titration a blank correction is necessary with either the platinum–10% rhodium or glass indicator electrodes, although the blank correction for the glass electrode is somewhat larger. The blank is constant for a given titrant flow rate and indicator electrode, remains constant from day to day, and is unaffected by concentration of reactant.

OXIDATION-REDUCTION TITRATIONS

Automatic differential potentiometric titrations of ferrous iron with standard dichromate and ceric solutions are very precise (24). Automatic titration results for another redox system are presented in this section.

The titration of hydrazine sulfate with standard potassium iodate by the Andrews method (2) is a rather complex oxidation-reduction reaction, which was considered worthy of investigation to determine whether the solution equilibrium was established sufficiently rapidly that the automatic differential titrator could be successfully employed. The potentiometric study of this reaction in at least 4F hydrochloric acid was found by Singh (31) to give accurate and reproducible results with a sharp inflection

point, and McBride and others (26), found it reproducible within 0.2% when done manually.

An electrode system of platinum vs. calomel, and a titrant flow rate of 2 ml. per minute were used for the titrations. Titrant delivery rates faster than 3 ml. per minute resulted in the formation of free iodine during the course of the titration. The potential change at the end point is large and the magnitude of the second derivative curve is also large as shown in Figure 5.

Eight samples of hydrazine sulfate of varying size were dissolved in about 15 ml. of water and 45 ml. of concentrated hydrochloric acid. These solutions were then titrated with a 0.02500M solution of potassium iodate. Four of these samples were titrated automatically at a flow rate of 2 ml. per minute and four manually using brilliant scarlet 3R as internal indicator (Table IV). There is no apparent lag in electrode response at a flow rate of 2 ml. per minute, because of the excellent precision obtained on the automatic titrations for the various sample sizes of hydrazine.

PRECIPITATION TITRATIONS

Chloride was accurately titrated with standard silver nitrate automatically at titrant flow rates up to 7 ml. per minute and with indicator electrodes of silver, platinum, or platinum–10% rhodium. A silver electrode with an area of approximately 7 sq. cm. was used, but this electrode had a slow potential response and gave results about 0.9% high. Upon substituting a No. 24 silver wire, this error was eliminated.
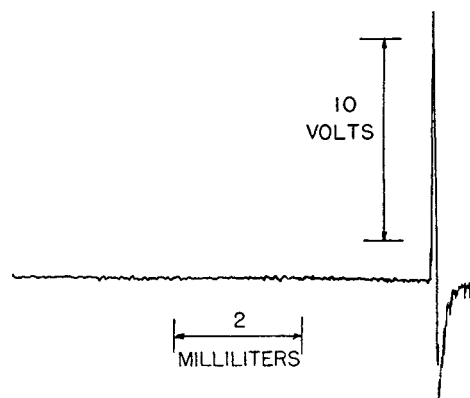
Figure 5. Recorded second derivative curve for titration of hydrazine sulfate with potassium iodate

The use of the platinum wire as an indicator electrode for silver ion is not new (13–17). It is generally agreed that a micro amount of silver on the surface of the platinum accounts for this phenomenon, which Allen and Hickling (1) recently demonstrated analytically. The mechanism for the appearance of this coating, however, is not clear. It has been suggested that it might be the result of a redox process involving a higher oxide of silver (14), or a trace of silver subnitrate rearranging to give the deposit of silver (29). Allen and Hickling (1) have shown the effects of pretreatment of the platinum and suggest that it is simply a reduction of the univalent silver ion.

In this laboratory the potential from a used platinum-calomel electrode pair was found to agree with that from a silver-calomel couple within 10 mv. at several points along the titration curve, especially in the end-point region and beyond. Kolthoff (22) has shown, however, that in dilute solutions the platinum wire may not assume a reproducible potential, and Druet (9) has claimed a variation in the end point using a silver electrode which depends on the age and nature of the silver electrode. These factors are insignificant when using the differential titrator, because the

titration is automatically terminated at the inflection point and not at an absolute preset potential.

Figure 6 shows a recorded second derivative titration curve for this titration, and is presented to illustrate considerable voltage fluctuation on the upswing of the second derivative curve. The broken line represents the thyratron firing level. The first two sharp voltage pulses to go above and then below this level do not, however, operate the buret solenoid or motor because condenser C_2, Figure 1, does not have sufficient time to charge to a value that will open relay 2. The titration curve in Figure 6 is typical for the titration of chloride with silver nitrate at a flow rate of 7 ml. per minute, but no false end points were experienced.

In all the titrations 10-ml. aliquots of approximately $0.065N$ sodium chloride were diluted with about 75 ml. of distilled water and titrated with $0.1N$ silver nitrate solution. The results tabulated in Table V show a precision within the reading error of the buret. No significant differences in the second derivative titration curves were noticed between the platinum–10% rhodium, platinum, and silver wire indicator electrodes, which were paired with a calomel reference electrode isolated from the solution with a salt bridge. Better precision might have been obtained if a buret with less reading error had been used.

COMPLEX FORMATION TITRATIONS

The automatic differential titrator was also applied to a soluble complex formation titration, for which case chloride was titrated with a standard mercuric nitrate solution.

The usual wet method employs sodium nitroprusside as internal indicator, and requires a slight excess of mercuric ion to cause a visual turbidity (21). The disadvantage to this method is the variable indicator blank, varying from 0.05 to 0.20 ml., depending upon the final volume, concentration of reagents, acidity, and the method of viewing the end point.

The high frequency titration technique has also been used for this titration (3), but it has the disadvantage of requiring a low foreign electrolyte concentration. Chloride has been titrated

Figure 6.   Recorded second derivative curve for titration of chloride with silver nitrate

Figure 7.   Recorded second derivative curve for titration of chloride with mercuric nitrate

potentiometrically with mercuric nitrate with a mercury indicator electrode (28), and it is possible to use a platinum wire as an indicator electrode (33). Although the potential of a platinum wire drifts greatly during a titration of chloride with mercuric nitrate, this is not serious in automatic differential titrations. Either a platinum or platinum–10% rhodium wire indicator electrode was found suitable for the automatic differential titrations, and a recorded titration curve is shown in Figure 7 for a titrant flow rate of 2.6 ml. per minute. Figure 7 shows the drift near the start of the titration, and although this initial drift does not affect the operation of the titrator, it can be eliminated by adding a little mercuric chloride at the start.

Table IV.   Titration of Hydrazine Sulfate with Potassium Iodate
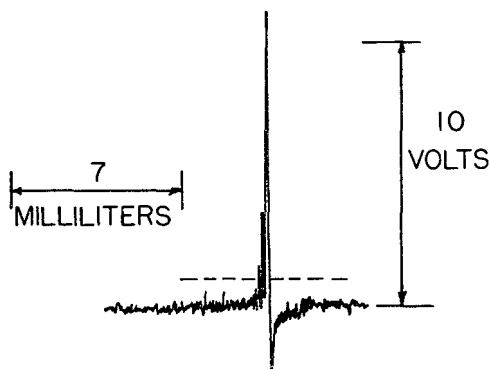
(Weighed samples of hydrazine sulfate were dissolved in 15 ml. of water and 45 ml. of concentrated hydrochloric acid and titrated with $0.02500M$ potassium iodate)

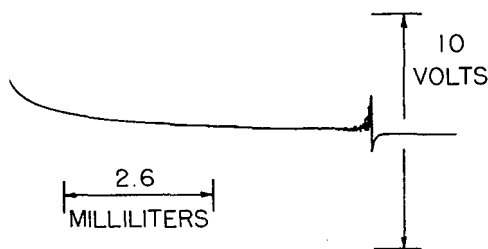| Conditions	$N_2H_4 \cdot H_2SO_4$, G.	KIO_3, Ml.	Purity of Sample, %
Manual titration with	0.1101	34.80	102.7
brilliant scarlet 3R	0.0824	26.60	105.1
as indicator	0.0542	16.90	101.5
	0.0748	23.29	101.3
Automatic	0.0996	30.42	99.36
Pt vs. Calomel elec-	0.1323	40.41	99.37
trode,	0.0584	17.83	99.32
2 ml./minute	0.1372	41.89	99.33

Table V.   Titration of Chloride with Silver Nitrate

(10 ml. aliquots of sodium chloride were diluted with 75 ml. of distilled water and titrated with silver nitrate at a flow rate of 7 ml. per minute)

	Silver Nitrate, Ml.		
		Automatic	
Manual Mohr method	Pt (10% Rh) vs. calomel	Platinum vs. calomel	Silver vs. calomel
6.53	6.57	6.54	6.55
6.57	6.57	6.57	6.57
6.56	6.56	6.58	6.57
6.60	6.55	6.55	6.58
	6.53		
Av. 6.57	6.59	Av. 6.56	6.57
	6.57		
	6.58		
	6.54		
	6.56		
	6.56		
	6.58		
	6.55		
	Av. 6.56		

The magnitude of the second derivative curve is not large because the potential break is not sharp, but it is sufficient to operate the buret relay. In fact, at a delivery rate of 2.6 ml. per minute the magnitude of the second derivative curve is just sufficient to trip the relay, and in this titration case the relay circuit should not have any delay inserted because of the sharp voltage spike on which it operates. Of course, with a noisy endpoint curve as shown in Figure 7, it is advantageous that the relay operates only near the peak of the derivative curve so as to prevent false end points just prior to the equivalence point. If routine titrations were to be performed with poor second derivative curves, it would be best to modify the relay circuit so the titration would not be terminated until the second derivative curve had swung slightly negative in the case shown in Figure 7. This would cause an insignificant overshoot of the inflection point and prevent false end points.

The titrant delivery rate was generally maintained below 3 ml. per minute to eliminate any blank correction from either slow solution equilibration or lag in electrode response. However, at faster delivery rates, it was found that the lag was reproducible and a blank correction could be applied as in the other types of titrations described.

Aliquots of approximately $0.065N$ chloride were diluted to

Table VI. Titration of Chloride with Mercuric Nitrate

(Various aliquots of sodium chloride solution were diluted to 75 ml. with distilled water and titrated with approximately 0.1N mercuric nitrate)

Conditions	Chloride, Ml.	Hg(NO$_3$), Ml.	
Manual potentiometric	10.00		6.04
			6.08
			6.04
			6.05
		Av.	6.05
Automatic, 2.6 ml./minute	10.00		6.06
			6.04
			6.03
			6.05
			6.05
			6.07
			6.07
			6.04
		Av.	6.05
	20.00		12.11
			12.10
			12.09
		Av.	12.10
	30.00		18.15
			18.17
			18.15
		Av.	18.16
Automatic, 7 ml./minute	10.00		6.30
			6.29
			6.28
			6.31
			6.32
		Av.	6.30

Table VII. Titration of Cyanide with Silver Nitrate

(10-ml. aliquots of sodium cyanide solution were diluted with about 50 ml. of distilled water and titrated with approximately 0.1N silver nitrate at a flow rate of 2.6 ml. per minute)

Conditions	Silver Nitrate, Ml.		
	First end point	Second end point	Average of 1st and 2nd end points
Automatic Pt (10% Rh) vs. calomel, 2.6 ml./minute	5.92	5.97	5.95
	5.90	5.95	5.93
	5.86	5.87	5.87
	5.93	5.91	5.92
	5.89	5.93	5.91
	5.86	5.92	5.88
	5.90	5.92	5.91
	Av. 5.89	5.92	5.91
Manual Liebig-Denigès method	5.90		
	5.90		
	5.88		
	5.91		
	Av. 5.90		

about 75 ml. with distilled water and titrated with approximately 0.1N mercuric nitrate by both the manual potentiometric technique and the automatic differential titrator. An electrode system of platinum indicator electrode vs. calomel isolated with a salt bridge was used. Automatic titrations were also performed with the sample size of chloride doubled and tripled, and gave results (Table VI) that show no time lag at the titrant flow rate of 2.6 ml. per minute.

MULTIPLE END-POINT TITRATIONS

The titration of cyanide with silver nitrate was performed in order to demonstrate the automatic differential titration of a system having two or more potential breaks without making any instrument adjustments throughout the titration. After the titration is automatically terminated at the first end point, the start button is merely pushed and the titration again automatically terminated at the second end point. The titrant delivery rate must be less than 3 ml. per minute because of the relatively slow rate that the solution equilibrates.

A platinum wire was used as the indicator electrode. Allen and Hickling (1) studied this electrode for this reaction and showed that during the formation of the soluble complex the silver ion concentration is so low that the platinum wire does not establish the potential of a silver electrode until some time near the first potential break. This was of no consequence when using the automatic differential titrator, because the platinum electrode responded rapidly and gave potential breaks at the end
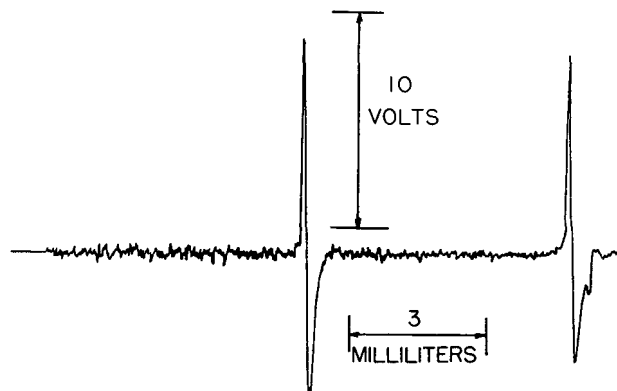
Figure 8. Recorded second derivative curve for titration of cyanide with silver nitrate

points of sufficient magnitude for differentiation and proper operation of the instrument. A typical recorded curve of this titration, with relay system disconnected, is shown in Figure 8.

Ten-milliliter aliquots of cyanide were diluted with about 50 ml. of distilled water and automatically titrated with 0.1N silver nitrate solution. The milliliter ratio was first determined manually by the Liebig-Denigès method. Results are tabulated in Table VII and show a precision about the same as the buret reading error.

REFERENCES

(1) Allen, P. L., and Hickling, A., Anal. Chim. Acta, 11 [5], 467 (1954).
(2) Andrews, L. W., J. Am. Chem. Soc., 25, 756 (1903).
(3) Blaedel, W. J., and Malmstadt, H. V., ANAL. CHEM., 22, 1410 (1950).
(4) Ibid., 24, 450 (1952).
(5) Boltunov, Y. A., and Krogius, E. A., J. Gen. Chem. (U.S.S.R.). 7, 2838 (1937).
(6) Boltz, D. F., "Selected Topics in Modern Instrumental Analysis," p. 21, Prentice-Hall, New York, 1952.
(7) Brünnick, J. C., Ind. Eng. Chem., 17, 631 (1925).
(8) Conant, J. B., and Hall, N. F., J. Am. Chem. Soc., 49, 3047 3062 (1927).
(9) Druet, Y., Compt. rend., 222, 1480 (1946).
(10) Fritz, J. S., "Acid-Base Titrations in Nonaqueous Solvents," G. Frederick Smith Chemical Co., Columbus, Ohio, 1952.
(11) Fritz, J. S., ANAL. CHEM., 22, 1028 (1950).
(12) Fritz, J. S., and Lisicki, N. M., Ibid., 23, 589 (1951).
(13) Furman, N. H., IND. ENG. CHEM., ANAL. ED., 2, 213 (1930).
(14) Ibid., 14, 367 (1942).
(15) Furman, N. H., ANAL. CHEM., 22, 33 (1950).
(16) Ibid., 23, 21 (1951).
(17) Ibid., 26, 84 (1954).
(18) Giunta, A., Chimica e industria (Milan), 33, 695 (1951).
(19) Holt, M. L., and Kahlenberg, L., Trans. Am. Electrochem. Soc., 57, 361 (1930).
(20) Kolthoff, I. M., and Furman, N. H., "Potentiometric Titrations," 2nd ed., Wiley, New York, 1931.
(21) Kolthoff, I. M., and Stenger, V. A., "Volumetric Analysis," Vol. II, p. 331, Interscience, New York, 1947.
(22) Kolthoff, I. M., and Wang, C., J. Phys. Chem., 41, 539 (1937).
(23) Lux, H., Z. Elektrochem., 45, 303 (1939).
(24) Malmstadt, H. V., and Fett, E. R., ANAL. CHEM., 26, 1348 (1954).
(25) Markunas, P. C., and Riddick, J. A., Ibid., 23, 337 (1951).
(26) McBride, W. R., Henry, R. A., and Skolnik, S., Ibid., 23, 890 (1951).
(27) Moss, M. L., Elliott, J. H., and Hall, R. T., Ibid., 20, 784 (1948).
(28) Müller, E., and Aarflot, H., Rec. trav. chim., 43, 874 (1924).
(29) Obruchera, A., J. Phys. Chem. (U.S.S.R.), 11, 473 (1938).
(30) Perley, G. A., and Godshalk, J. B., (to George Kent, Ltd.) Brit. Patent 567,722 (1945).
(31) Singh, B., and Ilani, I., J. Indian Chem. Soc., 14, 376 (1937).
(32) Tikhonov, A. S., Zavodskaya Lab., 8, 17 (1939).
(33) Vyakhirev, D. A., and Guglina, S. A., Ibid., 15, 1426 (1949).
(34) Willard, H. H., and Boldyreff, A. W., J. Am. Chem. Soc., 51, 471 (1929).

New Method for Determining Phthalate Esters in Propellants

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This work was undertaken in order to develop a convenient and reliable method for the determination of phthalate esters in propellants. The method described is based upon a titanous chloride reduction of nitroesters and nitroaromatic bodies, which allows a separation of phthalate esters, by a petroleum ether extraction, and their volumetric determination. This method has been applied to the determination of dimethyl phthalate, diethyl phthalate, dibutyl phthalate, diamyl phthalate, and diphenyl phthalate, in the presence of nitroglycerin, dinitrotoluene, diphenylamine, ethyl centralite, and ethyl acetate. The method allows one to determine the percentage of phthalate esters in smokeless powders with a precision within 0.02 to 0.03%. It is accurate and simple and requires a short operating time, and is therefore well suited for routine control work in industrial laboratories. A very convenient procedure for identifying the type of phthalate esters has also been developed.

DURING the past 10 years several papers have been published dealing with the determination of phthalates. Their authors suggested polarographic (14), spectrometric (9), volumetric (2), or gravimetric methods (8, 11).

However, there has been up to the present considerable need for a convenient and reliable method for determining phthalates in propellants which requires a specific procedure, because of the interference of other ingredients, such as stabilizers, nitroesters, and nitroaromatic bodies.

All the previously published methods for the determination of phthalates in propellants possess serious disadvantages. The earliest, the lead phthalate gravimetric method, proposed by Thames (12), is complicated and has been proved to be theoretically incorrect (10). The method of Lamond (5) applies an ammonium sulfide reduction procedure in order to separate the phthalates from the nitroesters and nitroaromatic bodies. It is, however, lengthy and time consuming because of the numerous operations involved in the separation of phthalates from the nitroesters and nitroaromatic bodies, as well as in the preparation of the ammonium sulfide reagent, according to the special requirements of the method. Moreover, its disadvantages are not outweighed by its accuracy. The iodometric method of ethyl phthalate determination, developed by Butts, Prine, Kouba, and Becker (1) is based on the oxidation of ethyl alcohol, which has been obtained from the phthalate, after its saponification. However, the traces of the residual solvents, if present in the powder, will interfere, causing too high results.

A partly indirect method has been adopted by an American Army specification (6) for diethyl phthalate determination. According to this method the phthalate together with the nitroglycerin is chromatographically separated from the ethyl centralite and the former is calculated from the difference after the titanometric determination of nitroglycerin. This procedure has been suited to only one definite type of powder and possesses the disadvantages of an indirect determination. The recently published infrared spectrophotometric method of Pristera (7) seems to outline a fast procedure. However, it requires expensive equipment which is not usually available for routine work in industrial laboratories. The latest acidimetric method of Tranchant (13) suggests the liberation of phthalic acid from potassium phthalate by the action of phosphoric acid on the latter, which has previously been formed as a result of the ether-extract

saponification of the powder. Then the free phthalic acid is separated and titrated with alkali according to the Kavanaugh (4) procedure. The exactness of this method is low, especially if applied to powders containing nitroaromatic bodies or a high percentage of nitroglycerin.

This work was undertaken in an effort to obtain a quick, reliable, and generally applicable method of determining phthalates in propellants, well suited for routine control analysis. This method has been based upon a titanous chloride reduction of nitroesters and nitroaromatic bodies which allows a separation of phthalates by a petroleum ether extraction. The phthalates are afterward saponified in an alcohol solution, previously neutralized, and the alkali is retitrated.
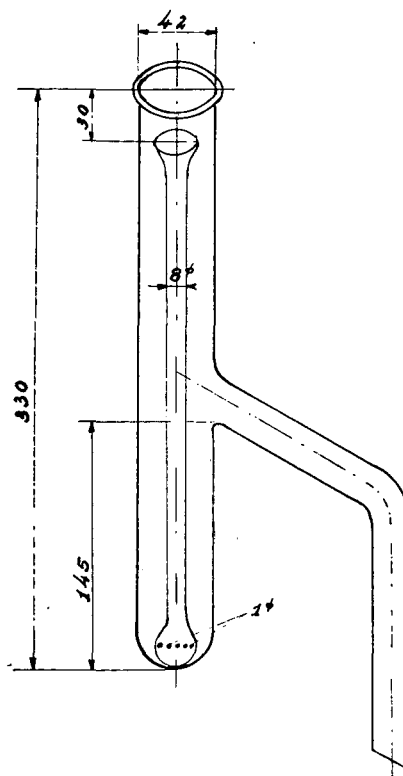
Figure 1. Liquid-liquid extraction apparatus

The method proposed in this paper has been applied to the determination of dimethyl phthalate, diethyl phthalate, dibutyl phthalate, diamyl phthalate, and diphenyl phthalate. Both the artificially prepared mixtures of phthalates with nitroglycerin, dinitrotoluene, diphenylamine, ethyl centralite, and ethyl acetate as well as powders of known composition have been tested. The method proved to be uncomplicated and exact.

APPARATUS AND REAGENTS

Liquid-liquid extraction apparatus (Fisher Scientific Co., Catalog No. 9-573 with slightly modified dimensions). The apparatus consists of an outer glass jacket with a bent side tube which is connected to an Erlenmeyer flask of 200-ml. capacity:

The jacket encloses a narrow inner tube with a funnel top and an enlarged bulb-shaped lower end with small openings.

Petroleum ether. Collect the fraction of boiling point 60° to 70° C. from the purified petroleum, boiling point, 60° to 90° C.

Titanium chloride solution, 20%.

Acetic acid, 84%.

Standard hydrochloric acid, 0.1N.

Alcoholic potassium hydroxide solution, approximately 0.2N. This should contain about 10% of water, which will prevent precipitation of the potassium phthalate formed in the reaction.

RECOMMENDED PROCEDURE

Weigh 2 grams of previously ground propellant into a 250-ml. flask. Add 20 ml. of 84% acetic acid, fit the condenser into the neck of the flask, place it on a water bath, and heat for 20 minutes at 100° C. Decant the liquid and filter into another 250-ml. flask. Repeat the extraction of the propellant with a second 20-ml. portion of 84% acetic acid. Filter the acetic acid solution and rinse the residue with several portions of acetic acid, using a total of 30 ml.

Table I. Hydrolysis of Phthalates during Reduction Procedure

	Time of Heating, Minutes, at ° C.			
	2 at 100	3 at 100	5 at 100	5 at 60, then 5 at 100
Reduced nitroesters, %	93.3	98.5	99.9	100.0
Hydrolyzed dimethyl phthalate, %	0.04	0.06	0.11	0.12
Hydrolyzed dibutyl phthalate, %	0.02	0.03	0.05	0.05

Table II. Summary of Results Obtained on Synthetic Samples

Sample	Phthalate Added, Gram	Phthalate Found, Gram	Recovery, %
Dimethyl phthalate + 1 gram of mixture A	0.1348	0.1347	99.9
	0.1375	0.1375	100.0
	0.1845	0.1841	99.8
Diethyl phthalate + 1 gram of mixture A	0.1280	0.1283	100.2
	0.1562	0.1568	100.4
Dibutyl phthalate + 1 gram of mixture A	0.1220	0.1221	100.1
	0.1917	0.1923	100.3
	0.1766	0.1769	100.2
Dibutyl phthalate + 1 gram of mixture B	0.1624	0.1624	100.0
	0.1625	0.1626	100.1
	0.1773	0.1780	100.4
Diphenyl phthalate + 1 gram of mixture B	0.1735	0.1736	100.1
	0.1561	0.1555	99.6
	0.1842	0.1836	99.7
	0.1673	0.1670	99.8

Composition of mixture A. 90% nitroglycerin, 10% ethyl centralite.
Composition of mixture B. 60% nitroglycerin, 35% dinitrotoluene, 3% ethyl acetate, and 2% diphenylamine.

After all of the phthalate solution in acetic acid is collected in the second flask, pass in carbon dioxide to expel the air. Add 10 ml. of titanium trichloride solution for each 100 to 125 mg. of nitroglycerin or 75 to 100 mg. of dinitrotoluene present in the solution (the solution must remain intensively violet after the reduction is completed as well as in the course of the extraction). Place the bottle on a water bath and heat the solution successively 5 minutes at 60° C. and 5 minutes at 100° C.; cool the flask, and add 30 ml. of petroleum ether and 20 to 50 ml. of water. Insert a rubber stopper and shake the contents of the flask vigorously for 5 minutes. Transfer the contents of the flask into the outer jacket of the liquid-liquid extraction apparatus and add 30 to 50 ml. of water. Rinse the flask first with 3 to 4 ml. of acetic acid and then with 50 ml. of petroleum ether and add these solutions to the contents of the jacket. Put the inner tube into the jacket. Take care that no acetic acid solution passes into the receiving flask. Connect the jacket with the condenser, and place the receiving flask on the heater. Isolate the upper part of the jacket by means of a thick glass-wool layer to prevent the condensation of the petroleum ether vapors on the walls of the outer jacket.

Start the heating and adjust the boiling rate of the petroleum ether in the Erlenmeyer flask so that 4 to 5 drops of petroleum ether pass per second through the aqueous solution (for 8 hours). The 8 hours of continuous extraction under the stated conditions are sufficient for complete extraction of quantities of phthalate up to 200 mg. Then remove the receiving bottle, connect with a

deflegmator and condenser, and evaporate the petroleum ether on a water bath. Rinse the deflegmator with 10 ml. of ethyl alcohol and transfer the alcohol into the receiving bottle. Add 4 to 5 drops of phenolphthalein, cool the alcoholic solution to between 0 and 5° C., add the potassium hydroxide solution dropwise, until a slight pink coloration appears. Neutralize the excess of alkali with 1 to 2 drops of hydrochloric acid solution. Add exactly 20 ml. of the potassium hydroxide solution, fit a reflux condenser, and heat 45 minutes on a water bath at 100° C., then rinse the condenser with 50 ml. of distilled water free from carbon dioxide. Titrate the excess of alkali with hydrochloric acid solution. Carry out a blank titration.

$$\frac{(B - S) \times N \times K \times 100}{\text{Grams of sample} \times P} = \% \text{ phthalate ester}$$

where

B = ml. of HCl required for blank determination
S = ml. of HCl required for back titration
N = normality of HCl
$K = \dfrac{\text{molecular weight of phthalate ester}}{2 \times 1000}$
P = purity of estimated phthalate expressed as a decimal. This is calculated from the saponification number; if the latter is not known, P is assumed to be 1.000.

DISCUSSION AND RESULTS

Owing to a very rapid extraction procedure [adapted from Hirschhorn (3)], and to the comparatively small number of operations involved, the time of determining phthalates in propellants is greatly shortened, as compared with most other available methods. The extraction is directly followed by a titanous chloride reduction. The reduction of nitroesters and nitroaromatic bodies extracted from the powder is quantitative under prescribed conditions. During the reduction procedure the hydrolysis of phthalates is negligible (Table I).

A summary of results obtained by analyzing the artificially prepared mixtures of phthalates with the usual smokeless powder ingredients is presented in Table II. For all the analyzed samples the difference between the given amount of phthalates and that found by the analysis does not exceed ±0.4%.

Table III. Summary of Results for Some Typical Smokeless Powders

Sample No.	Principal Ingredients	Phthalate Calculated from Difference, %	Phthalate Found, %	Average
1	Diethyl phthalate Ethyl centralite Nitroglycerin Nitrocellulose	2.6	2.61 2.63 2.63	2.62
2	Dibutyl phthalate Diphenylamine Nitroglycerin Nitrocellulose	6.2	6.19 6.15 6.14	6.16
3	Dibutyl phthalate Diphenylamine Dinitrotoluene Nitrocellulose	5.0	5.00 5.01 5.03	5.01
4	Diamyl phthalate Ethyl centralite Dinitrotoluene Nitrocellulose	3.9	4.02 4.05	4.03
5	Diphenyl phthalate Diphenylamine Dinitrotoluene Nitroglycerin Nitrocellulose	5.2	5.03 5.01 4.99	5.01

Some of the results obtained for the analyzed propellants are contained in Table III. For all the analyzed samples the results are reproducible within ±0.03% of the phthalate contents in the powder, and are also in accordance with those calculated from the difference between the total weight of the sample and that of all the other ingredients.

The method permits the determination of the percentage of phthalates in practically all smokeless powders, now in use, with a precision within 0.02 to 0.03, by a simple procedure, which requires only a short operating time.

IDENTIFICATION OF TYPE OF PHTHALATE ESTER

The following two methods dealing with the identification of the type of phthalate ester in propellants may be found in the literature on the subject: the infrared spectrophotometric method of Pristera (7) and the method of Tranchant (13), which is based on a rather lengthy procedure consisting of the separation of free alcohols from the phthalate esters and identification of the former by their p-nitrobenzoic acid esters.

By the present method it is possible to separate the phthalate ester qualitatively from the other propellant ingredients by a simple and convenient procedure. The separated phthalate esters may be conveniently identified by their refractive indices, by their saponification number, or by one of the other usually applied methods.

The following procedure for the separation of phthalate esters from the other propellant ingredients is recommended.

Reagents. Hydrochloric acid–acetic acid solution. Mix 500 ml. of concentrated hydrochloric acid with 500 ml. of glacial acetic acid.

Potassium dichromate solution. Dissolve 5 grams of U. S. Pharmacopoeia potassium dichromate in 370 ml. of distilled water and pour this solution slowly into 350 ml. of sulfuric acid (specific gravity, 1.84; A.C.S.).

Sodium bicarbonate solution, 0.5%.

Carbon tetrachloride, U.S.P.

Table IV. Refractive Indices of Phthalate Esters[a]

No.	Type	Ingredients	Type	n_D^{20}	n_D^{20}	n_D^{74}
		Sample			Refractive Index Found for Separated Phthalate Ester	
			Phthalate Ester			
1	Mixt.	Pht, NG, C	Dimethyl phthalate	1.5138	1.5135	...
2	Mixt.	Pht, DNT, DFA	Dimethyl phthalate	1.5138	1.5135	...
3	Mixt.	Pht, NG, C, DNT, DFA	Diethyl phthalate	1.5019	1.5020	...
4	Prop.	Pht, NG, C	Diethyl phthalate	1.5010	1.5010	...
5	Mixt.	Pht, NG, DFA, C, DNT	Dibutyl phthalate	1.4900	1.4900	...
6	Mixt.	Pht, NG, M.J., C	Dibutyl phthalate	1.4900	1.4885	...
7	Prop.	Pht, NG, M.J., C	Dibutyl phthalate	1.4925	1.4905	...
8	Prop.	Pht, DNT, DFA	Dibutyl phthalate	1.4925	1.4920	...
9	Mixt.	Pht, NG, DNT, DFA, C	Diisoamyl phthalate	1.4860	1.4860	...
10	Mixt.	Pht, NG, DNT, DFA, C	Diphenyl phthalate	$n_D^{74} = 1.572$	1.571	

[a] Abbreviations. Mixt., synthetic mixture. Prop., propellant. Pht, phthalate ester. NG, nitroglycerin. C, ethyl centralite. DFA, diphenylamine. DNT, dinitrotoluene. M.J., mineral jelly.

Procedure. Extract the propellant with acetic acid and reduce the extracted nitroglycerin or dinitrotoluene, as previously described, but without the precautions observed in the quantitative determination. After the reduction has been completed, transfer the solution to a 250-ml. separatory funnel, and add 30 ml. of concentrated hydrochloric acid and 40 ml. of carbon tetrachloride. Shake vigorously, allow the two layers to separate, and transfer the carbon tetrachloride layer to a 150-ml. separatory funnel. The procedure then depends on the type of stabilizer present in the propellant.

If the propellant has been stabilized only with diphenylamine proceed as follows:

Rinse the carbon tetrachloride solution with two to three portions of hydrochloric acid–acetic acid mixture, 10 ml. each, so as

to wash out completely the remaining traces of diphenylamine (a strip of filter paper moistened with 1 to 2 drops of the carbon tetrachloride solution and of the potassium dichromate solution should give no traces of blue coloration). Then successively rinse the carbon tetrachloride solution with 10 ml. of sodium bicarbonate solution and with two 10-ml. portions of distilled water, and transfer to a 100-ml. distilling flask. Concentrate the solution to 5 to 6 ml. If it is colored, add a pinch of activated carbon and filter into a small beaker. Evaporate the carbon tetrachloride completely. The phthalate ester remains on the bottom of the beaker.

This procedure must be modified for a propellant containing ethyl centralite. In this case the rinsing with hydrochloric acid–acetic acid mixture may be omitted as inefficient, the rest of the procedure remaining unchanged. Then in order to eliminate the ethyl centralite, the following additional operations are required. Add 25 ml. of the potassium dichromate solution, cooled previously to between 5° and 10° C., to the phthalate ester mixed with ethyl centralite, which remained in the beaker. Transfer the solution to a 150-ml. separatory funnel. Rinse the beaker with several portions of petroleum ether to a total of 50 ml. and add the petroleum ether solution to the separatory funnel. Shake it and separate the petroleum ether layer. Rinse with two to three 5-ml. portions of potassium dichromate solution, cooled to between 5° and 10° C., so as to wash out the ethyl centralite completely. (The last portion of the potassium dichromate solution should not give any change in its original coloration after having been vigorously shaken with the petroleum ether solution.) Then rinse and evaporate the petroleum ether solution as previously described (for the rinsing and evaporation of the carbon tetrachloride solution). The phthalate ester remains on the bottom of the beaker.

CONCLUSIONS

The isolated phthalate ester may now be identified by its refractive index.

Refractive indices of phthalate esters, isolated by the described procedure from artificially prepared mixtures and from propellants of known composition, have been determined in a Spencer Abbe type refractometer. Results are presented in Table IV.

As is evident from Table IV, the pure phthalate esters have been separated from all the synthetic mixtures and propellants examined, except from those containing mineral jelly (for those the refractive indices are slightly diminished because of the traces of mineral jelly contained in them).

Considering the differences in the refractive indices of the phthalate esters, normally used in the production of propellants, the purity of all phthalate esters separated is sufficient to identify them by their refractive indices.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Butts, P. G., Prine, G. B., Kouba, D. L., and Becker, W. W., ANAL. CHEM., 20, 1066–7 (1948).
(2) Goldberg, A. I., Ibid., 16, 198–200 (1944).
(3) Hirschhorn, I. S., ANAL. CHEM., 19, 880–2 (1947).
(4) Kavanaugh, F., IND. ENG. CHEM., ANAL. ED., 8, 397–8 (1936).
(5) Lamond, J., Analyst, 73, 674–7 (1948).
(6) Office of Chief of Ordnance, Washington 25, D. C., Military Specification, MIL-P-10557 (Ord.).
(7) Pristera, F., ANAL. CHEM., 25, 844–55 (1953).
(8) Shaefer, W. E., and Becker, W. W., Ibid., 25, 1226–31 (1953).
(9) Shreve, O. D., and Heether, M. R., Ibid., 23, 441–5 (1951).
(10) Smith, S. B., and Strempfer, J. F., Ibid., 16, 416 (1944).
(11) Swann, M. H., Ibid., 21, 1448–53 (1949).
(12) Thames, F. C., Ibid., 8, 418–9 (1936).
(13) Tranchant, J., Mém. poudres, 35, 303 (1953).
(14) Whitnack, G. C., and Gantz, E. St. C., ANAL. CHEM., 25, 553 (1953).
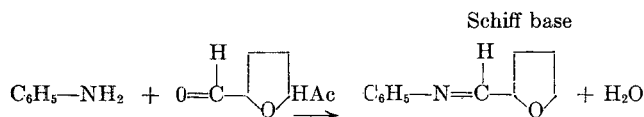
Rapid Detection of Aniline Vapors in Air

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Aniline vapor in concentrations above 5 p.p.m. (by volume) in air is known to be toxic. Current applications of aniline made it imperative that a fast and simple field method be developed. The concentration of aniline vapor in the air can be determined easily and rapidly by use of paper strips impregnated with the liquid or vapor of a furfural–acetic acid mixture (4%, by volume, in glacial acetic acid). The test paper turns from white to pink or red in the presence of aniline vapor, having a range of determination from 5 to approximately 150 p.p.m. The speed and intensity of color development increases with the concentration of aniline vapors. The test is specific for aniline in the presence of many other organic vapors. The reagent mixture has a life of at least 1 month at room temperature.

ORGANIC amines, such as aniline and its homologs, are known to be poisonous in both the liquid and vapor phases— e.g., concentrations higher than 5 p.p.m. (by volume) of aniline should be avoided in working areas (3, 4, 6–8). A rapid and extremely simple field method of analysis of aniline vapor in this concentration range is not available. The method probably in most common use is the official British method (1). A known volume of air has to be drawn through an acid solution and this solution subsequently analyzed using standard colorimetric techniques. Recommended spot test methods also require the presence of a liquid phase, and thus also require absorption of the vapors in a liquid.

It has been found that the approximate concentration of aniline vapor in the atmosphere can be detected rapidly by use of paper strips impregnated with the liquid or vapor of a furfural–acetic acid mixture (4%, by volume, in glacial acetic acid). The test is based upon the condensation to colored Schiff bases, as follows:

Schiff base

$C_6H_5-NH_2 + O=C-\underset{O}{\overset{H}{\underset{\diagdown}{\diagup}}}HAc \longrightarrow C_6H_5-N=C-\underset{O}{\overset{H}{\underset{\diagdown}{\diagup}}} + H_2O$

The prepared strips turn pink to red in the presence of aniline vapors, having a lower limit of detection at about 5 p.p.m. The speed and intensity of color development increases with the concentration of aniline vapors.

REAGENTS AND EQUIPMENT

Furfural, technical
Glacial acetic acid
Silica gel, 6 to 16 mesh
Filter paper strips, 115 × 5 mm.
Glass tube, approximately 178 × 25 mm. outside diameter.
Perforated porcelain disk, approximately 20 mm.

PROCEDURE

Preparation of Reagent Mixture. Dilute 2 ml. of technical grade furfural to 50 ml. in a volumetric flask with glacial acetic acid. Saturate approximately 13 grams of silica gel with this solution and drain off excess liquid for 3 to 5 minutes.
Preparation of Tube and Strips. Constrict a glass tube (28 mm. outside diameter), approximately 178 mm. long, with four indentations 76 mm. from one end. Cut about two dozen strips of filter paper (115 × 5 mm.) and fasten to cork by means of masking tape. Insert strips and cork into long end of tube. Place perforated porcelain disk in position on short end. Place the reagent mixture charge in the lower end and close tight with cork covered with aluminum foil. Allow to stand for at least 15 minutes before use.

SENSITIVITY

The effect of aniline concentrations from 1 to 155 p.p.m. was determined by use of known samples. A 20-liter glass jug was used as a test chamber, into which known amounts of aniline were added and allowed to evaporate. Concentrations of aniline were calculated by use of

$$\text{Mg./liter} = MP/T \times 1.61 \times 10^{-5} \text{ p.p.m. (by volume) } (3, 5)$$

where

M = molecular weight
T = temperature in ° K.
P = pressure in millimeters

Assuming standard temperature and pressure, 20 liters of a 5 p.p.m. solution of aniline in air will then contain 0.38 mg. of aniline.

Table I. Effect of Aniline Vapors on Furfural–Acetic Acid Strips

Concn., P.P.M. (Vol.)	Exposure, Min.		Relative Intensity
	First formation	Definite color	
0	10	..	No observable change
	12	..	
1	10	..	No observable change
5	6	8	Pale pink
	4	5	
6	4.5	8	
	5		
	4.5		
10	2	3	Pink
	3	5	
	2.75		
55	Sec. 15	Sec. 60	Red
60	20	30	
61	15	60	
	35	85	
110	15	30	Deep red
111	15	30	
		45	
155	20	45	
	5	5	

Table II. Sensitivity of Furfural–Acetic Acid Strips

Concn., P.P.M.	Exposure, First Formation to Definite Color	Relative Intensity
1	10 min.	No observable change
5	5 to 8 min.	Pale pink
10	3 to 5 min.	Pink-red
60	30 to 60 sec.	Red
110	15 to 45 sec.	Deep red
155	5 to 45 sec.	Deep red

A standard solution was prepared fresh daily by diluting 1.9 ml. (approximately 1.9 grams) of aniline to 500 ml. with acetone. Each milliliter of the latter solution when evaporated in the 20-liter chamber then gave 5 p.p.m. of aniline.

Upon addition of the standard solution the chamber was stoppered, inverted for 2 minutes, and shaken to mix the aniline vapor into the air. A test paper was then suspended from the stopper by use of masking tape so that it hung about one third from the bottom (to allow for the fact that aniline vapor is over three

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times heavier than air). Times were then noted at which the color first formed and when it became prominent. The relative intensity was also estimated. Results are shown in Table I.

Although the results are not so nearly reproducible as customarily expected, or desired, for an ordinary quantitative method, they are quite satisfactory to serve as a basis for a semiquantitative determination, or an exceptionally good estimation.

Times and colors of normally expected results are listed in Table II.

SPECIFICITY

No observable change of the test strips occurred in a 10-minute exposure to the vapors of the substances listed in the left-hand column of Table III. All tests were made by suspending the strips from the cap of each reagent bottle without direct contact with the reagent itself.

Frehden and Goldschmidt reported results obtained with over 50 amines (2). In only a few cases was no coloration obtained. However, those authors' tests were made in solution rather than the vapor phase, and in most cases it was necessary to heat to dryness.

STABILITY

Since acids catalyze the polycondensation of furfural, it was expected that the reagent mixture should lose its effectiveness within a short time. Tests were made to determine the storage life of the furfural–acetic acid strips at room and elevated (74° C.) temperatures. Using 10-minute exposures at 5 p.p.m. it was found that after 30 to 34 days at room temperature the strips failed to give an easily distinguishable color. The strips stored at 74° C. began to fail after only 12 to 13 days. Thus, storage in a cool location will lengthen the life of the papers considerably.

Table III. Specificity of Furfural–Acetic Acid Strips

(10-minute exposure to vapors)

No Observable Change	Weak Color	Positive Color
Furfuryl alcohol		
Hydrazine, 95%		
Red fuming ⎫		
White fuming ⎬ nitric acid		
Concentrated ⎭		
Hydrogen peroxide, 76%		
Ethyl alcohol		
Kerosine		
m-Nitroaniline	o-Nitroaniline (yellow)	
	p-Nitroaniline (yellow)	
p-Aminophenol		o-Aminophenol (purple)
m-Aminophenol		
2,4,6-Tribromoaniline		p-Bromoaniline (purple)
Sulfanilic acid		p-Anisidine (magenta)
1-Naphthylamine	Diphenylamine (gray)	p,p'-Diaminodiphenyl- methane (red)
2-Naphthylamine		
2-Aminoanthraquinone		
Benzylamine		
Dimethylaniline		
p-Aminodimethylaniline		
p-Nitrosodimethylaniline		
p-Phenylenediamine	o-Phenylenediamine (yellow)	
m-Phenylenediamine		

However, when the kit remained closed at ambient temperature for 16 months, the limit of detection increased to only 25 p.p.m. At 100 p.p.m. a pink color then formed within 3 minutes.

The pink to red color formed on the strips is not fast, and fades within several hours, even when sealed under nitrogen.

CONCLUSIONS

This device offers an easily portable, pocket size method for the qualitative and semiquantitative determination of aniline vapors in the atmosphere. The procedure is extremely simple and rapid.

It is necessary only to remove a strip of paper from the tube, and expose it to the air in question for 1 to 10 minutes. The concentration may be estimated from the time necessary for development of a definite color. However, it would be preferable to compare the color obtained in a 10-minute exposure with those of standards.
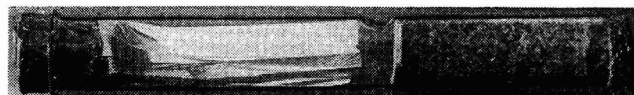

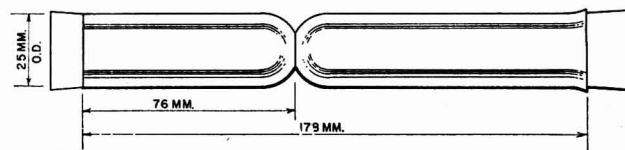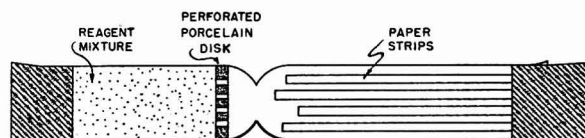**Figure 1.   Aniline test kit ready for use**

Figure 2.   Schematic diagram of prepared tube

Reagents required are inexpensive and readily available. The range of determination appears to be 5 to 150 p.p.m. This is fortuitous, inasmuch as the maximum allowable concentration in air is 5 p.p.m. and above 100 to 150 p.p.m. the toxic symptoms are serious (3, 6, 7). Further, the test is specific for aniline in the presence of other vapors such as indicated in Table III. In daily use at ambient temperature the reagent mixture has a life of at least 1 month, and may be replaced by a fresh charge, if desired.

ACKNOWLEDGMENT

LITERATURE CITED

(1) *Dept. Sci. Ind. Research (Brit.)*, Leaflet No. 11 (1939).
(2) Frehden, O., and Goldschmidt, L., *Mikrochim. Acta*, **1**, 388 (1937).
(3) Jacobs, M. B., "Analytical Chemistry of Poisons, Hazards, and Solvents," 2nd ed., vol. 1, pp. 76, 709, 760, Interscience, New York, 1949.
(4) Ordnance Corps, Dept. of the Army, "Ordnance Safety Manual," **ORD-M 7-224**, 15–19 (1951).
(5) Prentiss, A. M., "Chemicals in War," p. 701, McGraw-Hill, New York, 1937.
(6) Rumpel, W., *Mitt. Chem. Forsch.-Insts. Ind. Österr.*, **4**, 113–17 (1950).
(7) Sax, N. I., "Handbook of Dangerous Materials," p. 25, Reinhold, New York, 1951.
(8) Sayers, R. R., "International Critical Tables," vol. 2, p. 318, McGraw-Hill, New York, 1927.

Coprecipitation of Thallium(I) with Silver Chloride

Precipitation from Homogeneous Solution

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This investigation was undertaken to develop a method for the precipitation of silver chloride from homogeneous solution and to study the coprecipitation of thallium(I) with this carrier. Large crystals of silver chloride were produced. The thallium-silver ratio in the crystal was found to be dependent on the solution concentration of thallium. Under the experimental conditions used the mole ratio of thallium to silver in the precipitate is of the order of 10^{-7}. The results indicate adherence to neither the homogeneous nor heterogeneous distribution laws. However, a pseudohomogeneous distribution is obtained because so small a fraction of thallium is coprecipitated from a solution initially $10^{-6}M$ in thallium that the thallium-silver ratio in the crystal remains essentially constant throughout the entire precipitation process. The gross picture is thus one of an apparent homogeneous distribution of thallium within the crystals of silver chloride.

RECENT studies (*2, 3, 16, 20, 21*) in coprecipitation have utilized the technique of precipitation from homogeneous solution (*1, 22*). This paper reports a study of the coprecipitation of thallium(I) with silver chloride precipitated from homogeneous solution.

Silver ions were slowly released from the silver ammonia complex in the presence of chloride. The complex is destroyed with hydrogen ions produced by the slow hydrolysis of β-hydroxyethyl acetate (*18*). Photomicrographs of silver chloride produced by this technique are shown in Figure 1.

Thallium(I) chloride is known to coprecipitate with silver chloride in the ordinary analytical precipitation (*8*); adsorption studies have also been made with thallium(I) on silver halides (*6, 7, 11*). Although thallium(I) chloride does not mix isomorphously with silver chloride (*12*), it is only slightly soluble; the thallous ion does not precipitate in ammoniacal medium. These and other factors led to the choice of thallium(I) as the ion to be used in this coprecipitation study.

pylene chlorohydrins were found to produce chloride ion at a desirable rate and to result in complete precipitation of the silver. To obtain good results with these chlorohydrins (Matheson Co)., these reagents were first purified by distillation and then passed through a moist ion exchange column containing IRA-400 resin (Rohm and Haas) in the free base form. The initial water-containing eluant was discarded, and the chloride ion–free reagents were used.

These chlorohydrins hydrolyze very slowly at room temperature; only a small amount of precipitate will be obtained in a week's time from 100 ml. of a solution containing 0.1 gram of silver and 10 ml. of the reagent. However, at 60° C. quantitative precipitation can be effected within a few hours. The precipitate is white in the absence of light and consists of fine crystalline particles 2 to 8 microns in diameter. The method shows promise for the determination of silver, as was indicated by a limited gravimetric study.

Another approach to the problem resulted in the production of large crystals of silver chloride, some of which were of the order of 0.2 mm. in diameter. In this method the silver cation was homogeneously released from its ammonia complex, in contrast to the previous method whereby only chloride ion was released from a reagent. Ethylene chlorohydrin was first tested, because it produces upon hydrolysis the necessary hydrogen ion for the destruction of the silver ammonia complex as well as chloride ion for precipitation.

However, a more desirable method of precipitation was evolved by adding chloride ion to the solution containing the silver ammonia complex and effecting the latter's destruction through the hydrolysis of a water soluble ester such as β-hydroxyethyl acetate. The use of the ester is advantageous in that it results in simpler control of chloride ion concentration. It was this method which was adopted for the coprecipitation study.

Determination of Thallium in Silver Chloride Precipitate. Preliminary work showed that the amount of coprecipitated thallium was small. The molar ratio of thallium to silver in the precipitate was of the order of 5×10^{-7}. This necessitated a determination of thallium in the precipitate by the use of radioactive thallium.

PRELIMINARY STUDIES ON PRECIPITATION OF SILVER CHLORIDE FROM HOMOGENEOUS SOLUTION

Several organic compounds containing chlorine were tested to determine whether they would release chloride ion into an aqueous solution by hydrolysis. These were ethylene chlorohydrin, 3-chloro-1,2-propanediol (propylene chlorohydrin), chloroacetic acid, dichloroacetic acid, trichloroacetic acid, chloroacetamide, triglycol dichloride, methylene chloride, chloral hydrate, and ethyl chlorocarbonate. Of these, only ethylene and pro-
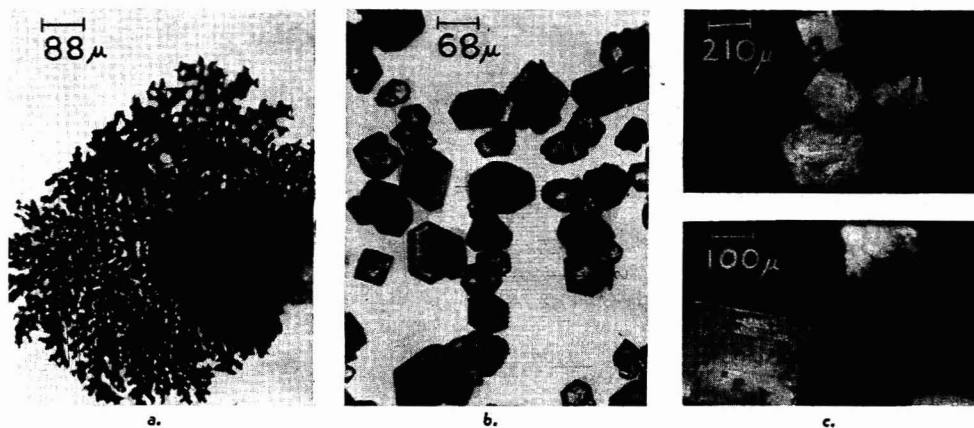
Figure 1. Photomicrographs of silver chloride precipitated from homogeneous solution

a. Direct illumination, 60° C. in 0.0118*M* chloride
b. Direct illumination, 25° C. in 1.00*M* chloride
c. Vertical illumination, 25° C. in 0.0118*M* chloride

THALLIUM-204. Thallium-204 nitrate in better than 99% radiochemical purity, but not carrier-free, was obtained from the Oak Ridge National Laboratory for this work. A solution was treated with sulfur dioxide to ensure that all the thallium was in the unipositive state. After removal of excess sulfur dioxide by boiling, the thallium solution was diluted and stored in a polyethylene bottle. Total thallium was determined by spectrophotometric comparison (4) with a gravimetrically standardized (9) thallium solution.

RADIOACTIVE ASSAY OF STANDARD SOLUTION. The assay of the radioactive thallium solution was carried out by an adaptation of the procedure of Berg and coworkers (17) for the determination of thallium. The procedure was as follows:

An aliquot of the standard radiothallium solution was delivered with a calibrated micropipet and diluted with 1 ml. of water. Then 5.00 ml. of standard thallium(I) nitrate solution (1 ml. = 1 mg. of thallium) were added followed by 1.0 ml. of 10M sodium hydroxide solution. The solution was diluted to 10 ml. and heated to 60° C. Then 2.0 ml. of thionalide solution, 1% in acetone, freshly prepared, were added and the solution was stirred and allowed to cool to room temperature by standing for 1 hour. Next, 1 to 2 drops of Anti-Creep solution, Schleicher and Schuell, were added and the solution was again stirred. A fine jet of water served to stir the solution whenever necessary.

The precipitate was filtered through a Royal Berlin A-3 porcelain crucible, washed with water, then with acetone, and finally air-dried. This method of precipitation leaves less than 0.1% of the original thallium in the filtrate as was determined by the spectrophotometric method (4). The crucible with its thionalide precipitate was then positioned under a Geiger-Müller tube and the activity determined.

The precision of this method is shown in Table I. Because the subsequent determination was to be that of thallium in silver chloride, the latter was added in some of the experiments given in Table I. The silver salt was dissolved with potassium cyanide and the thallium determined as in the preceding procedure. The observed specific activity of samples of the standard radiothallium solution measured by this method was found to be 187 × 10^2 counts per minute per microgram of thallium.

Table I. Precision of Radioactive Method for Thallium

No.	Counts per Minute[a]	Average	Deviation from Over-all Average, %
1[b]	7253, 7266, 7202, 7238	7240	1.1
2[b]	7256, 7360, 7345, 7365	7381	0.8
3[b]	7519, 7439, 7480, 7441	7470	2.1
4[b]	7452, 7395, 7357, 7365, 7421	7398	1.1
5[b]	7371, 7354, 7290, 7240	7315	0.0
6[c]	7153, 7226, 7137, 7180	7174	2.1
7[c]	7340, 7345, 7316, 7229	7307	0.3
8[c]	7274, 7282, 7377, 7188	7280	0.5

[a] All counts were made over a 10-minute period.
[b] These samples contained thallium but no silver chloride.
[c] These samples contained 0.1 gram of silver chloride as well as thallium.

Table II. Precipitation at 60° C.

Time of Precipitation, Hours	Yield of AgCl, Gram	Tl/Ag in Precipitate[a] × 10^5	Fraction of Ag Precipitated	Fraction of Tl Precipitated × 10^3
3.0	0.1114	0.0955	0.838	0.081
4.0	0.1232	0.140	0.928	0.131
5.5	0.1224	0.150	0.922	0.139
23.0	0.1312	0.201	0.988	0.200

[a] Weight ratio.

Determination of Thallium in Precipitate. The precipitate of silver chloride containing the radiothallium was dissolved by the addition of 0.5 gram of potassium cyanide and 1 ml. of water. Five milligrams of stable thallium were then added and the determination was made as before. The amount of radiothallium in the precipitates obtained from the coprecipitation experiments was then determined by reference to the standard value previously obtained.

In these determinations, and in the previous ones as well, cor-

rections were made for dead time and for radioactive decay of the thallium.

PRECIPITATION PROCEDURE

Precipitations were carried out in 250-ml. polyethylene beakers in a water bath controlled to ±1° C. Polyethylene vessels were used to minimize small adsorption losses of thallium on glass. The solutions were stirred by means of a glass rod passed through a stopper inserted in the beaker. The entire precipitation assembly was enclosed in a light-tight box.

The solutions contained, in a 100-ml. volume, 0.1 gram of silver as nitrate, ammonium chloride, ammonium hydroxide, β-hydroxyethyl acetate, and thallium sulfate. All chemicals were of reagent grade and were used without purification except in the case of β-hydroxyethyl acetate. The latter (Distillation Products Industries) was distilled and the fraction boiling between 180° and 190° C. was used.

For the coprecipitation studies solutions were prepared as described above; the reaction was allowed to proceed for various periods of time as noted in the subsequent tables. In this manner different fractions of the total silver in solution were precipitated. The precipitate was filtered through a porcelain crucible, washed with sufficient water to remove mother liquor, dried, and weighed. A known weight of the solid phase was then analyzed for thallium. A previous experiment showed that washing the precipitate did not affect the thallium-silver ratio of the crystal. The fractions of silver precipitated were in general above 0.25 since it was observed especially at 25° C. that when fractions less than this were precipitated a supersaturation effect caused the spontaneous formation of small new particles during filtration; this did not occur at 5° and 15° C.

The thallium-silver ratios were determined for the various fractions of total silver precipitated at several temperatures and in solutions of different initial chloride ion concentration.

RESULTS

Coprecipitation at 60° C. Precipitations were made in a 100-ml. volume containing 0.0998 gram of silver as nitrate, 0.00118 mole of ammonium chloride, 99.3 γ of radiothallium, 2.0 ml. of 14M ammonium hydroxide, and 6.0 ml. of β-hydroxyethyl acetate. The data are shown in Table II.

The limited data indicate a rather rapid increase in thallium content of the crystal as a function of fraction of silver precipitated. This was not observed in the subsequent work at lower temperatures. This observation is apparently intimately connected with the physical characteristics of the precipitate, as shown by Figure 1 where the spongy structure is in sharp contrast with the cubes obtained at the lower temperatures.

Coprecipitation at 25° C. at Initial 0.0118M Chloride Ion Concentration. Precipitations were next performed at 25° C. in order to obtain crystals of silver chloride with improved physical characteristics and to coprecipitate increased amounts of thallium(I) chloride because of the large decrease in solubility of the latter with temperature. In these experiments the solutions were identical with those used at 60° C., except that either 0.0998 or 0.1003 gram of silver and 76.0 γ of thallium were used. Figure 1 shows a photomicrograph of the crystals. The data (Nos. 1 to 19) are given in Table III.

In this series of experiments the composition of the precipitate, as expressed by the ratio of micrograms of thallium per gram of silver, changed very slightly with increase in fraction of carrier precipitated. The ratio decreased somewhat, but as might be expected it was very slight; because so little thallium is coprecipitated, its concentration remains essentially constant regardless of the fraction of carrier which has been precipitated. However, the concentration of silver can increase almost fivefold as chloride is removed from solution by precipitation. Thus, a competitive adsorption effect between silver and thallium during crystal growth could explain the apparent slight decrease in thallium coprecipitation as the fraction of carrier precipitated becomes larger.

Coprecipitation at 25° C. at Initial 0.100M and 1.00M Chloride Ion Concentrations. In order to determine if a competitive adsorption effect caused the slight decrease in thallium coprecipitation with increase in fraction of carrier precipitated as noted in

the previous experiments, the concentration of initial chloride ion was increased. This resulted in a decrease of the silver ion concentration during precipitation according to the solubility product relationship. However, as the initial chloride ion concentration increased, the change in chloride during precipitation—and thus the change in silver ion concentration—was not so great.

Precipitations were made with solutions containing the following in 100 ml.: 0.0998 gram of silver, 0.0100 mole of ammonium chloride, 76.0 γ of thallium, 4.0 ml. of 14M ammonium hydroxide, 12 ml. of β-hydroxyethyl acetate; and 0.998 gram of silver, 0.100 mole of ammonium chloride, 76.0 γ of thallium, 7.0 ml. of ammonium hydroxide, and 12.0 ml. of β-hydroxyethyl acetate. The crystals are shown in Figure 1.

At these increased chloride ion concentrations—i.e., at 0.100M

Table III. Coprecipitation Results

No.	Reaction Time, Hr.	Yield of of AgCl, Gram	Tl/Ag in Ppt.[a] × 10^5	Fraction of Ag Pptd.	Fraction of Tl Pptd. × 10^3
		Initial Chloride Ion Concentration = 0.0118M; t = 25° C.			
1	5.1	0.0596b	0.107	0.450	0.621
2	6.0	0.0569b	0.126	0.429	0.710
3	6.3	0.0561	0.112	0.420	0.622
4	6.9	0.0589	0.134	0.441	0.783
5	9.7	0.0797b	0.100	0.601	0.788
6	10.0	0.0900b	0.106	0.679	0.945
7	13.0	0.0976	0.0968	0.731	0.937
8	16.2	0.0972b	0.101	0.734	0.965
9	20.0	0.1103	0.121	0.826	1.33
10	22.0	0.1204b	0.0877	0.909	1.04
11	24.0	0.1144b	0.0838	0.863	0.935
12	25.0	0.1183	0.0969	0.886	1.14
13	30.2	0.1255	0.108	0.940	1.35
14	43.5	0.1293b	0.0823	0.969	1.05
15	47.3	0.1318	0.0985	0.987	1.28
16	61.3	0.1338	0.0815	1.001	1.08
17	67.0	0.1328b	0.0754	1.001	0.988
18	75.3	0.1337	0.0950	1.001	1.26
19	96.3	0.1335	0.112	1.000	1.48
		Av. 0.101 ± 0.012			
		Initial Chloride Ion Concentration = 0.100M; t = 25° C.			
20	2.8	0.0435c	0.144	0.327	0.570
21	4.3	0.0735c	0.113	0.553	0.810
22	6.0	0.0923c	0.140	0.694	1.27
23	8.5	0.1088c	0.126	0.818	1.34
24	11.1	0.1153c	0.138	0.868	1.55
25	19.3	0.1273c	0.130	0.959	1.60
26	30.0	0.1311c	0.136	0.987	1.75
27	46.0	0.1325c	0.155	0.997	2.02
		Av. 0.135 ± 0.009			
		Initial Chloride Ion Concentration = 1.00M; t = 25° C.			
28	4.2	0.0346c	0.146	0.261	0.352
29	6.3	0.0646c	0.133	0.486	0.830
30	10.0	0.0877c	0.167	0.660	1.42
31	23.3	0.1126c	0.0995	0.847	1.11
		Av. 0.136 ± 0.021			
		Initial Chloride Ion Concentration = 0.0118M; t = 15° C.			
32	3.9	0.0099c	0.121	0.075	0.109
33	5.0	0.0329c	0.116	0.248	0.378
34	7.0	0.0525c	0.133	0.395	0.692
35	9.4	0.0681c	0.128	0.512	0.862
36	17.3	0.0942c	0.136	0.709	1.26
37	24.2	0.1085c	0.137	0.816	1.46
38	42.3	0.1265c	0.132	0.952	1.64
39	42.3	0.1261c	0.132	0.950	1.65
		Av. 0.129 ± 0.006			
	Initial Chloride Ion Concentration = 0.0118M, also 0.100M in NaNO₃; t = 15° C.				
40	5.5	0.0606c	0.127	0.456	0.762
41	11.4	0.0921c	0.123	0.693	1.12
42	21.8	0.1131c	0.114	0.851	1.28
43	45.3	0.1310c	0.113	0.985	1.47
		Av. 0.119 ± 0.006			
		Initial Chloride Ion Concentration = 0.0118M; t = 5° C.			
44	9.0	0.0265d	0.162	0.199	0.541
45	11.9	0.0384d	0.103	0.289	0.500
46	24.5	0.0815d	0.234	0.613	2.40
47	31.0	0.0939d	0.179	0.707	2.12
48	47.5	0.1108d	0.219	0.833	3.07
49	70.0	0.1231d	0.181	0.927	2.81
50	86.0	0.1276d	0.166	0.960	2.66
51	194.0	0.1339d	0.162	1.008	2.73
		Av. 0.176 ± 0.026			

a Weight ratio.
b Initial silver = 0.0998 gram; all others above = 0.1003 gram of silver.
c Initial silver = 0.0998 gram.
d Initial silver = 0.1000 gram.

and 1.00M—the respective silver ion concentrations are decreased by large factors. Table III (Nos. 20 to 31) shows that the thallium content of the precipitate is slightly greater than was obtained with the experiments with 0.0118M chloride solutions. However, the increase in coprecipitation of thallium is small compared to the decrease in silver ion concentration, and, if anything, one must conclude that the amount of thallium coprecipitated is essentially independent of both silver and chloride ion concentrations within the limits studied. The data also show an increasing trend of thallium coprecipitation with fraction of silver coprecipitated.

Coprecipitation at 15° C. at Initial 0.0118M Chloride Ion Concentration. The solutions in this series of experiments contained the following in 100 ml. of solution: 0.0998 gram of silver, 0.00118 mole of ammonium chloride, 2.0 ml. of 14M ammonium hydroxide, 10.0 ml. of β-hydroxyethyl acetate, and 76.0 γ of thallium. The rate of precipitation at 15° C. is not appreciably different from that at 25° C. Table III (Nos. 32 to 43), shows that the coprecipitation of thallium is essentially the same as at 25° C. Some of the precipitations at 15° C. were carried out in 0.100M sodium nitrate, but again the data are essentially unchanged.

Coprecipitation at 5° C. at Initial 0.00118M Chloride Ion Concentration. These precipitations were carried out in 100.0-ml. volumes containing 0.1000 gram of silver, 0.00118 mole of ammonium chloride, 2.0 ml. of 14M ammonium hydroxide, 10.0 ml. of β-hydroxyethyl acetate, and 59.6 γ of thallium. The data are shown in Table III (Nos. 44 to 51). The crystals are shown in Figure 1.

The data indicate a somewhat greater thallium coprecipitation than in the experiments at 15° and 25° C. The variability in the data makes it impossible to determine whether there is an increase or decrease in thallium coprecipitation with fraction of silver precipitated.

Coprecipitation at 25° C. with Varying Thallium Concentrations. These experiments were performed in a manner identical with the others carried out at 25° C. in 0.0018M initial chloride solution, except that the thallium content was varied. The data are shown in Table IV. All of the precipitations were allowed to proceed for about the same length of time, so that the fraction of silver precipitated was in the range 0.77 to 0.79. The data show that the ratio of thallium to silver in the crystal is approximately proportional to the concentration of thallium in solution.

Evidence of Internal Distribution of Thallium within Silver Chloride. Two precipitations were performed at 15° C., as previously described for the work at that temperature. While the precipitates were on the filtering crucible they were partially dissolved with dilute ammonia, while suction was applied. After 70% of the original weight had been dissolved away, the remaining crystals contained, respectively, 0.125 and 0.154 γ of thallium per gram of silver as compared to 0.132 (cf. Table III). This indicates internal distribution of thallium rather than adsorption on the outer surface of the crystals of silver chloride.

DISCUSSION

Because silver and thallium(I) chloride are not isomorphous, it would be expected that neither the homogeneous nor heterogeneous distribution laws would be followed. However, Hahn's classification (5) of coprecipitation considers anomalous mixed crystal formation as a possibility. That this is not the present case is shown in Table V, which summarizes the distribution coefficients (19) calculated for the present work.

The values of the homogeneous distribution coefficient, D, were calculated in the usual manner from

$$\left(\frac{\text{Tracer}}{\text{carrier}} \right)_{\text{ppt.}} = D \left(\frac{\text{tracer}}{\text{carrier}} \right)_{\text{soln.}} \tag{1}$$

Likewise, the values of the heterogeneous distribution coefficient, λ, were calculated from the Doerner-Hoskins equation.

$$\left(\frac{\text{Tracer}}{\text{carrier}}\right)_{\substack{\text{surface of ppt.}\\\text{during its growth}}} = \lambda\left(\frac{\text{tracer}}{\text{carrier}}\right)_{\text{soln.}} \quad (2)$$

In view of the equilibria involved, the carrier ion was considered to be free silver ion rather than total silver (or the ammonia complex).

The usual integrated form of the Doerner-Hoskins equation

$$\text{Log} \frac{(\text{tracer})_{\text{initial}}}{(\text{tracer})_{\text{final}}} = \lambda \log \frac{(\text{carrier})_{\text{initial}}}{(\text{carrier})_{\text{final}}} \quad (3)$$

would erroneously lead to a' negative distribution coefficient, since the concentration of silver ion increases, because of removal of chloride, as the fraction of total silver precipitated increases.

Thus, for the present system, Equation 2 should be:

$$\frac{d\text{Tl}}{d\text{Ag}} = \lambda \frac{\text{Tl}_0 - \text{Tl}}{\text{Ag}_{\text{soln.}}} = \frac{\lambda}{K_{\text{sp}}V^2}(\text{Tl}_0 - \text{Tl})(\text{Cl}) =$$

$$\frac{\lambda}{K_{\text{sp}}V^2}(\text{Tl}_0 - \text{Tl})(\text{Cl}_0 - \text{Ag}) \quad (4)$$

Integration leads to:

$$\ln \frac{\text{Tl}_0}{\text{Tl}_0 - \text{Tl}} = \frac{\lambda}{K_{\text{sp}}V^2}\left(\text{Cl}_0\text{Ag} - \frac{\text{Ag}^2}{2}\right) \quad (5)$$

where

$(\text{Tl}_0 - \text{Tl})$	= moles Tl in solution
$(\text{Cl}_0 - \text{Ag})$	= moles Ag in solution
Tl	= moles Tl in precipitate
Ag	= moles Ag in precipitate
Cl_0	= moles Cl initially added
K_{sp}	= solubility product of silver chloride (10)
λ	= distribution coefficient
Tl_0	= moles Tl initially added
V	= volume in liters

The values of λ in Table V were calculated using Equation 5. The data of Table V show conformity to neither distribution law. This is especially evident from the data at 25° C., where an extensive range of chloride ion concentration was employed. Further calculations have shown that neither distribution law is obeyed even if "total silver" is considered, thus permitting the use of Equation 3. The present system might have conformed to one of the distribution laws—and thus be anomalous mixed crystal formation according to Hahn's classification—had it been possible to work with thallium concentrations of the order of $10^{-10}M$. One of the unique aspects of the present system is that the concentration of the minor constituent, thallium, is much greater than that of the carrier ion, uncomplexed silver. This is in sharp contrast with other distribution studies in which the carrier ion concentration is much greater than that of the tracer.

For the present system, coprecipitation can be considered to be internal adsorption as specified by Hahn (5), where the thallium is adsorbed by the silver chloride as the latter is being formed during the precipitation process. As the crystal continues to grow, each succeeding layer covers the adsorbed layer. This process is essentially that of occlusion, as Kolthoff (13, 14) uses this term, except that the aging process which, according to his views perfects the imperfect crystals first formed, probably assumes a much lesser role here in precipitation from homogeneous solution.

The Langmuir equation (15) predicts that the adsorption of an ion will be proportional to its solution concentration if the fraction of surface covered by this ion is small. This apparently is the case here with thallium, where the observed mole ratio of thallium to silver in the precipitate is of the order of 5×10^{-7}. Likewise, in the case of silver which should be strongly attracted to the silver chloride crystal, the Langmuir equation predicts that its adsorption would be nearly independent of its solution concentration. This essentially occurs in the present case where the silver ion concentration was varied by altering the chloride ion concentration. If the competitive adsorption of silver and thallium were dependent on the silver ion concen-

Table IV. Coprecipitation at 25° C. with Varying Thallium Concentration

Concentration of Tl, γ/ml.	Tl/Ag in Precipitate[a] $\times 10^5$	Fraction of Tl Precipitated $\times 10^3$
1.52	1.36	0.695
0.760	1.01	1.05
0.456	0.503	0.86
0.304	0.20	0.40
0.152	0.11	0.54
0.0760	0.092	0.95
0.0152	0.021	1.1
0.00152	0.007	3.8
0.000152	0.003	15.0

[a] Weight ratio.

Table V. Summary of Distribution Coefficients[a]

Temperature, ° C.	[Cl⁻] Molarity	$\lambda \times 10^9$	$D \times 10^9$
60	0.0118	3.3 to 5.3	4.3 to 10
25	0.0118	3.1 to 10	2.8 ± 0.3
25	0.100	0.64 ± 0.05	0.32 ± 0.02
25	1.00	0.045 to 0.073	0.30 ± 0.04
15	0.0118	1.3 ± 0.2	0.96 to 4.0
15	0.0118[b]	1.3 ± 0.05	1.5 to 3.7
5	0.0118	0.4 to 1.1	0.4 to 1.6

[a] Calculated from data given in previous tables.
[b] Also $0.100M$ in sodium nitrate.

tration, a variation in the extent of thallium coprecipitation should have been expected. However, thallium coprecipitation remained essentially constant with variation in silver ion concentration.

The data of Table V indicate adherence to the equation:

$$\left(\frac{\text{Tl}}{\text{Ag}}\right)_{\text{crystal}} = K_1[\text{Tl}]^{1/n} \cong K_1[\text{Tl}]$$

For experiments under a given set of conditions, such as in Table III, where but a small fraction of the initial thallium is coprecipitated so that the concentration of thallium remains essentially constant throughout the entire precipitation process, this equation reduces to:

$$\left(\frac{\text{Tl}}{\text{Ag}}\right)_{\text{crystal}} = K_2$$

This equation actually describes a "homogeneous" distribution of the thallium, such as would normally characterize a system adhering to the homogeneous distribution law (19). However, in the present case, homogeneous distribution is attained because each layer of silver chloride adsorbs essentially the same small amount of thallium from the solution which contains an essentially unvarying thallium concentration. The gross picture is thus one of an apparent homogeneous distribution of thallium within the crystals of silver chloride. However, it is possible that a heterogeneous distribution will be observed in the case where the solution concentration of thallium decreases significantly during the precipitation process.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Gordon, L., ANAL. CHEM., 24, 459 (1952).
(2) Gordon, L., Reimer, C. C., and Burtt, B. P., Ibid., 26, 842 (1954).
(3) Gordon, L., Teicher, H., and Burtt, B. P., Ibid., 26, 992 (1954).
(4) Haddock, L. A., Analyst, 60, 394 (1935).
(5) Hahn, O., "Applied Radiochemistry," pp. 64–164, Cornell University Press, Ithaca, N. Y., 1935.
(6) Hahn, O., Z. angew. Chem., 43, 871 (1930).
(7) Hahn, O., and Imre, L., Z. physik. Chem., A144, 161 (1929).

(8) Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoff-
    man, J. I., "Applied Inorganic Analysis," 2nd ed., p. 205,
    Wiley, New York, 1953.
(9) *Ibid.*, p. 478.
(10) Hodgman, C. D., "Handbook of Chemistry and Physics," 34th
    ed., p. 1559, Chemical Rubber Publishing Co., Cleveland,
    1952-3.
(11) Imre, L., *Z. physik. Chem.*, A146, 41 (1930).
(12) "International Critical Tables," vol. IV, p. 53, McGraw-Hill,
    New York, 1926.
(13) Kolthoff, I. M., *J. Phys. Chem.*, 36, 860 (1932).
(14) Kolthoff, I. M., and Sandell, E. B., *Ibid.*, 37, 723 (1933).
(15) Prutton, C. F., and Maron, S. H., "Fundamental Principles of
    Physical Chemistry," pp. 652-4, Macmillan, New York, 1944.

(16) Salutsky, M., Stites, J. F., and Martin, A. W., ANAL. CHEM.,
    25, 1677 (1953).
(17) Sandell, E. B., "Colorimetric Determination of Traces of Met-
    als," 2nd ed., p. 562, Interscience, New York, 1950.
(18) Stine, C. R., and Gordon, L., ANAL. CHEM., 25, 1519 (1953).
(19) Wahl, A. C., and Bonner, N. A., "Radioactivity Applied to
    Chemistry," pp. 105-7, Wiley, New York, 1951.
(20) Weaver, B., ANAL. CHEM., 26, 477 (1954).
(21) *Ibid.*, p. 479.
(22) Willard, H. H., *Ibid.*, 22, 1372 (1950).

Titrimetric Assay of Trichloroacetate

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A simple and accurate assay method based upon the
decarboxylation of trichloroacetate is presented. A
known amount of standard acid is added to the neutral
sample, the solution is refluxed for at least an hour,
and the residual acid is titrated. The acid consumed
is a direct measure of the trichloroacetate content.
Contaminants usually present in commercial grades
of the acid or salt do not interfere and need not be de-
termined. The proposed method requires much less
time than the classical alkaline hydrolysis procedure.

THE accepted method for the assay of trichloroacetate is es-
sentially a chloride determination corrected for the chloride
contributed by the major contaminant, dichloroacetate. The
total chloride is determined following a Parr peroxide bomb de-
composition or an alkaline hydrolysis. The latter process also
converts any dichloroacetate present to oxalate, which is then
determined by the usual calcium oxalate precipitation and sub-
sequent permanganate titration. This method, which may be
credited to Pool (*4*), has been largely developed by industrial
laboratories without publication.

In this laboratory, it has been noted that different analysts
could not always agree on trichloroacetate assay, the usual point
of difference being the dichloroacetate determination. A study
by Dalin and Haimsohn (*1*) discussed the errors of dichloroace-
tate determination as applied to the assay of monochloroacetate,
but these authors found that low results were related to the quan-
tities of reagents used. However, in the present work, high
values of dichloroacetate were the major concern.

Table I. Standardization of Decarboxylation Method

Standard Sample	% Compound Found
Trichloroacetic acid	99.4, 99.8, 99.5, 99.7
Ethyl trichloroacetate	100.2, 99.6, 99.9, 99.9, 99.6
Sodium trichloroacetate	99.7, 99.6, 99.7, 99.6
Sodium trichloroacetate	99.7, 99.7, 99.6, 99.5

Samples of trichloroacetic acid, known to be free of dichloro-
acetate by infrared and freezing point data, invariably showed a
dichloroacetate content of from 2 to 3%, which was found to be
a function of the sodium hydroxide concentration used for hydrol-
ysis. The conclusion reached was that the alkaline hydrolysis
also converts some trichloroacetate to oxalate, thus causing spuri-
ously high values for dichloroacetate. Clearly, a new assay
method was needed for trichloroacetate.

Several kinetic studies have been made of the decarboxylation

Table II. Effect of Contaminants

Substance Added	% of Mixture	NaTCA Taken,[a] G.	NaTCA Found, G.	% Recovery
None	...	2.053	2.051	99.9
	...	2.492	2.492	100.0
	...	2.492	2.492	100.0
NaHCO₃	1.98	2.508	2.510	100.1
	4.63	2.508	2.510	100.1
Na₂HPO₄	1.07	2.492	2.495	100.1
	2.05	2.508	2.510	100.1
	5.43	2.508	2.510	100.1
NaDCA	11.6	2.053	2.051	99.9
	31.9	2.053	2.049	99.8
	50.2	2.492	2.473	99.2
NaMCA	9.5	2.053	2.049	99.8
	19.2	2.492	2.484	99.6
	40.5	2.492	2.468	99.0
	51.7	2.492	2.380	95.5

[a] Weights of NaTCA taken were computed on the basis of assayed salt
purity of 99.6%.

of trichloroacetate in aqueous solution. In some of these, the
reaction rate was followed by titration of the residual acid (*2, 3*).
The present method is essentially a refinement of this principle
and is defined by the following equation:

$$CCl_3CCO^- + H_3O^+ \xrightarrow[H_2O]{100^\circ C.} CHCl_3 + CO_2 + H_2O$$
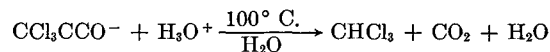
Although the procedure described was developed specifically
for the assay of technical sodium trichloroacetate, the general
method is not restricted to the salt, as it has been used with equal
success for the acid and the ethyl ester.

APPARATUS AND REAGENTS

Flasks, 250 ml., flat-bottomed, short-necked $24/40.
Condenser, 50-cm., water-cooled, with glass joint $24/40.
Methyl red indicator, 0.1% solution in 95% ethyl alcohol.
Sulfuric acid, approximately $1N$ solution.
Sodium hydroxide, $1N$ standard solution.
Dioxane, freshly distilled.

PROCEDURE

Dissolve a 25-gram sample of sodium trichloroacetate in water
and dilute to 100.0 ml. (It is best to take such a relatively large
quantity to ensure a representative sample.) After dissolving,
select an aliquot which will cause the final titration volume to be
about half of the blank titration volume.
Pipet duplicate 10.00-ml. aliquots of sample solution into 250-
ml. reflux flasks, add 1 drop of methyl red, and neutralize by

titrating with $1N$ sulfuric acid. As the solution is usually alkaline due to bicarbonate, it is important to titrate to a distinct orange-pink, which is about pH 5.5 to 5.3 and indicates neutralization of the bicarbonate. The usual requirement is less than 0.15 ml. of $1N$ sulfuric acid. In the case of an acid sample, neutralization is accomplished with $1N$ sodium hydroxide, and the end point (methyl red is used) is the transition from light orange to yellow.

Add 25.00 ml. of $1N$ sulfuric acid, 35 ml. of dioxane and a few glass beads. Boil under reflux for at least 60 minutes. This time is not critical, but less than 60 minutes may not be sufficient for complete reaction.

To the cooled solution add 2 drops of methyl red, and titrate with standard $1N$ sodium hydroxide. The end point is taken at the transition from light orange to yellow. In this titration, the indicator is influenced by the relatively basic solvent, and the orange transition color appears long before the end point; however, a sharp change from orange to yellow clearly marks the proper end point.

Because of acid contained even in freshly distilled dioxane, run duplicate blanks with each series of determinations. These are identical to the test solutions except that they contain no sample. This practice also bases the determination on a single standard solution, the sodium hydroxide.

The sources of error in this method are primarily those which are common to all volumetric methods: calibration and drainage of glassware, estimation of end points, and buret readings. In addition, low results may be caused by incomplete reaction due either to insufficient reflux time or nonvigorous boiling, which is essentially the same thing.

CALCULATION

Expressed as per cent sodium trichloroacetate:

$$\% \text{ NaTCA} = \frac{(\text{net titration volume})}{\text{aliquot weight}} \times 0.1854 \times 100$$

RATE OF DECARBOXYLATION

The first trials of this method were conducted in water solutions. Using 0.25-gram aliquots and $0.1N$ reagents in a total volume of 50 ml., the rate of reaction was such that refluxing for 1 hour seemed to be sufficient for completion. However, when applied to recrystallized trichloroacetic acid (99.57 mole %), results were found to be low by about 3%. With larger samples and a higher sulfuric acid concentration, even 3 hours of refluxing time was not enough.

A review of the literature revealed that the reaction rate is a function of the trichloracetate ion concentration and is increased in a solvent such as dioxane. According to Salmi and Korte (5), the rate of decarboxylation of trichloroacetic acid is most rapid in 62% dioxane–water solutions. However, the actual concentration of dioxane does not appear critical. Since in this work identical and rapid rates were found in both 40 and 50% solutions, the 62% concentration is considered unnecessarily large.

For the procedure, 50% dioxane was selected, because of a secondary influence on the rate. The reaction product, chloroform, is not expelled during boiling under reflux. With samples as large as 2.5 grams in 70 ml. of solution, the accumulation of chloroform as a second liquid phase seriously cools the system, thus slowing the reaction rate. In water alone, the effect of the chloroform is very dramatic, practically stopping active boiling. A solution of 40% dioxane is barely enough to keep the chloroform dissolved, but 50% solutions remain single phase throughout the procedure.

Data were obtained comparing reaction rates for two alternate sample sizes, 0.25 and 2.5 grams. The smaller sample with $0.1N$ reagents would seem preferable, because only 30 minutes are needed for completion; however, this advantage is offset by a greater uncertainty in selection of the end point and by a potentially greater sampling error. As a control procedure, the $1N$ reagents are better; but for special work, good precision and accuracy can be obtained with small samples and $0.1N$ reagents.

In the latter case an electrometric detection of the end point is recommended.

STANDARDIZATION OF PROCEDURE

The procedure has been tested with pure samples of trichloroacetic acid, ethyl trichloroacetate, and sodium trichloroacetate (each entry in Table I is an independent single determination). The acid, prepared by recrystallization six times from carbon tetrachloride, was dried in a vacuum, and contamination of atmospheric moisture was avoided. Freezing point data indicated a purity of 99.74 mole %; total chloride determination showed 99.7%.

By virtue of careful preparation and fractional distillation through a 40-plate column, the ethyl ester was thought to be nearly 100% pure, and infrared analysis showed that is was free of ethyl dichloroacetate. A freezing point determination reported 99.45 mole %, and a saponification titration at 15° C. showed 99.9%. This substance seems to be an inadequate standard in that the total chloride determinations were unsatisfactory. By Parr peroxide bomb, the assay was 99.2%, whereas hydrolytic chlorides ranged from 98.7 to 99.4% giving about the same average. In terms of precision, the proposed decarboxylation method is superior, in this case, to the chloride methods, and the results agree with those of the freezing point and saponification determinations.

Two different lots of sodium trichloroacetate were purified by repeated crystallization from water at temperatures between 30° and 0° C. This was a drastic procedure because of the solubility of the salt. From 10 pounds of the salt in the first solution, about 0.5-pound yield was obtained. The products were dried, ground fine, and then dried in a vacuum desiccator for at least 10 days to remove water of crystallization. By total chloride determination, the purity of each was 99.6%.

EFFECT OF CONTAMINANTS

Technical grades of sodium trichloroacetate, containing bicarbonate and dichloroacetate, also may contain disodium phosphate in concentrations up to 1% of the dry salt as an added corrosion inhibitor. Monochloroacetic acid is not usually encountered in trichloroacetic acid preparations, but its behavior in this procedure is of interest.

The results of the influence of these four substances are listed in Table II, but large concentrations of bicarbonate and phosphate were not considered, as the procedure is presented as an assay method. However, it is probable that concentrations greater than 10%, especially of phosphate, would seriously affect the titration end points.

It was expected that dichloroacetate (NaDCA) and monochloroacetate (NaMCA) would cause low results by yielding acid in hydrolysis, but this laboratory found that these can be tolerated up to concentrations of 32 and 9.5%, respectively, which is an important point in favor of this new method.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Dalin, George, and Haimsohn, Jerome, ANAL. CHEM., 20, 470 (1948).
(2) Johnson, P., and Moelwyn-Hughes, E. A., Proc. Roy. Soc. (London), A175, 118 (1940).
(3) Kappanna, A. N., Z. physik. Chem., A158, 355 (1932).
(4) Pool, J. F. A., Pharm. Weekblad, 42, 165 (1905); J. Chem. Soc., 88, 425 (1905).
(5) Salmi, E. J., and Korte, Raymond, Ann. Acad. Sci. Fennicae, A54, No. 10, 22 (1940); Chem. Zentr., 1942 I, 328.

Colorimetric Determination of Iridium with *p*-Nitrosodimethylaniline

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Even though iridium has extensive commercial uses, few methods of determination are available and these are not sensitive. It was found that *p*-nitrosodimethylaniline produced an intense color with small quantities of iridium. In heated alcohol-water solution buffered to pH 7.2 to 7.3, a cherry-red colored complex is formed which absorbs over a broad band of wave length. The method fails in the presence of nonvolatile acids or salts, but in such cases iridium may be obtained as the chloro salt after hydrolytic precipitation using nickel as a collector. The nickel is removed on a cation exchange resin. In samples from which the other platinum metals have been removed, precise results can be obtained for iridium in the range 1.5 to 10 p.p.m.

O NLY two acceptable colorimetric methods for iridium other than direct measurement of the absorbance of hexachloroiridate have been developed and published to date. Ayres and Quick (*1*) used a mixed acid reaction, and Maynes and McBryde (*4*) used ceric sulfate. Both involve fuming with acid, the final color depending on the care with which this operation is carried out. These methods are not particularly sensitive.

Several platinum metals can be determined with *p*-nitrosodimethylaniline. Although the method presented for the determination of iridium using this reagent enjoys no specificity among the platinum group, it is sensitive and precise. Various procedures exist for the separation of iridium from micro amounts of all the platinum metals except rhodium, and a procedure for the separation of traces of this metal is currently being investigated in this laboratory.

APPARATUS AND SOLUTIONS

Optical Instruments. A Klett-Summerson photoelectric colorimeter using matched tubes was used for most of the work. A Beckman Model DK spectrophotometer was used to examine the variation of absorbance with wave length.

Standard Iridium Solution. A solution of sodium iridium chloride prepared in $0.05N$ hydrochloric acid was standardized gravimetrically using 2-mercaptobenzothiazole according to the method of Barefoot, McDonnell, and Beamish (*2*). The mean result of four determinations was 0.143 mg. per ml. The stock solution was diluted to one tenth of this concentration with water, and such solutions were stable for at least a 2-week period.

Color Reagent. A solution of *p*-nitrosodimethylaniline (Eastman Kodak Co.) was prepared by dissolving 150 mg. in 100 ml. of 95% ethyl alcohol and filtering. The solutions tended to deposit a dark powder if kept more than a week or two. Alcoholic solutions were required in order to form the color.

Buffer Solutions. Analytical grade disodium hydrogen phosphate and potassium dihydrogen phosphate were dissolved together in water in varying amounts to prepare buffers $1.0M$ in phosphate, the pH of these being determined with a Beckman Model G pH meter. The buffer used in the final procedure contained 10 grams of disodium hydrogen phosphate and 4.1 grams of potassium dihydrogen phosphate in 100 ml. of aqueous solution.

COLOR REACTION

When an iridium chloride solution is heated with an alcoholic solution of *p*-nitrosodimethylaniline the solution develops a red color superimposed upon the yellow color of the reagent. It may be noted that *p*-nitrosodiphenylaniline and *p*-nitrosodiethylaniline did not produce an intense color with iridium chloride solutions.

A plot of the absorbance of the complex and of the blank reagent compared with water appears in Figure 1. It can be seen from the plot of the difference of these that there is a broad band
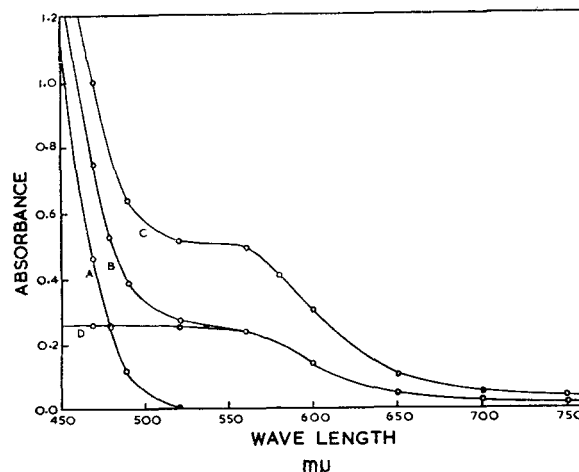

Figure 1. Variation of absorbance with wave length

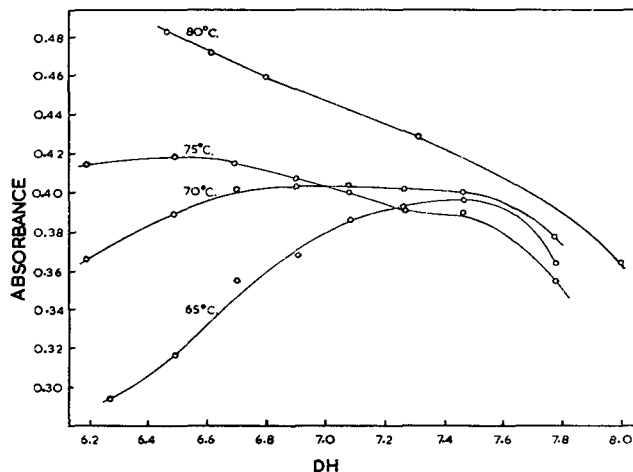|	
A.	5.8 p.p.m. of iridium
B.	2.9 p.p.m. of iridium
C.	Blank plus reagent
D.	*B-C*

Figure 2. Effect of pH and temperature

of absorption due to the complex extending over the whole wavelength range studied, with a maximum at 530 mμ.

The absorbance is dependent upon various factors such as temperature, pH, time of heating, and ionic strength. The first three factors are interrelated in a rather complex manner.

A study of these relations was carried out using samples containing 0.115 mg. of iridium in a 4-ml. volume to which were added 2 ml. of reagent and 2 ml. of buffer. The samples were contained in test tubes suspended in a bath of hot water, the temperature of which was controlled manually to $\pm 1°$ C. After heating for a given period the tubes were cooled in running water, the pH was measured, the samples were diluted to 25-ml. volume with water, and the absorbance was determined.

Temperature. Solutions buffered at various values of pH were heated for 40 minutes at various temperatures. From the results plotted in Figure 2 it can be seen that the absorbance remained constant over a fairly broad range of pH only when the samples

were heated at 70° C. At this temperature the absorbance was nearly constant over the pH range 6.7 to 7.5. At higher values the iridium is probably hydrolyzed. At 80° C. a brown precipitate appeared. This was sometimes seen also at 70° C., but if the sample was made up to volume with 6N hydrochloric acid the precipitate dissolved. No change in the absorption due to the complex was caused by this treatment, but the excess reagent color was bleached. This is fortunate, in. that it reduced the considerable absorption of the blank.

The appearance of a precipitate during heating was not attended by any discrepancy in the results. The relative heights of the various curves are not significant, since the sensitivity frequently varied from one simultaneously prepared set of samples to the next. On the basis of the increased proportion of hydrolysis of chloroiridate, one may account for the anomalous position of the 75° C. curve. Such factors as the temperature of the water used for dissolving the sample and the time of standing before color formation can govern the effect.

Table I. Effects of Quantity of Reagent and Volume on Absorbance

Iridium Taken, 5.7 P.P.M.		Iridium Taken, 3.43 P.P.M.	
Reagent, ml.	Absorbance	Volume, ml.	Absorbance
0.5	0.262	7	0.301
1.0	0.374	8	0.301
1.5	0.432	9	0.299
2.0	0.505	10	0.296
		11	0.286

Table II. Precision and Adherence to Beer's Law

Final Concentration, P.P.M.	Absorbance	Absorbance/P.P.M.
1.42	0.141	0.0993
2.87	0.279	0.0973
4.29	0.424	0.0988
5.72	0.570	0.0995
7.16	0.714	0.0996
10.01	1.000	0.0999

Time of Heating. Color developed more rapidly in samples of low pH than in those of high, so that heating times at various values of pH were studied. The plots in Figure 3 show that color development was complete after 36 minutes' heating at 70° C. A heating period of 40 minutes was chosen.

Choice of Buffer. A buffer yielding a solution having a pH of 7.2 to 7.3 was used in subsequent work, because at this pH the absorbance was less dependent on variations in temperature, heating time, and residual acidity in the sample.

Quantity of Reagent Added. The absorbance of the solution depends upon the quantity of reagent added. As can be seen from Table I, the absorbance was greater for higher reagent concentrations. More than 2 ml. of reagent in a volume of 10 ml. was considered undesirable because of the high reagent color. Correspondingly, as can be seen from the table, the absorbance was somewhat dependent upon the volume of solution heated.

Treatment of Acidic Samples. As iridium is usually handled in acidic solutions, a study directed at finding the best method of preparing such solutions for analysis was made. It was found that if the solutions were neutralized or merely brought to pH 5 or 6 before adding the buffer, low results were obtained. This is probably due to partial hydrolysis of the iridium in regions of high hydroxyl ion concentration occurring as the base is added. Slow addition with stirring overcame this trouble in part only. Because of the high temperature, homogeneous neutralization by boiling with urea resulted in the development of no color whatever, when samples were evaporated to less than 0.5-ml. volume, 2 ml. of reagent were added, and the solution was diluted to volume with a 1M phosphate buffer with pH of 7.8 acceptable results were obtained with stock solutions.
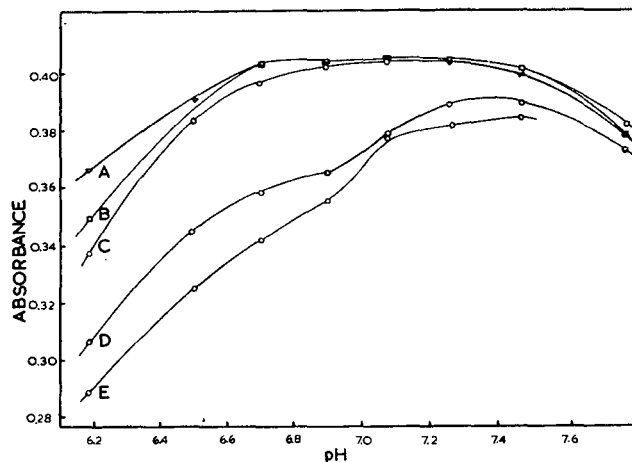

Figure 3. Effect of heating time

A. 40 minutes
B. 36 minutes
C. 32 minutes
D. 28 minutes
E. 24 minutes

In the authors' opinion, however, a better method of eliminating acid consists in evaporating to dryness solutions containing a little sodium chloride. Evaporations were carried out in 50-ml. beakers on a hot plate until the solution just barely covered the bottom of the beaker, at which time the beaker was placed on a steam bath and allowed to go thoroughly dry. The absorbance of the solution was found to decrease with increasing ionic strength of the solution; hence the amount of salt present should be controlled. Samples containing 4.29 p.p.m. of iridium and 40, 80, and 120 mg. of sodium chloride gave absorbances of 0.428, 0.416, and 0.404, respectively.

The amount of color developed in iridium solutions which had been treated by different procedures was unpredictable, unless some means were taken to standardize the form of dissolved constituent immediately prior to the determination. Low results were obtained with aliquots of stock solution which had been diluted to a 200-ml. volume and evaporated to dryness with salt. From this it appears that even under these conditions iridium is partially hydrolyzed. This trouble was overcome by making such solutions at least 0.5N with hydrochloric acid before evaporating, then treating the residue with aqua regia and hydrochloric acid in the usual manner, and again evaporating to dryness.

As the sensitivity was not always reproducible under the conditions of the procedure, simultaneous standards were necessarily employed. An indication of precision and conformity to Beer's law is represented in Table II. The same Klett tube was used to contain the samples and absorbances were obtained by dividing the Klett unit by the factor 519 (3).

Recommended Procedure. An acid sample of iridium to which are added 2 ml. of 2% sodium chloride is evaporated to dryness in a 500-ml. beaker on a hot plate and finally on a steam bath. The residue is treated with 4 ml. of aqua regia, and this is then evaporated to dryness. The crystals are moistened with concentrated hydrochloric acid and this is evaporated three times, being dried thoroughly to get rid of residual acid. A minimum of water is added to dissolve the salts, and the solution is rinsed into a 15 × 150 mm. test tube containing 2 ml. of buffer and 2 ml. of color reagent. The solution is diluted to 8-ml. volume with water, and the test tube suspended in a 70° C. bath for 40 minutes. The test tubes are then cooled in running water and the solutions diluted to 10-ml. volume with 6N hydrochloric acid.

SEPARATION OF TRACES OF IRIDIUM FROM NONVOLATILE ACID

If the solution contains much sulfuric acid or fixed salt, it is not feasible to apply the determination directly. It was found

that small quantities of iridium can be separated from such solutions by hydrolytic precipitation if a carrier precipitate is formed as well. Thus, if nickel is added to the solution, quantitative removal of the hydrated iridium oxide can be effected. The nickel can then be removed by passing the redissolved precipitate through a column of Dowex 50 ion exchange resin. To ensure that the iridium in the column process is entirely anionic, the precipitate must be heated with strong acid containing chloride.

Table III. Recovery of Iridium by Hydrolytic Precipitation

Number	Iridium Taken, γ	Iridium Recovered, γ	Recovery, %
1	58	57.5	99
2	116	116	100
3	580	580 ± 3	100
4	1160	1160 ± 6	100
5	29	29	100
6	58	57.5	99
7	116	108	93
8	14.5	14.3	99
9	29	26.5	91
10	58	57.5	99

Aliquots of iridium solution were fumed with various quantities of sulfuric acid until the final volume was about 2 ml. or less. The solution was allowed to cool, diluted to 10 ml. with water, and 50 mg. of nickel in the form of a solution of its chloride and 5 ml. of 10% sodium bromate were added. The solution was nearly neutralized with sodium hydroxide and finally adjusted to pH 6.7 to 7.5 by means of dilute sodium bicarbonate and dilute hydrochloric acid. The nickel hydroxide turns black in this pH range. The solution was finally boiled gently for 0.5 hour and filtered through a porous-bottom crucible of 5-ml. capacity.

The precipitate was washed with a few milliliters of 1% ammonium chloride solution. The crucible was returned to the original beaker, and the precipitate was dissolved in 8 ml. of aqua regia and then evaporated to about 2-ml. volume. Two milliliters of concentrated hydrochloric acid were added, and this was evaporated slowly to 2-ml. volume. The solution was transferred to another vessel and the crucible leached on the steam bath with 5 ml. of slightly acidified water. This was added to the main bulk of the solution and the crucible was leached a second time. The solution was diluted to a 100-ml. volume and passed at a rate of about 2 ml. per minute through a 10-cm. deep bed of Dowex 50. The column was washed with 50 ml. of water, 10 ml. of concentrated hydrochloric acid, and 2 ml. of 2% sodium chloride solution were added to the effluent, and this was evaporated to dryness. The residue was treated with aqua regia and hydrochloric acid as described above and the determination was carried out.

Results of samples subjected to this procedure appeared in Table III. Nos. 1 to 4 contained no sulfuric acid; Nos. 5 to 7 were fumed with sulfuric acid to about 2-ml. volume, and Nos. 8 to 10 were fumed to less than 0.5 ml. in 50-ml. beakers so that a green oily film remained.

LITERATURE CITED

(1) Ayres, G. H., and Quick, Q., ANAL. CHEM., 22, 1403 (1950).
(2) Barefoot, R. R., McDonnell, W. J., and Beamish, F. E., Ibid., 23, 514 (1951).
(3) Currah, J. E., Fischel, A., McBryde, W. A. E., and Beamish, F. E., Ibid., 24, 1980 (1952).
(4) Maynes, A. D., and McBryde, W. A. E., Analyst, 79, 230 (1954).

Determination of Chlorine or Chlorine Dioxide in Dilute Aqueous Solutions Containing Oxidizing Ions

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Chlorine or chlorine dioxide may be determined in aqueous solutions in the presence of many oxidizing ions by extracting the chlorine or chlorine dioxide into a measured volume of carbon tetrachloride and measuring the absorbance of the extract with a spectrophotometer at 3270 A. for chlorine or 3550 A. for the dioxide. Extracts need not be filtered and photochemical decomposition is not serious if reasonable care is taken. The procedure is suitable for quantities of chlorine between 1 and 12 mg. in 5 ml. of sample, and for quantities of chlorine dioxide between 0.1 and 5 mg. in 1 ml. of sample.

D URING the course of certain experiments in these laboratories it was necessary to determine chlorine and chlorine dioxide in aqueous solutions at concentrations of a few hundredths molar. The problem is relatively simple by iodometry in the absence of interfering ions, but with substantial quantities of ferric iron, cupric copper, ceric cerium, dichromate, etc., the determination becomes complicated. A simple solution to this problem arises from the fact that both chlorine and chlorine dioxide can be extracted from aqueous solution into carbon tetrachloride, and there estimated directly by spectrophotometry. Losses by gas partition and photochemical decomposition are not serious and a very rapid technique which is suitable for routine analytical work can be used. The need for such an analysis may arise infrequently but may prove useful.

The great advantage of the procedure is its wide applicability to almost any aqueous solution free from solid matter or extractable organic material. Under the conditions recommended no appreciable quantities of metals in a cationic or anionic form can be extracted, and interference is limited to the other halogens and, possibly, a relatively few nonionized inorganic substances. Direct tests in the presence of $0.1N$ ferric iron, cupric copper, ceric cerium, antimony(V), and dichromate gave no difference in the absorbance of a standard chlorine extract. The method is not highly sensitive and cannot replace trace methods such as the o-tolidine procedure. In carbon tetrachloride solution both chlorine and chlorine dioxide give well-defined absorption peaks at 3270 A. and 3550 A., respectively. The band for the dioxide reaches just into the visible region of the spectrum, giving a pale yellow solution, but the chlorine solutions are quite colorless in the dilutions used. The peaks are too broad and too close together for any useful differentiation between chlorine and chlorine dioxide to be possible spectrophotometrically.

PROCEDURE

Chlorine. Exactly 10.0 ml. of carbon tetrachloride are measured from a buret into a 30-ml. separating funnel with a short stem drawn out to a narrow tip. Next 5.0 ml. of $5M$ hydrochloric acid are added, followed by 5.00 ml. of sample solution. The funnel is shaken vigorously for 1 to 2 minutes, and the layers are then allowed to separate for exactly 1 minute. The lower layer of carbon tetrachloride is run into a 1-cm. quartz absorption cell, filling it to the brim. Then the cell is stoppered. The absorbance
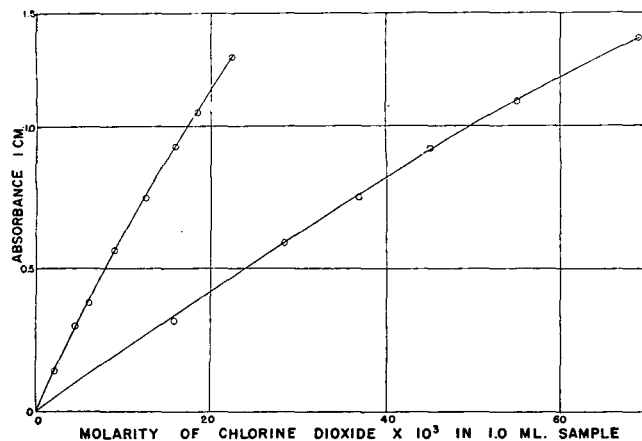
MOLARITY OF CHLORINE DIOXIDE X 10³ IN 1.0 ML. SAMPLE

Figure 1. Calibration curve

of this solution is measured without delay against water using a spectrophotometer with light of wave length 3270 A. The cell containing the chlorine solution should not be exposed to the light in the spectrophotometer for longer than about 30 seconds.

A blank determination is made on 5.0 ml. of solution of the same composition as the sample solution, but which contains no chlorine. The preparation of such a solution is not difficult, but depends on the exact nature of the samples being analyzed.

The carbon tetrachloride used in this method is of reagent quality and freed from any material oxidizable by chlorine by shaking the solvent with about 2 to 3 ml. of saturated chlorine water per liter of carbon tetrachloride. The solution should then have an absorbance of 0.05 or less. If it is greater, nitrogen is passed through the liquid until an absorbance of 0.02 or less results.

The solvent containing the chlorine must not be exposed to direct sunlight or to bright lighting from mercury fluorescent discharge tubes; however, moderate diffuse lighting is satisfactory, especially if the separating funnel is painted black on the exterior.

This method is directly suitable for amounts of chlorine between 1 and 12 mg., or 3 and 30 $\times 10^{-3}M$ chlorine in 5 ml. of sample, and a linear relation is found between concentration and absorbance. The range can be increased by using a slightly larger funnel and 20 ml. of carbon tetrachloride when exactly one half of the absorbance results. For concentrations of chlorine below about $10^{-2}M$ (3.5 mg. of chlorine in 5 ml.) 5.0 ml. of carbon tetrachloride may be used, but it is then better to use a calibration curve for the lower concentrations as a straight-line relation is not strictly obeyed. The limit of sure detection using 5 ml. of carbon tetrachloride lies around 15 p.p.m. of chlorine in the aqueous solution.

Chlorine Dioxide. The method is, in essentials, the same as that used for chlorine except that, for convenience of absorbance measurement, the relative volumes of carbon tetrachloride and sample solution are altered. For amounts of chlorine dioxide up to 1.5 mg., 1.00 ml. of sample solution is used together with 5.0 ml. of $5M$ hydrochloric acid and 15 ml. of carbon tetrachloride. If the chlorine dioxide is present between 1.5 and 5 mg., 1.00 ml. of sample solution is again used, but 50 ml. of carbon tetrachloride. The absorbance is measured in a 1-cm. cell using light of wave length 3550 A. For both quantities of carbon tetrachloride a calibration curve must be constructed as the concentration-absorbance relationship is not linear.

DISCUSSION

To suppress hydrolysis of chlorine solutions to hypochlorite the pH should be below 1, but for safety and to ensure a fairly uniform electrolyte concentration, irrespective of the composition of the sample solution, the extraction is made from solution $2.5M$ with respect to hydrochloric acid. The use of a similar molarity of sulfuric acid appears to be satisfactory, but this point has not been tested extensively. The hydrolysis of chlorine dioxide in acid solutions is not dependent upon acidity unless the pH exceeds 2, but for uniformity a procedure has been used similar to that employed for chlorine.

The partition coefficient for chlorine into carbon tetrachloride

exceeds 90% under the conditions used in this method. It is seen from Table I that with 10 ml. of solvent, a straight-line relationship can be assumed with an accuracy comparable with most routine absorptiometric methods. The same is found with 20 ml. of solvent, but in using 5 ml. (Table II) serious errors occur in the lower range. If a calibration curve is drawn, however, these can be largely eliminated. The partition coefficient for chlorine dioxide is much less than for chlorine (below 75%) and a calibration curve must be constructed (see Figure 1). The precision of the two methods is then comparable. As the molar absorbance coefficient for chlorine dioxide is some ten times that of chlorine, the method can be made much more sensitive on a molarity basis, although on a normality basis the difference is less marked.

Table I. Extraction of Chlorine Using 10 Ml. of Carbon Tetrachloride

Mean factor, assuming linear relationship. Molarity = absorbance $\times 2.34 \times 10^{-2}$

Molarity $\times 10^{-2}$ of Sample	Absorbance	Calculated Molarity $\times 10^{-2}$	% Error
0.535	0.235	0.550	+3
1.095	0.470	1.10	+0.5
1.77	0.750	1.76	−0.5
1.97	0.865	2.02	+2.5
2.67	1.120	2.62	−2
2.86	1.215	2.85	<0.5

Table II. Extraction of Chlorine Using 5 Ml. of Carbon Tetrachloride

Mean factor, assuming linear relationship. Molarity = absorbance $\times 12.9 \times 10^{-3}$

Molarity $\times 10^{-3}$ of Sample	Absorbance	Calculated Molarity $\times 10^{-3}$	% Error
1.65	0.145	1.87	+13
2.65	0.220	2.85	+7.5
5.50	0.437	5.63	+2.5
7.80	0.620	8.00	+2.5
9.40	0.732	9.45	+0.5
12.10	0.925	11.95	−1.0
13.25	1.015	13.10	−1.0
17.60	1.315	17.0	−3.5

Because errors from photochemical decomposition are not serious if the method is carried out rapidly in subdued light, no great precautions need be taken. The solutions decompose when irradiated by ultraviolet light in the spectrophotometer, but the decomposition is negligible if the exposure does not exceed the few seconds required to take a reading.

The complications arising from making extracts to a known volume and filtering the extracts are eliminated by using the method as described, where a known volume of solvent is accurately measured before extraction and the solution cleared by allowing the layers to separate in the funnel for a standard time. The slight haze of aqueous solution left in the organic layer is reproducible and contributes a blank of only 0.01 to 0.02 absorbance in a 1-cm. cell. If the aqueous solution contains a high concentration of ions absorbing light of wave length around 3000 A. this blank may increase a little, but is reproducible. If chloroform is substituted for carbon tetrachloride, a more favorable partition results, but separation of the organic and inorganic layers is much less satisfactory.

Dehydration of Orthophosphoric Acid

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The behavior of phosphoric acid when heated at 100° and 176° C. was important to other studies. Selective elution from anion exchange resin served to separate the acids formed when labeled phosphoric acid was heated. Labeling with phosphorus-32 aided materially in the analysis of the fractions, each of which was identified by coelution of the reaction mixture with unlabeled carriers. Heating phosphoric acid at 100° C. yielded ultimately 4% of the phosphorus-32 activity as pyrophosphate. Heating phosphoric acid at 176° C. yielded in the steady state a mixture that contained 49% of the phosphorus-32 as orthophosphate, 40% as pyrophosphate, 10% as triphosphate, 2% as tetraphosphate, and 0.2% as pentaphosphate. The combination of phosphorus-32 labeling with the selective elution procedure should prove useful for studying the more complex phosphorus compounds.

L ABELING of the tributyl phosphate molecule with radioactive phosphorus-32 (1) has practical significance for such applications as solubility determinations, and may be an aid in the investigation of fundamental problems in solvent extraction.

A new and more efficient method for preparing the phosphorus-32 labeled compound has been developed utilizing ester interchange between tributyl phosphate and labeled orthophosphoric acid, and will be submitted as a separate paper. When the partial esters of phosphoric acid resulting from such interchange were separated by selective elution from anion exchange resin, more products were found, under certain reaction conditions, than could be explained by the ester interchange itself. Inorganic phosphates were naturally suspected. A knowledge of all constituents present in the reaction mixture was desirable for the understanding of the reaction paths, and for devising methods to purify the reaction products. Therefore, orthophosphoric acid (labeled with phosphorus-32) was heated at the temperatures of the reaction (100° and 176° C.) for periods of time that included those used for the exchange reaction itself.

Bell (2) thoroughly analyzed mixtures of dehydrated phosphates over a wide range of phosphorus pentoxide concentrations by colorimetric, precipitation, and titration methods then available; thus knowledge of the phosphorus pentoxide concentrations at the temperatures and times used gave an indication of the number and apparent concentration of major components. However, since the combination of radioactive phosphorus labeling with selective elution from anion exchange resins suggested itself as possibly a more accurate and sensitive means of analysis, it was mandatory to confirm identity of each component. In addition to conventional methods of identification, another method involving coelution of authentic carrier with each labeled component was employed in separate column runs.

During the course of this work Beukenkamp, Rieman, and Lindenbaum (5) published fundamental data for several phosphates which predicted separation and which improved the column separations of this laboratory. Lindenbaum, Peters, and Rieman (10) later published an effective scheme for a column separation which does not require the use of phosphorus-32. The results, obtained in this laboratory from analysis of a radioactive mixture of unknowns, agree very well with their findings and demonstrate the applicability of tracer technique to analysis of phosphorus compounds by ion exchange.

APPARATUS AND REAGENTS

Resins. Anion exchanger, Dowex 1 (100 to 200 mesh, 8% cross-linked, in the chloride form) was used in columns to separate the mixtures of phosphorus acids. Cation exchanger, Nalcite HCR (50 to 100 mesh, in the hydrogen form), was used for the determination of the equivalent weight of salts. Fine particles were removed from the resins by decantation. The anion exchange resin was washed with the eluting solution and then with distilled water before use. The cation exchange resin was washed with hydrochloric acid to convert it to the hydrogen form, then excess acid was washed out with distilled water.

Column. The resin column was an adaptation of that described by Tompkins, Khym, and Cohn (13). A continuous delivery tube (polyethylene surgical tubing, 0.16-cm. inside diameter and 0.21-cm. outside diameter, looped once and attached to an end-window Geiger-Müller tube) was a modification of that described by Boyd, Myers, and Adamson (6). The radioactivity in the flowing eluate was recorded, and each fraction was caught separately in volumetric flasks.

Interstitial volume was determined in a manner similar to that of Rieman and Lindenbaum (11). The resin was treated with a large volume of eluent and the liquid was drained to the level of the resin. After drying the walls above the resin with absorbent paper, the eluent in the resin and delivery tube was removed with water and the amount determined by titration with standard base in the case of hydrochloric acid eluent, and by electrometric titration with standard silver nitrate in the case of potassium chloride. The total volume thus found, less the holdup in the delivery tube, gave the volume of eluent in the resin bed.

Column Statistics

Bed	0.85 sq. cm. × 15.5 cm. containing 13.2 ml. Dowex 1, 100 to 200 mesh, 8×, Cl⁻ form
Column volume	5.6 ml.
Delivery tube	3.3 ml. to counter

Solution Counter. Solutions containing radioactive phosphorus were counted at 7% geometry with a thin-walled glass Geiger-Müller tube (C, Figure 1). Aliquots (15 ml.) of a suitable dilution were measured into the sample holder, A, which was placed in a fixed position on the movable platform, B, raised around the tube, and the activity measured with a scaler. The tube and sample were enclosed in a light-tight lead pig to minimize the background count.

Titrimeter. All titrations were followed electrometrically using a Fisher Titrimeter.

Reagent grade chemicals were employed except as noted below.

Orthophosphoric Acid. Carrier-free phosphorus-32 labeled acid was obtained from the Oak Ridge National Laboratory Iso-
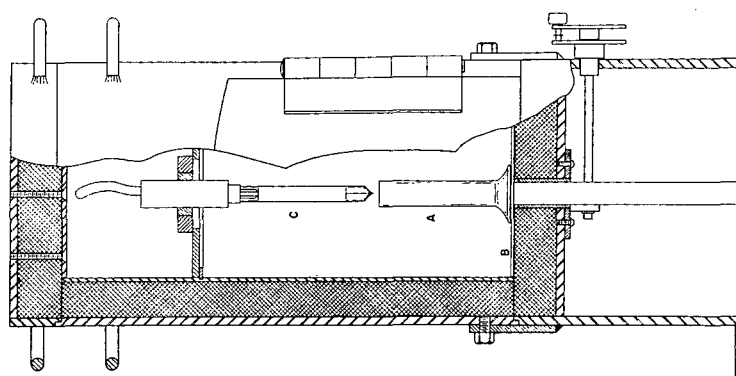

Figure 1. Solution counter for assay of solutions containing phosphorus-32 (1.7 m.e.v. β^-)

Sample (15 ml. of suitable dilution) in container, A, on movable platform, B, is raised around GM counting tube, C, in lightproof lead shield; activity is recorded on scaler
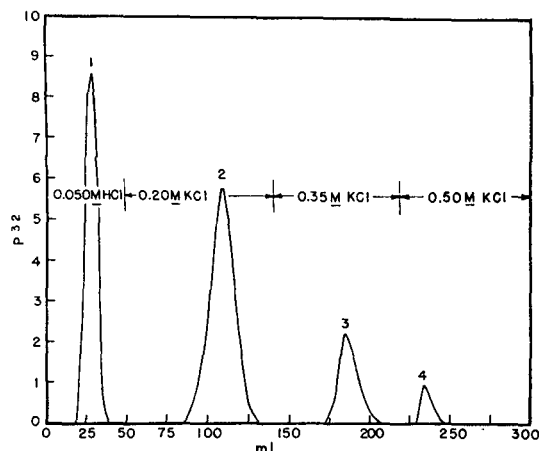
Figure 2. Elution of phosphate mixture resulting from heating orthophosphoric acid at 176° C. for 8 hours

Resin loaded with 0.05 mg. atom phosphorus, 14 μc. phosphorus-32

1. Orthophosphate
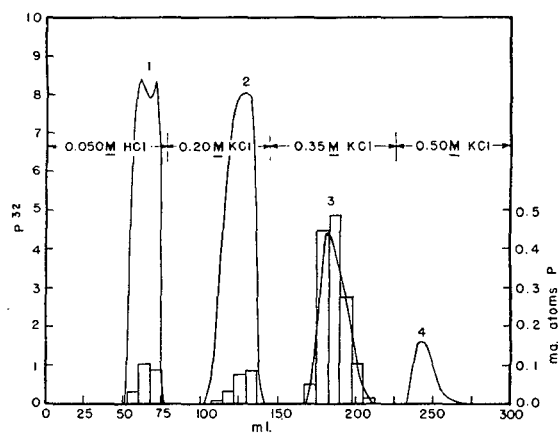2. Pyrophosphate
3. Triphosphate
4. Tetraphosphate

Figure 3. Identification of fraction 3 as triphosphate

Blocks, total phosphorus by titration; curve, phosphorus-32; carrier, potassium triphosphate (232 mg., 1.54 mg. atoms phosphorus); tracer, orthophosphoric acid heated for 24 hours at 176° C. (0.39 mg. atom phosphorus, 58 μc. phosphorus-32);

1. Orthophosphate
2. Pyrophosphate
3. Triphosphate
4. Tetraphosphate

topes Production Division. Reagent grade orthophosphoric acid (used for carrier) was shown to be free from pyrophosphoric acid by analysis using the ion exchange column.

Pyrophosphate. Both phosphorus-32 labeled and unlabeled salts were prepared by heating dibasic sodium or potassium phosphates for several days at 300° C.

Potassium Triphosphate and Sodium Trimetaphosphate. Samples of these salts were obtained from Victor Chemical Co., Chicago Heights, Ill. Purity of the triphosphate was determined by passing a weighed sample dissolved in water through Nalcite HCR (the hydrogen form in a column 10 cm. × 1 sq. cm.) and titrating the effluent and washes with carbonate-free sodium hydroxide. The equivalent weight found was 151; calculated for potassium triphosphate ($K_5P_3O_{10}$), 149.5.

Table I. Elution Order and Effect of Concentration on Location of Peak

Orthophosphate, Phosphite, Hypophosphate, and Pyrophosphate

Eluent, $0.10M$ HCl

Compound	Concn., mg., atoms, P	Column volumes to peak
H_3PO_4	0.01	1.6
	1.00	5.5
H_3PO_3[a]	0.76	5.5
	1.01	7.1
	1.03	6.8
$H_4P_2O_6$[a]	0.81	13
	2.00	21
$H_4P_2O_7$	0.01	10.5
	0.82	22

[a] Elution order established by elution with radioactive ortho- and pyrophosphates.

Sodium Tetrametaphosphate. This was prepared with a modification of the method of Bell, Audrieth, and Hill (4). After the reaction of the phosphorus pentoxide with water below 15° C., neutralization to pH 7 with sodium hydroxide, and filtration, the solution was concentrated to half its volume by drawing air through a sintered-glass filter over the solution's surface instead of adding sodium chloride and allowing to stand. The crystals formed were filtered, washed with a small amount of cold water, and dried 2 days in an evacuated desiccator over sodium hydroxide. An equivalent weight determination as described yielded only one inflection, showing the absence of polyphosphates, and gave an equivalent weight of 120.7. Theoretical for sodium tetrametaphosphate tetrahydrate ($Na_4P_4O_{12}\cdot 4H_2O$) is 120.0.

Sodium Tetraphosphate. Using the method of Thilo and Rätz (12), an aqueous solution of sodium tetraphosphate (Na_6-

P_4O_{13}) was obtained which, after 21 hours in an evacuated desiccator over sodium hydroxide, contained 58% water. When an equivalent weight analysis was made on 355 mg. of this solution, 1.28 meq. of standard sodium hydroxide were required to the first inflection and an additional 0.63 meq. to the second inflection. Sodium and phosphorus analyses gave a ratio of 6 sodium to 4.02 phosphorus.

Sodium hypophosphate ($Na_2H_2P_2O_6\cdot 6H_2O$) was furnished by Elmer Leininger (9).

Sodium hexametaphosphate was obtained from Blockson Chemical Co., Joliet, Ill.

EXPERIMENTAL

Dehydration of Orthophosphoric Acid. Phosphoric acid (85%, 0.5 ml.) and phosphorus-32 labeled phosphoric acid in aqueous solution (2 to 5 mc., 0.5 to 1 ml.) were mixed in a 2-ml. platinum crucible [orthophosphoric acid reacts with glass at elevated temperatures (7, 8)], which was then placed in a test tube in a suction flask and the mixture concentrated by drawing air through a sintered-glass filter and over the surface of the liquid for 24 hours. Final drying was accomplished in an empty drying pistol with a vacuum pump at 2 to 3 mm. mercury for 16 hours at room temperature. At the end of this period a sample was removed for analysis and the crucible was immediately lowered into a test tube in the constant temperature bath. This consisted of a 300-ml. round-bottomed flask half filled with p-cymene for the 176° C. (observed) temperature and water for 100° C. The flask was fitted with a condenser and the sample holder was inserted through a stopper in such manner that the crucible was submerged to its own depth in the already boiling liquid. The open end of the test tube was covered with an inverted filter cone to prevent contamination by foreign matter. Samples (25 to 50 μl.) were removed at intervals, dissolved in 50 ml. of distilled water, and immediately adjusted to pH 10 with $1.00M$ sodium hydroxide, observing the total phosphorus content by this titration. After titration the sample was transferred to a 100-ml. volumetric flask, electrode and beaker rinsings were added, and the volume was adjusted by diluting to the mark with distilled water.

Analysis of Mixtures. Aliquots of the mixtures resulting from the dehydration treatment described were separated by ion exchange. The resin was loaded with a solution (at pH 10) containing 3 to 30 μc. phosphorus-32, 0.02 to 0.1 mg. atoms of phosphorus, and was washed with 4 to 5 interstitial volumes of distilled water. Using a flow rate of 0.5 to 0.7 ml. per minute, the orthophosphate was removed with $0.05M$ hydrochloric acid or $0.15M$ potassium chloride, pyrophosphate was removed by using $0.20M$ potassium chloride, triphosphate using 0.30 or $0.35M$ potassium chloride, and tetraphosphate using 0.40 to $0.50M$ potassium chloride. Cleaner separation was effected by using dilute hydrochloric acid for the removal of orthophosphate. A typical elution curve is shown in Figure 2. The holdup volume has not been subtracted.

An aliquot of each fraction was assayed for total phosphorus-32 activity in the solution counter. Results are expressed as the per

cent of the total activity in the solution used to load the column (see Figure 6).

Identification of Components. The identity of the compound causing each peak in the elution graph was established by the elution of known compounds, one at a time, with the radioactive mixture. The carrier (0.8 to 2 mg. atoms of phosphorus) was added to an aliquot of the phosphorus-32 labeled dehydration mixture and the resin was loaded at pH 10 at such dilution ($\sim$0.001M) as to minimize self-elution. The separation was performed at a flow rate of 0.5 ml. per minute and the carrier phosphorus was measured in separate aliquots ($\sim$ one interstitial volume) by the amount of alkali required to titrate between the first and second inflection points. When the eluent was a salt, it was necessary to acidify to a pH of about 3 before titration, and when the known was a metaphosphate it was necessary to hydrolyze to orthophosphate in order to measure the phosphorus by titration. This was accomplished by adding excess hydrochloric acid and taking to near dryness twice.

Using the above technique, fraction 1 was proved to be orthophosphate; 2, pyrophosphate; 3, triphosphate; and 4, tetraphosphate. Typical plots of the carrier phosphates against the elution curve of the phosphorus-32 labeled mixture are shown in Figures 3 and 4. Figure 5 shows the position of tetrametaphosphate relative to the polyphosphates.

Several other phosphorus carriers were tested individually with the radioactive mixture and did not coelute with any of the labeled fractions, but the tests did establish the elution order of these compounds. Phosphorous acid and hypophosphate are shown in Table I, which also demonstrates the effect of the concentration of the compound on the location of its peak. By elution under the same conditions as used for tetrametaphosphate (Figure 5) it was proved that trimetaphosphate was not a constituent of the radioactive mixture. After 51 ml. of 0.50M hydrochloric acid and 77 ml. of 0.20M potassium chloride, it required 72 ml. of 0.35M potassium chloride to the tetraphosphate peak, whereas 88 ml. of 0.35M potassium chloride were required to the trimetaphosphate peak. The main component of the so-called "sodium hexametaphosphate" mixture was found to elute following the fifth fraction of the radioactive mixture. The concentration of the eluent was increased to 0.50M potassium chloride after fraction 5 to accomplish this removal.

Fraction 5. Because a carrier amount of a component spreads the elution curve of that component, a larger quantity of tracer had been used for the identification of the tetraphosphate (Figure 4). After elution of the tetraphosphate a fifth fraction (0.2% of the starting activity) was found in the reaction mixture where $H_3P^{32}O_4$ was heated at 176° C. for 21 hours The fifth fraction did not coelute with any of the carriers mentioned. It has been shown that each successive fraction contained a phosphate whose chain was one phosphorus atom longer than the previous, the yield progressively became less, and the rate at which each compound was eluted from the column became slower (see Figures 2 to 5). It therefore appears probable that fraction 5 contains a five phosphorus chain, or pentaphosphate.

Reaction between Zinc Sulfate and Tetraphosphate. Triphosphate is known to react with zinc sulfate solutions at pH 3.8 to increase the hydrogen ion concentration, and this fact has been utilized for its determination (3). It was not known whether tetraphosphate reacts similarly; thus, a test was made on 272 mg. of sodium tetraphosphate (0.58 millimole). After adjustment to pH 3.8 and addition of 70 ml. of 12.5% zinc sulfate heptahydrate at pH 3.8, 0.82 meq. of sodium hydroxide was required to bring the pH up to 3.8 again. The solution remained
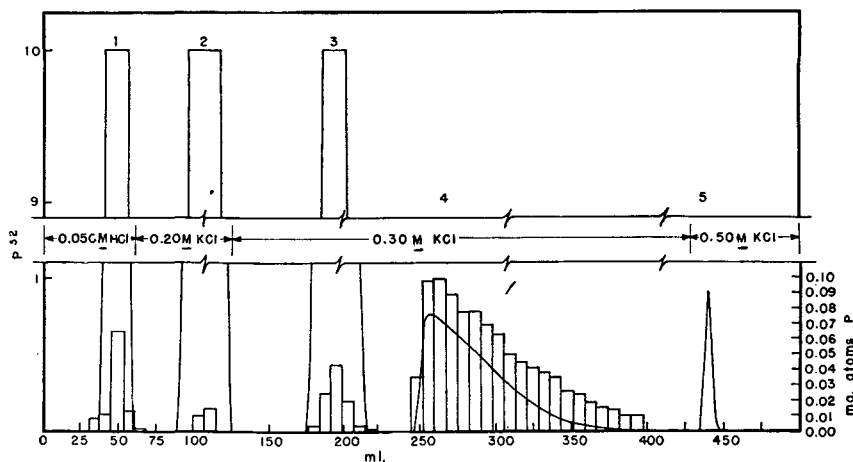

Figure 4. Identification of fraction 4 as tetraphosphate

Blocks, total phosphorus by titration; curve, phosphorus-32; carrier, sodium tetraphosphate (154 mg., 1.31 mg. atoms phosphorus); tracer, orthophosphoric acid heated for 21 hours at 176° C. (0.10 mg. atom phosphorus, 277 μc. phosphorus-32);

1. Orthophosphate
2. Pyrophosphate
3. Triphosphate
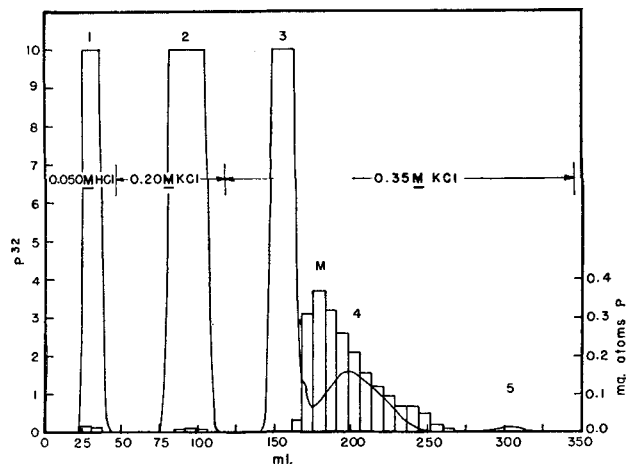4. Tetraphosphate
5. Fraction 5

Figure 5.   Elution position of tetrametaphosphate relative to polyphosphates

Blocks, total phosphorus by titration; curve, phosphorus-32; carrier, sodium tetrametaphosphate tetrahydrate (270 mg. 2.23 mg. atoms phosphorus); tracer, orthophosphoric acid heated for 21 hours at 176° C. (0.05 mg. atom phosphorus, 186 μc. phosphorus-32)

1. Orthophosphate
2. Pyrophosphate
3. Triphosphate
M. Tetrametaphosphate
4. Tetraphosphate
5. Fraction 5

clear. It is evident that the presence of tetraphosphate would introduce error in the determination of triphosphate.

RESULTS

The selective elution from anion exchange resin proved to be a valuable tool for the separation, identification, and estimation of polyphosphorus compounds. The amount of compound present influenced the shape of the elution curve and the location of the maximum on this curve, but coelution with carriers served for identification.

The order of elution of the phosphorus-32 labeled compounds in the mixture of unknowns resulting from heating orthophosphoric acid at 176° C. (observed temperature) was found to be: orthophosphate, pyrophosphate, triphosphate, tetraphosphate, and the fifth fraction, presumably pentaphosphate. The order
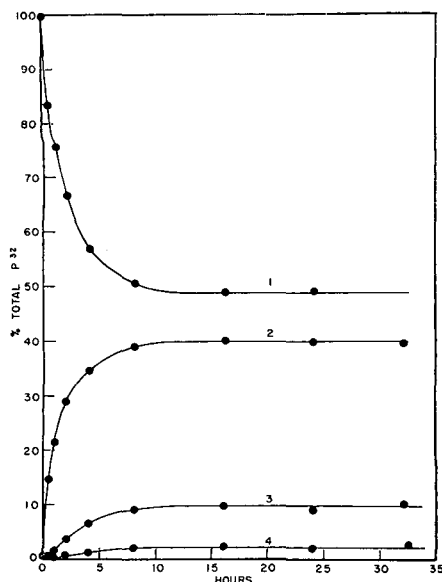
Figure 6. Composition of orthophosphoric acid as function of heating time at 176° C.

1. Orthophosphate
2. Pyrophosphate
3. Triphosphate
4. Tetraphosphate

of elution including various phosphates tested individually during identification of the labeled fraction is: orthophosphate, phosphite, hypophosphate, pyrophosphate, triphosphate, tetrametaphosphate, tetraphosphate, trimetaphosphate, fraction 5, and the main component of a commercial sodium hexametaphosphate.

The orthophosphoric acid solutions (used for the heating tests) that had been dried in an air stream and under vacuum at room temperature were found to contain 0.3% of the phosphorus-32 activity as pyrophosphate. Heating of this dried acid at 100° C. yielded after 24 hours 4% of the phosphorus-32 activity in the

pyrophosphoric acid and 96% as orthophosphoric acid. No change was noted on prolonged heating up to 150 hours at 100° C. At 176° C. the equilibrium mixture (Figure 6) contained 49% of the phosphorus-32 as orthophosphoric acid, 40% as pyrophosphoric acid, 10% as triphosphoric acid, 2% as tetraphosphoric acid, and 0.2% as a component believed to be pentaphosphoric acid.

A combination of phosphorus-32 labeling and the separation and identification procedure described might well be used for the analysis of concentrated phosphoric acids, and even the so-called hexametaphosphate mixture might be separated into its components.

LITERATURE CITED

(1) Baldwin, W. H., and Higgins, C. E., *J. Am. Chem. Soc.*, 74, 2431 (1952).
(2) Bell, R. N., *Ind. Eng. Chem.*, 40, 1464 (1948).
(3) Bell, R. N., ANAL. CHEM., 19, 97 (1947).
(4) Bell, R. N., Audrieth, L. F., and Hill, O. F., *Ind. Eng. Chem.*, 44, 568 (1952).
(5) Beukenkamp, J., Rieman, W., and Lindenbaum, S., ANAL. CHEM., 26, 505 (1954).
(6) Boyd, G. E., Myers, L. S., and Adamson, A. W., *J. Am. Chem. Soc.*, 69, 2849 (1947).
(7) Hautefeuille, P., and Margottet, J., *Compt. rend.*, 96, 1052 (1883).
(8) Hüttner, K., *Z. anorg. Chem.*, 59, 216 (1908).
(9) Leininger, E., and Chulski, T., *J. Am. Chem. Soc.*, 71, 2385 (1949).
(10) Lindenbaum, S., Peters, T. V., and Rieman, W., *Anal. Chim. Acta*, 11, 530 (1954).
(11) Rieman, W., and Lindenbaum, S., ANAL. CHEM., 24, 1199 (1952).
(12) Thilo, E., and Rätz, R., *Z. anorg. Chem.*, 260, 255 (1949).
(13) Tompkins, E. R., Khym, J. X., and Cohn, W. E., *J. Am. Chem. Soc.*, 69, 2769 (1947).

Determination of Formic Acid in Presence of Acetic Acid

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A convenient and precise means for determining formic acid is described. The method is based on the azeotropic distillation of formic acid with chloroform. Quantitative separation of formic acid from other organic acids is easily accomplished by a method which involves no high temperatures or complicated reactions. Recovered formic acid is directly titrated with standard base. The method is not subject to the interferences of the usual oxidation methods.

FOR the determination of formic acid in the presence of acetic acid, a general procedure has been selective oxidation of the formic acid. Oxidizing agents are usually mercuric oxide (*2*), mercuric salts (*1, 3, 6*), and lead tetraacetate (*7*). These methods can be in error if other oxidizable materials are present.

The method described utilizes the fact that formic acid can be distilled as an azeotrope with chloroform (*4*), whereas acetic and higher acids cannot (*5*). By this means the formic acid can be

separated and quantitatively determined by titration with base. The advantages are that the formic acid is quantitatively separated, no high temperatures are involved, no complicated reactions occur, and the acid is recovered unchanged.

APPARATUS AND MATERIALS

Materials. ACS grade formic acid, glacial acetic acid, chloroform, salicylic acid, and methanol were used. Stock solutions of formic acid and acetic acid were made up in chloroform and diluted as desired. Titrations were carried out with $5 \times 10^{-3}N$ methanolic sodium hydroxide solution, prepared by diluting a $1N$ aqueous solution with methanol.

Apparatus. The solutions were distilled in a column 100 cm. long, 20-mm. internal diameter, and packed with 4-mm. spherical glass beads. The column was surrounded by a 50-mm. glass tube, which in turn was surrounded by a 100-mm. column for insulation.

Titrations. Titrations of the chloroform solutions were made after adding 10 ml. of methanol and 2 ml. of water to 10 ml. of the solutions being analyzed. A Model G Beckman potentiometer with glass and calomel electrodes was used in the titration.

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[1] Deceased.

Table I. Analysis of Formic and Acetic Acid Solutions

Formic Acid Added, Mg.	Acetic Acid Added, Mg.	Recovered Acid (as Formic), Mg.	% Formic Acid Recovered
2.10	7.50	2.05	97.6
1.70	7.00	1.65	97.0
1.30	12.50	1.28	98.5

EXPERIMENTAL

Stock solutions of formic acid in chloroform were prepared and aliquots were titrated. Fifty milliliter aliquots of these solutions were distilled after addition of 50 ml. of chloroform and 2 grams of salicylic acid. Slightly less than 50 ml. of distillate were collected and made up to volume in 50-ml. volumetric flasks. Aliquots were titrated and compared with the known formic acid content before distillation.

Table II. Recovery of Formic Acid from Sodium Formate Solutions

Sodium Formate as Formic Acid, Mg.	Formic Acid Recovered by Distillation, Mg.	% Recovery
10.2	9.8	96
10.2	9.6	94
10.2	9.9	97
113.0[a]	107.5	95

[a] Titrated with 0.1N standard sodium hydroxide.

Solutions containing known quantities of formic and acetic acids were mixed and distilled with chloroform in the presence of salicylic acid. The distillates were titrated against standard base.

Aqueous sodium formate solutions were analyzed for formate content, which was done by neutralizing an aqueous formic acid solution with sodium hydroxide (0.1N) and removing the water by azeotropic distillation with chloroform. (Drying was required, because the water-chloroform azeotrope boils below the chloroform–formic acid azeotrope.) After acidification of the water-free sample with salicylic acid, the procedure followed was as described for formic acid.

RESULTS

Table I shows the recovery of formic acid in the presence of acetic acid. Distillation of chloroform solutions containing only formic acid indicated 95 to 100% recovery. Distillation of chloroform solutions of mixed formic and acetic acid showed that no acetic acid was present in the distillate.

CONCLUSIONS

The isolation of formic acid by distillation with chloroform affords a convenient and precise means for determining formic acid. The method does not appear to be subject to the interferences to which the oxidation methods are subject. An aqueous solution of formic acid can be analyzed by evaporating the neutralized solution and distilling with chloroform after liberating the acid.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Ahlen, L., and Samuelson, O., ANAL. CHEM., 25, 1263 (1953).
(2) Arthur, W. J., and Struther, G. W., Ibid., 21, 1209 (1949).
(3) Hopton, J. W., Anal. Chim. Acta, 8, 429 (1953).
(4) Horsley, L. H., and others, Advances in Chem. Ser., No. 6, 20, No. 920 (1952).
(5) Ibid., No. 928.
(6) Marconi, M., Chimica (Milan), 6, 315 (1951).
(7) Perlin, A. S., ANAL. CHEM., 26, 1053 (1954).

Determination of Total Carbon in Organic Materials by a Wet-Dry Combustion Method

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An improved simple and rapid wet-dry combustion method has been developed for estimating small amounts of organic matter in water. Provisions have been made for analyses of aqueous samples containing high-boiling, steam-distillable, or volatile substances. There is no interference from halogens. Solids or liquids up to 100 ml. in volume containing organic matter equivalent to 1 to 150 mg. of carbon dioxide are taken for analysis. As little as 0.5 mg. of carbon per 100 ml. of water can be determined with a precision and accuracy within 1% relative.

R ELIABLE analytical methods are needed for determining organic matter in aqueous systems. This is reflected by the increasing literature pertaining to problems of sewage disposal, water pollution by industrial wastes, recovery of industrially important materials, and water treatment (4). Relatively nonvolatile organic compounds have been analyzed successfully by wet combustion using chromic acid–sulfuric acid (1, 3) and oxidizing agents such as dichromate (5, 7), iodic acid, permanganate (5), and persulfate (2). All of the laboratory procedures reported are limited in their applicability. Some are not suitable for all types of compounds, whereas others lack the sensitivity necessary for determining a few parts per million of carbon. Consequently, there is still a definite need for a simple and rapid general laboratory method of analysis for organic matter in aqueous systems.

Oemler and Mitchell (6) developed a wet-dry combustion method which overcame the difficulties often encountered in the usual wet combustion procedures. Organic material was oxidized in the presence of aqueous chromic acid and concentrated sulfuric acid. High-boiling, steam-distillable compounds, which escaped from the reaction flask into the condenser, were washed back into the flask by concentrated sulfuric acid added dropwise at the top of the condenser. Halogen acids produced by the oxidation were removed in a gas-washing bottle containing 0.1N hydro-
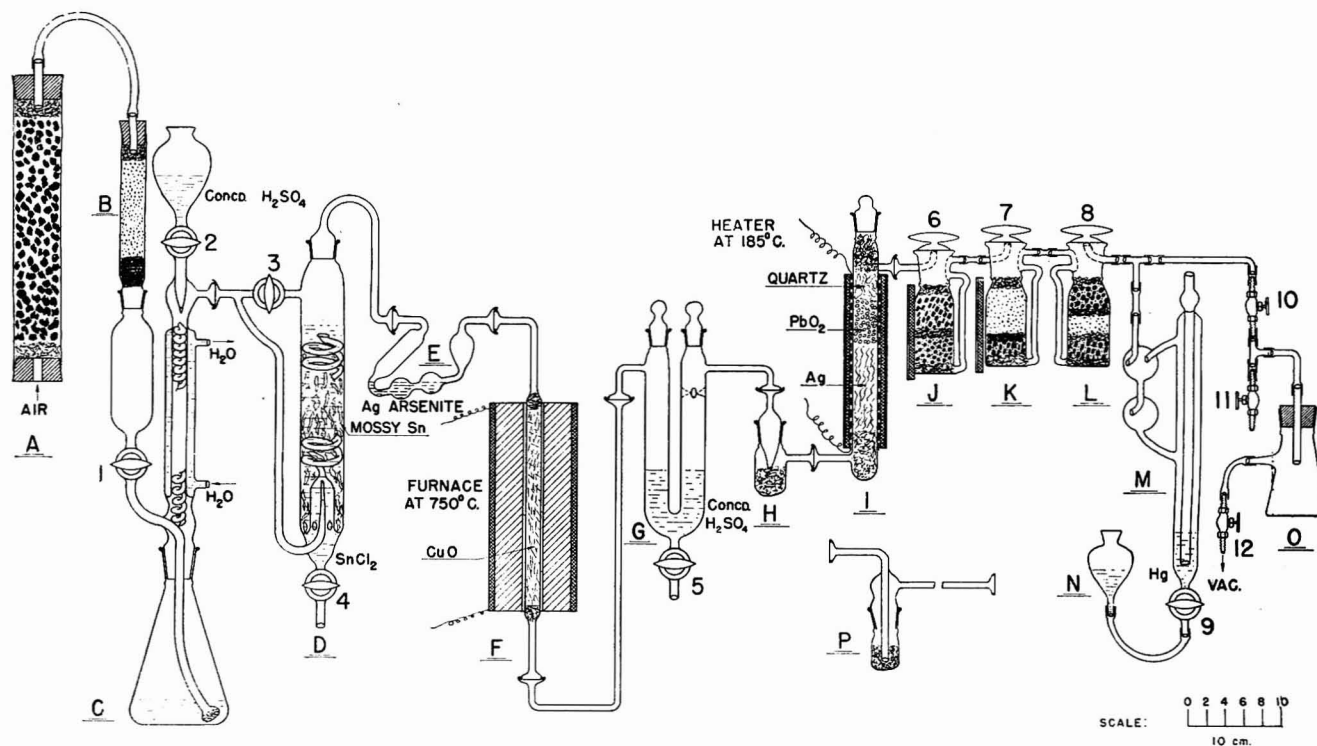
Figure 1.   Details of wet-dry combustion apparatus

chloric acid followed by an easily replaced borosilicate glass tube containing copper oxide heated at 250° C.   Organic vapors which escaped from the condenser were oxidized in a copper oxide combustion tube at 750° C.   Total carbon dioxide produced was absorbed on Ascarite and determined gravimetrically.   However, the halogen scrubber system was found to be impractical for analyses of waste stream samples containing large amounts of halides.   The dilute hydrochloric acid had to be changed after each determination, and the 750° C.-copper oxide tube was rapidly plugged in spite of the 250° C. tube.

The present method, which is closely patterned after that described, was developed to eliminate more effectively the interference of halogens.   Some improvements also have been made in the apparatus.   The halogen scrubbing system used has been replaced by scrubbers containing metallic tin and stannous chloride and silver arsenite.   An increase in maximum sample volume from 50 to 100 ml. has decreased the limit of detection from 10 to less than 5 p.p.m. carbon.   Analysis time has been reduced considerably.   The procedure is suitable also for the determination of total carbon in liquid and solid organic materials, which cannot be analyzed readily by the conventional dry combustion carbon and hydrogen method.

APPARATUS

The combustion apparatus is shown in detail in Figure 1. With the exception of stopcocks, joints, stoppers, and connections, all items were drawn approximately to scale.

Moisture and carbon dioxide absorbers, A and B, are open cylinders, and one end of B is equipped with a $\mathbb{S}$ 19/22 inner joint. Reaction assembly C is composed of a reagent funnel equipped with stopcock 1 and a bent delivery tube ending in a round bulb perforated with six 2-mm. holes, a water-cooled condenser (which contains a glass helix made of a 2-mm. rod, a $\mathbb{S}$ 24/40 inner joint, and a 12/5 ball joint) a dropping funnel connected to the condenser through stopcock 2, and a detachable 500-ml. Erlenmeyer reaction flask with a $\mathbb{S}$ 24/40 outer joint.

Primary halogen scrubber, D, consists of an outer cylinder which contains 12 turns of a spiral made of 3-mm. inside diameter glass tubing.   The spiral is connected to an inner chamber which is perforated with four 3-mm. holes, and the inner chamber is sealed to the bottom of the cylinder.   The upper inlet tube is

connected to the condenser by a 12/5 socket joint and contains stopcock 3.   The gas inlet tube of the scrubber leads into the inner chamber up to where the spiral is connected to the top of the chamber and is drawn out to form a capillary of 1-mm. inside diameter.   The outer cylinder has a $\mathbb{S}$ 29/26 joint at the top and stopcock 4 at the bottom.   Secondary halogen scrubber, E, is a Norris potash bulb connected to the system by 12/5 ball and socket joints.

Combustion tube, F, may be either quartz or Vycor, including the two 12/5 ball joints.   The furnace tube is Alundum (Catalog No. RA 1139, Norton Co., Worcester, Mass.) 8.5 inches long and $^7/_8$-inch in inside diameter, wound with a length of $^1/_{16} \times 0.0045$-inch Nichrome ribbon having a resistance of about 17 ohms.   The end coils of the Nichrome ribbon are placed closer together than are those in the center to compensate for the greater heat losses at the ends of the furnace.   The heating unit is enclosed in a stainless steel shell 9 inches long and 2.5 inches square.   The space between the furnace tube and the shell is packed with Eagle 66 asbestos insulation.   Each end of the shell is closed with a Marinite block 2.5-inches square and 0.25-inch thick, which has a $^7/_8$-inch diameter hole in the middle, and the steel shell is then wrapped with a 0.25-inch layer of wet asbestos paper which is allowed to dry.

Sulfuric acid scrubber, G, is a simple stoppered U-tube with two 12/5 ball joints on the side arms.   Stopcock 5 serves as a drain.   Several fingerlike indentations are made in the outlet section of the U-tube to break the film of the acid bubbles formed by the passage of gas.   Spray trap H is equipped with $\mathbb{S}$ 29/26 joints and 12/5 ball and socket joints.

Absorption tube I is connected to the system by 12/5 ball and socket joints.   The tube is wound with a 10.4-foot length of $^1/_{16} \times 0.0089$ inch chrome ribbon having a resistance of 9.4 ohms.   This tube is covered with electrical insulation tape and a 0.25-inch layer of wet asbestos paper, which is allowed to dry.

Absorption bulbs, J, K, and L, are Nesbitt type carbon dioxide absorbers and are used to absorb moisture and carbon dioxide.

Mercury spill, M, is equipped with stopcock 9.   Leveling bulb, N, has a capacity of 125 ml.   Vacuum trap O is a 125-ml. filter flask equipped with valves 10, 11, and 12.   Valve 10 should be a needle valve, but valves 11 and 12 may be either stopcocks or needle valves.

Trap, P, is used in place of scrubbers, D and E, for analyses of samples which do not contain halides.

A compact arrangement of the apparatus is shown in Figure 2.

PREPARATION OF APPARATUS

The absorbers, A and B, which provide dry carbon dioxide–free air for sweeping out the apparatus, are filled, respectively, with

indicating Drierite (8 mesh) and Ascarite (8 to 20 mesh) held in place with glass wool plugs.

The primary halogen scrubber, D, is filled with mossy tin up to the outlet of the spiral, and then some $3N$ stannous chloride ($SnCl_2.2H_2O$) in $2N$ hydrochloric acid solution is added to about 5 mm. above the outlet of the spiral. The stannous chloride removes halogens from the gas stream, and the tin regenerates the spent solution by reducing stannic ions to stannous ions.

The secondary halogen scrubber, E, is charged with saturated silver arsenite solution which reduces and precipitates as silver salts any gaseous halogens that may escape from D.

The combustion tube, F, which completes the oxidation of any organic material remaining in the gas stream, is filled with cupric oxide wire held in place with glass wool plugs.

The sulfuric acid scrubber, G, which is partly filled with concentrated sulfuric acid, absorbs most of the water vapor in the gas stream and also serves as a bubble counter for observing the rate of gas flow. The glass indentations in this scrubber and the glass wool in the spray trap, H, both serve to prevent any acid spray from entering the absorption tube, I.

I is filled with glass wool up to about 1 cm. above the inlet tube. Small twisted pieces of clean silver gauze (40 mesh) are inserted to form a 10-cm. layer followed by a compact layer of glass wool. Enough pelletized lead peroxide is added, with gentle tapping to form an 8-cm. layer. This is followed by a 1-cm. layer of silicon carbide (12 to 30 mesh, acid washed) and enough glass wool to fill up the rest of I. (It has been demonstrated that glass wool alone is not sufficient to prevent lead peroxide dust from being carried over into the absorption bulb, J.) Silver gauze removes traces of halogens, whereas lead peroxide absorbs the oxides of sulfur and nitrogen from the gas stream.

The remaining moisture in the gas stream is removed by the guard bulb, J, which is filled with a 3-cm. layer of phosphorus pentoxide–silicon carbide mixture (1 + 3 mixture by weight) held in place with glass wool plugs followed by a 3-cm. layer of Drierite and another glass wool plug. J is closely fitted with a circular asbestos and aluminum shield, which protects it from the heat given off by I. The weighing bulb, K, is filled with a 1-cm. layer of Drierite held in place with glass wool plugs followed by a 6-cm. layer of Ascarite and a glass wool plug. K is protected from the heat given off by the electric furnace and I by another arc-shaped asbestos and aluminum shield. The second guard bulb, L, which protects K against back diffusion of atmospheric moisture and carbon dioxide, is filled with successive 2-cm. layers of Drierite, Ascarite, and Drierite, each separated with glass wool plugs. J, K, and L are then connected with $^3/_4$-inch lengths of Tygon plastic tubing of $^3/_{16}$-inch bore and $^1/_{16}$-inch wall thickness.

About 50 ml. of mercury are introduced into the leveling bulb, N, which is attached to the lower outlet of M with a 12-inch length of heavy rubber tubing. The mercury spill, M, safety trap, O, and absorption bulb, L, are then connected as shown in Figure 1.

All the joints, stoppers, and stopcocks, with the exception of stopcocks 1 and 2 and the inner joint of the water condenser, are well lubricated with stopcock grease. The halogenated polychlorotrifluoroethylene Halocarbon stopcock grease, supplied by Halocarbon Products Corp., 2012 88th St., North Bergen, N. J., has been found to be superior to all other types of stopcock greases tried for this purpose. The higher temperature grade grease, 85 to 220° F. range, is preferred for use in this method, because its heavier texture permits a more permanent lubrication.

Before the apparatus is placed in service, it must be checked for leaks, and the new reagents with the exception of Ascarite in absorbers, K and L, conditioned to saturate them with carbon dioxide. During conditioning a piece of glass tubing is used temporarily in place of absorbers, K and L. The suction rate is regulated primarily by the depth of mercury in M and should not be higher than 5 bubbles per second through G when stopcock 1 is fully open. The plug of stopcock 1 has a tapered groove to permit more sensitive regulation of air suction.

PROCEDURE

At the beginning of the analysis, the temperature of the absorption tube, I, and of the electric furnace for the copper oxide tube, F, are brought up to 190° ± 5° C. and 750° ± 10° C., respectively. These temperatures are maintained throughout the analysis. The halogen scrubbers, D and E, are installed if the sample to be analyzed contains appreciable amounts of halides; otherwise only the spray trap, P, is used. Stopcocks 1 and 2, as well as the inner joint of the condenser, are lubricated with a few drops of orthophosphoric acid lubricant (85% orthophosphoric acid saturated with metaphosphoric acid) and the funnel above stopcock 2 is partly filled with concentrated sulfuric acid. The apparatus is opened to the source of a controlled vacuum and swept out with carbon dioxide–free air at the rate of 2 or 3 bubbles per second through G for 5 minutes. After the sweep-out, the vacuum in the apparatus is released, the weighing bulb, K, is removed, weighed to ±0.0001 gram, and replaced.
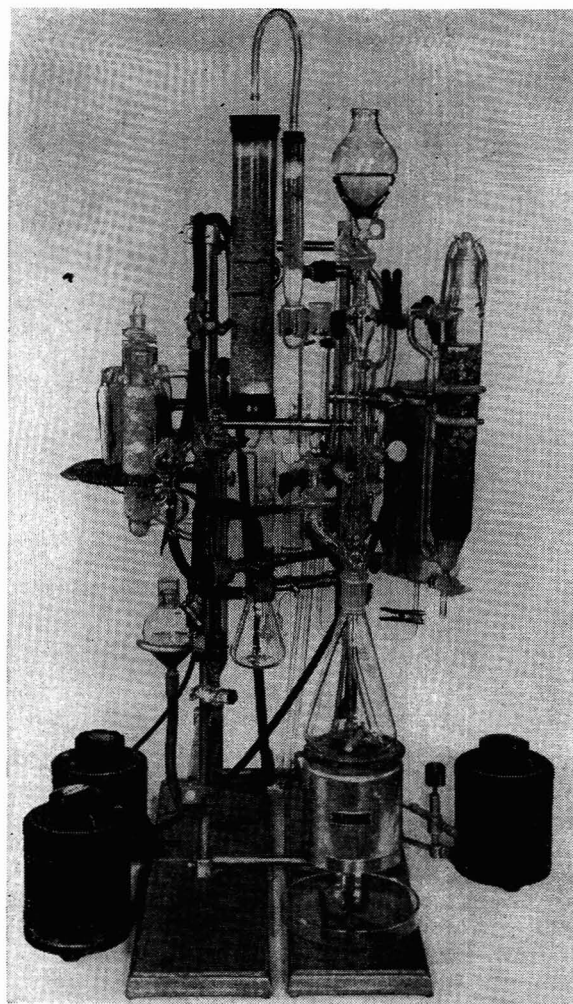

Figure 2. Compact arrangement of apparatus

A sample containing organic material equivalent to 1 to 150 mg. of carbon dioxide and not more than 100 ml. in volume is weighed or pipetted into a clean Erlenmeyer flask containing one or two boiling granules and a Teflon (Du Pont Co.) tetrafluoroethylene resin covered magnetic stirring bar. Samples of low density solids are weighed into a microbeaker which is wrapped in 5-cm. platinum gauze squares (80 mesh) to prevent floating of the sample. The flask is washed down with a few milliliters of water, and if necessary, more water is added to obtain a minimum liquid volume of about 25 ml.

The Erlenmeyer flask used in the sweep-out is replaced by the flask containing the sample, and vacuum is applied to the apparatus, keeping stopcock 1 closed. An amount of chromic acid solution (50% aqueous chromic anhydride) is drawn into the flask through stopcock 1 and washed down twice with 2-ml. portions of water. Usually, 10 ml. of acid solution is satisfactory for solid and aqueous samples of less than 50 ml., while 15 ml. is needed for aqueous samples of 50 to 100 ml. If the sample contains any volatile material, the apparatus is first flushed with carbon dioxide–free air for 10 minutes. Then the flask contents are stirred and the concentrated sulfuric acid in the funnel is allowed to drip slowly down the condenser spiral into the flask.

An amount of concentrated sulfuric acid as shown is slowly added to the flask through stopcock 1.

Sample Size	Acid Volume, Ml.
1 to 25 ml.	50
25 to 50 ml.	75
50 to 100 ml.	150
All solid samples	50
50-ml. samples or diluted to 50 ml. containing not more than 1 gram of halides	75
Samples containing both high halides and low carbon	Volume equal to sample volume, with a minimum volume of 50 ml. of acid

The flask contents are thoroughly mixed, and the mixture is boiled gently and steadily for 10 minutes. Then the apparatus

is flushed with carbon dioxide–free air at the rate of 2 or 3 bubbles per second through G for 15 minutes. After the sweep-out, the vacuum is then released, and absorption tube, K, is weighed. The increase in weight of the absorption tube is equal to the carbon dioxide evolved from the sample plus blank.

At least one blank is run substituting an equal volume of carbon-dioxide–free distilled water for the sample and the same volumes of reagents as used for oxidation of the sample.

For consecutive analyses on the same day, the preliminary 5-minute sweep-out step may be eliminated after the first determination.

Dissolved carbon dioxide, carbonate, and bicarbonate ions interfere if samples are analyzed specifically for organic materials, as carbon dioxide is released in acid solution. In the absence of low-boiling materials, dissolved carbon dioxide can be removed by aspiration. The total of these materials can be determined quantitatively by displacement with hot dilute sulfuric acid and aspiration. In most cases they can be precipitated with barium hydroxide solution.

Absolute blank values vary not only with the amount of reagents used and dissolved carbon dioxide present, but also depend on the correct handling of absorber, K, and the efficiency of the moisture absorbers. The blank is in the range from less than 0.5 mg. to a maximum of 2.5 mg. of carbon dioxide. During a single day using the same lots of reagents, however, the absolute value of the blanks vary by no more than ±0.2 mg. of carbon dioxide.

The per cent of total carbon is calculated as follows:

$$\% \text{ carbon} = \frac{(A - \text{blank}) \times 0.2729}{B} \times 100$$

where A = weight of total carbon dioxide collected for sample and B = weight of sample

DISCUSSION

In early analyses by the usual wet combustion procedure the authors observed that low recoveries were obtained from low-boiling compounds and from steam-distillable materials. Further studies indicated that quantitative analyses for carbon in volatile materials could be made by introducing into the train a dry combustion unit containing cupric oxide at 750° C., and aerating aqueous samples prior to addition of the wet oxidation reagent. If a sample which contained volatile materials was not aerated, addition of the acid to the aqueous solution released sufficient heat to evolve the low-boiling components so rapidly that they were not completely burned in the copper oxide tube. This is illustrated in Table I.

Steam-distilled materials were observed to collect in the condenser above the gently boiling wet oxidation mixture. Drop-wise addition of concentrated sulfuric acid down a spiral inserted into the condenser was found effective. The acid continuously washed the unreacted organic matter back into the wet combustion mixture until oxidation was complete. The effect of counter-current addition on analyses for small amounts of dichlorobutene is shown in Table II.

After the new wet-dry combustion unit was completed with provision for removing halogens, the precision and accuracy of the method were determined on dextrose in the absence and presence of sodium chloride. For the former, 50-mg. samples of Bureau of Standards dextrose, weighed on a semimicrobalance, were dissolved in 25 ml. of water. Analyses were made using the combustion apparatus, Figure 1, equipped with spray trap, P, in place of halogen scrubbers, D and E. For the latter, 50-mg. samples of dextrose were dissolved in 50 ml. of 3% sodium chloride solution and the apparatus was equipped with halogen scrubbers, D and E. In both series a macrobalance, sensitive to 0.1 mg., was used in weighing the carbon dioxide absorption bulbs. Results are given in Table III.

These data indicate that accuracy of the determination is unaffected by sodium chloride or by the halogen scrubbers. Precision, however, appears to be somewhat better with salt-free samples in the apparatus equipped with a simple spray trap in place of the halogen scrubbers. This may be due to the slightly higher blanks observed with the latter system.

Several organic compounds were analyzed by the new procedure. Usually 50 to 100 mg. of sample were dispersed in 25 to 50 ml. of water and then analyzed for carbon. Results are given in Table IV.

A variety of aqueous samples containing small concentrations of organic matter also were analyzed. Results are given in Table V. The sample size indicated in column 2 is the weight of the aqueous sample taken.

Table I. Effect of Preliminary Aeration of Volatile Materials

Compound	Mg. in Sample	Recovery, % Not aerated	Recovery, % Aerated
Ethyl ether	51	85	99
	255	73	101
Cyclohexane	25	91	98
	56	87	99

Table II. Effect of Countercurrent Addition of Sulfuric Acid

Dichlorobutene Added, Mg.	Recovery, % Without H_2SO_4	Recovery, % With H_2SO_4
8.3	88	100
5 to 5.5	81	100 to 107
1 to 2.6	69, 65	105

Table III. Analytical Data for Carbon in Dextrose

	A^a	B^b
Carbon, wt. %, calcd.	40.00	40.00
No detn.	18	24
X	39.98	39.98
Std. dev., σ	0.09	0.12

^{a} A = 50-mg. sample in 25 ml. of H_2O.
^{b} B = 50-mg. sample in 50 ml. of 3% NaCl soln.

Table IV. Analytical Data for Carbon in Organic Compounds

Compound	Sample Wt., Mg.	Carbon, Wt. % Calcd.	Carbon, Wt. % Found
Acetic acid	100, 50, 100	40.0	40.7, 39.4, 38.3
n-Butyric acid	35	54.5	54.7, 54.5
Cyclohexanol	42, 37	71.9	71.7, 70.3
Dichlorobutene	42, 77, 66	38.4	38.4, 38.7, 38.3
Cystine	80	30.0	29.7, 30.0
Benzoic acid	50, 32	68.8	69.0, 68.7
p-Cymene	94^a, 28, 47	89.5	89.4, 88.6, 89.9
Acetanilide	50	71.1	70.9, 71.4
Benzyl isothiourea hydrochloride	60	47.4	47.2, 47.2

^{a} Because of large amount of CO_2 evolved (ca. 300 mg.), the unit was aspirated an additional 15 minutes.

Table V. Analytical Data for Carbon in Aqueous Solutions

Material	Sample Size, G.	Carbon, P.P.M. Calcd.	Carbon, P.P.M. Found
Acetic acid	10	420	440
Stearic acid	100	167	161
		295	285
		350	346
Glycine	100	67	67, 71
Cycloheptane	10	3400	3200
Benzene	100	103	102
		110	109
Dextrose	100	27.4	27.0, 27.3
		13.6	13.9, 13.9
		5.5	5.7, 6.3
Ethyl ether	5	3300	3200
n-Butylamine	100	66	62
Benzylamine	100	100	98, 100
Brine soln., 15 to 20% NaCl	4	Unknown	2000, 1900
	5, 28	Unknown	660, 600
Brine soln., 2% NaCl	8	Unknown	3400, 3400
Aqueous waste	50	Unknown	9, 9
			40, 37
Sewer water	50	Unknown	235, 235

The data shown in Tables I through V demonstrate that this new wet-dry combustion method is suitable for the determination of total carbon in aqueous systems containing a wide variety of organic materials. The method can be used over a considerable range of carbon concentrations and is applicable for analyses of numerous industrial products as well as waste streams.

In the course of this investigation two compounds were studied which failed to react quantitatively. Tribromobenzene tended to steam distill and precipitate on the sides of the condenser where the countercurrent stream of sulfuric acid did not contact it. Only about a 50% recovery was obtained. A slight change in design of the condenser system will overcome this problem. For compounds of relatively high melting point, which steam distill, a baffle is necessary to force the sulfuric acid down the sides of the condenser. In this way the solid is washed back into the wet oxidation mixture. Pyridine reacted only partially. This material is quite stable. Only about a 20% recovery of carbon was observed after as much as 30 minutes of boiling. Therefore, several hours of boiling may be necessary before pyridine and similar compounds are oxidized completely.

The efficiency of moisture absorbers preceding the weighing bulb, K, Figure 1, was found to have influence on the magnitude and constancy of the blanks obtained by this method. Calcium sulfate was not a suitable predesiccant. Although not as desirable from a physical standpoint, phosphorus pentoxide was chosen over the more dangerous, but more efficient magnesium perchlorate.

The frequency in refilling absorber, J, with fresh reagents is low if the acid scrubber, G, is charged with fresh concentrated sulfuric acid after about five analyses when the halogen scrubbers are employed. The sulfuric acid should last for at least 25 analyses when only the spray trap, P, is used.

A sufficiently high concentration of acid in solution permits steady boiling without any danger of bumping and loss of sample. However, when samples contain large amounts of halides, trouble may be encountered, because treatment with concentrated sulfuric acid sometimes forms chromyl chloride which quickly exhausts the halogen scrubbers and plugs the copper oxide tube. The evolution of chromyl chloride can be prevented by reducing

the effective salt concentration by high dilution with water. A chloride content of not more than 1 gram per 50 ml. of sample or diluted sample gives satisfactory results, using 75 ml. of concentrated sulfuric acid. Samples containing both high chlorides and low carbon, which necessitate large sample volumes, can be run at the expense of exhausting the scrubbers and eventual plugging the copper oxide tube. In such samples, the evolution of chromyl chloride can be reduced by limiting the amount of sulfuric acid to not more than 1 volume of acid to 1 volume of sample, with a minimum volume of 50 ml. of acid.

The wet-dry combustion procedure has been used successfully for the determination of total carbon in a number of polymeric materials. Polyamides, acrylates, and cellulose acetate gave quantitative results by the standard procedure. Other polymers required several hours boiling of the wet combustion mixture before oxidation was complete.

The method measures total carbon in the sample—i.e., organic matter, carbonates, and carbon dioxide. Where only the organic components are desired, a separate determination can be made for carbonates. This is made conveniently by simple displacement from dilute mineral acid solution, after which the evolved carbon dioxide is absorbed on Ascarite and weighed. Kieselbach (3) removed carbonates by reaction with barium hydroxide followed by filtration in his continuous organic compound analyzer patterned after the laboratory technique described.

LITERATURE CITED

(1) Archer, E. E., *Analyst*, **79**, 30 (1954).
(2) Gertner, A., and Ivekovic, H., *Z. anal. Chem.*, **142**, 36 (1954).
(3) Kieselbach, R., ANAL. CHEM., **26**, 1312 (1954).
(4) Love, S. K., and Thatcher, L. L., *Ibid.*, **24**, 294 (1952); **25**, 65 (1953).
(5) Moore, W. A., Ludzack, F. J., and Ruchhoft, C. C., *Ibid.*, **23**, 1297 (1951).
(6) Oemler, A. N., and Mitchell, J., Jr., "A Universal Combustion System," Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., March 1952.
(7) Schulz, N. F., ANAL. CHEM., **25**, 1762 (1953).
(8) Van Slyke, D. D., *Ibid.*, **26**, 1706 (1954).

Gaseous Phase of Cigarette Smoke
Isolation and Analysis for Total Aldehydes

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A simple method for isolating smoke gases from the liquid-solid components during the automatic smoking of cigarettes was developed. The isolation was accomplished by using a specially designed Cambridge filter behind the cigarettes on a smoking apparatus. This filter removed over 99% of the liquid-solid phase of the smoke and allowed the gases to pass through into absorption flasks for analysis. A method for analyzing cigarette smoke gases for total aldehyde content also was developed. The analysis was accomplished by reaction of the aldehydes with dimedon (5,5-dimethyl-1,3-cyclohexanedione) in the gas-absorption flasks to form their bis(dimedon) derivatives as the precipitate. The method was found to be highly efficient for recovering aldehydes of low molecular weight from known solutions. The "apparent acetaldehyde" content of the smoke gases from four brands of cigarettes was found to be 8 to 9 mg. per 10 cigarettes, or 1.9 to 2.2 mg. per liter of smoke gases.

THIS report concerns a simple technique for isolating the gaseous phase of cigarette smoke. It also offers a method for determining the total aldehyde content of the gaseous phase.

King-size cigarettes were smoked by an automatic smoking machine which pulled the smoke through a specially designed filter (4) and a gas-absorption train. The filter removed the liquid-solid phase of the smoke while the absorption train removed the aldehyde vapors by reaction with dimedon (5,5-dimethyl-1,3-cyclohexanedione.)

The volatile aldehydes of the gaseous phase of cigarette smoke were considered first for analysis, because their presence in tobacco smoke has been frequently mentioned in the literature (1, 2, 5, 7, 11). However, little quantitative information on the subject is available.

Dimedon was chosen as the aldehyde reagent, because under the conditions selected it does not react with ketones, which are also present in the gaseous phase of cigarette smoke (7, 8, 11).

SELECTION OF CIGARETTES FOR SMOKING

Typical American brands of unfiltered, king-size cigarettes were used in the smoking tests. They were conditioned to $12 \pm 0.5\%$.
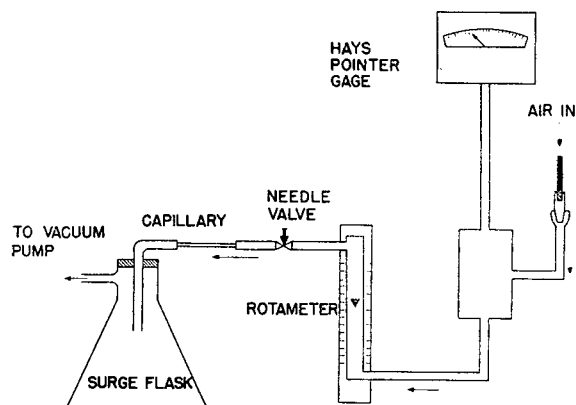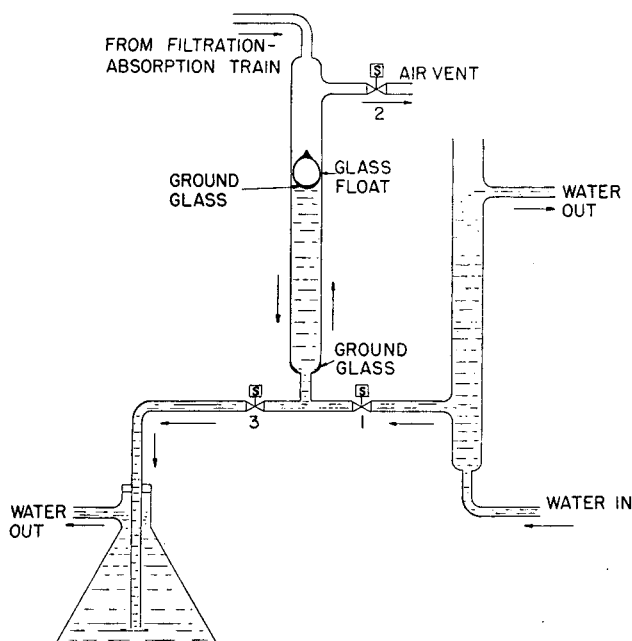
Figure 1. Pressure-drop apparatus

Figure 2. Puffing machine

moisture content and selected on the basis of pressure drop (resistance to air flow) and weight.

Two hundred cigarettes of each of the four brands investigated were conditioned to 12% moisture content and weighed, and their average weight was calculated. Each cigarette was then separately weighed and only those cigarettes weighing within 2% of the average weight of the 200 cigarettes were selected for further screening.

The cigarettes having the proper weight were then tested for pressure drop at an air flow rate of 1050 ml. per minute using a Hays pointer gage calibrated in inches of water (see Figure 1). The average pressure drop of the cigarettes was then calculated. Those cigarettes having a pressure drop within 4% of the average pressure drop of the lot were chosen to represent the brand of cigarette being tested.

Table I. Properties of Standardized Cigarettes

Brand	Av. Wt., G.	Av. Moisture Content, %	Av. Pressure Drop, Inches
A	1.224	11.8	2.3
B	1.211	12.1	3.0
C	1.208	12.2	3.1
D	1.256	11.7	3.2

The cigarettes, thus screened for uniformity in weight and pressure drop, were designated as "standardized" cigarettes. They were stored at 55 to 60% relative humidity at 75° F. until they were smoked.

Table I lists the average weight, moisture content, and pressure drop of the four brands of cigarettes tested.

APPARATUS FOR ISOLATION AND ANALYSIS OF SMOKE GASES

The smoking experiments were carried out in an air-conditioned room maintained at a relative humidity of 53 to 58% at 75° F. The apparatus for the collection and analysis of the smoke gases for aldehydes consisted of three parts: An automatic smoking machine for regulating the volume and rate at which the cigarettes were smoked and for recording the number of puffs taken.

A filter for removing the liquid-solid phase of the smoke as it leaves the butt end of the cigarette.

Two gas-absorption flasks containing a reagent for removing the aldehydes in the gaseous phase of the smoke.

Puffing Machine. The automatic puffing machine employed for pulling the smoke through the filter and absorption flasks was similar in operation to the machine described by Bradford, Harlan, and Hanmer (*3*). A diagram of the machine is shown in Figure 2. It pulled a 35-ml. puff of a 2-second duration through the system at the rate of one puff a minute. The suction for the puff was created by a falling 35-ml. column of water in a buret and was terminated when a glass float in the water column seated in a ground-glass constriction in the bottom of the buret. A constant-level reservoir refilled the buret, and a series of solenoid valves, properly timed, closed and opened the water lines to and from the buret.

Filter for Removing the Liquid-Solid Phase of Smoke. The filter for removing the liquid-solid phase of the smoke consisted of a sheet of Cambridge filter material $1/16$ inch thick inserted in glass funnels directly behind the cigarette (*4*). This is shown in Figure 3. It was prepared by cutting the feltlike material into the form of a disk 2.5 inches in diameter and sealing the disk between the glass funnels with a wrapping of 0.75-inch elastic tape (Scotch tape No. 33). The funnels were also 2.5 inches in diameter with stems 0.25 inch in diameter and 1.5 inches in length. The funnel stems terminated in glass socket connections, which held the cigarette holder on one side of the filter and sealed into the gas absorption flask on the other side.
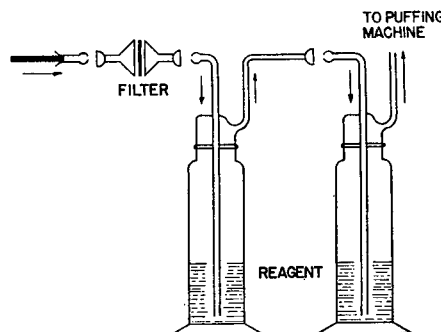

Figure 3. Filtration-absorption train

The Cambridge filter mat, which is a soft, specially treated paper impregnated with fine asbestos fibers, is reported to be capable of removing particles in the range of 0.1 to 1.0 micron at extremely low pressure drops. Previous work with the filter indicated that it removed over 99% of the liquid-solid phase of cigarette smoke as it left the butt end of a cigarette under normal smoking conditions. As used in the apparatus, the filter had a pressure drop of only 0.2 inch of water.

Gas-Absorption Flasks. The two gas-absorption flasks, shown in Figure 3, were placed on the apparatus in series and directly behind the Cambridge filter. They were standard-type scrubbing flasks, 8 inches tall and 1.5 inches in diameter with 29/40 standard taper joints and with inlet tubes extending to within $1/8$ inch of the bottom. Glass ball-and-socket joints were employed for sealing the flasks to each other and to the Cambridge filter; the usual pinch clamps completed the seal.

Before the cigarettes were smoked, 35 ml. of a 0.4% aqueous dimedon solution having a pH of 5.8 was added to each flask and the flasks were then immersed in an ice bath. This amount of cold reagent was sufficient to trap quantitatively up to 35 mg. of formaldehyde, acetaldehyde, or propionaldehyde when such

amounts of the vapors were pulled through the system by the puffing machine.

DIMEDON REAGENT FOR ALDEHYDE DETERMINATION

The 0.4% aqueous solution of dimedon (5,5-dimethyl-1,3-cyclohexanedione) in the absorption flasks was prepared by dissolving 4.0 grams of the reagent grade material in 1 liter of a solution composed of 1 volume of $0.3M$ acetic acid and 17.3 volumes of $0.3M$ sodium acetate. The pH of the reagent solution was 5.8.

Dimedon has been frequently reported as a reagent for the gravimetric determination of aldehydes of low molecular weight under various conditions (*3, 10, 12*). Gaffney, Williams, and McKennis recently studied the reaction of acetaldehyde with the reagent at various pH levels (*6*). At pH 5 to 6, a saturated (0.4%) solution of dimedon absorbed acetaldehyde rapidly and quantitatively from a mixture of gases. They also noted that by acidification of the resulting solution to pH 3.9 high yields of the bis-(dimedon) derivative of acetaldehyde were obtained.

Tests with dilute solutions of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and isobutyraldehyde of known concentration indicated that the dimedon reagent formed their insoluble bis(dimedon) derivatives in high yields. The technique employed in analyzing these known solutions was similar to that used in analyzing the collected smoke gases:

A 3.0-ml. sample of the solution containing 11 to 12 mg. of the aldehyde was pipetted into 60 ml. of the cold (5° C.) dimedon solution. This mixture was stored in an open 250-ml. Erlenmeyer flask in an ice bath for 2 hours, diluted with an equal volume of water, and adjusted to pH 3.9 with dilute hydrochloric acid. Upon acidification, the bis(dimedon) derivative precipitated. This mixture was allowed to stand at room temperature for 24 hours. The precipitate was then collected on a tared, sintered-glass crucible, dried at 100° C. for 30 minutes, cooled over calcium chloride, and weighed. The efficiency of the analytical procedure is shown in Table II.

SMOKING PROCEDURE

The two gas-absorption flasks, each containing 35 ml. of the 0.4% buffered dimedon solution at pH 5.8, were placed in the smoking train directly behind the Cambridge filter. Ice was packed around the flasks and the smoking machine was standardized for a 35-ml. puff of a 2-second duration. After five puffs of air were pulled through the smoking train, the puff counter was adjusted to zero. The apparatus was then ready for operation.

The smoking of 10 cigarettes to butt lengths of 35 mm. constituted a run. Duplicate runs were made on all the brands tested. At the completion of each run of 10 cigarettes, the reading on the puff counter was recorded and the system was purged with 30 puffs of air. After the purging operation, the puffing mechanism was stopped and the absorption flasks were removed. The apparatus was then reassembled with a fresh filter and another set of absorption flasks for the next run.

DETERMINATION OF ALDEHYDES IN SMOKE GASES

Procedure. The solution in the second absorption flask was washed into the first flask, and the total volume was brought to 100 ml. with water. This solution was adjusted to pH 3.9 with 10% hydrochloric acid and allowed to stand 24 hours at room temperature. The pH adjustment was accomplished by adding 5 drops of bromophenol blue indicator to the solution and slowly adding the acid until the deep purple color of the solution turned to pale green. A blank solution of 70 ml. of the dimedon solution, 25 ml. of water, and 5 drops of indicator, adjusted to pH 3.9 with a Beckman pH meter, served as the color standard in the operation.

After the precipitate had remained 24 hours in the solution at pH 3.9, it was filtered onto a tared, sintered-glass crucible and washed with 30 ml. of water. The crucible was then dried at 100° C. for 30 minutes, cooled over calcium chloride, and reweighed.

In transferring the precipitate from the absorption flask to the crucible, the filtrate obtained during the operation was used in place of water for washing out the flask. This precaution minimized the possibility of losing traces of the precipitate by solution in water.
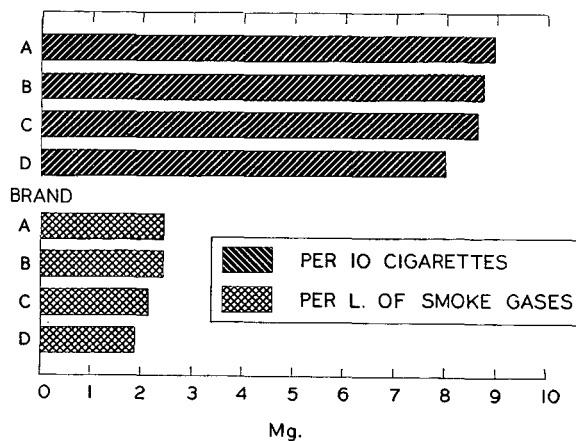

Figure 4. Apparent acetaldehyde in smoke gases

Table II. Determination of Aldehydes in Known Solutions

Aldehyde	Present, Mg.	Found, Mg.	Recovery, %
Formaldehyde	11.9	11.8	99.5
Acetaldehyde	12.6	12.4	98.5
Propionaldehyde	11.4	10.4	91.1
Butyraldehyde	6.9	5.9	85.5
Isobutyraldehyde	11.6	9.3	80.0

Calculations. The amount of aldehyde present in the smoke gases was calculated as "apparent acetaldehyde." This basis was chosen because a study of the structure of the bis(dimedon) precipitates indicated that they were derived mainly from acetaldehyde.

The milligrams of apparent acetaldehyde was found by multiplying the milligrams of precipitate by 0.1438, the factor representing the molecular weight ratio of acetaldehyde to its corresponding bis(dimedon) derivative.

The milligrams of apparent acetaldehyde per liter of smoke gases for each run was calculated as follows:

$$\frac{\text{Mg. apparent acetaldehyde}}{\text{per liter of smoke gases}} = \frac{\text{mg. of ppt.} \times 0.1438}{\text{total number of puffs} \times 35} \times 1000$$

Results. The results of the tests are summarized in the bar chart of Figure 4. The precision was within 2% of the values shown. At the top, the milligrams of apparent acetaldehyde in the smoke from ten king-size cigarettes of the four brands tested is shown. At the bottom, these values are presented as milligrams of apparent acetaldehyde per liter of smoke gases.

The apparent acetaldehyde content of the smoke gases from four brands of cigarettes was found to be 8 to 9 mg. per 10 cigarettes, or 1.9 to 2.2 mg. per liter of smoke gases.

REFERENCES

(1) Bailey, C. F., and Petre, A. W., *Ind. Eng. Chem.*, **29**, 11–9 (1937).
(2) Bogen, E., *J. Am. Med. Assoc.*, **93**, 1110–4 (1929).
(3) Bradford, J. A., Harlan, W. R., and Hanmer, H. R., *Ind. Eng. Chem.*, **28**, 836–9 (1936).
(4) Cambridge Corp., Syracuse, N. Y., "Cambridge Absolute Filter," A.I.A. Nos. 35-K-1 and 30-D-3, 1952.
(5) Derr, R. B., Reismeyer, A. H., and Unangst, R. B., *Ind. Eng. Chem.*, **29**, 771–6 (1937).
(6) Gaffney, G. W., Williams, W. H., and McKennis, H., Jr., ANAL. CHEM., **26**, 588–9 (1954).
(7) Neuberg, C., and Burkhard, J., *Biochem. Z.*, **243**, 472–84 (1931).
(8) Schmalfuss, H., and Barthmeyer, H., *Z. Untersuch. Lebensm.*, **63**, 283–8 (1932).
(9) Vorlander, D., *Z. anal. Chem.*, **77**, 241 (1929).
(10) Walker, J. F., "Formaldehyde," pp. 263–6, Reinhold, New York, 1944.
(11) Wenusch, A., *Österr. Chem.-Ztg.*, **42**, 226–31 (1939).
(12) Yoe, J. H., and Reed, L. C., IND. ENG. CHEM., ANAL. ED., **13**, 238 (1941).

Factors Influencing Quantitative Determination of Methylpentoses and Ketohexoses with Anthrone

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This work was carried out to determine why published anthrone methods fail to yield reproducible results when applied to methylpentoses and ketohexoses. One important reason is the assumption that 100° C. is optimum for developing the anthrone color of any carbohydrate; the optimum temperature for developing methylpentose and ketohexose color was found to be 70° and 60° C., respectively. Optimum heating temperature appears to be more characteristic of a given type of monosaccharide than the optimum heating time. Furthermore, the reagent solutions and the developed color of methylpentoses and ketohexoses are much less stable than those of aldohexoses. Therefore, the time factor is much more critical in the present application than in most previous ones. However, by controlling time and temperature within indicated tolerances, methylpentoses and ketohexoses can be estimated with a precision comparable to that reported for glucose.

THIS study arose from efforts to ascertain why quantitative anthrone methods, devised primarily for glucose, do not yield reproducible results when applied to methylpentoses and ketohexoses. The answer to this problem centers chiefly about two factors: temperature and stability.

The importance of temperature as a factor in the anthrone-carbohydrate color reaction has been indicated increasingly in the literature. Morse (9) in 1947 observed that the anthrone-carbohydrate color is sensitive to temperature; and although he made no recommendations for controlling this factor, later investigators did. Morris (8) recommended a particular manner of mixing the reactants as well as the use of test tubes of uniform bore. Viles and Silverman (17) introduced the idea of stopping the color reaction after a suitable time interval by immersing the reaction mixture in a cold-water bath. Rather than attempt to control the heat of mixing Seifter, Seymour, Novic, and Muntwyler (13) and Trevelyan and Harrison (16) chose to minimize it by chilling either the sample solution or the anthrone reagent prior to mixing the two; full color was then developed by heating the reaction mixture in a boiling-water bath. Finally, the heat of mixing was completely eliminated by Black (1), who used 60% (by volume) sulfuric acid as solvent for both anthrone and solid carbohydrate sample. In this case the color was developed entirely by heat from a controlled source—viz., a boiling-water bath.

With the development of quantitative methods which minimized or eliminated the heat of mixing, the study of the influence of the duration of applied heat upon color intensity became both possible and necessary. McCready, Guggolz, Silviera, and Owens (7) published time-intensity data for glucose, while Black (1) did so for cellulose and carboxymethylcellulose. The most comprehensive work in this regard is that of Koehler (6), who published data for a number of the more common classes of monosaccharides as well as for some related polymers and derivatives. Finally, Scott and Melvin (12) published time-intensity data for glucose at several temperatures between 80° and 100° C., thus introducing consideration of the influence of temperature intensity—a matter which had been largely neglected. In this investigation the influence of temperature intensity upon the anthrone color of methylpentoses and keto-

hexoses has been examined. The results were of such interest that this portion of the study was extended to aldohexoses, pentoses, and uronic acids.

Both the reagent solutions and the developed anthrone-carbohydrate color were found to be rather unstable. This lack of reagent and color stability is one of the largest potential sources of variation in the method, as applied to methylpentoses and ketohexoses. However, by suitably standardizing the conditions of time and temperature, it has been possible to elaborate a quantitative method with a useful degree of precision.

MATERIALS AND APPARATUS

Sulfuric Acid. Commercially available c.p. reagent was diluted with water to $27.5 \pm 0.1N$. This acid concentration was employed for most of the determinations reported in this paper.

The cap liner employed by some manufacturers gives a trace anthrone test for carbohydrates. When this source of contamination is present, anthrone blanks are variable, particularly at higher temperatures (80° to 100° C.). In this laboratory the concentrated acid is routinely transferred to glass-stoppered bottles.

Anthrone. About 100 grams of this material was prepared according to a published method (3); two recrystallizations yielded a substance with a melting point range of 155.0° to 157.0° C. (anthrone-M). One 25-gram specimen from each of two commercial sources was also used. The melting point ranges of these materials were as follows: Eastman Organic Chemicals (white label), 156.0° to 158.0° C. (anthrone-E); Nutritional Biochemicals Corp., 156.5° to 158.0° C. (anthrone-NBC).

The anthrone solutions were prepared by dissolving 0.160 gram of anthrone in 100 ml. of $27.5N$ sulfuric acid. Such solutions were usually made up 1 to 2 hours prior to use. If, however, the reagent was kept for longer periods, it was stored in the dark, because Schönberg, Mustafa, and Zayed (10, 11) have observed that bianthrone, which is thermochromic, is produced by a photochemical reaction in anthrone solutions.

Carbohydrates. The various monosaccharides employed in this study were from commercial sources and agreed well in optical rotation with published values. The fucose was chromatographically pure.

Carbohydrate solutions were prepared by dissolving a quantity of solid sample in $27.5N$ sulfuric acid so that each milliliter of solution contained either 50 or 100 γ of carbohydrate. The time required to effect solution varied from 1 to 20 minutes, and no readily measurable amount of heat was evolved.

Apparatus. Test samples were heated in a thermostatically controlled, circulating water bath. At and below 70° C. the temperature was constant within less than $\pm0.1°$ C.; above 70° C. it was constant within $\pm0.2°$ C. Test samples were cooled in a cold-water bath maintained at $4° \pm 1°$ C.

Wire racks were used to hold the test tubes (150 × 20 mm. outside diameter, 1 mm. wall) containing the test solutions in the heating and cooling baths. The racks were so constructed that each test tube was held at least 1 cm. from any other. At temperatures above 50° C., it was found necessary for uniform heating to use only alternate rows of these racks. Mohr-type pipets, to deliver 5 ml., were used. The rate of free flow of $27.5N$ sulfuric acid was about 1 ml. per second.

All measurements of absorbance were made at $23° \pm 2°$ C. on a Beckman spectrophotometer, Model DU, using 1-cm. Corex cells. The solvent acid was used as the reference standard.

PROCEDURE

Four milliliters of anthrone reagent were pipetted into each test tube, followed by 2 ml. of the carbohydrate solution. If less than 2 ml. of the carbohydrate solution were used, $27.5N$ sulfuric acid was added to make a final volume of 6 ml. Since methylpentoses and especially ketohexoses developed appreciable amounts of color with anthrone at room temperature, carbohydrate solutions were added last in order to minimize color de-

velopment prior to heating. Carbohydrate solutions were aged 30 to 60 minutes and anthrone solutions 1 to 2 hours prior to use.

Heating time was measured to ±2 seconds. After heating, the test samples were immediately placed in the cold-water bath for 3 minutes, then allowed to equilibrate at room temperature for 20 to 25 minutes. Spectral measurements were made promptly thereafter and were completed within 30 minutes.

EXPERIMENTAL

Selection of Wave Length for Measuring Anthrone-Mono-saccharide Absorbance. Figure 1 shows the absorbance spectra in the visible region for representative monosaccharides after two or three intervals of heating. The temperature employed was 97° C.; the concentration of monosaccharide in the reaction mix-

ture was 100 γ per 6 ml.; all other conditions were as indicated in the sections above on materials and procedure.

All of the principal maxima lie at or very near to 625 mμ. Consequently this wave length was selected for all subsequent absorbance readings. Although the maxima for arabinose and rhamnose deviate from 625 mμ, the curves are sufficiently broad so that no appreciable loss of sensitivity or precision results when readings are taken at this wave length. As heating time is increased, the rhamnose maximum migrates to 610 mμ. This hypsochromic shift was not observed with any of the other sugars examined.

Spectra, not reported here, of the anthrone-fructose color developed at 40° and 60° C. and the anthrone-rhamnose color

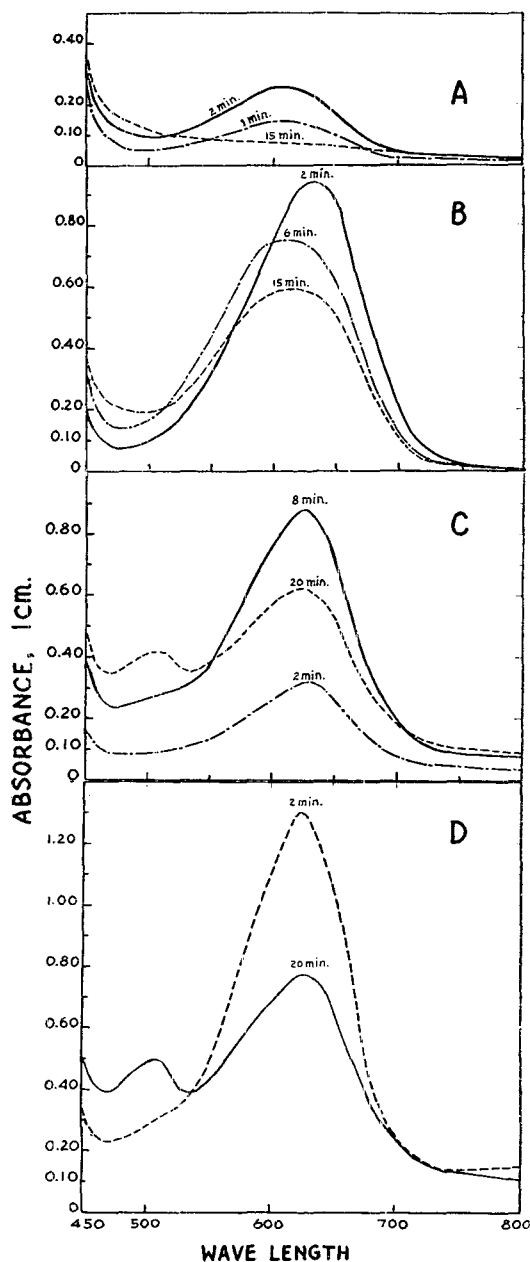Figure 1. Spectra developed by heating at 97° C.

A.   Anthrone-arabinose
B.   Anthrone-rhamnose
C.   Anthrone-glucose
D.   Anthrone-fructose

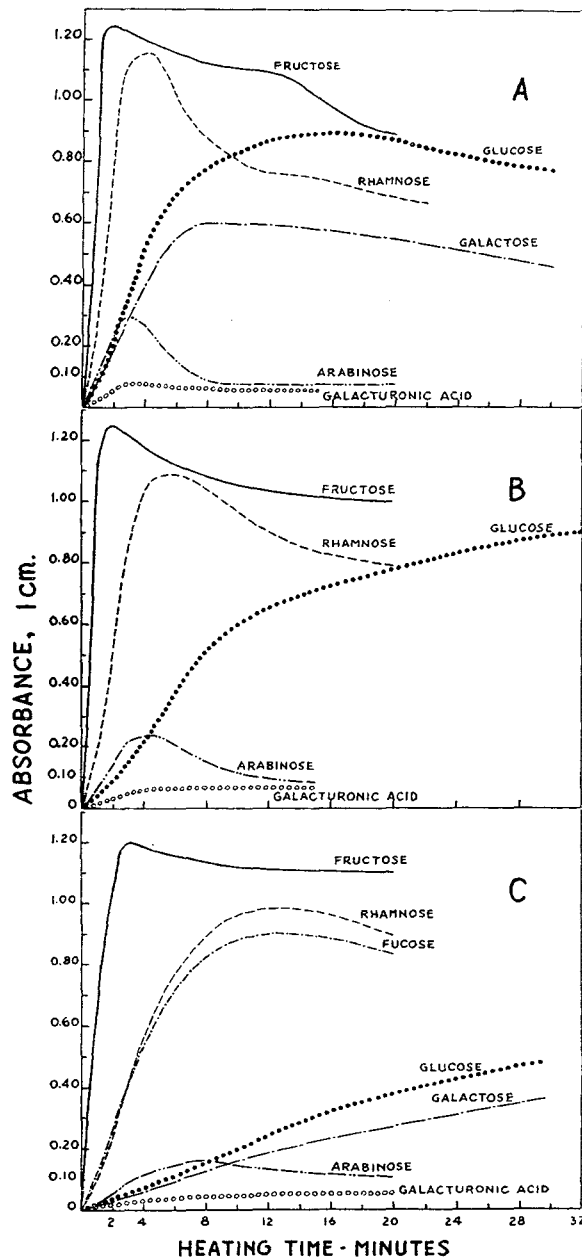

Figure 2. Influence of temperature on time vs. absorbance at 625 mμ

A.   90° C.
B.   80° C.
C.   70° C.

Monosaccharide concn. = 100 γ/6 ml. of reaction mixture

developed at 70° C. indicate that the wave length of maximum absorbance is independent of the temperature at which the color is developed.

Time-Absorbance Data from 90° to 30° C. The influence of temperature on the time-absorbance relationship of monosaccharides is delineated in Figures 2 through 4. The monosaccharide concentration employed in every case was 100 γ per 6 ml. of reaction mixture.

Observations were also made at 100° C., but they have been omitted inasmuch as the findings at this temperature chiefly confirm the observations of Koehler (6). In general, as the temperature is lowered from 100° C., the absorbance maxima of monosaccharides tend to diminish and to require a longer time
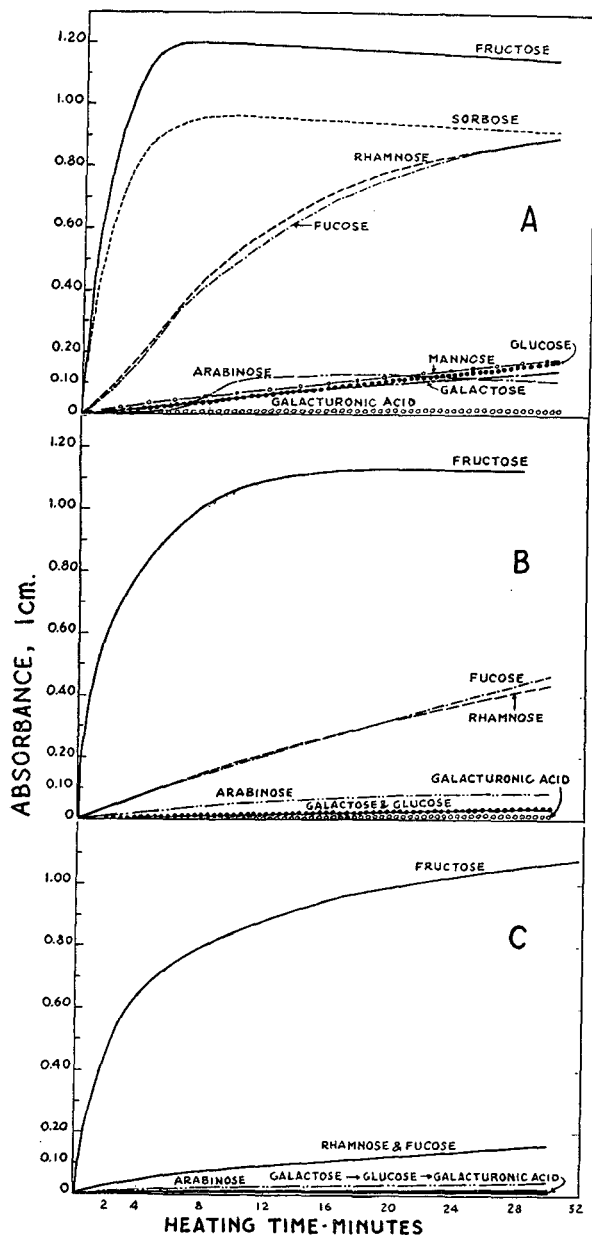
to develop. The behavior of rhamnose and fructose is somewhat exceptional, since the maximum absorbance of both of these sugars increases rather than decreases as the temperature is lowered from 100° to 90° C. Furthermore, as the temperature is lowered fructose suffers relatively little loss in maximum absorbance. At temperatures in the vicinity of 60° C. fructose, sorbose, and presumably ketohexoses in general, are again atypical; the time-absorbance curves rise to a maximum which is effectively sustained for heating times up to at least 30 minutes
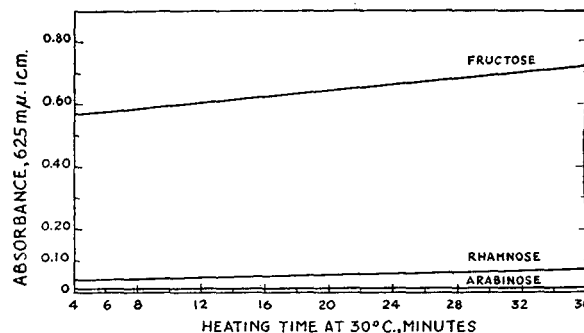

Figure 4. Time vs. absorbance at 30° C.

Monosaccharide concn. = 100 γ/6 ml. of reaction mixture

On the basis of the curves for α-D-galacturonic acid it seems reasonable to conclude that the anthrone method has limited value for either detecting or estimating this class of carbohydrates. Optimum heating times and temperatures for the other carbohydrates considered are given in Table I. In selecting these conditions as optimum, a small amount of absorbance usually has been sacrificed in order to use curves with broader maxima. The time element is thus rendered less critical, and more is gained in precision than is lost in sensitivity. Table I and Figures 2 through 4 indicate that monosaccharides of a given type tend to behave in an analogous manner. Considering the findings of Morris (8) and Koehler (6) on this point with those presented here, it seems very likely that the optimum temperature conditions indicated will be valid for the other methylpentoses, ketohexoses, etc., as well as for oligo- and polysaccharides composed predominantly of a single monomer type.

Table I. Optimum Heating Conditions for Developing Anthrone-Monosaccharide Color

Monosaccharide	Temperature, ° C.	Duration, Min.
Glucose	90	16
Galactose	90	8
Arabinose	80	4
Rhamnose	70	13
Fucose	70	13
Fructose	60	7
Sorbose	60	8

Further perusal of Figures 2 through 4 suggests the possibility of resolving multicomponent mixtures by the use of various combinations of heating times and temperatures. In a subsequent paper, on which work is currently in progress, the authors propose to develop this point. The improved precision attainable at temperatures below 100° C. makes the application of this technique seem feasible.

Although this procedure differs from that of Scott and Melvin (12), the data for glucose at 90° C. (Figure 2A) are in complete

Figure 3. Influence of temperature on time vs. absorbance at 625 mμ

A. 60° C.
B. 50° C.
C. 40° C.

Monosaccharide concn. = 100 γ/6 ml. of reaction mixture

accord with theirs. Also, they observed that when fructose was determined under conditions optimum for glucose—viz. 16 minutes at 90° C.—the standard deviation for fructose was somewhat more than five times that for glucose. The slope of the fructose curve at 16 minutes and 90° C. affords at least a partial explanation for their results with fructose.

Stability of Reagent Solutions. The visible spectra of both monosaccharide and anthrone solutions aged at room temperature were determined at various times over a 10-day period. In all cases the absorbance increased continuously throughout the period investigated.

Fructose, rhamnose, and glucose developed a single maximum at 460 to 65 mμ; arabinose did so at 440 mμ. The rapidity with which such maxima appeared was as follows: fructose, 1 hour; rhamnose, 24 hours; arabinose, 48 hours; glucose, between 5 and 10 days. This behavior correlates well with the ease with which these sugars form the anthrone color (Table I). Anthrone solutions on the other hand did not exhibit a maximum in the visible region, though their absorbance rose sharply below 500 mμ.

Influence of Reagent Age. Because the reagent solutions are time-variable, the influence of their age upon the intensity of the anthrone-carbohydrate color was investigated. Solutions of fructose and anthrone-M, respectively, were aged 1 hour and 24 hours prior to use. The color was developed by heating at 40° C. for 20 minutes. The absorbances obtained when each fructose solution was used with each anthrone solution are given in Table II. The statistical significance or nonsignificance of aging effects was determined from an analysis of variance (Table III). A full explanation of the terminology and computational procedures for this type of statistical analysis can be found in any standard reference or textbook on the subject (2, 4, 15, 18).

Table II. Influence of Reagent Age on Anthrone-Fructose Color

(Fructose concn. = 50 γ/6 ml. of reaction mixture)

| Anthrone-M | Fructose | |
	1 hr.	24 hr.
1 hr.	0.529[a]	0.386[a]
24 hr.	0.519[a]	0.370[a]

[a] Mean of 4 replicates.

Table III. Analysis of Variance

Source	Degrees of Freedom	Sum of Squares $\times 10^5$	Mean Square $\times 10^5$	F	Significance at 0.99 Probability Level
Fructose	1	8526	8526	3552	Very high
Anthrone	1	68	68	28.3	High
Interaction	1	4	4	1.67	Not significant
Residual	12	29	2.4		
Total	15	8627			

Although the effect produced by a fructose solution aged for 24 hours is evident by inspection, the effect of an anthrone solution aged for a like period is not so apparent. However, the magnitude of the F value for anthrone (Table III) indicates that an anthrone solution aged for 24 hours produces a highly significant effect.

The aging of either a fructose or anthrone reagent solution causes a diminution of the developed color, and the separate effects of these reagents are additive, as indicated by the nonsignificance of the interaction term (Table III). Similar experiments using sorbose, rhamnose, and fucose gave analogous results, except that the effect of aged anthrone was much less pronounced with methylpentoses than with ketohexoses. A second series of experiments using the four monosaccharides, but with color developed at 60° and 70° C., yielded data similar in character to that obtained at 40° C. Therefore, for maximum sensitivity, methylpentoses or ketohexoses should be determined with reagent solutions as freshly prepared as possible. Experi-

ence indicates that, because of the time required to effect complete solution, the minimum age of reagent solutions cannot be much less than 30 minutes for methylpentoses or ketohexoses and 60 minutes for anthrone.

The influence of reagent age upon precision was considered next. The variances of the subgroups represented by the means in Table II were tested and found to be effectively equal; such was also the case in the experiments with sorbose, rhamnose, and fucose. Thus, the color produced by either fresh or aged reagent solutions is equally reproducible, although the level of color and therefore the sensitivity differs significantly. Good run-to-run precision therefore requires precise duplication of the age of reagent solutions.

The color produced by a solution of a ketohexose or methylpentose aged 2 to 3 hours was just significantly different from that produced by such a solution aged 30 minutes. With ketohexoses, an anthrone solution aged 4 to 5 hours produced effects just significantly different from one aged 1.5 hours, while with methylpentoses, the effects of anthrone solutions aged 1.5 and 12 hours, respectively, did not differ significantly. Statistical significance in this study was always ascertained at the 0.99 probability level.

Because the individual differences noted are significant, though barely so, and because the aging effects of carbohydrate and anthrone are additive, the tolerance limits on reagent age are relatively narrow. For the sake of uniformity and in order to maximize both sensitivity and run-to-run precision, the tolerance limits for age of reagent solutions in this procedure have been standardized as follows: methylpentoses and ketohexoses, 30 to 60 minutes; anthrone, 1 to 2 hours.

Viles and Silverman (17), Shetlar (14), and others have indicated that anthrone reagent less than 2 to 4 hours old causes inconsistent results. From the evidence cited above, it appears that such is not the case. In this regard the present findings are in agreement with those of Scott and Melvin (12).

Stability of Developed Anthrone-Carbohydrate Color. Although earlier investigators, working with glucose and glucose polymers, reported that the anthrone-carbohydrate color is stable for as long as 3 hours, Scott and Melvin (12) found the color stability with glucose and dextran to be variable: Sometimes it was stable for about an hour, while at other times it diminished as much as 1% per hour. Methylpentoses and ketohexoses did not at any time exhibit a period of stability such as that reported for glucose.

The color developed with rhamnose or fucose, heated for 13 minutes at 70° C., showed an average decrease of 2% per hour over a 20-hour period. Over a like period, the diminution of the fructose and sorbose colors, developed by heating for 8 minutes at 60° C., averaged 0.5% per hour. Further experimentation, however, indicated that the methylpentose and ketohexose colors do not deteriorate at a constant rate; rather, the process is one of deceleration. Consequently the average per cent change per hour gives an erroneously good picture of the stability of the color immediately after its formation. During the first hour following the removal of test samples from the cooling bath, methylpentose and ketohexose color was found to diminish 4 to 7% and 1 to 2.5%, respectively. However, the rate of color change appears to level off rapidly; for, if test samples are allowed to equilibrate at room temperature (23° ± 2° C.) for 20 to 25 minutes after removal from the cooling bath, the subsequent rate of change is relatively constant for at least 1.5 hours. Within this period the standard deviation of replicate determinations read within any 15- to 20-minute interval seldom exceeds 0.005 absorbance unit (cf. Scott and Melvin's standard deviation of about 0.003 for dextran). Therefore, to obtain good run-to-run precision, it was found necessary to adhere rather strictly to a preselected time table for taking spectral readings, and to use the means of replicates run simultaneously, rather than individual values.

Two other observations pertinent to color stability are noteworthy. Fading, by which is meant color deterioration due to exposure to light, is very definitely a factor to be considered. Color deterioration in samples exposed to daylight was about 50% greater than that in protected samples. Consequently, test samples were protected at all times from direct sunlight. Illumination of methylpentose and ketohexose color by fluorescent lamps for several hours produced no discernible effect.

When such time-temperature conditions are used that maximum color is not attained—e.g., fructose heated 20 minutes at 40° C. (Figure 3)—the developed color does not diminish but slowly continues to increase. Although such behavior might not normally be encountered, it would almost certainly have to be considered in the resolution of mixtures.

Adherence to Beer's Law. The anthrone-fructose color developed at both 40° and 60° C. obeys Beer's law up to concentrations between 75 and 100 γ per 6 ml. of reaction mixture. The deviation from linearity at 100 γ per 6 ml. was in both cases greater than could be accounted for by experimental error.

Table IV. Precision of Anthrone Method

Monosaccharide	Time-Temperature Conditions		No. of Runs	Total No. of Detn.	Std. Dev. (σ)
	Min.	° C.			
Rhamnose	20 at	60	3	9	0.004
	13 at	70	15	60	0.003
Fucose	13 at	70	15	60	0.004
Fructose	20 at	40	8	28	0.004
	8 at	60	18	100	0.006
Sorbose	8 at	60	22	96	0.005

The anthrone-sorbose color developed at 60° C. also obeys Beer's law, but in this case up to concentrations of at least 100 γ per 6 ml., though probably not much beyond that. The anthrone-rhamnose and anthrone-fucose colors developed at 70° C. likewise conform to Beer's law at concentrations up to at least 100 γ per 6 ml.

Comparison of Anthrone from Three Sources. The three samples of anthrone mentioned in the section on materials were compared in terms of the color produced by each with fructose at 60° C. and with rhamnose at 70° C. None of the anthrone specimens produced a significant variation in color with either monosaccharide, after correction for the appropriate blank was made. However, the blanks themselves did differ significantly: Anthrone-E was consistently highest and anthrone-NBC consistently lowest. Blanks with the latter material very seldom exceeded 0.003 after being heated 8 minutes at 60° C. or 13 minutes at 70° C.

Influence of Acid Concentration. Reagent solutions of rhamnose, fucose, fructose, sorbose, and anthrone were each prepared in 26.5, 27.5, and 28.5N sulfuric acid. Acid concentration was determined by titration and was adjusted to within ±0.1N of the indicated value. Anthrone-methylpentose color was developed by heating for 13 minutes at 70° C., and anthrone-ketohexose color by heating for 8 minutes at 60° C. Carbohydrate concentration was 50 γ per 6 ml. of reaction mixture. At the higher normality the monosaccharides tended to be difficultly soluble, while the same was true for anthrone at the lower normality. A normality of 27.5 provides a good compromise.

The three levels of acid concentration caused significant variation in the color intensity produced by each of the monosaccharides tested. Consequently, some care must be exercised in the preparation of the solvent acid to ensure a constant concentration level. A tolerance of ±0.1N gives very satisfactory results.

The methylpentoses, like the aldohexoses, gained in color intensity as the acid concentration increased. The ketohexoses, however, gained in color intensity as the acid concentration diminished. These observations are consistent with those reported by Johanson (5)—viz., that anthrone is specific for ketosugars when mild rather than strong acid conditions are used.

Influence of Anthrone Concentration. The results of experiments dealing with the influence of anthrone concentration on the absorbance of methylpentoses and ketohexoses are given in Figure 5. From these graphs it can be seen that the optimum concentration of the anthrone reagent for use with methylpentoses is about 0.13% (w./v.); while with ketohexoses, it is in the vicinity of 0.25% (w./v.). The concentration of 0.16% (w./v.) used throughout this study is a compromise introduced for the sake of uniformity.

Comparison of Methods. The method developed here is basically like Black's (1) in that a solid sample, rather than an aqueous solution, is taken as the starting material. However, as it is sometimes more convenient to work with aqueous solutions, determinations of fructose have been made using a procedure similar to that of Scott and Melvin (12), except that the reactants were cooled and mixed in a slurry of crushed ice and salt. This modification was necessary in order to keep the temperature from exceeding 60° C. when the layered reactants were mixed. Uniformity of handling, particularly with respect to time and temperature, is very important. As above, full color was developed by heating for 8 minutes at 60° C.
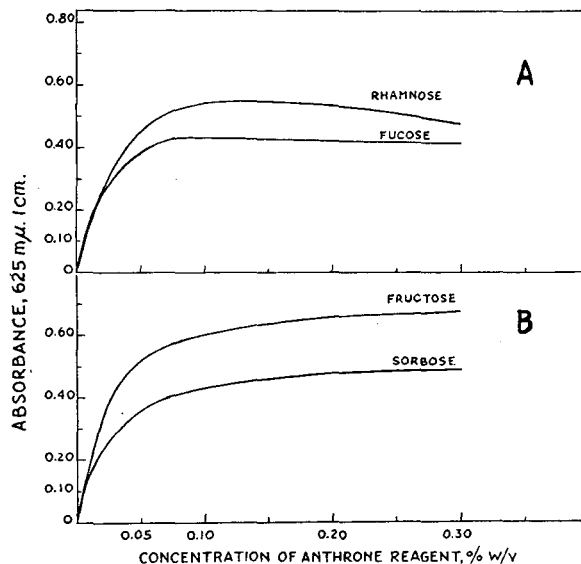

Figure 5. Influence of anthrone concentration on absorbance at 625 mμ

A. Methylpentoses
B. Ketohexoses
Monosaccharide concn. = 50 γ/6 ml. of reaction mixture

The variability of the method employing an aqueous solution of fructose tended to be greater than that of the method using fructose dissolved in 27.5N sulfuric acid. Also, the aqueous method produced a somewhat higher level of color, so that at present the two methods are not interchangeable. Results thus far do indicate, however, that a satisfactory aqueous method for methylpentoses and ketohexoses can be elaborated without much difficulty, if due consideration is given to those factors which have been shown to be important for good precision.

PRECISION

The run-to-run precision obtained with methylpentoses and ketohexoses is summarized in Table IV. The number of replicates per run was not always the same in successive runs, but for reasons of convenience or necessity varied from 3 to 8. The

pooled estimate of the standard deviation, $\hat{\sigma}$, for each monosaccharide was calculated as follows:

$$\hat{\sigma} = \sqrt{\frac{n_1 s_1^2 + n_2 s_2^2 + \ldots + n_k s_k^2}{n_1 + n_2 + \ldots + n_k - k}}$$

where n is the number of determinations comprising a run, s^2 is the variance of a run, and k is the number of runs pooled.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Black, H. C., Jr., ANAL. CHEM., 23, 1792 (1951).
(2) Dixon, W. J., and Massey, F. J., Jr., "Introduction to Statistical Analysis," McGraw-Hill, New York, 1951.
(3) Gilman, H., and Blatt, A. H., "Organic Syntheses," vol. 1 (Coll.), Wiley, New York, 1941.
(4) Hoel, P. G., "Introduction to Mathematical Statistics," Wiley, New York, 1947.
(5) Johanson, R., Nature, 172, 956 (1953).
(6) Koehler, L. H., ANAL. CHEM., 24, 1576 (1952).
(7) McCready, R. M., Guggolz, Jack, Silviera, Vernon, and Owens, H. S., Ibid., 22, 1156 (1950).
(8) Morris, D. L., Science, 107, 254 (1948).
(9) Morse, E. E., ANAL. CHEM., 19, 1012 (1947).
(10) Schönberg, A., and Mustafa, A., J. Chem. Soc., 1945, 657–60.
(11) Schönberg, A., Mustafa, A., and Zayed, S., Science, 119, 193 (1954).
(12) Scott, T. A., Jr., and Melvin, E. H., ANAL. CHEM., 25, 1656 (1953).
(13) Seifter, S., Seymour, Dayton, Novic, B., and Muntwyler, Edward, Arch. Biochem., 25, 191 (1950).
(14) Shetlar, M. R., ANAL. CHEM., 24, 1844 (1952).
(15) Snedecor, G. W., "Statistical Methods," Iowa State College Press, Ames, Iowa, 1946.
(16) Trevelyan, W. E., and Harrison, J. S., Biochem. J. (London), 50, 298 (1952).
(17) Viles, F. J., Jr., and Silverman, L., ANAL. CHEM., 21, 950 (1949).
(18) Youden, W. J., "Statistical Methods for Chemists," Wiley, New York, 1951.

Rapid Determination of Carbon Dioxide in Silicate Rocks

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In the development of rapid methods for silicate rock analysis, a simpler and faster means was needed for the determination of carbon dioxide than the conventional "train" procedures. With the method presented here, which involves measurement of the volume of carbon dioxide evolved, the time required for a determination is about 5 minutes per sample. It provides a saving of 30 to 40 minutes for each determination without significant loss of accuracy.

CARBON dioxide in silicate rocks is usually determined by absorbing and weighing the carbon dioxide evolved when the sample is decomposed by acid (2). Methods of this type usually require about 45 minutes for a single determination.

In the scheme for rapid analysis of silicate rocks developed by Shapiro and Brannock (4) an ignition loss determination is used in lieu of water and carbon dioxide determinations. In many instances rock analyses are more useful if values are given for water and carbon dioxide. A rapid method for the determination of water has been developed (3), and this paper describes a procedure for the determination of carbon dioxide that requires about 5 minutes per determination.

The procedure presented is similar to that described by Fahey (1), in which the volume of gas evolved by acid attack on the sample is measured. The well-known qualitative test for carbonate, in which the formation of bubbles is observed when hydrochloric acid is added to a test tube containing the ground sample and hot water, has been made quantitative by the use of a tube with a side arm for catching and measuring the gas evolved.

APPARATUS

The special tube designed for the determination of as much as 2% carbon dioxide, the range common in silicate rocks, is shown in Figure 1. It consists of a borosilicate glass test tube, 18 by 150 mm., to which is attached a side arm of borosilicate glass, 200 mm. in length and 10 mm. in outside diameter, with a closed end.

An electric heater of the Gilmer type with a means of regulating the power input is used to heat the sample and liquid in the lower portion of the carbon dioxide tube. The heater should be fitted with a cover of asbestos board, about 0.25 inch in thickness, with a hole about 20 mm. in diameter in its center.

REAGENTS

Hydrochloric acid, 1 + 1.
Mercuric chloride, 3%.
Motor oil, S.A.E. No. 10. Addition of a small amount of antifoaming agent is desirable although not essential.

CALIBRATION OF CARBON DIOXIDE TUBE

1. Weigh 1.03 grams of National Bureau of Standards standard sample No. 79 and transfer, by means of a dry funnel, to the bottom of the carbon dioxide tube.
2. Add 2 ml. of the mercuric chloride solution and tap the tube to free entrapped air bubbles.
3. Add oil to the oil-level mark.
4. Tilt the tube so that the oil completely displaces the air from the side arm. Then return the tube to a position such that the main part of the tube is vertical.
5. Add 2 ml. of 1 + 1 hydrochloric acid, and tilt the tube so that the side arm is vertical to allow any carbon dioxide produced to enter the side arm.
6. Mount the carbon dioxide tube in a clamp attached to a support so that the side arm is vertical, and insert the lower part of the tube through the hole in the cover of the heater with the interface of the aqueous and oil phases just at the level of the cover. (The heater should be preheated so that the temperature around the lower part of the tube can be maintained at about 185° C.) Allow the aqueous phase to boil for 2.5 minutes.
7. Remove the tube from the heater and allow tap water to flow down the outside of the side arm for 15 seconds. (Tap water temperature should be between 15° and 25° C.)
8. Remove from the stream of tap water, hold the tube with the side arm upright, and mark the position of the meniscus on the side arm. This is the 1% mark.
9. Repeat steps 1 through 8 using 0, 0.206, 0.412, 1.54, and 2.06 grams of the standard sample to obtain calibration marks equivalent to 0, 0.2, 0.4, 1.5, 2.0% carbon dioxide, respectively.
10. The tube can now be marked off by interpolation to give marks for each 0.1% carbon dioxide.

PROCEDURE

Transfer 1.000 gram of sample powder to the bottom of the carbon dioxide tube by means of a dry funnel.
Proceed as described in steps 2 to 7 of "Calibration of Carbon Dioxide Tube."

Remove from the stream of tap water and estimate the per cent carbon dioxide from the scale on the side arm.

EXPERIMENTAL

A number of experiments were made to determine the effects of several pertinent variables on the evolution and measurement of carbon dioxide from samples of silicate rocks: Three different supernatant liquids were tested, three tubes with different side-arm lengths were tried, the time required for sample decomposition was studied, and the feasibility of using mercuric chloride to inhibit the formation of hydrogen, which might result from the reaction between hydrochloric acid and "tramp iron," was determined.
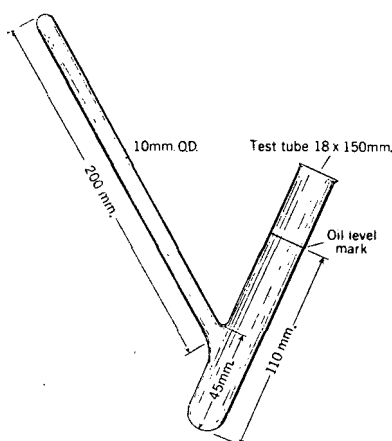

Figure 1. Carbon dioxide tube

Tests of Three Supernatant Liquids. Distilled water, 20% sodium chloride solution, and motor oil were compared as supernatant liquids. Distilled water and sodium chloride solution were usable but were inferior to oil, especially at low levels (below 0.5%), because of the greater solubility of carbon dioxide in water and sodium chloride solutions. It was also found that with oil it was easier to control the boiling of the solution in the lower part of the tube.

Selection of Suitable Side-Arm Length. Three carbon dioxide tubes were tried. One had the dimensions shown in Figure 1. Another was the same except that the side-arm length was 100 mm. The third had a side-arm length of 400 mm. Two runs were made with each tube using a sample of rock powder containing about 0.5% carbon dioxide. The results are shown in Table I.

These results indicate that the longer the oil path through which the gas bubbles must travel, the less gas recovered. This may be explained as the solubility effect of the carbon dioxide in the oil. It is evident that the longer the side arm used, the less precisely the readings could be made, and that the shorter the side arm, the less the range covered for a given sample size. Taking these facts into consideration, in addition to the fact that the tube with the long side arm was difficult to manipulate, the 200 mm. side-arm length was chosen as the best compromise.

Time Required for Decomposition of Finely Ground Samples. Four portions of a sample containing about 1% carbon dioxide were carried through the procedure using boiling periods of 1, 2, 3, and 5 minutes. The lengths of the gas columns obtained were 91, 103, 102, and 102 mm., respectively.

Use of Mercuric Chloride to Eliminate Interference of Tramp Iron. Rock samples which are prepared for analysis by grinding with steel grinding apparatus usually contain a small amount of metallic iron introduced as a contaminant by the grinding apparatus.

The results for carbon dioxide in samples contaminated in this way are about 0.1 to 0.2% high if nothing is done to prevent the evolution of hydrogen resulting from the reaction of the metallic iron with the hydrochloric acid added to decompose the carbonates. This difficulty can be overcome by the addition of mercuric chloride solution, which converts the metallic iron to ferrous iron without formation of hydrogen.

Table II gives a comparison of results obtained for a granite sample when mercuric chloride is added and when it is omitted for the determination of carbon dioxide in portions of sample powder with a normal amount of iron contaminant, with portions of sample from which iron had been removed by a magnet, and with portions of sample to which an abnormally large amount of metallic iron had been added. The data show that metallic iron can cause errors in results for carbon dioxide by volumetric methods unless precautions are taken to prevent the formation of gaseous hydrogen and that the difficulty can be overcome very simply by use of mercuric chloride.

Table I. Effect of Length of Side Arm on Length of Gas Column

Length of Side Arm, Mm.	Length of Gas Column, Mm.	
100	50	49
200	38.5	38
400	29	29

Table II. Effect of Mercuric Chloride on Evolution of Gas from Granite Sample Containing Metallic Iron

	Apparent CO_2, %[a]	
Treatment of Sample Powder	With $HgCl_2$	Without $HgCl_2$
None	0.06	0.20
Iron removed by magnet	0.09	0.09
8-mg. iron filings added	0.09	0.39

[a] Using a conventional train procedure, five analyses averaged 0.07% CO_2.

Table III. Carbon Dioxide Determinations by Conventional Train Method and by Rapid Method

	CO_2, %	
Conventional method	Rapid method	Difference
0.19	0.22	+0.03
0.67	0.68	+0.01
0.27	0.28	+0.01
0.05	0.05	0.00
0.32	0.32	0.00
0.00	0.06	+0.06
0.75	0.75	0.00
1.7	1.6	−0.10
1.9	1.9	0.00
0.06	0.06	0.00

The experiments indicate that the optimum procedure would be one in which oil is used as the supernatant liquid, the boiling time is at least 2 minutes, the length of the side arm on the carbon dioxide tube is about 200 mm., and mercuric chloride is used to eliminate the interference of metallic iron.

RESULTS

In Table III results obtained by this rapid method for a series of samples containing from 0 to 2% carbon dioxide are compared with results obtained by conventional train procedures. The results are in good agreement.

LITERATURE CITED

(1) Fahey, J. J., U. S. Geol. Survey, Bull. 950, 139 (1946).
(2) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 623, Wiley, New York, 1929.
(3) Shapiro, L., and Brannock, W. W., ANAL. CHEM., 27, 560 (1955).
(4) Shapiro, L., and Brannock, W. W., U. S. Geol. Survey, Circ. 165 (1952).

Integrated Set of Laboratory Fractionators for Routine Analytical Distillations

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A new design for a routine, all-glass laboratory fractionator makes possible precisely controlled 15/5 fractionation. This is achieved using a compact reflux divider, together with the high thermal resistance of a silvered vacuum jacket plus electric compensation. Heat losses are closely controlled, and speed of operation under vacuum is markedly improved. An atmospheric fractionation can be completed in one 8-hour shift. Five sizes of these 15-plate distillation units having capacities ranging from 1 to 80 liters are operating satisfactorily up to 950° F. equivalent atmospheric vapor temperature. They have been found ideal for crude oil assays and laboratory workup of refinery operations.

BECAUSE the true reflux ratio in a fractionating tower has so great an effect on the operating efficiency of the fractionator, efforts have been made in these laboratories to design a laboratory fractionating tower in which the actual reflux ratio is accurately known and controlled. Simply because a reflux dividing device is operating on a fixed cycle it cannot be assumed that the actual reflux ratio is the same as the cycle. This would be true if there were no heat losses below the reflux dividing apparatus.
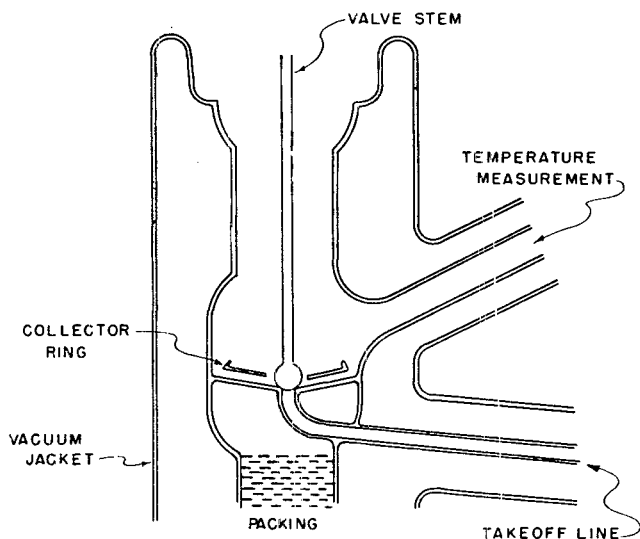

Figure 1. Vertical section of fractionator

In many designs, heat losses below the reflux dividing device are large and thus have considerable effect on the actual reflux ratio. In atmospheric fractionation where the boilup is high, these heat losses may not have a great effect on the actual reflux ratio. However, in vacuum distillation where the boilup is much lower, this heat loss can prevent a large percentage of the vapors from reaching the liquid dividing device, thus altering the actual reflux ratio by many times the nominal ratio. In some cases, these heat losses are sufficient to quench the distillation completely at 800° F. atmospheric vapor temperature.

Heat losses in a fractionating tower and their final effect on the operating efficiency of the fractionator have led to studies in these laboratories designed to find a tower design in which heat losses are very small. Added reflux due to heat losses has greatly limited fractionation under vacuum in old-fashioned lagged fractionators. Electrical compensation by incorporating resistance heaters in the insulation of metal or glass stills without vacuum jackets requires expensive instrumentation and maintenance. In addition, many of the popular designs on the market were found to be unable to handle the full boilup potential of the recently developed high-speed packing (*3, 10*) and did not lend themselves to construction out of glass in the larger sizes. Steel flasks are used for sizes larger than 5 liters.

DEVELOPMENT

Attempts to approach an adiabatic condition by the use of heated insulation depend on the uncertainty of a tiny thermocouple bead to indicate the true temperature of its surroundings.

Table I. Comparative Capacities of Packings (*8–10*)
(Suitable for 1-inch inside diameter towers)

Type	Maximum Rate, Ml./Hr.-Sq. Cm.
Oldershaw	850
Cannon protruded metal	825
Podbielniak Octapak, 0.20 inch	685
Fenske helices, 1/8 inch	575
Podbielniak Helipak, 2918	500
Podbielniak Heligrid	500
Stedman 30-gage cone	355
Berl saddles, 3/8 inch	340
Raschig rings, 1/4 inch	245

Coupled with this uncertainty and the high heat conductivity of metal as construction material for the tower, minor jacket temperature errors will have a large effect on heat transfer and will introduce uncontrolled variation, either cooling or superheating the vapors in the tower. For several years, this type of equipment has been used with its poor reproducibility of both yield and quality. Distillation rates are very low, and it was very difficult to distill beyond approximately 650° F. equivalent atmospheric vapor temperatue on crude oil which had a slope of 10° F. per per cent distilled.

The problem of the control of heat losses seemed to be divided into two main parts. The first phase indicated a need for increasing the thermal resistance, thus reducing heat losses from the tower; the second required the control of radiation losses by reducing the temperature gradient across the thermal resistance.

The first part of the problem, that of a high resistance to heat flow, seemed to preclude any massive design incorporating large amounts of material to be brought to thermal equilibrium and to eliminate the use of highly conductive materials such as metal. Small laboratory columns had been made of glass and this material seemed to be attractive because of its low thermal conductivity plus the fact that it could be cheaply and readily fabricated into small complicated shapes. Its low mechanical strength places a limitation on the maximum size of designs, but this deficiency can be overcome to a large extent by the use of modern methods of glass fabrication. Glass can easily be built with necessary expansion bellows in the form of an evacuated envelope, which is an efficient resistance to thermal conductivity. Such a

jacket can also be coated with a highly reflective silver surface which will control heat losses by radiation. However, because glass has some thermal conductance, the unit should be designed so that it has no dead ends or joints between the tower and reflux dividing device. The reflux dividing device should be as compact as possible, very close to the packed section of the tower, and enclosed completely in a silvered evacuated jacket integral with the packed section.

To reduce extreme temperature gradients across the vacuum jacket, a heating mantel around the jacket is used. The external temperature of the jacket is raised to approximately the vapor temperature within the tower. With the combination of a high thermal resistance and a low temperature gradient, as nearly adiabatic conditions as possible are maintained within the fractionator; thus, no extra reflux is added in the tower.

DESCRIPTION OF FRACTIONATOR

The Sarnia Mark II fractionator, as the design is called, will satisfy the requirements as outlined above. It has been designed for 15 theoretical plates at infinite reflux ratio with an operating holdup of approximately 1 to 2% of the charge. This design will work equally well at any other tower efficiency and can be adjusted by increasing or decreasing the length of the packed section. In all these towers, Cannon protruded metal packing (3, 5, 10) is used because it is self-wetting and has the highest throughput capacity of all except Oldershaw, as shown in Table I. The latter was rejected on the basis of high holdup per theoretical plate. A combination of the Sarnia MK II fractionator and Sarnia Hivac pot still will cover a distillation range from 75° to 1200° F. (These two pieces of apparatus are manufactured under exclusive license by H. S. Martin & Co., Evanston, Ill.)
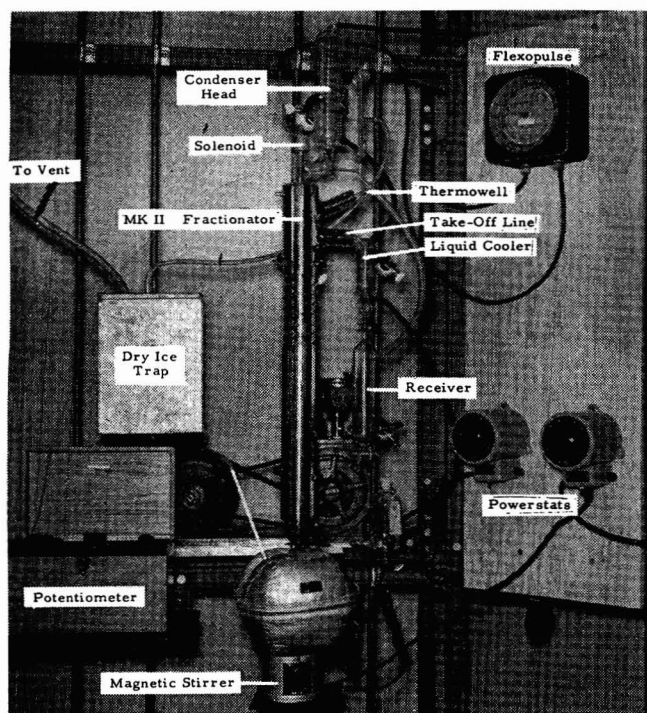
running down the walls from the condenser is collected in a ring, integral with the wall of the tower, and led through two tubes from opposite sides of the ring to the take-off point at the center of the tower. Here, the total condensate is either taken off as distillate or returned to the tower as reflux. The cyclic operation of the valve is controlled by an electric timer and solenoid, which lifts the valve, allowing distillate to be taken off. When the valve is closed, reflux overflows the low pouring lips onto the packing. In towers 50 mm. or less in diameter, two pouring lips are used, but in larger towers four lips are used to ensure uniform distribution of reflux to the packing. As the tower is on either total take-off or total reflux, a short time cycle is used to maintain smooth and uniform operation of the tower.
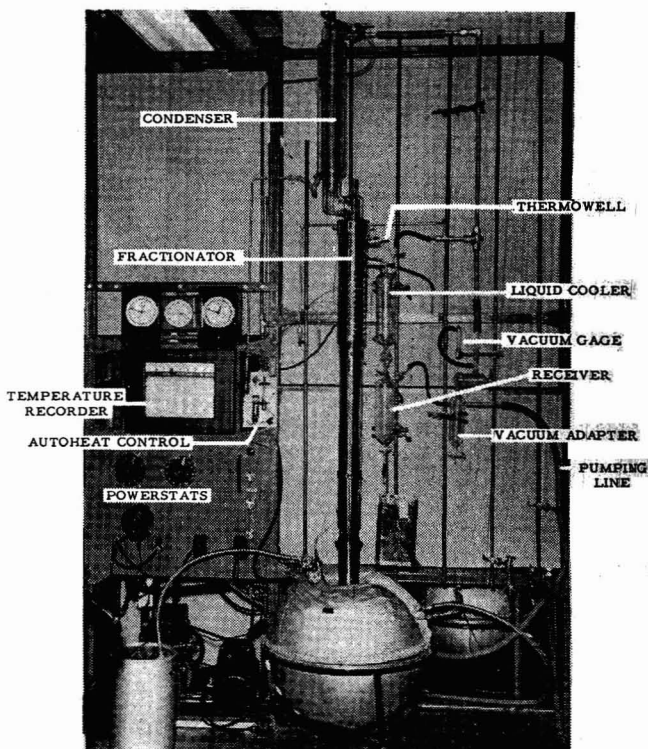
Figure 3. Fractionator arranged for vacuum operation

40-liter unit shown without mantle

Figure 2. Fractionator arranged for atmospheric fractionation

4-liter unit, shown without mantle

In this design, as shown in Figure 1, the reflux dividing device is compact and is built immediately above the packed section. With this arrangement, there is little heat leak below the reflux divider, and as substantially all the reflux is produced above this point, it cannot effect the actual reflux ratio. Condensate

In older designs, small amounts of water in a charge could give very uneven temperature indication by dropping off the condenser onto the packing and flashing. The new design overcomes much of this difficulty by offsetting the condenser head, which causes any water drops to fall onto the hot sloping neck of the condenser and flash at a point above the temperature measuring zone, thus eliminating wild temperature fluctuations. The development of a small, dependable, and accurate temperature-measuring device is being carried on in an effort to overcome the disadvantages of the massive mercury bulb or the undependable thermocouple. Currently thermocouples are checked frequently, together with their potentiometers, with melting point baths of pure metals. The McLeod gage in a dry atmosphere is used as the primary pressure standard. Figures 2 and 3 are photographs of the 4- and 40-liter units and show the simple arrangement of equipment.

Future development associated with these units includes a thorough study of fundamental measuring devices for temperature and pressure, automatic instruments for simultaneous recording of these two variables, and ultimately a mechanical record of the temperature corrected to 760 mm. of mercury from

operation at any pressure. This temperature will be recorded as a function of the volume per cent distilled using a device recently developed elsewhere, thus making the units completely automatic.

PERFORMANCE

Several towers have been evaluated with two sizes of Cannon protruded metal packing. In towers 36 mm. or smaller in diameter, 0.16 × 0.16 inch packing was used, in the 50- and 70-mm. towers, 0.24 × 0.24 inch packing. These towers were designed to approximate 15 theoretical plates at infinite reflux ratio.

Table II. Evaluation of Cannon Protruded Metal Packing in Sarnia Mark II Fractionators

Time after Preflooding, Hours	Pressure Drop, Mm. of Hg	Refractive Index Pot.	Refractive Index Overhead	Plates, Difference	HETP, Inches
Tower. 25 mm. i.d. × 24 inches. Packing. 0.16 × 0.16 inch stainless steel. Charge. 1 liter of 50% n-heptane–50% methylcyclohexane					
0.5	5.5	1.4088	1.3991	16.5	1.45
0.75	5.5	1.4088	1.3992	16.5	1.45
0.9	7	.....	1.3991	16.5	1.45
Second Test					
0.5	8	1.4002	1.3925	18.0	1.33
0.8	3	1.4000	1.3929	18.0	1.50
Tower. 50 mm. i.d. × 36 inches. Packing. 0.24 × 0.24 inch stainless steel. Charge. 5 gal. of 50% n-heptane–50% methylcyclohexane					
0.5	..	1.4032[a]	1.3953	16.0	2.4
1.0	8	1.4032[a]	1.3954	16.0	2.4
Tower. 70 mm. i.d. × 44 inches. Packing. 0.24 × 0.24 inch stainless steel. Charge. 8.5 gal. of 50% n-heptane–50% methylcyclohexane					
0.5	12	1.4048[a]	1.3945	20.5	2.1
1.0	14	1.4040[a]	1.3948	19.0	2.3
2.0	14	1.4041[a]	1.3942	20.0	2.2

[a] Vapor sample, one plate added for kettle.

Table III. Distillation Rates for Sarnia MK II Fractionators[a]

(Protruded metal packing, 15-plate towers at atmospheric pressure)

Tower Diameter, Mm.	Cross-Sectional Area, Sq. Cm.	Normal Charge, Liters	Max. Take-off at 5:1 R.R., Ml./Hr.[b]	Full Boilup, Ml./Hr. Total	Full Boilup, Ml./Hr. Per sq. cm.
18[c]	2.5	2	375	2,250	900
25	4.9	4	620	3,700	760
50	18.1[d]	20	2,400	14,400	795
70	38.4	40	5,500	33,000	860
110[e]	95	80	13,400	80,500	850

[a] Protruded metal packing; 15-plate towers at atmospheric pressure.
[b] Observed rates considered to be about 95% of flood point.
[c] Calculated from 48-inch 36-plate tower.
[d] Actual inside diameter 48 mm.
[e] Estimated performance—not yet in operation.

Evaluation of the towers is shown in Table II when a synthetic mixture of 50% n-heptane and 50% methylcyclohexane was employed using the method of Fenske (1–4). The results show a minimum height equivalent to one theoretical plate (HETP) of 1.33 inches for the 0.16 × 0.16 inch packing, and 2.1 inches for the 0.24 × 0.24 inch packing, which agrees with the published data of the manufacturer (10). These towers when operating at 5 to 1 reflux ratio and 90 to 95% of maximum boilup have efficiencies of approximately five to six theoretical plates at atmospheric pressure. A check of the efficiency of this design at subatmospheric pressures will be undertaken soon, but it has been stated (11) that the efficiency of Cannon packing is reasonably insensitive to pressure, running n-decane–trans-Decalin. In any case, the effect of reduced pressure, while variable (6, 7), is not great above 10 mm. of mercury absolute pressure and has primarily to do with the change in relative volatility with pressure.

Distillation rates have been determined for the three sizes of

tower when operating at 5 to 1 reflux ratio and at pressures varying from 1 mm. of mercury to atmospheric pressure and are plotted in Figure 4. Total boilup has been calculated by multiplying the take-off by a factor of 6. Boilup rates of 750 to 850 ml. per hour per sq. cm. of cross-sectional area have been achieved on towers larger than 18-mm. diameter, and a boilup of 900 ml. per hour per sq. cm. and higher has been measured several times on an 18-mm. diameter tower of older design at atmospheric pressure, but this extremely high rate requires further examination. Table III shows take-off and boilup rates at atmospheric pressure for the MK II fractionator using protruded stainless steel packing. These rates are for towers with heating mantles and are 70% above the maximum rates published by Podbielniak, Inc., for Helipak (8). They are notably higher than rates observed for some 20- and 48-mm. towers of popular design which can handle only 320 to 350 ml per hour per sq. cm. without flooding. The maximum rate attainable is a function of the material being distilled. It has been observed with other charges that the maximum rate appears to be only 75% of that for Redwater, a mixed base crude.

Table IV gives similar data for operation mostly on Redwater crude at 100, 10 and 1 mm. of mercury absolute pressure, respectively, in the condensing region of towers commonly used in crude assay and product workup analysis. The data for 100-mm. operation represent corrected atmospheric vapor temperatures up to approximately 650° F. and the 10 mm. data up to about 800° F. depending on the charge material. At 1 mm. of mercury head pressure, fractions boiling up to 900° F. can be distilled in the larger stills having 3 feet of packing while the 24-inch towers can handle liquids boiling up to 1000° F.

The accuracy of the reflux dividing valve has been checked by pumping liquid at a constant rate into the top of the condenser head while the valve was operating. By collecting the product from the take-off line and from the bottom of the tower separately and weighing, the actual reflux ratio can be determined directly. Results show that in only one case did the actual reflux ratio fall below 4.6 to 1 at near full atmospheric boilup. In practice, the timer setting is adjusted to give an actual reflux ratio of 5 to 1.
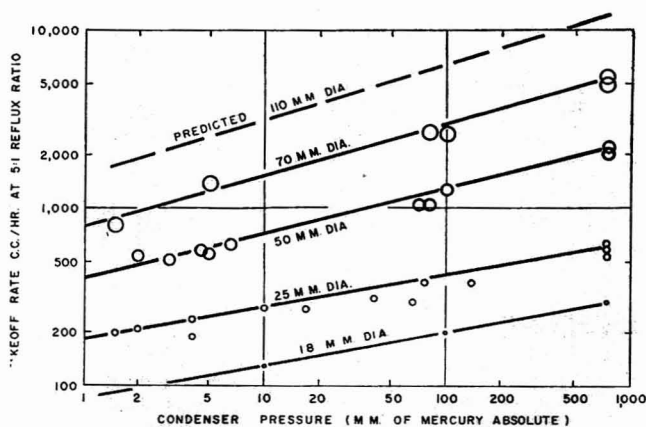
Figure 4. Maximum take-off rates of fractionators

Running Redwater crude

Heat losses have also been studied by boiling up pure compounds under total take-off conditions. All the boilup which reaches the valve is thus removed and any condensate which forms in the tower below is removed at the bottom through a special nonreturning trap in the insulated neck of the kettle. A blank run is necessary to account for heat losses occurring below the bottom of the tower. In this manner, the MK II fractionator was compared with an Oldershaw column of the

same size, equipped with a standard vapor-dividing head of Shell design. Table V summarizes the results obtained using pure compounds of three different boiling ranges. No comparable information is available for the Podbielniak design at this writing.

The boilup will be effectively increased by the use of a heating mantle, especially at 10 mm. of mercury absolute pressure and lower. Additional data indicate a possible increase in take-off for the 50-mm. tower of 150 ml. per hour or an increase in total boilup of 900 ml. per hour. At this high temperature, without a heating mantle, the actual reflux ratio in the tower is increased to not over 7.5 to 1 for nominal 5 to 1 reflux ratio, whereas in older designs the reflux ratio is actually 20 to 1 to 50 to 1, and some designs are completely inoperative.

Table IV. Distillation Rates for Sarnia MK II Fractionators

Tower Diameter, Mm.	Cross-Sectional Area, Sq. Cm.	Normal Charge, Liters	Max. Take-off at 5:1 R.R. Ml./Hr.[b] •	Boilup, Ml./Hr. Total	Per sq. cm.
At 100 Mm. of Mercury Absolute Pressure[a]					
25	4.9	4	425	2,550	520
50	18.1[c]	20	1300	7,800	430
70	38.4	40	3000	18,000	470
At 10 Mm. of Mercury Absolute Pressure[a]					
25	4.9	4	275	1,650	335
50	18.1[c]	20	750	4,500	250
70	38.4	40	1559	9,300	240

[a] Pressure in condensing region. Add 10 mm. of mercury pressure drop to get pressure at boiling surface of 50- and 70-mm. sizes, 7 mm. for 25-mm. size.
[b] Considered to be about 95% of flood point.
[c] Actual inside diameter 48 mm.

Nine runs have been made on Redwater (Canadian) crude to determine reproducibility and interchangeability of data from 25-, 50-, and 70-mm. fractionators. Table VI contains the data for these runs and indicates an average standard deviation of approximately 0.5% or 5° F. The samples of Redwater crude used in this series of nine runs were not identical but similar; some contained small amounts of water and others contained no water. Minor changes in operating technique were made. For these reasons, the standard deviations are higher than they would be for a uniform sample. More recent data on wholly vacuum operation indicate a standard deviation of about 4° F. for these stills, and it is expected that atmospheric operation has also improved.

The cross contamination at a cut point has been determined for these fractionators by rerunning fractions from controlled 15/5 operation in a 36-plate tower operating at 30 to 1 reflux ratio. The 36-plate tower under these conditions is the equivalent of approximately 21 theoretical plates at infinite reflux ratio. Table VII contains cross-contamination data for a 50-mm. fractionator with tower heating mantle and indicates a "leader"—i.e., the material boiling below the actual cut point of 12.5 to 18% and a "tail" or the material boiling after the cut point of 3 to 6% of the fraction.

ADVANTAGES

High Speed. Speed is from over 50% faster at atmospheric pressure to several times that of most popular stills at low pressures. Handling is so easy that one man can accomplish a complete turn-around, including a change of packing, in 20 minutes. Because of its very low mass, heating and cooling rates are very fast.

Reproducibility is at least as good as other designs currently in use. Accuracy depends largely on the measurement of temperature and pressure.

Maximum temperature attainable is higher than any other known design; 15/5 distillations have been carried up to 1000° F. (atmospheric equivalent) on distillates without thermal cracking in the charge.

Cost is competitive with the comparable existing designs in the smaller sizes. In larger sizes, where older steel stills are replaced, the over-all cost resulting from cheaper tower and related parts plus cheaper instrumentation and installation is reduced from $10,000 to $15,000 per unit to $3000 to $5000. Maintenance is also lower.

Space requirement is small—about 20 square feet per unit proper for 10-liter and larger sizes with a 10-foot ceiling.

DISADVANTAGES

Temperature and Pressure Limitations restrict application of the fractionator in pilot unit operation.

Table V. Heat Losses in Bare 1-Inch, 15-Plate Towers with Silvered Vacuum Jackets

	Cal./Min. Running 3.5 to 4 Liters/Hr. Total Boilup		
	n-Heptane, 210° F.	Aniline, 365° F.	Tetralin, 405° F.
MK II	70	165	240
			212[a]
Oldershaw	121	485[b]	680[b]

[a] With unheated mantle in place.
[b] About 2.5 liters per hour was maximum rate without flooding.

Table VI. Reproducibility of Sarnia MK II Fractionators[a]

	Yield—Cumulative LV % on Crude				
Diam., Mm.	Atmospheric			Vacuum	
	250° F.	350° F.	430° F.	530° F.	650° F.
25	18.4	27.7	34.5	...	...
	18.2	28.2	35.8	45.7	57.3
	18.3	28.4	35.6	46.9	...
	18.2	28.5	35.4	45.9	56.4
	18.0	27.4	34.3	44.9	56.1
50	18.2	28.8	35.1	...	...
	18.2	27.8	34.7	44.6	56.6
	17.3	27.5	34.4	44.8	57.2
70	18.4	27.4	34.4	44.4	56.5
Average, X	18.1	28.0	34.9	45.3	56.7
Std. deviation, σ	0.33	0.52	0.58	0.89	0.47

[a] Charge. Redwater crude; slope approximately 10° F. per 1% distilled

Table VII. Cross-Contamination Corresponding to 15/5 Operation[a]

15/5 Cut Range, ° F.	C₅– 200° F.	200– 281° F.	281– 355° F.	355– 435° F.	435– 515° F.	515– 600° F.
C₅-200	97.0	3.0	...	...	...	...
200-281	14.1	80.2	5.7	...	...	...
281-355	...	12.5	81.7	5.8	...	...
355-435	...	...	18.1	76.1	5.8	...
435-515	...	...	...	16.8	79.9	3.3
515-600	...	...	...	...	13.0	87.0[b]

[a] Charge. 5 gallons of Redwater crude; tower: 50 mm. × 36 inches at 5 to 1 reflux ratio (found later to be tower whose valve gave actual reflux ratio of 4.0 to 1). MK II design packed with 0.24 × 0.24 inch cannon packing.
[b] Similar data have been obtained on 70-mm. fractionator without heating mantle and show approximately same cross-contamination relationship of about 15% "leader" and 4.5% "tail" at cut point.

Fragility. The stills are relatively fragile. In spite of this, experience of many years both here and elsewhere has shown that large glass vessels can be handled safely by trained laboratory personnel. As the distillation can be quenched from full boilup to zero in less than 15 seconds, it is easy to make an automatic device to avoid the release of large quantities of vapor in case of accident. Glass fabric mantles are themselves good insurance against the spread of fire and will not initiate it.

Safety Note. Because of the presence of evacuated glass

vessels, the usual safety precautions regarding safety shields or face protection should be emphasized.

LITERATURE CITED

(1) Beatty, H. A., and Colingaert, G., *Ind. Eng. Chem.*, **26**, 504 (1934).
(2) Berg, L., and Popovac, D. O., *Chem. Eng. Progr.*, **45**, 683 (1949).
(3) Cannon, M. R., *Ind. Eng. Chem.*, **41**, 1953–5 (1949).
(4) Fenske, M. R., *Ibid.*, **24**, 482 (1932).
(5) Heinlein, A. C., Manning, R. E., and Cannon, M. R., *Chem. Eng. Progr.*, **47**, 344–6 (1951).
(6) Miles, M., Feldman, J., Wender, I., and Orchin, M., *Ind. Eng. Chem.*, **43**, 1452 (1951).
(7) Peters, M. S., and Cannon, M. R., *Ibid.*, **44**, 1452 (1952).
(8) Podbielniak, Inc., 341 East Ohio St., Chicago 11, Ill., Bull. A-4D, 3.
(9) Reed, T. M., Pennsylvania State College, M.S. thesis, 1948.
(10) Scientific Development Co., Box 795, State College, Pa., Bull. 12, 3, 8.
(11) Struck, R. T., and Kinney, C. R., *Ind. Eng. Chem.*, **42**, 77 (1950).

Extraction of Uranium by 8-Quinolinol and Its Derivatives

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The extraction of uranium(VI) with 8-quinolinol and several of its derivatives has been studied. The pH ranges most suitable for extraction into chloroform are 5.8 to 8.0 for uranyl oxinate, 5.4 to 7.2 for uranyl dichlorooxinate (derived from 5,7-dichloro-8-quinolinol), and 5.6 to 7.3 for uranyl dibromooxinate (derived from 5,7-dibromo-8-quinolinol); the resulting bright orange colored metal complex solutions can be best measured spectrophotometrically at 430, 420, and 420 mμ, respectively.

EXTRACTION of a trace constituent from aqueous solution by an immiscible organic solvent is frequently the ideal method for isolating it from large amounts of foreign substances and concentrating it in a small volume of solution. Suitable chelating agents play a very important role in such extraction procedures, not only because of possible selectivity in their complexing action, but also because of the possible formation of metal complexes sufficiently strongly colored in organic solvents to permit the metals to be separated and prepared for spectrophotometric determination in one operation.

8-Quinolinol (8-hydroxyquinoline, oxine) and its derivatives form stable complexes with uranium(VI) [present as dioxouranium(VI) or uranyl ion, UO_2^{++}], which are extractable into organic solvents. Although the quinolinols are not selective reagents, it is possible to effect separation of uranium from certain other metals by solvent extraction at controlled pH. Moreover, the chloroform solutions of the quinolinol complexes of uranium (present in milligram quantities) are strongly orange colored. Consequently, in a quest for a suitable reagent for the extraction of uranium present in micro quantities, it was thought worth while to evaluate 8-quinolinol and its available derivatives. Complete information about 8-quinolinol in this respect is lacking and no work has been reported on derivatives of 8-quinolinol.

Rodden (5) summarizes the methods proposed on the Manhattan Project for the determination of uranium involving extraction by 8-quinolinol. Colorimetric methods based upon extraction of uranyl oxinate into chloroform and measurement at 400, 420, or 440 mμ (6) or at 425 mμ (3) have been proposed recently. Silverman, Moudy, and Hawby (6) have ably summarized the separation of uranium from elements which interfere in the extraction by 8-quinolinol. After a considerable portion of the present work was completed, Moeller and Ramaniah (4) described the absorption spectra of nonaqueous solutions of the pure 1 to 3 and 1 to 2 type chelates of uranium and oxine, and uranium and 5,7-dichlorooxine, and Dyrssen and Dahlberg (2) published a study of the extraction of uranyl ion by oxine-chloroform from unbuffered solution. Moeller (4) and Silverman

(6) and their coworkers have summarized many pertinent data on uranium(VI) 8-quinolinate and on the extraction of other 8-quinolinol chelates.

The present report deals with the investigation of the extraction behavior over the pH range of 2 to 12 of the species formed by uranyl ion with oxine (8-quinolinol), dichlorooxine (5,7-dichloro-8-quinolinol), and dibromooxine (5,7-dibromo-8-quinolinol). The experimental conditions were selected so as to simulate those usually encountered in standard analytical practice—e.g., the standard and analytical photometric curves were obtained under conditions which gave reproducible amounts of quinolate reagent in the reference and sample solutions. The presence of such excess reagent and the other working conditions were sufficient to minimize or at least to compensate for the effects due to traces of water in the chloroform solution of uranium oxinate, photochemical effects, and other potential causes of error (4, 6). In view of the study by Silverman, Moudy, and Hawby (6) on the preparation of uranium solutions for extraction by oxine-chloroform and the possible interferences, no attempt was made to gather detailed information on these topics; the present study emphasizes the optimum conditions for the extraction separation and the photometric analytical measurement.

8-Hydroxy-5-quinolinesulfonic acid and 8-hydroxy-7-iodo-5-quinolinesulfonic acid were found to be of little value as reagents for the extraction of uranium, as they are insoluble in chloroform, ether, and benzene.

EXPERIMENTAL

Apparatus. Spectrophotometric data were obtained with a Cary Model 11 recording spectrophotometer and two Beckman Model DU quartz spectrophotometers. A Beckman Model G pH meter was used for pH measurement. Glass-stoppered separatory funnels, burets, and volumetric flasks were used for solution extraction and preparation.

Reagents and Chemicals. All the chemicals used, unless otherwise mentioned, were chemically pure or analytical reagent grade materials.

Approximately 1% solutions in chloroform of 8-quinolinol (Mallinckrodt), 5,7-dichloro-8-quinolinol [prepared as described by Berg (1)], and 5,7-dibromo-8-quinolinol (Eastman Kodak) were employed. The 5,7-dichloro-8-quinolinol (melting point 180–181° C.; reported in literature 183° C.) gave on analysis 50.7% carbon and 2.3% hydrogen (calculated, 50.2 and 2.8%); the commercial product (melting point 172° to 178° C.) could not be used for extraction, as it gave a precipitate with uranium over the whole pH range of 2 to 10, which seemed to be due to the presence of an impurity.

A stock solution of uranyl sulfate, prepared by dissolving 18.45 grams of the trihydrate (Fisher Scientific Co.) in 12 liters of 1% sulfuric acid solution, was standardized by taking an aliquot, reducing to uranium(IV) by a Jones reductor, aerating to oxidize any uranium(III) to uranium(IV), and then titrating

with a standard dichromate solution; 1.00 ml. contained 0.85 mg. of uranium. For extraction studies, this solution was diluted fourfold so that the uranium content was 0.21 mg. per ml.

Approximately $1M$ buffer solutions were prepared as follows: 110 grams of dichloroacetic acid (Eastman Kodak practical grade) neutralized to pH 2.5 with ammonia and diluted to 1 liter; 60 grams of acetic acid and 13.9 grams of ammonium acetate adjusted to pH 4.0 by addition of ammonia and diluted to 1 liter; 77 grams of ammonium acetate dissolved in water, acidified with acetic acid to pH 5.9 and diluted to 1 liter; 54 grams of ammonium chloride with ammonia added to pH 8.0 and diluted to 1 liter; pH of the last solution adjusted to 7.0 by adding $1N$ hydrochloric acid; 61 grams of boric acid dissolved in water, the pH adjusted to 10.1 with hydrochloric acid and sodium hydroxide, and the volume brought to 1 liter; $1N$ hydrochloric acid added to a solution of $1N$ sodium hydroxide until a pH of about 12.5 was obtained.
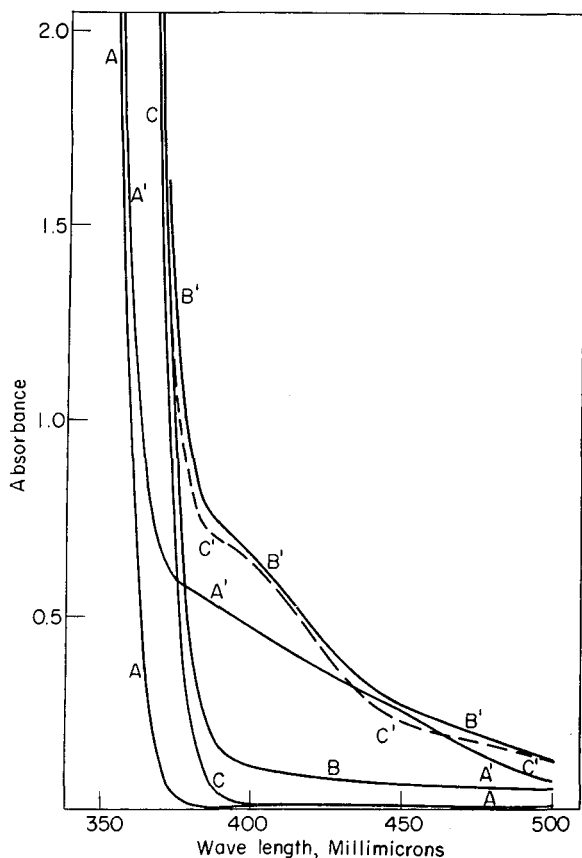

Figure 1. Absorption spectra

Chloroform solution
A. 8-Quinolinol
B. 5,7-Dichloro-8-quinolinol
C. 5,7-Dibromo-8-quinolinol
Uranyl complexes
A'. 8-Quinolinol
B'. 5,7-Dichloro-8-quinolinol
C'. 5,7-Dibromo-8-quinolinol
Solutions prepared by extracting 0.2% quinolinol solutions with buffer solution of pH 6 in absence and presence of 2 mg. U(VI) in buffer solution

Procedure. The general extraction and measurement procedures were as follows: A mixture of 10 ml. of uranyl sulfate solution (uranium = 2.1 mg.) and 25 ml. of buffer solution was shaken for 6 to 8 minutes with 20 ml. of a chloroform solution of the quinolinol (1%). The chloroform layer was separated and saved; the aqueous layer was then rinsed twice with 5-ml. portions of chloroform. The pH of the final residual aqueous layer was measured. The three chloroform extracts were combined and diluted to 100 ml. with chloroform; the absorbance of this solution was then measured at 430 mμ for 8-quinolinol and at 420 mμ for the dihalo-8-quinolinols, using matched 1-cm. Cortex

cells and a Beckman quartz spectrophotometer. The blank used was 20 ml. of the quinolinol solution (1%) in chloroform, which was extracted with 25 ml. of the same buffer solution as that used in the regular extraction, and then diluted to 100 ml. with chloroform.
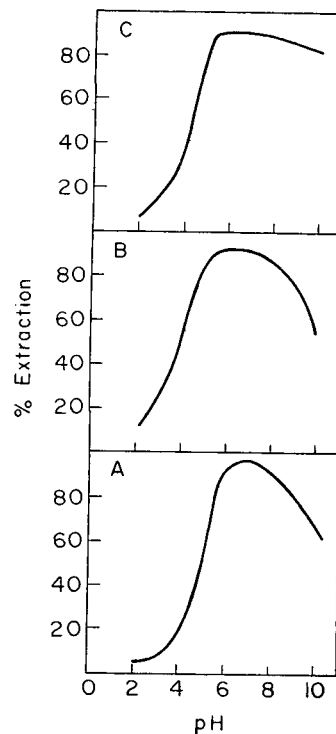

Figure 2. Extraction of uranyl complexes with chloroform as function of pH

A. 8-Quinolinol
B. 5,7-Dichloro-8-quinolinol
C. 5,7-Dibromo-8-quinolinol

Values of K_D (the working distribution constant for the experimental conditions used) were calculated from the extraction curves by the relation:

$$1 - \frac{\% \text{ extracted}}{100} = \frac{V}{K_D V' - V}$$

where V = volume of the aqueous phase, V' = volume of the organic phase, and K_D = distribution constant.

Calibration Data. Calibration curves of absorbance vs. percentage extraction were prepared as follows: 10 ml. of uranyl sulfate solution (uranium = 8.5 mg.) plus 25 ml. of buffer of pH 8.0 were exhaustively extracted three times with 10-ml. portions and then four times with 5-ml. portions of oxine solution (1%) in chloroform. Any precipitate produced was completely dissolved and the aqueous layer was colorless. The chloroform extracts were combined and diluted to 250 ml. with chloroform. This was used as the standard solution (100% extraction). The procedure followed in the case of the 5,7-dihalooxines was the same except that the buffer used was of pH 6.0. The standard solutions were diluted two-, four-, five-, and tenfold with chloroform and the absorbances of these solutions were measured at 430 mμ for the oxinate and at 410 mμ for the dihalooxinates.

ABSORPTION SPECTRA

For orientation purposes, absorption curves of chloroform solutions of the three oxines (about 0.2% solution) were run in the ultraviolet and visible regions against chloroform blanks on the Cary recording spectrophotometer, using 1-cm. quartz cells, and on the Beckman DU spectrophotometers, using 1-cm. Cortex cells. The absorption curves obtained on the directly prepared chloroform solutions of the oxines were compared with the curves ob-

tained on solutions prepared by extracting 20 ml. of a 1% oxine solution in chloroform with 25 ml. of pH 6 buffer solution, washing the aqueous phase twice with 5-ml. portions of chloroform, and then combining the three chloroform phases and diluting to 100 ml. with chloroform.

In the case of 8-quinolinol, the absorbance dropped to negligible values (circa 0.01 or less) at wave lengths longer than 390 mμ. The absorbance of the extracted solutions was a few thousandths greater than that of corresponding unextracted solutions. The values obtained on the Beckman spectrophotometer were usually a few thousandths greater than those found on the Cary. At 430 mμ, the analytical wave length used, the absorbances for the different instruments and conditions used were in the range of 0.000 to 0.007.

Unextracted chloroform solutions of the dichlorooxine had stronger adsorption with a drop in absorbance at wave lengths greater than 380 mμ, but still had appreciable absorption at 420 mμ ($A = 0.36$). The absorbance of the extracted solutions was considerably less—e.g., $A = 0.08$ at 420 mμ. The absorption of the dibromooxine solutions was considerably less—e.g., at 420 mμ, $A = 0.21$ for the original chloroform solution and 0.025 for the extracted solution. This is at least in part because of the higher molecular weight of the dibromooxine.

In Figure 1 are given pertinent portions of the spectrophotometric curves obtained on the Cary spectrophotometer for extracted chloroform solutions of the three oxines (about 0.2%) and of the corresponding uranyl oxinates (uranium = 2.1 mg. per 20 ml. of original buffer solution).

EXTRACTION DATA AND DISCUSSION

The extraction data are graphically shown in Figure 2; values of K_D, calculated from these curves, are tabulated in Table I.

Table I. Distribution Constants of Uranyl Oxinate, Dichlorooxinate, and Dibromooxinate between Chloroform and Buffer Solution as Function of pH

pH	Oxinate	Dichloro-oxinate	Dibromo-oxinate
		K_D	
2.0	0.1	0.3	0.2
2.5	0.1	0.3	0.2
3.0	0.2	0.5	0.4
3.5	0.3	0.7	0.5
4.0	0.4	2.1	1.1
4.5	0.9	5	2.4
5.0	2	9	6
5.5	6	18	16
6.0	16	20	16
6.5	27	20	16
7.0	85	20	16
7.5	33	18	14
8.0	16	14	13
8.5	10	8	12
9.0	7	6	11
9.5	7	3	9
10.0	4	2	8

8-Quinolinol seems to be a better extracting agent than its dichloro and dibromo derivatives. The pH ranges for 90% or greater extraction are 5.8 to 8.0 for uranyl oxinate, 5.4 to 7.2 for the dichlorooxinate, and 5.6 to 7.3 for the dibromooxinate; maximum extraction occurs at pH 7.0 (98%, $K_D = 85$), 6.0 to 6.6 (92%, $K_D = 20$), and 5.6 to 7.3 (90%, $K_D = 16$), respectively. This is to be compared to the pH range of 7 to 9 recommended for uranyl oxinate (5). There are significant drops in percentage extraction above pH 8. Above pH 12 the aqueous layers become yellow and turbid; the corresponding chloroform layers are colorless and exhibit no absorption.

The results obtained by different workers at different times are generally reproducible to within ±1%, except for those at pH 2 where the extent of extraction was small and the reproducibility was within ±5%. The total operations for an oxine extraction require about 15 to 20 minutes.

The chloroform solutions obtained were bright orange in color; measurements of absorbances, made at 430 mμ for the oxinate and 420 mμ for the dihalooxinates, followed Beer's law up to 0.03 mg. of uranium per milliliter in a 1-cm. cell. As little as 0.003 mg. of uranium per milliliter can be measured. Since the wave lengths selected for photometric measurement are on relatively sharply descending branches of the absorption curves, the sensitivity of the observed absorbance value to wave-length setting was checked by inserting a chloroform extract of uranyl oxinate in a Beckman DU spectrophotometer, taking an absorbance reading at 430 mμ, spinning the wave-length setting away by a considerable margin, resetting to 430 mμ, remeasuring the absorbance, and then repeating the process several times. The spread of each of two such series of six readings each was 0.001 for average absorbances of 0.352 and 0.356. A more stringent test of the reproducibility of wave-length setting is reflected in the concurrent results obtained by several different individuals working with two different Beckman spectrophotometers.

The extraction of oxine, 5,7-dichlorooxine, and 5,7-dibromooxine (0.004% solution in chloroform) at various pH values from 2 to 12 was followed spectrophotometrically in the ultraviolet region and gave results, which correlated nicely with the known pK values of the compounds. The suitable pH ranges for extraction of the oxines themselves are 4 to 8, 3 to 7, and 3 to 7, respectively.

To determine the effect of the presence of a complexing agent on the extraction of uranyl oxinate, a tartrate buffer solution was used (23 grams of sodium tartrate was dissolved in 100 ml. of water and the pH adjusted with 1N hydrochloric acid). The per cent extraction was 3 at pH 4.4; under the same conditions, but in the absence of tartrate, the extraction was 36%, indicating that tartrate strongly inhibits the extraction. Phosphate- and citrate-containing buffers were avoided in the work reported.

To determine the effects of time of standing, and of daylight and laboratory illumination upon the chloroform extracts of the uranyl oxinates, a uranium solution at pH 6 was extracted with 8-quinolinol while working rapidly in a fairly dark room. The absorbance of the combined and diluted chloroform solution was measured. The solution was then placed on the desk top under electric lights and daylight for about 15 minutes, and then the absorbance was remeasured; This process was repeated four times. The absorbance for the six measurements over a period of 75 minutes was 0.356 ± 0.001. A repetition of the experiment in which 16 minutes were consumed in the extraction process gave essentially identical results; a portion of the chloroform solution, which had stood for 60 minutes before being measured, had at the end of 130 minutes an absorbance within 0.003 unit of a portion which was measured immediately on the completion of extraction. Apparently, under the usual analytical conditions advocated in the present study, the presence of the excess quinolinol stabilizes the solution.

LITERATURE CITED

(1) Berg, R., "Die Analytische Verwendung von O-Oxychinolin ("Oxin") und seiner Derivative," p. 102, F. Enke Verlag, Stuttgart, 1938.
(2) Dyrssen, D., and Dahlberg, V., Acta Chem. Scand., 7, 1186 (1953).
(3) Hok, B., Svensk Kem. Tidskr., 65, 106 (1953).
(4) Moeller, T., and Ramaniah, M. V., J. Am. Chem. Soc., 76, 5251 (1954).
(5) Rodden, C. J., "Analytical Chemistry of the Manhattan Project," National Nuclear Energy Series VIII-I, pp. 116–120, McGraw-Hill, New York, 1950.
(6) Silverman, L., Moudy, L., and Hawby, D. W., ANAL. CHEM., 25, 1369 (1953).

Continuous Detection of Hydrogen or Hydrogenous Materials in Gases

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The concentration of hydrogen in any form must be rigidly controlled in intermediate energy nuclear reactors because of the high thermalizing cross section of hydrogen. This paper describes an instrument based on the dew point principle for the continuous detection of low concentrations of hydrogen or hydrogenous materials in the inert blanket gases required with liquid alkali metal coolants. A sensitivity of 0.001 volume % of hydrogen is claimed.

THE concentration of hydrogen in any form, hydrogen or hydrogen-containing compounds, in the inert gas blanket must be rigidly controlled in intermediate energy reactors employing liquid alkali metal coolants. If the concentration of hydrogen or hydrogenous compounds is too high, the neutrons will be thermalized with an accompanying increase in reactivity. Accordingly it is essential that the total hydrogen content of the inert blanket gas be continuously monitored for hydrogen or hydrogenous materials to assure the compliance of the blanket gas with specifications.

In a recent article (*2*) an instrument for continuous detection and measurement of low concentrations of oxygen in gases was described, based on the dew point principle. The continuous oxygen detector was constructed by additions to and modifications of the General Electric dew point recorder. A small quantity of hydrogen is added to the gas stream and the oxygen is converted to water over a platinum catalyst. The resulting water concentration is measured by determining the dew point of the gas on a continuous basis. By a simple valving arrangement the hydrogen injector and catalyst bed can be bypassed and the straight dew point of the gas determined. The difference between this value and the dew point after the oxygen has been converted to water is a measure of the oxygen concentration in the test gas. A sensitivity within 0.0005 volume % of oxygen is attained, with a mean deviation for precision within ±0.0001 volume %. A simple modification of this instrument will convert it to the detection and estimation of hydrogen instead of oxygen on a continuous basis.

Figure 1. Copper oxide furnace tube for continuous hydrogen detector

The hydrogen monitor is identical to the oxygen analyzer (*2*), except that a copper oxide bed is substituted for the hydrogen metering assembly and platinum catalyst bed. The copper oxide bed is mounted vertically and heated to 350° to 400° C. with a resistance furnace. The best results were obtained with the arrangement shown in Figure 1. The entering gas is thus heated before it comes in contact with the copper oxide and the entrance and exit connections are located at the bottom, resulting in a simplification and shortening of the copper tubing arrangement. The connection between the glass tubing and copper tubing at the exit end is made with silicone rubber tubing which will withstand the elevated temperature. Tygon tubing is adequate for the copper-to-glass entrance connection. The open leg on the stopcock is provided for flushing the assembly before use. In normal use with inert atmospheres the copper oxide bed is depleted very slowly because of the low concentrations of hydrogen involved. In the present installation the instrument has been in constant use for over a year without any regeneration of the copper oxide bed. However, if the copper oxide is depleted, it is easily regenerated in place by passing oxygen through the heated bed until the bright copper color at the top of the bed disappears.

The operation of the instrument is the same as for the detection and measurement of oxygen (*2*), except for the injection of hydrogen. A sensitivity within 0.001 volume % is attained by using the differential method (*2*) of setting the mirror temperature at any desired dew point corresponding to the specification concentration of total hydrogen in the gas when converted to water. At all hydrogen concentrations below this value the mirror will remain clear and the transient fluctuations in hydrogen concentrations below this value will not actuate the alarm system. At higher hydrogen concentrations, frost will form on the mirror and the alarm circuit will be energized.

The total hydrogen equivalent in the gas may be estimated by determining the dew point of the gas by bypassing the copper oxide bed. When this value has been determined, the gas is passed through the copper oxide bed. The value thus obtained will be the total dew point. The difference between these two values is equivalent to the total hydrogen concentration. The conversion from temperature to hydrogen concentration can be made by using dew point tables (*1*), remembering that 1 mole of hydrogen is equivalent to 1 mole of water.

The effectiveness of the instrument as a hydrogen detector was determined with a variety of materials of various vapor pressures and with gas mixtures. The response of the apparatus was followed by noting the time and intensity of a 60-watt light bulb plugged into the alarm outlet. The mirror temperature was set at the dewpoint of the helium carrier gas (−40° F.) obtained directly from a gas cylinder. The liquids were tested by bubbling the helium through the material at room temperature at the rate of 0.05 cu. foot per minute. The gases were made up in 1-liter bulbs fitted with stopcocks at each pole by diluting the sample with helium to 1 atmosphere, so that the concentration of the hydrogenous material was 0.002 volume %. The bulb or bubbler was attached with Tygon tubing in a bypass arrangement, so that equilibrium could be attained with the sweep gas and then the bulb or bubbler turned into the line, so that the sweep gas could carry the sample into the instrument. Accordingly, the actual concentration of sample was less than 0.002 volume % because of the dilution by the sweep helium.

On this basis the sensitivity is conservatively claimed to be within 0.001 volume %. The inherent sensitivity is undoubtedly lower than this, as 0.0005 volume % was easily attained in the case of the oxygen detector. Positive responses were attained within 5 seconds, although the gas had to pass through some 5 feet of tubing.

The materials tested are as follows: water, methanol, ethyl alcohol, benzene, chloroform, trichloroethylene, petroleum ether, toluene, hydrogen, methane, propane, butane, ethylene, propylene, and monomethylamine.

LITERATURE CITED

(1) General Electric Co., Schenectady, N. Y., "General Electric Dewpoint Recorder Instructions," GEI-40444.
(2) Pepkowitz, L. P., ANAL. CHEM., 27, 245 (1955).
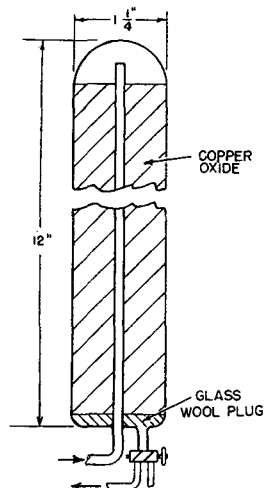
Dumas Nitrogen Determination on the Decimilligram Scale

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A procedure for the determination of nitrogen on the decimilligram scale using volatilization at high temperature and combustion over nickel oxide is described. Its accuracy, which mainly appears to be determined by the weighing accuracy, is equal to that of the ordinary microprocedures on the 5-mg. scale. One operator can easily carry out 10 complete analyses in a 7-hour working day. A procedure for the preparation of very pure carbon dioxide is given, and a few important points on the use of nickel oxide are discussed.

K IRSTEN (2) has described a method for the Dumas determination of nitrogen which involves volatilization of the sample in carbon dioxide at high temperature using the reaction $C + CO_2 \rightarrow 2CO$ and combustion of the gases obtained over nickel oxide. The procedure provides for a complete combustion of the samples and avoids the errors arising from retention of nitrogen in the combustion tube caused by formation of metal-oxygen-nitrogen compounds. [Copper and nickel oxides obtained by heating their respective nitrates have been investigated by x-ray diffraction at the Institute of Chemistry of this university. The samples gave exactly the same pattern as the ordinary oxides (9). This fact, together with the facts earlier known concerning these compounds (1, 2, 7), make it probable that the metal-oxygen-nitrogen compounds are oxides in which variable numbers of oxygen atoms have been replaced by nitrogen atoms.]

The high accuracy and simplicity of the new procedure made it interesting to try its applicability to the determination of nitrogen in very small samples.

The original equipment (2) was modified only slightly in the initial stages of this investigation. The diameters of the tubes were about half of those previously described. An ultramicro modification of the weight nitrometer described by Koch, Simonson, and Tashinian (3) was used. When a sample had been burned, most of the nitrogen was quickly swept out. However, when blank determinations were carried out, high blanks were immediately obtained. These high blanks decreased only very slowly, even though the apparatus was not opened after combustion. When the blank was subtracted from the sample reading, rather low results were obtained. An investigation showed that the silicone stopcock grease absorbed nitrogen in the apparatus and released it only very slowly.

Ungreased joints and rubber connections have previously (2, 4) been found still less satisfactory than greased joints, so it was necessary to reconstruct the apparatus to eliminate all joints or other connections in places where the grease could pick up nitrogen and give it off to the carbon dioxide which passes into the nitrometer.

APPARATUS

The apparatus which resulted after some experiments is shown in Figures 1 and 2 and a photograph in Figure 3. The combustion tube consists of the empty parts, A and B (Figure 1), divided by the side tube, C, and the wide part, L, filled with nickel oxide and held at 1000° C. by furnace M. The narrower end, O, is filled with Hopcalite. Capillary P has an inner diameter of 1 mm. and ends right in the joints, S, of the nitrometer tubes, Q. The rate of the carbon dioxide flow in the tube is regulated with the T-tube type pressure regulator, U, which is filled with mercury, and the capillary, AB. A small part of the carbon dioxide always passes

out the back through capillary H (Figure 2), and sweeps out any atmospheric nitrogen which has been absorbed by the grease of joint D during the introduction of the sample. Furnace K (Figure 1) is held at 1050° C. The foot, R, of the nitrometer is made of Lucite and filled with mercury. The nitrometer tubes are made of borosilicate glass and fit into the joints, S, of the foot. Joints S are lubricated with silicone grease. The calibrated capillaries of the nitrometers have a volume of 120 μl. in a length of 300 mm. The funnel, AF, of the nitrometers is made of Lucite and cemented
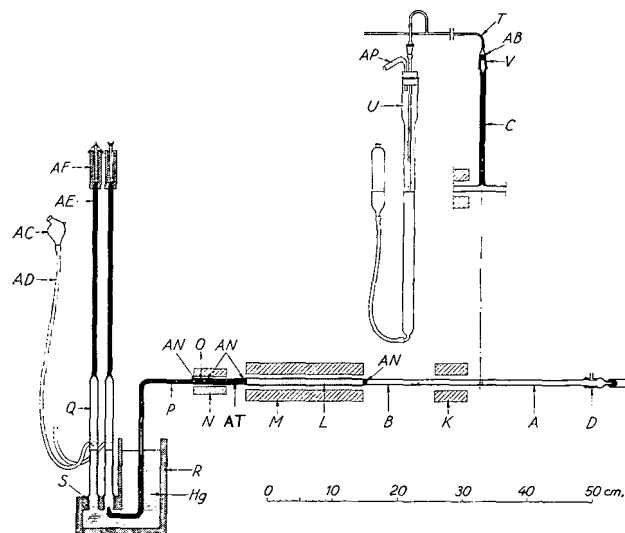

Figure 1. Setup for nitrogen determination on decimilligram scale

|	
A.	Back end of combustion tube, inner diameter 6 mm., outer 10 mm., length 180 mm.
B.	Tube in which sample is volatilized, diameters as A, length 240 mm.
C.	Side tube where carbon dioxide enters combustion tube, inner diameter 1 mm., outer 8 mm., length 180 mm.
D.	Ground joint through which sample is introduced, widest diameter of ground surface 12.5 mm., length 20 mm.
E.	Score in joint D which allows carbon dioxide to pass into side tube G.
F.	Female joint cap on joint D
G.	Side tube on cap F
H.	Capillary on joint cap F, diameter ca. 0.03 mm.
K.	Movable split-type furnace, held at 1050° C., length 70 mm.
L.	Wide part of combustion tube filled with nickel oxide, inner diameter 8 mm., outer 12 mm., length 180 mm.
M.	Split-type combustion furnace held at 1000° C., length 180 mm.
N.	Split-type heating furnace held at 130° C., length ca. 50 mm.
O.	Hopcalite, length of filling 10 mm.
P.	Capillary, inner diameter 1 mm., outer diameter ca. 6 to 7 mm., length suitable for combustion stand used
Q.	Nitrometer tubes, inner diameter 17 mm., outer 20 mm., length 210 mm.
R.	Foot of nitrometer made of Lucite
S.	Joints of nitrometer tubes; widest diameter of ground surface 18.8 mm., length 20 mm.
T.	Thin, flexible copper tubing
U.	Pressure regulator, T-tube type with glass tube connected to copper tubing T with tapered standard joint lubricated with silicone grease
V.	Tapered metal joint, widest diameter of ground surface 10 mm.
AB.	Capillary fixed in joint V with rubber tubing, diameter ca. 0.05 mm.
AC.	Leveling bulbs for potassium hydroxide
AD.	Surgical polyethylene tubing connection to leveling bulbs, inner diameter 3 mm.
AE.	Capillary of nitrometer, calibrated to contain 120 μl. on length of 300 mm.
AF.	Lucite funnel cemented to capillary of nitrometer
AG.	Screws in funnel AF for holding rubber cord AH
AH.	Rubber cord
AK.	Rubber stopper cemented into glass tube, AL, with Krönig's glass cement
AL.	Glass tube
AM.	Ground tapered end of capillary AE
AN.	Quartz wool
AP.	Drying tube containing iodine adsorbed on carbon
AR.	Rubber tubing holding capillary H
AS.	Glass tube which prevents orifice of capillary H from being contaminated with grease, etc.
AT.	Quartz rod
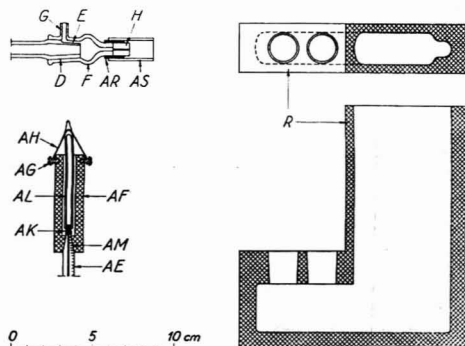
Figure 2. Detail of Figure 1

See legend under Figure 1

to the tapered joint, AM (Figure 2), of the capillary with plastic cement. AK is a rubber stopper which is cemented into the glass tube, AL, with Krönig's glass cement. It is held with the rubber cord, AH. In order to be able to use the two nitrometer tubes alternately, the Lucite foot, R, is mounted upon a mechanical manipulation device which allows one to raise it, move it sideward, and lower it again. The carbon dioxide used comes from a tank. The copper tube, T (Figure 1), is welded to the reducing valve of the tank. Joint V is a long tapered metal joint lubricated with silicone grease. AD are polyethylene tubing connections to the leveling bulbs, AC.

REAGENTS

Carbon Dioxide. Royer, Norton, and Foster (8) recommended tank carbon dioxide from which the top half has been blown off. This method was tested, with irregular results. Experiments revealed that the quality of the remaining gas is unsatisfactory when the first half of the filling is blown off slowly, but that the quality is good when the filling is blown off quickly, with a fully opened valve. It appears important to lower the pressure sufficiently over the liquid carbon dioxide so that any entrapped nitrogen greatly expands and is blown off. Much of the remaining liquid is probably precipitated as solid carbon dioxide. In order to obtain a quick lowering of the pressure and thereby decrease the loss of carbon dioxide, the tube may be cooled to a low temperature before the blowing off process.

The following procedure for the preparation of pure carbon dioxide is used in this laboratory:

The tank containing 10 kg. of liquid carbon dioxide is placed overnight in the freezing room at $-20°$ C. When it is removed, it is placed upright and the valve is at once opened fully. The valve is kept open until the sound of the escaping gas indicates that the pressure inside the tank has greatly decreased. The valve is then closed and the gas is ready for use. About 5 kg. of gas have been blown off. The remaining gas is sufficient for about 7 to 10 months of daily use in nitrogen determinations according to the procedure (2). No investigation has been made to determine whether satisfactory results could be maintained if less gas were blown off.

The microbubbles obtained with this gas in the present procedure seem to disappear completely. Even in the ultramicronitrometer no detectable blank is obtained during a single analysis.

Nickel Oxide. Approximately 15 setups employing nickel oxide in nitrogen determination are in use in Sweden. Several similar setups are in use in other countries. Most investigators report that these apparatus give complete combustion without formation of methane. Parks, Bastin, Agazzi, and Brooks (6) report incomplete combustion with a nickel oxide obtained by oxidation of nickel powder. In this laboratory precipitated nickelous oxide powder has been used for the preparation of the granulated nickel oxide according to the description given previously (2). Most of the users of the Dumas apparatus in Sweden have used the granulated nickelous oxide prepared in the same manner which is supplied by Nicroma (Klara Västra Kyrkogata

7, Stockholm). It appears probable that the particle size of the nickel oxide is an important factor. The authors attempted in an earlier experiment to use nickel oxide from wire, but the results were unsatisfactory, probably because nickel, unlike copper, does not allow a free diffusion of oxygen through it. Also, finely divided nickel is pyrophoric, while compact nickel is extremely resistant to oxidation. Precipitated nickel oxide should, therefore, be used for the preparation of the granulated reagent according to (2). For the use in the ultramicroprocedure, a granule size of about 20 to 30 mesh is most satisfactory.

Hopcalite. The commercially available product is satisfactory. Fine dust should be sieved off.
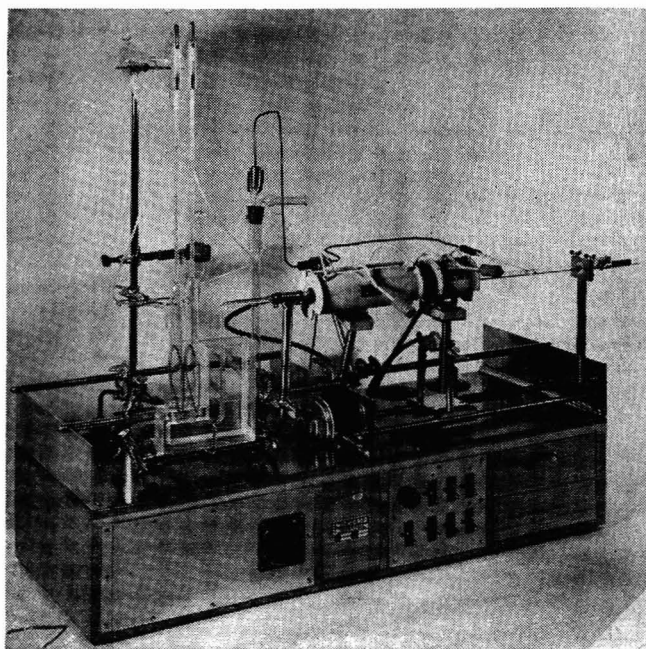
Quartz wool, C.P.

Figure 3. Equipment for nitrogen determination, with manipulator for nitrometers mounted upon automatic combustion carriage

Apparatus is available from Nicroma, Klara Västra Kyrkogata 7, Stockholm Sweden

Potassium hydroxide, 50% solution. About 2 liters are prepared and allowed to stand until any precipitations have settled. Then the potassium hydroxide is filtered through a sintered-glass filter. The amount used in every nitrometer is mixed by shaking with a few drops of isomyl alcohol before it is introduced (5).

ADJUSTING APPARATUS

The apparatus is assembled as shown in Figures 1, 2, and 3. The Hopcalite is introduced into the tube through a long glass tube in order to avoid contamination of the other parts of the combustion tube. The nickel oxide is glowed in a flow of oxygen just before filling it into the tube. The capillaries, AB and H, and the carbon dioxide pressure are adjusted to permit a slow flow back through H. The rate of the flow into the nitrometers should be adjusted so that the big gas bubbles obtained in an ordinary size combustion begin to decrease markedly 5 minutes after the arrival of furnace K to furnace M, or about 12 minutes after the beginning of the combustion. A combustion, carried out according to the procedure given below, is made to determine the necessary sweeping time. The blank is then determined for the obtained time. In the present work the blank obtained was zero.

PROCEDURE

The sample is weighed out in a small platinum boat. Joint D is opened and the boat is introduced with a glass rod into tube A, about 3 cm. from side tube C. The sample and the glass rod remain there for 5 minutes. The sample is then slowly pushed

into the middle of tube B. The glass rod is slowly drawn out and joint D is put on so that the gas passes out through side tube G, for 3 minutes. D is then turned so that the side tube is closed. Gas bubbles should now appear in the nitrometer. If no gas bubbles appear, the carbon dioxide pressure is increased for a moment by raising the leveling bulb of the pressure regulator, U. When the bubbles appear, the ordinary pressure is restored. Furnace K is now drawn over the tube and the motor is switched on. The next sample can now be weighed out.

After 30 minutes, joint D is opened, the boat is taken out, and the next sample is introduced. The nitrometer is moved so that the gas passes into the other tube and the gas collected in the nitrometer is moved to a suitable place for reading by loosening stopper AK. If there are several bubbles of gas in the capillary, the leveling bulb of the nitrometer is lowered and potassium hydroxide is allowed to flow down by loosening stopper AK. The gas bubbles and the potassium hydroxide move down together and are combined at the place where capillary AE widens. The leveling bulb is then raised again and the gas bubble is moved back into the capillary for reading. In the few instances when the bubbles do not combine, a small amount of mercury from R can be pipetted into the funnel and drawn down into the capillary. The bubbles will then combine below the mercury. The mercury is then allowed to fall down into the wide part of the nitrometer and the gas bubble is moved up again. There must be sufficient potassium hydroxide in the funnel to fill the capillary after the mercury falls. Care must be taken that no gas bubbles adhere to the mercury after its introduction into the funnel. Such gas bubbles can be removed with a thin glass rod or a wire.

The gas bubble now remains in the nitrometer for 30 minutes to drain. The next combustion is, in the meantime, carried out using the other nitrometer tube. The carbon dioxide bubbles which leave capillary P of the combustion tube do not pass up into the potassium hydroxide one by one, but they are collected below the upper surface of the mercury as a very big bubble which then breaks up and passes into the potassium hydroxide. This avoids all risk of the sticking of nitrogen bubbles to the mercury surface.

EXPERIMENTAL RESULTS

A series of analyses was carried out with this procedure. The necessary correction for the film of potassium hydroxide and the necessary waiting time for the drainage were unknown. After

the combustion, the gas bubble was drawn into the capillary so that the upper meniscus was situated at the line for 10 λ. The position of the lower meniscus was then read after several intervals of time. The results of the readings of some analyses, given in Table I, show that small volumes of nitrogen can be read almost immediately after combustion, while larger volumes require a considerable time for drainage. The 30 minutes for drainage reported above was chosen as a safe period of time under any circumstances. Analyses have been carried out with up to 0.5 mg. of lauric acid. The analyses gave absolutely no nitrogen reading.

It appeared that the most suitable procedure for the correction of the results for all systematical errors was to calculate the nitrogen results obtained without any correction and to calculate the average relative deviation according to the formula:

Average % relative deviation =

$$\frac{\dfrac{(\% \text{ N found} - \% \text{ N calcd.}) \times 100}{\% \text{ N calcd.}}}{n}$$

in which n is the number of analyses carried out.

The result was $+2.5\%$. This average relative deviation contains all systematical errors involved in the method, and it was subtracted from the "volume of nitrogen read" given in Table II. The values given in the column "volume of nitrogen corrected" were thus obtained. The final results were then calculated with these values, using the nomograph published by Koch, Simonson, and Tashinian (3).

The standard deviation of the results is 0.22 absolute %, which is not more than what is considered satisfactory for the ordinary Dumas method on the 5-mg. scale.

It appeared interesting to investigate the nature of the empiric correction obtained, 2.5 relative %. The corrections generally applied in the Dumas micro determination are a constant blank, a correction of 0.3% of the volume read for the water vapor tension over the potassium hydroxide solution (10) and a correction of 0.5% of the volume read for the volume of the potassium hydroxide film on the inner surface of the nitrometer (10). In the method reported the constant blank is zero. The tension of the water vapor must be the same as in the ordinary micromethod. However, in the ultramicronitrometer used, the ratio

$$R = \frac{\text{inner glass surface of capillary}}{\text{volume of capillary}}$$

is about five times that of an ordinary micronitrometer. A necessary correction of $5 \times 0.5\% = 2.5\%$ of the volume read should therefore be expected for the volume of the potassium hydroxide film. This gives a total correction of 2.8 relative %, which agrees very well with the 2.5% experimentally obtained. The difference of 0.3% lies beyond the limits of accuracy with which the theoretical calculation of the correction can be made. It appears therefore, that the correction of 2.5% is also theoretically fully justified.

It also appeared interesting to calculate the standard weighing error which would cause the standard deviation of 0.22% obtained for the analyses given in Table II. The calculation showed

Table I. Influence of Drainage Time on Volume of Nitrogen Read in Capillary Nitrometer

Minutes	Volume of N₂ Read, μl.					
0	3.0	9.0	26.6	36.4	63.8	96.9
5	3.0	9.0	26.1	36.3	63.8	96.9
10	3.0	8.9	26.0	35.8	63.8	96.9
15	3.0	8.9	26.0	35.7	63.7	96.8
20	..	8.9	25.9	35.6	63.6	96.2
25	..	8.9	25.9	35.5	63.4	95.7
30	3.0	8.9	25.9	35.5	63.3	95.7
40	..	..	..	35.5	63.3	95.7

Table II. Analyses of Nitrogen-Containing Compounds

No.	Compound	Wt. of Sample, γ	Corrected Barometer Reading, Mm. Hg	Temp., ° C.	Volume of Nitrogen, μl.		Nitrogen Content, %		Deviation, %
					Read	Corrected	Found	Calcd.	
1	p-Nitrobenzoic acid	172	754	29	13.3	13.0	8.50	8.38	+0.12
2	p-Nitrobenzoic acid	274	754	28	20.7	20.2	8.32	8.38	−0.06
3	Cyanacetamide	106	754	27	31.6	30.8	32.89	33.33	−0.44
4	3-Nitrophthalic acid	212	757	28	12.6	12.3	6.57	6.64	−0.07
5	Cyanacetamide	274	757	28	81.6	79.4	32.80	33.33	−0.53
6	Behenamide	94	757	28	3.7	3.6	4.35	4.12	+0.23
7	Cystine	106	757	29	11.0	10.7	11.39	11.66	−0.27
8	Taurine	206	757	30	20.4	19.9	10.87	11.20	−0.33
9	Taurine	136	757	28	13.4	13.1	10.89	11.20	−0.31
10	Taurine	224	759	30	22.9	22.3	11.23	11.20	+0.03
11	p-Nitrobenzoic acid	115	759	30	9.0	8.8	8.63	8.38	+0.25
12	dl-Serine	278	759	31	33.7	32.8	13.26	13.33	−0.07
13	4-Amino, 5-bromo, 4-methyl-pyrimidine	111	759	31	22.9	22.3	22.58	22.34	+0.24
14	Behenamide	85	761	32	3.3	3.2	4.25	4.12	+0.13
15	3-Nitrophthalic acid	178	761	32	10.7	10.4	6.56	6.64	−0.08
16	Cyanacetamide	135	761	30	40.8	39.8	33.31	33.33	−0.02
17	Cystine	248	762	28	25.8	25.1	11.53	11.66	−0.13
18	p-Nitrobenzoic acid	362	763	31	27.5	26.8	8.36	8.38	−0.02
19	Serine	288	763	29	34.2	33.7	13.30	13.33	−0.03
20	Taurine	518	763	32	51.7	50.4	10.96	11.20	−0.24
21	p-Nitrobenzoic acid	175	763	30	13.3	13.0	8.42	8.38	+0.04
22	p-Nitrobenzoic acid	241	759	29	18.4	17.9	8.40	8.38	+0.02

Standard deviation, σ = 0.22 absolute %.

that a standard error of 3.5 γ in the weight of the samples would cause this deviation. As every sample weight is the difference of two weighings it appears that not even the best tested balances could be expected to give much better results (11). The Ainsworth balance Model F.D.J. on which the weighings were carried out has been in daily use in the laboratory since 1948. So, if it were assumed that the errors in the determinations were caused by the weighing errors alone—which of course is impossible—the results would still be a good proof of the excellent performance of this balance. The method is so fast and convenient that one operator easily and without having to hurry can carry out 10 complete analyses, including weighing, running the analysis, and calculating the results, in a 7-hour working day. Experiments to apply the method to the determination of submicrogram amounts of nitrogen by the use of an ultramicrobalance and finer nitrometers are planned.

LITERATURE CITED

(1) Duval, Clement, *Anal. Chim. Acta*, 2, 438 (1948).
(2) Kirsten, W. J., *Mikrochemie*, 40, 121 (1952).
(3) Koch, C. W., Simonson, T. R., and Tashinian, V. H., ANAL. CHEM., 21, 1133 (1949).
(4) Lindner, Josef, "Mikromassnalytische Bestimmung des Kohlenstoffes und Wasserstoffes mit grundlegender Behandlung der Fehlerquellen in der Elementaranalyse," Verlag Chemie, Berlin, 1935.
(5) Pagel, H. A., and Oita, I. J., ANAL. CHEM., 24, 756 (1952).
(6) Parks, T. D., Bastin, E. L., Agazzi, E. J., and Brooks, F. R., *Ibid.*, 26, 229 (1954).
(7) Richards, Th. W., *Z. anorg. Chem.*, 1, 187 (1892).
(8) Royer, G. L., Norton, A. R., and Foster, F. J., IND. ENG. CHEM., ANAL. ED., 14, 79 (1942).
(9) Schönberg, N. E., Söderfors Bruk, private communication.
(10) Trautz, O. P., *Mikrochemie*, 9, 300 (1931).
(11) Waber, J. T., and Sturdy, G. E., ANAL. CHEM., 26, 1177 (1954)

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Complexometric Determination of Sulfide

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A complexometric method for the determination of sulfide ion is described. The method is based on the observation that an alkaline sulfide solution added to a neutral or slightly acid solution containing an excess of metal perchlorate gives a stoichiometric precipitate with copper but not with zinc or cadmium.

THE modern volumetric methods of analysis employing ethylenediaminetetraacetic acid [(ethylenedinitro)tetraacetic acid, EDTA] and related compounds are characterized by a high degree of accuracy and also by the fact that the titer of the standard solutions is extraordinarily stable. In seeking a complexometric method for the determination of sulfide ion in which the principle of difference could be used, the following results were discovered. When the alkaline sulfide solution to be analyzed was added to a neutral or slightly acid solution containing an excess of a metal perchlorate, zinc and cadmium gave colloidal precipitates and erroneous results. The cupric ion, on the other hand, gave a stoichiometric precipitate of copper sulfide which is known to be extremely slightly soluble (2) and was easily filterable.

The sulfide solution to be analyzed was prepared from analytical grade sodium sulfide nonahydrate using oxygen-free water, and was kept under hydrogen atmosphere. Samples were taken by means of a buret connected to the sulfide flask and the standardization was done iodometrically (3, 4). The titration of the standard solution and the excess of copper was performed according to Flaschka (1). The reagent solutions were of the order of 0.05M.

Table I lists a few results obtained with the iodometric and the new methods.

From the results obtained one can judge that the new method is as accurate as the classical iodometric one. However, in addition to the advantages mentioned above, the new method is also characterized by the fact that, in the medium used, the sulfide only will be precipitated as copper sulfide, leaving the sulfite and thiosulfite ions in the filtrate. As is known, iodine oxidizes all of these ions. A possible disproportionation of the cupric sulfide into cuprous sulfide and sulfur obviously does not affect the final result.

REAGENTS

Disodium salt of EDTA (Complexon III), 0.05M.
Cupric perchlorate, 0.05M.
Ammonia, 1M.
Acetate buffer (0.67M acetic acid + 0.33M sodium acetate), 1M.
Saturated water solution of murexide.

PROCEDURE

Pipet 25 ml. of the copper solution into a 150-ml. Erlenmeyer flask, rinse it down with little water, and add 15 ml. of the acetate buffer. Add slowly the sodium sulfide solution (10 to 40 ml. of an approximately 0.02M solution or equivalent), constantly shaking the flask. Filter off the copper sulfide using a fine-porosity (G4) fritted-glass funnel and a 250-ml. suction flask. Wash with 20 to 30 ml. of hot water. Add a few milliliters of ammonia to the filtrate until the deep blue copper solution is clear, and dilute to 100 to 120 ml. Add 3 to 6 drops of murexide indicator and titrate to a reddish violet color.

LITERATURE CITED

(1) Flaschka, H., *Mikrochemie*, 39, 38 (1952).
(2) Goates, J. R., Gordon, M. B., and Faux, N. D., *J. Am. Chem. Soc.*, 74, 835 (1952).
(3) Kolthoff, I. M., *Z. anal. Chem.*, 60, 451 (1921).
(4) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., p. 2183, Van Nostrand, New York, 1947.

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Table I. Standardization of Sodium Sulfide Solution Using Iodometric and Complexometric Methods

(All reagents approximately 0.05M)

| I2, Ml.	Na2S, Ml.	Molarity of Na2S Found	Cu, Ml.	Na2S, Ml.	Molarity of Na2S Found
25	10	0.01710	25	10	0.01700
25	10	0.01710	25	15	0.01696
25	15	0.01711	25	20	0.01709
25	20	0.01696	25	30	0.01695
50	40	0.01696	25	40	0.01700
		Av. 0.01704			Av. 0.01700

Volumetric Determination of Soluble Silicates in Detergents

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A rapid volumetric method has been developed for the determination of soluble silica in alkaline detergents containing various compositions of the soluble silicates. Sodium oxide can also be determined on the same portion of the sample. Modifications necessary for the accurate volumetric determination of the soluble silica in the presence of carbonates, phosphates, and wetting agents are described and discussed. The results obtained are in agreement with the gravimetric values to within ±0.05% of silicon dioxide.

THE gravimetric methods for the determination of soluble silica consist of evaporating the solutions containing the silicates with either hydrochloric, sulfuric, or perchloric acid in order to dehydrate the silica. The silica is filtered off, washed, dried, and subsequently ignited and weighed. For very accurate results it is also necessary to treat the final weighed silica with hydrofluoric acid and to subtract the residue from the total weight to obtain the actual silica present. These procedures are tedious and time-consuming. Since this laboratory is interested in the rapid determination of silica in various alkaline silicates and detergents for plant control during manufacture, a rapid procedure for the determination of silica which can be used successfully by nontechnical laboratory personnel is desirable.

Vail (3) mentions that soluble silica can be determined in alkaline silicates by first titrating a suitable aliquot with 2N hydrochloric acid to a methyl red end point and then completing the titration after the addition of sodium fluoride.

$$SiO_2 + 6F^- + 6H^+ \rightarrow 2H^+ + SiF_6^{--} + 2H_2O$$
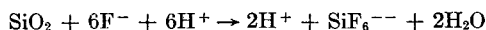
The above reaction is not instantaneous, making it very difficult to obtain the proper end point.

Halfter (1, 2) determined silica in alkaline silicates by adding to a measured volume of 0.33N sodium fluoride or potassium fluoride solution, 0.1N hydrochloric acid to a pH of 4.9. This solution was then mixed with a measured quantity of an alkaline silicate solution containing no cation other than alkali. The solution was again titrated back to a pH of 4.9 with 0.1N hydrochloric acid in the presence of bromocresol green or purple as indicators. The calculation is based on the equation:

$$6NaF + SiO_2 + 4HCl = Na_2SiF_6 + 2H_2O + 4NaCl$$
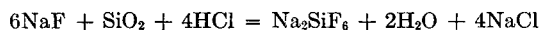
This procedure is also slow and does not give the quick reliable results necessary for plant control. Consequently the following rapid volumetric method was developed. With the following modifications, accurate and quick determinations of silica can be made on compositions containing carbonates, phosphates, and surfactants. The sodium oxide may also be determined on the same portion of the sample.

REAGENTS

Sodium fluoride, reagent grade, Merck & Co., Inc.
Hydrochloric acid, standardized, 1N.
Sodium hydroxide, standardized, 1N.
Methyl red indicator (1 gram dissolved in 600 ml. of 95% ethyl alcohol diluted with 400 ml. of water).
Methyl red–xylene cyanol FF indicator, 0.8 gram of methyl red and 0.2 gram of xylene cyanol FF dissolved in 1000 ml. of 95% ethyl alcohol.
Methyl orange, 0.1% in water.
Ethyl alcohol, 95%.
Foamex, Glyco Products Co., 26 Count St., Brooklyn 2, N. Y.

PROCEDURE AND RESULTS

Analysis of Sodium Silicates. A 20-gram sample of sodium silicate is dissolved in distilled water, transferred to a 1000 ml. volumetric flask, cooled, and diluted to the mark. A 50-ml. aliquot is transferred to a 250-ml. beaker, and approximately 0.5 ml. of methyl red indicator is added. The solution is titrated with 1N hydrochloric acid to the first color change and the volume of 1N hydrochloric acid at this point is noted. Approximately 5 grams of sodium fluoride are added to the titrated solution and as much as possible is dissolved by agitation. After the addition of 25 ml. of ethyl alcohol, the titration with 1N hydrochloric acid is continued until the color of the solution is definitely red, indicating an excess of hydrochloric acid. An excess of about 2 ml. of 1N hydrochloric acid is sufficient. About 0.5 ml. of the methyl red–xylene cyanol FF indicator is added at this point and the solution is titrated back with 1N sodium hydroxide until the end point is reached. The end point color is an intermediate gray or the color immediately after the disappearance of the pink and before the appearance of the green.

Calculation.

a = ml. of 1N hydrochloric acid to first methyl red end point
b = ml. of 1N hydrochloric acid after the addition of sodium fluoride and ethyl alcohol
c = ml. of 1N sodium hydroxide used for back-titration

$$\% \ SiO_2 = (b\text{-}a\text{-}c)\ (0.01502)\ (100)$$

$$\% \ Na_2O = (a)\ (0.03100)\ (100)$$

The authors analyzed samples of Orthosil (Pennsylvania Salt Manufacturing Co.) and other soluble sodium silicates by the volumetric method and compared the results (Table I) with the gravimetric method, using sulfuric acid to dehydrate the soluble silicic acid to insoluble silica.

Analysis of Sodium Silicates in Presence of Carbonates. The sample of the detergent to be analyzed is prepared as above and a 50-ml. aliquot is transferred to a 250-ml. beaker. Approximately 0.5 ml. of methyl orange is added and the solution is

Table I. Per Cent of Silica in Sodium Silicates

Sample	Gravimetric Method			Volumetric Method			Difference, %
	1	2	Av.	1	2	Av.	
Orthosil[a]	29.92	29.89	29.91	29.91	29.94	29.93	+0.02
	29.03	29.00	29.02	29.04	29.04	29.04	+0.02
	28.70	28.75	28.73	28.73	28.70	28.72	−0.01
	29.80	29.83	29.82	29.85	29.80	29.83	+0.01
	29.00	29.10	29.05	29.04	29.00	29.02	−0.03
Sodium silicate	27.65	27.66	27.66	27.63	27.63	27.63	−0.03
	4.87	4.87	4.87	4.86	4.86	4.86	−0.01
Liquid sodium silicate	25.39	25.37	25.38	25.35	25.35	25.35	−0.03
Metso[b]	28.44	28.53	28.49	28.45	28.50	28.48	−0.01
Sodium disilicate (GD)[b]	55.03	55.17	55.10	55.20	55.10	55.15	+0.05

[a] Pennsylvania Salt Manufacturing Co.
[b] Philadelphia Quartz Co.

Table II. Per Cent of Silica in Orthosil–Sodium Carbonate Compositions

Composition		Gravimetric Method			Volumetric Method			Difference, %
Orthosil, %	Na₂CO₃, %	1	2	Av.	1	2	Av.	
99.00	1.00	29.03	29.00	29.02	29.04	29.04	29.04	+0.02
95.00	5.00	27.47	27.38	27.43	27.37	27.41	27.39	−0.04
90.00	10.00	26.05	25.84	25.95	25.85	25.85	25.85	−0.10
50.00	50.00	14.40	14.42	14.41	14.44	14.44	14.44	+0.03

Table III. Per Cent of Silica in Orthosil–Sodium Carbonate–Surfactant Compositions

Composition, %			Gravimetric Method			Volumetric Method			Differ-ence, %
Orthosil	Na₂CO₃	Surfactant, 2%	1	2	Av.	1	2	Av.	
98	...	Nonic	28.50	28.55	28.53	28.46	28.50	28.48	−0.05
98	...	Kreelon	28.52	28.52	28.52	28.46	28.50	28.48	−0.04
98	...	Santomerse	28.52	28.56	28.53	28.52	28.56	28.54	+0.01
97	1.00	Nonic	28.50	28.55	28.53	28.46	28.50	28.48	−0.05
90	8.00	Nonic	25.66	25.71	25.69	25.63	25.67	25.65	−0.04
90	8.00	Kreelon	25.65	25.68	25.67	25.70	25.65	25.68	+0.01

Table IV. Per Cent of Silica in Orthosil–Sodium Carbonate–Phosphate Compositions

Composition, %			Gravimetric Method			Volumetric Method			Differ-ence, %
Orthosil	Na₂CO₃	Phosphate	1	2	Av.	1	2	Av.	
66.67	21.67	11.66 as Quadrafos	19.03	19.04	19.04	19.07	19.07	19.07	+0.03
60.00	25.00	15.00 as sodium tripolyphosphate	18.10	18.12	18.11	18.10	18.10	18.10	−0.01

titrated to the end point with 1N hydrochloric acid. The carbon dioxide is removed from the solution by air-lancing with carbon dioxide–free air for 5 minutes. The incomplete removal of carbon dioxide will give low results. Approximately 5 grams of sodium fluoride are added to the solution and as much as possible is dissolved by agitation. About 0.5 ml. of methyl red–xylene cyanol FF indicator is added and after the addition of 25 ml. of ethyl alcohol the titration is continued until an excess, or about 2 ml. of hydrochloric acid, are present. The excess hydrochloric acid is back-titrated with 1N sodium hydroxide to the end point.

Calculations.

a = ml. of 1N hydrochloric acid to first methyl orange end point

b = ml. of 1N hydrochloric acid after addition of NaF and C_2H_5OH

c = ml. of 1N sodium hydroxide used for back-titration

$$\% \ SiO_2 \ = \ (b\text{-}a\text{-}c) \ (0.01502) \ (100)$$

$$\% \ Na_2O \ = \ (a) \ (0.03100) \ (100)$$

Mixtures of Orthosil and sodium carbonate were prepared in the laboratory in order to show the effect of the presence of carbonates. The results and compositions of the mixtures are listed in Table II.

Analysis of Sodium Silicates in Presence of Carbonates and Surfactants. The procedures described above are followed, except that it is necessary to add 2 or 3 drops of Foamex during the air-lancing to prevent excess foaming. The presence of surfactants—such as Nonic (Sharples Division, Pennsylvania Salt Manufacturing Co.), Kreelon (Wyandotte Chemical Co.), and Santomerse (Monsanto Chemical Co.) does not interfere in the titration for soluble silica. The detergent compositions analyzed were prepared in the laboratory. The results are given in Table III.

Analysis of Sodium Silicates in the Presence of Carbonates, Phosphates, and Surfactants. The reagents required are identical to those above, except that methyl orange indicator is used instead of methyl red. A suitable aliquot is transferred to a 250-ml. beaker and titrated to the methyl orange end point with 1N hydrochloric acid. The titrated solution is air-lanced for about 5 minutes with carbon dioxide-free air. Five grams of sodium fluoride are dissolved in the titrated solution and the resulting solution is titrated with 1N hydrochloric acid after the addition of 25 ml. of ethyl alcohol and 0.5 ml. of xylene cyanol FF indicator. An excess of about 2 ml. of the 1N hydrochloric acid is added and back-titrated with 1N sodium hydroxide to the end point.

Calculations.

a = ml. of 1N hydrochloric acid to methyl orange end point

b = ml. of 1N hydrochloric acid after addition of sodium fluoride and ethyl alcohol

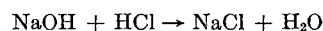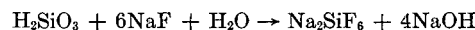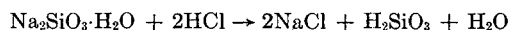c = ml. of 1N sodium hydroxide used for back-titration

$$\% \ SiO_2 \ = \ (b\text{-}a\text{-}c) \ (0.01502) \ (100)$$

$$\% \ Na_2O \ = \ (a) \ (0.03100) \ (100)$$

The analyses of mixtures containing Orthosil, sodium carbonate, and phosphates are listed in Table IV.

DISCUSSION

The reactions

$$Na_2SiO_3 \cdot H_2O \ + \ 2HCl \rightarrow 2NaCl \ + \ H_2SiO_3 \ + \ H_2O$$

$$H_2SiO_3 \ + \ 6NaF \ + \ H_2O \rightarrow Na_2SiF_6 \ + \ 4NaOH$$

$$NaOH \ + \ HCl \rightarrow NaCl \ + \ H_2O$$

involved in this analytical procedure show that 4 moles of sodium hydroxide are liberated for each mole of sodium silicate contained in the detergent. The resulting liberated sodium hydroxide can then be titrated with the standardized acid. In preliminary work the authors attempted to titrate the liberated sodium hydroxide directly but found that the reaction was slow and the end point obscure. Near the end point it was necessary to add the standardized acid in small increments and wait until the reaction was completed before continuing to the end point. Incorrect results could be obtained if the time factor was not considered. The authors found that by adding an excess of standard acid and ethyl alcohol the reaction rate was increased, so that no waiting period was necessary to obtain a sharp and accurate end point. The use of xylene cyanol FF–methyl red indicator increases the accuracy of the end point so that it can be readily observed by nontechnical personnel.

REFERENCES

(1) Halfter, George, *Angew. Chem.*, **61**, 413 (1949).
(2) Halfter, George, *Z. anal. Chem.*, **128**, 266–9 (1948).
(3) Vail, J. G., "Soluble Silicates, Their Properties and Uses," vol. 1, pp. 40–1, Reinhold, New York, 1952.
(4) Willard, H. W., and Diehl, H., "Advanced Quantitative Analysis," p. 186, Van Nostrand Co., New York, 1943.

Infrared Analysis of Commercial Diethyl Ethylmalonate

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A simple infrared analysis designed for production control of diethyl ethylmalonate containing small amounts of diethyl malonate and diethyl diethylmalonate is described. The method is based on the determination of the absorbances at 11.18, 11.85, and 13.51 microns of a 70% solution of the sample in carbon tetrachloride. These bands are characteristic of diethyl ethylmalonate, diethyl malonate, and diethyl diethylmalonate, respectively.

DIETHYL ethylmalonate is an important intermediate in the production of several barbituric acids. The ester is usually prepared by the ethylation of diethyl malonate, and the reaction product contains variable amounts of diethyl malonate and diethyl diethylmalonate. The customary method of purification is by fractional distillation. In large scale operations it is difficult to separate the diethylated ester cleanly from the desired monoethylated ester. If sufficient care is not exercised in the distillation operation, significant amounts of unalkylated ester may also be found in the distillate. As the purity of the barbituric acids and the economies of process yields are dependent on the quality of malonate esters used in the syntheses, it is desirable to control closely the purity of monoethylated ester. To this end, a relatively simple and speedy method of analysis was sought.

It has been reported (4) that mixtures of these esters may be analyzed by selective saponification with alkali. This method proved to be too erratic to be used as an effective control procedure. A study of the infrared spectra of the purified esters indicated the presence of suitable absorption bands, and a simultaneous three-component analysis of relatively pure mixtures of the three malonate esters was developed.

EXPERIMENTAL

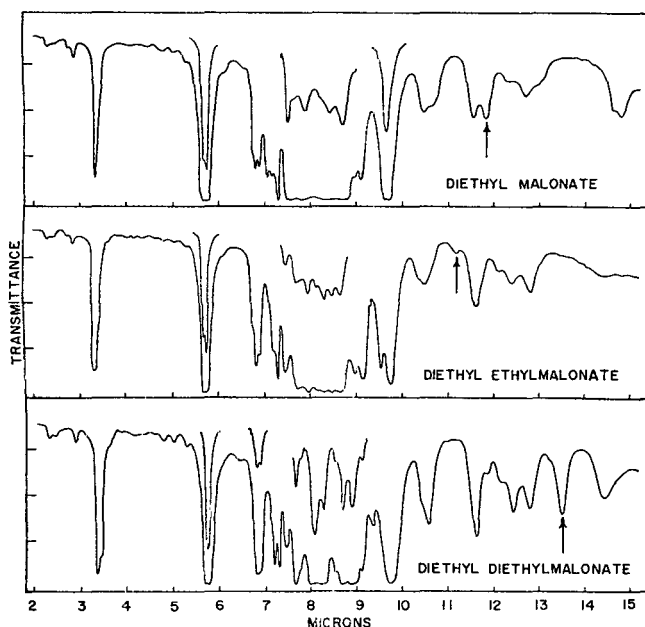The pure esters used in obtaining the qualitative absorption spectra and in preparing the working curves were obtained by repeated fractional distillation, using a 90-cm., glass-helix packed column at a reflux ratio of 10 to 1. Heart-cuts of those fractions showing no change in boiling point were chosen. The boiling points and refractive indices of the esters were: (a) diethyl malonate, b_{20}, 96°; $n_D^{26.2}$, 1.4118 (b) diethyl ethylmalonate, b_{20} 104.5°; $n_D^{25.8}$, 1.4143 and (c) diethyl diethylmalonate, b_{20}, 116°; $n_D^{27.2}$, 1.4222.

The qualitative absorption spectra (Figure 1) were obtained using a Perkin-Elmer Model 21 spectrophotometer. A Perkin-Elmer Model 12C spectrometer was used for all quantitative measurements.

Working curves of absorbance vs. concentration were established in the usual manner for a three-component analysis (2). The ranges studied corresponded to compositions of 70 to 100% of diethyl ethylmalonate and 0 to 15% each of diethyl malonate and diethyl diethylmalonate. A linear relationship between absorbance and concentration was found for each component.

Procedure. Adjust the amplifier gain of the Perkin-Elmer Model 12C spectrometer to obtain full scale deflection with a 4-μv. test signal. Transfer exactly 7.0 ml. of sample to a suitable flask, add 3.0 ml. of carbon disulfide (analytical reagent grade), stopper tightly, and mix well. Transfer the solution to a 0.1-mm. sodium chloride cell. Using the "cell in, cell out" technique, determine the absorbance of the solution at 11.18, 11.85, and 13.51 microns vs. a 0.1-mm. sodium chloride cell containing carbon disulfide. The cells need not be matched perfectly.

Table I. Results of Analyses by Proposed Method

	Sample	Diethyl Ethylmalonate, % Theory	Found	Diethyl Malonate, % Theory	Found	Diethyl Diethylmalonate, % Theory	Found
Synthetic blends	1	92.8	93.8	3.6	3.3	3.6	3.0
	2	85.7	86.3	7.2	9.2	7.2	6.0
	3	85.7	86.5	7.2	9.3	7.2	5.7
Commercial preparations	1	...	94.0	...	2.0	...	4.0
		...	93.8	...	2.8	...	3.8
	2	...	96.7	...	1.1	...	3.3
		...	95.6	...	2.0	...	3.4
	3	...	94.0	...	2.8	...	4.3
		...	93.3	...	3.5	...	4.2
	4	...	92.7	...	4.0	...	2.9
		...	92.7	...	4.5	...	3.0
	5	...	88.8	...	0.0	...	11.0
		...	89.9	...	0.0	...	10.5
	6	...	96.0	...	0.0	...	5.0
		...	96.5	...	0.0	...	4.9

The difference between absorbances of solvent-filled blank and sample cells is checked frequently and the necessary corrections are applied to the absorbance determinations. Refer the corrected absorbances to the working curves and calculate the percentage of esters graphically (2).

DISCUSSION

Figure 1 shows the qualitative curves for the pure esters. The analytical bands are designated by arrows. The band chosen for diethyl ethylmalonate is relatively weak. This proved to be a desirable feature inasmuch as commerical preparations usually contain 90% or more of this component. Conversely, the stronger bands chosen for diethyl malonate and diethyl diethylmalonate provided for a satisfactory determination of these minor components.

Table I shows the results obtained on several synthetic mixes of known ester composition, and on several samples of commercial diethyl ethylmalonate. While the relative error of the method is large, especially for the minor components, the absolute error is sufficiently small so that all the information necessary for adequate plant control may be obtained.

Figure 1. Qualitative absorption spectra of pure esters

The method is satisfactory for preparations containing no significant amounts of impurities. Occasional lots have been encountered containing entrained ethylating agents or other substances which invalidate the method. Preliminary investigation has shown that in these cases the diethyl ethylmalonate and diethyl diethylmalonate content may be estimated by resort to the base line method (1, 3, 5), using the bands at 11.18 and 13.51 microns, respectively. This method is less sensitive to impurities than the three-component spot method, but is not satisfactory for estimating diethyl malonate.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Barnes, R. B., Gore, R. C., Williams, E. F., Linsley, S. G., and Petersen, E. M., ANAL. CHEM., 19, 620 (1947).
(2) Carol, J., Molitor, J. C., and Haenni, E. O., J. Am. Pharm. Assoc., Sci. Ed., 37, 173 (1948).
(3) Friedel, R. A., and Pierce, L., ANAL. CHEM., 22, 418 (1950).
(4) Gamas, A. S., Zavodskaya Lab., 10, 155 (1941).
(5) Heigl, J. J., Bell, M. F., and White, J. U., ANAL. CHEM., 19, 293 (1947).

Beta-Mercaptopropionic Acid as Colorimetric Reagent for the Determination of Cobalt

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β-Mercaptopropionic acid is a useful reagent for the colorimetric determination of cobalt in dilute solution. The presence of nickel or copper in the same order of concentration is not detrimental, as an excess of ammonium hydroxide which does not affect the cobalt test, will discharge any color due to these metals.

THIOGLYCOLIC acid (2) was found to be a very useful reagent for the colorimetric determination of iron. β-Mercaptopropionic acid (B. F. Goodrich Co., Cleveland, Ohio), although of no particular value in the test for iron, was found to give excellent results with cobalt. Its use in the determination of nickel has been studied by Uhlig and Freiser (3) and Lear with Mellon (1).

COLOR REACTION WITH COBALT

When a drop of mercaptopropionic acid is added to a concentrated solution of a cobaltous salt and the resulting mixture is made alkaline with ammonium hydroxide, a deep green solution is obtained. The depth of color depends on the concentration of cobalt and fails to appear when the concentration of cobalt is less than about 2.5 p.p.m. Prepared "unknowns" in concentration of 2.5 to 20 p.p.m. lend themselves readily to matching with known standards, so that the method may be used for quantitative determination.

The green color, particularly in the more concentrated solutions, soon begins to turn dark and ultimately the color becomes brown. It was found that oxidizing agents speed up this change. In the absence of metal ions such as mercury (II) or gold (III) (which may reduce to the metal), a "pinch" of sodium hyposulfite ($Na_2S_2O_4 \cdot 2H_2O$) added to the mixture prior to the addition of the ammonium hydroxide, retards the oxidation. The resulting brilliant bluish green tint makes the matching of color easier and it is stable over many hours.

COLOR REACTION WITH NICKEL

Because nickel is frequently associated with cobalt, the effect of its presence was studied. With nickel the color obtained (when the test is carried out as previously described for cobalt) is deep red. Obviously, it was found to interfere with the cobalt test and vice versa. However, in dilute solution (concentration as given for cobalt), the red color due to nickel is readily discharged by an excess of ammonium hydroxide. Consequently, in carrying out the test on nickel one must add only enough hydroxide just to neutralize the solution. Prepared unknowns were thus readily compared with all dilutions of known concentrations, so that here too the proposed method may be used for a quantitative determination of nickel.

In testing for cobalt in the presence of nickel in equal concentration, the addition of 4 or 5 drops of ammonium hydroxide per drop of mercaptoacid will cause the color due to the nickel to be discharged. The green color due to the cobalt is not affected by the excess hydroxide used. On the other hand, cobalt may seriously interfere with the test for nickel.

COLOR REACTION WITH PALLADIUM

Acid palladium salts in concentration greater than 1000 p.p.m. impart a yellow color to the solution. Solutions of lesser concentration are colorless. If to such colorless solution containing not less than about 40 p.p.m. of palladium is added a drop of mercaptopropionic acid and the resulting mixture is made alkaline with ammonium hydroxide, the yellow color is again brought out.

METAL IONS WHICH DO NOT GIVE COLORED SOLUTIONS WITH β-MERCAPTOPROPIONIC ACID

The following metal ions in solution do not give characteristic color reactions with the reagent: silver, gold(III), bismuth(III), calcium, chromium(III), copper(II), mercury(II), magnesium, manganese(II), lead(II), platinum(IV), rhodium(III), tin(II), thorium(IV), titanium(III), and zirconium(II).

Copper(II) gives a yellow colored precipitate with thioglycolic acid and β-mercaptopropionic acid, which dissolves in ammonium hydroxide or sodium hydroxide with formation of a colorless solution. Therefore, copper should not interfere with the test for iron or cobalt when these mercapto acids are used.

LITERATURE CITED

(1) Lear, J. B., with M. G. Mellon, ANAL. CHEM., 25, 1411 (1953).
(2) Lyons, E., J. Am. Chem. Soc., 49, 1916 (1927).
(3) Uhlig, L. J., and Freiser, H., ANAL. CHEM., 23, 1014 (1951).

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Titrations of Weak Acids and Bases Related to Nitroguanidine

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The acidic and basic character of nitroguanidines and related compounds was studied by titration with sodium methoxide in dimethylformamide and ethylenediamine and by titration with perchloric acid in trifluoroacetic acid. In the basic solvents, azo violet was satisfactory as a visual indicator. The strength of the acids could not be differentiated potentiometrically (antimony–calomel electrode system) although the pK$_a$'s ranged from 8.1 to 12.40. A platinum–platinum electrode system was used for titrations in trifluoroacetic acid. Excellent titrations were obtained for nitroguanidine in trifluoroacetic acid.

THE use of various acidic and basic solvent systems for potentiometric titrations has opened numerous channels for systematizing the knowledge of acids and bases. In connection with the study of the chemistry of nitrogen compounds in these laboratories it has been important to have a measure of acid and base strengths of the nitroguanidines in various media. The purpose of the present work was to show the scope of nonaqueous acid-base titrations as applied to nitroguanidines, substituted nitroguanidines, and their derivatives, all being weak acids and bases in aqueous media (1, 5).

The acidic character of trifluoroacetic acid and its unique solvent characteristics suggested its use as a titration medium. No previous application of trifluoroacetic acid for this purpose has been reported. Both dimethylformamide and ethylenediamine have been shown to be excellent solvents for titrations of weak acids (2–4, 6). These materials, then, offered an exceptional range of conditions for the titration of nitroguanidines, which are not sufficiently strong acids or bases to be titrated in aqueous media.

REAGENTS

Trifluoroacetic acid, commercial product.
Benzoic acid, primary standard grade.
Dimethylformamide, technical grade.
Ethylenediamine, 95 to 100% commercial product.
Sodium methoxide solution. Add 5 grams of sodium metal piecemeal to 100 ml. of methanol. Then add 150 ml. of methanol and 1500 ml. of benzene.
Azo violet, saturated solution in benzene.
Perchloric acid, 0.1N, in trifluoroacetic acid. Add 8.2 ml. of 72% perchloric acid to 850 ml. of trifluoroacetic acid, then add 50 grams of trifluoroacetic anhydride, and finally dilute to 1 liter with trifluoroacetic acid.
Trifluoroacetic acid solutions were saved and purified in the following manner. The impure trifluoroacetic acid was diluted with water by at least 30% of its volume. An azeotropic mixture of trifluoroacetic acid and water was distilled, using care not to let the volume of the residue get too small, because of the perchloric acid present. The water was removed by dropping the azeotropic mixture onto hot phosphoric pentoxide, distilling off the trifluoroacetic acid, and then redistilling.

PROCEDURE

The basic solvents, dimethylformamide and ethylenediamine, contained acidic impurities and were neutralized just before use. Fifty milliliters of solvent was neutralized with sodium methoxide using 5 drops of azo violet indicator. This neutralization and subsequent titration of sample were carried out in a flask equipped for continuous nitrogen flushing, as carbon dioxide in the air interferes. The weighed sample was introduced into the neutralized solvent and then titrated with sodium methoxide solution to the same blue end point established for the blank. Mixing was accomplished with a magnetic stirrer. Because the amount of the compounds available was limited, small samples had to be titrated, for which 10-ml. burets were used. The sodium methoxide was standardized against benzoic acid. The titrations in ethylenediamine were most satisfactory when done in 5 to 1 ethylenediamine-water mixtures.

For potentiometric titrations to accompany visual titrations, the usual pH meter system was used, with readings taken on the millivolt scale. A calomel–antimony electrode pair was used, the glass-antimony pair being insensitive. An end-point break of 250 to 300 mv. was commonly obtained.

For acidic solvent, trifluoroacetic acid, the weighed sample was transferred to the titration flask and dissolved in 70 ml. of trifluoroacetic acid. A magnetic stirrer was used for mixing throughout the procedure. The titration flask, buret, and electrode assembly were designed to exclude moisture and carbon dioxide; the arrangement was substantially that of a Machlet buret. The potentiometric titration was carried out in the usual fashion, using a Rubicon potentiometer. A Leeds & Northrup Cherry amplifier was used to increase the sensitivity of the measuring system. Of several electrode systems tried, including antimony-antimony, glass-antimony, glass-calomel, and calomel-antimony, only the bimetallic platinum–platinum electrode system was found to be satisfactory. The indicating electrode was in the solution and the reference electrode was in the titrant stream. The perchloric acid in trifluoroacetic acid was standardized against sodium trifluoroacetate.

RESULTS

The compounds that have been titrated are listed in Tables I and II along with a description of each titration. The recovery values, all the result of duplicate titrations, are not highly accurate, but indicate that the reactions are essentially stoichiometric. The end points of titrations of nitroguanidines in ethylenediamine were not sharp, but these compounds were all titrated in dimethylformamide with excellent end points.

Table I. Titrations of Nitroguanidines in Basic Media

Compound[a]	Solvent	Purity Found, %
Nitroguanidine	DMF	
1-Amino-3-	DMF	102.3
1-Phenyl-3-	DMF	100.8
1-(p-Anisyl)-3-	DMF	103.5
1-(2-Pyridyl)-3-	DMF	100.7
1-Benzoyl-3-	DMF	100.2
1-Benzyl-3-	DMF	105.3
1-Methyl-3-	DMF	98.3
1-(β-Phenylethyl)-3-	DMF	99.9
1-(α-Phenylethyl)-3-	DMF	100.5
1-(p-Dimethylaminophenyl)-3-	DMF	100.1
1-(p-Cyanophenyl)-3-	DMF	100.0
Nitroguanylmorpholine	DMF	100.7
Benzalnitroaminoguanidine	DMF	100.1
p-Isopropylbenzalnitroaminoguanidine	DMF	99.1
p-Methoxybenzalnitroaminoguanidine	DMF	102.8
p-Hydroxybenzalnitroaminoguanidine[b]	DMF	110.5
Benzalaminoguanidine nitrate	EN to H$_2$O	99.6
Aminoguanidine hydrochloride	EN to H$_2$O	100.1
Diaminoguanidine nitrate	EN to H$_2$O	100.8
Dimethylhydrazine sulfate	EN to H$_2$O	100.4

[a] All compounds furnished by Ronald A. Henry of these laboratories.
[b] Calculation based on 2 equivalents per mole.

Table II. Titrations in Trifluoroacetic Acid

Compound	Titration Description
Sodium trifluoroacetate Potassium acid phthalate Guanidine carbonate Guanidine nitrate Nitroguanidine (NO$_2$G)	Smooth curve, sharp break at end point, precipitate forms during titration, stoichiometric
Nitroaminoguanidine	Smooth curve gently decreasing potential, no sharp break at end point
Diaminoguanidine nitrate Diphenylguanidine	No equilibrium potentials could be determined
Urea	Smooth curve, good end point, not so sharp a break at end point as NO$_2$G
Thiourea	Smooth curve, sharp break at end point, stoichiometric
m-Nitrodimethylaniline p-Anisidine	No suitable equilibrium potentials could be determined

Ethylenediamine-water proved superior for titrations of salts both in rate of dissolution of sample and in sharpness of end points. Although no particular effort was made to refine the procedural details, such as temperature corrections in the volume measurements, purification of solvents, repurification of compounds, and increasing sample size for larger titration volumes to decrease the buret reading error, the titrations as made were essentially stoichiometric, with the exceptions indicated. Titration volumes ranged from 2.5 to 9 ml., being limited by availability of compounds.

The relative strengths of acids, as exhibited in the acidic and basic solvents here used, were not differentiated potentiometrically even where there was a spread in pK_a values from 12.40 for 1-methyl-3-nitroguanidine to 8.10 for 1-benzoyl-3-nitroguanidine.

Of particular interest were the unusually good titrations obtained for nitroguanidine and thiourea in trifluoroacetic acid. These materials are not titratable in glacial acetic acid. Trifluoroacetic acid has thus proved extremely promising as a solvent for a new system of acids and bases in these preliminary experiments. The neutralizations are apparently facilitated by the removal of perchloric acid salts, which are only slightly soluble in this medium of low dielectric constant.

LITERATURE CITED

(1) De Vries, J. E., and Gantz, E. S. C., *J. Am. Chem. Soc.*, 76, 1008 (1954).
(2) Fritz, J. S., ANAL. CHEM., 24, 306 (1952).
(3) *Ibid.*, p. 674.
(4) Fritz, J. S., and Keen, R. T., *Ibid.*, 24, 308 (1952).
(5) Henry, R. A., De Vries, J. E., and Boschan, R., to be published in *J. Am. Chem. Soc.*
(6) Moss, M. L., Elliott, J. H., and Hall, R. T., ANAL. CHEM., 20, 784 (1948).

Flame Photometry of Organic Phosphorus

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A rapid method for determining phosphorus based upon flame emission measurement at 540 mμ was developed, and ten representative organic phosphorus compounds in alcoholic solution were determined in the range 0.01 to 0.03M with an average error of 0.0006M. Sodium and calcium cause positive errors because of interfering emission. Nitrogen, sulfur, iodine, and chlorine do not interfere at concentrations equivalent to that of the phosphorus.

THE recent expansion of the flame photometric field of analysis to include the determination of elements other than the alkalies and the alkaline earths has been welcomed by cost-conscious analytical chemists, owing to the savings often realized in time and expense upon adoption of a flame photometric method. Gilbert (*3*) has reported quantitative measurements of 46 elements by use of the flame photometer. The effect of nonmetals on the emission spectrum of metallic elements in some cases makes possible the determination of the nonmetal by flame photometry. An example is to be found in a report by Dippel, Bricker, and Furman (*2*) of determination of phosphorus by its effect on the emission of calcium. A continuous or band spectrum may be used advantageously in certain cases to allow the direct determination of a nonmetallic element, as in the determination of organic nitrogen described by Honma and Smith (*4*).

An investigation has been made of the continuous emission of phosphorus from solutions of organophosphorus compounds in alcohol or kerosine. It has been found that the emission of phosphorus is suitable for the determination of these compounds over a wide range of concentrations in these solvents with adequate sensitivity for many applications.

APPARATUS

A Beckman Model DU spectrophotometer equipped with Beckman No. 9200 flame photometry attachment, hydrogen burner, and Beckman No. 4300 photomultiplier attachment was used for making the measurements reported.

PROCEDURE

Prepare standard solutions of a stable organophosphate compound, such as tributyl phosphate, in ethyl alcohol in the range 0.005 to 0.04M. Prepare solutions of the samples in ethyl alcohol such that they fall in this same range with respect to phosphorus concentration. Measure the emission at 540 mμ of the standards and the samples relative to the emission of the 0.04M phosphorus standard. Construct a calibration curve by plotting phosphorus concentration *vs.* per cent relative emission. Obtain the phosphorus content of the sample aliquots from the calibration curve.

EXPERIMENTAL RESULTS

A straight-line plot was obtained by following the above procedure. The range may be extended down to the limit of detection, which was found to be about $10^{-4}M$ phosphorus. Before ethyl alcohol was selected as a solvent because of its ability to dissolve a large number of organic compounds, several calibration curves were constructed for tributyl phosphate in kerosine covering the entire range of 0 to 100% tributyl phosphate. Figures 1 and 2 show the manner in which the standard curves vary with range. These curves demonstrate the wide choice of phosphorus concentration ranges possible in standardizing the method and show that the curves are no longer linear in higher

Table I. Determination of Organic Phosphorus

Compound Analyzed	Phosphorus Added, M	Phosphorus Found, M	Error
Tributyl phosphite	0.0147	0.0146	−0.0001
	0.0220	0.0222	+0.0002
Dioctyl benzene phosphonate	0.0097	0.0100	+0.0003
	0.0145	0.0146	+0.0001
Butyl octyl phenyl phosphate	0.0115	0.0112	−0.0003
	0.0172	0.0169	−0.0003
Dibutyl ethoxyethyl phosphate	0.0141	0.0137	−0.0004
	0.0210	0.0217	+0.0007
Tri (β-chloro)ethyl phosphate	0.0192	0.0205	+0.0013
	0.0284	0.0304	+0.0020
Ethyl di(chlorophenyl) phosphinate	0.0126	0.0118	−0.0008
	0.0191	0.0189	−0.0002
Tricresyl phosphate	0.0119	0.0115	−0.0004
	0.0178	0.0169	−0.0009
Dibutyl phosphate	0.0191	0.0183	−0.0008
	0.0281	0.0267	−0.0014
Phosphoric acid	0.0172	0.0176	+0.0004
	0.0285	0.0279	−0.0006
Benzene phosphonic acid	0.0253	0.0254	+0.0001
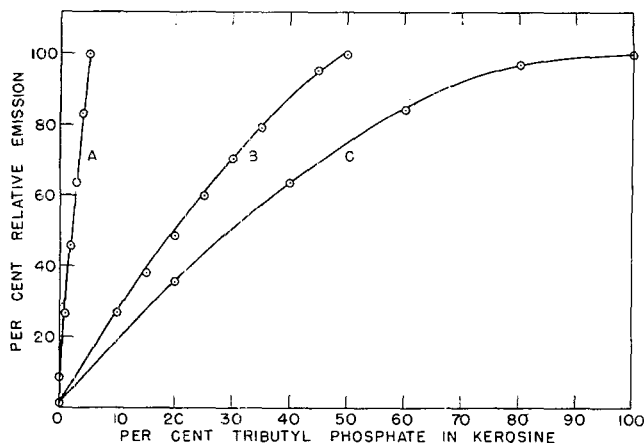
Figure 1. Emission at 540 mμ vs. tributyl phosphate concentration in kerosine

High Range

Curve	Slit width, mm.
A	0.06
B	0.02
C	0.01

Figure 2. Emission at 540 mμ vs. tributyl phosphate concentration in kerosine

Low Range

Curve	Slit width, mm.
A	0.14
B	0.10
C	0.10
D	0.08

concentrations of phosphorus, because of changes in the physical properties of the mixture. The qualitative observation was made that approximately equal intensities of radiation resulted from solutions of identical concentrations of tributyl phosphate in ethyl alcohol and in kerosine.

A study was made of the effect of the state of chemical combination of the phosphorus on the flame emission. Ten different phosphorus compounds were obtained and alcoholic solutions were prepared in the range 0.01 to 0.04M. Upon analyzing the solutions flame photometrically by the procedure given above, an average error of 0.0006M resulted. The compounds analyzed, named in conformance with the scheme of nomenclature presented by Daasch and Smith (1), are listed in Table I, together with the phosphorus concentrations of the solutions used for analysis, the phosphorus concentration found, and the absolute error.

Table II. Effect of Various Compounds on Determination of 0.0184M Phosphorus

Materials Added	Molarity	Phosphorus Found, M	Error
Pyridine	0.063	0.0186	+0.0002
Chloroacetic acid	0.053	0.0183	−0.0001
m-Nitrobenzoic acid	0.030	0.0183	+0.0001
Urea	0.083	0.0187	+0.0003
Thiourea	0.066	0.0185	+0.0001
Iodoform	0.013	0.0187	+0.0003
Thioglycolic acid	0.054	0.0188	+0.0004
Calcium ricinoleate	0.018	>0.04	>0.02
Sodium benzoate	0.008	>0.04	>0.02

Some of the compounds were commercial preparations of unknown purity, and the deviations listed in Table I reflect any lack of purity of the compounds tested.

The effect of the presence of other elements frequently found in organic molecules was also investigated. A solution of tributyl phosphate in ethyl alcohol was prepared such that 20-ml. aliquots diluted to 50 ml. resulted in a phosphorus concentration of 0.0184M. Known amounts of the materials tested for interference were added to the solutions, which were then analyzed by the above procedure for phosphorus. The compounds tested and their effect on the determination of phosphorus are shown
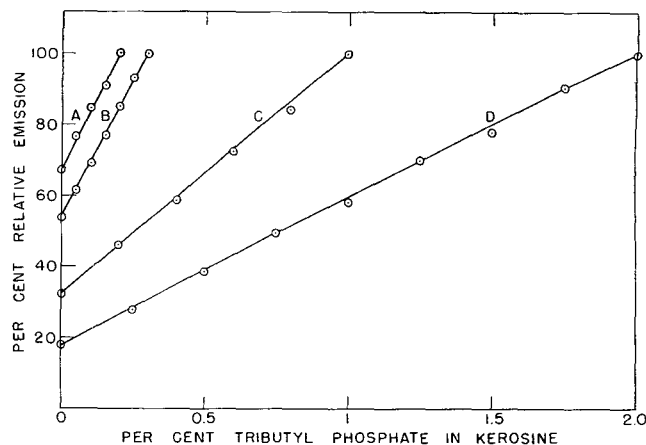
in Table II. Of the elements studied, which include calcium, sodium, nitrogen, sulfur, chlorine, and iodine in several forms, only calcium and sodium cause interference with the method at the concentrations chosen.

The reproducibility of the flame photometric measurements was estimated using hydrocarbon solutions of tributyl phosphate.

Table III. Reproducibility of Measurement

	Tributyl Phosphate Found, %	
	Solution 1	Solution 2
	46.3	1.71
	44.3	1.69
	46.6	1.68
	45.8	1.72
	46.6	1.71
	46.0	1.68
	45.2	1.70
	45.4	1.68
	44.4	1.72
	47.0	1.70
Mean	45.8	1.70
Standard deviation of mean	±0.88	±0.015

Ten measurements were made alternately on each of a 45 and 1.70% tributyl phosphate solution. Before each measurement it was necessary to reset the slit width and sensitivity of the instrument to give a reading of 100% relative emission for a reference standard. The precision figures found in this manner should therefore include any error for nonreproducibility in setting the slit width or in standardizing the instrument. The resulting dial reading was converted to per cent tributyl phosphate from a previously prepared standard curve. The results of this study are shown in Table III.

LITERATURE CITED

(1) Daasch, L. W., and Smith, D. C., Naval Research Laboratory, Report 3657 (April 1950).
(2) Dippel, W. A., Bricker, C. E., and Furman, N. H., Anal. Chem., 26, 553 (1954).
(3) Gilbert, Paul F., Jr., Ind. Labs., 3, 41 (1952).
(4) Honma, M., and Smith, C. L., Anal. Chem., 26, 458 (1954).

Gravimetric and Titrimetric Determination of Titanium, Zirconium, and Hafnium with Cupferron

Application to Fluoride Solution

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Gravimetric and titrimetric determination of titanium, zirconium, and hafnium by precipitation with cupferron (N-nitrosophenylhydroxylamine) in 10% sulfuric acid has been investigated. The metals can be titrated with a precision of better than 0.3% (relative) when employing amperometric equivalence point detection. The procedures are simple, subject to few interferences, and can usually be applied without any or with a minimum of preliminary treatment after sample dissolution in hydrofluoric and sulfuric acids, which are efficient solvents for the metals, and their alloys and compounds. Fluoride, phosphate, and other complexing species do not interfere. The ready titrability of suspended zirconium and hafnium phosphates indicates a rapid way of completing the analysis after separation of the metals as the phosphate from sulfuric acid–hydrogen peroxide solution.

THE growing importance of the metals of Periodic Group IVB (titanium, zirconium, hafnium, and thorium) in commercial alloys and in the atomic energy program has increased interest in their analytical chemistry.

These metals are quadrivalent in aqueous solution although titanium exhibits unstable bi- and trivalent states. Their high oxidation potentials (18), coupled with the high hydrogen ion concentration necessary to prevent hydrolysis, make their electrolytic reduction difficult; the polarography of titanium is well worked out, whereas the reported polarographic reduction of zirconium (19) is of little value since it is preceded by hydrogen ion reduction. Polarographic reduction of hafnium and thorium has not yet been achieved.

Thorium is sufficiently different in chemical behavior from the other Group IVB metals to make its separation simple (31, 36)—e.g., solubility of thorium phosphate in strongly acid solution containing excess phosphate in which zirconium and hafnium are insoluble. The latter may be separated from titanium by precipitation of their phosphates from strongly acid solution containing hydrogen peroxide and excess phosphate (4).

None of the methods proposed for separating zirconium and hafnium (1–3, 10, 15, 17, 35) serve for quantitative separation, but rather as concentration procedures for preparation of pure compounds, usually in poor yield. Their quantitative determination in presence of each other has been accomplished with varying degrees of success by activation analysis (11, 28, 30), emission spectroscopy (30), x-ray fluorescence (30), mixed oxide density procedures (35), and methods based on ratio of oxide residue to isolated precipitate (8). However, because of the lack of precision of these methods, the difficulty of performing many of them, and the expensive equipment often required, the sum of zirconium plus hafnium is determined where ever possible.

Determination of zirconium and hafnium, alone or together has largely been done gravimetrically—e.g., precipitation by cupferron (21, 22, 33), mandelic acid and its derivatives (7, 12, 16, 23, 26, 27), phosphates (20, 37), selenous acid (32, 34), substituted arsonic acids (29), or hydrolysis (36), followed by ignition to the oxide. Proposed titrimetric methods include an amperometric one using p-nitrophenylarsonic acid (13); the exacting conditions necessary to obtain even fair results on pure zirconium solutions and the apparent large solubility of the precipitate cited by the authors (who did not investigate interferences) appear to make the method of limited value. Titration with ethylenediaminetetraacetic acid [(ethylenedinitrilo)tetraacetic acid] (5) is satisfactory, but cannot be applied in the presence of fluoride, phosphate, or organic hydroxy acids; in addition, a considerable number of metallic ions interfere. Other than these titration procedures, little has appeared on rapid methods for zirconium and hafnium, particularly in the presence of hydrofluoric acid in which these elements, and their alloys and oxides are most readily dissolved. Therefore, development of a rapid method which would be effective in the presence of anions, which generally interfere by their complexing action, appeared desirable.

Successful application of cupferron precipitation for determination of zirconium (25) in presence of fluoride suggested examination of the applicability of the method to the determination of titanium and hafnium in the presence of complexing agents as well as further survey of the use of cupferron as a reagent for zirconium, especially in reference to possible interference.

EXPERIMENTAL

Reagents. The following test solutions were used in developing and testing the methods: (1) 1.5817 grams of National Bureau of Standards titanium dioxide (sample 154) dissolved in sulfuric acid–potassium sulfate mixture according to directions supplied with the sample and diluted to 1000 ml. with water to give a solution which was 10% sulfuric acid by volume; (2) 1.7241 grams of pure zirconium metal dissolved in 100 ml. of boiling concentrated sulfuric acid and diluted to 1000 ml. with water; (3) 1.7375 grams of hafnium dioxide (purified as described below) dissolved in a mixture of 50 ml. of concentrated sulfuric and 10 ml. of 48% hydrofluoric acids in a platinum dish and, after several alternate fumings and dilutions, diluted to 500 ml. with water. Test solution compositions in millimoles per milliliter were (1) 0.0196 of titanium, (2) 0.0189 of zirconium, and (3) 0.0165 of hafnium.

Standard cupferron solutions, 0.05 to 0.1M, were prepared by dissolving cupferron, purified as previously described (25), in water and diluting to volume. Nitrogen used for deoxygenating was passed through chromous sulfate solution to remove residual oxygen and then through 10% sulfuric acid to remove any spray and to obtain vapor pressure equilibration. All other chemicals were of reagent or c.p. grade and were used without further purification. Sulfuric acid (specific gravity 1.84) was diluted 1 to 10 by volume with distilled water.

Purification of Hafnium. The hafnium source was a dioxide sample produced from ignition of an organic hafnium compound and known to contain ca. 95% hafnium oxide (HfO_2). This sample was analyzed by determining titanium colorimetrically with hydrogen peroxide (36), and zirconium and hafnium by Hahn's ratio method (8); other constituents were reported as carbon, inasmuch as other metallic substances were substantially all removed in the original preparation. The results were 1.45% titanium oxide (TiO_2), 3.35% carbon, 0.8% zirconium oxide (ZrO_2), and 94.4% hafnium oxide (HfO_2).

Hafnium was further concentrated and purified as follows. Some dioxide was dissolved in a sulfuric-hydrofluoric acid mixture; most of the fluoride was removed by fuming. Zirconium and hafnium phosphates were precipitated from 10% sulfuric acid solution containing hydrogen peroxide and were washed by centrifugation with a sulfuric-phosphoric acid solution containing hydrogen peroxide until the supernatant liquid was colorless. The solid phosphates were suspended in 5% aqueous cupferron solution and, after about 30 minutes of stirring, the cupferrates, into which the phosphates had been transformed, were filtered,

dissolved in chloroform, and washed with several portions of 10% sulfuric acid. After evaporation of the chloroform layer to dryness, the organic matter was destroyed with aqua regia, followed by fuming with perchloric acid. An excess of hydrofluoric acid was added, followed by dilute sodium hydroxide which caused precipitation of sodium hexafluohafnate. This precipitate was isolated, dissolved in concentrated sulfuric acid, and heated to sulfur trioxide fumes to remove fluoride. After dilution, the p-bromomandelate was precipitated according to Hahn (8). The per cent of hafnium oxide was determined on a portion of this precipitate; the remainder was ignited to the oxide which was used for preparation of the hafnium solution. Three analyses of the p-bromomandelate showed the precipitate to contain 99.8, 99.7, and 100.3% hafnium oxide.
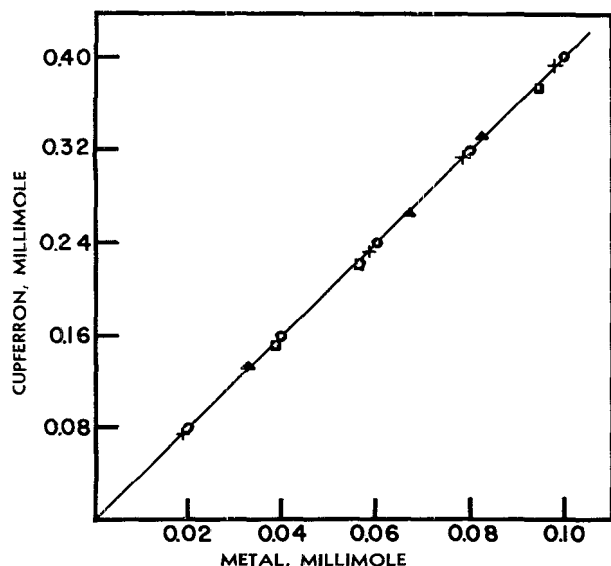

Figure 1. Relation of millimoles of cupferron consumed to millimoles of Group IVB metal present

O.   Theoretical points for 4 to 1 ratio
+.   Titanium
□.   Zirconium
△.   Hafnium

p-Bromomandelic acid was prepared as follows (9). A half mole (100' grams) of p-bromoacetophenone was dissolved in 250 ml. of glacial acetic acid; 80 grams of bromine, dissolved in 100 ml. of glacial acetic acid, was added with constant stirring. After the bromine color had disappeared, the solution was warmed to 60° to 80° C. and another 80 grams of bromine in 100 ml. of glacial acetic acid was slowly added with constant stirring. The solution was cooled to room temperature and poured into 1 liter of water. The crude ω,ω,p-tribromoacetophenone was filtered by suction and recrystallized from 200 ml. of 95% ethyl alcohol. The crystals were filtered and added slowly (over a period of an hour) with constant mechanical stirring to 1 liter of cold 5% sodium hydroxide. Stirring, at room temperature, was continued for 12 to 14 hours, after which any insoluble residue was filtered and discarded. The filtrate was made strongly acidic by adding concentrated hydrochloric acid; the solution was then extracted with several 250-ml. portions of ether. The combined ether phases were dried over anhydrous sodium sulfate for about 12 hours, filtered, and then evaporated to about 50 ml. Five hundred milliliters of benzene was added; the p-bromomandelic acid crystals produced were filtered by suction and recrystallized from benzene (melting point of product, 116° to 118° C.). Yield was 84% of theory.

Apparatus. Amperometric titration, using a Fisher Electropode or a Sargent Ampot, was performed in a 150-ml. beaker, fitted with a four-hole rubber stopper to accommodate the dropping mercury electrode, saturated calomel electrode, buret, and nitrogen inlet; the beaker lip served as nitrogen outlet. A 10-ml. buret graduated to 0.05 ml. and a 5-ml. buret graduated to 0.02 ml. were used.

Amperometric Titration Procedure. Transfer the sample solution of 5 to 10 ml. (0.02 to 0.1 millimole of one or a mixture of Group IVB metal ions) to a 150-ml. beaker, add 1 ml. 1% gelatin solution (see discussion concerning omission of gelatin), and dilute

to 50 ml. with 10% sulfuric acid. Remove oxygen by purging with nitrogen (about 10 minutes). Add oxygen-free standard cupferron solution in 1-ml. increments (if the titration is expected to consume less than 5 ml. of titrant, add 0.5-ml. increments) with nitrogen bubbling after each addition until the end point is reached; then add smaller increments. Read the current 1 to 1.5 minutes after each addition at a potential of −0.84 volt vs. S.C.E.; plot the points in the usual manner to obtain two straight lines intersecting at the equivalence point. ·

If the sample fluoride content is very high, use a wax-coated or polyethylene beaker and add prior to dilution of the sample solution a suitable volume of aluminum solution (5.0 grams of aluminum chloride hexahydrate added to 50 ml. of 10% sulfuric acid) (25).

Gravimetric Procedure. Transfer the sample (5 to 10 ml. containing 0.1 to 1 millimole of Group IVB metal) to a 250-ml. beaker (polyethylene is advisable if the fluoride concentration is high) and dilute to 100 ml. with 10% sulfuric acid. Slowly add 5% cupferron solution from a buret with vigorous stirring until the precipitate coagulates; then add a few more milliliters. Wash (0.1% cupferron solution) the precipitate several times by decantation, filter it through ashless paper, and wash on the paper 15 to 20 times with a total of 250 ml. of 0.1% cupferron solution. Transfer to a weighed crucible, char cautiously, ignite to constant weight, and weigh as the metal dioxide.

DISCUSSION

In strongly acidic solution cupferron gives a single well-defined polarographic wave with a pronounced maximum which is suppressed by gelatin (14, 24). The Group IVB metals, except thorium, are quantitatively precipitated by cupferron from 10% sulfuric acid solution (6). Solubilities of the cupferrates of titanium, zirconium, and hafnium in 10% sulfuric acid are in the range of $1 \times 10^{-7}M$ (24).

Table I.   **Gravimetric Determination of Titanium and Hafnium in Presence of Fluoride**

Millimoles Present		Molar Ratio	MO₂, Mg.		
Metal	F	F to Metal	Taken	Found	Error,%
Titanium					
1.648	0	0	131.7	132.0	+0.23
1.648	0	0	131.7	132.3	+0.46
1.648	0	0	131.7	131.5	−0.15
1.648	3.3	2	131.7	131.1	−0.46
1.648	6.6	4	131.7	131.9	+0.15
1.648	9.9	6	131.7	132.4	+0.53
1.648	13.2	8	131.7	132.2	+0.38
1.648	16.5	10	131.7	131.9	+0.15
1.648	82.5	50	131.7	132.5	+0.61
1.648	165.0	100	131.7	132.4	+0.53
				Av.	±0.37
Hafnium					
0.694	0	0	146.2	146.6	+0.27
0.694	0	0	146.2	145.9	−0.20
0.694	0	0	146.2	147.0	+0.54
0.694	1.2	1.7	146.2	146.7	+0.34
0.694	2.4	3.4	146.2	146.0	−0.12
0.694	3.6	5.1	146.2	146.5	+0.20
0.694	4.8	6.8	146.2	146.8	+0.40
0.694	6.0	8.5	146.2	145.9	−0.20
0.694	30.0	42.5	146.2	146.4	+0.12
0.694	60.0	85.0	146.2	147.1	+0.61
				Av.	±0.30

Gravimetric Determination. Data for gravimetric determination of titanium and hafnium with and without fluoride present are given in Table I; similar data for zirconium have been published (25). Even a very large excess of fluoride has no effect on the accuracy; the effects of a high fluoride-metal ratio upon the physical characteristics of the precipitates are similar to those discussed for zirconium (25). Extensive washing is necessary to remove all foreign ions; even then a slight positive error may appear, which is believed due to traces of potassium sulfate. Dissolution of the cupferrate and reprecipitation from hydrochloric acid solution would, no doubt, eliminate this trend; the resulting slight improvement in accuracy hardly justifies such a procedure except where results of the highest accuracy are necessary.

Amperometric Titration. Using information obtained in preliminary studies, pure titanium, zirconium, and hafnium solutions of varying metal content were titrated; the results [Table II; zirconium in (25); Figure 1] were calculated on the basis that the cupferron was 100% pure and that Equation 1 is stoichiometric:

$$M(SO_4)_3^{--} + 4Cup^- = M(Cup)_4 + 3SO_4^{--} \quad (1)$$
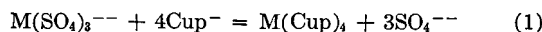
The data show the assumptions to be valid; consequently, standard solutions may be prepared by weighing out purified cupferron.

The data are uncorrected for dilution owing to reagent addition; the correction (0.01 to 0.02 ml.) is of the same order of magnitude as that inherent in the graphical location of the equivalence point. The data show average deviations of ±0.00044 millimole for titanium and hafnium, and ±0.00056 millimole for zirconium (25) over the range of 0.02 to 0.10 millimole of metal; these deviations amount to approximately 0.01 ml. of cupferron solution and are also comparable to the error in graphical end-point location. The precision—i.e., average of precision figures for each sample size and each metal over the same sample range—is ±0.00013 millimole or ±0.32 relative %.

Table II. Amperometric Titration with Cupferron of a Single Group IVB Metal in 2M Sulfuric Acid Solution

Titanium, Millimole			Hafnium, Millimole		
Taken	Found	Dev.	Taken	Found	Dev.
0.0196	0.0192	−0.0004	0.0165	0.0163	−0.0002
0.0196	0.0199	+0.0003	0.0165	0.0166	+0.0001
0.0196	0.0200	+0.0004	0.0165	0.0169	+0.0004
0.0196	0.0222	+0.0006	0.0165	0.0169	+0.0004
0.0392	0.0387	−0.0005	0.0330	0.0334	+0.0004
0.0392	0.0393	+0.0001	0.0330	0.0330	0.0000
0.0392	0.0396	+0.0004	0.0330	0.0337	+0.0007
0.0392	0.0398	+0.0006	0.0330	0.0326	−0.0004
0.0588	0.0587	−0.0001	0.0495	0.0490	−0.0005
0.0588	0.0587	−0.0001	0.0495	0.0493	−0.0002
0.0588	0.0590	+0.0002	0.0495	0.0495	0.0000
0.0588	0.0589	+0.0001	0.0495	0.0485	−0.0010
0.0784	0.0780	−0.0004	0.0660	0.0660	0.0000
0.0784	0.0789	+0.0005	0.0660	0.0667	+0.0007
0.0784	0.0776	−0.0008	0.0660	0.0665	+0.0005
0.0784	0.0788	+0.0004	0.0660	0.0658	−0.0002
0.0980	0.0971	−0.0009	0.0825	0.0814	−0.0011
0.0980	0.0985	+0.0005	0.0825	0.0823	−0.0002
0.0980	0.0988	+0.0008	0.0825	0.0838	+0.0013
0.0980	0.0974	−0.0006	0.0825	0.0819	−0.0006
			0.1650	0.1644	−0.0006
			0.1650	0.1649	−0.0001
Av. dev.	±0.00044				±0.00044

Measurements involving the complete polarographic wave pattern of cupferron usually have to be made in the presence of gelatin to avoid maxima. Consequently, gelatin has been used as a safeguard in most amperometric titrations involving cupferron. However, since the applied potential is well past that at which the maxima occur, gelatin may be omitted if desired. After deoxygenating, titration requires 10 to 15 minutes; by purging one sample while titrating another, one sample in triplicate or three individual samples can be analyzed in an hour or less.

The appreciable decomposition rate of cupferron in strongly acid solution introduces error into a procedure involving direct measurement of cupferron, consumed on adding Group IVB metal to a known excess of cupferron and measuring polarographic diffusion currents before and after metal addition. A procedure based on addition of a known excess of cupferron to the metal-containing solution and subsequent determination of the cupferron wave height would be unsatisfactory, as it would involve the difference of two measurements, each precise to only about 2%.

Anionic Interferences in Titration. Satisfactory results were obtained in the presence of phosphate and several organic acids known to form stable complex ions with titanium, zirconium, and hafnium [Table III; (25)].

Appreciable thiocyanate concentrations result in considerable error (Table IV). Thiocyanate complexes of zirconium and

Table III. Survey of Anionic Interferences in Amperometric Titration of Titanium and Hafnium

	Added Species		Metal, Millimole		
Anion	Milli-mole	Salt added	Taken	Found	Dev.
		Titanium			
Oxalate	0.74	$(NH_4)_2C_2O_4 . H_2O$	0.0735	0.0736	+0.0001
Citrate	0.74	$(NH_4)_2HC_6H_5O_7$	0.0735	0.0731	−0.0004
Tartrate	0.74	$K_2C_4H_4O_6 . {}^1/_2H_2O$	0.0735	0.0735	0.0000
Phosphate	0.74	$(NH_4)_2HPO_4$	0.0735	0.0734	−0.0001
		Hafnium			
Oxalate	0.83	$(NH_4)_2C_2O_4 . H_2O$	0.0825	0.0825	0.0000
Citrate	0.83	$(NH_4)_2HC_6H_5O_7$	0.0825	0.0821	−0.0004
Tartrate	0.83	$K_2C_4H_4O_6 . {}^1/_2H_2O$	0.0825	0.0827	+0.0002
Phosphate	0.83	$(NH_4)_2HPO_4$	0.0825	0.0828	+0.0003

Table IV. Effect of Thiocyanate on Amperometric Titration of Titanium, Zirconium, and Hafnium

Millimole Present		Molar Ratio, CNS to Metal	Metal Found, Millimole	Error, %
Metal	KCNS			
		Titanium		
0.1541	0	0	0.1540	− 0.07
0.1541	10	65	0.1408	− 8.6
0.1541	100	650	0.1297	−15.8
		Zirconium		
0.1494	0	0	0.1496	+ 0.13
0.1494	10	67	0.1431	− 4.2
0.1494	100	670	0.1309	−12.7
		Hafnium		
0.0437	0	0	0.0433	− 0.91
0.0437	10	230	0.0399	− 8.7

hafnium have been studied (2, 3), but dissociation constants were not reported. The present study indicates these constants to be comparable in magnitude to those of the fluoride complexes, since thiocyanate interference occurs at about the same mole ratio of metal to anion as does fluoride interference. Thiocyanate interference was encountered during an investigation whose objective was to eliminate the interference of iron(III). Although the latter was not achieved, it was found that iron(III) in amounts up to about 1 millimole can be kept in solution in the presence of 100 millimoles of thiocyanate even on addition of a considerable excess of cupferron. Unfortunately, this fact was here unusable, because thiocyanate forms stable complexes with the Group IVB metals.

In spite of the insolubility of zirconium and hafnium phosphates, phosphate does not interfere; titration of phosphate suspensions is satisfactory if time (5 to 10 minutes) is allowed for equilibrium to be established after each cupferron addition. This phenomenon is important—e.g., after separation as phosphate, the determination of zirconium and hafnium could be rapidly completed by an amperometric cupferron titration. This fact was utilized in the hafnium purification described; following the sulfuric acid–phosphate–hydrogen peroxide separation of hafnium and titanium, interconversion of hafnium phosphate to cupferrate gave rapid quantitative separation of hafnium and phosphorus.

Interference due to fluoride occurs only at greater than a 30- to 35-fold molar excess of fluoride ion with zirconium, 10- to 15-fold excess with hafnium and about a fivefold excess with titanium, providing the points immediately following the equivalence point are chosen for extrapolation [Table V; (25)]. The titration curves for titanium and hafnium in the presence of fluoride are similar to those observed for zirconium (25). The earlier zirconium studies (25) revealed the minimization, and even elimination, of the interference caused by relatively large amounts of fluoride by the addition of aluminum; similar results were obtained on adding aluminum to solutions of titanium and hafnium containing fluoride.

The noninterference of fluoride is significant in that, although it can usually be removed by efficient fuming, such a process is time-consuming and in the case of radioactive solutions may be highly undesirable.

Cationic Interferences in Titration. Possible cationic interferences in the amperometric titration of Group IVB metals with cupferron are evident from the earlier zirconium studies (*24, 25*). Iron(III), vanadium(V), niobium(V), uranium(IV), and large amounts of tin(IV) are the only common interfering metals.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Connick, R. E., and McVey, W. H., *J. Am. Chem. Soc.*, **71**, 3182 (1949).
(2) Fischer, W., and Chalybeaus, W., *Z. anorg. Chem.*, **255**, 79 (1947).
(3) Fischer, W., Chalybeaus, W., and Zumbusch, M., *Ibid.*, **255**, 277 (1947).
(4) Fresenius, W., and Jander, G., "Handbuch der analytischen Chemie," vol. IVB, Springer-Verlag, Berlin, 1950.
(5) Fritz, J. S., and Fulda, M. O., ANAL. CHEM., **26**, 1206 (1954).
(6) Furman, N. H., Mason, W. B., and Pekola, J. S., *Ibid.*, **21**, 1325 (1949).
(7) Hahn, R. B., *Ibid.*, **21**, 1579 (1949).
(8) *Ibid.*, **23**, 1259 (1951).
(9) Hahn, R. B., private communication.
(10) Hansen, R. S., Gunner, K., Jacobs, A., and Simmons, C. R., *J. Am. Chem. Soc.*, **72**, 5043 (1950).
(11) Harris, S. P., Rasmussen, S., Schraeder, H., and Thomas, G., U. S. Atomic Energy Commission, ANL-4277.
(12) Klingenberg, J. J., and Papucci, R. A., ANAL. CHEM., **24**, 1861 (1952).
(13) Kolthoff, I. M., and Johnson, R. A., *J. Electrochem. Soc.*, **98**, 138 (1951).
(14) Kolthoff, I. M., and Liberti, A., *J. Am. Chem. Soc.*, **70**, 1885 (1948).
(15) Kraus, K., and Moore, G. E., *Ibid.*, **71**, 3182 (1949).
(16) Kumins, C. A., ANAL. CHEM., **19**, 376 (1947).
(17) Larsen, E. M., *J. Chem. Educ.*, **28**, 529 (1951).
(18) Latimer, W. M., "Oxidation States of Elements and Their Potentials in Aqueous Solutions," 2nd ed., Prentice-Hall, New York, 1952.
(19) Laubengayer, A. W., and Eaton, R. B., *J. Am. Chem. Soc.*, **62**, 2704 (1949).

Table V. Effect of Fluoride on Amperometric Titration of Titanium and Hafnium

Millimole		Molar Ratio, F to Metal	Metal, Mg.	
Metal	KF		Taken	Found
Titanium				
0.0735	0	0	3.12	3.11
0.0735	0	0	3.12	3.12
0.1470	0	0	6.24	6.27
0.0735	0.37	5	3.12	2.72
0.0735	0.74	10	3.12	2.34
0.0735	1.48	20	3.12	2.46
Hafnium				
0.0825	0	0	14.73	14.71
0.0825	0	0	14.73	14.76
0.0825	0	0	14.73	14.75
0.0825	0.82	10	14.73	14.79
0.0825	1.64	20	14.73	12.40
0.0825	2.46	30	14.73	11.21

(20) Lundell, G. E. F., and Knowles, H. B., *Ibid.*, **41**, 1801 (1919).
(21) *Ibid.*, **42**, 1439 (1920).
(22) Lundell, G. E. F., and Knowles, H. B., *J. Ind. Eng. Chem.*, **12**, 562 (1920).
(23) Oesper, R. E., and Klingenberg, J. J., *Ibid.*, **21**, 1509 (1949).
(24) Olson, E. C., Ph.D. thesis, University of Michigan, Ann Arbor, Mich., 1955.
(25) Olson, E. C., and Elving, P. J., ANAL. CHEM., **26**, 1747 (1954).
(26) Papucci, R. A., Fleishman, D. M., and Klingenberg, J. J., *Ibid.*, **25**, 1758 (1953).
(27) Papucci, R. A., and Klingenberg, J. J., *Ibid.*, **27**, 835 (1955).
(28) Pomerance, H., U. S. Atomic Energy Commission, ORNL 366 and 577.
(29) Rice, A. C., Fogg, H. C., and James, C., *J. Am. Chem. Soc.*, **48**, 895 (1926).
(30) Rodden, C. J., "Analytical Chemistry of the Manhattan Project," McGraw-Hill, New York, 1950.
(31) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., Van Nostrand, New York, 1939.
(32) Simpson, S. G., and Schumb, W. C., *J. Am. Chem. Soc.*, **53**, 921 (1931).
(33) Smith, G. F., "Cupferron and Neocupferron," G. F. Smith Chemical Co., Columbus, Ohio, 1938.
(34) Smith, M. M., and James, C., *J. Am. Chem. Soc.*, **42**, 1764 (1920).
(35) Street, K., and Seaborg, G. T., *Ibid.*, **70**, 4268 (1948).
(36) Willard, H. H., and Diehl, H., "Advanced Quantitative Analysis," Van Nostrand, New York, 1943.
(37) Willard, H. H., and Hahn, R. B., ANAL. CHEM., **21**, 293 (1949).

Detection of Gold(III) in Nonaqueous Solution

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An investigation has been made to establish the feasibility of using color tests for inorganic compounds dissolved in organic solvents. A test for gold is now proposed which consists of the isolation of chloroauric acid by means of extraction with *n*-butyl alcohol with subsequent color development with 1-naphthylamine. As little as 1.0 γ of gold can be detected and the only interferences are mercury(I) and dichromate. Cyanide represses the test.

THE present investigation was undertaken for the purpose of establishing the feasibility of color development reactions for use in detecting and determining inorganic materials in nonaqueous solutions. Although the effect of the reaction medium in controlling analytical specificity has never been subjected to extensive study, it undoubtedly plays a prominant role in establishing reactions or reaction characteristics. Where efficient extraction procedures are found, added selectivity and simplicity of analytical procedures should result if suitable color development reagents are available for use in the nonaqueous systems. Such reagents would eliminate the necessity for transferring the inorganic materials to an aqueous system before final analytical study and might possibly exhibit advantageous characteristics over comparable reagents which react only in aqueous solutions.

The isolation of gold by extraction into *n*-butyl alcohol has been studied and 1-naphthylamine is proposed as a reagent for the direct detection of gold in the alcoholic solution. This study illustrates the advantageous selectivity and sensitivity to be gained by solvent extraction combined with direct spot tests applied to nonaqueous systems.

EXPERIMENTAL

Chemicals, Solutions, and Reagents. Chloroauric acid, C.P. Salt was dried prior to use.

Potassium chloride, C.P.
Acetone, reagent grade.
Benzene, reagent grade.
n-Butyl alcohol, reagent grade.
Diethyl ether, reagent grade.
Ethyl alcohol, reagent grade absolute.
Glacial acetic acid, reagent grade.
Methanol, reagent grade.
Aniline. Commercial product was purified by distillation.
Methylaniline.
n-Butylamine.
1-Naphthylamine. Commercial product purified by recrystallization from warm petroleum ether, Grade B. 1% solutions of amine used in each solvent studied.
2-Naphthylamine.

Preliminary Studies. The experimental approach used in developing analytical procedures involving nonaqueous systems was concerned initially with determining the effect of utilizing different classes of solvents as the reaction media for the reaction between gold(III) and aniline. The solvents employed may be classed as ionizable, nonionizable but polar, and inert. A very pronounced solvent influence on the reaction was noted (Table I).

Table I. Reaction of Chloroauric Acid with Aniline in Various Solvents

Solvent	Observations
Water	Very rapid development of dark red color followed by precipitation
Methanol	Rapid development of dark red color with no subsequent precipitation
Ethyl alcohol	Similar to methanol, but requiring slightly longer time for color development
n-Butyl alcohol	Similar to methanol, but requiring considerably longer time for color development
Acetone	Faint orange color forms immediately, with development of red color on long standing
Ethyl acetate	Similar to acetone
Glacial acetic acid	Similar to acetone
Diethyl ether	Similar to acetone
Benzene	Chloroauric acid insoluble in benzene; not applicable

While no analyses were made of the final products of the reaction, the fact that all test solutions were ultimately dark red in color is evidence that the same reaction occurred in the several solvents. No reaction was realized in benzene because of the insolubility of the gold (chloroauric acid) in this solvent. Of the solvents investigated, n-butyl alcohol was considered to be especially suited to the present study, because it was observed that a chloroauric complex was efficiently extracted into this solvent from an aqueous solution saturated with potassium chloride.

Table II. Reaction of Chloroauric Acid with Various Amines in a n-Butyl Alcohol Solution

Amine	Observations
Aniline	Dark red color develops slowly
Methylaniline	Dark red color develops slowly
1-Naphthylamine	Intense violet color develops
2-Naphthylamine	Faint orange color develops with no change in color on standing
n-Butylamine	Similar to 2-naphthylamine

The possibility of using amines other than aniline for the reaction with gold(III) in a n-butyl alcohol solution was investigated. Only monodentate type of reagents was studied, so that a similar type of reaction would be expected in all cases. It was observed that the use of 1-naphthylamine resulted in the development of a highly colored solution (Table II). The reaction

was reasonably rapid and the color produced was found to be stable.

From the studies of solvent and reagent influence on reactions with chloroauric acid it is proposed that gold(III) be detected in an n-butyl alcohol solution using 1-naphthylamine as the reagent.

Recommended Spot Test Procedure. Saturate one or more drops of the test solution with potassium chloride and, if necessary, make acidic (pH 2 to 6) with dilute hydrochloric acid. Extract the resulting solution with 1 or 2 ml. of n-butyl alcohol and place a drop of the alcoholic extract on a white spot plate. Add 1 drop of 1% 1-naphthylamine in n-butyl alcohol. The development of a violet color within 2 or 3 minutes indicates the presence of gold.

Limit of identification = 1γ.

DISCUSSION

The limit of identification was determined in accordance with the method described by Feigl (1). No limiting concentration was established because the extraction procedure makes such values meaningless. Traces of gold can be isolated from relatively large quantities of original sample and thereby readily concentrated for final identification.

Interferences were studied by the procedure discussed by West (2), with the modification that aqueous solutions containing each of the possible interference ions were first saturated with potassium chloride, extracted with n-butyl alcohol, and the resulting organic solutions were then tested with alcoholic 1-naphthylamine. This procedure was repeated for the possible interfering ions with the addition of chloroauric acid to the aqueous solutions prior to the extraction operation.

The results of the interference studies may be summarized as follows. Positive interferences are given by mercury(I) and dichromate ions. It was observed that an aqueous solution saturated with potassium chloride and containing mercury(I) ion when extracted with n-butyl alcohol yielded an alcoholic phase that produced a violet color upon the addition of 1-naphthylamine. The intensity of the color increased on standing. The mercury interference can be eliminated by adding hydrogen peroxide to the test solution and heating so as to oxidize mercury(I) to mercury(II). Dichromate ion in an aqueous solution saturated with potassium chloride caused the alcoholic phase resulting from the n-butyl alcohol extraction to be colored orange. Treatment of this alcoholic solution with 1-naphthylamine resulted in the development of an intense blue color. A masking effect on the reactions with gold was observed when cyanide ion was present.

CONCLUSION

Not only is the proposed method for the detection of gold(III) sensitive but it also serves as a means for detecting the inorganic material in a nonaqueous system by a color development reaction. The spot test procedure is very simple, requiring no elaborate conditioning treatments.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Feigl, F., "Chemistry of Specific, Selective, and Sensitive Reactions," pp. 8–10, Academic Press, New York, 1949.
(2) West, P. W., *J. Chem. Educ.*, **18**, 528 (1941).

Determination of Furfural in Petroleum Stocks

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Furfural can be determined photometrically in furfural refined lubricating oil stock by reaction with aniline in glacial acetic acid–benzene medium. The color is unstable, but the maximum intensity that develops can be measured and related to furfural content. The method is reliable for the determination of furfural concentrations as low as 0.0001%, and is applicable to both dark and colorless oils.

TRACES of furfural which may remain in lubricating oil stocks that have been refined by furfural extraction can catalyze oxidation, causing discoloration during storage. It is therefore important to maintain close control of the residual furfural content of the raffinate. A number of methods for determining small quantities of furfural have been reported (1–7). These methods, however, require a relatively expensive spectrophotometer (4), or cannot be used directly on petroleum stocks, thus necessitating preliminary isolation of the furfural.
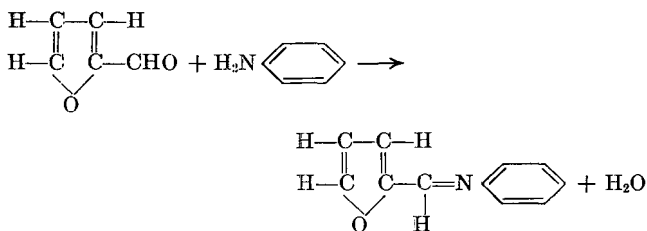
Table I. Effect of Age of Aniline Acetate

Sample	Aniline Acetate Prepn. No.	Aniline Acetate Age, days	Absorption Coefficient, Absorbance Units/Mg. Furfural	Furfural Found, P.P.M.[a]
A	1	0	680	14.4
	1	13	742	15.5
	2	0	658	15.0
B	2	0	658	104
	2	7	698	104
C	1	0	680	286
	1	7	705	270
	2	0	658	276
	3	0	700	272

[a] 1 p.p.m. = 0.0001 wt. %.

The method described in this paper is free from both of the above objections, and has the following additional features. It is simple. It may be used on colored samples. An analysis can be completed in 15 to 30 minutes. Accurate results can be obtained on concentrations as low as 1 p.p.m.

The method is based on the familiar reaction of furfural with aniline to form a colored Schiff's base according to the equation:

$$\text{H—C—C—H} \quad \text{(furan ring with CHO)} + H_2N\text{—}C_6H_5 \longrightarrow$$

$$\text{H—C—C—H} \quad \text{(furan ring with C=N)—}C_6H_5 + H_2O$$

However, in this case, the reaction is carried out in glacial acetic acid–benzene medium, rather than in aqueous medium as in the conventional method. This permits one to work directly with the oil and avoids the need for distillation, extraction, or similar preparatory operations common to most other available methods.

COLOR CHARACTERISTICS

It was found that when a hydrocarbon sample containing furfural was mixed with aniline acetate under the conditions described in the analytical procedure, a red color developed. Examination of the spectral curve showed that the color was similar to that obtained by the conventional test for furfural in aqueous solution. However, in the anhydrous medium, the color develops only slowly over the course of several minutes to a maximum intensity, then fades gradually. Because of this instability, as well as the fact that the reaction rate varies with furfural concentration, the customary procedure of allowing a fixed or minimum reaction time before measuring color intensity cannot be followed. However, if the solutions are mixed and rapidly transferred to a colorimeter cell, the development of the color can be followed quantitatively by rapidly and continuously adjusting the dial of the instrument. The maximum absorbance that is attained before fading begins is directly proportional to the furfural content of the oil.

REFRACTION EFFECTS

In attempting to correct for the initial color of dark oils by considering the absorbance value of the unreacted benzene-oil solution, an apparent anomaly was observed. The aniline acetate, which has a slight color, gave a lower reading than benzene alone. This was shown to be caused by differences in the refractive indices of the two solvents. With cylindrical colorimeter cells, the amount of light reaching the detecting surface of the photocell is altered significantly by a "focusing effect." With cells having plane-parallel faces, the effect is insignificant; however, if aberrations of the optical path are produced—e.g., by marked displacement of the light source—a similar effect is obtained even with parallel surfaces. It is therefore recommended that parallel-faced cells be used; as a precaution, the light source of the instrument should be adjusted so that equal readings are obtained with benzene and glacial acetic acid. Under these conditions, by taking into consideration the absorbance of each component of the system, it is possible to make a simple correction for any color of the original oil solution.

STABILITY OF REAGENT

The aniline acetate reagent gradually darkens on standing, but retains its effectiveness for at least several weeks. In fact, the intensity of the color developed for any given concentration of furfural gradually increases with the age of the aniline acetate. If exposure of the reagent to light is kept to a minimum, the increase in absorbance of the reaction product amounts to less than 1% for each day that the aniline acetate has aged (Table I). However, for precise work, it is necessary to analyze a standard furfural solution on the same day that the test samples are run, and to establish the absorption coefficient for that particular day. Despite this change in the reactivity of the aniline acetate with time, results within the precision limitations of the method are obtained when the appropriate absorption coefficient is used (Table I).

ANALYTICAL PROCEDURE

Reagents. STANDARD FURFURAL SOLUTION, 0.01 mg. per ml. Reagent grade furfural, freshly redistilled, was dissolved in benzene and stored in the dark.

ANILINE ACETATE SOLUTION. One hundred milliliters of reagent grade aniline was diluted to 1 liter with glacial acetic acid.

Colorimeter. A Klett-Summerson colorimeter with No. 52 filter (maximum transmittance at approximately 520 mμ) and 2-cm. light path was used.

Standardization. Appropriate volumes of the standard furfural solution containing 0.01 to 0.06 mg. of furfural were transferred to dry 50-ml. volumetric flasks and diluted to about 20 ml. with benzene. At the same time a reagent blank was prepared by adding 25.00 ml. of aniline acetate to a 50-ml. volumetric flask and diluting to the mark with benzene. The colorimeter was adjusted with the blank, and each of the standards was treated in turn by adding 25.00 ml. of aniline acetate, then adjusting to volume and transferring to the colorimeter cell within 1 minute. The colorimeter reading was noted at 10- to 15-second intervals, and when maximum intensity was reached after 1 or 2 minutes the reading was recorded. An absorption coefficient, K, expressed as milligrams of furfural per scale division, was calculated for each of the standards by dividing the instrument reading into furfural content. Because no individual K value deviated by more than 1% from the average, the average value was used.

Processing of Samples in Benzene. SAMPLES GIVING COLORED SOLUTIONS. The weighed sample was dissolved in benzene and the volume was adjusted to 100 ml. Equal aliquots (estimated to contain 0.01 to 0.06 mg. of furfural) were transferred to 50-ml. volumetric flasks. One aliquot was diluted to volume with benzene, and the absorbance (A) determined against benzene. The second aliquot was diluted to about 20 ml. with benzene, and treated with aniline acetate as in the standardization. Maximum absorbance relative to benzene was recorded as B. A separate 25.00-ml. portion of aniline acetate was diluted in a 50-ml. volumetric flask with benzene, and its absorbance (C) was measured.

Table II. Furfural in Lubricating Oil Blends

(All values as parts per million)

Added	Present	Found
0	..^{a}	9
10	19	17
20	29	31
30	39	37
50	59	59
100	109	109

^{a} Furfural content not known.

Table III. Furfural in Dewaxing Tower Charge Stock

Sample	Furfural Found, P.P.M.
Distilled raffinate	11, 10, 11
Distilled raffinate + 20 p.p.m. furfural	31, 29, 31
Distilled raffinate + 50 p.p.m. furfural	61

SAMPLES GIVING COLORLESS SOLUTIONS. A single aliquot was taken and the absorbance of the aniline acetate reaction product was determined exactly as in the standardization.

Redetermination of K. Each day that samples were analyzed, the absorption coefficient was redetermined with 0.05 mg. of furfural. This new value was used in the final calculation. So that no gross error was made by basing calculations on a single standard, the recalculated value of K was compared with previous values, allowance being made for the slight change with the age or batch of aniline acetate (Table I).

Calculation. For colorless oils, the instrument reading was converted directly to weight of furfural.

For dark oils, a corrected instrument reading was first calculated by the equation:

$$R_c = B - (C + A)$$

ACCURACY AND PRECISION

The reliability of the method has been evaluated in several ways. In the first case, a refined lubricating oil stock, found to contain only 9 p.p.m. of furfural by the described method, was used as a base to prepare blends containing 10 to 100 p.p.m. of added furfural. As the base oil was rather dark, the procedure described for use with colored oils was followed. Results are given in Table II.

In a second series of tests, a colorless, undewaxed, stripped raffinate was analyzed by the simplified procedure used for colorless oils. At the same time, analyses were run on the same sample containing known added amounts of furfural. Results are given in Table III.

The data in Tables II and III indicate a reliability of about 1 p.p.m. for the method as applied to samples containing of the order of 100 p.p.m., or less. However, since the replicates in these tests were run simultaneously, the precision of the method has also been computed from other data shown in Table IV. In accumulating these latter data, all check analyses were made on different days to obtain a more reliable estimate of the repeatability of the test.

Table IV. Repeatability of Test for Furfural

Sample	No. of Tests	Av., P.P.M.	Range, P.P.M.	Std. Dev., $\sqrt{\frac{\Sigma d^2}{(n-1)}}$ P.P.M.	% of amt. present
1	3	1.70	1.3 to 2.0	0.36	21
2	2	4.15	4.0 to 4.3	0.21	5.1
3	12	15.1	13.5 to 16.7	1.09	7.0
4	6	99	93 to 104	5.1	5.2
5	4	276	270 to 286	7.3	2.6
6	2	456	442 to 469	19.1	4.2

These results indicate that, except at the lower sensitivity limit of about 1 p.p.m., the percentage error is relatively independent of concentration and corresponds to an average standard deviation of about 5% of the amount present.

INTERFERENCES

As the method is intended for use on a well-defined system in which other aldehydes would not be expected to be present, no comprehensive investigation of interferences was made. The reaction is generally considered specific for the furaldehyde structure; methylfurfural and hydroxymethylfurfural react, but absorb only very slightly at the wave length used for the measurement (6). Tests were made on the effect of formaldehyde, acetaldehyde, propionaldehyde, and crotonaldehyde in concentrations of the order of a hundred times that of the furfural. No interference was found.

Occasionally, one may encounter a relatively insoluble oil which forms a haze on addition of the aniline acetate. This is overcome by taking a smaller aliquot, thus obtaining a final solution that has a higher concentration of benzene.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 6th ed., Method 9.20, 1950.
(2) deChalmont, G., *Am. Chem. J.*, **15**, 276 (1893); *Ibid.*, **16**, 218 (1894).
(3) Duncan, I. J., IND. ENG. CHEM., ANAL. ED., **15**, 162 (1943).
(4) Gent, L. L., Pomatti, R. C., and Levin, H., ANAL. CHEM., **26**, 413 (1954).
(5) Javes, A. R., *Proc. Am. Petroleum Inst.*, III, **29M**, 39 (1949).
(6) Stillings, R. A., and Browning, B. L., IND. ENG. CHEM., ANAL. ED., **12**, 499 (1940).
(7) Woelfel, W. C., Good, W. D., and Neilson, C. A., *Petroleum Engr.*, **24**, No. 7, C42 (1952).

Use of a Monochromator in Refractometry

Refractive Indices and Dispersions of High Molecular Weight Hydrocarbons

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A monochromator with a tungsten filament lamp may be used as a source of monochromatic light suitable for refractive index and dispersion measurement. The index of refraction of 14 hydrocarbons of high molecular weight is reported at 25° and 30° C. at a series of seven wave lengths.

THE usual procedure for measuring index of refraction at different wave lengths is to use standard lamps, together with suitable filters, to obtain monochromatic light (8). The Bausch & Lomb Precision Abbe-type refractometer is calibrated by the manufacturer for measurements at the following wave lengths:

Wave Length, A.	Usual Spectral Source
6678.1	Helium red
6562.8	Hydrogen C
5892.6	Sodium D_1, D_2
5460.7	Mercury e
5015.7	Helium blue
4861.3	Hydrogen F
4358.3	Mercury g

Commercially available sodium vapor and mercury vapor lamps are satisfactory sources: They give intense light, are stable, and are easy to operate. On the other hand, the hydrogen and helium sources are clumsy in operation and the former is difficult to maintain in constant operating form. This paper reports the use of a monochromator coupled with a high intensity tungsten filament lamp as a source of radiation of narrow wavelength spread. It is an especially suitable replacement for the troublesome helium and hydrogen lamps, and may be used for any wave length in the visible region with ease.

The index of refraction of 14 hydrocarbons of high molecular weight (American Petroleum Institute Research Project 42) is reported at the above wave lengths at 25° and 30° C.

EXPERIMENTAL

Instruments. A Bausch & Lomb Precision, Abbe-type refractometer was used, covering the range $n = 1.323$ to 1.657 and calibrated at seven wave lengths. The sodium vapor lamp which is normally an integral part of the instrument was removed and mounted on a separate stand.

A Gaertner high dispersion wave length spectrometer, Model No. L231, was used as a monochromator. The eyepiece of the instrument was replaced by a calibrated bilateral slit. The instrument disperses light from 800 to 400 $m\mu$. The instrument dial was graduated in units of 1 $m\mu$ and could be estimated to 0.1 $m\mu$ (1 A.).

The light sources used for calibration and measurements consisted of the sodium vapor lamp provided with the refractometer, a Bausch & Lomb Model H4 mercury vapor lamp, and a Bausch & Lomb microscope illuminator (No. 31-33-78) with an aspheric condenser and 6-volt tungsten ribbon filament lamp. These lamps were mounted on a single stand in such a manner that any lamp could be rotated into place without dismounting any other lamp.

The temperature of the refractometer prisms was maintained constant and controlled to ±0.02° C. by the use of a constant temperature bath (Precision Scientific Co., No. 6600). The refractometer thermometer, graduated in 0.2° divisions, was checked against a National Bureau of Standards certified thermometer.

The arrangement of the instruments is shown in Figure 1.

Calibrations. The accuracy of the monochromator dial was checked by reading the wave lengths of the sodium and mercury

spectral lines and comparing these values with tabular values. The dispersion characteristics were also checked and compared with the values provided by the manufacturer. The dispersion characteristics are plotted in Figure 2.

The accuracy of the refractometer was checked first with the glass test piece provided with the instrument. It was checked against one or more of the NBS standard refractive index liquids, toluene, methylcyclohexane, and 2, 2, 4-trimethylpentane, at each of the seven wave lengths and at the given temperature.

Hydrocarbons. All of the hydrocarbons studied were prepared at The Pennsylvania State University under the direction of one
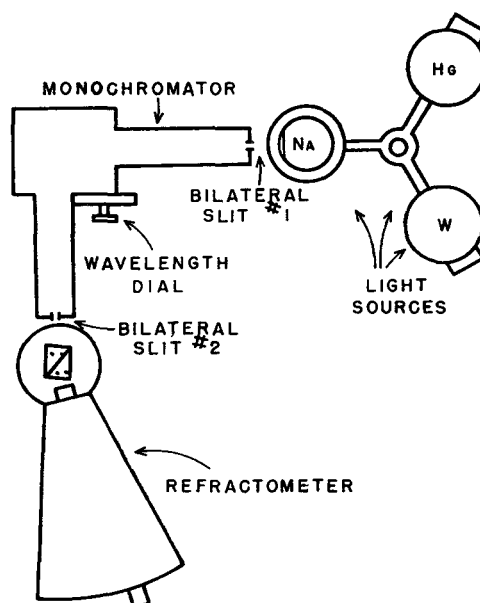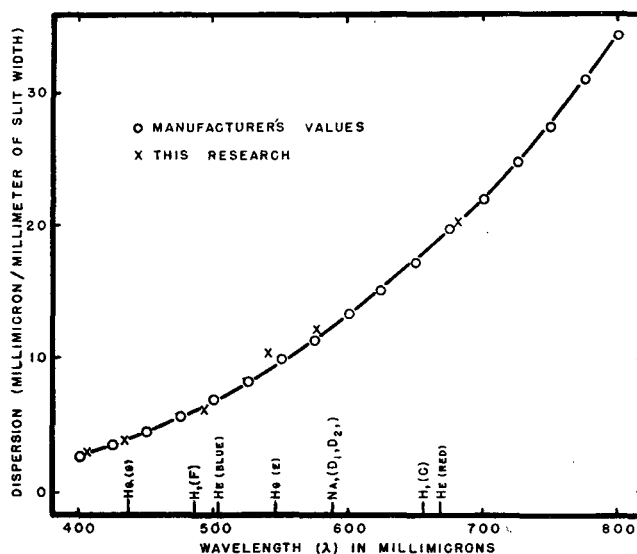

Figure 1. Arrangement of instruments

Figure 2. Dispersion characteristics

Table I. Compounds Studied

PSU No.	Empirical Formula	Name	Structure
8	$C_{31}H_{64}$	11-n-Decylheneicosane	C_{10}—C—C_{10} / C_{10}
18	$C_{25}H_{36}$	1-Phenyl-3(2-phenylethyl)hendecane	[⟨phenyl⟩—C_2—]$_2$ C—C_8
19	$C_{25}H_{48}$	1-Cyclohexyl-3(2-cyclohexylethyl)hendecane	[⟨ S ⟩—C_2—]$_2$ C—C_8
25	$C_{25}H_{52}$	9-n-Octylheptadecane	C_8—C—C_8 / C_8
110	$C_{25}H_{50}$	9(3-Cyclopentylpropyl)heptadecane	C_8—C—C_8 / C_3 / (cyclopentyl)
111	$C_{25}H_{48}$	1-Cyclopentyl-4(3-cyclopentylpropyl)dodecane	[⟨cyclopentyl⟩—C_3—]$_2$ C—C_8
113	$C_{25}H_{46}$	1,7-Dicyclopentyl-4(2-cyclohexylethyl)heptane	[⟨cyclopentyl⟩—C_3—]$_2$ C—C_2—⟨ S ⟩
122	$C_{31}H_{56}$	1,1-Di(alpha-Decalyl)hendecane	[⟨ S / S ⟩—]$_2$ C—C_{10}
134	$C_{38}H_{78}$	13-n-Dodecylhexacosane	C_{12}—C—C_{13} / C_{12}
500	$C_{19}H_{40}$	7-n-Hexyltridecane	C_6—C—C_6 / C_6
503	$C_{19}H_{24}$	1,1-Diphenylheptane	[⟨phenyl⟩—]$_2$ C—C_6
516	$C_{14}H_{14}$	1,1-Diphenylethane	[⟨phenyl⟩—]$_2$ C—C
549	$C_{10}H_{22}$	4-n-Propylheptane	C_3—C—C_3 / C_3
567	$C_{11}H_{10}$	1-Methylnaphthalene	CH$_3$ (naphthalene)

of the authors (*5, 6*), except PSU 567, which was supplied by C. E. Boord and K. W. Greenlee (*1*). (Preparations of most of the compounds have been described in the literature or will be published soon.) All samples were of high purity and had been stored under nitrogen in sealed glass ampoules.

A list of the compounds with their empirical and structural formulas is given in Table I. Other physical properties of many of the hydrocarbons studied here have been reported (*6*).

Procedure. The refractometer was used in the usual manner. The exit slit of the monochromator was set at 0.10-mm. opening and the wave length of the light passing through the slit was set to the proper range by adjusting the dial of the wave-length spectrometer. The index of refraction was then read starting with the light of λ = 6678 A. and then proceeding stepwise to the other wave lengths. Having made a set of measurements, the instrument was again set at 6678 A. and the measurements were repeated by a different observer.

To test the validity of the procedure, the index of refraction measurements at 5893, 5461, and 4358 A. were read twice, first with the appropriate standard lamp and then with the tungsten light source. The light beam always passed through the monochromator. The readings made with the two sources, standard lamp and tungsten lamp, agreed within ±0.00003 refractive index unit. At 4358 A. the light intensity is weak and to obtain suffi-

cient light for the measurements it was necessary to open the exit slit of the monochromator to 0.15 or 0.20 mm. The dispersion curve in Figure 2 indicates the justification for this procedure.

Accuracy and Precision. The daily instrument checks using the NBS standard refractive index liquids, 2,2,4-trimethylpentane, methylcyclohexane, and toluene, duplicated the values certified for these liquids to within ±0.00006 unit. A careful review of the daily calibration checks showed that no instrument corrections were necessary. The average difference between the refraction readings of the two observers was ±0.00003 unit. Thus the values reported here are self-consistent to ±0.00005 unit and are probably accurate to ±0.0001 refractive index unit.

The temperature variation was about ± 0.02° C. From a consideration of the dn/dT characteristics of the hydrocarbons, this would lead to a variation of only 0.00001 refractive index unit, which is insignificant.

RESULTS

Table II lists the refractive indices for the 14 hydrocarbons at 25° and 30° C. at the seven wave lengths.

A number of equations have been proposed to relate refractive index with wave length. The constants of two of these equations have been evaluated for the hydrocarbons used in this study.

The first equation, known as the Sellmeier-Drude equation (2, 7, 8) is

$$n^2 - 1 = \frac{C}{\nu_0^2 - \nu_\lambda^2}$$

in which n is the measured index of refraction at wave length λ, corresponding to frequency ν_λ, and ν_0 and C are constants which were evaluated by the method of least squares. From the C and

ν_0 the index of refraction extrapolated to infinite wave length was calculated by the formula

$$n_\infty = \sqrt{\frac{C}{\nu_0^2} + 1}$$

The second equation is the modified Hartmann equation suggested by Forziati (3)

$$n_\lambda = n_\infty + \frac{C}{(\lambda - \lambda^*)^{1.6}}$$

where n_∞, C, and λ^* are constants. The evaluation of these con-

Table II. Index of Refraction of High Molecular Weight Hydrocarbons as a Function of Wave Length and Temperature

PSU No.	T, °C.	$\lambda =$ 6678.1 A.	6562.8	5892.6	5460.7	5015.7	4861.3	4358.3
8	25	1.44911	1.44943	1.45176	1.45371	1.45631	1.45638	1.46186
	30	1.44731	1.44764	1.44986	1.45182	1.45442	1.45551	1.45991
18	25	1.51257	1.51313	1.51693	1.52033	1.52483	1.52677	1.53469
	30	1.51069	1.51122	1.51505	1.51837	1.52286	1.52476	1.53268
19	25	1.47090	1.47124	1.47368	1.47580	1.47852	1.47962	1.48441
	30	1.46908	1.46943	1.47187	1.47395	1.47666	1.47778	1.48249
25	25	1.44413	1.44445	1.44671	1.44864	1.45123	1.45225	1.45660
	30	1.44228	1.44261	1.44482	1.44680	1.44931	1.45039	1.45474
110	25	1.45459	1.45494	1.45729	1.45926	1.46188	1.46296	1.46750
	30	1.45274	1.45305	1.45535	1.45734	1.45998	1.46106	1.46552
111	25	1.46591	1.46630	1.46872	1.47079	1.47343	1.47461	1.47922
	30	1.46422	1.46452	1.46694	1.46894	1.47165	1.47277	1.47732
113	25	1.47821	1.47857	1.48103	1.48313	1.48593	1.48705	1.49179
	30	1.47643	1.47676	1.47924	1.48132	1.48407	1.48521	1.49001
122	25	1.49945	1.49981	1.50238	1.50463	1.50756	1.50870	1.51376
	30	1.49771	1.49810	1.50064	1.50287	1.50577	1.50700	1.51193
134	25	1.45316	1.45351	1.45586	1.45788	1.46047	1.46149	1.46604
	30	1.45134	1.45168	1.45396	1.45590	1.45855	1.45961	1.46403
500	25	1.43624	1.43653	1.43879	1.44074	1.44317	1.44422	1.44853
	30	1.43431	1.43466	1.43685	1.43873	1.44125	1.44226	1.44648
503	25	1.53256	1.53321	1.53769	1.54163	1.54694	1.54916	1.55869
	30	1.53052	1.53112	1.53562	1.53956	1.54485	1.54710	1.55657
516	25	1.56401	1.56477	1.57022	1.57503	1.58154	1.58426	1.59597
	30	1.56186	1.56259	1.56801	1.57278	1.57925	1.58198	1.59363
549	25	1.40884	1.40918	1.41127	1.41296	1.41531	1.41624	1.42017
	30	1.40672	1.40700	1.40900	1.41081	1.41306	1.41399	1.41761
567	25	1.60575	1.60686	1.61498	1.62225	1.63238	1.63677	1.65606
	30	1.60351	1.60463	1.61264	1.61986	1.62995	1.63435	1.65356

Table III. Dispersion Equation Constants of High Molecular Weight Hydrocarbons

PSU No.	T, °C.	Sellmeier Equation			Hartmann Equation		
		$C \times 10^{-30}$	$\nu_0^2 \times 10^{-30}$	$n_\infty{}^a$	$n_\infty{}^a$	C	λ^*, μ
8	25	9.30310	8.66090	1.44019	1.43910	0.004153	0.0905
	30	9.08793	8.50472	1.43825	1.43780	0.003836	0.1012
18	25	6.97350	5.61829	1.49707	1.49693	0.005985	0.1196
	30	6.96403	5.63503	1.49527	1.49522	0.005874	0.1217
19	25	9.65363	8.50097	1.46137	1.46009	0.004559	0.0847
	30	9.44020	8.35317	1.45950	1.45839	0.004507	0.0852
25	25	9.04674	8.53661	1.43519	1.43430	0.004099	0.0889
	30	8.98529	8.52107	1.43334	1.43242	0.004135	0.0872
110	25	9.19904	8.44641	1.44537	1.44450	0.004199	0.0904
	30	9.17709	8.46722	1.44355	1.44272	0.004143	0.0914
111	25	9.38844	8.37346	1.45644	1.45522	0.004578	0.0808
	30	9.42241	8.43962	1.45480	1.45378	0.004353	0.0876
113	25	9.68561	8.37515	1.46849	1.46752	0.004466	0.0887
	30	9.62455	8.36029	1.46670	1.46577	0.004414	0.0910
122	25	10.06137	8.26247	1.48920	1.48808	0.004765	0.0869
	30	10.04430	8.28218	1.48753	1.48654	0.004669	0.0888
134	25	9.16364	8.44512	1.44398	1.44262	0.004547	0.0768
	30	9.19363	8.51215	1.44224	1.44168	0.003934	0.0982
500	25	8.85755	8.53685	1.42743	1.42589	0.004527	0.0702
	30	8.86344	8.58555	1.42561	1.42484	0.003949	0.0905
503	25	6.44085	4.97916	1.51445	1.51470	0.006654	0.1287
	30	6.40082	4.97179	1.51242	1.51242	0.006787	0.1256
516	25	5.97332	4.33444	1.54211	1.54239	0.007966	0.1320
	30	5.95894	4.34393	1.54006	1.54050	0.007812	0.1341
549	25	8.42361	8.75487	1.40077	1.40008	0.003647	0.0916
	30	8.57994	8.96676	1.39888	1.39676	0.004696	0.0419
567	25	4.53398	3.07763	1.57264	1.57652	0.009601	0.1691
	30	4.52264	3.08368	1.57055	1.57488	0.009315	0.1723

$$^a\; n_\infty = \sqrt{\frac{C}{\nu_0^2} + 1}.$$

stants is accomplished by the use of the tables in the Forziati paper.

Table III lists the values of the constants for the 14 hydrocarbons.

DISCUSSION AND CONCLUSIONS

The use of a monochromator together with a tungsten filament lamp as a means of obtaining monochromatic light for index of refraction measurements is satisfactory and convenient. The index values obtained by the monochromator method and those determined using the sodium or mercury vapor lamp agree within about ± 0.00003 unit.

REFERENCES

(1) American Petroleum Institute Research Project 45, Ohio State University, Columbus, Ohio.
(2) Drude, P., *Ann. phys.*, 14, 677 (1904).
(3) Forziati, A. F., *J. Research Natl. Bur. Standards*, 44, 373 (1950).
(4) Kurtz, S. S., Jr., and Ward, A. L., *J. Franklin Inst.*, 222, 563 (1936).
(5) Schiessler, R. W., and McLaughlin, R. L., "Synthesis and Purification of High Molecular Weight Hydrocarbons," from "Chemistry of Petroleum Hydrocarbons," vol. I, Reinhold, New York, 1954.
(6) Schiessler, R. W., and Whitmore, F. C., *Ind. Eng. Chem.*, 47, 1660 (1955).
(7) Sellmeier, W., *Pogg. Ann.*, 143, 272 (1871); 145, 399, 520 (1872); 147, 389 (1872).
(8) Weissberger, A., "Physical Methods of Organic Chemistry," pp. 1141–240, 2nd ed., Interscience, New York, 1949.

Titration of Basic Copolymers of Acrylonitrile in Nonaqueous Solution

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The methods of nonaqueous titration for the determination of organic bases have been extended to include the determination of these bases when copolymerized with acrylonitrile. Amines, amine salts, and quaternary ammonium salts in the polymer may be titrated with 0.05N perchloric acid when the polymers are dissolved in a mixture of nitromethane and formic acid. The salts of pyridine derivatives but not those of aliphatic amines may be determined in polymers by titration with 1,3-di-o-tolylguanidine.

MANY simple organic compounds are readily titrated in nonaqueous solution by acid-base technique. Fritz (2) and Pifer, Wollish, and Schmall (6) have done extensive work in this field, and have described methods for the quantitative determination of amines, amine salts, and quaternary ammonium salts. These methods with appropriate modifications have proved suitable for the work described in this paper.

When acrylonitrile is copolymerized with an unsaturated organic molecule containing either an amino or quaternary ammonium nitrogen, the resulting polymer retains the basic characteristics of the comonomer. This property may be used to determine the amount of basic material which has been copolymerized. As mineral acids are used in the polymerization process, the amines contained in the polymer may occur as salts as well as free amine. Any means of analysis must then be capable of determining both free amine and amine salt in order to give the total amount of amine present. This is also true of quaternary ammonium compounds, as they, too, occur as salts in the polymers.

SOLVENTS AND TITRANTS

Acrylonitrile copolymers are all insoluble in acetic acid and acetonitrile, but show complete solubility in dimethyl formamide. Unfortunately, it is not possible to titrate amine salts (with perchloric acid) in this solvent, because of the basicity of the solvent.

The polymers are also partially soluble in nitromethane, a reagent recommended by Fritz (3), and are completely solubilized by addition of small amounts of formic acid, maleic anhydride, or water to the suspension. The addition of acetic acid or acetonitrile to the nitromethane suspension does not aid solubilization.

A 2 to 3% solution of 98% formic acid in nitromethane shows the best solvent properties of the mixtures tested. Solution will also occur if 90% formic acid is used, but the presence of the increased amount of water tends to give poorer end points in the perchloric acid titration.

Either 0.1 or 0.05N perchloric acid in dioxane was used as titrant for the nitromethane–formic acid solutions of the polymers. Amines, amine salts, and quaternary ammonium salts are all titrated under these conditions, provided that the salt is not a halide. The use of the more dilute titrant increases the precision of the determinations. The end point is detected potentiometrically through the use of a glass-calomel electrode combination.

Sodium methylate, the usual titrant for the acid portion of amine salts, cannot be used to determine the amine salts in the polymers, as this reagent attacks the acrylonitrile portion of the polymer, apparently by hydrolysis. No definite end point can be obtained in such a titration.

1,3-Di-o-tolylguanidine, designated by Davis and Hetzer (1) as one of the stronger substituted guanidine bases, was found capable of titrating heterocyclic amine salts in the presence of the free amine, but could not be used successfully with aliphatic amine salts. This is undoubtedly due to the nearly equal basic strength of the amines and the titrant. This base combines with the acid portion of the salt and is, therefore, useful in determining that part of the compound. It does not attack the nitrile portion of the polymer.

TITRATION OF QUATERNARY AMMONIUM SALTS

All of the polymers containing quaternary compounds were prepared by the copolymerization of acrylonitrile and a quaternary ammonium chloride in the presence of mineral acids. The chloride ion is not completely displaced, so that some of this anion remains in the polymer.

The presence of the halide ion necessitates the use of Pifer and Wollish's (5) mercuric acetate modification. Their work was done by dissolving an amine or quaternary ammonium halide in glacial acetic acid and adding a solution of mercuric acetate. The formation of the mercuric halide removes the halide ion from solution and the excess mercuric acetate, being essentially undissociated, does not interfere with the perchloric acid titration.

As the polymers were insoluble in acetic acid, it was first neces-
sary to show that this method would be equally successful in
another solvent. Accordingly an essentially pure sample of
tetrabutyl ammonium iodide was dissolved in the nitromethane–
formic acid mixture, a 6% solution of mercuric acetate in glacial
acetic acid was added, and the sample was then titrated with
0.1N perchloric acid in dioxane. Six determinations gave an
average value of purity of 98.8 ± 0.5%. Four Volhard determi-
nations of the salt run as a check gave an average value of 98.9
± 0.4%. The excellent agreement of results shows that Pifer
and Wollish's method works as well in the nitromethane–formic
acid mixture as in acetic acid. Other simple compounds analyzed
in this manner gave equally accurate results.
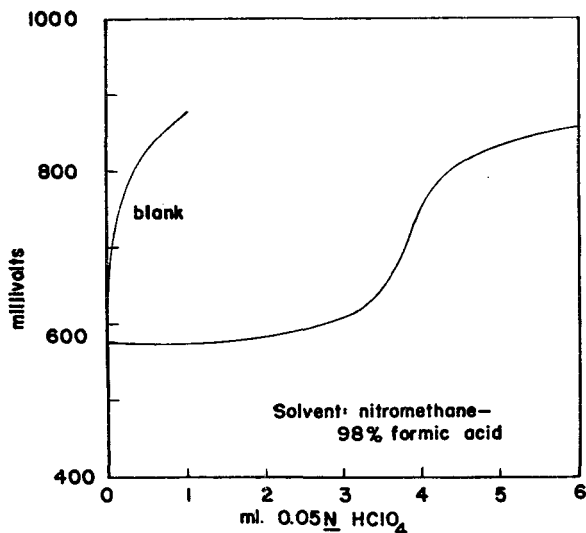
**Figure 1. Titration of quaternary ammonium
chloride in acrylonitrile copolymer**

A number of polymer samples were then analyzed. A typical
titration curve is given in Figure 1. Polymers containing from
2 to 5% of the salts may be analyzed by this method with a
relative precision within 4%.

The accuracy of the method was checked by preparing a poly-
mer which contained chloride salts exclusively. Nonaqueous
data for this polymer indicated that 4.78 ± 0.08% quaternary
ammonium chloride was present. Bomb combustion of the poly-
mer followed by a Volhard analysis for chloride gave 4.7 ± 0.2%
as the quaternary ammonium chloride content.

TITRATION OF AMINES AND AMINE SALTS

These polymers were formed by copolymerizing acrylonitrile
and an unsaturated amine in the presence of a mineral acid;
the amines included both tertiary aliphatic amines and pyridine
derivatives. As these salts are completely titrated by perchloric
acid in nonaqueous solution (2), the mercuric acetate modifica-
tion is unnecessary.

A typical titration curve for the amine-acrylonitrile copolymer
is shown in Figure 2. Both the aliphatic and heterocyclic amine-
containing polymers give essentially the same titration curve.
When the total amine content of the polymer is about 5%, the
relative precision of determination is 4%; the absolute precision
0.2%.

The pyridine-containing copolymers may also be titrated with
aqueous acid and alkali when the polymer is dissolved in concen-
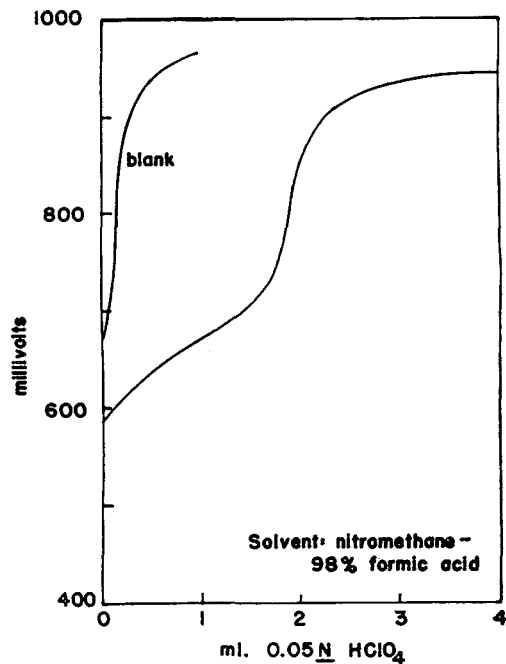trated thiocyanate solution. This method, developed by Leus-

**Figure 2. Titration of amine and amine salt
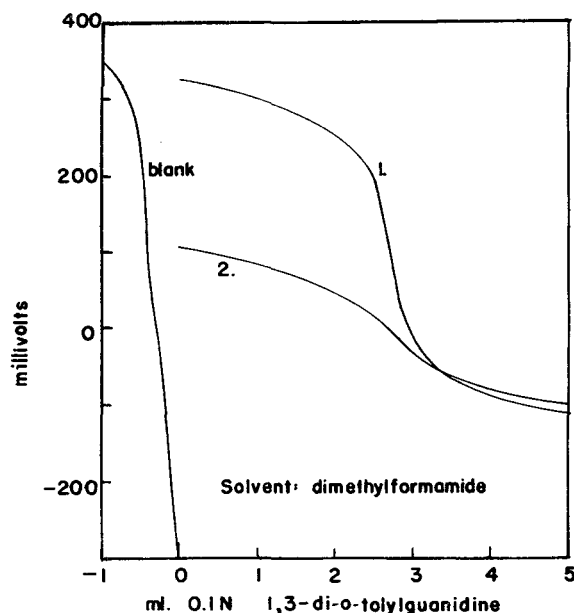in acrylonitrile copolymer**

**Figure 3. Titration of amine salts in acrylonitrile
copolymers**

sing (4), gave agreement with nonaqueous results within 0.2%
when the sample contained about 5.0% base.

The titration of the acid portion of the amine salts in the poly-
mers with 1,3-di-o-tolylguanidine is illustrated in Figure 3.
Curve 1 is typical of the polymers containing salts of pyridine
derivatives and shows a clearly recognizable end point. Curve
2 is representative of salts of aliphatic amine–containing poly-
mers. The titration break is too poor to be of much use analy-
tically. Dimethylformamide makes an excellent solvent for
this titration. Glass and calomel electrodes are used to detect

the end point. The addition of standard perchloric acid to the solvent corrects the small negative blank.

REAGENTS

Perchloric acid, 0.05N. Dissolve ca. 4.2 ml. of 72% perchloric acid in 1 liter of dioxane and standardize against potassium acid phthalate according to the method of Seaman and Allen (7).

Mercuric acetate solution. Dissolve ca. 6 grams of mercuric acetate in 100 ml. of hot glacial acetic acid (5).

Nitromethane, c.p. Fisher Scientific Co.

Formic acid, 98%, Fisher Scientific Co.

1,3-Di-o-tolylguanidine, 0.1N. Dissolve 24 grams of reagent in 100 ml. of methanol and 900 ml. of methylchloroform. Standardize potentiometrically against 20-ml. portions of standard 0.1N aqueous hydrochloric acid or 40 ml. of the 0.05N perchloric acid in dioxane. Methanol is used as sample solvent.

Dimethylformamide, E. I. du Pont de Nemours & Co.

PROCEDURE

Sample weights are based on an expected base content of the polymer of 5%.

Determination of Total Base. Accurately weigh 0.3 to 0.5 gram of the polymer into a tared 150-ml. beaker and stir with 20 ml. of nitromethane until the material is well dispersed. Add 2 ml. of 98% formic acid, and heat until the suspension dissolves completely. Dilute with 50 ml. of cold nitromethane and allow the solution to come to room temperature. If the amine or quaternary salts present contain halide ion, add 2 ml. of 6% mercuric acetate solution.

Introduce the glass and calomel electrodes into solution, set the pH meter to + mv., and titrate the solution potentiometrically with standard 0.05N perchloric acid. A microburet should be used.

The end point of the titration is the maximum value of $(\Delta E/\Delta V)$. Appropriate plots may be made.

A blank must also be titrated and the titer of the sample corrected for the solvent titration.

Determination of Heterocyclic Amine Salt. Dissolve a weighed 0.5-gram sample of polymer in 70 ml. of dimethylformamide. Using a glass and calomel electrode combination titrate the solution potentiometrically with the standardized solution of 1,3-di-o-tolylguanidine. The end point is determined by the maximum value of $(\Delta E/\Delta V)$.

The negative solvent blank is determined by titrating 70 ml. of dimethylformamide with standard perchloric acid and adding this correction to the sample titer.

LITERATURE CITED

(1) Davis, M. M., and Hetzer, H. B., *J. Research Natl. Bur. Standards*, **48**, 381 (1952).
(2) Fritz, J. S., "Acid-Base Titrations in Nonaqueous Media," G. Frederick Smith Chemical Co., Columbus, Ohio, 1952.
(3) Fritz, J. S., and Fulda, M. O., ANAL. CHEM., **25**, 1837 (1953).
(4) Leussing, D. L., unpublished data.
(5) Pifer, C. W., and Wollish, E. G., ANAL. CHEM., **24**, 300 (1952).
(6) Pifer, C. W., Wollish, E. G., and Schmall, M., *J. Am. Pharm. Assoc.*, **42**, 509 (1953).
(7) Seaman, W., and Allen, E., ANAL. CHEM., **23**, 592 (1951).

Fluorometric Determination of 0.1 to 10 Micrograms of Cholesterol

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A fluorometric method is described for the measurement of as little as 0.1 γ of cholesterol in animal tissues. The simplicity of the procedure and the stability of the fluorescence facilitate the measurement of a large number of samples. The effect on the reaction of other substances likely to be present in lipide extracts of animal tissues has been studied. No substances have been encountered which seriously affect the results. Free and esterified cholesterol produce nearly the same final fluorescence on an equimolar basis.

IN CONNECTION with a quantitative histochemical study of brain (6) a method was needed for measuring as little as 0.1 γ of cholesterol, or 1000 times less than the amount required in the Sperry method (9), for example. The reliability of the colorimetric Liebermann-Burchard reaction prompted an investigation of its fluorescence possibilities. A simple procedure resulted, which is based upon the measurement of a stable fluorescent product and which has the required sensitivity when used with a photomultiplier-type fluorometer (5).

Glick (3) has recently adapted the colorimetric Sperry method to the measurement of as little as 0.2 γ of cholesterol. Nevertheless, the fluorometric method proposed may have certain advantages including greater ultimate sensitivity. Chen has reported a fluorometric method for hydroxy steroids (2), but no details have been published.

METHOD

Reagents and Equipment. Acetic anhydride, analytical grade, is used without further purification.

Sulfuric acid, analytical grade. If the sulfuric acid contributes to the reagent blank, it may be purified by heating with 5% by volume of 70% perchloric acid until the solution becomes colorless and fuming subsides.

1,1,2-Trichloroethane. The solvent obtained from Distillation Products Industries (No. T851) is suitable after further purification. The fraction boiling at 111–113° C. is washed four times with 0.1 volume of concentrated sulfuric acid, twice with water, and is finally dried over anhydrous sodium sulfate.

Redistilled absolute ethyl alcohol.

Standard cholesterol solutions are prepared in trichloroethane at concentrations of 0.02 to 0.4 mg. per ml.

Solutions of 0.01 to 0.1 mg. % safranine O (National Aniline Co.) or rhodanine B (Distillation Products Industries) in 0.01N hydrochloric acid. These are not essential, but may be convenient to control instrument settings during fluorescence measurements.

Lang-Levy pipets (6, 7) are used throughout. For the initial extraction the tip must be rather slender (7).

Fluorometer tubes are selected from 7 × 70 mm. serological tubes (Kimble, No. 45060-S181, A. S. Aloe Co., St. Louis). Tolerances are kept within 1 or 2% for both inner and outer diameter. The fluorometer tubes need to be very carefully cleaned before use. The following procedure is recommended: two rinses with a detergent, two with water followed by heating in half concentrated nitric acid at 100° C. for 45 minutes, then three rinses with distilled water.

The Farrand fluorometer is suitable for measuring the fluorescence after adaptation to hold tubes of 7 mm. in diameter with 150-μl. volumes (7).

Centrifuge evaporator (Figure 1). The motor, M, 280 r.p.m., Type KCI-23, Bodine Electric Co., 2254 West Ohio St., Chicago, is suspended from desiccator plate, P, and is fitted with a 9-inch Lucite rotor, R. The rotor is drilled with 50 holes 8 mm. in diameter at a 45° angle. A half-inch strap of metal, S, permits easy removal of the assembly, which is mounted in a 10-inch vacuum desiccator. The electrical leads are stripped bare at the point of emergence and sealed in with a De Khotinsky type of cement. The low speed motor for the small centrifuge constructed within the vacuum desiccator provides centrifugal force to prevent boiling or creeping of the solvent up the walls of the tubes during evaporation. With the vacuum from a water aspirator and a little heat from an infrared lamp, the samples (alcohol)

evaporate to dryness within 15 minutes; however, with higher boiling solvents a better vacuum is required. If desired, the tubes may be loosely capped with aluminum foil during the operation.

Procedure. The description given below is applicable to 1 to 25 γ of frozen-dried sections of brain or other tissue containing 0.1 to 2 γ of cholesterol. A general description of analytical tools and techniques suitable for measurements at this scale has been given (7).
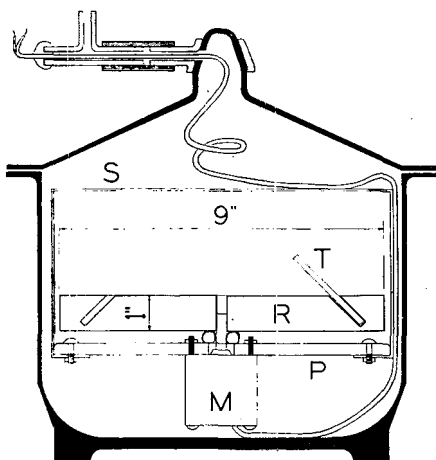

Figure 1. Centrifuge evaporator

Extraction. In tubes of 2-mm. bore and 40-mm. length (7), the samples are extracted directly with 15 to 20 μl. of redistilled absolute ethyl alcohol. With the thin porous frozen-dried tissue sections two short extractions at room temperature have proved adequate. Extraction is accomplished by mixing with a "buzzer" (7), followed by centrifugation and removal of most of the solvent with a pipet. The extracts are transferred directly to fluorometer tubes which are kept in an ice bath during the extraction procedure to prevent the solvent from creeping up the walls of the tubes. (When analyzing wet samples or when it is desirable to measure various acid-soluble constituents or protein in the same sample (8), the extraction procedure is modified as follows: The tissue samples are extracted with 10 μl. of 5% trichloroacetic acid. They are buzzed and allowed to stand in the acid for at least 10 minutes, then centrifuged for 15 minutes at 3000 r.p.m. The centrifuge is allowed to coast to a halt. Of the supernatant fluid 8 μl. are removed and saved for other analyses. The residue is then extracted once with 12 to 15 μl. of 0.1N potassium acetate in ethyl alcohol and twice with absolute ethyl alcohol. The three alcoholic extracts are combined for analysis.)

The extracts are evaporated to dryness with precautions to keep the residue in the bottom of the tube. This may be accomplished with 50 samples at once in the centrifuge device described, or with one sample at a time by heating in a water bath while directing a gentle stream of nitrogen into the tube from a glass capillary.

Development and Measurement of Fluorescence. The dried lipide residues are dissolved in 150 μl. of a fresh 5 to 1 mixture of trichloroethane and acetic anhydride. About 20 minutes with two or three mixings are allowed for complete solution of the sample and to permit any moisture present to react with the acetic anhydride. Six microliters of concentrated sulfuric acid are added to each sample with prompt and thorough mixing. The samples are capped with aluminum foil and read in the fluorometer after standing at room temperature for 1 to 2 hours.

Standards are prepared by adding 0.1 to 2 γ of cholesterol in 5 μl. of trichloroethane to fluorometer tubes and carrying them through the analytical procedure. An appropriate small volume correction is applied before calculating the unknown samples. It may be necessary to calculate from a curve, since fluorescence is not strictly proportional to concentration. The λ546 mμ mercury line isolated with Corning filters No. 4010 and 5120 is used as the exciting source. The secondary filter is the Corning No. 2424, a cutoff filter with 37% transmittance at about λ590 mμ. All samples may be read against one of the standards or against a fluorescent dye solution (safranine O or rhodanine B).

The procedure may be readily adapted to larger samples by

appropriate selection of tube size and reagent volumes. For example, 2 to 20 γ of cholesterol may be extracted with 0.1-ml. volume of ethyl alcohol in 7 × 70-mm. tubes and the fluorescence developed at a volume of 1 ml. in standard 3-ml. fluorometer tubes.

DISCUSSION AND RESULTS

The strong fluorescence of the reaction products of cholesterol in concentrated sulfuric acid (Salkowski reaction) was investigated in preliminary experiments. Although the fluorescence was found to be much more stable than the color, it was difficult to achieve precision. Various modifications of the method of Trappe (10) were explored. Strong fluorescence could be obtained, but the blank fluorescence could not be reduced to a workable level. The Liebermann-Burchard reaction in chloroform yielded more reproducible results; however, a higher boiling solvent than chloroform was necessary for use on a micro scale.
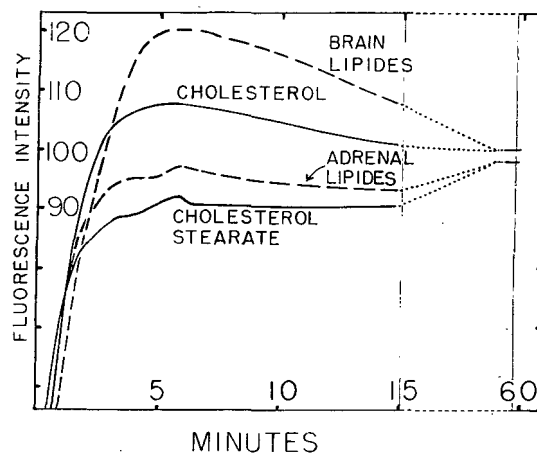

Figure 2. Rate of fluorescence development

Trichloroethane proved to be the best of a long series of solvents tested. The time course of the reaction is influenced by the solvent. Thus, with trichloroethane fluorescence reaches a peak value in 5 to 10 minutes, then falls off slightly to a steady value after about 45 minutes (Figure 2), whereas in tetrachloroethane a slow development of fluorescence continues for over 3 hours.

The fluorescence intensity is recorded as per cent of the final fluorescence obtained with cholesterol. Cholesterol and cholesterol stearate are compared on an equimolar basis. Brain cholesterol, which is chiefly nonesterified, is plotted relative to free cholesterol. Adrenal cholesterol, which is chiefly esterified, is plotted relative to cholesterol stearate.

In the presence of brain lipides a higher early peak value is reached (Figure 2). This is ascribed to the effect of other lipides on the course of the reaction rather than to the presence of other steroids. In spite of the fact that the kinetics of the fluorescence development with free and esterified cholesterol are somewhat different (Figure 2), the final fluorescence intensity is only 3% less with the ester.

The composition of the reagent is such that the components must be reasonably anhydrous to avoid turbidity. Beyond this moisture has not been found to be a critical factor. The fluorescence intensity increases markedly with increasing acid concentration. Increasing amounts of acetic anhydride decrease the fluorescence somewhat. The reagent chosen was that which produced the highest ratio of cholesterol fluorescence to reagent blank fluorescence. The choice of filters was dictated by the same criterion. The loss of sensitivity entailed by measuring only the red fluorescence is compensated by a greater specificity.

The visible fluorescence excited by the λ546 mμ line is red-orange, whereas that resulting from λ365 mμ excitation is blue-white. When the concentration of cholesterol in the reagent is high enough to produce a visible color, the color sequence is from pink to rose to blue within 30 seconds; the phase from blue to green requires about 10 minutes, whereas that from green to yellow occurs slowly over 2 or 3 hours. The development of fluorescence nearly parallels that of the yellow color as measured by the λ420 mμ absorption, although the yellow color continues to develop slowly after the fluorescence has attained a stable value.

Quenching becomes marked at concentrations greater than 100 γ per ml., but the fluorescence-concentration curve is practically linear up to 25 γ per ml.

Validation of Method. The coefficient of variation with the proposed procedure is about 2% down to a few tenths of a microgram of cholesterol (Tables I and III). Even with 0.1 γ of cholesterol, the coefficient of variation is only about 5% (Table I). Rabbit brain and rat adrenal samples were analyzed with and without amounts of added cholesterol (Ch) as follows:

A.	88.4 γ brain, 1.58 γ Ch
B.	20.8 γ brain, 0.377 γ Ch
C.	5.10 γ adrenal, 0.40 γ Ch
D.	2.55 γ adrenal, 0.20 γ Ch
E.	1.28 γ adrenal, 0.10 γ Ch
F.	no tissue, 0.20 γ Ch

Recovery of cholesterol added to brain homogenate is satisfactory (Table I).

Table I. Precision and Recovery

	Cholesterol Found, γ				Cholesterol Found, γ		
	Tissue alone	After addition	Calcd. Total		Tissue alone	After addition	Calcd. Total
A.	1.91	3.38	3.48	D.	0.237	0.401	0.420
	1.88	3.53	3.48		0.215	0.398	0.420
	1.92	3.43	3.48		0.206	0.386	0.420
	1.91	3.37	3.48		0.220	0.411	0.420
B.	0.314	0.726	0.695	E.	0.116	0.224	0.210
	0.319	0.695	0.695		0.102	0.212	0.210
	0.322	0.703	0.695		0.113	0.213	0.210
					0.110	0.201	0.210
C.	0.440	0.839	0.840	F.	...	0.197	0.200
	0.435	0.846	0.840		...	0.197	0.200
	0.446	0.835	0.840		...	0.210	0.200
	0.438	0.845	0.840		...	0.195	0.200

Table II. Comparison of Fluorometric and Colorimetric Methods

Age of Rabbits, Months	Grams of Cholesterol per Kg. of Protein			Age of Rabbits, Months	Grams of Cholesterol per Kg. of Protein		
	Fluor.	Color	Difference		Fluor.	Color	Difference
8	244	231	+13	11	261	264	− 3
8	248	245	+ 3	11	263	264	− 1
8	254	261	− 7	14	299	317	−18
8	276	280	− 4	14	273	289	−16
11	265	268	− 3	14	298	320	−22
11	243	248	− 5	14	263	269	− 6
11	287	292	− 5	Av.	269	273	− 4 ± 2.5[a]

[a] Standard error of difference. Coefficient of variation for fluorometric determinations was 2.0%; for colorimetric determinations it was 1.1%.

A comparison was made between the proposed fluorometric procedure and a modification of the colorimetric method of Sperry (9) by means of parallel determinations of the total cholesterol in lipide extracts of 13 rabbit brains of three age groups. For the colorimetric procedure an amount of the dried residue of the lipide extract containing about 50 γ of cholesterol was dissolved in 0.25 ml. of glacial acetic acid; 0.50 ml. of acetic anhydride was then added to each sample and mixed, followed by the addition of 25 μl. of concentrated sulfuric acid with immediate and thorough mixing. The reaction was timed from the addition of sulfuric acid and read at 620 mμ between 27 and 37 minutes. The fluorometric analyses were performed on samples containing 4 to 8 γ in a final volume of 175 μl. Protein was determined on the extracted residues by a colorimetric method (10). No

significant difference was found between the values obtained by the two procedures (Table II).

A number of steroids were examined for interference (Table III). Many steroids fluoresce in the reagent, but there fluorescence differs both quantitatively and qualitatively. With the filter combination recommended most of the steroids tested produced considerably less fluorescence than cholesterol. Of those tested only Δ⁵-pregnenolone and Δ⁷-cholestenol were equal to cholesterol. The fluorescence with other filter combinations may be of interest (Table III). The fluorescences are recorded as per cent of that obtained with the same weight of cholesterol and with the same filter combination. The filter combination in the first column is that recommended in the proposed procedure. Data should be considered only approximate. The secondary filter was the Corning No. 2424 in all cases. Since in most unfractionated lipide extracts the amount of cholesterol present is greater than other steroids by several orders of magnitude, interference would not be a serious problem.

Table III. Fluorescence of Other Steroids Relative to That of Cholesterol

Primary filter[a]	4010 + 5120	5113	5860
Exciting wave length	546 mμ	436 mμ	365 mμ
Cholesterol	100	100	100
Dihydrocholesterol	30	28	22
Δ⁷-Cholestenol	135	158	187
Cortisone	0	20	0
Pregnandiol	1	8	3
Dihydroepiandrostane	10	70	60
Alloprenanolone	12	45	28
Androsterone	22	105	123
Estrone[b]	26	45	16
Progesterone	35	135	120
Desoxycorticosterone	66	470	250
Δ⁵-Pregnenolone	115	495	210

[a] Corning filter numbers.
[b] Estrone fluorescence faded rapidly on irradiation.

Purified samples of phosphatidyl choline, phosphatidyl ethanolamine, and sphingomyelin do not interfere when present in amounts 10 times greater than the cholesterol. Cholic acid produces about one tenth the fluorescence of an equal weight of cholesterol.

The substitution of fluorometry for colorimetry in the measurement of cholesterol has several advantages. Not only is it easy to obtain high sensitivity without sacrifice of precision, but the readings are relatively stable for some time, which is not true of present colorimetric methods. In addition, free and esterified cholesterol give almost the same readings (Figure 2). No attempt has been made to attain maximal sensitivity. Merely reducing volumes tenfold—a completely feasible change—would increase the useful sensitivity 10 times (since the reagent blank is the present limiting factor).

REFERENCES

(1) Bessey, O. A., Lowry, O. H., and Brock, M. J., *J. Biol. Chem.*, 164, 321 (1946).
(2) Chen, C., *Federation Proc.*, 10, 25 (1951).
(3) Glick, D., ANAL. CHEM., 24, 1839 (1952).
(4) Levy, M., *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 21, 101 (1936).
(5) Lowry, O. H., *J. Biol. Chem.*, 173, 677 (1948).
(6) Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L., Farr, A. L., and Albers, R. W., *Ibid.*, 207, 39 (1954).
(7) Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L., Farr, A. L., *Ibid.*, 207, 1 (1954).
(8) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *Ibid.*, 193, 265 (1951).
(9) Sperry, W. M., *Am. J. Clin. Pathol., Tech. Suppl.*, 2, 91 (1938).
(10) Trappe, W., *Hoppe-Seyler's Z. physiol. Chem.*, 273, 177 (1942).

Separation of Primary and Secondary Thiols from Tertiary Thiols in Liquid Ammonia

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A method is described for separating a thiol extract in the C_6-C_{12} range into two groups, one containing the primary and secondary alkane- and cycloalkanethiols; the other containing the tertiary alkanethiols. Compounds of the first group form insoluble ammonium mercaptides in liquid ammonia, whereas those of the second group do not. Separation efficiencies ranged from 60 to 98%, depending upon molecular weight and structure of the carbon chain. 5-Ethyl-2-nonanethiol could not be separated under the general conditions chosen for the method.

THE problems of identifying the sulfur compounds present in petroleum distillates become more complex as the boiling point of the distillate increases. Below 111° C. the sulfur compounds can be identified and quantitatively estimated after concentration by adsorption and distillation (2). Above this temperature additional methods of separation, chemical and physical, must be used to simplify the character of the material sufficiently for it to be successfully identified.

A method for extracting thiols from a distillate using sodium aminoethoxide in ethylenediamine has been reported (1). When a distillate boiling between 111° and 150° C. was treated by this method and the thiol extract fractionally distilled, a series of fractions was obtained in which seven thiols were identified and two tentatively identified. Additional simplification of such mixtures is desirable and can be realized by using the method described in this paper. This should result in a more rapid and accurate characterization, particularly in the higher boiling ranges.

This paper describes a method of separating primary and secondary alkane- and cycloalkanethiols from tertiary alkanethiols in the C_6 through C_{10} range, with limited application through C_{12}. The separation is based on the reaction of the primary and secondary thiols with liquid ammonia to form sparingly soluble ammonium mercaptides (3), whereas the tertiary thiols either react incompletely or form noncrystalline soluble mercaptides. There is evidence that low reactivity, high solubility, and the noncrystalline nature of tertiary ammonium mercaptides are all involved in the failure of the tertiary thiols to yield a precipitate.

DEVELOPMENT OF METHOD

Various thiols were tested qualitatively to determine the molecular types and molecular weight range which yield precipitates with liquid ammonia. The following generalizations were inferred from these tests: Straight-chain ammonium mercaptides are less soluble than branched-chain mercaptides of the same molecular weight; primary mercaptides are less soluble than secondary mercaptides of the same chain structure; the $n-1$-alkane mercaptides decrease in solubility with increase in molecular weight with the exception of methylammonium mercaptide which is very sparingly soluble in ammonia, whereas ethylammonium mercaptide is readily soluble; and aromatic thiols form readily soluble mercaptides, and phenyl alkanethiols form mercaptides whose solubility depends on the proximity of the ring to the mercaptide group. Thus phenylmethanethiol and 2-phenylethanethiol form soluble mercaptides, whereas 3-phenyl-1-propanethiol forms a mercaptide which readily crystallizes from solution. Cyclohexanethiol is very reactive with liquid ammonia and the mercaptide is very sparingly soluble.

It was found that the first two members of the tertiary thiol series give at least partial yields of mercaptide; the method is therefore not applicable below C_6 or 100° C., the boiling point of 2-methyl-2-butanethiol. Higher tertiary thiols seldom produce any crystalline mercaptide and if so the amount is very small. No *gem*-alkyl cycloalkanethiols were available for testing, so their behavior cannot be predicted.

The use of a mutual solvent is necessary in this procedure because of the low solubility of thiols in liquid, ammonia. Approximately 80 solvents have been screened, but only a few have any practical value. Methylal and tetrahydrofuran are satisfactory for the thiols of low molecular weight but lack enough solvent power for the C_{11} and C_{12} thiols. Several C_3, C_4, and C_5 alcohols show fair solvent power but exhibit an inverse temperature-solubility relationship in that their solvent power in liquid ammonia increases with decrease in temperature. Crystallization is more difficult under these conditions, and especially difficult crystallizations may fail completely.

The glycol ethers—*n*-hexyl Cellosolve, *n*-hexyl Carbitol, and 2-ethylbutyl Cellosolve—have the highest solvent power of all compounds tested. They also show the inverse temperature-solubility relationship which is unfavorable for crystal formation from some thiols. Their high boiling point and low solubility in water are also disadvantageous because of the difficulty of recovering the portion of sample remaining in the filtrate. Dimethyl ether has been selected as the mutual solvent for this method because it has good solvent power and low boiling point and generally allows good crystal formation.

DESCRIPTION OF METHOD

The sample is dissolved in 50 volumes of dimethyl ether and added slowly with stirring to 100 volumes of liquid ammonia (50 volumes for C_{12}). The mixture of sample, dimethyl ether, and ammonia is allowed to stand in a dry ice bath for 30 minutes and filtered on a precooled, vacuum-jacketed filter.

Before the filtration is begun, some sodium aminoethoxide in excess of that required for neutralization of the tertiary thiol is prepared in the flask which is to be used as a receiver by dissolving sodium in liquid ammonia and adding monoethanolamine until the blue color is discharged. The filtrate is collected and the solvents are evaporated. When evaporation is complete

Table I. Application of Procedure to Thiol Mixtures

Component		Volume Recovered, % Theor.		Analysis			
				A		B	
A (pri.-sec.)	B (tert.)	Ppt.	Filt.	In ppt.	In filt.	In ppt.	In filt.
1-Hexanethiol	*tert*-Hexylmercaptan	110	70	73	40	27	60
2-Hexanethiol	*tert*-Hexylmercaptan	100	86	87	21	13	79
Cyclohexanethiol	*tert*-Hexyl mercaptan	120	72	82	17	18	83
1-Heptanethiol	*tert*-Heptylmercaptan	140	50	85	24	15	76
tert-Heptylmercaptan	(Blank)	10	78	No analysis available			
1-Octanethiol	*tert*-Octylmercaptan	102	100	92	13	8	87
2-Octanethiol	*tert*-Octylmercaptan	90	92	89	9	11	91
2-Ethyl-1-hexanethiol	*tert*-Octylmercaptan	80	90	79	23	21	77
5-Ethyl-2-nonanethiol	*tert*-Dodecylmercaptan	Unsuccessful (see text)					
1-Dodecanethiol	*tert*-Dodecylmercaptan	100	100	98	5	2	95

water is added and the solution is acidified with dilute sulfuric acid. A trap (1) is attached to the flask and the tertiary thiol fraction is collected by steam distillation and measured.

The precipitate is warmed on the filter by blowing a stream of air through a long test tube inserted into the filter to dissociate the ammonium mercaptide to thiol and ammonia. Dissociation is ordinarily complete before room temperature is reached. The regenerated primary and secondary thiol fraction is washed through the filter with pentane and recovered by distilling off the pentane. The receiver for the washings consists of a 15-ml., conical, graduated centrifuge tube sealed to the bottom of a round-bottomed flask. This permits distillation of the pentane and measurement of the residue in the same vessel.

In a few instances the ammonium mercaptides have been difficult to dissociate. In this event a small amount of dry ice is placed on the filter, allowed to stand a few minutes, and washed with pentane. A residue of ammonium carbamate remains.

The procedure should be carried to conclusion without interruption after the filtration has been made to prevent oxidation of the thiols after the ammonia has evaporated and air gains access to the sample under basic conditions. If the filtrate residue is diluted with acidified water immediately after the ammonia has evaporated, there is little chance for oxidation.

Table I summarizes the results of tests on a number of binary mixtures of primary or secondary thiols with a tertiary thiol. A mixture of 0.5 ml. of each component was dissolved in 50 ml. of dimethyl ether and added to 100 ml. (50 ml. for C_{11}–C_{12} mixtures) of liquid ammonia. The precipitate was filtered after about 30 minutes' standing in a dry ice bath. The fractions from the precipitate and filtrate were recovered as described above. All of the tertiary thiols were commercial; each is a mixture of isomers of unknown structures. The high results for component A in the case of the 1-heptanethiol–tert-heptylmercaptan mixture

are attributable to a substance in the tert-heptylmercaptan which yields a precipitate, but this substance has not yet been identified. A second determination using the filtrate from a blank run on tert-heptylmercaptan gave normal yields of precipitate and filtrate.

The fractions were analyzed by infrared spectroscopy to determine the amounts of primary or secondary and tertiary thiols in both the filtrate and precipitate. The absolute accuracy of analysis is thought to be ±5%. The availability of pure standards would better this value considerably.

CONCLUSIONS

Primary and secondary alkanethiols may be separated from tertiary alkanethiols as an aid in identification of individual compounds in the mixture. The efficiency of the separation increases with increase in molecular weight for those mixtures on which data are given. On the other hand the mixture of 5-ethyl-2-nonanethiol and tert-dodecylmercaptan can be separated only under ideally chosen conditions. The separation failed under the conditions adopted for the method.

LITERATURE CITED

(1) Hopkins, R. L., and Smith, H. M., ANAL. CHEM., 26, 206 (1954).
(2) Thompson, C. J., Coleman, H. J., Rall, H. T., and Smith, H. M., Ibid., 27, 175–85 (1955).
(3) Williams, F. E., with Gebauer-Fuelnegg, E., J. Am. Chem. Soc., 53, 352 (1931).

Absorption of Organic Vapors by Anhydrous Magnesium Perchlorate

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Anhydrous magnesium perchlorate has been used as an absorbent for various organic vapors from mixtures of these with inert gases. For all the polar compounds tested, the absorption was quantitative. No explosions have occurred in 4 years, but adequate safety precautions are recommended.

DURING the past 4 years, anhydrous magnesium perchlorate has been used as a quantitative absorbing agent for a number of organic vapors. The procedure is completely analogous to that used in the determination of water vapor in air or other permanent gases, and appears to be equally quantitative for all but two of the vapors so far tested. A sample of inert gas containing organic vapor is passed through a Nesbitt absorber containing ca. 50 grams of magnesium perchlorate, and the increase in weight of the absorber is measured. Gas flow rates ranged up to approximately 2 liters per hour at 1 atmosphere, with the organic vapor usually present at its saturation pressure.

Some sample data illustrating the quantitative nature of the absorption are shown in Table I. In experiments with methanol, ethyl alcohol, acetone, and dioxane, air was first bubbled through the liquids and then passed through two Nesbitt absorbers connected in series. In experiments with pyridine, acetonitrile, nitromethane, and chloroform, the second Nesbitt absorber was replaced by a cold trap at dry-ice temperature. Ammonia was drawn directly from a commercial cylinder and was not diluted with an inert gas. In this case, the second absorber contained an aqueous solution of bromothymol blue. For dioxane and chloroform, absorption is not quantitative. On the basis of these data, it is reasonable to suppose that magnesium perchlorate could be a general reagent for the vapors of alcohols, aldehydes, ketones, amines, nitriles, and nitro compounds, or, more generally, polar rather than nonpolar compounds.

Table I. Absorption of Organic Vapors by Anhydrous Magnesium Perchlorate at 25° ± 2° C.

Compound	Weight Increase, Grams	
	Absorber A	Absorber B
Methanol	1.3890	−0.0002[a]
Ethyl alcohol	0.7180	−0.0003[a]
Acetone	0.4968	−0.0006[a]
1,4-Dioxane	2.4497	0.0367[a]
Pyridine	1.408	0.0001[b]
Acetonitrile	5.574	0.0000[b]
Ammonia	0.175	...[c]
Nitromethane	1.080	0.0002[b]
Chloroform	0.051	0.4448[b]

[a] Magnesium perchlorate absorber.
[b] Cold trap.
[c] Aqueous bromothymol blue solution, colored yellow by dissolved CO_2. No color change during experiment.

As magnesium perchlorate is known to give explosive mixtures with organic materials (1), it is significant that the authors have not had a single explosion. The adsorbent was always kept at room temperature, and no attempts were made to regenerate it after use. This, however, should not be taken to imply that there is no explosion hazard under these conditions, and adequate precautions should be taken when this absorbent is used for organic vapors.

LITERATURE CITED

(1) Stross, M. J., and Zimmerman, G., Ind. Eng. Chem., News Ed., 17, 70 (1939).

Estimation of Acetate in Zinc Plating Baths

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A method is described for the estimation of acetate in zinc plating baths. Acetate is separated by a double distillation procedure and estimated in the distillate by a colorimetric method using lanthanum nitrate, iodine, and ammonia as reagents. The final solution is carefully buffered to pH 9.0 with ammonium chloride–ammonia solution. The procedure is very sensitive and can estimate milligram quantities of acetate, but the accuracy and precision vary by 10% of the amount of acetate present on the average.

QUANTITATIVE analyses of solutions from which metals are electroplated are highly important in the control of such processes. Methods for the estimation of all cations and anions in typical zinc-plating baths, except acetate ion, have been reported in the literature (*1*). Acetate ion is difficult to estimate quantitatively, because of the interfering substances which are normally present in a zinc-plating bath. A typical solution for zinc plating is:

Zinc sulfate, $ZnSO_4.7H_2O$	360 grams per liter
Ammonium chloride, NH_4Cl	30 grams per liter
Sodium acetate, $NaC_2H_3O_2.3H_2O$	15 grams per liter
Glucose, $C_6H_{12}O_6$	120 grams per liter

Scott reported (*10*) that acetate may be separated from such a bath by distillation and titrated as acetic acid with a standard solution of strong base. In cases where other acids also distill, a conductometric titration has been reported (*3*). The first method must be carefully standardized, because hydrochloric acid usually distills with the acetic acid, and the second method requires a considerable period of time to perform it, plot the results, and calculate the final concentration.
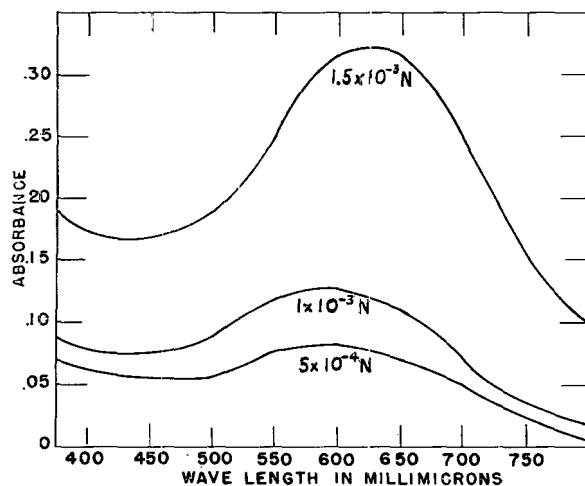

Figure 1. Absorbance *vs.* concentration of acetate

The present paper presents a colorimetric method, which is based upon methods for the qualitative detection of acetate in water solutions (*2-9*). Feigl (*2*) describes this test as follows:

[1] Present address, Carbide and Carbon Chemicals Co., Oak Ridge, Tenn.

"A drop of 5% solution of lanthanum nitrate is mixed with a drop of 0.02N iodine on a spot plate. One drop of the solution containing acetate ion is added. The test is positive, if, when this mixture is alkalized with ammonia, a blue color is formed or a blue ring is formed around the edge of the mixture."

BASIS OF PROCEDURE

Kruger and Tschirch (*7*) stated that the formation of the blue color seems to be due to a basic lanthanum-iodo complex with acetate ion rather than to adsorption phenomena. The results of this study are in agreement with this assumption. This system is far from ideal for a quantitative method, because it is time-sensitive, pH-sensitive, and varies with dilution. Consequently all of these factors must be controlled carefully. Reproducible color formation takes place between pH values of 8.6 and 9.4 at 20° C. At lower pH values, the color is less intense, and at pH values above 9.4 a precipitate forms. The optimum value is pH 9.0 at 20° C.
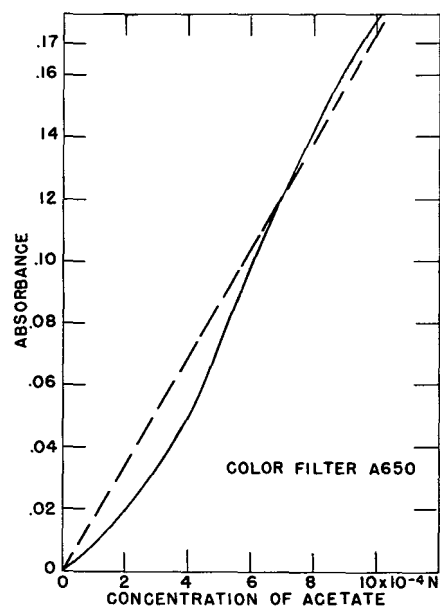

Figure 2. Calibration curve for acetate

A spectrophotometric study of this system was made with a Coleman Universal spectrophotometer at intervals of 5 mμ over a range of 350 to 800 mμ. These data are plotted in Figure 1 and show that maximum absorbance occurs in the range 600 to 625 mμ with a slight shift toward the longer wave length as the concentration of acetate ion is increased.

According to the procedure, measurements were made on solutions of pure acetic acid varying from 10^{-3} to $10^{-4}M$ with a Fisher Electrophotometer using color filter A-650. Typical data are plotted in Figure 2, which show the divergence from Beer's law

and the necessity for preparing a calibration curve before attempting to use this method for any given type of zinc-plating bath.

Sulfate ion interferes seriously with the color formation as does furfural, which is formed by decomposition of glucose during distillation. However, the addition of phosphoric acid followed by careful distillation, precipitation of any sulfate with barium chloride, and a second distillation removed the interference from sulfate and furfural. With this method small amounts of chloride ion do not interfere in the estimation.

DISTILLATION APPARATUS

The distillation train required uses conventional apparatus with some modification. The first distilling flask is a 500-ml. Kjeldahl flask closed with a three-hole rubber stopper through which pass a thermometer, a glass delivery tube, and the stem of a 60-ml. separatory funnel. This flask is connected to the second distilling flask by the glass delivery tube that dips almost to the bottom of a 250-ml. Claisen distilling flask. The delivery tube between the two flasks is joined by a short length of rubber tubing over which a pinch clamp is fitted. The Claisen flask is closed with a two-hole rubber stopper through which pass a delivery tube and the stem of a 60-ml. separatory funnel; the second neck of the flask is closed with a one-hole rubber stopper carrying a thermometer. The stem of the Claisen flask is connected to a Liebig condenser in the usual manner. The final distillate is caught from the condenser in an Erlenmeyer flask of 200-ml. capacity, dipping in an ice bath.

OTHER APPARATUS

Fisher Electrophotometer, AC Model, No. 1697 with color filter A-650 and 23-ml. cuvettes (or an equivalent instrument).
Beckman pH meter, Model G, No. 10074 with shielded glass electrode and saturated calomel half-cell (or equivalent instrument).

REAGENTS

Lanthanum Nitrate Solution. Dissolve 50 grams of reagent grade lanthanum nitrate in distilled water to make 1 liter.
Iodine Solution. Dissolve 1.2692 grams of reagent grade iodine in 95% ethyl alcohol to make 500 ml. of solution.
Buffer Solution. Dissolve 2.70 grams of reagent grade ammonium chloride in 1 liter of distilled water, immerse the electrodes of the Beckman pH meter in this solution at 20° C. Adjust the pH of the solution to 9.0 with 7.5M ammonia by adding it dropwise and stirring. After several hours check the pH and adjust to pH 9.0 if necessary.

EXPERIMENTAL PROCEDURE

Pipet into the Kjeldahl flask 50 ml. of the plating solution and 5 ml. of 85% phosphoric acid. Pipet into the Claisen flask 50 ml. of a saturated aqueous solution of barium chloride and 5 ml. of 85% phosphoric acid.
Pour 50 ml. of distilled water in each of the separatory funnels with their stopcocks closed. Connect the distillation train tightly and heat the Kjeldahl flask with a Bunsen burner or electric hot plate. Continue distillation until the thermometer in the Kjeldahl flask reads 105° C. Remove the heat then, and as soon as active boiling ceases, open the stopcock on the separatory funnel and allow the water to run into the flask. Next quickly close the stopcock of the separatory funnel and resume heating the flask. Again distill until the temperature reaches 105° C. Remove the heat, close the rubber tubing of the delivery tube with the pinch clamp, and open the stopcock of the separatory funnel. Apply heat to the Claisen flask and repeat the operations just performed on the Kjeldahl flask—that is, conduct two distillations.
Transfer the distillate from the Erlenmeyer flask to a 400-ml. beaker, wash the flask with several 10-ml. portions of distilled water, and add them to the beaker. Immerse the electrodes of the Beckman pH meter in the distillate and adjust the pH value to 8.0 at 20° C. with 7.5M ammonia. Remove the electrodes, rinse them with distilled water, and allow the rinsings to go into the beaker. Transfer the solution from the beaker to a 250-ml. volumetric flask, rinse the beaker, and add the rinsings to the flask. Then make up to 250 ml. with distilled water at 20° C.

Pipet 5 ml. of lanthanum nitrate solution into a 50-ml. beaker which also contains the electrodes of the Beckman pH meter. Pipet 3 ml. of iodine solution and the proper aliquot of the distillate (normally 5 ml.). Add distilled water to make a total volume of approximately 15 ml., and rapidly adjust to pH 9.0 at 20° C with 7.5M ammonia.
Allow 5 minutes for the color to develop, then transfer the solution from the beaker to a 100-ml. volumetric flask. Rinse with the ammonium chloride buffer solution and make to 100-ml. volume at 20° C. with the buffer solution. Shake well and measure the light absorbance of the solution with the Fisher Electrophotometer using the A-650 color filter.
From the calibration curve, calculate the amount of acetate ion in the solution, and, knowing the dilutions which have been made and the size of the aliquots taken, calculate the acetate concentration of the original plating solution. The calibration curve should be obtained from different aliquots of the distillates.

DISCUSSION OF RESULTS

Provided the calibration curve has already been obtained, the procedure described requires about 1.5 hours to make a complete estimation. The precision and accuracy leave much to be desired as deviations as high as 15% of the amount of acetate present were obtained in some cases. On the average, results can be reproduced on similar plating baths within 10% of the amount of acetate present. For example, three analyses run on the plating bath cited in this paper and using the dilutions described gave results of 6.00, 5.61, and 5.92 mg. of acetate when the correct amount was 6.51 mg. of acetate. If greater accuracy than this is required, this method is not satisfactory; however, for most purposes in electroplating this is sufficiently exact. It eliminates interference from furfural, sulfate ion, and hydrochloric acid. These impurities seriously interfere with procedures which depend upon titration with standard base.

The order of adding reagents is important and must be followed exactly as described or wide variations in color will result.

LITERATURE CITED

(1) Blum, W., and Hogaboom, G. B., "Principles of Electroplating and Electroforming," McGraw-Hill, New York, 1930.
(2) Feigl, F., "Qualitative Analyse mit Hilfe von Tupfel-Reaktionen," Akademische Verlagsgesellschaft, Leipsig, 1935.
(3) Kolthoff, I. M., and Laitinen, H. A., "pH and Electro Titrations," Wiley, London, 1947.
(4) Kruger, D., and Tschirch, E., Ber. deut. chem. Ges., 62-B, 2776 (1929).
(5) Ibid., 63-B, 826 (1930).
(6) Kruger, D., and Tschirch, E., Chem.-Ztg., 54, 42 (1930).
(7) Kruger, D., and Tschirch, E., Mikrochemie, 2, 337 (1930).
(8) Kruger, D., and Tschirch, E., Pharm. Acta Helv., 5, 25 (1930).
(9) Kruger, D., and Tschirch, E., Pharm. Zentralhalle, 71, 145 (1930).
(10) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., p. 2251, Van Nostrand, New York, 1939.

Colorimetric Determination of Sulfate Ion—Correction

In the article on "Colorimetric Determination of Sulfate Ion" [Lambert, J. L., Yasuda, S. K., and Grotheer, M. P., ANAL. CHEM., 27, 800 (1955)], the first sentence under "Preparation of Reagent" should read:

Thorium borate is obtained by the reaction of 1 liter of 0.01M thorium nitrate solution and 1 liter of 0.05M sodium tetraborate solution, the latter being added dropwise with constant stirring.

JACK L. LAMBERT

Determination of Free Phenol in Polyoxyethylene Phenyl Ethers

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Small amounts of free phenol in polyoxyethylene phenyl ethers can be determined by alkaline extraction and use of the 288 mμ absorption of phenolate ion. The precision is within about 1% with an average accuracy within 5% in a concentration range of 0.008 to 0.8% phenol.

THE use of polyoxyethylene phenyl ethers as plasticizers and surfactants makes it imperative from a safety standpoint that the level of free phenol in these products be determined. The equation for the reaction of ethylene oxide and phenol is given below:

$$\text{OH} \qquad\qquad \text{O(CH}_2\text{CH}_2\text{O)}_x\text{H}$$
$$\bigcirc + X(\text{CH}_2\text{---CH}_2) \rightarrow \bigcirc$$
$$\diagdown\!_O\!\diagup$$
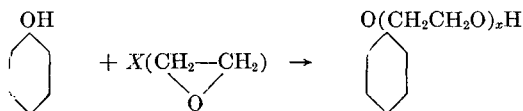
It is generally believed that, on addition of ethylene oxide to phenol, the first mole reacts quantitatively (*3, 5*) with the phenolic hydroxyl before any polymerization occurs. This is attributed to the fact that the phenolic hydrogen is more reactive than the resulting primary alcohol hydrogen at the end of the chain. To verify this contention, a simple, reliable method for the determination of any residual phenol was desired.
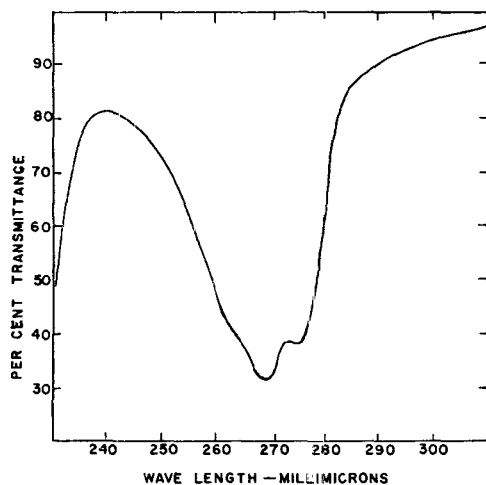

Figure 1. Typical ultraviolet absorption spectrum of polyoxyethylene phenyl ether

The literature describes many methods (*6*) for the determination of small quantities of phenol in various mixtures. The simplest, the least time-consuming, and probably the most reliable method is based upon the ultraviolet absorption of phenol in an alkaline solution. Applications of the ultraviolet spectrophotometric method have been made to such materials as gasoline, cresylic acid (*4*) and recently lignin preparations (*1*) and polyphenolic tanninlike materials (*2*).

The polyoxyethylene phenyl ethers used (in this work) contained from 1 to 5 moles of ethylene oxide per mole of phenol. Phenol-free polyethers exhibited an absorption maximum at about 270 mμ (Figure 1) and low general absorption at 288 mμ, where the phenolate ion shows its maximum (Figure 2). As the free phenol content was too low for direct measurement, because of interference from general absorption, a quantitative separation of the phenol from the ether was required; this was realized by extracting an alkaline solution of the sample with chloroform (Figure 3).

APPARATUS AND MATERIALS

The ultraviolet absorption curves were determined with a Beckman quartz spectrophotometer, Model DU. Matched 1-cm. quartz absorption cells were used with aqueous 0.4% sodium hydroxide as the solvent.
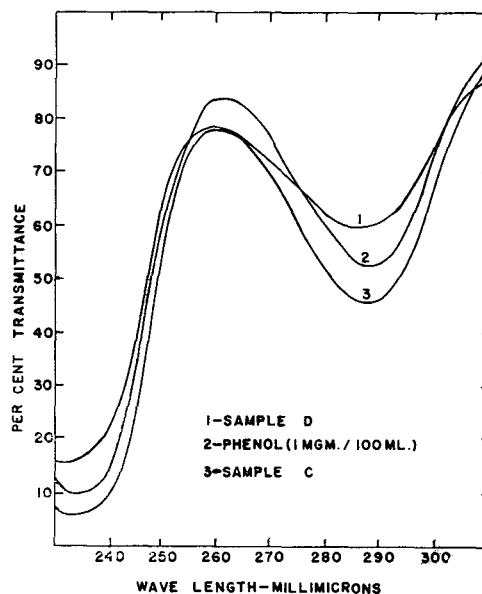

Figure 2. Ultraviolet absorption spectra of alkaline phases obtained in the analysis of various polyoxyethylene phenyl ether samples

Chloroform, Baker and Adamson, reagent grade, washed with aqueous 0.4% sodium hydroxide solution.

Sodium hydroxide, aqueous, 0.4% saturated with chloroform. The 0.4% aqueous sodium hydroxide was shaken with washed chloroform and filtered.

Phenol, Baker analyzed reagent grade.

Polyoxyethylene phenyl ethers. The Atlas Powder Co.'s products used represented various mole ratios (1 to 5) of ethylene oxide to phenol.

PROCEDURE

A 10- to 25-gram sample (optimum weight of phenol is 1.4 mg.), weighed by difference to the nearest centigram, is transferred to a 500-ml. separatory funnel which contains 100 ml. of 0.4% sodium

Table I. Determination of Free Phenol in Polyoxyethylene Phenyl Ethers

Sample	Moles Ethylene Oxide Per Mole Phenol[a]	Sample Weight, Grams	Dilution Factor	T, %	Concn. of Phenol, Mg./100 Ml.	Total Weight Phenol in Sample, Mg.	Free Phenol, %	Dev. from Mean
A	a	24.86	...	28.9	2.020	2.020	0.0081	0.0003
		10.16	...	60.1	0.803	0.803	0.0079	0.0001
		10.47	...	60.5	0.802	0.802	0.0077	0.0001
		10.18	...	61.1	0.801	0.801	0.0079	0.0001
		24.48	...	31.7	1.880	1.880	0.0077	0.0001
		10.32	...	60.2	0.803	0.803	0.0078	0.0000
		10.37	...	60.2	0.803	0.803	0.0078	0.0000
						Mean	0.0078	0.0001
B	a	10.21	5	59.6	0.845	4.225	0.041	
		22.44	10	59.0	0.806	8.060	0.036	0.0025
						Mean	0.0385	
C	b	10.31	62.5	45.9	1.270	79.40	0.770	
		10.24	100.0	61.8	0.785	78.50	0.766	0.002
						Mean	0.768	
D	c	10.28	5	58.2	0.885	4.425	0.043	
		9.94	5	59.8	0.840	4.200	0.042	0.0005
						Mean	0.0425	
E	d	10.20	5	51.2	1.095	5.475	0.054	
		10.45	5	51.0	1.100	5.500	0.053	0.0005
						Mean	0.0535	

[a] Representative of values, indicating different ratios.

Table II. Estimation of Accuracy

(Sample A used in analyses. Free phenol in sample, 0.0078%)

Sample Weight, Grams	Phenol in Sample, Mg.	Phenol Added, Mg.	Total Phenol in Sample, Mg.	Phenol Recovered, Mg.	Relative Error on Total Phenol, %
10.08	0.79	0.20	0.99	1.06	+7
9.92	0.77	0.50	1.27	1.30	+2
10.10	0.79	0.75	1.54	1.51	−2
10.13	0.79	1.00	1.79	1.80	+1
10.16	0.79	0.00	0.79	0.80	+1
		Phenol-Free Sample A			
25.32	0.0	1.00	1.00	1.02	+2.0
25.32	0.0	1.00	1.00	1.04	+4.0
22.06	0.0	2.00	2.00	1.86	−7.0
24.94	0.0	3.00	3.00	3.09	+3.0
22.06	0.0	5.01	5.01	4.95	−1.2

hydroxide (measured with a transfer pipet). The sample is dissolved by swirling gently. One hundred milliliters of washed chloroform are added and the contents are shaken for about 1 minute. After the layers have separated, the chloroform layer is discarded and the extraction is repeated with two additional 100-ml. portions of chloroform. The alkaline layer is filtered through a dry, hand-folded analytical paper, into a flask. A 1-cm. quartz absorption cell is filled with the filtered solution and the per cent transmittance is measured at 288 mμ, slit width 0.5 mm., with the 0.4% aqueous sodium hydroxide saturated with chloroform in the reference cell.

If the transmittance is less than 25%, a suitable aliquot is diluted to 100 ml. in a volumetric flask with aqueous 0.4% sodium hydroxide saturated with chloroform.

The phenol content is estimated from a standard curve of per cent transmittance vs. phenol concentration, obtained by measuring the transmittance of series of phenol standards, 0.1 to 2.0 mg. per 100 ml.

ESTIMATION OF ACCURACY

Known quantities of phenol, 0.2 to 1.0 mg., were added to the 100-ml. portion of 0.4% aqueous sodium hydroxide used in the initial extraction. A 10-gram sample of polyoxyethylene phenyl ether (sample A of Table I) was transferred into the aqueous alkaline solution, weighing by difference. The resulting mixture was analyzed according to the procedure and the observed versus the calculated standard values are shown in Table II. The phenol content of sample A was taken as 0.0078%.

An additional portion of sample A was analyzed by the general procedure. The chloroform extracts (300-ml. total) which contained the phenol-free derivative were re-extracted with three 100-ml. portions of 0.4% sodium hydroxide. The absorption spectrum of this second alkaline extract is the upper curve

of Figure 3. The determination was repeated, using the general procedure and the purified sample in chloroform, except that, before the extraction, a portion of the 300 ml. of 0.4% sodium hydroxide was replaced with an equivalent amount of alkaline solution containing 1.0 to 5.0 mg. of phenol. After the original 300 ml. of chloroform had been separated, the aqueous phase was re-extracted with two 300-ml. portions of chloroform. The recovery of phenol and the calculated relative error are shown in Table II. The absorption spectrum of the standard phenolate extractions is shown by the lower curve of Figure 3.

RESULTS AND DISCUSSION

A plot of the calibration data shows that, in the range of 0.1 to 2.0 mg. of phenol per 100 ml., Lambert-Beer's law is applicable. The published (4) specific extinction coefficient, E, at 290 mμ is 27.5 liters per gram-cm. for phenol in 0.4% aqueous sodium hydroxide. The E value, calculated from the authors' data at 288 mμ is 27.2 liters per gram-cm.
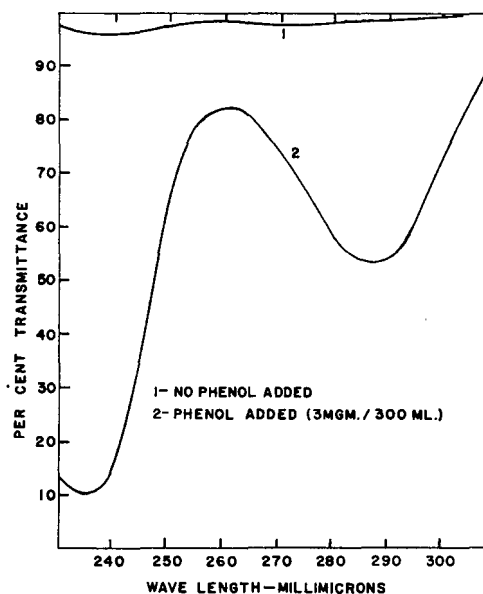

Figure 3. Accuracy study of ultraviolet absorption spectra of alkaline phases obtained in analysis of "stripped" sample

The data in Table I indicate that the precision of the method is approximately 1% for most samples.

The accuracy, as indicated by the results in Table II, is on the order of 5% or better. The spectrum of the second alkaline extraction of the "stripped" polyoxyethylene phenyl ether does not exhibit any absorption peaks but shows a general absorption slightly less than 100%. This observation tends to indicate that, on extraction of the polyoxyethylene derivative, the phenolate ion is quantitatively held in the alkaline phase.

The procedure which has been developed for the determination of free phenol in polyoxyethylene phenyl ethers is reliable and rapid. The derivatives that were investigated were ones which the values of x in the formula

$$O \quad (CH_2CH_2O)_xH$$
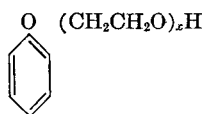
ranged from 1 to 5. The procedure may apply to the analysis of higher polymers ($x > 5$) but as the chain length is increased a possible limit may be reached where the procedure may not be applicable. This procedure, also may have possible application to the determination of free phenollike compounds in their polyoxyethylene aryl ethers. These base materials, to which ethylene oxide is added, are nonylphenol, octylphenol, pentachlorophenol and others.

LITERATURE CITED

(1) Goldschmidt, O., Anal. Chem., **26**, 1421 (1954).
(2) Maranville, L. F., and Goldschmidt, O., Ibid., **26**, 1423 (1954).
(3) Mayhew, R. L., and Hyatt, R. C., J. Am. Oil Chemists' Soc., **29**, 357 (1952).
(4) Murray, M. J., Anal. Chem., **21**, 941 (1949).
(5) Patat, F., Cremer, E., and Bobleter, O., Monatsh. Chem., **83** (2), 322 (1952).
(6) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," vol. III, p. 104, Van Nostrand, New York, 1953.

Spot Test Reaction for Detection of Elementary Sulfur

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Hydrogen sulfide is formed if free sulfur is heated with fused benzoin. This is the basis of a new and sensitive test for free sulfur in mixtures with organic or inorganic substances. The heating is done in a test tube whose mouth carries lead acetate paper. The identification limit is 0.5 γ. Selenium does not interfere. Tests on a large variety of commercial products gave satisfactory results.

VARIOUS procedures have been recommended for the spot test detection of elementary sulfur in solid materials or dissolved in organic liquids (1). These tests are reliable and some of them are very sensitive; however, they are not particularly rapid. None of them involves the formation of hydrogen sulfide from the sulfur, a product which can be detected with high specificity and sensitivity. A convenient formation of hydrogen sulfide from sulfur is found in a study by Zymaczynski (2) of a macro test for free sulfur and several ketonic alcohols. The basis of the test is that glycerol containing iron yields black iron sulfide when heated with sulfur and ketonic alcohols. (The reagent is prepared by allowing glycerol to stand with iron filings for several weeks with access to air.) Zymaczynski states that 0.1 mg. of sulfur dissolved in 1 ml. of such glycerol can be detected if 1 ml. of glycerol containing 5 to 10 mg. of ketonic alcohol is added and the mixture is heated over a free flame.

The chemical basis of the test is that a redox reaction between sulfur and the ketonic alcohol produces hydrogen sulfide, which in turn yields iron sulfide with the iron contained in the reagent. Zymaczynski offers as proof of this interpretation that much hydrogen sulfide is released when 10 mg. of sulfur is heated with 70 mg. of benzoin. Accordingly, the redox reaction in this case would lead to the formation of benzyl (diphenyl diketone) and hydrogen sulfide:

$$C_6H_5CHOHCOC_6H_5 + S^\circ \rightarrow C_6H_5COCOC_6H_5 + H_2S$$
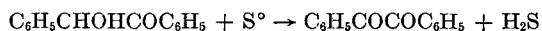
This test for sulfur was checked with respect to its applicability in spot test analysis and it was found that the production of iron sulfide in glycerol cannot be recommended. On the other hand, the authors have observed that direct fusion with benzoin (melting point 137° C.) at 140° to 150° C. provides a very sensitive and rapid test for free sulfur, since the hydrogen sulfide formed can be readily detected by the simple procedures of spot test analysis.

PROCEDURE

The test is conducted in a micro test tube (5 × 0.5 cm.). A few grains of the solid sample, or 1 to 3 drops of a solution in carbon disulfide, benzene, etc., evaporated to dryness, suffice. Several hundredths of a gram of benzoin is added, the mixture is stirred with a glass rod if necessary, and the open end of the test tube is covered with a disk of moist lead acetate paper. The tube is then plunged into a glycerol bath previously heated to 130° C., and the temperature is raised to between 140° and 150° C. Depending on the quantity of free sulfur in the sample, a black or brown stain appears on the reagent paper at once or within 1 to 2 minutes.

When testing for sulfur dissolved in low-boiling solvents, a is best to place the benzoin in the test tube first and then to add it drop or two of the solution. The test tube should then be carefully warmed by suspending it in the glycerol bath whose temperature is gradually raised.

LIMIT OF IDENTIFICATION, 0.5 γ

This procedure is adequate for the majority of instances in which it is necessary to detect the presence of free sulfur. A greater sensitivity is attained by the use of a capillary tube, the open end of which is closed, after introducing the charge, with a loose plug of cotton moistened with lead acetate solution.

The test was tried on agricultural insecticides, pharmaceutical preparations, black gunpowder, match heads, vulcanized rubber, and gas purification material. The results were excellent.

Selenium does not undergo a similar redox reaction with benzoin. Neither does it interfere with the tests for sulfur. A satisfactory response was obtained with a mixture of 10 γ of sulfur and 25 mg. of selenium (1 to 2500).

The fusion reaction with sulfur can be reversed and thus serve for the detection of benzoin. This procedure can be used for the detection of many compounds containing the secondary alcohol group. A detailed report of this finding will be given later.

LITERATURE CITED

(1) Feigl, F., "Spot Tests," 4th ed., vol. I, p. 334; vol. II, pp. 334 336, Elsevier, New York, 1954.
(2) Zymaczynski, E. W., Z. anal. Chem., **106**, 32 (1936).

Spectrophotometric Determination of Tetrathionate

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The spectrophotometric method of Robinson for the determination of tetrathionate has been modified to yield greater sensitivity and accuracy. The procedure depends on the production of thiocyanate equivalent to the tetrathionate and determination of thiocyanate with an excess of ferric iron. By developing the color in opaque cylinders, the rapid decomposition of the ferric thiocyanate color is eliminated. The use of ferric nitrate and nitric acid instead of ferric chloride and hydrochloric acid has decreased the absorbance of the reagent blanks. By measuring the final color at 460 mμ instead of at 525 mμ a twofold increase in sensitivity has been achieved.

THE Analytical Department of this laboratory was presented with the problem of determining concentrations of tetrathionate ion as low as 0.005 gram per liter in the presence of amounts of sulfate on the order of 30 grams per liter. A literature survey was conducted for methods of determining tetrathionate. The published methods for macro amounts of polythionates have recently been reviewed by Jay (2), but none of these procedures approached the required sensitivity. Murayama (4) has reported a well defined polarographic reduction wave for tetrathionate ion in a supporting electrolyte of 0.1N potassium chloride and 0.1N barium chloride, and Furness and Davies (1) reported a similar wave in phosphate buffers. A spectrophotometric method proposed by Robinson for the determination of low concentrations of tetrathionate ion (5) is based on the formation of thiocyanate from tetrathionate (and higher polythionates) by reaction with cyanide in an alkaline medium, and the subsequent formation of a red ferric thiocyanate complex with excess ferric chloride.

Because of the need for a very sensitive procedure, this investigation was confined to an evaluation of possible polarographic and spectrophotometric techniques based on the methods mentioned above. The polarographic approach was abandoned after preliminary experiments indicated that the desired degree of sensitivity could not be obtained. An investigation of Robinson's spectrophotometric method proved to be productive. The present paper reports the results of this investigation and a modified spectrophotometric procedure.

APPARATUS AND REAGENTS

Beckman Model DU spectrophotometer with 1- and 10-cm. Corex cells. The bodies of the 10-cm. cells were covered with Scotch black electrical tape.

One hundred-milliliter mixing cylinders coated with Krylon black acrylic spray, except for small vertical areas on opposite sides near the top graduation.

All chemicals used were of analytical reagent grade purity.

Sodium tetrathionate dihydrate was prepared according to the procedure of von Klobukoff as given by Mellor (3). This salt was analyzed for tetrathionate ion by the volumetric procedure of Jay (2), and was found to contain 73.2% tetrathionate, which is the theoretical value. Stock solutions were prepared as required, since aqueous solutions of sodium tetrathionate are unstable.

Amberlite IR-120 cation exchange resin. Columns of 15 ml. of resin in 25-ml. burets were employed. The resin was converted to the hydrogen form by repeated washes with 5% sulfuric acid. The exhausted resin was regenerated by washing with 100 ml. of 5% oxalic acid followed by 200 ml. of 4N hydrochloric acid.

RECOMMENDED PROCEDURE

Pass an aliquot of the sample, which contains 0.05 to 5 mg. of tetrathionate ion in no more than 25 ml., through 15 ml. of the hydrogen form of Amberlite IR-120 resin. Collect the effluent in a 100-ml. beaker. Wash the column with 15 ml. of water, collecting the washings in the beaker. In order to develop the ferric thiocyanate color, neutralize the effluent and washings from the column to phenolphthalein with 1 to 10 ammonium hydroxide. Add 5 ml. of 5% sodium cyanide solution. After 15 minutes, add 10 ml. of 1 to 1 nitric acid (in a fume hood). After the evolution of hydrocyanic acid has ceased, transfer the solution quantitatively into a Krylon-coated 100-ml. mixing cylinder, add 5 ml. of 2M ferric nitrate, dilute the solution to 100 ml., and mix well.

For samples known to contain thiocyanate prepare a sample blank. Pass another equal aliquot of the sample over a fresh resin bed, and wash in the same manner. In order not to convert the tetrathionate to thiocyanate, add the 10 ml. of 1 to 1 nitric acid first and then the 5 ml. of 5% sodium cyanide. After the evolution of hydrocyanic acid has ceased, transfer the solution quantitatively into a Krylon-coated 100-ml. mixing cylinder, add 5 ml. of 2M ferric nitrate, dilute the solution to 100 ml., and mix well.

Transfer a portion of the sample and sample blank to either 1-cm. or 10-cm. absorption cells. Measure the absorbances of the sample and sample blank at 460 mμ (0.020-mm. slit) against a reagent blank. Subtract the absorbance reading obtained with the sample blank from that obtained for the sample. Determine the amount of tetrathionate ion in the aliquot by reference to a calibration curve or by calculation using the average absorbance index.
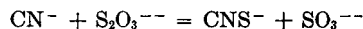
EXPERIMENTAL

Preliminary Work. The spectrophotometric approach of Robinson (5) using the reaction

$$S_4O_6^{--} + 3CN^- + H_2O = S_2O_3^{--} + SO_4^{--} + 2HCN + CNS^-$$
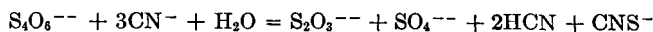
followed by the colorimetric determination of the thiocyanate with ferric iron was examined. Employing the procedure of Robinson, a calibration curve was prepared. Beer's law was obeyed over the range of 0.5 to at least 5.0 mg. of tetrathionate ion per 100 ml., and the average absorbance index was 0.0844 per mg. of tetrathionate ion per 100 ml. in 1-cm. cells.

For the analysis of solutions, Robinson (5) recommends taking a 25-ml. aliquot. In order to get an absorbance reading of about 0.1 the sample would have to contain 0.045 gram of tetrathionate ion per liter. As this sensitivity was 10 times less than desired, an attempt was made to obtain the necessary increase by using 10-cm. cells. With the more dilute solutions, straight-line calibration curves were obtained, but the absorbance index varied from 0.024 to 0.06. It was thought that in the extremely dilute solutions of tetrathionate being studied the formation of thiocyanate from tetrathionate was not stoichiometric. Attempts were made to drive the reaction to completion by varying the cyanide concentration, pH, reaction time, and temperature. In no case was the same absorbance index obtained with 10-cm. cells as with 1-cm. cells. In a few instances, when the hydrolysis reaction was allowed to take place overnight, the absorbance index was higher than that obtained in 1-cm. cells. This was shown to be caused by a secondary reaction of cyanide on the thiosulfate produced in the first decomposition

$$CN^- + S_2O_3^{--} = CNS^- + SO_3^{--}$$

During one experiment, it was noticed that the absorbance of a sample which was left in the sample compartment of the spectrophotometer for 10 minutes remained constant while the absorbance of a sample left in daylight for the same time decreased to one half the value of the unexposed sample. It then became apparent that the instability of the ferric thiocyanate color to light was the cause of the inconsistent absorbance indices obtained with the most dilute solutions rather than an incomplete hydrolysis with cyanide. This fading of the ferric thiocyanate

color has been recognized (6) as one of the limitations of the colorimetric thiocyanate method for iron. However, the fading observed in this experiment was about five times greater than previously reported. The fading is believed to be due to a reaction between the nitric acid and thiocyanate, and is considered to be promoted by the presence of ferric ions. This theory is supported by the authors' observation of an increased fading with high ferric ion concentration and low thiocyanate concentration.

Stabilization of Ferric Thiocyanate Color. In an attempt to intensify the color of the ferric thiocyanate complex the samples were prepared in 50% acetone solutions, but no pink color was apparent, indicating that very little ferric thiocyanate complex had formed. It was felt that the acetone was causing the ferric chloride, which was used as a source of ferric ions, to be undissociated in solution. Therefore, ferric nitrate and nitric acid were employed in the procedure as the source of ferric ions and acid, respectively. When the nitrate reagents were used in 50% acetone, the average absorbance index per milligram of tetrathionate ion per 100 ml. was 0.093 with 1-cm. cells and 0.088 with 10-cm. cells. There was still a decrease in absorbance in a sample exposed to light for 10 minutes before being read, but the rate of color fading was not so fast as in the aqueous-chloride system.
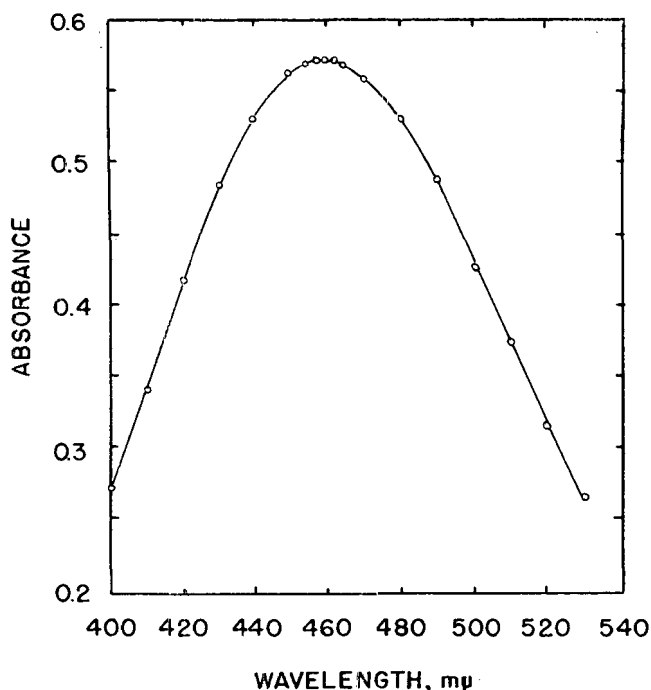

Figure 1. Absorption spectrum of ferric thiocyanate complex obtained from 2.93 mg. of tetrathionate

Color developed according to recommended procedure, and measured in 1-cm. absorption cells

The instability of the color to light was corrected by developing the complex in 100-ml. mixing cylinders coated with Krylon black acrylic spray. When the reagents were mixed in these vessels, the color was stable for at least 4 hours, and in 18 hours the absorbance decreased by only 3%, as shown in Table I. Therefore by employing 50% acetone, nitrate reagents, and opaque mixing vessels, adequate color stability was obtained.

Since the use of 50 ml. of acetone per 100 ml. of final volume limited the amount of sample which could be taken, a study was made to determine the minimum amount of acetone necessary to obtain a stable color. As the percentage of acetone was decreased from 50 to 0%, only a 15% decrease in absorbance was observed with no decrease in stability, provided the color was

Table I. Effect of Acetone on Sensitivity and Stability

(All samples contained 0.50 mg. of $S_4O_6^{--}$ per 100 ml. The color was developed in opaque mixing cylinders, and the absorbance was measured at 460 mμ in 1-cm. cells)

Acetone Concn., %	Hours				
	0.25	0.50	1	4	18
			Absorbance		
50	0.114	0.114	0.114	0.114	0.111
25	0.105	0.105	0.105	0.105	0.101
0	0.097	0.097	0.097	0.097	0.094

Table II. Calibration Data

$S_4O_6^{--}$ Taken, Mg.	Absorbance	Absorbance Index
	1-Cm. Cells	
0.732	0.143	0.195
1.464	0.286	0.195
2.196	0.427	0.194
2.928	0.570	0.195
3.660	0.711	0.194
7.32	1.42	0.195
Average absorbance index		0.195
Standard deviation		±0.00052
Coefficient of variation, %		±0.25
	10-Cm. Cells	
0.050	0.097	0.194
0.100	0.194	0.194
0.150	0.294	0.195
0.200	0.390	0.195
0.250	0.485	0.194
0.500	0.973	0.195
Average absorbance index		0.195
Standard deviation		±0.00055
Coefficient of variation, %		±0.28

developed in opaque cylinders, as shown in Table I. Since the slight decrease in sensitivity obtained in the absence of acetone is more than compensated for by the ability to use larger sample aliquots, further use of acetone was discontinued.

Acid Concentration. A study of the color development as a function of acid concentration revealed that the absorbance is constant over a final concentration of 0.35 to 1.0N nitric acid, provided that the same amount of acid is present in the blank. Therefore, the use of 10 ml. of 1 to 1 nitric acid to give a final concentration of 0.8N nitric acid is recommended in the proposed procedure.
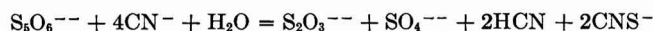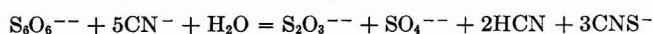
Ferric Nitrate Concentration. A similar study revealed that the absorbance is constant over a final concentration of 0.1 to 0.3M ferric nitrate. Since excess ferric nitrate merely increases the background absorbance, 5 ml. of 2M ferric nitrate to give a final concentration of 0.1M ferric nitrate is recommended in the color-developing procedure.

Optimum Wave Length. The spectrum of the complex, as shown in Figure 1, indicates that the optimum wave length is 458 to 462 mμ instead of 525 mμ as employed by Robinson (5). Use of this lower wave length results in a twofold increase in sensitivity, without any change in cell length.

Calibration Data. The calibration data, shown in Table II, were obtained by developing the color on aliquots of a standard solution of sodium tetrathionate. Identical absorbance indices were obtained in the 1-cm. and 10-cm. cells. Excellent reproducibility was achieved in each instance. A practical lower limit of about 0.05 mg. of tetrathionate ion per 100 ml. was attained, indicating that a sample containing as little as 0.002 gram of tetrathionate ion per liter can be analyzed by the recommended procedure.

Interferences. No formal study was made of interferences. Cations which form complexes or precipitates with thiocyanate and could compete with the ferric ion for the available thiocyanate must be absent. Therefore, Robinson recommended passing such samples over a strong cation exchange resin in order to remove these interferences. This procedure has been retained.

In addition to tetrathionate, the higher polythionates also react with cyanide to form thiocyanate.

$$S_6O_6{}^{--} + 5CN^- + H_2O = S_2O_3{}^{--} + SO_4{}^{--} + 2HCN + 3CNS^-$$

$$S_5O_6{}^{--} + 4CN^- + H_2O = S_2O_3{}^{--} + SO_4{}^{--} + 2HCN + 2CNS^-$$

Therefore the method as developed is suitable for the three polythionates, although each ion will interfere in the determination of the other. In practice, it is probable that all three ions will exist to some degree in any sample suspected to contain polythionates. As any thiocyanate in the liquor is a serious interference, in the recommended procedure a sample blank is prepared by adding the reagents in such a manner that tetrathionate is not converted to thiocyanate. Robinson measured the sample against the sample blank, but this procedure required working with a variable slit width. In the recommended procedure both the sample and sample blank are measured against a reagent blank at constant slit width. The difference in absorbance is proportional to the tetrathionate concentration.

Test of Method. Neither standard samples nor an independent method of analysis of sufficient sensitivity was available to test the method. Therefore, it was decided to add various amounts of a standard tetrathionate solution to a solution of unknown tetrathionate composition and attempt to determine the added tetrathionate accurately. Several 10-ml. aliquots of a liquor known to contain tetrathionate, with and without 0.05 to 2.5 mg. of added tetrathionate, were analyzed by the recommended procedure using both 1-cm. and 10-cm. absorption cells. The absorbance obtained for an aliquot containing no added tetrathionate was subtracted from the absorbance obtained on the other aliquots. From these corrected values the amount of tetrathionate ion found was calculated. In these calculations an absorbance index obtained by passing pure solutions over the ion exchange resin (1.4% lower than the value reported in Table II) was employed. An average error of only 1% was observed between the amount of tetrathionate added and recovered (Table III). The general

Table III. Test of Method

Tetrathionate Added, Mg.	Tetrathionate Found, Mg.	Error, %
	1-Cm. Cells	
0.500	0.501	+0.2
1.00	1.01	+1.0
1.50	1.51	+0.7
2.00	2.00	0.0
2.50	2.48	−0.8
	10-Cm. Cells	
0.050	0.049	−2.0
0.100	0.097	−3.0
0.150	0.150	0.0
0.200	0.197	−1.5
0.250	0.245	−2.0
		Av. ±1.0

tendency for the answers to be slightly low seems to indicate some holdup of the tetrathionate on the resin. If the calibration curve is obtained by passing pure solutions over the ion exchange columns in the same manner as the samples are to be treated, this holdup of tetrathionate should be automatically compensated for.

LITERATURE CITED

(1) Furness W. and Davies, W. C., *Analyst*, 77, 697 (1952).
(2) Jay, R. R., ANAL. CHEM., 25, 288 (1953).
(3) Mellor, J. W., "Comprehensive Treatise on Inorganic and Theoretical Chemistry," vol. 10, p. 617, Longmans, Green, London, 1930.
(4) Murayama, T., *J. Chem. Soc. Japan, Pure Chem. Sect.*, 74, 349 (1953).
(5) Robinson, R. E., unpublished data.
(6) Sandell, E. B., "Colorimetric Determination of Traces of Metals," 2nd ed., p. 367, Interscience, New York, 1950.

CRYSTALLOGRAPHIC DATA

100. Procaine Penicillin G

HARRY A. ROSE, Lilly Research Laboratories, Indianapolis 6, Ind.
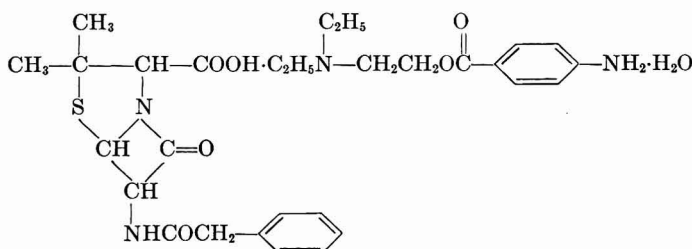
Structural Formula for Procaine Penicillin G

Procaine penicillin G is a salt of penicillin which is highly insoluble in water. The compound has found great use in medicine, but the crystallography has been very incompletely described.

Crystals suitable for crystallographic work may be obtained by slow evaporation of methanol-water solutions (Figure 2). Good crystals may also be obtained by slow mixing of aqueous solutions of procaine hydrochloride and sodium penicillinate.

The crystals obtained from methanol-water were exception-

X-Ray Powder Diffraction Data

d	I/I₁	hkl	d(Calcd.)
13.83	0.33	100	13.92
9.49	0.27	001	9.54
8.38	0.33	110	8.37
7.38	0.13	11$\bar{1}$	7.36
7.03	0.27	011	7.05
6.59	0.13	101	6.60
6.05	0.53	21$\bar{1}$	6.07
5.77	0.07	210	5.80
5.55	0.20	111	5.58
5.22	0.67	020	5.23
4.90	0.20	120	4.90
4.71	0.07	002	4.77
4.62	1.00	31$\bar{1}$, 300	4.66, 4.64
4.47	0.07	30$\bar{2}$	4.50
4.27	0.13	22$\bar{1}$	4.28
4.12	0.53	31$\bar{2}$	4.13
3.68	0.33	32$\bar{1}$, 22$\bar{2}$	3.69, 3.68
3.51	0.27	320	3.47
3.38	0.33	130	3.39
3.28	0.33	31$\bar{3}$	3.28
3.16	0.07		
3.12	0.07		
3.03	0.07		
2.954	0.07		
2.888	0.03		
2.793	0.07		
2.701	0.03		
2.582	0.03		
2.459	0.20		
2.334	0.03		
2.281	0.03		
2.078	0.03		
2.050	0.03		
2.022	0.03		

ally clear, and interfacial angles calculated from x-ray data were checked against those measured optically using the Unicam rotation x-ray camera as a single circle goniometer with results as noted in the crystal morphology section.
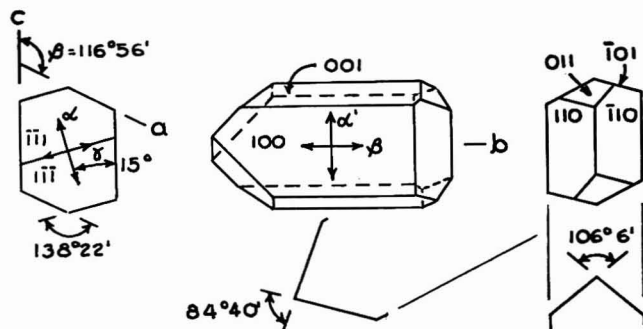

Figure 1. Orthographic projection of procaine penicillin G

The x-ray powder diffraction data were obtained using vanadium-filtered chromium radiation and a camera 114.6 mm. in diameter. A wave-length value of 2.2896 A. was used in the calculations.

CRYSTAL MORPHOLOGY

Crystal System.   Monoclinic hemimorphic.

Form and Habit.   Massive crystals elongated parallel to the b axis and lying either on the 100 or 001 faces. The usual forms are the orthopinacoid {100}, basal pinacoid {001}; right prism {110} with left positive monoclinic sphenoid {$\bar{1}11$} and right clinodome {011} or left prism {$1\bar{1}0$} with right negative monoclinic sphenoid {$1\bar{1}1$} and left clinodome {$0\bar{1}1$}; and the positive hemiorthodome {$10\bar{1}$}.

Axial Ratio. $a : b : c = 1.491:1:1.022$ (x-ray).

Interfacial Angles (Polar).   110 ∧ $\bar{1}10$ = 73°54' (calculated x-ray), 73° 54' (observed optical). $1\bar{1}1$ ∧ $\bar{1}11$ = 90° 40' (calculated x-ray), 90° 24' (observed optical). $\bar{1}01$ ∧ 001 = 41° 38' (calculated x-ray), 41° 42' (observed optical). 011 ∧ $01\bar{1}$ = 95° 20'.

Beta Angle.   116° 56'.

X-RAY DIFFRACTION DATA

Cell Dimensions. a_0 = 15.61 A., b_0 = 10.47 A., c_0 = 10.70 A.
Formula Weights per Cell.   2.
Formula Weight.   588.71.

Density.   1.255 grams per cc. (displacement and flotation), 1.256 grams per cc. (x-ray).

Space Group. $C_2^2 - P2_1$ (based on the facts that the only regular x-ray extinctions show $0k0$ present only when $k = 2n$ and that the external morphology is obviously hemimorphic).

OPTICAL PROPERTIES

Refractive indices. α = 1.545, β = 1.570, γ = 1.685 (1). α' (in 100) = 1.546, γ' (in 001) = 1.610.

Optic Axial Angle. $2V$ = 52° (calculated from α, β, and γ).

Optic Axial Plane.   010.

Acute Bisectrix. γ.

Optic Sign.   Positive(1).

Extinction. α ∧ c = 15° in acute β.
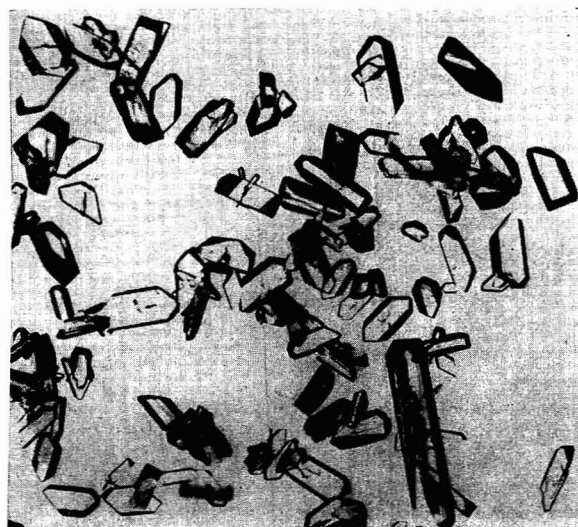

Figure 2.   Crystals of procaine penicillin G
Recrystallized from methanol-water on microscope slide

FUSION BEHAVIOR.   Procaine penicillin G melts in the range 106° to 110° C. with decomposition. The melt does not crystallize on cooling.

LITERATURE CITED

(1) Tillson, A. H., and Eisenberg, W. V., *J. Am. Pharm. Assoc.*, **43**, 760–7 (1954).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

SCIENTIFIC COMMUNICATION

Determination of Minute Traces of Water by Use of Methylene Blue

SIR: The determination of minute traces of water, especially in cases where drying methods cannot be used, has been difficult and has involved the use of long procedures and complicated and unwieldy apparatus. The basis of most routine methods, other than drying, involves distillation or absorption on anhydrous magnesium perchlorate, calcium sulfate, phosphorus pentoxide, or cobaltous chloride.

Various miscellaneous methods involving measurements of physical constants have been used, such as the measurement of specific gravity for the determination of water in alcohols; the measurements of electrical conductivity, refractive index, viscosity, and critical solution temperature; centrifugation for suspended moisture; measurements of heat of hydration, dew point, heat of dilution in sulfuric acid, and pressure differential after exposure to lithium chloride; and near infrared absorption spectra.

Other methods involve condensation into a tared cold trap, where the water is weighed, or into a buret, where the volume change is recorded. In fuel gas, moisture has been detected by the color change of cobaltous bromide on hydration. Water

in aniline has been estimated from a cloud point with rapeseed oil or a cottonseed oil–heavy mineral oil composition and in furfural with a hexanol–cottonseed oil reagent; in textiles and dehydrated foods by equilibrium humidity; in ammonium nitrate by the boiling of the melt at reduced pressure; in acetic acid by a polarimetric method; in gases by the absorption of the water in an organic solvent on which the conductance was determined; and in textiles, ceramics, paper, tobacco, and petroleum oils by electrical resistance.

Chemical methods used include manometric ones, such as the measurement of hydrogen evolved after treatment with sodium; titrimetric methods, such as the reaction between water and magnesium nitride to give ammonia, which is absorbed and titrated with sulfuric acid; the sodium ester method for small quantities of water in alcohol; hydrolysis of acetyl chloride in the presence of pyridine, which detects 0.02% water but is subject to much interference; and anhydrous cupric sulfate and potassium permanganate. The most widely used method for the determination of small quantities of water is the Karl Fischer, which uses a solution of iodine, sulfur dioxide, and pyridine in methanol. This method has a sensitivity as low as 0.005% with an accuracy of within ±0.001%.

Here a simple and very sensitive method, which makes use of the extreme solubility of methylene blue in water, has been developed in connection with the determination of moisture in hexachloroethane, which has a limit of 0.05% water.

One volume of the sample is dissolved in two volumes of carbon tetrachloride, technical grade, Octagon Process Inc., and a small quantity (approximately 10 mg.) of dry methylene blue, water-soluble, technical grade, Fisher Catalog No. A-766 is added. Water present in the sample tends to be released owing to decreased solubility in the mixture of hexachlorethane and carbon tetrachloride, and coagulates in globules. In the globules, the methylene blue, which remains as a reddish brown powder on the surface of the carbon tetrachloride, turns a deep blue, indicating the presence of the water. This indication appears with as little as 0.03 mg. of water, although when the water content goes below 0.1 mg. the globules may not appear on the surface, but may adhere to the sides of the beaker, in which case vigorous stirring and policing are required to disperse the methylene blue.

The sensitivity was determined by using samples of a jet fuel, (Navy Grade JP-5, or heavy end aviation fuel, HEAF), saturated with water. The saturation point of this jet fuel had been carefully studied in this laboratory and determined to be at 0.011% water by means of the classical Karl Fischer method. Various volumes of this water-saturated jet fuel were dissolved in carbon tetrachloride and methylene blue was added. As little as 0.08 mg. of water, representing 0.000016% on the basis of a 500-gram sample, was indicated by the methylene blue. On a sample of turbine oil as little as 0.03 mg. of water was detected. The dry jet fuel when dissolved in the carbon tetrachloride gave no reaction with the methylene blue. Because this technical grade of carbon tetrachloride showed no reaction at all with the methylene blue, it was assumed to be anhydrous.

It is believed that this method could be made applicable to other substances with only traces of water, so long as the substance itself gives no reaction with methylene blue. Both dry acetone and dry benzene give no reaction with methylene blue, and thus may be used also as media. It is possible to develop strips, made of materials inert to methylene blue and impregnated with dry methylene blue, which detect traces of water on dipping. This method, of which systematic investigation is under way at this laboratory is applicable also to moisture detection in gases.

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MEETING REPORT

Symposium on Microscopy

JOHN W. SHELL

The Upjohn Co., Kalamazoo, Mich.

THIS Symposium on Microscopy, the sixth in a series sponsored by the Armour Research Foundation of Illinois Institute of Technology, was held June 16 to 18 in Chicago. It covered aspects of electron microscopy, x-ray microscopy, organic qualitative microanalysis using the microscope, teaching of microscopy, and unsolved problems in the microscopy laboratory. There were no prepared talks, and the discussions were wholly spontaneous.

The topic for the first session was: "Should the Light Microscopist Buy an Electron Microscope?" Jack Kelsch, Interchemical Co., served as panel chairman; panel members were F. Gordon Foster, Bell Telephone Laboratories; A. G. Huckle, Imperial Paper and Color Corp.; Alan Kirkpatrick, American Cyanamid Corp.; F. F. Morehead, American Viscose Corp.; and C. F. Tufts, Sylvania Produc's Co.

The arguments for the expected affirmative answer covered fairly well the general application of electron microscopy. Many advantages of a combination of light and electron microscopy were presented. C. F. Tufts noted the relationship between degree of resolution and cost and pointed out that whether or not cost is commensurate with the answers expected over the years depends upon a given company's specific problems.

Among the applications where electron microscopy is particularly adept are studies of micro structure and grain boundaries, deformation, and etch pits, and determination of particle size. In all these applications, the light microscope is limited by low resolving power.

In a discussion of the advantages of electron microscopy in the biological fields, Mary Rollins, Southern Regional Research Laboratory, pointed out that in a study on problems of high speed propulsion, the detection of a double, rather than single lining to walls of pulmonary arteries was possible only with the increased resolving power of electron microscopy. G. J. Socha, University of Wisconsin, reported on the use of electron microscopy by cytologists in studies of chromosome structure. Its use in virology is well known.

Considerable attention was given to specific instances in which the so-called overlap region of light and electron microscopy is needed. J. J. Kelsch cited the study of the effect of particle size, shape, and efficiency of grinding on pigments, where important subtle differences are just beginning to be detectable as the limit of resolution of light microscopy is reached.

The topic for the second session was x-ray microscopy. Serving as chairman was Sterling Newberry, General Electric Co., with panel members: Albert Baez, University of Redlands; Jackson Clemmons, University of Wisconsin; Harold Sherwood, Eastman Kodak Co.; and Thomas Turnbull, North American Phillips Co.

Newberry presented a brief history of x-ray microscopy, and defined the present status of this rapidly developing field. Advantages include the large gain in resolving power due to the great decrease in wave length, the large depth of field, and the high penetrating power. Moreover, wave-length-dependent

absorption characteristics of matter are such that x-ray microscopy may be used for microchemical analysis. Figures 1 and 2 show the General Electric x-ray microscope; typical radiomicrographs are shown in Figures 3 and 4.

Three methods of x-ray microscopy are now in use. The simplest, contact radiography, records microscopic information by the irradiation of an object in contact with the film. Magnification is entirely due to photoenlarging. The advantage is simplicity; limitations lie in the enlargement process. H. F. Sherwood reports increased definition by the use of a vacuum exposure holder, which ensures good contact between the specimen and the film emulsion. When double-emulsion film is used, such as Kodak Type M, the back emulsion is either removed or restrained from development.
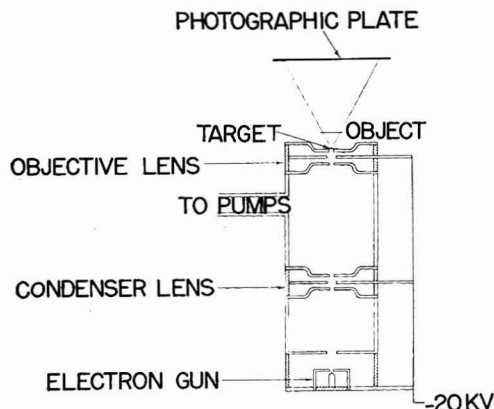
Figure 1. Basic design of General Electric
x-ray microscope

The limitations of this method are overcome, to some extent, by a second method, by which a shadow of an object very near a point source of x-rays is projected onto a film at some distance from the object. Magnification is achieved by virtue of divergence of the x-rays before the image is formed on the film. This minimizes the limitation of film resolution, as little photoenlarging is necessary. Magnification is increased as the distance from the source to the object decreases, and the final resolving power is a function of the diameter of the source. A. V. Baez, reporting on his work at the University of Redlands, improves resolution by using a tube designed by Cosslett and Nixon at Cambridge, in which electrons are magnetically focused on a very thin metal target. The width of the electron beam at convergence on the target is of the order of 1 micron. Thus, this is essentially the width of the "point" source, provided the target metal is thin enough. A great advantage lies in the fact that the target is also the tube window, which permits close proximity of the object to the target at atmospheric pressures. The ability to work at normal pressures, thus obviating the problem of specimen desiccation, is particularly appealing to the biologist. The General Electric Laboratories have devised an x-ray microscope which uses electrostatic rather than magnetic focusing.

A third method of x-ray microscopy utilizes focusing by reflection. Total reflection does occur, even with x-rays, at angles approximating grazing incidence. Use is made of a system of curved "mirror" surfaces: A single pair of reflectors produces a line image from a point. A second pair of reflectors, whose effective surfaces remain perpendicular to those of the first, produces a point from this line image. Thus, divergent beams may be focused into convergence. At present, the resolving power of existing instruments of this type is estimated at 0.5 micron.

Considerable attention has been given the method originally

proposed by Gabor, which may be termed microscopy by reconstructed wave fronts. An imaging system is devised such that diffraction fringes are plainly evident. The image is known as a hologram. Illuminating this with a source of radiation, and reversing it through an optical system, an image of the original object is reconstructed of the same size as the object. However, by changing wave lengths, the reconstructed image will be larger by a ratio of the two wave lengths. Thus, employing x-rays initially, and reconstructing with visible light, a magnification of 5000 to 1 may be achieved.

An important application of x-ray microscopy in chemical analysis was discussed by Baez. The mass absorption coefficients of x-rays for an absorber of fixed atomic number follow a curve which has the general shape illustrated in Figure 5. The discontinuity in the mass absorption coefficient as a function of wave length is associated with the binding energy of the electrons in their orbits. Considering a specific example, copper exhibits a discontinuity at about 1.38 A. If a microscopic object containing copper is illuminated with monochromatic radiation of 1.30 A., the absorption will be high. It will be low with radiation of 1.5 A. If two pictures are made using each of these wave lengths, the copper in the sample will be dark in the first and light in the second. This is the qualitative aspect of the method; densitometry techniques permit its quantitation. Engström claims the detection of chemicals to a concentration of about 10^{-13} gram.

Jackson Clemmons discussed an adaptation of the above method to biological materials. Such an adaptation, termed quantitative historadiography, involves the calculation of mass absorption constants for thin tissue slices, using nitrocellulose as a reference standard. The concentration of water as well as organic matter may be determined on a given portion of a tissue section.
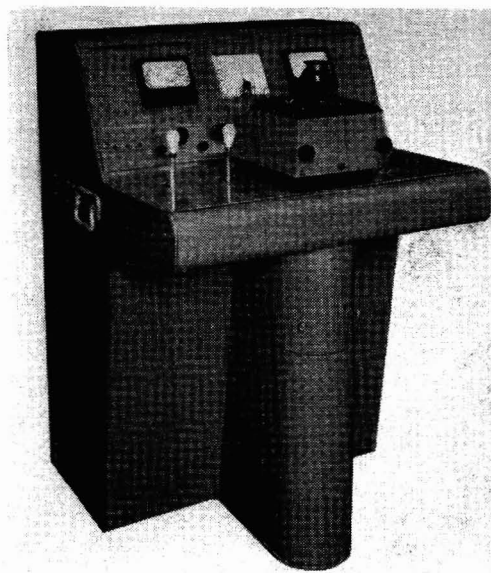

Figure 2. General Electric x-ray
microscope

H. F. Sherwood announced the availability of an extensive bibliography on microradiography and soft x-ray radiography from the Medical Division, Eastman Kodak Co., Rochester 4, N. Y.

"Organic Micro-qual. Using the Microscope" was the topic of the next session. D. E. Laskowski, Armour Research Foundation, was chairman; panel members were W. C. McCrone, Armour Research Foundation; D. G. Grabar, Industrial Rayon Co.; and Ralph Johnson, University of Illinois.

McCrone emphasized convenience, the advantages of observing directly the results of tests, permitting a higher degree of confidence, as well as greater insight as to what really happens, and the small sample required. An example cited was the application to the identification of chromatographic fractions, particularly significant to the organic chemist. A more subtle but powerful advantage was pointed out by Kirkpatrick: Microscopists have a habit of retaining a vast storehouse of mental images which are of great value in the solution of many difficult problems of identification or behavior.

A consideration of separation and purification methods gave rise to discussions of (1) recrystallization on the microscope slide, whereby the best crystals, say for x-ray purposes, are "teased out" using a needle, (2) microsublimation, with the use of high vacuum for compounds that decompose at elevated temperatures, (3) microdistillation, (4) mechanical separation on the microscope stage, to obtain samples for infrared, ultraviolet, and other analytical methods, and (5) thermal diffusion. Impurities in a melt will migrate to or away from the cooler portion of a microscope slide on a Kofler stage (that section directly over the optical aperture). This was suggested by McCrone as the basis for a purification micromethod. Tufts suggested that zone recrystallation for purification of organic compounds might be successful if the thermal diffusion mechanism can be utilized.

Figure 3. Potato chip, showing salt crystals

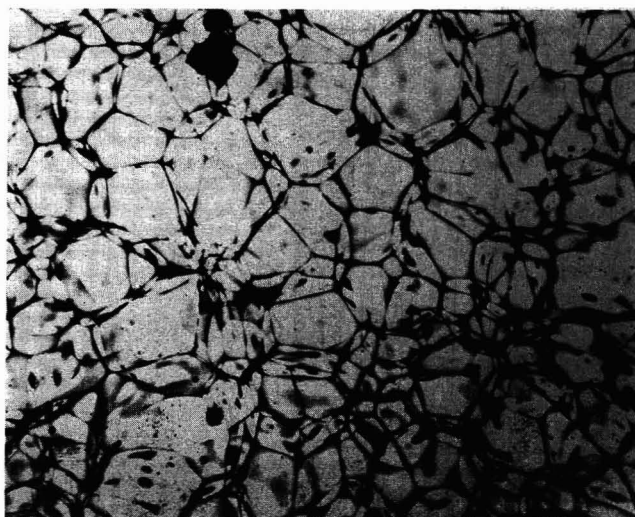Taken with General Electric x-ray microscope (×43)

Only brief mention was given the methods of optical crystallography in organic qualitative analysis.

Ralph Johnson discussed an electronic melting point device in which use is made of a hot stage with a very slow rate of temperature increase. Light passes through the crystals on the stage through the microscope into a photoelectric cell above the eyepiece. The variation in light transmittance as the crystals melt triggers an electronic device which records the temperature.

In a discussion of classification reactions, considerable attention was given to mixed fusion techniques. The method is exemplified by the use of 2,4,7-trinitrofluorenone (TNF), which forms molecular addition compounds with polynuclear aromatic compounds. Whether or not an unknown forms such an addition compound serves as a first classification; further classifications are made on the basis of melting point values for the unknown, the addition compound, the eutectic, if present, between the unknown and

the addition compound, and the eutectic, if present, between the addition compound and the TNF. Should the unknown not form an addition compound, aromatic derivatives of the unknown may often be made, which will then react to form an addition compound.

Many of the classical methods of qualitative organic chemistry are readily adapted to micro levels, the reactions being carried out on a microscope slide. An example is the 2,4-dinitro- or p-nitrophenylhydrazone formation as a test for the carbonyl group. As C. W. Mason, of Cornell, observed, the product of a lengthy reflux reaction very often may be quickly obtained by simply melting the reactants together.

Figure 4.   Aluminum-tin alloy (95 to 5) (×43)

Taken with General Electric x-ray microscope

Two half-day sessions were devoted to discussions of problems having an incomplete or unsatisfactory answer. Chairmen were Nick Galitzine, General Electric Co., and Charles Maresh, American Cyanamid Co. The combined panels included G. G. Cocks, Battelle Memorial Institute; John Facq, Toni Co.; F. B. Rosevear, Procter and Gamble; F. Gordon Foster, Bell Laboratories; Fred Morehead, American Viscose Corp.; Oscar Richards, American Optical Co.; Mary Willard, Pennsylvania State University; and H. W. Zieler, W. H. Kessel Co.

One of the first problems presented was that of making stereomicrographs at high (400 to 500×) magnification. Among solutions suggested was the half-aperture method, whereby a photograph is taken with half the condenser aperture covered; the other is taken with the other half covered. A slight loss of resolving power accompanies this procedure. Other suggestions were shifting of a decenterable iris diaphragm, and the use of crossed polaroids, each covering only half the field of the condenser and with the two photomicrographs taken with the analyzer alternately parallel to each sector in the condenser.

A problem of viscosity determination on a micro level was presented by McCrone. This specific problem required a determination involving little or no motion of a supercooled liquid melt. No promising suggestions were presented except perhaps magnetic movement of 1 to 5 micron carbonyl iron particles.

Difficulties in photographing moving objects, such as bacteria, or particles which exhibit Brownian movement, were mentioned by Cocks, who described a photoflash illuminator. In connection with the procedures of flash photomicrography was a discussion of problems of light intensity and shape of light source,

the intensity becoming more significant in phase work. O. W. Richards reported excellent results with his arrangement. McCrone mentioned the use of the cold stage in certain systems to "freeze" Brownian movement, permitting the use of standard exposures.

The problem of sectioning fibers for electron microscopy received considerable attention. Mary Rollins suggested embedding the fibers in a 4 to 1 mixture of methyl methacrylate and ethyl methacrylate. Following sectioning and mounting on the grid, the methacrylate is dissolved out. The fiber may then be shadowed, and the electron micrograph taken.

The advantages of pressure, rather than heat, in forming replicas for electron microscopy were mentioned. F. G. Foster pointed out the use, when microtomy is not applicable, of wax replication studied with the metallograph. This versatile instrument, though designed for the study of metal surfaces, may be applied to many other surfaces. In resinography, fillers and curing cracks are readily seen. Methods of surface preparation such as mild grinding and lapping are applicable, even on nonmetal surfaces. Dark-field and polarized light may also be used to advantage and are readily available with most metallographs.

In connection with the measurement of sample thickness, disadvantages of the microtome setting method were mentioned.
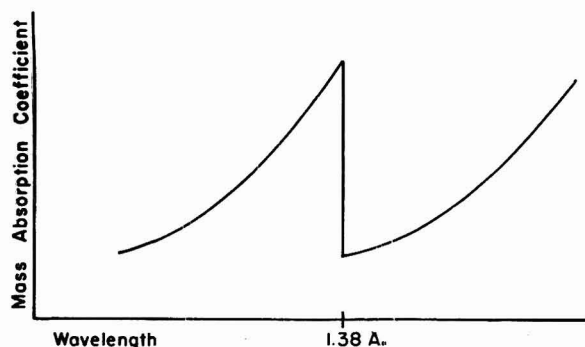

Figure 5. Mass absorption coefficient of copper

Among the methods presented were use of the micromanipulator to rotate a section on edge for measurement with the filar ocular, and the most common method, that of focusing from lower surface to upper with a calibrated fine focus. The instrumental, optical, and human errors involved were discussed. The interference microscope works very well for measurement of sample thickness when the refractive index of the sample is known.

The question of versatility in instruments received considerable attention; opinions were divided as to the advantages of a "universal" microscope. The differences in opinion could be resolved, however, if the so-called universal microscope were not too badly cluttered with attachments and the price were low enough to permit several in each laboratory. The best universal microscope would seem to be a stand on which all possible accessories—i.e., interference, fluorescence, phase, polarized light, dark field, etc.—could be fitted as needed without disturbing the specimen.

Other problems discussed involved projection of crystal images onto the slit of a spectrophotometer, for purposes of quantitating pleochroism, and methods of correcting for the various aberrations of lens systems. R. L. Seidenberg, Bausch & Lomb, discussed distortion, astigmatism, coma, and field curvature. Flatfield photomicrography may be achieved by introducing negative power into the lens system with a special eyepiece. This is an approach to the ideal, which could be surpassed, but appears to be optimum considering the cost of a more elaborate correction.

Another consideration is the sacrifice of resolution with gain in flatness of field, due to the decrease in the numerical aperature.

The last session of the symposium was on the teaching of chemical microscopy. Chairman was F. Gordon Foster, Bell Laboratories. Panel members were C. W. Mason, Cornell University; Mary Willard, Pennsylvania State University; Alan Kirkpatrick, American Cyanamid Co.; and C. F. Tufts, Sylvania Products Co.

A considerable portion of the discussions concerned the defining of features possessed in common by all good microscopists. These traits from the academic standpoint were outlined by Willard and Mason, and were generally coincident with those from the industrialist's viewpoint, as presented by Kirkpatrick and Tufts. A good microscopist is an "idea man," well trained in the physical sciences. He is inquisitive, and as he is often the one who must bridge the gap between the other scientific fields, he is adaptable.

The microscopy courses at Cornell University and Pennsylvania State University were outlined, and examples of industrial training programs were presented. C. W. Mason, whose teachings and writings have probably influenced the training of more chemical microscopists than those of any other man, summarized his feelings on the teaching of the subject. His emphasis was on supervised self-teaching, where one learns by doing. In teaching fiber identification, for instance, only an introduction should be presented. In ferreting out the rest of the information, the student develops his own technique, and is caused to admit ignorance and ask questions. This latter characteristic continues to identify the best graduate microscopist. The importance of precise language was also stressed: Poor communication is no less a barrier in teaching microscopy than in other fields.

W. C. McCrone emphasized the need in industry for more microscopists, which reflects the need for more teaching of microscopy. The teaching plant must be adequate; many phenomena must be shown, as well as described. As Mason stated, it is one thing to study phase diagrams—another actually to see that a eutectic is fine-grained.

Most schools that teach microscopy merely integrate the work with other courses, such as instrumental analysis or mineralogy. Only a few, such as Cornell University, Pennsylvania State University, the University of Colorado, and Illinois Institute of Technology, give specific courses.

EXHIBITS

Features of interest were exhibits of photomicrographs and various instruments available commercially. H. W. Zieler, W. H. Kessel Co., demonstrated a number of these instruments. Of interest was a Baker interference microscope with a light-shearing system, American Optical equipment for phase microscopy, and a Zeiss Winkel polarizing microscope with built-in illumination system, quintuple nosepiece with individually centerable objectives, and an interchangeable binocular tube. A new Zeiss Winkel assembly for electron flash photomicrography included facilities for determining and setting the light intensity by means of a variable neutral density filter and a photoelectric measuring device. The Leitz Ortholux microscope with photomicrographic equipment and the Reichert research microscope, Zetopan, with features for visual observation as well as photomicrography, were demonstrated. Also shown were the Reichert heating stage and hot bar, both Kofler designed. Attached to a single upright was a photomicrographic unit with built-in light source (Orthophot) suitable for negative size 4 × 5 inches, as well as 35 mm., a time-lapse unit, in the low-price range, with beam splitter, auxiliary lens system, and a Bolex 16-mm. movie camera, with range of exposure intervals from 1 to 1200 seconds.

As a result of informal discussions, a committee was formed to plan future microscopy symposia with meetings in the East, perhaps alternating with Chicago meetings.

Simplified Operating Dead-Stop Magic-Eye End-Point Indicator

C. A. McCauley and W. J. Gresham,
Monsanto Chemical Co., Monsanto, Ill.

The use of an instrumental dead-stop end-point indicator for Karl Fischer determination of moisture is well known. Numerous literature references describe the construction and application of this type of instrument (1–3). For application in an industrial control laboratory, where various products must be analyzed in a number of solvent systems, several objections are encountered with most instruments:

Operation is too complex for routine control work, where the analysis of different types of materials frequently makes it necessary to change the adjustment of the instrument.

Commercially available instruments are rather expensive for wide laboratory use.

Many models are alternating-direct current–powered with the ever-present shock hazard in case of insulation or part failure.
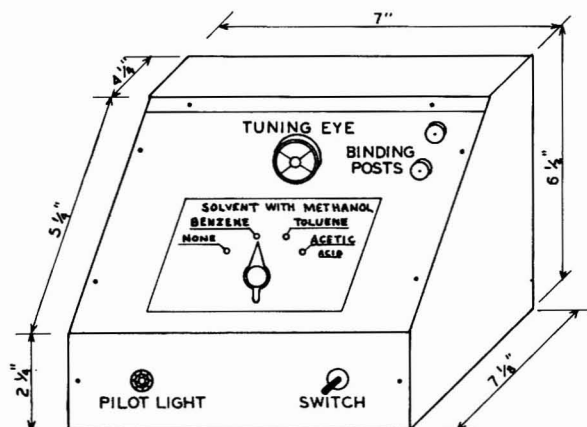

Figure 1.   Optical view of instrument cabinet

With the increasing shortage of technically trained analysts, it is becoming more and more desirable to tailor analytical techniques to suit the available personnel, minimizing as many variables as possible. To answer this demand, an instrument described in the literature (1) has been adapted to combine the following desirable features.

All variable controls are eliminated from the operating panel.

A twin-indicator tuning-eye tube is employed to widen the effective viewing angle and sufficient brightness is obtained for accurate observation under almost all lighting conditions, including daylight or bright fluorescent light without shading the tube.

No critical matching of tubes or other parts has been found necessary on 14 instruments so far constructed.

Accommodations are provided for the selection of numerous solvent systems without readjustment, so that a variety of materials can easily be analyzed with the same instrument.

Exceptional precision in detecting the same equivalence point repeatedly is possible with the circuit employed and adequate sensitivity is provided for all normal analytical requirements.

The instrument is effectively stabilized against line voltage fluctuations up to at least ±15%.

Shock hazard is almost entirely removed, as this type of instrument features a transformer power supply.

The complete instrument can easily be constructed from standard radio parts costing less than $25.

The instrument is very compact (8 × 8 × 8 inch sloping front cabinet, Figure 1), and the cost of operation is negligible.

APPARATUS

The circuit (Figure 2) was adapted from one described by Kieselbach (1). Refinements include the substitution of a twin-indicator "magic-eye" tube for a single indicator, a resistor step switch in place of a continuously variable control, and changes in resistor values to provide greater brightness of the magic-eye tube. These changes also appear to have minimized the occasional need for the critical matching of the 6SL7-GT duo-triode (1).
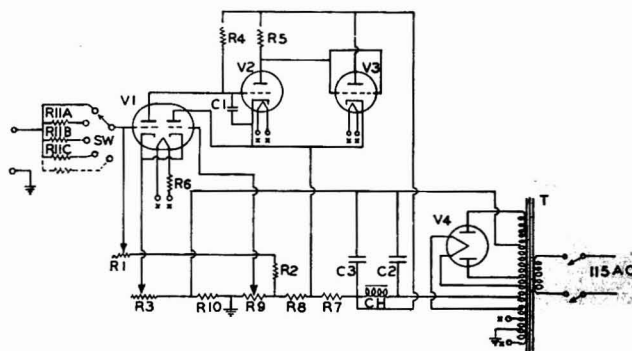

Figure 2.   Circuit diagram

V1.	6SL7	R5.	1-meg.
V2.	6F5-GT	R6.	3.3-ohm 1-watt
V3.	6AF6-G	R7.	20,000-ohm 10-watt
V4.	5Y3-6T	R8.	6000-ohm 10-watt
T.	Transformer Stancor. 6010	R9.	25-ohm wire-wound
CH.	12 Hy Choke Triad C-5X	R10.	6000-ohm 10-watt
C1.	0.01-mfd. ceramic	R11, A, B, C.	Adjust for solvent
C2.	8-mfd. 450-volt electrolytic		system and titration cell used
C3.	30-mfd. 450-volt electrolytic		Chassis, Bud Radio Co. No. C-B-38
R1.	250,000-ohm		Cabinet, Bud Radio Co. No. C-1584
R2.	4700-ohm		Switch, CRL No. 2503
R3.	1-meg.		Magic-eye bracket, Amphenol No.
R4.	2.2-meg.		58-MEA-8

All parts are mounted on a 7 × 6 × 2 inch chassis, except for the solvent selector switch with its resistor, R11, the magic-eye tube mounting clamp, and the binding post terminals for the electrode leads, all of which are mounted on the front panel.

If interference from stray electrical fields makes it desirable to employ shielded leads, the binding posts may be replaced with a coaxial connector. Alternatively, adequate shielding can be secured more economically by the use of conventional microphone connectors, such as the Amphenol 75-PCIM chassis unit and 75-MCIF cable connector.

The instrument is adjusted initially as follows:

1. After a 5-minute warm-up period, with the leads disconnected from the electrodes, turn the adjusting control, R9, to the stop nearest the junction of R2 and R8. Turn the solvent selector switch to "None."

2. Adjust control R3 so that the eye just closes.

3. Connect the leads to the electrodes (mounted in the titration vessel) and make a temporary parallel connection from the binding posts to a potentiometer or vacuum tube voltmeter.

4. With methanol in the titration vessel adjust R1 to read approximately 350 mv. on the potentiometer or voltmeter.

5. Titrate with Karl Fischer reagent to a sudden large change in e.m.f.

6. Adjust R9 so the eye just opens at this point. The instrument is now ready for use. No further adjustment should be required unless the 6SL7 tube is replaced.

Resistors R11A, B, C, etc., are selected to compensate for differences in cell resistance, at the end point, for various solvent

systems as compared to the cell resistance for methanol alone. As many solvent systems may be provided for as are desired, by the addition of suitable resistors to the selector switch.

Preliminary values can be obtained easily by measuring endpoint cell resistance through the electrodes with an ohmmeter. Values for $R11$ are equal to the difference between the cell resistance for the solvent used and that for methanol. In preparing the various solvent systems for measuring comparative resistance, the end points may be established by measuring the largest break with a potentiometer or voltmeter as was done with methanol in steps 3, 4, and 5.

In most cases, standard radio-type 0.5-watt resistors singly or in combination can be employed to obtain the required values in ohms.

Representative Values of $R11$ for a Particular Cell Assembly

	Ohms
Methanol	0
Methanol-benzene	680
Methanol-toluene	1050
Methanol–acetic acid	200

Electrodes used were No. 16 gage platinum wire sealed in glass. Interelectrode spacing was not found to be critical.

LITERATURE CITED

(1) Kieselbach, R., ANAL. CHEM., **21**, 1578 (1949).
(2) McKinney, C. D., Jr., and Hall, R. L., IND. ENG. CHEM., ANAL. ED., **15**, 460 (1943).
(3) Serfass, E. J., *Ibid.*, **12**, 536 (1940).

Modified Beckman-Type Atomizer in the Perkin-Elmer Flame Photometer

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IN RECENT years, flame photometry has become an important technique in analytical chemistry, but the equipment has not been perfected in every detail to achieve the best results with the least inaccuracy and inconvenience, particularly in regard to the atomizers.

The Perkin-Elmer flame photometer has two types of atomizer: a metal atomizer containing a needle valve for regulating the rate of atomization, and a glass atomizer without a valve. Both have a "funnel" on top for introducing the sample and a tube on the bottom through which the compressed air enters. Neither atomizer has been entirely satisfactory in this laboratory, because the glass one requires a large amount of sample to obtain an accurate reading; the metal one is subject to frequent air leakage and clogging; and with either one it is necessary to wait until the remainder of the sample in the funnel has been atomized before introducing a new sample.

Figure 1

A Beckman-type atomizer, with slight modifications, was tried in the Perkin-Elmer flame photometer and found to be very convenient. This type of atomizer is similar to that used with the early model of Beckman flame photometer. It was modified as follows:
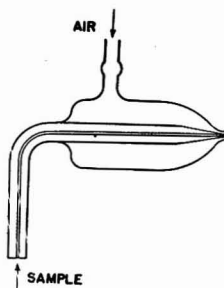
The length of the capillary inlet tubing, after bending, was extended to about 20 mm. and the inlet orifice was not constricted, but left the full 0.5-mm. diameter of the bore of the capillary tubing. The outlet orifice was constricted, but to only about half the diameter of the bore (Figure 1). This permitted an atomization rate of about 2.5 ml. per minute with an air pressure of 10 pounds per square inch—several times that of the regular Beckman atomizer, which has very constricted inlet and outlet orifices. In the Perkin-Elmer flame photometer this atomizer is held in place with a rubber stopper, and the liquid samples are supplied to it from a 5-ml. beaker that is held under it by a spring steel attachment fastened to the photometer by the right front screws (Figure 2).
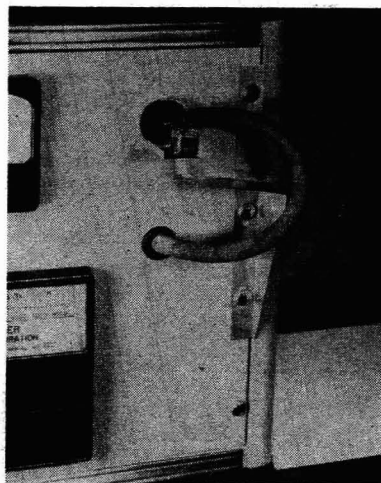

Figure 2

This type of arrangement has been in use in this laboratory for the past 3 years and has been found most satisfactory and timesaving for determination of sodium, potassium, and calcium in plant material, soil extracts, and water. No loss of instrument sensitivity or precision occurs with this modification, and there is no difference in instrument readings between the Perkin-Elmer atomizer and the modified Beckman atomizer. Small samples may be accurately read, very little clogging occurs, there is no air leakage of the atomizer so that a uniform rate of atomization is obtained, and it is possible to change samples immediately after a reading has been made, without waiting for the remainder of the sample to atomize. Readings can be made for about two samples per minute.

This modification is less expensive and simpler than other modifications, such as that of Dubbs. [ANAL. CHEM., **24**, 1654 (1952)]. Approximate dimensional measurements of modified atomizer (in millimeters) are:

Capillary tubing	
O.D.	6
Bore	0.50
Orifice at outlet	0.25
O.D. at outlet	1.0
Orifice at inlet	0.5
Length of inlet extension after bending	20

Air chamber	
O.D.	12
Length	30
Orifice at outlet	2.0
Air inlet tubing	
O.D.	5
Length	40

Modified Flame Photometer for Microdetermination of Sodium and Potassium

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IT IS necessary to determine very small quantities of sodium and potassium in connection with studies on the perfusion of single kidney tubules in the Necturus in vivo, which are currently under way in this laboratory. The collected fluid after perfusion amounts to 1 µl. or less, and contains, as a maximum, 95 millimicromoles of sodium and 2.8 millimicromoles of potassium. These quantities are too small to be measurable in normal commercial flame photometers. Consequently, a new detector for the Beckman flame photometer (Model DU) has been designed to make the necessary microdetermination of sodium and potassium.

The availability of the RCA Type 6217 photomultiplier tube has made it possible to measure both elements with a single detector, using the most favorable flame emission lines, 589 mµ for sodium and 768 mµ for potassium. The photomultiplier has an S-10 spectral response, high (approximately 76%) in the range of sodium flame emission, and low (approximately 2%) but still usable in the range of potassium emission. Unfortunately, the tube is available only with a 1.5-inch photocathode, much larger than is required for the present service, and the unused area of the photocathode contributes unwelcome noise to the signal.

DESCRIPTION OF APPARATUS

Figure 1 shows a block diagram of the complete equipment.

A Beckman DU spectrophotometer with Model 9200 flame attachment is used to provide the signal. The box which normally contains the phototubes and amplifier in the spectrophotometer has been modified to hold the 6217 tube. The high voltage for the tube is supplied by a Nuclear Instrument and Chemical Corp. Model 1090 power supply. As the phototube voltage should never normally exceed 800 volts because of phototube noise limitations, a smaller power supply may be used, provided it has equally good regulation and stability characteristics. The output of the photomultiplier, a voltage drop across a 40-megohm Victoreen resistor, is impressed across the input terminals of an Applied Physics Corp. Model 30 vibrating reed electrometer. The electrometer in turn feeds a Brown 10-mv., 4-second, recording potentiometer which provides a written record of the flame output.
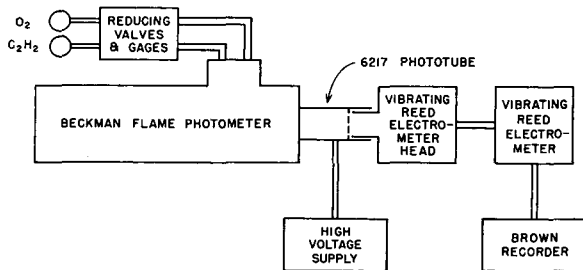

Figure 1. Block diagram of flame photometer assembly

The oxyacetylene flame attachment of the Beckman operates in the normal fashion, except for regulation of the gas supply. The regulation valves built into the flame control panel are kept constantly open, and the pressure is controlled instead by Kendall precision regulators (Model 10A). As these valves normally obtain their good regulation by bleeding a small fraction of the input gas to the ambient atmosphere, it is necessary to specify that the acetylene valve be modified to omit this feature.

The modifications to the Beckman amplifier box to enable it to hold the 6217 are straightforward. The shutter has been re-

tained, but all other parts within the box have been discarded. Although the shutter is not required in normal operation, its use makes it easy to segregate tube noise from flame noise when finding the optimal conditions of operation. The box is extended by a brass cylinder which completely encloses the photomultiplier tube, socket, and chain of resistors, making the entire assembly insensitive to external light and signal interference. The circuit diagram is shown in Figure 2. All resistors are precision, although there is no evidence that this precaution is essential.

As the G terminal of the electrometer is not at ground potential, the positive lead (ground) of the high voltage supply must not be grounded to the rest of the system and a 1 to 1 isolation transformer is desirable in the 110-volt input of the high voltage supply. The Brown recorder should be grounded to the electrometer-monochromator ground, which should be connected to a good building ground.

Care must be exercised in obtaining a good light seal, as the modified instrument is very sensitive to extraneous light. Because of this, it was found advisable to run an additional black painted brass tube inside the instrument from the output of the monochromator to the shutter. The tube is machined to fit on one end into the recess surrounding the exit port of the monochromator, and on the other end into the recess around the shutter opening. All exposed inside parts are painted black, and all outside apertures are covered with black Scotch electrical tape.

A smaller section of brass tubing fastened onto the end of the large brass phototube enclosure shields the anode-output conductor. This smaller cylinder and central conductor are fashioned to allow direct mounting of the electrometer preamplifier head, thus providing a minimum signal loss and maximum shielding to the electrometer input.
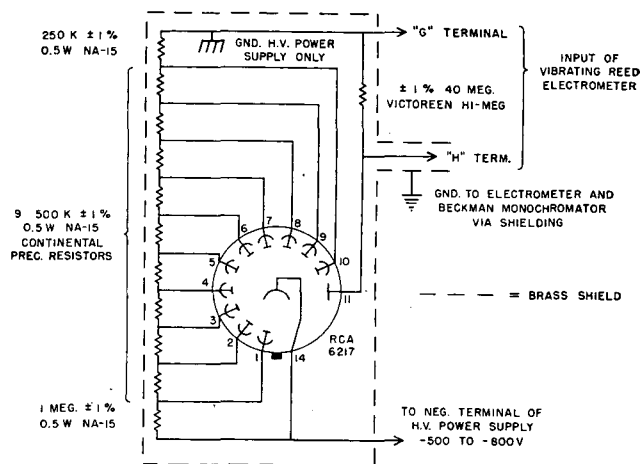

Figure 2. Circuit diagram for 6217 tube

The vibrating reed electrometer provides absolutely stable amplification of the moderately high impedance signal. The Brown recorder is an essential part of the system, as a written record is necessary for the measurement of samples of 0.5 ml. or less. Samples of 0.5 ml. can be measured satisfactorily using the small beakerlets supplied with the flame photometer. Smaller samples require the use of special cups which can be machined from Lucite to fit the specific needs of the problem. The sample holder can be adjusted so that the aspirator can suck the Lucite cups almost entirely dry.

RESULTS

The performance of the modified flame photometer is completely satisfactory for sodium. The standard deviation of a set of 10 determinations on 1.0-ml. samples of 100 micromoles of sodium per liter (100 millimicromoles of sodium per sample) is 0.6%, when the same cup is used for all samples (instrument

settings: slit width, 0.01 mm.; phototube, 620 volts; and electrometer sensitivity, 100 mv.). The standard deviation of a set of 10 determinations on 0.5-ml. samples of 1 micromole of sodium per liter (500 micromicromoles of sodium per sample) is 1.4%. In practice, the limit of detectability seems to be set by the sodium contamination from deionized water and glass containers.
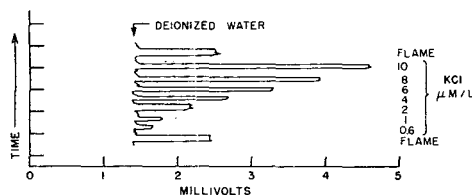
Figure 3. Tracing of Brown recorder of signal from potassium chloride solutions

Rate of consumption of fluid is 1.1 ml. per scale division, and the record moves at 50 seconds per scale division. The notation "flame" refers to the signal when no solution is being aspirated into the burner and the base line is taken as the signal when water purified by passage through an ion exchange column (Barnstead Bantam demineralizer) is aspirated

It is more difficult to obtain satisfactory microdeterminations of potassium than sodium. The potassium concentration in the solutions under study is much smaller than that of sodium, and the photomultiplier response is very poor at 768 mμ. Whereas tube noise and flame noise are effectively absent in the sodium measurements, each contributes significantly to the error in the potassium measurement. Although it is possible to get a much larger signal when the vibrating reed electrometer is set at the 10-mv. sensitivity for the potassium determinations, the records appear to be more easily measurable when the electrometer is set at 100-mv. sensitivity.

Figure 3 shows a tracing of the record obtained on samples of 0.5 ml. or less of solutions containing graded amounts of potassium running from 0.6 up to 10 micromoles per liter. These values are plotted against potassium concentration in Figure 4 (instrument conditions: slit width, 0.24 mm.; phototube, 720 volts; and electrometer sensitivity, 100 mv.). The standard deviation of sets of 10 duplicate samples measured under these conditions ranges from 1.5% for 6 micromoles per liter up to 8% for samples containing 0.6 micromole per liter. As these samples contained about 0.3 ml. of solution, the smallest sample represents a measurement of 200 micromicromoles of potassium.

Under the experimental conditions, there is no interference effect when potassium chloride in concentrations of 0 to 16 micromoles per liter is added to sodium chloride at a concentration of 100 micromoles per liter. There is a small increase in signal when sodium chloride in concentrations of 50 to 100 micromoles per liter is added to potassium chloride in concentrations of 2 to 4 micromoles per liter. At a concentration of 4 micromoles of potassium and 100 micromoles of sodium per liter, the increase amounts to 0.067%/(micromoles of sodium per liter), so that changes in sodium concentration of 10 micromoles per liter or less may be neglected in this concentration range.

The results obtained with the modified flame photometer may be compared with the manufacturer's specifications for the Model DU photometer with photomultiplier attachment. The detection limits are given as 0.0002 p.p.m. for sodium at 589-mμ wave length, and 0.001 p.p.m. for potassium at 766.5-mμ [Beckman Instruments, Instruction Manual No. 334, June 1954; Gilbert, P. T., Jr., *Ind. Labs.*, **3**, 41 (August 1952)]. These

are equal to concentrations of 8.7 millimicromoles of sodium and 25.6 millimicromoles of potassium per liter. The manufacturer further states that the detection limit is generally equivalent to the error in concentration measurement, and that the detection "limits indicated may be reached only under optimum conditions." From these figures it may be calculated that the Model DU with photomultiplier attachment will be able to measure concentrations of 0.6 micromole of sodium per liter to an accuracy within ±1.4% and 0.3 micromole of potassium to an accuracy within ±8%. These calculations may be compared with the accuracy obtained in the present study: for sodium, concentrations of 1 micromole per liter have been measured to ±1.4% and for potassium, concentrations of 0.6 micromole per liter have been measured to ±8%. The authors' results are slightly less accurate than the values calculated from the manufacturer's optimum performance data.
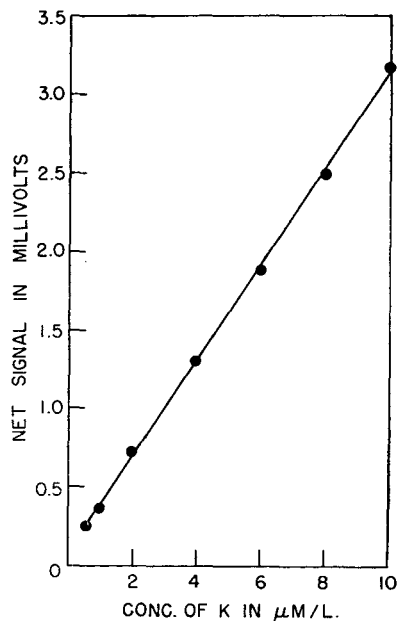
Figure 4. Response of flame photometer to potassium chloride solutions of graded concentrations

Points taken from record shown in Figure 3

However, the present modification was designed to measure very small quantities of sodium and potassium. The quantity of solution used to obtain the results given above comprised 0.5 ml. for sodium and 0.3 ml. for potassium. In practice, samples of 0.25 ml. are routinely used with no apparent sacrifice in accuracy. These volumes may be compared with the capacity of the standard beaker used in the photometer with photomultiplier attachment, which contains 5 ml. of solution. Thus, the modified spectrophotometer makes it possible to measure samples of one tenth or less of the normal volume with an accuracy comparable to the best that can be obtained with samples of normal volume under optimum conditions.