ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editorial Director

Pittsburgh Conference and the Analyst

QUITE NATURALLY we have followed the progress of the Pittsburgh Conferences on Analytical Chemistry and Applied Spectroscopy with keen interest, for they are one of the most convincing manifestations of the ever-increasing importance of a large segment of the chemical profession which we have been privileged to serve for many years.

In reading through the 5-day program published in this issue, our curiosity was aroused to the point where we referred back to the January 1950 issue of this journal in order to see the splendid growth experienced by analytical chemistry in seven short years. We wanted to compare in some detail the first conference with the one which will require the entire week of February 27–March 2, and will just about occupy every facility in Pittsburgh's huge William Penn Hotel.

Before making such a direct comparison we feel, however, that some reference to the conferences sponsored in Pittsburgh prior to 1950 is worth recalling. This is particularly important for the younger analytical chemist who, perhaps for the first time, will experience the unique thrill of attending a modern Pittsburgh conference cosponsored by the analysts and spectroscopists in the greater Pittsburgh industrial area.

In the early 40's the Pittsburgh analysts and spectroscopists started sponsoring conferences catering to their respective needs. These meetings were on a local and very modest scale. After several years of following the practice of holding separate conferences, the idea of joining forces was born in the late 40's. By 1950, the first of the Pittsburgh conferences as we know them today was staged in the William Penn Hotel. In three days, some 800 registered, much to the amazement of those in attendance and probably even more so to the cosponsors.

What seemed like a daring and very uncertain venture proved to be an instantaneous success scientifically and financially. Personally we believe much of the success of all of the six conferences held so far can be traced to the happy thought of combining a scientific meeting with an exposition where the tools of the analyst would be explained in papers and demonstrated in exhibits. Neither the scientific nor the show aspects have suffered by the presence of the other—indeed they constitute a "packaged deal" which has proved highly attractive to more and more who are engaged in the broad field of analysis, quality control, analytical research, instrumentation, etc.

Even a casual comparison of the 1950 and 1956 conference programs strikingly emphasizes the dependence most analysts today place on instrumentation and are quite happy to acknowledge it. It is a growing dependence that has helped materially to improve the professional and economic status of the analyst. In a somewhat different fashion much the same observation can very well be deduced from the impressive increase in the number of manufacturers of scientific apparatus who will be demonstrating their newest equipment in the exposition area at the William Penn Hotel.

Still another innovation of the conferences (initiated in 1950) and one which has added to the attendance and prestige of these gatherings, has been the staging of ASTM committee meetings.

At the first conference and exposition in 1950, an important meeting of E-2 (Emission Spectroscopy) was held. This group again will assemble in Pittsburgh on Wednesday afternoon of the meeting and will cosponsor a panel discussion and open forum on x-ray spectroscopy. On Monday in an all-day session, Committee E-13 (Absorption Spectroscopy), will hold four important subcommittee meetings and a general meeting late in the day with all sessions open to conferees. Some very long-range decisions may result from these deliberations.

It is difficult to point out all of the features scheduled for the seventh conference because the needs and interests of analysts vary widely. However, we feel we would be remiss if we did not point out among the special features the full-day Symposium on Chemical Corps Methods scheduled for Monday, February 27, and the conference dinner on Wednesday evening. The speaker is the internationally known associate director of the National Bureau of Standards, Wallace R. Brode, who will discuss "Ethical Problems in Scientific Publications."

And speaking of publication problems, we are duly impressed and somewhat concerned when we note 187 papers are scheduled for presentation. As editors, most of our trials and tribulations will be just beginning late in the afternoon of March 2 when we feel certain that new records for the conference in both attendance and number of papers will be history and when more than 2000 tired but wiser analysts will be on their way home. As editors, it will be up to us to find ways and means of publishing many of these papers. We have met this challenge before, we expect to meet it now and in the future.

Fisher Award Address

Precipitation of Sulfides from Homogeneous Solutions by Thioacetamide

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Rate measurements have shown that the hydrolysis of thioacetamide to acetamide and hydrogen sulfide in dilute acid solutions is first order with respect to both thioacetamide and hydrogen ion concentrations; only a small fraction of the acetamide formed was hydrolyzed to acetic acid and ammonia. The second-order rate constant for the hydrolysis reaction was found to be 0.21 ± 0.023 liter per mole per minute at 90° C. and the energy of activation was calculated to be 19.1 kcal per mole through the temperature range from 60° to 90° C. The assumption that thioacetamide hydrolyzes rapidly and quantitatively in hot acid solutions and can be substituted for hydrogen sulfide without modification of procedure is not valid. In solutions having pH values less than 3 the precipitation of lead sulfide by thioacetamide proceeds through hydrolysis of the latter; the rate of precipitation quantitatively follows the rate of hydrolysis. In solutions having pH values from 5.1 to 3.5 the precipitation is first order with respect to both thioacetamide and lead ion concentrations and inversely half order with respect to hydrogen ion concentration. The velocity constant for the expression $-d[Pb(II)]/dt = k[Pb(II)][CH_3CSNH_2]/[H^+]^{1/2}$ was found to be 1.13×10^{-3} liter $^{1/2}$ mole$^{-1/2}$ minute^{-1} at 90° C. in 0.081*M* sodium formate, and the energy of activation was calculated to be 15.5 kcal per mole. At pH 5 the rate of precipitation of lead sulfide can be a thousand times greater than the rate of formation of hydrogen sulfide by hydrolysis of the thioacetamide.

IN SPITE of the tremendous importance and extensive use of sulfide reactions in analytical work, most of the methods used are still based upon empirically developed procedures which in many cases originated in the earlier eras of analytical chemistry. Even today there is surprisingly little fundamental information available concerning sulfide reactions in general.

This lack of information is true regarding the mechanism of the precipitation of metal sulfides from aqueous acid solutions by hydrogen sulfide, the formulas of the initial precipitates and the changes which these precipitates undergo on aging in solution or on being dried under various conditions, the formulas of the thio anions existing in alkaline sulfide solutions, and the stability constants of these anions.

Deterrents to investigations of sulfide phenomena, and to more wide-spread use of sulfide separations, have been the unpleasant and toxic nature of hydrogen sulfide and the annoying physical characteristics of most sulfide precipitates. For this reason the use of thioacetamide as a substitute for hydrogen sulfide is a development which could have great potential significance in the future use of sulfide separations.

The first use of thioacetamide for analytical purposes was made by Iwanof (*10*) in 1935, the first application to a qualitative system for instructional purposes by Barber and his colleagues (*1, 2*), and the first application to quantitative procedures by Flaschka and coworkers (*6*). However, in the procedures in which thioacetamide has been employed to date it has been assumed that thioacetamide hydrolyzes quantitatively and rapidly at elevated temperatures in acid solutions to give acetic acid, ammonium ion, and hydrogen sulfide. The advertising literature from certain commercial sources of the reagent has carried categorical statements that thioacetamide can be substituted for hydrogen sulfide without modification of procedure and that exactly the same result is achieved as would be achieved with hydrogen sulfide gas.

Because of the general acceptance of the above assumption there has been a lack of critical investigations of the mechanism and rates of hydrolysis of thioacetamide in aqueous solutions of various pH values and temperatures, and of the mechanism of the precipitation of metal sulfides by thioacetamide under various conditions. In addition there has been no work on the use of thioacetamide as a homogeneous phase precipitant for effecting the quantitative separations of various elements as sulfides.

Interest in the reagent was aroused when, in the course of experiments made to determine the applicability of thioacetamide to an analytical system, observations showed that quantitative precipitation of certain sulfides was not obtained under conditions which had been satisfactory with hydrogen sulfide. In certain cases the sulfide precipitate appeared to form more slowly and in others more rapidly with thioacetamide than with hydrogen sulfide. In other cases the rates of precipitation of the sulfides appeared to be significantly faster than seemed compatible with preliminary measurements on the rate of hydrolysis of thioacetamide under similar conditions.

As a result of these observations a series of investigations was initiated of the mechanisms and rates involved in the hydrolysis of thioacetamide and in the precipitation of various metal sulfides by thioacetamide. It is hoped that these studies will provide a basis for the prediction of the optimum conditions for the use of this reagent in sulfide precipitations and separations.

HYDROLYSIS OF THIOACETAMIDE IN ACID AQUEOUS SOLUTIONS

Thioacetamide has found recent use in systems of qualitative analysis (*1, 2, 9, 13*) for the precipitation of the conventional hydrogen sulfide group elements and also has been proposed as a reagent for the quantitative precipitation and determination of certain metals (*6*). In most of these procedures it is assumed that conventional procedures based on the use of solutions saturated with hydrogen sulfide gas can be used with only minor modification. Even if this were true, effective use of the reagent as a homogeneous phase precipitant would be expedited by information concerning the rates of hydrolysis of thioacetamide in solutions at various temperatures and having various pH values. The results obtained from a study of the hydrolysis of thioacetamide in acid aqueous solutions are presented below.

EXPERIMENTAL

Reagents. Thioacetamide solutions, 1.0*VF* (volume formal, formula weights per liter), were prepared from Eastman white

label reagent. The solid material was white and was found to have a melting point range of 109.5° to 110.6° C. The trace of white, insoluble matter was filtered from the solutions. The solutions were colorless and gave no indication of change over a period of 2 to 3 weeks. At no time was the odor of hydrogen sulfide detected above the solutions.

Reagent grade chemicals were used unless otherwise specified. Hydrochloric acid solutions, 0.2 and $4VF$, were prepared from concentrated acid and standardized against standard sodium hydroxide. These hydrochloric acid solutions were diluted appropriately to give the desired concentrations for specific experiments.

Collecting solutions for hydrogen sulfide were prepared by dissolving cadmium chloride in $6VF$ ammonium hydroxide to give solutions which were $0.4VF$ in cadmium chloride.

Standard $0.01VF$ potassium iodate and potassium dichromate solutions were prepared by weight.

A $0.1VF$ sodium thiosulfate solution was standardized against the potassium iodate solution.

Formic acid, 90%, and $6VF$ sodium hydroxide solution were used for buffered solutions.

A $0.5VF$ lead nitrate solution was used.

A $2VF$ sodium hydrogen sulfide solution was prepared by saturating $4VF$ sodium hydroxide with hydrogen sulfide and then diluting this with an equal volume of water.

Table I. Rates of Evolution of Hydrogen Sulfide from Thioacetamide Solutions and Calculated First-Order Velocity Constants for Hydrolysis of Thioacetamide at 90° C.

	$0.10VF$ HCl			$0.05VF$ HCl		
CH_3CSNH_2, VF	Determinations Made	$-\frac{d[CH_3CSNH_2]}{dt}$ Moles ($\times 10^3$)/Liter Min.	k, Min.$^{-1}$	Determinations Made	$-\frac{d[CH_3CSNH_2]}{dt}$ Moles ($\times 10^3$)/Liter Min.	k, Min.$^{-1}$
0.0063	4	0.12	0.019			
0.0125	4	0.24	0.019	3	0.11	0.0088
0.025	7	0.40	0.016	3	0.18	0.0072
0.050	5	0.78	0.016	4	0.40	0.0080
0.10	4	1.7	0.017	5	0.75	0.0075
0.20	2	3.2	0.016			
		Average	0.017 ± 0.0015			0.0079 ± 0.0007

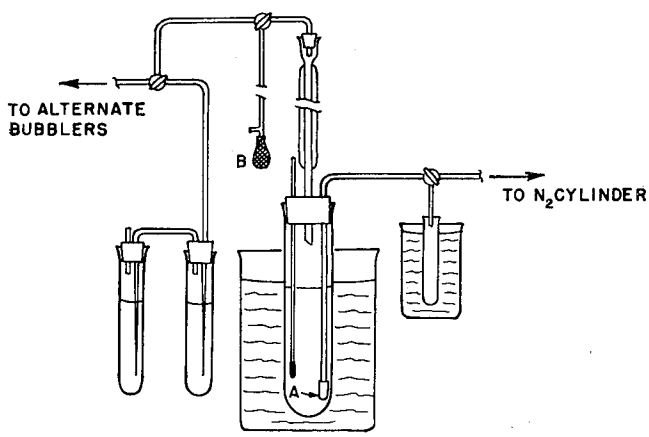

Figure 1. Hydrolysis apparatus

A. Sintered glass bubbler
B. Rubber pressure bulb

Apparatus. The apparatus is shown in Figure 1. The reaction tube consisted of a stoppered 38 × 200 mm. lipless test tube. Leading into and reaching to the bottom of the reaction tube was a sintered-glass gas bubbling tube, through which nitrogen was passed. Attached vertically above the reaction tube was a 60-cm. Liebig condenser. Tubing led from the condenser through a two-way stopcock to collecting tubes. The two-way stopcock permitted the changing of collecting tubes at will. The gas was led into the collecting tubes (22 × 175 mm. test tubes) through drawn capillary tubing. A two-way stopcock on the nitrogen inlet tube permitted the reaction solution to be sampled by being forced back through the sintered glass bubbler.

The reaction tube was surrounded by a water bath which maintained the temperature of the reaction solution at 90° ±0.5° C. Tests of the effluent gas showed that the collecting solutions effectively retained the hydrogen sulfide.

Procedure. Forty milliliters of solution were prepared in the reaction tube from water and the standard stock solutions of hydrochloric acid and thioacetamide. The pH of the solution was determined before and after the hydrolysis with a Beckman Model G pH meter in those cases in which the pH was 2 or greater. The reaction solution was heated to 90° C., and the flow of nitrogen was started. Timed portions of the effluent gas were passed through successive collecting solutions. The periods of gas collection ranged from 3 to 45 minutes, depending upon the reaction solution.

Each collecting solution was added to a 200-ml. flask containing a solution which consisted of approximately 0.5 gram of potassium iodide dissolved in the appropriate volume of standard potassium iodate solution. An amount of $12VF$ hydrochloric acid sufficient to neutralize the solution and make it $3VF$ in excess hydrochloric acid was used to rinse the collecting tube and was then added to the solution in the flask. This relatively high acid concentration was used to ensure the solution of all cadmium sulfide. The hydrogen sulfide was oxidized to sulfur. The excess of iodine in the resulting solution was titrated with standard sodium thiosulfate solution.

The velocity constant and order of the hydrolysis reaction of thioacetamide were calculated from data from series of such experiments.

Experiments to determine the effect of lead ion and lead sulfide upon the rate of hydrolysis of thioacetamide were conducted in the same manner as were the hydrolysis experiments, with the modification that lead nitrate was added to the reaction solution. In the cases in which the effect of lead ion was being studied, the precipitation of lead sulfide was prevented by the use of acid concentrations of 0.1 to $0.3VF$ and lead nitrate concentrations of 0.001 to $0.01VF$. Higher lead nitrate concentrations or lower acid concentrations would have resulted in partial precipitation of the lead as sulfide.

When the effect of lead sulfide was under consideration a hydrogen ion concentration of the order of $10^{-3}M$ was used. The lead sulfide precipitate was obtained by reaction with thioacetamide; therefore an initial thioacetamide concentration of $0.10VF$ and an initial nitrate concentration of $10^{-4}VF$ were used in order that the thioacetamide concentration would be virtually unaffected by the precipitation of the lead sulfide.

DISCUSSION AND RESULTS

The reactions were carried out at 90° C. in order that the hydrolysis would proceed at a rate that could be followed readily. Initially, experiments were conducted in which the hydrolysis was continued until about 50% of the thioacetamide had reacted. However, with hydrogen ion concentrations less than $0.02M$ the hydrolysis required several hours and, as is discussed later, the subsequent hydrolysis of acetamide took place to an appreciable extent in this time. Moreover, despite the use of a condenser, some loss of water by evaporation resulted. Because of these two factors there was an appreciable net change in the acid concentration. Since the rate of the thioacetamide hydrolysis is dependent upon hydrogen ion concentration, initial rates of hydrolysis were determined from solutions of known concentration by continuing the reaction for a period of time sufficiently short to ensure that changes in concentrations of species initially present would be small with respect to experimental errors. In each experiment, with the exception indicated in Table II, the differences in the initial and final thioacetamide and hydrogen ion concentrations were less than 5%.

Velocity Constant and Order of Reaction. The data from series of experiments conducted at 90° C. in 0.10 and $0.05VF$ hydrochloric acid solutions with concentrations of thioacetamide ranging from 0.0063 to $0.20VF$ are shown in Table I, together with

the calculated first-order velocity constants for the hydrolysis reaction of thioacetamide. Standard deviations are shown for the hydrogen sulfide analyses for each series and for the velocity constants.

The calculated velocity constant, k, for the first-order expression

$$-\frac{d[CH_3CSNH_2]}{dt} = k[CH_3CSNH_2]$$

is constant, as shown by Figure 2 in which $-d[CH_3CSNH_2]/dt$ is plotted against the thioacetamide concentration.

Table I shows that, for the two hydrochloric acid concentrations considered, the ratio $k/[H^+]$ is essentially constant, which implies that the hydrolysis is also first order with respect to hydrogen ion concentration. This matter was investigated with solutions ranging in hydrogen ion from 10^{-4} to 0.08 molal, and with the thioacetamide concentration kept constant at $0.10VF$. The data are assembled in Table II, where average values from a series of experiments are shown in each case.

Table II shows that the total hydrogen sulfide evolved in the experiments of Series 1 and 2 was small; as a result the reproducibility of the analyses is not better than 15 to 20%. In Series 5 and 6 the hydrogen ion activities were calculated from the formal hydrochloric acid concentrations by the use of the data of Randall and Young (*12*). In the other series a pH meter was used.

The ratio $k/[H^+]$ remains constant through the range of hydrogen ion activities considered, and the kinetic equation for the hydrolysis to acetamide may be expressed as

$$-\frac{d[CH_3CSNH_2]}{dt} = k'[H^+][CH_3CSNH_2]$$

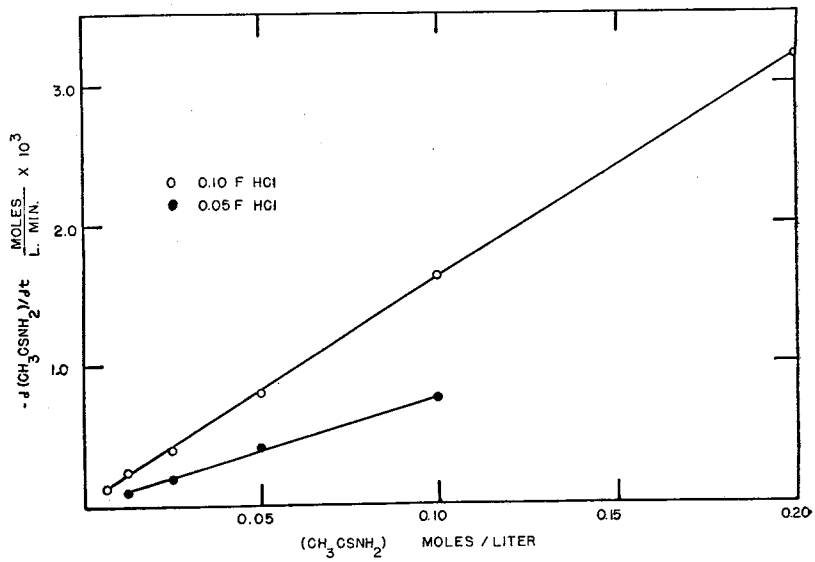

Figure 2. Rate of hydrolysis of thioacetamide at 90°C. *vs.* thioacetamide concentration

where $k' = k/[H^+]$. Figure 3 shows the rate of hydrolysis of thioacetamide as a function of the pH.

Temperature Dependence of Hydrolysis of Thioacetamide. The rates of the hydrolysis in solutions $0.100VF$ in thioacetamide and $0.100VF$ in hydrochloric acid were determined through the temperature range from 60° to 90° C. In Table III are values of k' for the various temperatures as calculated from the measurements. The activation energy is calculated from the slope of Figure 4, which is a plot of log k' against $1/T$. The value obtained is 19.1 kcal. per mole.

Hydrolysis of Acetamide. Analytical separations depending upon the use of thioacetamide would be considerably restricted in their application if, as has been assumed (*2*, *9*, *13*), the hydrolysis of acetamide were appreciable under the conditions and during the periods of time required for sulfide precipitations; the ammonia produced by the acetamide hydrolysis would make uncertain the final acid concentration of the solution. Experiments were conducted in order to determine the extent of the acetamide hydrolysis.

In one such experiment, a solution 40 ml. in volume and 0.10 VF in thioacetamide was maintained at 90° C. for 180 minutes. The initial and final hydrogen ion activities were 2.0×10^{-3} and 1.7×10^{-3}, respectively. The total hydrogen sulfide evolved was 0.24 millimole.

The change in total hydrogen ion is calculated to be 0.012 millimole. Thus, the results indicate that of the 0.24 millimole of acetamide formed, only 0.012 millimole or 5% was hydrolyzed.

These results are in approximate agreement with the data given by Crocker (*3*). Extrapolation from Crocker's data gives a second-order velocity constant of 0.2 liter per mole per minute for the hydrolysis of acetamide at 90° C. If the average concentrations of acetamide and hydrogen ion in the above experiment are used, the value 0.3 liter per mole per minute for the velocity constant is obtained. The difference between the calculated value and Crocker's value is of the magnitude expected from the approximations involved.

Under the conditions usually prevailing when thioacetamide is used in systems of qualitative analysis for the precipitation of the hydrogen sulfide group, the secondary hydrolysis of acetamide does not proceed to such an extent as to cause difficulty in the adjustment and control of the pH. On the other hand, in work involving successive operations recognition should be made of the continued presence of acetamide, as well as excess thioacetamide, in the solution.

Table II. Effect of Hydrogen Ion upon Hydrolysis of Thioacetamide and Second-Order Velocity Constant for Hydrolysis[a]

Series	Determinations Made	a_{H^+}	$-\frac{d[CH_3CSNH_2]}{dt}$, Moles ($\times 10^4$)/Liter Min.	k', Liter/Mole Min.
1	2	8.0×10^{-5}[b]	0.018	0.23
2[c]	4	1.7×10^{-4}	0.041	0.24
3	5	1.1×10^{-3}	0.22	0.20
4	3	2.0×10^{-3}	0.38	0.19
5	5	4.1×10^{-2}	7.4	0.18
6	4	8.0×10^{-2}	17	0.21
			Average	0.21 ± 0.023

[a] Thioacetamide concentration was $0.10VF$ in all experiments.
[b] Change in hydrogen ion concentration in experiments of this series amounted to approximately 12%.
[c] In experiments of Series 2 a formic acid–formate buffer was used. In other cases hydrochloric acid was used.

Table III. Variation of Second-Order Hydrolysis Constant with Temperature[a]

T, °C.	k', Liter/Mole Min.
90	0.21
80	0.098
70	0.039
60	0.019

[a] Thioacetamide $0.100VF$; hydrochloric acid $0.100VF$.

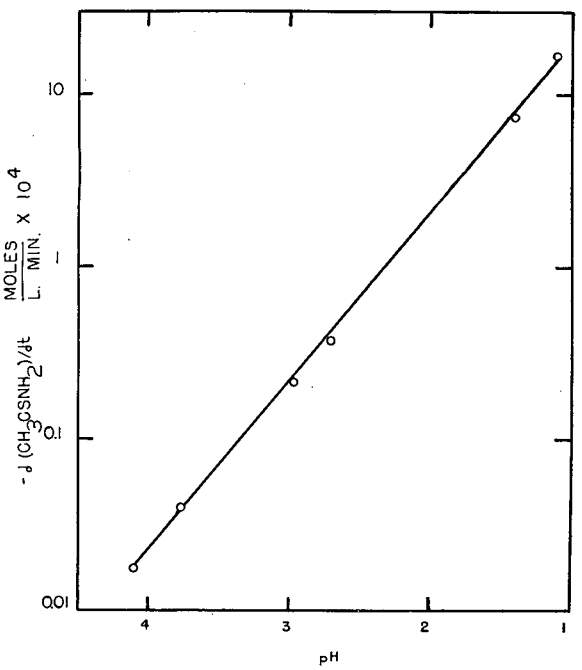

Figure 3. Rate of hydrolysis of thioacetamide at 90° C. *vs.* pH

Thioacetamide concentration 0.10*VF*

Effect of Lead Sulfide on Rate of Hydrolysis of Thioacetamide. Flaschka (*6*) has suggested that, in certain cases at least, the newly precipitated inorganic sulfide may catalyze the hydrolysis of thioacetamide. This possibility was investigated for the case of lead sulfide. In each experiment, a solution of 40-ml. volume and initially 0.10*VF* in thioacetamide, $10^{-4}VF$ in lead nitrate, and with a hydrogen ion activity of 2×10^{-3} was heated to 90° C., and the subsequent rate of evolution of hydrogen sulfide was determined. The lead was quantitatively precipitated as the sulfide by the time the reaction temperature was reached. The average rate of evolution of hydrogen sulfide from five experiments was 0.0015 ± 0.0002 millimole per minute. Table II shows that the rate found in the presence of the lead sulfide is the same, within the accuracy of the measurements, as that calculated for the case in which no lead sulfide was present.

Effect of Lead Ion on Rate of Hydrolysis of Thioacetamide. Lead ion could accelerate the hydrolysis of thioacetamide by catalysis or inhibit the hydrolysis by the formation of a relatively stable complex with thioacetamide. These possibilities were investigated. Two similar experiments were made with solutions 0.05*VF* in thioacetamide and 0.3*VF* in hydrochloric acid. One solution was also 0.01*VF* in lead nitrate. The data obtained are shown in Table IV. No lead sulfide precipitated in the solution containing lead nitrate. The close agreement of the data of the last three time periods indicates the absence of lead ion catalysis. The discrepancy in the first period is probably due to a slight difference in the rates at which the two solutions were heated.

Table IV. Effect of Lead(II) upon Rate of Hydrolysis of Thioacetamide

Experiment	H_2S Evolved in Successive 3-Min. Periods, Mmole/Min.			
	1	2	3	4
A[a]	0.089	0.070	0.063	0.051
B[b]	0.075	0.071	0.063	0.049

[a] Solution A. 0.05*VF* thioacetamide, 0.3*VF* HCl.
[b] Solution B. 0.05*VF* thioacetamide, 0.3*VF* HCl, 0.01*VF* $Pb(NO_3)_2$.

Other experiments were made in which lead ion was in excess of the thioacetamide. Two solutions were maintained at 90° C. and swept with nitrogen as in the hydrolysis experiments. Each was 40 ml. in volume, 0.60*VF* in hydrochloric acid, and 0.05*VF* in thioacetamide, and one was also 0.06*VF* in lead nitrate. The rate of evolution of hydrogen sulfide as a function of the thioacetamide concentration is shown in Figure 5. There is no indication of a decrease in the rate of evolution of hydrogen sulfide from the solution which contains the lead nitrate, such as would be expected if a stable complex of lead and thioacetamide were formed.

In a solution 0.05*VF* in thioacetamide, 0.1*VF* in hydrochloric acid, and 0.001*VF* in lead nitrate, the average rate of evolution of hydrogen sulfide was found to be 0.030 ± 0.002 millimole per minute from five determinations. This rate agrees within experimental error with the data shown in Table I for a similar solution with no lead ion.

Under the conditions of these experiments neither lead sulfide nor lead ion catalyzes the hydrolysis of thioacetamide. The possibility of catalysis by other metal ions is not precluded by these experiments.

Analytical Implications of Rate Data. If the assumption is valid that the precipitation of sulfides by thioacetamide is preceded by hydrolysis to hydrogen sulfide, the above rate data can be used to calculate certain limiting conditions.

For example, Flaschka (*5*) has recommended that the pre-

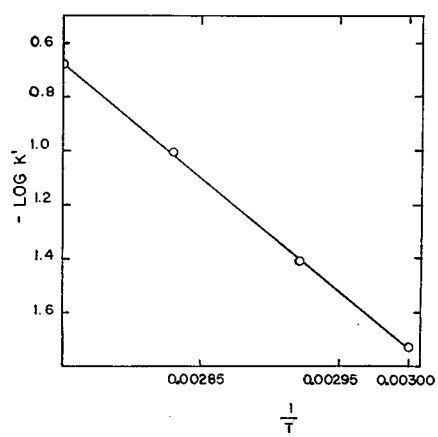

Figure 4. Rate of acid-catalyzed hydrolysis of thioacetamide as function of temperature

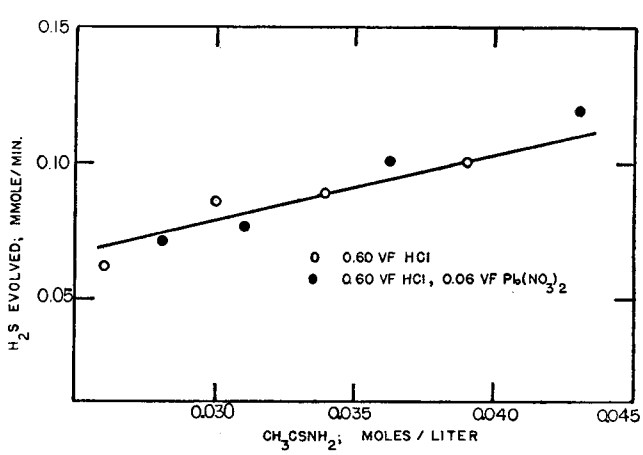

Figure 5. Effect of excess lead upon rate of evolution of hydrogen sulfide from thioacetamide solution at 90° C.

cipitation of lead sulfide be made from a solution 0.1VF in hydrochloric acid and having a fivefold "excess" of thioacetamide. Therefore, if the initial concentration of lead salt is 0.01VF, the initial thioacetamide should be made 0.05VF. Calculations made with the above rate data indicate that such a solution would have to be heated at 90° C. for approximately 15 minutes in order for hydrolysis of the thioacetamide to furnish a quantity of hydrogen sulfide just equivalent to the lead present. At 60° C. the time required becomes approximately 2 hours.

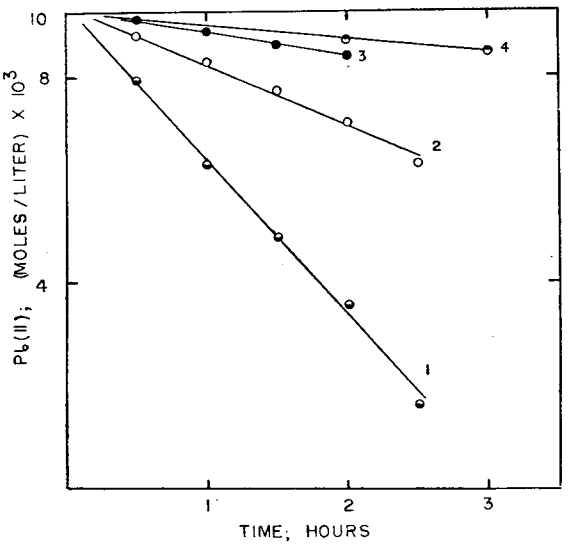

Figure 6. Effect of concentration of thioacetamide upon rate of precipitation of lead sulfide at 90 °C.

Hydrogen ion concentration = 1.6 × 10^{-1}
1. 0.10VF thioacetamide
2. 0.050VF thioacetamide
3. 0.020VF thioacetamide
4. 0.010VF thioacetamide

When sulfide precipitations are made by means of gaseous hydrogen sulfide, the general practice is to saturate the solution with the gas at atmospheric pressure, which provides an aqueous hydrogen sulfide concentration approximately 0.1VF. Complete hydrolysis of the thioacetamide under the above conditions without loss of hydrogen sulfide would cause the solution to be only half saturated at 1-atm. pressure; furthermore, the time required for half of the thioacetamide to be hydrolyzed at 90° C. is calculated to be 40 to 45 minutes.

As a second case, consider the precipitation of zinc sulfide. This precipitation is usually made from solutions having pH values from 2 to 3 and saturated with the gas. One would predict from the above rate data that at a pH of 2.7, a solution 0.01VF in zinc salt and 0.1VF in thioacetamide would require at 90° C. a time of 4.5 hours for hydrolysis to produce a quantity of hydrogen sulfide equivalent to the zinc present. A qualitative experiment was made in which 100 ml. of a solution having a pH of 2.7, and being initially 0.1VF in thioacetamide and 0.01VF in zinc salt was heated to 90° C. for 1 hour; the precipitate which formed corresponded to less than 1 mg. of zinc. Upon passage of a rapid stream of hydrogen sulfide through a similar solution containing no thioacetamide, the precipitation began within 30 seconds and appeared to be complete within 3 to 4 minutes.

In systems of qualitative analysis the precipitation of the hydrogen sulfide group by hydrogen sulfide is frequently begun in a small volume of a hot solution with a relatively high acid concentration in order to secure more rapid precipitation of arsenic(V). These conditions are favorable for the hydrolysis of thioacetamide at a rate which will produce within a reasonable time a quantity of hydrogen sulfide equivalent to the metals to be precipitated. However, this is not likely to be true if, as has been recently recommended (*4*), only a "slight excess" of thioacetamide is added. After this initial treatment, the solution is usually diluted to give a solution 0.2 to 0.3VF in acid, and a second saturation is made in order to obtain quantitative precipitation of lead, tin(II), and cadmium. A similar dilution of a thioacetamide solution would leave a solution which was not saturated with hydrogen sulfide and from which, unless care is observed in any subsequent heating, hydrogen sulfide can be expelled more rapidly than it is produced by hydrolysis. Thus, lead sulfide precipitates produced by such a dilution with cold water have dissolved when the solution was subsequently heated to boiling, even though an excess of thioacetamide was present. Finally, in an attempt to adapt thioacetamide to the precipitation of the hydrogen sulfide group elements, the precipitation of lead was found to be incomplete under conditions which had been satisfactory with gaseous hydrogen sulfide.

PRECIPITATION OF LEAD SULFIDE BY THIOACETAMIDE

EXPERIMENTAL

Reagents. Standard solutions of potassium dichromate were prepared by weight, and sodium thiosulfate solutions were standardized against these.

A standard solution of sodium perchlorate for control of the ionic strength of solutions was prepared by the neutralization of a measured volume of standard perchloric acid with sodium hydroxide to pH 7.0, as measured with a pH meter. The neutral solution was then diluted to a known volume.

Solutions of thioacetamide, lead nitrate, and sodium hydrogen sulfide were prepared as described in the preceding section.

Table V. Precipitation of Lead Sulfide by Hydrogen Sulfide from Hydrolysis of Thioacetamide[a]

Time, Min.	Lead(II), Mole/Liter	
	Found	Calcd.
0	0.0088	....
3	0.0083	0.0082
6	0.0077	0.0076
18	0.0051	0.0051

[a] Initial thioacetamide 0.10VF; hydrochloric acid 0.01VF; 90° C.

Eastman white label thioacetamide (Lot 34) and Matheson, Coleman and Bell thioacetamide (Lot 209518) were used in the experiments and were found to give identical results.

Sodium formate–formic acid buffer solutions with constant sodium formate concentration were prepared from sodium hydroxide solution and 90% formic acid. Solutions of formic acid of various concentrations were prepared and to each of these was added the same amount of sodium hydroxide solution. The solutions were adjusted by addition of water to the same final volume.

Apparatus. The reaction apparatus and sampling device were the same as those used in the hydrolysis study.

Procedure. The reaction solutions were prepared by mixing measured volumes of stock solutions of thioacetamide, buffer, sodium perchlorate, and lead nitrate and diluting to 100 ml. The reaction solution was placed in the constant temperature bath, and, when measurements of the direct reaction were being made, a slow stream (one to two bubbles a second) of nitrogen was passed through the solution. The nitrogen kept the reaction solution stirred and forced solution from the sampling outlet tube after a sample had been taken.

At timed intervals about 10 ml. of solution were forced through the sintered-glass bubbler from the reaction tube into a sample tube surrounded by a cooling bath which quenched the reaction.

The sample was left in the cooling bath for 1 to 2 minutes, by which time it had reached room temperature, then was centrifuged to remove the small amount of lead sulfide which passed through the sintered glass. A 5.00-ml. portion of sample was taken from the centrifugate by pipet and transferred to a 15 × 125 mm. test tube which contained 1.5 millimoles of sodium hydroxide. The lead in solution was rapidly and quantitatively

precipitated in the alkaline solution by the thioacetamide. In experiments in which equivalent amounts of lead and thioacetamide were present, 0.2 millimole of sodium hydrogen sulfide was added to ensure that the lead was completely precipitated. The precipitate of lead sulfide was removed by centrifugation and washed twice with 2-ml. portions of hot water which contained 0.1 millimole of sodium hydrogen sulfide.

The lead sulfide was then treated with 1.5 ml. of $2VF$ nitric acid, and the mixture was heated in boiling water. If, after 5 minutes, any black residue remained, the mixture was heated over a direct flame until the residue dissolved. Dilute nitric acid and moderate heating in a water bath were used to lessen danger of oxidation of sulfide to sulfate, which takes place in hot concentrated nitric acid. There was occasionally evidence of some sulfur formation.

To the nitric acid solution of the lead were added 6 meq. of ammonium acetate; then it was heated in boiling water for 1 to 2 minutes to dissolve any lead sulfate which may have formed and was transferred to a 125-ml. conical flask. Standard potassium dichromate in slight excess was added by pipet, and the mixture was heated to boiling to coagulate the lead chromate. The mixture then was cooled and filtered, and the excess chromate in the filtrate was determined iodometrically. The titration with standard sodium thiosulfate was made from a microburet.

To determine the extent of complex formation by lead and formate, excess solid lead chloride was added to solutions which had constant pH and ionic strength values but various sodium formate concentrations. The mixtures were rotated in a constant temperature bath until equilibrium was reached (equilibrium was approached from both above and below saturation); then the lead concentration was determined by precipitation of the lead with excess standard potassium dichromate and iodometric titration of the excess with sodium thiosulfate.

DATA AND DISCUSSION

Precipitation of Lead Sulfide by Hydrolysis of Thioacetamide. Semiquantitative experiments indicated that in acid concentrations of the order of $0.01M$ the rate of precipitation of lead by thioacetamide corresponded to the rate of hydrolysis of thioacetamide, but that at pH 4 the precipitation proceeded much faster than the hydrolysis.

Quantitative experiments were made to determine if the rate of hydrolysis of thioacetamide in $0.01VF$ hydrochloric acid could be followed by measuring the change in concentration of lead(II).

Table VI. Effect of Concentration of Thioacetamide upon Rate of Precipitation of Lead Sulfide at Constant pH[a]

CH_3CSNH_2, Mole/ Liter	Pb(II) Initial/ Pb(II) Final	Total Time, Min.	k_1, Liter/ Mole Min.
0.10	3.50	150	0.083
0.05	1.69	150	0.070
0.02	1.18	120	0.069
0.01	1.14	180	0.073
		Average	0.074 ± 0.006

[a] Calculated constant for expression $-\frac{d[Pb(II)]}{dt} = k_1[Pb(II)][CH_3CSNH_2]$. Initial lead(II) = $0.010VF$; hydrogen ion = $1.6 \times 10^{-4}M$; 90° C.

A solution initially $0.10VF$ in thioacetamide, $0.01VF$ in hydrochloric acid, and $0.01VF$ in lead nitrate was maintained at 90° C., and the lead concentration was determined at various time intervals. The resulting data are shown in Table V. No nitrogen was bubbled through the solution in this case. The values in the last column in the table were calculated from the concentration of lead(II) found at time = 0 and by use of the second-order hydrolysis constant for thioacetamide; it was assumed that all the hydrogen sulfide from the hydrolysis reacted rapidly with lead(II). The rate of precipitation of lead sulfide agreed, within the limits of experimental accuracy, with the calculated rate of hydrolysis of thioacetamide.

These data support the conclusions drawn from the study of the hydrolysis of thioacetamide, that neither lead ion nor lead sulfide catalyzes the hydrolysis and that lead ion does not inhibit the hydrolysis. Thus there is no evidence for the formation of a stable intermediate between lead and thioacetamide.

Precipitation of Lead Sulfide by Direct Reaction with Thioacetamide. Subsequent experiments were made in solutions of lower hydrogen ion concentration, where the rate of precipitation of lead is much greater than is the rate of hydrolysis of thioacetamide. The data from these experiments are discussed below and show the effect on the rate of lead sulfide precipitation of the thioacetamide, lead, and hydrogen ion concentrations over a pH range from 3.5 to 5.1.

Effect of Thioacetamide Concentration. Experiments made at constant initial concentrations of lead nitrate and formic acid–sodium formate buffer, but with various initial thioacetamide concentrations, demonstrated that the precipitation reaction is first order with respect to the concentrations of both thioacetamide and lead(II). The experiments were so designed that in no case had more than 8% of the thioacetamide reacted with lead by the end of the run. At the pH value which obtained, the hydrolysis of thioacetamide proceeded to the extent of only 0.2% an hour; so changes in concentration due to this effect may be disregarded.

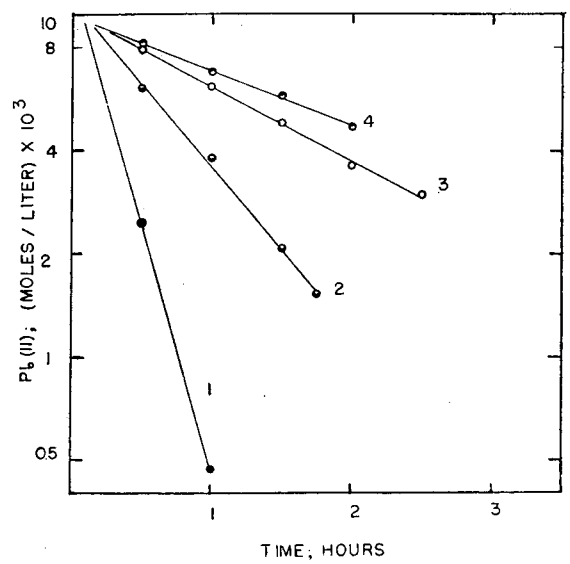

Figure 7. Effect of hydrogen ion concentration upon rate of precipitation of lead sulfide at 90°C.

1. Hydrogen ion concentration = $8.0 \times 10^{-6}M$
2. Hydrogen ion concentration = $4.0 \times 10^{-5}M$
3. Hydrogen ion concentration = $1.6 \times 10^{-4}M$
4. Hydrogen ion concentration = $3.1 \times 10^{-4}M$

In Figure 6 are shown plots of log lead(II) concentration *vs.* time for various thioacetamide concentrations. The plots are linear, indicating that the reaction is first order with respect to the concentration of lead(II). In Table VI are calculations, from the slopes, of the second-order velocity constant for the expression

$$-\frac{d[Pb(II)]}{dt} = k_1[Pb(II)][CH_3CSNH_2]$$

The consistency of the calculated rate constants indicates that the rate is first order with respect to thioacetamide.

Experiments at constant ionic strength in which the initial lead concentration was varied, showed, as does the linearity of the curves in Figure 6, the first-order dependence of the precipitation reaction upon the lead concentration.

Effect of Hydrogen Ion Concentration. The effect of the concentration of hydrogen ion was studied in solutions in

which the concentrations of lead ion, thioacetamide, sodium perchlorate, and sodium formate were maintained essentially constant. The hydrogen ion concentration was found to have a half-order inhibition effect upon the rate of precipitation of lead sulfide. Figure 7 shows the results of experiments in which the hydrogen ion was maintained at concentrations from 8×10^{-6} to $3.1 \times 10^{-4}M$. Calculated values of the velocity constants for the expression

$$-\frac{d[Pb(II)]}{dt} = k\frac{[Pb(II)][CH_3CSNH_2]}{[H^+]^{1/2}}$$

are presented in Table VII and are constant within the limits of experimental accuracy.

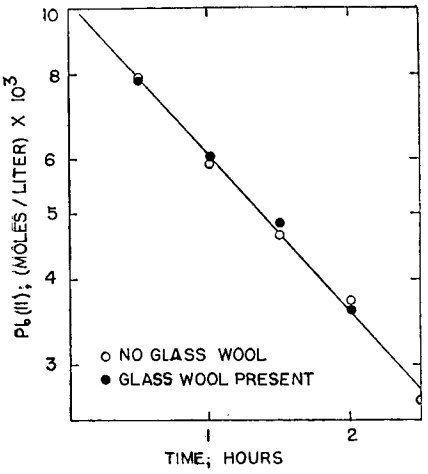

Figure 8. Effect of surface upon rate of precipitation of lead sulfide by thioacetamide at 90° C.

Solution $0.10VF$ in thioacetamide and $1.6 \times 10^{-4}M$ in hydrogen ion

Throughout the range of acid concentrations considered the hydrolysis of thioacetamide is not significant; in the most acid solution the ratio of the rate of the hydrolysis to the rate of the direct reaction is 0.05.

The inhibition effect of hydrogen ion might be explained by the assumption that there is catalysis by hydroxide ion through the formation of a lead hydroxide complex which is the reactive species for the direct reaction with thioacetamide. The ratio of lead ion to lead monohydroxide ion is approximately 10 (*8*) at pH 5.1 and 4.5×10^2 at pH 3.5. The assumption that lead monohydroxide is the reactive species would explain the apparent hydrogen ion inhibition, but not the half order found. The concentrations of such proposed polynuclear species as $[Pb_2OH]^{+++}$ and $[Pb_4(OH)_4]^{++++}$ (*11*) are such that even in the solutions with the highest pH considered here, less than 0.1% of the total lead is in such forms. Thus far, a satisfactory explanation of the half-order hydrogen ion inhibition effect has not been found.

Effect of Formate Concentration. A formic acid–formate buffer system was selected for control of the pH during the direct reaction experiments because of the appropriate value of the ionization constant and because no evidence of lead(II)-formate complex formation was found in the literature. However, in preliminary experiments made to develop a suitable analytical procedure, the rate of precipitation of lead sulfide by thioacetamide was found to be dependent upon the concentration of the sodium formate. When the sodium formate concentration was doubled and the pH kept constant, the rate of precipitation was reduced by about 50%. Accordingly, experiments were made on the solubility of lead chloride in solution buffered with formic acid and sodium formate. These indicated that lead and formate ions form a complex of significant stability. In addition, calculations indicated that the effects noted in the precipitation rate experiments were of the order of magnitude to be expected from the strength of the complex. Throughout the experiments reported in this work, the sodium formate concentration was kept constant at $0.081M$.

Effect of Surface Area. Increase of the glass surface area by about 500% by the addition of 0.275 gram of dry Pyrex glass wool (No. 800) did not have a measurable effect upon the rate of precipitation. The surface area of the reaction vessel, bubbling tube, and thermometer in contact with the solution was about 125 sq. cm. The surface area of the glass wool was estimated from the diameter of the fibers (8×10^{-4} cm.) and the density of glass (2.2 grams per cc.) to be 630 sq. cm. Figure 8 shows the agreement of experimental determinations of the rate of precipitation of lead sulfide with and without the presence of the glass wool. The concentrations of all species were identical in the two experiments; it is concluded that the precipitation reaction is not surface catalyzed.

Temperature Effect. Knowledge of the effect of temperature upon the rate of precipitation of lead sulfide is of value in the calculation of suitable conditions for obtaining complete precipitation of lead and for effecting quantitative separations from other elements. The rate of precipitation was found to vary by a factor of approximately 2 for a 10° C. change in temperature through the range from 60° to 90° C. Figure 9 is a plot of log k *vs.* $1/T$ and shows an Arrhenius temperature dependence. The activation energy is calculated from the slope to be 15.5 kcal. per mole.

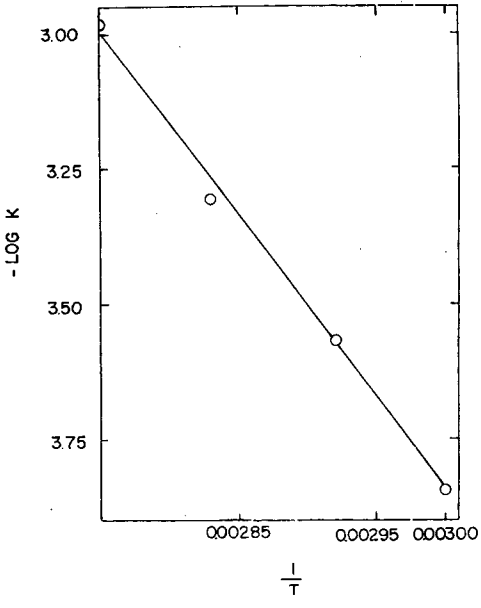

Figure 9. Rate of precipitation of lead sulfide as function of temperature

The lead sulfide precipitate obtained at the very slow rate at 60° C. was finely granular in appearance. Microscopic examination of the precipitates obtained at 60° and 90° C. showed that discrete and regular shaped particles could be detected in the former case but not in the latter. This emphasizes a potential analytical advantage in the use of thioacetamide—the possibility of control of the rate of formation and hence of the physical characteristics of the sulfide being precipitated.

Ionic Strength Effect. The Brønsted-Bjerrum-Christiansen equation (7)

$$\ln k = \ln k_0 + 2Z_a Z_b \alpha \sqrt{\mu}$$

predicts that the effect of ionic strength upon the rates of hydrolysis of thioacetamide and of the direct reaction of lead and thioacetamide will be small except at high ionic strengths, as presumably one of the reactants in each of the reactions is a neutral molecule.

No measurable effect upon the rate of the direct reaction resulted from decreasing the ionic strength from 0.14 to 0.11; however, the effect of larger variations in ionic strength was not investigated.

Analytical Considerations. Figure 10 shows the rates of precipitation of lead sulfide by thioacetamide through hydrolysis of the latter and by direct reaction as calculated from the rate constants. Only the rate of the direct reaction depends upon the concentration of lead; a change in lead concentration results in vertical displacement of this curve.

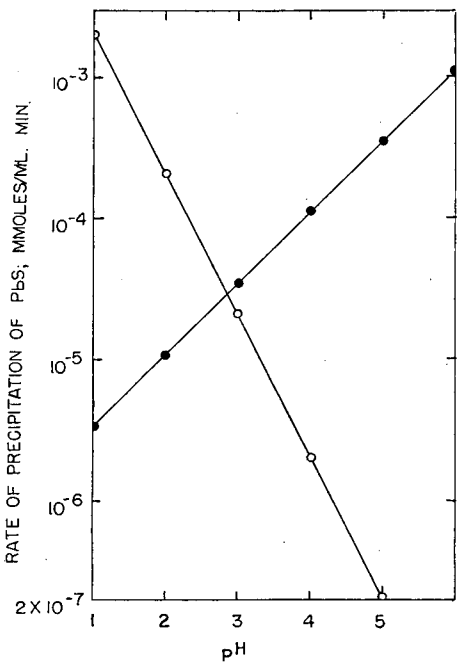

Figure 10. Rate of precipitation of lead sulfide by thioacetamide as functions of pH

0.10VF thioacetamide; 0.010VF lead(II); 90° C.
○. Hydrolysis of thioacetamide
●. Direct reaction

The following observations are significant concerning a 0.01VF lead(II) solution:

$R_d = R_h$ at pH 2.8, where R_d is the rate of direct reaction and R_h is the rate of precipitation of lead sulfide by hydrogen sulfide from hydrolysis.

$R_d = R_h/600$ at pH 1.

$R_d = 1000\ R_h$ at pH 5.

Thus, in solutions of pH 2 and lower, the precipitation of lead by thioacetamide takes place almost exclusively through hydrolysis of the latter. As the pH is raised, the direct reaction becomes increasingly important until at pH 4 and above [for 0.01VF lead(II)] the hydrolysis reaction is obscured by the much faster direct reaction. Barber and Taylor (2) state that "the hydrolysis (of thioacetamide) is more rapid in an alkaline solution than in an acid solution of the same strength." Preliminary experiments on the hydrolysis of thioacetamide in alkaline solutions suggest that what was actually observed was more rapid precipitation of metal sulfides because of a change of mechanism from hydrolysis to direct reaction such as has been observed for the case of lead.

Table VII. Effect of Hydrogen Ion Concentration upon Rate of Precipitation of Lead Sulfide[a]

aH^+	Pb(II) Initial/ Pb(II) Final	Total Time, Min.	$k \times 10^3$, Liter$^{1/2}$ Mole$^{-1/2}$ Min.$^{-1}$
3.1×10^{-4}	2.15	120	1.12
1.6×10^{-4}	3.50	150	1.05
4.0×10^{-5}	6.25	105	1.10
8×10^{-5}	4.06	30[b]	1.32
		Average	1.15 ± 0.12

[a] Calculated constant for expression $-\frac{d[Pb(II)]}{dt} = \frac{k[Pb(II)][CH_3CSNH_2]}{[H^+]^{1/2}}$. Initial lead(II) = 0.010 VF; thioacetamide = 0.10VF; 90° C.
[b] Point at 30 minutes was used because of uncertainties involved in determination of very low concentration of lead after 60 minutes.

This investigation has illustrated that the indiscriminate use of thioacetamide as a substitute for hydrogen sulfide is not justified because, in certain cases, the reactions involved in its use are complicated by mechanisms that change with the pH of the solution. Moreover, new uses for thioacetamide are indicated. Thus, the controlled rate of precipitation which can be achieved by its use should be of value in studies of precipitation and coprecipitation mechanisms. Moreover, separations may be possible which depend upon variations in the rates of the direct reaction between thioacetamide and metal ions. However, there must first be a thorough investigation of the precipitation of other sulfides by thioacetamide.

ACKNOWLEDGMENT

Preliminary qualitative experiments showing the behavior of various metals with thioacetamide made by Fred Anson and Glenn Crabbs have been of value in planning the above measurements. Eliot Butler is indebted to E. I. du Pont de Nemours & Co. for summer research assistance and to the Corning Glass Works Foundation for a fellowship for the academic year 1954–1955.

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Received for review July 25, 1955. Accepted October 22, 1955. Division of Analytical Chemistry, 127th meeting, ACS, Cincinnati, Ohio, March 1955. Contribution No. 2021 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena 4, Calif.

Quality Control in Petroleum Research Laboratory

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Because of the expense and time required for most petroleum research projects, it is imperative that all possible information be extracted from data obtained. A system of statistical quality control applied to both the process and analytical data has been a useful tool in accomplishing this. From this program has come information about the precision of the results, the sources of variance, and the measure of statistical control maintained. This has been invaluable in planning and controlling experimental work, and in the interpretation of the results.

IT IS well recognized that the technology of petroleum refining has matured to the point that new developments need to be evaluated even more precisely than in the past. Incremental yields of a few per cent gasoline, for instance, are spelling the difference between success and failure of a new process, a new catalyst, or a modification to an existing process. This means that the tools for measuring differences must be sharper—i.e., more accurate and more precise.

In any group of measurement of a property a certain amount of variation can be expected owing to chance causes alone. Under carefully controlled conditions the values may be expected to fall within a stable pattern of variation. Values which fall outside this pattern usually have an assignable cause, which may be discovered and corrected. To determine small differences, then, it is necessary first to determine as far as possible the assignable causes for the variations and to eliminate these causes. After this has been done, the system then will be subject to only chance or random variation which can never be completely eliminated. A knowledge of the magnitude of the random unassignable variations is then useful in planning experimental work, controlling the experimental work, and interpreting the results. This paper describes a statistical quality control program being used at this laboratory to help solve problems of the type indicated.

DISCUSSION

The end result of an experimental program is often a quantitative measure of yields and qualities of the products or the effect of some variable on these properties. An example of this in a petroleum research laboratory is the relative effects of catalyst *A* and *B* on yields and qualities of the products when reforming a naphtha under a given set of conditions. To interpret intelligently any measured differences resulting from using the two different catalysts, it is necessary to know the precision of the experimental operation. For example, if catalysts *A* and *B* result in gasoline yields of 80 and 75%, respectively, and if each yield can be guaranteed only with the broad limits of ±10% because of random error of measurement, then the apparent difference of 5% gasoline yield is not very firmly established. Thus, the apparent difference can be explained readily on the basis of the random error of the system and is, therefore, not statistically significant. On the other hand, if each yield can be guaranteed within ±1%, then there is obviously a cause for the 5% difference over and above the random error of the system. Since the imposed difference in experimental procedure is the use of two different catalysts, it would appear safe to assume that the two catalysts do give significantly different results.

One approach to such a problem of interpretation is the design of a completely randomized experiment to compare the two catalysts, taking into account all of the variables known to affect the results, and introducing sufficient replication to produce the necessary precision. This approach has been used very successfully in the agricultural and biological fields, but only to a limited extent in the petroleum laboratory. Another effective way to gain the information desired is through the use of a statistical quality control program as practiced at these laboratories. In this program, samples of typical feeds and products are used to learn, on a continuing basis, the precision of analytical methods and the degree of statistical control of these methods being maintained with time. To gain this same type of information on pilot units, control runs are made at regular intervals using standard feeds and catalysts at standard operating conditions. The values of precision thus obtained may be confidently used to apply to normal runs in these units, because they were obtained over a long period of time and represent many measurements. A statistical quality control program instead of conventional designed experiments has been used, because it gives an index of statistical control maintained over any period of time and because materials to be compared are not necessarily available at the same time for a planned program. For example, a catalyst tested last year may be compared with one tested this year. In addition, a quality control program allows more flexibility of operation—i.e., the program may be modified at any time data indicate this to be desirable.

In order to make the statistical quality control program most effective, all of the technical personnel were given a short course in the basic fundamentals of industrial statistics. The following paragraphs describe the system used and discuss the use of data therefrom.

Description of Program. It is recognized that the final data resulting from any research program reflect the quality of two equally important and equally indispensable phases of the work—namely, the experiment itself and the analysis of the feed and products involved. To be in position to guarantee the over-all results, it is necessary to know the precision of the over-all operation. Also, in order to direct a program intelligently toward improved and controlled precision it is necessary to know the distribution of the total variance—that is, how much of the total error is born in the pilot plant building and how much in the analytical and testing laboratories. Statistical quality control programs covering both of these phases have been in practice at the Esso laboratories as discussed below.

Analytical and Testing Quality Control Program. A quality control program as applied to analytical work must be concerned with two facets, accuracy and precision. Accuracy is usually determined by the use of synthetic samples or, as is often the case in empirical test methods, by the use of typical samples, the "true" value of which is assumed to be the average of many analyses by different analysts or laboratories. In analytical work lack of accuracy is usually due to some bias, such as calibration drift, which often can, and should, be corrected before reporting data. Accuracy is usually determined before beginning a precision control program. As is shown later the precision control program then indicates any changes in accuracy with time.

The precision control program has several integral parts.

METHOD OF SUBMITTING SAMPLES. Samples are submitted for analysis at regular intervals. The more often this is done, the better the control is maintained. As a large part of the analyses require considerable time, it is usually not possible to

get as many runs as desired. One sample of each type per week is the normal rate at these laboratories.

It is necessary that the analytical data on the control samples be unbiased to serve the intended purpose. For this reason the samples are of typical streams and are submitted on a repetitive basis in such a manner that they are indistinguishable from the regular plant samples. This is done by labeling each sample with a mock run designation (pilot plant, research project, etc.). The code is known only to the control group personnel and to the group among whose samples the control sample is hidden. Thus, a truly unbiased estimate of the precision is obtained, and this estimate can be confidently assumed to apply to the plant data.

SAMPLES. The samples used in the program are typical of those being tested in the normal operation of the laboratory. Occasionally, synthetic samples are used where plant samples are unstable, but synthetics often give precision values which are optimistic. This may be because of the knowledge of the synthesis or because of the necessary simplicity of the synthetic as compared with plant samples.

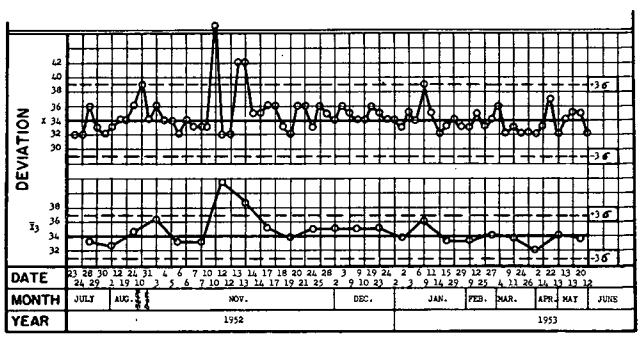

Figure 1. Variability in test procedure

Test, Na_2CO_3
Unit of measure, grams per liter

Large samples are used which allow 100 or more individual analyses to be made. Some samples are kept in large containers and samples are removed as needed. Others are placed in individual containers when prepared. Under either circumstance it is imperative that each sample submitted for analysis be representative of the total sample. Proper precautions are taken to eliminate, as far as possible, any change in composition with time. In some cases, where samples are unstable, it is necessary to submit a new sample each time for replicate analyses. This has several shortcomings, among which are: loss of statistical degrees of freedom; no measure of level shifts; and the fact that precision values are for short times only and do not include all sources of variance. Submission of different samples each time is avoided wherever possible.

CONTROL CHARTS. A control chart is a plot of the individual data points against time. When a sufficient number of values have been accumulated (at least 10, and preferably 20 or 30) the parameters of the distribution of these numbers are calculated—namely, the average and the standard deviation [$\sigma = \pm\sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$ where x represents an individual value, $\bar{x}$ is the average of the individual values, and n is the number of values used to obtain the average]. Lines are then drawn on the chart, parallel to the abscissa, to denote the average value and the values of the upper and the lower control limits. Control limits are usually chosen at ± 2 or ± 3 standard deviations from the average, depending upon the purpose to be served by the chart. In using the chart each new value is studied in terms of the average value and the variance pattern of the population from which the new value is presumed to have come. By this method it is possible to determine on a probability basis whether the system has remained constant (within the limits of chance causes) or whether it has changed, and such observations provide the basis for control action. A typical control chart is shown in Figure 1.

It is normal practice to use the ± 3 sigma limits for the rejection of individual data points. Lack of control may be due to shifts in the average value as well as to changes in the spread of the data points. Control charts based on the average of several individual points (see $\bar{X}_3$ in Figure 1, for example) are sensitive to shifts of this kind. Usually, significant shifts in level also result in several points in a row above or below the average.

The average and standard deviations are recomputed at regular intervals, usually after each 10 additional data points. Unless significant changes are observed in the results, these computations should be made using all of the data points, except those rejected because of nonconformity as indicated above. The standard deviation thus calculated represents the precision of the analysis on a routine basis at the average level indicated. A significant shift in the average level may be due to a change in accuracy of the method, or to change in composition of the sample. In either case an investigation into the cause of the change should be made.

COOPERATION OF PERSONNEL. The idea of using "blind" repetitive samples to check on the quality of analytical and testing data may not be popular with those performing the tests. Successful operation of such a system requires cooperation of all concerned. This can usually be obtained by explaining the motives of such a program and the value of knowing what the precision is on a given test. Being able to guarantee certain precision limits based on blind repetitive samples can eliminate any unwarranted aspersions being cast toward the analytical group in cases where over-all quality is not satisfactory.

In addition to the statistical quality control program described above, it is advantageous with some methods to check the procedure with a "known" sample each day before reporting any data. This helps to eliminate gross errors caused by some malfunctioning of the analytical method, such as spent scrubbers, bad solutions, or indicators, etc. The control program above catches such errors, but not soon enough. In the case of the known sample the operator, himself, plots the control chart and is thus in a position to spot and eliminate many of the difficulties. This helps to promote cooperation of personnel. Methods for which the use of known samples have been found particularly advantageous include Dietert sulfur, carbon and hydrogen, and American Standards for Testing Materials distillations.

The precision data from the analytical and testing control program are recomputed at regular intervals and current values are published in a statistics handbook, copies of which are available to all personnel using the data. In addition, precision values are often included on the daily report sheets along with the respective analytical results.

Pilot Unit Quality Control Program. Along with the analytical and testing quality control program, similar data are being obtained on the small pilot units. The objective is to control by statistical methods all quantitative measurements which contribute to final yield and product quality data, and to determine the precision of these data.

An important end result of numerous of these measurements, and one which is common to all pilot plants, is the material balance. Accordingly, a control chart is maintained on preliminary and final material balances. These charts are used to indicate when the over-all daily operation is satisfactory and when it deviates from normal operation more than can be accounted for by random variations. In the latter case, inspection of all the values going into the material balance usually indicates the source of error and the steps which should be taken to correct it. When the reason for the variation is not apparent the

Table I. Precision of Various Gasoline Tests

Test	Unit of Measure	Approximate Level	95% Confidence Limits
Gravity	° API	40– 60	±0.3
Reid vapor pressure	P.s.i.g.	5– 8	±0.5
Aniline point	° F.	20–120	±2
Bromine no.	G./100 g.	2– 5	±1
Research octane no.	O.H.	85– 95	±1
ASTM Distillation			
% at 158° F.	Vol. %	5– 10	±1.4
% at 212° F.	Vol. %	20– 30	±2.6
% at 257° F.	Vol. %	55– 65	±2.1
% at 302° F.	Vol. %	85– 95	±1.7

Table II. Hypothetical Naphtha Reforming Unit

(Assumed feed and product measurements from hypothetical naphtha reforming run)

Feed		
Volume, ml.	1383	
Density	0.751	
Grams		1038.6
Products		
Carbon, grams		0.0
Exit gas, SCF	10.0	
Grams (average)		138.6 (13.3%)
Liquid, grams		900.0 (86.7%)
Total		1038.6
Material balance (average)		100.0

unit is operated concurrently with efforts to renew control conditions; however, data from these periods are not reported until control is regained.

To determine the over-all precision of yield and quality data, and to obtain an indication of the control being maintained on these properties, standard baseline or control runs are made at regular intervals. These runs are made with a standard feed and catalyst using normal operating conditions. The precision established from these data may be used for determining significance of differences when comparing catalysts, feed stocks, etc., from normal operations. Also, the over-all average value for each variable in the standard runs represents a firm value against which other catalysts, feed stocks, etc., may be compared.

Uses of Data from Statistical Quality Control Program. The data being obtained in the statistical quality control program are used for a number of purposes, among which are:

VARIABILITY OF PROCESS AND ANALYTICAL DATA. An illustration of variability in a test procedure is shown in Figure 1. This chart indicates that the test is in statistical control during this period of time and that results from this test are known to ±3 grams per liter at the two standard deviations or 95% confidence level. Table I lists a number of tests used at these laboratories with precision limits obtained in the quality control program.

VARIANCE DISTRIBUTION. To be able to improve the variance in measurement of yield or quality of a product it is necessary to know where the major part of the variance arises. As indicated above, the over-all variance of yields and qualities from pilot units is estimated from replicate standard control runs. An estimate of the total analytical variance is obtained by testing, in an analytical quality control program, the total products from a given run. The unit operation variance is estimated as the difference between the total variance and analytical variance. The total analytical variance is further broken down into the variance due to each testing method through the use of a quality control program.

To illustrate how this works, certain values have been assumed for feed and product measurements in a hypothetical unit as shown in Table II. These values were chosen to give a typical yield pattern in the results from the unit as shown in Table III. The variance shown in Table III can then be studied in terms of the over-all variance referred to above, and the distribution of the variance between the operation of the unit (including sampling of products) and the analytical work-up is computed. Use was made of the very valuable statistical tool known as propagation of error, and the resulting variance distribution is shown in Table IV. The error in material balance, for example, has its source primarily (99%) in the operation of the unit. Two thirds of the errors in yields are due to unit variation and one third to analytical tests. The errors in gasoline quality, on the other hand, are two thirds in analytical tests and one third in unit variations. As part of the quality control program, data are available on the error involved in the various steps of the analytical work-up. A breakdown of the total analytical variance into its component parts is also shown in Table IV. For example, the error in the mass spectrometer analysis accounts for only a small part of the total error. The various other testing laboratories contribute error as shown. With the above information, it is now possible to design an intelligent program of work, should the precision picture need improvement.

SIGNIFICANCE OF DIFFERENCES IN RESULTS. As mentioned, the end results of many research programs are measures of yields

Table III. Hypothetical Naphtha Reforming Unit

[Estimate of random error from total analytical work-up based on 20 replicate work-ups on given total product (gas and liquid)]

	Average	95% Confidence Limits	
		Raw data	Adjusted to 90 CFRR octane no.
Material balance, wt. %	100.0	±0.2	
Yields, %			
Carbon, wt.	0.0	...	...
C_3, wt.	10.3	±0.3	±0.6
C_4, vol.	6.2	±1.1	±1.1
C_5, vol.	4.5	±1.0	±1.0
C_5^+, vol.	79.5	±0.9	±1.2
10 lb. gasoline, vol.	81.5	±1.2	±1.4
Quality of C_5^+ Gasoline			
° API	44.8	±0.9	...
O.N. research clear	90.2	±1.0	...
R.V.P.	2.6	±0.6	...
% at 158°	2.6	±1.6	...
212°	15.7	±2.8	...
257°	41.7	±2.3	...
302°	74.4	±2.8	...

Table IV. Estimate of Variance Distribution[a]

(Small fixed bed naphtha reforming unit)

Measurement	Material Balance	Yields at 95 O.N., % C_5^+, vol.	C_5, vol.	C_4, vol.	C_3^-, wt.	C_5^+ Quality at 95 O.N., % at °API	212° F.	R.V.P.
Over-all error (95% confidence limits[b])	±3.0	±1.8	±1.5	±1.7	±1.1	±1.1	±3.5	±0.75
Variance distribution, % of total								
Unit operation (by difference)	99	60	60	60	70	35	35	35
Analytical work-up, total	1	40	40	40	30	65	65	65
Glass stills	0	20	35	28	0	58	Neg.	45
Podbielniak distillation	0	3	4	4	0		Neg.	
Inspections (including O.N.)	0	17	1	4	21	7	65	20
Mass spectrometer analyses	1	Neg.	Neg.	4	9	Neg.	Neg.	Neg.

[a] In the general case, if $u = f(x,y\ldots)$ then $\sigma_u^2 = \left(\frac{\partial u}{\partial x}\right)^2 \sigma_x^2 + \left(\frac{\partial u}{\partial y}\right)^2 \sigma_y^2 \ldots$ where σ_u^2, σ_x^2 and σ_y^2 are the variances of u, x, and y, respectively. In above case over-all variance (σ_u^2) and analytical variance (σ_x^2) were determined as previously described. The unit variance (σ_y^2) was obtained by difference using propagation of error formula above.

[b] 95% confidence limits for a given datum point. A correlation line is, of course, more precise, since it is an average of many points.

and qualities of the products, or the effect of some variable on these properties. To compare results from two such operations it is necessary to know the least differences which can be considered significant. The least significant difference is a function of the precision with which the results are known, and this may be computed by application of the t test.

$$LSD = tS_D$$

where

S_D = standard deviation of the difference
$= S\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$
S = standard deviation of the individual results
N_1 and N_2 = number of individual results in each operation
t = 1.96 for 95% confidence with infinite degrees of freedom

(The meaning of t is explained in any standard book on statistics and its values at any confidence level with various degrees of freedom are available in tables.)

Some typical examples of the least statistically significant differences of process results are shown in Table V. It may be seen that differences in C_5^+ yield, for instance, must exceed 1.2% before they may be considered statistically significant at the 95% confidence level, based on the data given in the table. This type of information has been invaluable in appraising differences in results from pilot plant work.

Table V. Least Significant Difference of Typical Process Results

Yield or Quality	Std. Dev. of Individual Detn.[a]	Least Significant Difference at 95% Confidence Level for Total of 8 Tests[b]
C_3^-, wt. %	±0.6	0.8
C_4, vol. %	±0.9	1.2
C_5, vol. %	±0.8	1.1
C_5^+, vol. %	±0.9	1.2
°API	±0.6	0.8
Vol. % at 212° F. (ASTM)	±1.8	2.5
Reid vapor pressure, lb./sq. inch gage	±0.4	0.6

[a] Based on many determinations made in quality control program.
[b] For example, in 8 total tests, 4 would be made on Catalyst A and 4 on Catalyst B. The "least significant difference" between Catalyst A and B, at the 95% confidence level, is calculated as:

$$L.S.D. = tS\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$$

where S = standard deviation of an individual determination, N_1 and N_2 = number of determinations in each operation and t = "Student's" t.

CONCLUSION

Some 10% or less of the analytical time and the small pilot unit time is spent in the statistical quality control program. This expenditure has been amply justified by the results obtained. In addition to the benefits indicated above, it has been possible, because of a knowledge of the variance distribution, to reduce the over-all variance of some of our units. This results in a reduction of runs necessary to achieve a given precision, or better precision with the same number of runs. The value of this to a research program is often inestimable. An intangible benefit of a statistical quality control program is an awakening of the technical and nontechnical personnel alike to the need of quality in all steps of a process to achieve the desired results. This often results in an automatic reduction in variance in a short period of time.

ACKNOWLEDGMENT

The authors wish to thank C. E. Kling for coordination of the analytical quality control program and for computation of the many precision values, and E. M. Charlet and L. M. Addison for their assistance in effectively applying the quality control system in the analytical laboratories.

RECEIVED for review June 16, 1955. Accepted October 28, 1955. Division of Petroleum Chemistry, 126th Meeting, ACS, New York, September 1954.

Determination of High Boiling Paraffin Hydrocarbons in Polluted Water

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Investigation of oil pollution in surface water has frequently been confused by analytical problems. The methods may have failed because of incomplete recovery of oily materials, lack of identification of the major components, or inadequate removal of interference. The proposed method includes an improved wet extraction procedure, infrared analysis for quantitative and classification purposes, and chromatographic separation to isolate mineral oils from animal or vegetable oils, soaps, and miscellaneous extractable components found in polluted water. This procedure makes it possible to determine the amount, type, and condition of oily residues in the presence of mixed components in polluted water.

THE American Petroleum Institute (*1*) includes as oily wastes the highly complex mixtures of petroleum and their sulfur, nitrogen, and oxygen derivatives. It classifies oily wastes according to the tendency to form films, emulsions, or sedimentary deposits. Kirschman and Pomeroy (*7*) define an oily waste as a relatively nonvolatile unsaponifiable liquid that contributes to the formation of oil films or deposits.

Other definitions of oil are based primarily upon the method of analysis and include whatever is recovered by a particular procedure. Definitions of this type (*2–4, 8, 10–12*) may lead to results that are lacking in specificity. The definition for oil used in this investigation includes the relatively nonvolatile components that exhibit the characteristic infrared curves of saturated aliphatic hydrocarbons.

This definition excludes materials such as fatty acid soaps, animal or vegetable oils, and coloring materials, which may be associated with oils in a stream. Interference due to associated colloidal solids in the extract is minimized. The analyst could estimate the magnitude of significant interference and the type of material that had to be removed to obtain a specific result.

Investigation of oil in surface water frequently is more complex than determining oil in an isolated industrial waste. Stream concentrations of oil are generally lower; interference is increased by domestic and other industrial wastes. Living and dead microorganisms in the stream not only absorb oil but also tend to stabilize emulsions between sample and solvent during most

extraction procedures. Several of the established oil determinations that were satisfactory on fresh industrial wastes failed when applied to samples containing mixed wastes and biological detritus.

Extraction difficulties were controlled by a modified liquid-liquid extraction of the sample with carbon tetrachloride, followed by infrared determination of oil in the same solvent. Interference revealed by the infrared curve was removed by chromatographic separation and a corrected curve was obtained on the specific class of hydrocarbons.

ANALYTICAL PROCEDURE

Apparatus (Figure 1). Liquid-liquid extractor, 1-liter, Sargent No. 31414.

Boiling flasks, 500-ml., with ball joint socket 25/35 mm.

Allihn-type condenser, 600-mm., water-cooled, 29/42 joint.

Glass air inlet line (8-mm. diameter) of sufficient length to extend from the top of the condenser to about 15 cm. from the bottom of the extractor column.

Separatory funnel, 2-liter capacity.

Soxhlet flask with condenser and distillate trap. A small bulb should be blown in the flask near the bottom to serve as a collecting well to facilitate extract removal with a transfer pipet.

Infrared spectrophotometer, sodium chloride optics used.

Liquid cell, path length approximately 0.35 mm.

Chromatographic column, 10 × 300 mm.

Fritted-glass filter apparatus for vacuum operation.

Air compressor of a type which will minimize oil contamination.

Activated carbon air filter, 4 inches × 4 feet.

Reagents. Analytical grade carbon tetrachloride. This material should have no significant infrared absorption from 4000 to 1700 $cm.^{-1}$

Hydrochloric acid, concentrated.

Anhydrous sodium sulfate, powder.

Chromatographic alumina, Fisher No. A 541/2, 80 to 200 mesh, was suitable.

Reference standard. This material should be representative of the oil to be determined. Eastman octadecane was used in this work.

Procedure. The assembled apparatus should be dry; use a current of warm air if necessary. After drying, clean the entire apparatus by using it as air condenser for solvent distillation. To clean apparatus connect the 500-ml. flask to the side arm and add approximately 300 ml. of solvent to the extractor column. Add Berl saddles to the column to a point slightly above the junction of the solvent return arm. Tamp glass wool (extracted if necessary) in place lightly to form a return solvent filter of 1 to 2 cm. in thickness above the Berl saddles.

Add the sample (1 liter or aliquot). The solvent-sample interface should be located 2 to 3 cm. above the glass wool. Do not permit the glass wool or the solvent vapor arm to be wet with water. Add concentrated hydrochloric acid to reduce the sample pH to 3.5 or below. Check the amount of acid required on a separate aliquot. A lower pH may be desirable for breaking a persistent emulsion. Connect the condenser to the column and lower the air inlet to within 2 to 6 cm. of the solvent-sample interface.

When solvent is refluxing into the column at a suitable rate, adjust the air flow into the column to break the surface film of solvent and reduce the size of solvent globules. Time the extraction from the point of air adjustment. If the oil emulsion is broken in less than 1 hour, 4 hours' total time should be adequate for extraction. At the end of extraction, rinse the air inlet line and condenser with fresh solvent. Pour the contents of the column into a 2-liter separatory funnel through the glass wool filter. Combine sample container rinse and residual solvent from the column with the extract. Add approximately 10 grams of anhydrous sodium sulfate to the combined extract and mix.

Filter the dried extract through fritted glass, using suction. Transfer the sodium sulfate to the filter and break up any lumps, before rinsing. Transfer the filtrate to a 125-ml. Soxhlet flask by increments and distill to approximately 10 ml. at a rapid rate. Use a steam bath for final concentration to approximately 2 ml. Sweep the vapors off with a gentle stream of nitrogen (preferred) or dry air. Adjust the final volume of the extract on the basis of infrared absorption results or prior sample information. The sample should never approach the solvent disappearance point at any stage of the operations and should be protected from moisture condensation.

Calibrate the infrared instrument for zero adjustment with glass in the sample position; adjust for 100% transmittance in the critical areas with solvent in the sample cell. Establish the absorbance of a known concentration of a reference standard *several times during a series of sample runs.* Use a low gain setting and slow speed at the critical absorption bands. The results of a sample determination at this stage includes everything absorbing at 2925 $cm.^{-1}$ in terms of the reference equivalent. Hydrocarbons and oxygenated extractables (Hc + Ox) may be shown. Qualitative data and a rough quantitative estimate of contamination can be obtained from the position and intensity of extra absorption bands.

After this infrared determination, concentrate the sample to 2 to 3 ml. Prepare a column of activated alumina (10 × 150 mm.) and prewet with two bed volumes of carbon tetrachloride (20 ml.). Allow the prewet solvent to drain to a dull top surface and add the sample. Rinse the sample container with solvent and add more solvent to collect 30 ml. of eluate. Concentrate the eluate to a suitable volume as indicated by the previous determination. Perform a second infrared determination on the chromatographed sample. This result includes only the mixed hydrocarbons (Hc) in terms of the reference equivalent. Appearance of interference in this fraction indicates improper technique in chromatographing, column overloading, or an unsuitable adsorbent.

SAMPLE PREPARATION

All fresh samples of hydrocarbons were dispersed in water with a Waring Blendor. The resulting emulsion was stable for an indefinite period in a sterile system. Appreciable dilution did not affect it. Other samples were homogenized in an Eppenbach Type QV6-1 vertical mill if an aliquot was required for analysis. Best results were obtained when the sample was taken in a separate bottle and the entire amount used for analysis.

EXTRACTION

The sample was extracted by continuous downflow application of freshly distilled solvent. Countercurrent air flow caused a decrease in solvent globule size and greater interface area, increased solvent retention in the column, frequent reformation of interface area by collision of the solvent and air, and better distribution of solvent, suspended solids, and sample. Without countercurrent air 70% extraction of an octadecane emulsion was obtained in 23 hours; with air, more than 90% extraction was obtained in 2 hours. The agitation described did not produce excessive emulsification of sample and solvent. Only clean extract returned to the boiling flask. The solvent filter broke the slight emulsion of sample and solvent and retained suspended solids in the sample column.

Success or failure of the extraction was indicated early in the procedure by observation of sample clarification. If it did not clarify within a reasonable period, additional chemical treatment, different treatment on a new aliquot, or a smaller aliquot could be started without an excessive time loss.

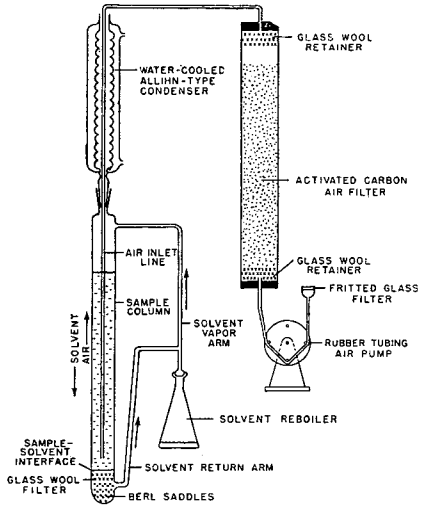

Figure 1. Extraction assembly

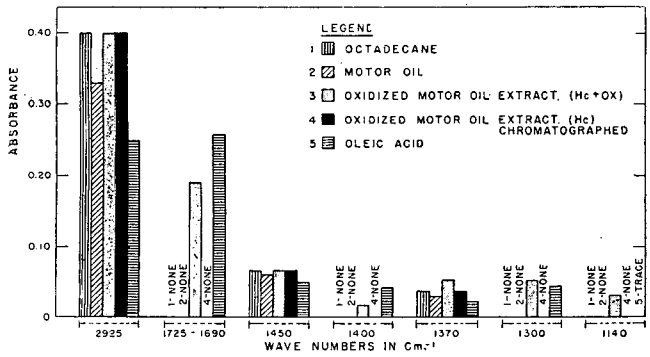

Figure 2. Relative infrared absorbance of samples at various band positions

15.0 mg. of sample per 5 ml. of carbon tetrachloride

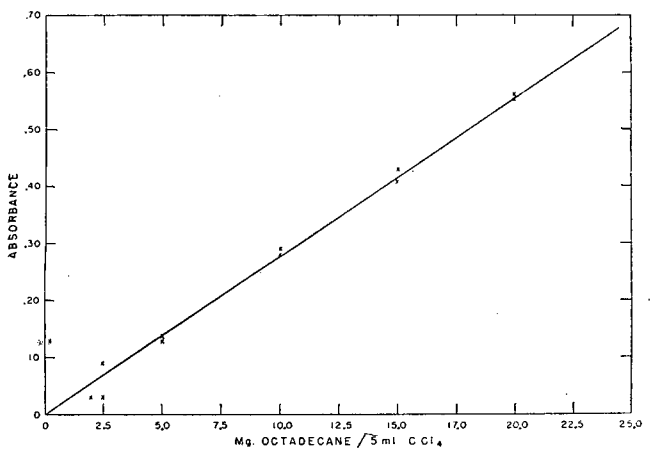

Figure 3. Calibration curve of octadecane absorption *vs.* concentration

Blank determinations, consisting of extraction of oil-free water should be made to check effectiveness of air and apparatus cleaning. A low and consistent blank requires careful operation.

Use of the carbon tetrachloride for both extraction and infrared measurement minimized errors associated with solvent removal, such as overheating, creeping, sample volatilization or oxidation, and identification of the solvent disappearance point. Loss of sample was insignificant as long as the temperature did not exceed the solvent boiling point or the sample vapor pressure was below 5 mm. of mercury at 100° C.

INFRARED DETERMINATION

A Baird recording spectrophotometer with sodium chloride optics was used as a single-beam instrument. A solvent curve was recorded on each chart with sample curves superimposed and color coded. Duplicate readings were obtained. The curve included the spectral region from 4000 to 1000 cm.$^{-1}$ in which carbon tetrachloride absorption introduced significant interference only at 1600 to 1500 and 1260 to 1190 cm.$^{-1}$ Drift or other instrument variables were detectable by the curves of the solvent, reference sample, or a polystyrene film. The path length of the cell was suitable for the desired sensitivity range for oil, but did not obscure significant sample information by excessive solvent absorption.

Octadecane, 99^{+} mole % purity, was selected as a reference material, so that all results could be expressed in terms of a definite material. The molecular weight of the reference was relatively close to that of the average for medium weight oil. Infrared absorptivity of the motor oil was appreciably less than that of octadecane. Absorptivity varied with different oil samples. Results expressed as an octadecane equivalent, were, therefore, minimum results. Other physical and chemical characteristics of the reference and oil were similar except for the additives, the nonparaffinic hydrocarbons, and the relatively wide volatility range of the oil.

Infrared absorption at 2925 cm.$^{-1}$ was the most suitable analytical band for the desired concentration range of oil. As this absorption also is characteristic of the carbon-hydrogen bands of alicyclic, olefinic, and aromatic hydrocarbons or their sulfur, nitrogen, or oxygen derivatives, many substances could interfere with the determination of paraffin series hydrocarbons. Significant interference was indicated and classified by the nature of the infrared curve.

Hydrocarbon absorption conformed with Beer's law. Base line technique similar to that of Heigl (*5*) and Simard (*13*) was used, except that the base line was obtained from the solvent curve. Sample calculation reduced to:

$$\text{Mg. sample/unit volume} = A_{\text{sample}} \times \left[\frac{\text{mg. reference/unit volume}}{A_{\text{reference}}}\right]$$

where A = sample or reference absorbance (*6*).

INTERFERENCE REMOVAL

Chromatographic technique was used to remove interference from the extract. Interference in the persistence study on motor oil (*9*) consisted mainly of the oxidation products of hydrocarbons. Biochemical oxidation of motor oil could result in the formation of peroxides, alcohols, aldehydes, acids, esters, ethers, and other oxygenated materials as intermediates in the path toward carbon dioxide and water. Alumina was the most satisfactory adsorbent for these intermediates. When a sample containing oxygenated compounds passed through the alumina column, hydrocarbons appeared first in the carbon tetrachloride eluate.

The results of the procedure can be illustrated by the behavior of a carbon tetrachloride solution of octadecane, methyl stearate, and oleic acid. The sample was concentrated to a volume of 3 ml., applied to a 10 × 150 mm. column of alumina, and developed with carbon tetrachloride. The first two bed volumes of eluate contained all of the octadecane. Methyl stearate appeared in the fifth bed volume of eluate. Oleic acid was not completely recovered by protracted development of the column. Shorter columns and overloading resulted in separation failure. Infrared was invaluable for the identification of the various fractions as well as in the control of technique.

RESULTS

Figure 2 is a bar graph of the absorbance *vs.* band position in cm.$^{-1}$ of fresh samples and oxidation system extracts. Each sample was based on 15 mg. of material, or the octadecane equivalent thereof, in 5 ml. of carbon tetrachloride. The analysis band for oil was located at 2925 cm.$^{-1}$; secondary bands appeared at 1450 and 1370 cm.$^{-1}$. The difference in absorptivity of oil and octadecane was apparent from samples 1 and 2. Absorbance at 1725 to 1690, 1400, 1300, and 1140 cm.$^{-1}$ (sample 3) was produced by sample components other than hydrocarbons. This type of absorption was common whenever motor oil was subjected to biochemical oxidation. Sample 5, oleic acid, shows similarity to sample 3. It also shows that an estimate of hydrocarbons could not be obtained without purification. Absorption at 1725 to 1690 cm.$^{-1}$ is a characteristic of carbonyl compounds. This absorption served only to indicate interference, not to correct for it. Sample 4, Figure 2, shows the effect of alumina chromatography on a sample similar to 3. Only the hydrocarbon absorption was found. Assuming equal concentration and absorptivity, the infrared curve was identical to that of fresh motor oil.

Figure 3 is a calibration curve of octadecane absorbance *vs.*

concentration. Within the region bounded by an absorbance of 0.10 to 0.55, linearity was good. Instrument response was poor and the results were erratic outside of this range. Extract concentration was regulated to use the optimum instrumental range, from 5.0 to 20 mg. of octadecane equivalent in 5 ml. of extract or 0.5 to 4.0 mg. in 1 ml. of extract.

Precision and recovery data on manipulation including extraction, concentration, and infrared on fresh octadecane emulsions are shown in Table I. Four replicates at three concentrations have a standard deviation of 1 mg. or less except when a dilution factor was involved as in series 3. Preliminary recovery data on extraction of motor oil or octadecane (16 samples, in concentrations from 35 to 140 mg. per liter) varied from 89.5 to 99%. There was no tendency for higher concentrations to show decreasing recovery, as shown in Table I.

Table I. Precision and Recovery Data on Fresh Octadecane Emulsions

Series	1	2	3
Added octadecane, mg./liter	4.0	25.7	64.5
Sample No.	Found, Mg./Liter		
X-1	3.9	25.8	59.4
X-2	3.2	24.3	60.9
X-3	3.6	24.3	57.9
X-4	5.0	23.5	56.1
$\overline{X}$, mean, mg./liter	3.9	24.5	58.6
S, standard deviation, mg.	0.8	0.9	2.4
Coefficient of variation = 100 ($S/\overline{X}$), %	21	4	4
Recovery, %	97.5	95.3	92.0

Table II. Precision of Hydrocarbon Analysis on Biochemical Oxidation Systems with Motor Oil Feed

(Hc + Ox reported)

Replicate No.	Found, Mg./Liter No. 1[a]	No. 2	No. 3
1	7.3	11.5	36.6
2	8.2	10.5	36.4
3	5.6	11.1	34.8
4	6.4	11.1	35.8
$\overline{X}$, mg./liter	6.9	11.1	35.9
S, mg.	1.1	0.4	0.8
Coefficient of variation, %	16.0	3 6	2.2

[a] Heavy growth of algae present.

Table III. Precision of Results on Oil Oxidation System Extracts before and after Alumina Chromatography

Sample No.	Found, Mg./Liter Before (Hc + Ox)	After (Hc)
X-1	24.7	15.8
X-2	23.7	16.0
X-3	23.7	15.2
X-4	22.4	14.8
$\overline{X}$, mg./liter	23.6	15.5
S, mg.	0.9	0.6
Coefficient of variation, %	4	4
Loss as octadecane equivalent, %		34.5

Table IV. Octadecane Equivalent on Samples before and after Alumina Chromatography

Sample No.		Found, Mg./Liter Before (Hc + Ox)	After (Hc)	Found, % Hc/(Hc + Ox)
	Motor oil or Octadecane (fresh preparations, 17 samples)	...	...	97–102 Min.-Max. Reported
1	Motor oil oxidation system extract	19.2	12.8	66.7
2		13.1	6.6	50.4
3		18.4	7.3	39.7
4		18.9	6.6	34.9
5		12.8	3.6	28.2
1	Fresh sewage extract	71.0	1.1	1.6
2		36.2	0.7	1.9
3		28.2	1.0	3.5
4		45.4	4.5	10.0
5		16.1	2.2	13.7

Table II shows the same type of data on motor oil oxidation system extracts. Results were similar to those in Table I.

Table III includes the results of oxidation extracts before and after chromatographic separation. Precision was similar but the results show the removal of more than one third of the octadecane equivalent. This would have appeared as a positive error in the results for oil, as defined here.

Results of alumina chromatography on fresh oil or octadecane, extracts of oil oxidation systems and of sewage are shown in Table IV. Seventeen samples of oil or octadecane were processed without significant loss of oil. Hydrocarbon in the extracts of the motor oil oxidation systems varied from 66 to 28%. Sewage extracts showed a relatively small percentage of hydrocarbon. The relatively large error possible in the determination of oil in a mixed system without suitable correction is obvious.

This analytical procedure was designed to obtain specific information on oil in the presence of relatively large concentrations of nonhomogeneous interference. It would be useful in the interpretation of results of the more rapid routine procedures used on a consistent type of sample. It was the authors' experience that anomalous results frequently could be resolved by the greater specificity of this method.

Alkane series hydrocarbons were separated from oxygenated products by alumina. Alumina was less effective for the separation of alkanes from aromatic interference. This and other separations would be possible by suitable selection of column technique, adsorbent, and developing solvent.

CONCLUSIONS

Continuous liquid-liquid extraction with air agitation was effective for recovery of oil from nonhomogeneous samples.

Infrared results provided a quantitative measure of oil concentration and information on the specificity of the result.

Chromatographic separation was effective for the conversion of mixed systems into homogeneous fractions.

ACKNOWLEDGMENT

The authors gratefully acknowledge the helpful suggestions by numerous individuals during the progress of this work. A. A. Rosen, Organic Analytical Unit, this laboratory, was particularly helpful on chromatographic and infrared technique.

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RECEIVED for review June 20, 1955. Accepted November 9, 1955. Division of Water, Sewage, and Sanitation Chemistry, Symposium on Problems in Stream Pollution, 127th Meeting, ACS, Cincinnati, Ohio, March-April 1955.

Determination of Anionic Detergents in Surface Waters and Sewage with Methyl Green

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The methyl green procedure was designed to eliminate or lower interferences encountered in the determination of anionic detergents by the methylene blue method. Interferences from thiocyanate, nitrate, and nitrite ions were reduced greatly. The methyl green procedure is not affected by high concentration of sulfate, chloride, sulfite, and phosphate. Recoveries of alkyl sulfate added to river and lake waters as well as to domestic sewage were more consistent and more accurate when the methyl green procedure was used.

THE literature shows that anionic detergents represent 90% of all detergents used. Several methods (*1, 2, 5, 9, 12, 13, 15, 16*) have been employed in the quantitative estimation of anionic detergents in sewage, but the Jones method (*11*) with its modifications (*3, 4, 6–8, 14*) appears to be the predominantly popular procedure. However, interference of thiocyanates, nitrates, nitrites, protein, and compounds found in urine causes inaccuracy. This paper presents a method which is more rapid and less affected by these interfering substances. It is designed only for anionic detergents and uses the principle of complexing the detergent with methyl green dye, extracting the complex with benzene, and measuring the absorbance of the benzene solution for comparison against a standard curve previously prepared. Sulfonated or sulfated surface active agents react with methyl green to form the same purple complex.

Methyl green was chosen from a group of 13 dyes, which were all tested for complex formation with detergents. Meldola blue, Capri blue, Alizarin green, Alizarin blue, patent blue, crystal violet, Victoria blue, and Azo carmine G proved unsatisfactory because they did not form the complex, were soluble in the extracting solvents, or were subject to interference from thiocyanate ions.

The methylene blue method (*11*) as used in this study is essentially the modified Jones method of Faust (*7*).

APPARATUS AND REAGENTS

Apparatus. Any spectrophotometer capable of accommodating 1.0-cm. cells and possessing a wave-length selection at 615 mμ may be used. A Beckman Model B was used in making all measurements in this study.

Pear-shaped separatory funnels (125-ml.), a 40.0-ml. volumetric pipet, and medicine droppers were also used.

Reagents. Methyl Green. Dissolve 0.5 gram of certified methyl green (obtainable from Hartman-Leddon Co., Philadelphia, Pa., Catalog No. 270) in distilled water and dilute to 100 ml.

Benzene, reagent grade.

Chloroform.

Glycine–Hydrochloric Acid Buffer (pH 2.5). Dissolve 7.5 grams of glycine and 5.8 grams of sodium chloride in distilled water and dilute to 1 liter. Dilute this solution with approximately 0.1*N* hydrochloric acid to produce a pH of 2.5. (The stability of this buffer is increased greatly by adding a few drops of benzene and keeping it refrigerated.)

Sodium lauryl sulfate, reagent grade (obtainable from Matheson, Coleman and Bell, Norwood, Ohio, Catalog No. 7003).

PROCEDURE

Standard Curve. Prepare a stock solution of sodium lauryl sulfate containing 1000 p.p.m. by dissolving 1.0 gram in distilled water and diluting to 1 liter. This stock solution should be made up every 4 weeks. Prepare an intermediate solution, which should be made up at least every 2 weeks by diluting 100 ml. of the stock solution to 1 liter. Dilute 50 ml. of this intermediate solution to 1 liter; 1 ml. contains 5 γ of sodium lauryl sulfate. From this standard solution set up a series of concentrations from 5 to 100 γ, dilute to 20 ml., and form the complex according to the procedure given below. Read the absorbance of these standards at 615 mμ against a blank containing all reagents except sodium lauryl sulfate.

Analysis of Samples. A sewage sample generally requires a volumetric dilution prior to the determination, owing to its high detergent content.

To the sample (20 ml. or aliquot diluted to 20 ml.) in a 125-ml. separatory funnel add the required buffer (for most samples 10 ml. is usually sufficient) and 2 ml. of 0.5% methyl green solution. Swirl the funnel to ensure complete contact of dye with sample. Add 40 ml. of benzene (accurately measured) and shake for 1 minute, or approximately 250 times. Allow to settle, then swirl to dissolve the complex completely in the benzene. Remove the water layer with care, so as not to lose any of the benzene layer. Add 15 ml. of distilled water buffered at pH 2.5 to the benzene layer and shake 75 to 100 times. Allow to settle, then swirl the funnel. After allowing water to settle out of the benzene layer (20 to 30 minutes), remove a sample with a clean, dry medicine dropper, and determine the absorbance at 615 mμ. Insertion of benzene into the cells by dropper usually introduces some air into the solvent, which escapes after about 1 minute. Read the concentration of detergent in terms of sodium lauryl sulfate from the standard curve.

Wash all glassware in acid to remove any complex adhering to it. Both the complex and dye are very soluble in chloroform and are removed from spectrophotometer cells by a chloroform washing after each reading. This is necessary because the complex and dye adhere readily to glass.

DISCUSSION

Standard. The anionic detergents found in sewage vary in molecular weight from approximately 250 to 350. Several available compounds were considered for a standard, but sodium lauryl sulfate with a molecular weight of 288 was chosen because it is procurable in pure form and is readily stable in 100 p.p.m. solution for 3 to 4 weeks. Solutions containing 100 p.p.m. of sodium lauryl sulfate and a specific preservative were tested for a period of 6 weeks. The preservatives used were 500, 1000, and 2000 p.p.m. of sodium borate, 1 p.p.m. of copper, 6 p.p.m of sodium azide, 100 p.p.m. of mercuric chloride, and 4000 p.p.m. of salicylic acid. The mercuric chloride and salicylic acid showed signs of interference at the concentration used and were discontinued. A sample not containing preservative was used as a control. After all samples had been tested at intervals of 3 days for a month, very little deterioration was observed. Between the fourth and sixth week, the sample without preservative showed a 20% breakdown, whereas the samples containing 500 p.p.m. of sodium borate and other preservatives showed a 10% loss of detergent.

Questions have arisen as to whether or not the vast number of detergents on the market all form color complexes having maximum absorptions at the same wave length. Gowdy (*8*) states that alkyl sulfates and alkyl aryl sulfonates represent the largest proportion of the synthetic detergents found in sewage. On the basis of this statement, absorption curves were determined for an alkyl aryl sulfonate and sodium lauryl sulfate. As shown in

Figure 1, maximum absorption occurs at the same wave length (615 mμ) for each compound.

CONCENTRATION RANGE. Figure 2 represents a standard curve using concentrations from 0 to 125 γ of sodium lauryl sulfate in 20 ml. of distilled water. The curve conforms to Beer's law to approximately 60 γ. Should concentrations lower than 5 γ be encountered, the sample size can be increased or the readings taken using a 5.0-cm. cell with reference to a standard curve made up for the larger cell.

DYE CONCENTRATION. The 2 ml. of 0.5% methyl green used for complexing the detergent is necessary in such high concentration because of a chemical phenomenon which has not as yet been investigated. The reaction is not stoichiometric, in the sense that 1 mole of added dye reacts with 1 mole of detergent. Sufficient dye must be added to the sample to develop an equilibrium at which the absorbance readings are constant. This was determined for a maximum permissible detergent concentration of 75 γ, to which the method is applicable. (A sample containing more than this amount of detergent should be diluted.)

To solutions containing 75 γ of sodium lauryl sulfate, 2 ml. of methyl green were added, in concentrations varying from 0.1 to 0.5%. As shown in Table I, the necessary dye concentration is 2 ml. of 0.4% methyl green. In this study, however, the 0.5% concentration was used to ensure an adequate excess.

Table I. Dye Concentration Necessary for Stabilization of Color

Concentration of Methyl Green, %	Absorbance
0.1	0.140
0.2	0.225
0.3	0.260
0.4	0.270
0.5	0.271

Table II. Effect of pH on Color Formation

pH	Absorbance
3.1	0.151
3.0	0.157
2.9	0.171
2.8	0.157
2.7	0.157
2.6	0.167
2.5	0.168
2.4	0.166
2.2	0.159
2.1	0.143

Table III. Effect of Interfering Substances on Methyl Green and Methylene Blue Methods

Interfering Substance	Concentration, P.P.M.	Recovery: Methyl Green Method P.p.m.	%	Methylene Blue Method P.p.m.	%
CNS^-	40	2.83	113	3.89	155
NO_3^-	10	2.54	101	2.77	111
NO_2^-	10	2.48	99	1.97	79
Peptone	5	2.45	98	2.39	96
Urine	0.5%	2.55	102	2.73	109
	2.0%	2.78	111	3.06	121

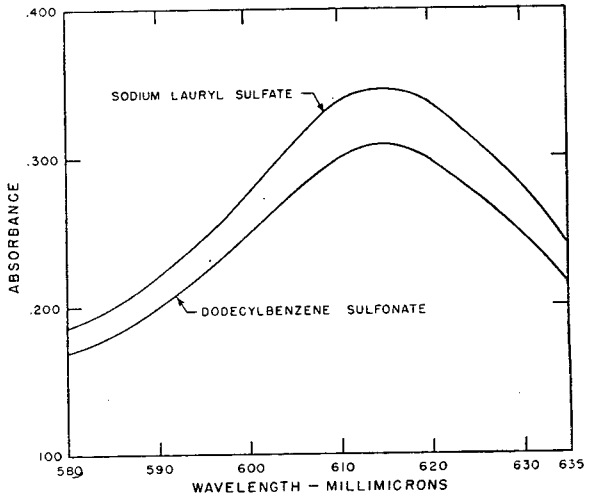

Figure 1. Absorption curves

EXTRACTING SOLVENTS. Several solvents were tried for the extraction of the complex, but the solubility of the dye in most of them precluded their use. Only xylene and benzene extracted the complex without dissolving the dye. Benzene proved to be the better extractant, because the complex was more soluble in it and it did not tend to form emulsions with strong sewage samples.

EFFECT OF pH. The intensity of the color formation is dependent upon the hydrogen ion concentration of the sample, as shown in Table II. The results given in this table show that even though a pH of 2.9 produces maximum color formation, less rigid control is necessary at pH 2.5. To maintain the latter pH, a glycine–hydrochloric acid buffer was used.

TIME FOR COLOR DEVELOPMENT. The time required for complexing the detergent with the methyl green dye is very short. Several samples containing 50 γ of sodium lauryl sulfate, buffer solution, and dye were allowed to stand for different time intervals prior to being extracted. The fact that there was no difference in the absorbances obtained on immediate extraction, and on extraction after 15 minutes, shows that the reaction between the dye and detergent is rapid.

COLOR STABILITY. The absorbance of the benzene-soluble complex formed with methyl green and a dilute solution of detergent was read at various time intervals to determine if color change was occurring with respect to time. The initial reading was taken after a 25-minute lapse in time to allow the water to settle out of the benzene layer after it had been washed. Even after 90 minutes, there was no change in the absorbance of the color complex.

Faust (7) has recommended that the complex formed using the methylene blue method be read immediately after wash-

Table IV. Recovery of Detergent from Sewage

Sample No.	Methyl Green Method: Detergent in Sewage	Detergent added, p.p.m.	Detergent recovered, p.p.m.	% recovered	Methylene Blue Method: Detergent in Sewage	Detergent added, p.p.m.	Detergent recovered, p.p.m.	% recovered
1	18.0	0.5	0.5	100	24.7	0.5	0.53	105
		2.0	2.01	101		2.0	2.12	106
2	14.1	0.5	0.5	100	21.1	0.5	0.18	36
		2.0	2.0	100		2.0	1.16	58
3	19:4	1.0	1.05	105	21.5	1.0	1.12	112
4	4.2	2.5	2.4	96	8.0	2.5	Less than blank	..
		50	48	96		50	52	104
5	3.3	0.3	0.27	90	4.0	0.3	0.43	143
		1.5	1.5	100		1.5	1.67	111

ing, because of its fading properties. Complexes formed by both methods using sewage were allowed to stand for a 24-hour period and compared. The fading of the methyl green complex was negligible as compared to that of the methylene blue complex.

Extraction of Complex. Benzene was used for all extractions of the methyl green–detergent complex. The use of this lighter than water solvent makes it difficult to use more than one extraction because of the error involved in transfer. The effectiveness of a single extraction is shown in Figure 3; 95% of the complex is removed in a single extraction with benzene. In this same figure, a curve is also given for the methylene blue method; using chloroform as the solvent, a single extraction removes about 84% of the color complex. Five extractions would be necessary to remove 95% of the complex, which is accomplished with one extraction in the methyl green procedure.

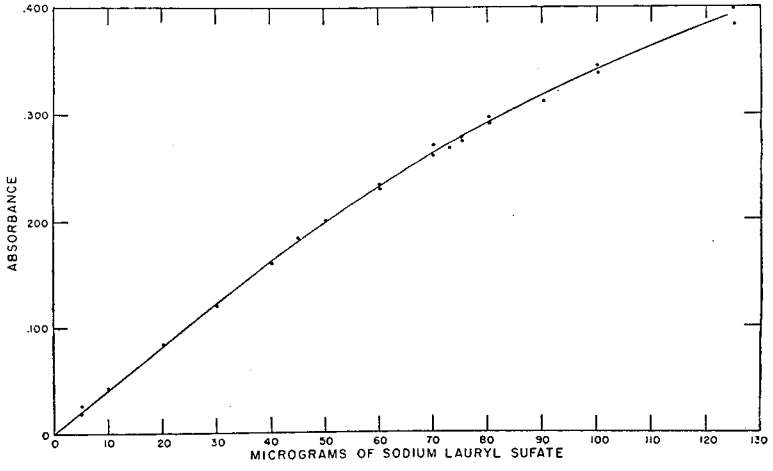

Figure 2. Standard curve for sodium lauryl sulfate

Effect of Washing Benzene and Chloroform. The solvents containing the complex are washed with water to remove interferences which form water-soluble dye complexes. The water wash is also used in the methylene blue method to remove excess dye.

Samples containing 50 γ of sodium lauryl sulfate were run by both methods. The results obtained show that the chloroform extract of the dye complex in the methylene blue method is affected more by the water washings than is the benzene extract in the methyl green method, probably because chloroform is approximately 12 times as soluble in water as is benzene.

Water Removal from Benzene. Washing the benzene with water causes a small amount of water to be temporarily suspended in it. Several types of drying materials were used to absorb the suspended water, but all failed because of the affinity of the complex for filter paper, cotton, glass wool, and chemical drying agents. The water was efficiently removed by allowing it to settle out for 20 to 30 minutes.

Precision and Accuracy. Ten replicates of sodium lauryl sulfate were run at two concentration levels, 10 and 50 γ, by the methyl green method. Using a 1-cm. cell the mean value obobtained at the 10-γ level was 10.02 with a standard deviation of ± 0.31; at the 50-γ level the mean was 49.77 γ with a standard deviation of ± 0.84.

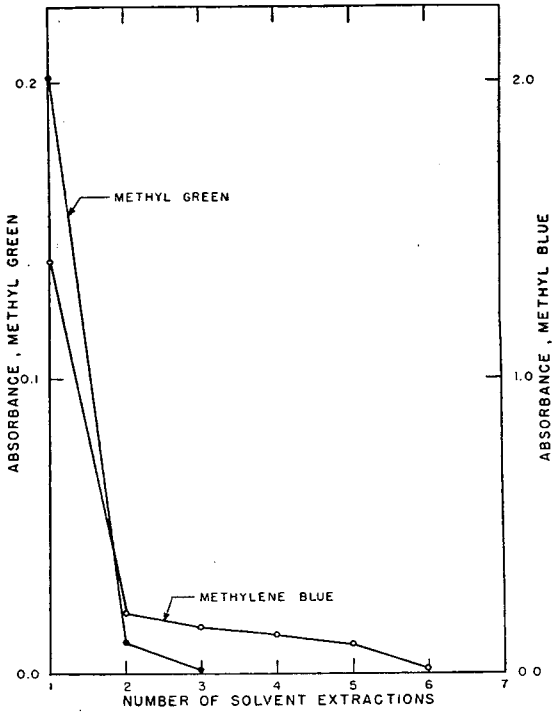

Figure 3. Solvent removal of detergent complex

Table V. Recovery of Detergent from Water

Sample		Methyl Green Method		Methylene Blue Method	
Water	Detergent added, p.p.m.	Detergent recovered, p.p.m.	% recovery	Detergent recovered, p.p.m.	% recovery
River water	0	0.05	..	0.16	..
	0.25	0.25	100	0.23	92
	0.5	0.51	102	0.5	100
	1.0	1.01	101	1.09	109
	2.0	2.05	103	2.09	105
	3.0	3.06	103	3.07	102
Lake water	0	0.01	..	0.16	..
	0.25	0.25	100	0.30	120
	0.5	0.50	100	0.66	132
	1.0	1.0	100	0.91	91
	2.0	1.93	97	2.06	103
	3.0	2.93	98	Absorbance reading off curve	

Interferences. Several ions and compounds were tested for interference in both the methylene blue and methyl green methods. Soap gave insignificant or no interference in concentrations likely to occur in sewage. Other compounds which did not interfere were an organic sulfonate (barium diphenylamine sulfonate), 1-naphthol-5-sulfonic acid, 2-naphthol-6,8-disulfonic acid, glycine, copper (1.0 p.p.m.), sodium borate (2000 p.p.m.), phosphate (10 p.p.m.), sulfate (300 p.p.m.), chloride (500 p.p.m.), and sulfite (5 p.p.m.).

The compounds that showed interference with both methods are compared in Table III. The data in this table, based on a standard sample of 50 γ of sodium lauryl sulfate and the indicated concentrations of interfering materials, show that thiocyanate, nitrate, nitrite, peptone, and urine have a greater effect on the results obtained by the methylene blue method than by the methyl green procedure.

Recovery. From Sewage. Experiments were run to check the recovery of sodium lauryl sulfate from fresh, predominantly domestic sewage using the methyl green and methylene blue methods. Because the sewage already contained detergent, a

dilution of 1 to 20 was necessary. Table IV shows the recovery of added increments of detergent from the diluted sewage. In this experiment, solutions containing 90% sewage were used with definite amounts of detergent. The solutions were then diluted 1 to 20 and the detergent was determined. The recovery data are averages of replicate determinations and thus do not reflect the wider variation found with the methylene blue method.

FROM RIVER WATER. Recovery of detergent from Ohio River water was determined following the same procedure as for sewage, but it was not necessary to dilute the river water, as its detergent content was low. Table V shows the recoveries of added detergent (corrected for the initial detergent present), ranging in concentrations from 0.25 to 3.0 p.p.m. The methylene blue method gave a higher initial detergent content than it did with sewage. The recovery of these added increments was comparable by both methods.

FROM LAKE WATER. The effect of vegetable decomposition products on the accuracy of a detergent analysis was tested by use of a sample of water taken from a small lake (approximately 0.75 acre), which had abundant vegetable growth. The possibility that this lake contained detergent compounds is very remote. The results of the tests indicate that the methylene blue may be reacting with some compound present other than detergent. The data on recovery of added increments of detergent show a much wider fluctuation in the case of the methylene blue method.

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RECEIVED for review June 20, 1955. Accepted December 8, 1955. Division of Water, Sewage, and Sanitation Chemistry, Symposium on Problems in Stream Pollution, 127th Meeting, ACS, Cincinnati, Ohio, March–April 1955. Other papers in this symposium appeared in the February 1955 issue of *Industrial and Engineering Chemistry*.

Determination of Low Chemical Oxygen Demands of Surface Waters by Dichromate Oxidation

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The desirability of a more sensitive evaluation of the chemical oxygen demand (C.O.D.) test in the range of 5 to 50 p.p.m. justified a thorough reinvestigation of the oxidizing efficiency of 0.025 and 0.05*N* potassium dichromate and comparison with reported values for 0.25*N* dichromate. Reproducible results were obtained with the weaker dichromate solutions in replicate series of determinations when the condenser outlets were kept plugged with glass wool, acidified distilled water was used for dilutions and washing, and the tapered-joint seals between flasks and condensers were cleaned by wiping with a damp cloth. Theoretical oxidation of chlorides and representative organic compounds compared favorably with the 0.25*N* dichromate reagent. When silver sulfate is used as a catalyst, only 40 p.p.m. correction should be subtracted from the C.O.D. found, where chlorides are from 40 to 300 p.p.m. Without silver sulfate, corrections are quantitative. The concentration of organic material should be regulated so that not more than 50% of the available dichromate is used. With varying stream flows the C.O.D.–B.O.D. ratio may vary by several hundred per cent; the farther from the source of pollution, the higher the ratio, which indicates a stream more biologically balanced. With stable stream conditions, where the ratios are relatively constant, B.O.D. ranges at representative stream points can be estimated from previous representative C.O.D.–B.O.D. data.

A METHOD proposed for estimation of the organic content of industrial wastes and sewage by a wet combustion method (*1*, *2*), using 0.25*N* potassium dichromate as the oxidant in a 50 volume % sulfuric acid solution, did not possess the desired sensitivity when applied to stream samples. In seeking to obtain greater sensitivity with samples containing a lower organic content (5 to 50 p.p.m.), use of 0.025 and 0.05*N* potassium dichromate was investigated. This study included the oxidation of representative organic compounds, chlorides, and the organic material contained in river waters.

PROCEDURE

In working with the weaker dichromate solutions, two possibilities are presented; 25 ml. of 0.025*N* potassium dichromate may be used or 2.5 ml. of 0.25*N* reagent can be substituted. In the latter case, the sample size may be increased from 50 to 70 ml.

Early in the course of this study, it became apparent that much more rigid control was necessary in working with 0.025*N* potassium dichromate than with the 0.2500*N* reagent. The procedure used was the same as that previously reported (*1*), except that in the titration of the excess dichromate present it was necessary to use two to three times as much indicator as for the stronger dichromate reagent. In many of the preliminary runs wide discrepancies in replicate samples or blanks, ranging from 0.50 to 1.50 ml., were encountered. These discrepancies were found to be due mainly to several factors.

The distilled water used to wash down the condensers sometimes contained algal growths which contributed to wide fluctu-

ation in replicate samples. This difficulty was overcome by adding 1 ml. of concentrated sulfuric acid per liter to the rinse water and keeping the wash bottle scrupulously clean.

The condenser outlets were kept plugged with glass wool, which was removed only to wash the condensers and promptly replaced. This prevented the entrance of organic material from the air during the reflux period.

Table I. Oxidation of Chlorides with 0.025*N* Dichromate

No. of Replicates	Cl^- Added, Mg.		Chlorides Oxidized: With Ag_2SO_4, Mg.	Without Ag_2SO_4, %	With Ag_2SO_4, %
3	0.443	Max.	...	108.0	132.0
		Min.	...	104.0	112.0
		Av.	...	106.0	119.3
6	0.886	Max.	...	102.4	104.4
		Min.	...	99.5	93.6
		Av.	...	100.8	100.8
6	1.77	Max.	1.68	102.5	95.0
		Min.	1.64	92.5	92.5
		Av.	1.67	97.3	94.0
6	4.43	Max.	2.02	101.0	...
		Min.	1.82	94.0	...
		Av.	1.92	98.1	...
6	8.86	Max.	2.14	98.4	...
		Min.	1.70	95.8	...
		Av.	1.82	96.9	...
3	13.29	Max.	1.99	97.3	...
		Min.	1.88	95.0	...
		Av.	1.95	96.5	...
3	17.72	Max.	2.38	97.9	...
		Min.	2.22	96.9	...
		Av.	2.30	97.6	...

The tops of the condensers and the area around the tapered-joint seals were wiped with a clean damp towel before the flasks were removed to introduce or remove samples.

RESULTS

Oxidation of Chlorides. Preliminary runs were made using 0.0025 and 0.025*N* standard sodium chloride solutions. While considerable variations among replicates of blanks and samples were encountered, chlorides were completely oxidized by the 0.025*N* potassium dichromate, when either 40 or 50 volume % sulfuric acid was used in refluxing. In some cases average depletions within a series varied from 0.5 to 2.5 ml., but in all series approximately 100% of the chloride was oxidized.

Chlorides in the lowest ranges gave the most erratic results, as variations of a few hundredths of a milliliter in titration figures became significant when multiplied by the necessary factors. A chloride content of 1 ml. of 0.0025*N* sodium chloride (0.089 mg. of chloride ion) would be less than 2 p.p.m. in a regular 50-ml. stream sample. With both 0.025 and 0.05*N* potassium dichromate the deviations from the theoretical depletions became progressively less and results more uniform as the chloride content increased from 0.1 to 0.5 ml. of 0.025*N* sodium chloride (0.089 to 0.443 mg. of chloride).

Characteristics of chloride oxidation are well illustrated in Table I. These results were obtained with glass wool plugs in the condenser openings, and using the acidified distilled water for dilutions and washings. Parallel series of replicates were analyzed simultaneously both with and without silver sulfate. Replicate series of blanks usually agreed within a few hundredths of a milliliter. Only two cases occurred in the seven replicate blank control series (33 total determinations) in Table I, where an individual result gave approximately 1% difference between the maximum and minimum of the control. When silver sulfate was used at the rate of 1 gram to each flask of the regular reflux mixture, colloidal silver chloride was precipitated. Coagulation of the suspended silver chloride proceeded rapidly, as in all cases the flasks became clear with a few minutes' boiling, and granular silver chloride remained when 2 ml. or more of 0.025*N* sodium chloride (1.77 mg. of chloride) had been added. Addition of from 5 to 15 ml. of 0.025*N* sodium chloride (4.43 to 13.29 mg. of chloride) resulted in the average oxidation of slightly more than the equivalent of 2 ml. of 0.025*N* sodium chloride, or approximately 1.9 mg. of chloride ion. With a 5-ml. stream sample, this would correspond to 38 p.p.m.

Based on the data of Table I, it is apparent that in regular determinations of oxygen consumed, quantitative chloride corrections are reliable in all ranges up to the limit of dichromate available, with the maximum expected deviations in the samples of lowest chloride content.

When silver sulfate is added to the regular reflux mixture, quantitative chloride corrections to samples containing less than 1.9 mg. of chloride ion may be made. With chloride concentrations up to 300 p.p.m. of chloride approximately 40 p.p.m. of the total chloride should be oxidized, and results corrected for this amount.

Oxidation of Organic Compounds. In Table II are shown the results obtained in the oxidation of organic compounds by 0.05

Table II. Oxidation of Organic Compounds

No. of Replicates	$K_2Cr_2O_7$ Normality	Ag_2SO_4 Used, Gram	Cl^- Added and Corr. for, Mg.	Sample Used, Mg.: 1.0	3.0	5.0	1.0	3.0	5.0
				C.O.D. found/1000 mg. of compound			% of theory		
Glucose									
12	0.050	0	0	1030	1020	1010	96.4	95.3	94.8
10	0.050	1.0	0	950	940	950	89.1	88.6	89.3
6	0.050	0	4.43	990	980	980	92.6	92.1	91.5
3	0.050	0	1.77	980	980	980	92.3	92.3	92.2
3	0.050	1.0	1.77	890	880	890	83.0	82.6	83.1
Resorcinol									
				(0.5)	(1.0)	(2.0)	(0.5)	(1.0)	(2.0)
6	0.025	0	0	1790	1780	1780	94.7	94.3	94.2
		1.0	0	1760	1750	1730	93.2	92.3	91.5
Acetic Acid									
				(1.0)	(3.0)	(5.0)	(1.0)	(3.0)	(5.0)
3	0.050	0	0	60	48	22	5.6	4.5	2.1
		1.0	0	1030	950	960	96.6	89.2	89.7
				(0.5)	(1.0)	(2.0)	(0.5)	(1.0)	(2.0)
3	0.025	1.0	0.89	970	930	930	90.9	87.5	87.3
		1.5	0	1020	990	1000	95.5	93.0	93.9
5	0.025	1.5	0.89	960	950	950	89.6	89.1	88.6
Butyric Acid									
3	0.025	0	0	1310	1290	1280	72.1	71.1	70.1
		1.0	0	1710	1690	1660	93.8	92.6	91.3
Alanine									
				(1.0)	(2.0)	(5.0)	(1.0)	(2.0)	(5.0)
3	0.025	0	0	175	186	176	13.9	14.8	14.0
3[a]	0.025	0	0	234	235	219	18.6	18.7	17.4
3	0.025	1.0	0	720	660	560	57.3	52.4	45.0
3[a]	0.025	1.0	0	800	740	640	63.2	58.7	51.2
Glutamic Acid									
				(1.0)	(2.0)	(5.0)	(1.0)	(2.0)	(5.0)
3	0.025	0	0	402	382	345	41.0	39.0	35.2
		1.0	0	922	895	782	94.1	91.3	79.8

[a] Refluxed 3 hours. All others regular 2-hour reflux time.

and 0.025*N* potassium dichromate. The solid organic compounds used were dried overnight in the oven and cooled, and 1 gram was dissolved in 1 liter of distilled water. Theoretical chemical oxygen demand (C.O.D.) values shown in the tables were calculated for these 1000 mg. per liter or 1000 p.p.m. solutions. As 1 ml. of these solutions contained 1 mg. of the organic compound, convenient amounts for each sample could be accurately measured by pipet, and by multiplying the results by the appropriate factor or 1000 divided by milligrams of sample used, the C.O.D. was obtained for comparison. Glucose solutions of various glucose contents were 95 to 97% oxidized with 0.05*N* dichromate by the regular reflux procedure. When 1 gram of silver sulfate was used as a catalyst, only 89% of the glucose was oxidized. Addition of 1.77 or 4.43 mg. of chloride, with the necessary corrections, resulted in approximately 92% oxidation in all glucose concentrations by the regular procedure. When silver sulfate was used, with chlorides added and corrected for, oxidation results were only 83% of theory in all cases. The silver ion lowered the oxidation efficiency approximately twice as much as the corrected chloride ion, and when both were present, the per cent glucose oxidized was 12 to 14% lower.

Resorcinol oxidation was approximately 2% lower when silver sulfate was used. A slight decrease in oxidation efficiency occurred as the resorcinol content was increased with silver sulfate present.

With both glucose and resorcinol, increasing the amount of material available for oxidation up to four or five times resulted in comparable oxidation values in practically all cases.

Acetic acid was very resistant to oxidation by the regular reflux conditions with 0.05*N* potassium dichromate. With 1 mg. of acetic acid present, 5.6% oxidation occurred. Three milligrams of acetic acid resulted in 4.5% oxidation, while when 5 mg. was present, only 2.1% was oxidized. When silver sulfate was used, corresponding oxidation values were 96 to 90% of theory. With 0.025*N* potassium dichromate and 1.5 grams of silver sulfate, average oxidation values were 96 to 93%. Addition of 1 ml. of 0.025*N* sodium chloride (0.89 mg. of chloride) and quantitative correction resulted in approximately the same per cent oxidation (90%) with either 1.0 or 1.5 grams of silver sulfate present.

Butyric acid was 70 to 72% oxidized by the regular procedure with 0.025*N* potassium dichromate. When silver sulfate was used, oxidation was increased to 91 to 94%. In both cases, the highest oxidation values were obtained with the series of lowest butyric acid content. With the butyric acid doubled and quadrupled, a slight decrease in oxidation values resulted.

The amino acids exhibited the most erratic behavior of any class of organic compounds used. Both alanine and glutamic acid were only partially oxidized with the regular Moore (*1*) procedure, alanine being the most resistant to oxidation. With the regular 2-hour reflux time, only 14 to 15% of the alanine was oxidized. Extending the reflux time to 3 hours resulted in oxidation of 17 to 19%. In both cases 1, 2, and 5 mg. of alanine gave practically the same amount of oxidation. Replicate series with 1 gram of silver sulfate added gave oxidations of 57, 52, and 45% in 2 hours and 63, 59, and 51% in 3 hours, a total decrease of 12% in each case in a practically uniform pattern for the 1-, 2-, and 5-mg. series.

Glutamic acid also showed a drop in per cent oxidation in the 1-, 2-, and 5-mg. series, both with and *without* silver sulfate. With the regular procedure, the total difference was 6% (41 to 35%). Where silver was used, a total difference of 14% (94 to 80%) was obtained using the same glutamic acid concentrations.

River Samples. Samples were collected from the Ohio and Little Miami rivers. Replicate 5-day biochemical oxygen demands (B.O.D.) were run on all samples to obtain the C.O.D.–B.O.D. ratios. Replicate series of C.O.D. determinations were made on each sample, with 0.025 and 0.05*N* potassium dichromate, both with and without silver sulfate. Chloride corrections were made on all river samples. The agreement between series of samples using 0.025 and 0.05*N* dichromate was slightly better in almost all cases when silver sulfate was used.

Results obtained on stream samples are shown in Table III. The first two samples of May 28 and June 2 were collected from the Ohio River at point 1 above Cincinnati. On both days, the Ohio River had been in the pool stage for several weeks. The C.O.D.–B.O.D. ratios were in a comparable range both with and without silver sulfate on May 28, while those on June 2 were slightly higher with silver sulfate. Samples taken June 18 during high water at this point were turbid and gave slightly higher C.O.D.–B.O.D. ratios. On July 1, the ratios dropped to approximately one half the June 18 value. Samples of July 7 gave the lowest C.O.D.–B.O.D. ratios of the series. In this series, samples of 25, 50, and 70 ml. were analyzed simultaneously, with and without silver sulfate. This series gave the highest B.O.D. and next to the lowest C.O.D. values found at point 1.

Point 2 on the Ohio River was located at the foot of Broadway

Table III. Oxidation of Ohio River Water

Date 1954	Ag_2SO_4 Added, Gram	$K_2Cr_2O_7$ Normality	C.O.D. Found, P.P.M. Max.	Min.	Av.	B.O.D., Av. P.P.M.	Ratio, C.O.D.–B.O.D.
			Sample Point 1				
May 28	0	0.025	15.6	11.9	13.9	1.08	12.9–1
	1	0.025	14.4	13.1	13.8	1.08	12.8–1
	0	0.05	12.9	11.6	12.4	1.08	11.5–1
	1	0.05	12.9	11.7	12.1	1.08	11.2–1
June 2	0	0.025	13.7	12.8	13.1	1.03	12.7–1
	1	0.025	14.7	13.3	13.8	1.03	13.4–1
	0	0.05	14.1	13.1	13.5	1.03	13.1–1
	1	0.05	14.8	14.3	14.5	1.03	14.1–1
June 18[a]	0	0.025	23.3	21.2	22.5	1.50	15.0–1
	1	0.025	26.1	21.7	23.3	1.50	15.6–1
	0	0.05	20.5	19.9	20.3	1.50	13.5–1
	1	0.05	26.8	20.5	23.7	1.50	15.8–1
July 1	0	0.025	8.0	7.6	7.8	1.20	6.5–1
	1	0.025	8.5	8.3	8.4	1.20	7.0–1
	0	0.05	7.8	7.4	7.6	1.20	6.3–1
	1	0.05	9.7	9.3	9.5	1.20	7.9–1
July 7							
25-ml. sample	0	0.025	11.5	8.9	10.1	2.12	4.8–1
	1	0.025	11.9	10.5	11.0	2.12	5.2–1
50-ml. sample	0	0.025	11.3	8.9	10.0	2.12	4.7–1
	1	0.025	10.0	9.1	9.3	2.12	4.4–1
70-ml. sample	0	0.025	9.6	9.2	9.5	2.12	4.5–1
	1	0.025	9.7	9.0	9.4	2.12	4.4–1
			Sample Point 2				
June 8	0	0.025	21.1	19.8	20.5	7.98	2.6–1
	1	0.025	22.2	21.4	21.9	7.98	2.7–1
	0	0.05	20.1	17.7	18.8	7.98	2.4–1
	1	0.05	22.1	21.5	21.8	7.98	2.7–1
June 21[a]	0	0.025	22.9	21.2	22.3	3.27	6.8–1
	1	0.025	23.3	22.3	22.7	3.27	6.9–1
	0	0.05	19.5	18.1	18.6	3.27	5.7–1
	1	0.05	24.1	22.4	22.9	3.27	7.0–1
June 23	0	0.025	16.4	15.7	16.0	8.14	2.0–1
	1	0.025	19.4	18.2	18.7	8.14	2.3–1
	0	0.05	17.3	16.2	16.8	8.14	2.1–1
	1	0.05	19.5	18.4	18.9	8.14	2.3–1

[a] Stream high and turbid.

Three replicate analyses on all B.O.D. and C.O.D. determinations. Averages in table include all three results. Chlorides were determined and quantitative correction made on all samples.

Street in Cincinnati. Several sewers discharge above the point sampled, so that when the river was in pool stage, this area was heavily polluted. The low average C.O.D.–B.O.D. ratios of 2.6 to 1 on July 8 and 2.2 to 1 on June 23 clearly indicate the presence of unstable organic material which was oxidized biochemically. The sample taken June 21 during high water, when the discharged sewage was diluted by the increased flow, resulted in an average ratio of 6.6 to 1, approximately three times as large as during the quiet pool stage.

Sampling of the Little Miami River was interrupted by high water on June 14 and 25, which flushed the river thoroughly and increased the C.O.D.–B.O.D. ratio from an average of 5.7 to 1 on June 3, to 10.2 to 1 on June 14, and 11.3 to 1 on June 25. The sample of June 3 was taken during low normal flow. On all of the Miami River samples, use of silver sulfate gave approximately 10% higher C.O.D. values than the regular Moore procedure.

Mill Creek, which was sampled on June 16, carried a large amount of raw sewage. This sample had a B.O.D. of 115 p.p.m. and an average C.O.D. of 256 p.p.m., giving approximately a 2 to 1 ratio of C.O.D.–B.O.D. Chlorides were 96 p.p.m.; correction for 40 p.p.m. of this amount was made on samples that contained silver sulfate.

DISCUSSION

In this study, chemical determinations of oxygen consumed with 0.025 and 0.05*N* potassium dichromate solutions gave reproducible, comparable results in replicate series of experiments.

Oxidation of chlorides and most of the representative organic compounds used compared favorably with results reported for the 0.25*N* dichromate (*1*, *2*). When silver sulfate was used, chlorides up to 1.9 mg. per sample, corresponding to 38 p.p.m. in a 50-ml. stream sample, were 94% oxidized. Increased chloride concentration up to the equivalent of 300 p.p.m. resulted in the oxidation of practically a constant amount (1.9 mg.), so that in the stream studies chlorides above 40 p.p.m. were corrected for this amount only. Without silver sulfate, chloride oxidation was quantitative up to the limit of dichromate available.

There was a tendency for the C.O.D. values found on some of the organic compounds to decrease with increased size of sample, both with and without silver sulfate. With glucose from 1 to 5 mg. and resorcinol from 0.5 to 2 mg., the decrease in C.O.D. values was less than 2%. The butyric acid decrease was 2 to 3%; the acetic acid decrease with silver sulfate added was 7% when the concentration was varied from 1 to 5 mg. Glutamic acid with silver sulfate gave 94.1% of theory with a 1-mg. sample, while a 5-mg. sample gave 79.8%. Alanine gave the lowest theoretical percentage recovery for the amino acids. When the reflux time was increased from 2 to 3 hours using silver sulfate, the average per cent of theory for a 1-mg. sample increased from 57.3% to 63.2%. With 5-mg. samples, the values were 45.0 and 51.2%, respectively.

The data on the oxidation of organic compounds definitely show that with increased concentration of C.O.D. a decrease in oxidation efficiency sometimes occurred. It is reasonable to suppose that this is due to a decrease in the redox potential in the system when 0.025*N* potassium dichromate is used. To secure uniformity in results, sample sizes should be arranged so so that no more than 50% of the dichromate is reduced. When a sample is sufficiently strong, it is desirable to use an aliquot that gives from 25 to 50% dichromate reduction. On samples of low C.O.D. (below 25 p.p.m.) 70 ml. of sample should be used.

In this investigation, the number of stream samples analyzed was limited. Changing stream conditions resulted in wide variations in the C.O.D.–B.O.D. ratios. Addition of silver sulfate usually gave slightly higher C.O.D. values in a comparable range with duplicate series, where the regular Moore procedure was used. In both of the Ohio River points sampled, the ratios were relatively constant, when the river was in pool stage. At point 1, high water may have disturbed unstable bottom sediments, resulting in release of material amenable to biochemical oxidation. The C.O.D. values at first increased and later dropped to much lower values than those found during pool stage. The B.O.D. values increased during this time, in spite of the high dilution, and continued to remain higher than those found at pool stage, resulting in the much lower C.O.D.–B.O.D. ratios.

Calculation of B.O.D. values from C.O.D. results does not appear feasible on natural stream samples. Seasonal changes, with increased or decreased biological activity, changing stream flows due to rains or melting snow, and changing industrial activities result in widely varying C.O.D. and B.O.D. values, and C.O.D.–B.O.D. ratios which may increase or decrease by several hundred per cent. Results of this investigation agree with the findings of Moore and Ruchhoft (*3*) on Lytle Creek and a stream polluted by oil refinery waste.

In normal stream surveys (*3*) the C.O.D.–B.O.D. ratios from stations located at representative points on the stream give valuable information on the general condition of the stream, location and probable extent of the pollution load, ability of the stream to oxidize satisfactorily the amount of waste carried, and degree of biological stability. Where records of C.O.D. and B.O.D. determinations are available for extended periods of time, and seasonal changes and effect of varying stream flows are considered, it should be possible to predict with reasonable accuracy the probable 5-day B.O.D. at a particular point from the results of the C.O.D. determination.

CONCLUSIONS

Results of determinations of chemical oxygen consumed with 0.025 and 0.05*N* potassium dichromate compared favorably with those reported for 0.25*N* on chlorides and most of the representative organic compounds used.

Uniform results in replicate series of oxidation experiments were obtained when all steps in the procedure were carefully controlled.

When the chloride content is between 40 and 300 p.p.m. and silver sulfate is used with the regular Moore method, correction for only 40 p.p.m. should be made on the C.O.D. obtained. Without the silver catalyst, chloride corrections are quantitative.

Calculation of B.O.D. values from C.O.D. results on stream samples may be unreliable. With weather conditions and stream flows relatively constant, the probable B.O.D. range at a particular stream point may be estimated from a series of previous representative C.O.D.–B.O.D. ratios.

In stream surveys, the C.O.D.–B.O.D. ratios from representative sampling points give information on general stream conditions, locations and extent of pollution, ability of the stream to oxidize the waste load carried, and relative degree of biological stability.

The sample size should be so selected that not more than 50% of the 0.025*N* potassium dichromate is used up during the oxidation.

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RECEIVED for review June 20, 1955. Accepted December 17, 1955. Division of Water, Sewage, and Sanitation Chemistry, Symposium on Problems in Stream Pollution, 127th Meeting, ACS, Cincinnati, Ohio, March–April 1955. Other papers in this symposium are published in the February 1956 issue of *Industrial and Engineering Chemistry*.

Chromatographic Separation and Determination of Straight-Chain Saturated Monocarboxylic Acids C_1 through C_{10} and Dicarboxylic Acids C_{11} through C_{16}

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A partition chromatographic method for the accurate analysis of mixtures of monocarboxylic acids C_1 through C_{10} and dicarboxylic acids C_{11} through C_{16} has been developed. The stationary phase is 2*M* aqueous glycine on silicic acid adjusted to the desired pH with either 0.5*N* hydrochloric acid or concentrated sodium hydroxide; the mobile phase is 1-butanol–chloroform in gradient elution. A series of three columns, with stationary phases of pH 2, 8.4, and 10, is employed for analysis of mixtures of monocarboxylic acids C_1 through C_{10} and a series of two columns, with stationary phases of pH 8.5 and 9, is necessary for analysis of mixtures of dicarboxylic acids C_{11} through C_{16}. Excellent resolution and analytical accuracy within ±1% are obtained for all components.

A SURVEY of existing methods of analysis for mixtures of fatty acids disclosed that no simple reproducible method had been devised which would separate a homologous series of monocarboxylic acids differing by one methylene group. Moyle's (*4*) phosphate-buffered column and Zbinovsky's (*9*) Cellosolve-water–Skellysolve B–*n*-butyl ether method proved to be unsatisfactory for quantitative work because of poor resolution and incomplete recovery. Other methods for separation of acids differing by two methylene groups by Silk (*8*), Peterson (*6*), Nijkamp (*5*), and Howard (*1*) were not adjustable to separation of acids differing by one methylene group. A need for such a method applicable on a small scale has long been felt. Current work in this laboratory required a method which would accomplish this purpose with an accuracy approaching 100%.

APPARATUS AND REAGENTS

The following apparatus is needed:

Beckman Model G pH meter with external electrodes.
Technicon fraction collector equipped with drop counter, Technicon, Inc.
Glass chromatographic columns, 18 mm. in inside diameter, 600 mm. long with delivery tube and stopcock.
Glass plunger.
Automatic 10-ml. microburet, Scientific Glass Apparatus Co.
Dry nitrogen, high purity.
Reducing gage equipped with double needle valve.
Volumetric pipets.
Analytical balance.
Vacuum desiccator.

These reagents are needed:

1-Butanol, C.P. grade.
Chloroform, reagent grade.
Silicic acid, Mallinckrodt chromatography grade, 100 mesh.
Standard methanolic sodium hydroxide, approximately 0.03 to 0.04*N*.
Glycine buffer, Pfanstiehl Chemical Co. aminoacetic acid. Adjust aqueous solution to proper pH with concentrated sodium hydroxide or 0.5*N* hydrochloric acid.
m-Cresol purple, 1% in 95% methanol.

PROCEDURE

Preparation of Silicic Acid. Silicic acid as prepared by Mallinckrodt according to the method of Ramsey and Patterson (*7*) proved unsuitable as a supporting medium for the aqueous phase of the chromatogram because of its adsorptive properties. Very pure gel (*3*) exhibits similar properties, suggesting that the adsorption is connected with the structure of the gel rather than with the presence of impurities.

The method described here for the preparation of a non-adsorbent silicic acid is similar to the method of Isherwood (*2*), and is based on the fact that treatment with 10*N* hydrochloric acid at room temperature for a period of 24 to 36 hours eliminates its ability to adsorb organic acids.

Mallinckrodt's silicic acid is suspended in 10*N* hydrochloric acid for the afore-mentioned period. The supernatant liquid is then decanted and the silicic acid washed with water to remove hydrochloric acid. When acid-free, the gel is washed with absolute methanol until the filtrate is neutral to litmus. It is then washed with anhydrous ether and finally dried in vacuo over phosphorus pentoxide. Two to three days and several changes of phosphorus pentoxide are necessary before the silicic acid is sufficiently dry. It is then stored in airtight glass jars.

Preparation of the Aqueous Phase. A 2*M* stock solution of the glycine stationary phase is prepared and stored in a refrigerator. The buffer at pH 2 is prepared by adding 0.5*N* hydrochloric acid to part of this solution, using the Beckman Model G pH meter and external electrodes. Inasmuch as pH is critical it is necessary to restandardize the meter before each titration.

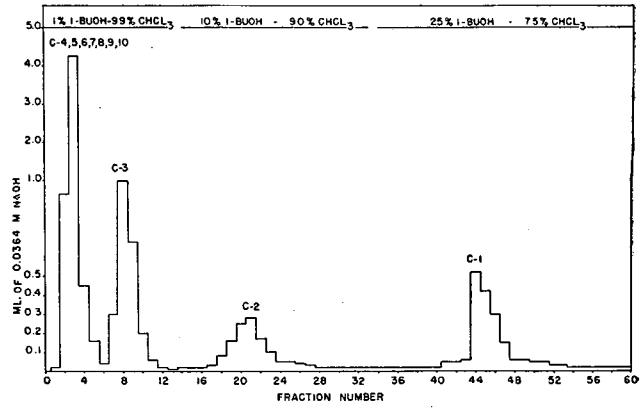

Figure 1. Analysis of monocarboxylic acids C_1 through C_{10} using glycine stationary phase at pH 2

The stationary phases higher in pH than 6.5 are prepared by adding concentrated sodium hydroxide to the 2*M* glycine solution. One drop of a 50% solution of Arquad HT (trimethyloctadecylammonium chloride) in isopropyl alcohol is added to each 150 ml. of the aqueous glycine as a fungicide and bactericide.

Preparation of the Column. In preparing the column, 22 ml. of the appropriate glycine phase and 25 grams of the hydrochloric acid–treated silicic acid are ground together in a beaker for 5 minutes, using a test tube as a pestle. Seventy-five milliliters of the first eluent, 1% 1-butanol–99% chloroform, are introduced gradually with stirring to form a smooth slurry. A pad of glass wool inserted in the neck of the chromatographic column retains the silicic acid. The slurry is added in small increments and packed with a glass plunger, and the excess solvent is allowed to drain. The usual precautions are observed to eliminate entrapped air.

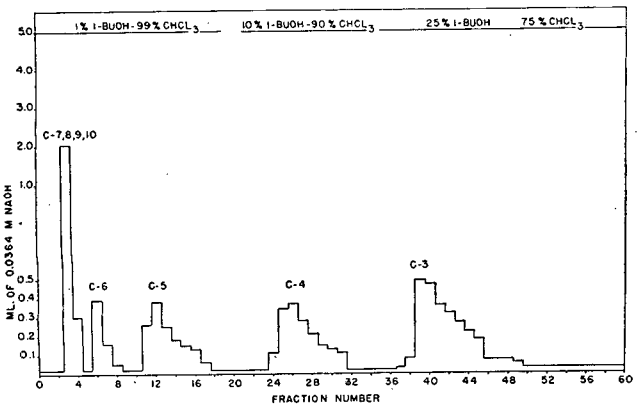

Figure 2. Analysis of monocarboxylic acids C_1 through C_{10} using glycine stationary phase at pH 8.4

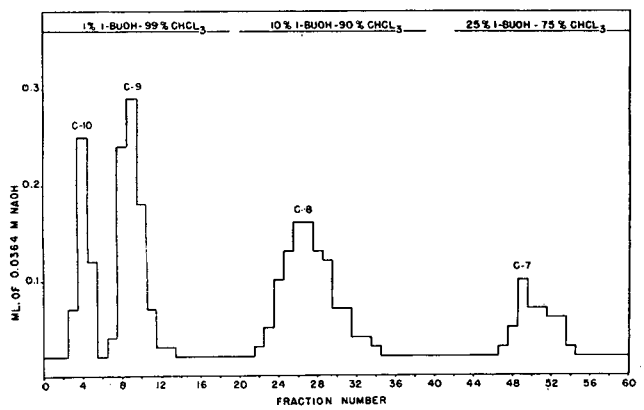

Figure 3. Analysis of monocarboxylic acids C_1 through C_{10} using glycine stationary phase at pH 10

Preparation of Sample and Operation of Column. Chloroform solutions of essentially pure monocarboxylic acids were prepared and a composite solution of all the acids was made, varying the concentration of each to simplify identification upon elution.

Table I. Results of Chromatographic Analysis of Monocarboxylic Acids C_1 through C_{10}

Monocarboxylic Acids	Charge		Recovery		Difference, Mg.
	Meq.	Mg.	Meq.	Mg.	
C_1	0.0512	2.36	0.0514	2.37	+0.01
C_2	0.0334	2.05	0.0331	1.99	−0.06
C_3	0.0774	5.72	0.0779	5.77	+0.05
C_4	0.0572	5.02	0.0578	5.09	+0.07
C_5	0.0444	4.53	0.0450	4.59	+0.06
C_6	0.0152	1.76	0.0153	1.77	+0.01
C_7	0.0198	2.58	0.0197	2.56	−0.02
C_8	0.0293	4.23	0.0298	4.29	+0.06
C_9	0.0236	3.74	0.0244	3.85	+0.11
C_{10}	0.0168	2.89	0.0168	2.89	0
Totals	0.3683	34.88	0.3712	35.17	

An aliquot of the composite sample equivalent to a maximum of 35 mg. of monobasic acid is carefully pipetted onto the top of the column and allowed to percolate into the silicic acid. Simultaneously, the Technicon fraction collector is set in operation and collection of 400-drop or 10-ml. fractions is begun. After the sides of the tube are washed several times with small quantities of the first eluent and the washings are allowed to percolate into the column, the tube is filled with the 1% 1-butanol–99% chloroform eluent and nitrogen pressure is applied (about 2 pounds per square inch), sufficient to maintain a drop rate of approximately 106 drops per minute. A total of 200 ml. of each of three eluents is used, the concentrations of butanol being 1%, 10%, and 25% in chloroform. Sixty fractions are collected and each fraction is titrated with approximately 0.03*N* sodium hydroxide in absolute methanol, with 1% *m*-cresol purple in 95% methanol used as the indicator. A stream of nitrogen is used for agitation during the titration to exclude carbon dioxide from the air. The time necessary for the completion of one column is approximately two hours.

RESULTS AND DISCUSSION

Analysis of a mixture of the series of ten monocarboxylic acids from formic through capric is effected by the use of three columns with pH 2, 8.4, and 10 (Figures 1, 2, and 3). Because of the relative strength of formic acid, 2 is the optimum pH at which it could be recovered in this system. Acetic and propionic acids are also recovered separately at this pH but acids from C_4 through C_{10} are eluted in one peak (Figure 1). Figures 2 and 3 illustrate the effect of increasing the pH of the stationary phase. The more strongly acidic constituents of the series are retained on the column. In each case, the same quantity of solvent and ratio of butanol to chloroform are maintained.

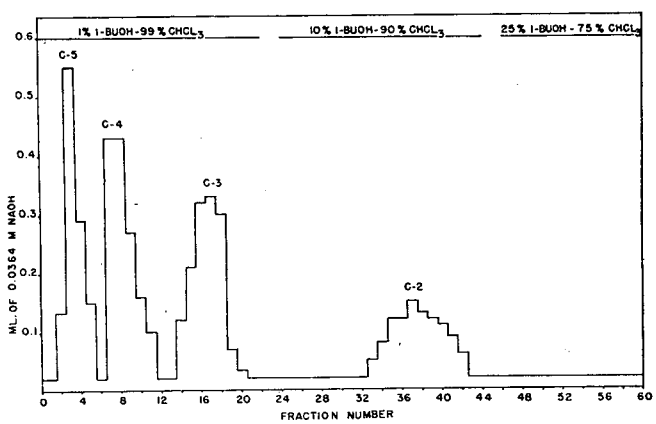

Figure 4. Analysis of monocarboxylic acids C_1 through C_6 using glycine stationary phase at pH 6.5

The versatility of the method is demonstrated in Figures 4 and 5. By varying the pH of the stationary phase, it is possible to resolve any desired group of the afore-mentioned acids. It is necessary only to determine the correct pH at which they may be recovered and resolved. In general, as molecular weight increases and acid strength decreases the pH of the column must be increased. The mixture chromatographed at pH 6.5

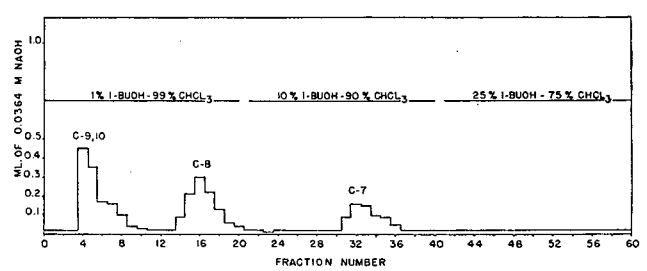

Figure 5. Analysis of monocarboxylic acids C_1 through C_{10} using glycine stationary phase at pH 9.6

(Figure 4) contained acids formic through valeric. Formic acid remained on the column.

The data obtained by analyzing the mixture of monocarboxylic acids formic through capric are shown in Table I.

Table II. Results of Chromatographic Analysis of Dicarboxylic Acids C_{11} through C_{16}

Dicarboxylic Acids	Charge		Recovery		Difference,
	Meq.	Mg.	Meq.	Mg.	Mg.
C_{11}	0.0066	0.72	0.0070	0.75	+0.03
C_{12}	0.0066	0.76	0.0073	0.84	+0.08
C_{13}	0.0367	4.48	0.0367	4.48	0
C_{14}	0.0276	3.56	0.0270	3.49	−0.07
C_{15}	0.0184	2.50	0.0180	2.45	−0.05
C_{16}	0.0176	2.52	0.0176	2.52	0
TOTALS	0.1135	14.54	0.1136	14.53	

The optimal load needed to obtain perfect resolution appears to be approximately 35 mg. On a total of 20 determinations the recovery slightly exceeded the charge. This may be attributed partially to the fact that solvents were not redistilled and partially to experimental error. Inasmuch as a total of 180 10-ml. fractions is collected for complete analysis of the C_1 through C_{10} series, an average titration error of 0.0016 ml. per fraction is indicated, which is within experimental error.

Because dibasic and monobasic acids are often present in the same mixture, it was necessary to determine the interferences contributed by dibasic acids. A typical mixture of C_4 with C_7 through C_{12} dibasic acids was prepared and chromatographed with the monobasic acids at the same pH ranges necessary for complete analysis of the monobasic acids (Figures 6, 7, and 8). At pH 2 (Figure 6) the C_4 through C_{10} monobasic acids were eluted in one peak in the same position as when mono acids alone were present.

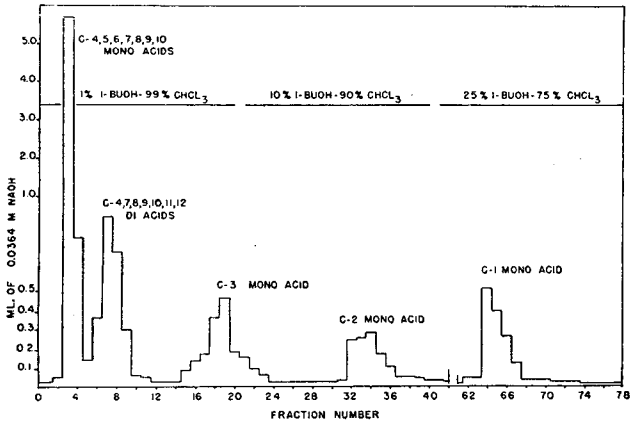

Figure 6. Analysis of a mixture of monocarboxylic acids C_1 through C_{10} and dicarboxylic acids C_4 and C_7 through C_{12} using glycine stationary phase at pH 2

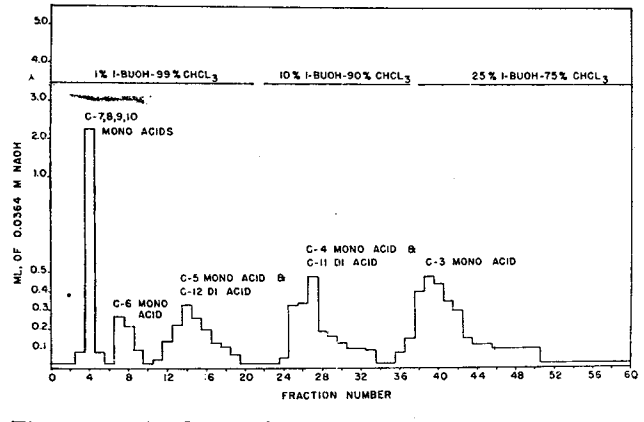

Figure 7. Analysis of a mixture of monocarboxylic acids C_1 through C_{10} and dicarboxylic acids C_4 and C_7 through C_{12} using glycine stationary phase at pH 8.4

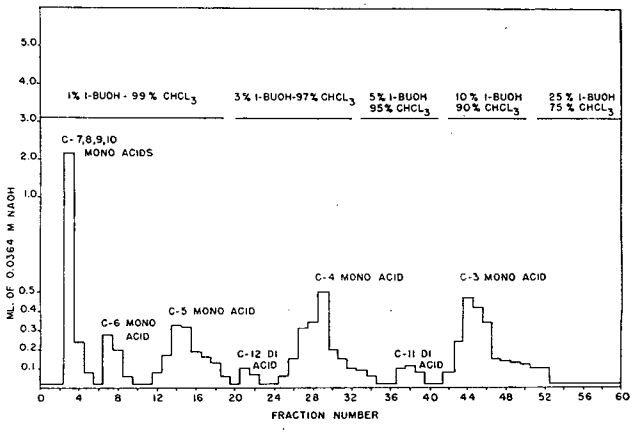

Figure 8. Analysis of a mixture of monocarboxylic acids C_1 through C_{10} and dicarboxylic acids C_4 and C_7 through C_{12} using glycine stationary phase at pH 8.5

The dicarboxylic acids were eluted in one peak followed by C_3, C_2, and C_1 monobasic acids which were completely resolved. The threshold volumes of these lower molecular weight acids are shifted as much as 220 ml. by the presence of dibasic acids, necessitating the collection of 78 fractions in order to elute formic acid completely.

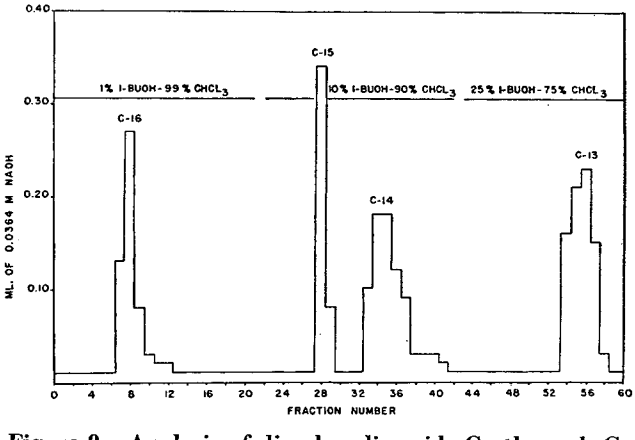

Figure 9. Analysis of dicarboxylic acids C_{13} through C_{16} using glycine stationary phase at pH 9

At pH 8.4 (Figure 7), the threshold volumes of the monocarboxylic acids combined with dicarboxylic acids are the same as those with only monocarboxylic acids present. C_{12} dicarboxylic acid, however, is eluted with C_5 monocarboxylic acid;

and C_{11} dicarboxylic acid with C_4 monocarboxylic acid. To separate the monobasic from the dibasic acids, it is necessary to increase the pH of the stationary phase to 8.5 and change the butanol ratios slightly (Figure 8). The change in pH of 0.1 gives excellent resolution of both mono- and dicarboxylic acids.

It was apparent that the glycine method was equally effective in separating both mono- and dicarboxylic acids; therefore, an attempt was made to determine the range of dicarboxylic acids which could be separated effectively. The available supply of higher molecular weight dibasic acids included those from C_{11} through C_{16}.

Excellent resolution and recovery are obtained with a column at pH 9 for the C_{13} to C_{16} dicarboxylic acids (Figure 9). C_{11} and C_{12} acids are resolved on a column at pH 8.5 (Figure 8). The conflicts of C_{13} to C_{16} dibasic acids with the monobasic acid peaks were not determined because of the extremely short supply of dibasic acids. Data pertaining to recovery of dibasic acids are shown in Table II.

The author believes this method is applicable to the determination of dicarboxylic acids through C_{20} merely by increasing the pH of the stationary phase.

ACKNOWLEDGMENT

The author is indebted to Stewart Leslie for preparation of the drawings.

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RECEIVED for review August 29, 1955. Accepted November 25, 1955.

Chromatography of Alpha-Keto Acid 2,4-Dinitrophenylhydrazones and Their Hydrogenation Products

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The 2,4-dinitrophenylhydrazones of a series of 35 α-keto acids (including the α-keto acid analogs of most of the common naturally occurring amino acids) have been prepared and studied in several paper chromatographic systems. The hydrazones (as prepared, or after elution from paper chromatograms) were hydrogenated under pressure in the presence of platinum oxide catalyst. Hydrogenation of the hydrazones of the α-keto acid analogs of all the amino acids studied (except cysteine) gave the corresponding amino acids, which were identified chromatographically. In several instances, more than one amino acid resulted from hydrogenation of a single keto acid hydrazone. The present results emphasize the value of combining chromatographic and hydrogenation procedures. The information presented here should serve as a useful basis for the identification of a wide variety of α-keto acids.

ALTHOUGH considerable attention has been given to the chromatographic identification of amino acids, relatively little chromatographic information has been collected concerning their α-keto analogs. Several α-keto acids have been found in nature—e.g., pyruvic, glyoxylic, oxalacetic, α-ketoglutaric, α-ketoisovaleric, α-keto-γ-methyleneglutaric—and it is probable that application of more sensitive procedures will disclose the presence of others.

A few studies on the paper chromatographic behavior of free α-keto acids have appeared (*13, 27, 31*). However, because of the instability of many α-keto acids during isolation procedures, and the fact that relatively large quantities of the free acids are usually required for identification on the chromatograms, a number of investigators (*1, 4, 20, 28, 29*) have resorted to the use of the corresponding 2,4-dinitrophenylhydrazone derivatives. Although chromatography of the 2,4-dinitrophenylhydrazones of α-keto acids is often a valuable tool for the identification and even for the quantitative determination (*3, 5, 12, 23, 26*) o- α-keto acids, several difficulties exist. For example, the chrof matographic behavior of certain 2,4-dinitrophenylhydrazones is very similar; furthermore, under some circumstances an α-keto acid 2,4-dinitrophenylhydrazone may give rise to two spots on one-dimensional paper chromatograms (and often to four spots on two-dimensional chromatograms). This is probably due to the presence of the *syn*- and *anti*-hydrazones (*10, 20, 23*). Kulonen (*11*) used a procedure for the hydrogenation of the hydrazones using aluminum amalgam, followed by paper chromatography of the resulting amino acids. Towers, Thompson, and Steward (*25*) independently developed a similar method based on catalytic hydrogenation with platinum oxide. These techniques have proved of value in the identification of several α-keto acids present in blood, urine (*11*), and certain plant tissues (*25*).

Studies in this laboratory on transamination and related problems have necessitated the use of a large number of α-keto acids, which have been prepared by synthetic organic techniques and by enzymatic oxidative deamination of the corresponding amino acid isomers (*15, 18*). In the course of these investigations the authors have had occasion to use paper chromatography for the identification of α-keto acid hydrazones, and to carry out hydrogenation of these derivatives. This paper reports paper chromatographic studies of the 2,4-dinitrophenylhydrazones of 35 α-keto acids, and of the products of hydrogenation of these compounds. This is a considerably larger series of α-keto acids than has previously been available for such study, and includes the α-keto analogs of most of the common naturally occurring amino acids. It may therefore be expected that the data presented here will be useful to those concerned with the identification of α-keto acids.

METHODS

References to the methods of preparation of the α-keto acids, and the solvents used for crystallization of the 2,4-dinitrophenyl-

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hydrazones, are given in Table I. Ascending paper chromatography was carried out on Whatman No. 4 paper, using the following solvents (*2*): (*a*) 77% ethyl alcohol; (*b*) liquefied phenol saturated with 10% aqueous sodium carbonate; (*c*) 1-propanol, ammonium hydroxide (28%), water (60:30:10); (*d*) 1-butanol, water, ethanol (50:40:10); and (*e*) methanol, benzene, 1-butanol, water (40:20:20:20).

A number of variations of these solvents were tried with no greater success than indicated in Table I. Although it cannot be maintained that the authors have found the best possible solvents, the following mixtures were found to be of relatively little value in the separation of these keto acid hydrazones: (*f*) *tert*-amyl alcohol saturated with 0.1*M* potassium phthalate buffer (pH 6.0); (*g*) lutidine, ethanol, water, diethylamine (55:20:25:1); (*h*) 1-butanol, glacial acetic acid, water (2:1:1); (*i*) phenol, 1-butanol, glacial acetic acid, water (25:12.5:50:25); (*j*) caprylic alcohol, ethanol, water, benzene (4:2:2:2); (*k*) methyl ethyl ketone, *tert*-butyl alcohol, formic acid, water (16:16:0.1:3.9); (*l*) diethyl ether, glacial acetic acid, water (50:40:10); (*m*) diethyl ether, ethyl alcohol (1:1).

The hydrazones were dissolved in ethyl acetate, ethanol, or glacial acetic acid and applied to the paper in quantities of 0.1 to 5 γ. Inspection of the chromatograms under ultraviolet light (a Hanovia Inspectolite lamp, Hanovia Chemical and Manufacturing Co., catalog No. SC-5041, with an EH-4 arc tube and filter was used) was found to be useful in detecting very small quantities (0.01 to 0.1 γ) of the hydrazones, not ordinarily visualized in white light. Where subsequent elution and hydrogenation were carried out, larger quantities of the hydrazones were applied to the paper as a series of spots.

Hydrogenation was carried out on pure samples of the 2,4-dinitrophenylhydrazones and on hydrazones eluted from specific spots on the paper chromatograms. In general, quantities of 40 to 200 γ were eluted with ethyl acetate or 10% sodium carbonate. When the latter eluting agent was used, the solution was acidified and the hydrazone extracted into ethyl acetate. The hydrazone solution was evaporated to dryness in a 12-ml. conical tube, to which 1 ml. of water and 2 mg. of platinum oxide catalyst (American Platinum Works, Newark, N. J.) were added. Several such tubes were usually placed together in a 250-ml. pressure-resistant bottle padded with cotton, and the bottle was attached to a Parr hydrogenation apparatus. After hydrogenation for 16 hours at 24° and 40 pounds per square inch, the catalyst was allowed to settle, and the clear solution was applied to paper chromatograms. Paper chromatography was carried out using the appropriate internal standards; the amino

Table I. Application of Chromatographic and Hydrogenation Procedures to α-Keto Acid 2,4-Dinitrophenylhydrazones

α-Keto Acid 2,4-Dinitrophenylhydrazone	Preparation References	Crystallization: Melting point, °C.	Crystallization: Solvent	$R_F \times 100$[a]: Solvent (*a*)	Solvent (*b*)	Solvent (*c*)	Solvent (*d*)	Solvent (*e*)	Amino Acids after Hydrogenation
Pyruvic	(*22*)	216	Water	54–63	68–74	71–78; 88–91	48–53	73–77; 79–83	Alanine
α-Ketoadipic	(*7*)	208	Water	53–58	53–60	45–51; 55–61	46–51	67–71	α-Aminoadipic acid
α-Ketobutyric	(*15*)	198	Water	72–77	61–66	84–86; 88–92	58–68	77–82	α-Aminobutyric acid
α-Ketoheptylic	(*15*)	130	Ethyl acetate, ligroin	77–86	75–88	89–94	77–86; 92–98	79–90; 98–100	α-Aminoheptylic acid
α-Keto-ε-hydroxycaproic	(*15*)	183	Water	69–73	63–69	84–87	52–59	76–79	α-Amino-ε-hydroxycaproic acid
α-Ketomalonic[b]		205	HCl	50–59	50–61	49–57	42–50	49–54; 58–63	α-Aminomalonic acid[c] glycine[c]
α-Ketophenylacetic	(*15*)	193	Water	67–75	70–76	85–91	58–68	77–82	α-Aminophenylacetic acid[c], cyclohexylglycine[c]
α-Keto-δ-guanidinovaleric	(*17*)	[d]		Streak	Streak	Streak	Streak	Streak	Arginine
α-Ketosuccinamic	(*16*)	183	Ethyl acetate	40–47	55–62	69–73	26–29; 32–36	58–66; 66–70	Asparagine[c], aspartic acid[c], alanine[c]
Oxalacetic	(*9*)	218	Water	42–51	22–34	55–72	26–40; 44–50	59–66; 74–77	Aspartic acid[c], alanine[c], β-alanine[c]
α-Keto-δ-carbamidovaleric	(*15*)	190	Water	52–61	60–72	71–76	25–28	70–74	Citrulline
β-Cyclohexylpyruvic	(*15*)	189	Water	76–83	75–84	87–91	87–94	79–87	β-Cyclohexylalanine
β-Sulfopyruvic	(*19*)	210	Alcohol	35–41	13–19	49–53	17–19; 20–23	58–65; 79–87	Cysteic acid, alanine[c]
β-Mercaptopyruvic	(*21*)	161–2	Water	64–68	60–63	79–83	42–51	74–79	Alanine
α-Keto-γ-ethiolbutyric	(*15*)	131	Water	70–76	73–82	83–90	64–73	77–82	Ethionine
α-Ketoglutaric[f]		220	Water	54–62	48–52	55–59	30–35	58–66	Glutamic acid
α-Ketoglutaramic	(*16*)	195[g]		44–53	23–30	70–75	29–32	58–63	Glutamine[c], glutamic acid[c]
Glyoxylic	(*30*)	203	Water	45–55	65–76	70–75	31–47	69–73; 76–78	Glycine
β-Imidazolylpyruvic	(*15*)	240	Ethyl acetate, ligroin	Streak	Streak	79–85	Streak	Streak	Histidine
d-α-Keto-β-methylvaleric[h]	(*14*)	176	Water	81–85	63–72	92–96	72–81	82–89	Isoleucine
α-Ketoisocaproic	(*15*)	162	Water	76–79; 81–85	63–71	93–97	79–86	84–92	Leucine
Trimethylpyruvic	(*8*)	180	Water	80–87	72–79	90–95	76–85	86–92	*tert*-Leucine
α-Keto-ε-aminocaproic	(*17*)	212	Water	71–76	75–85	79–85	67–75	91–98	Lysine[c], pipecolic acid[c]
α-Keto-γ-methiolbutyric	(*15*)	150	Water	66–79	72–79	85–88; 90–93	40–45	79–85	Methionine
α-Keto-γ-methylsulfonylbutyric	(*15*)	175	Water	52–61	65–72	74–76	30–33; 37–42	71–75	Methionine sulfone
α-Keto-δ-nitroguanidinovaleric	(*17*)	225	Glacial acetic acid	44–55	58–67	77–83	40–45	73–78	Nitroarginine[c], arginine[c]
α-Ketocaproic	(*15*)	153	Water	75–81	66–73	85–92	85–94	82–91	Norleucine
α-Ketovaleric	(*15*)	167	Water	75–81	66–72	88–93	76–85	82–87	Norvaline
α-Keto-δ-aminovaleric	(*17*)	211–2	Water	43–55	76–86	91–95	51–58	Streak	Ornithine[c], proline[c], pentahomoserine[c]
Phenylpyruvic	(*15*)	162–4; 192–4[i]		68–76; 79–83	73–83	83–89	82–93	81–91	Phenylalanine
β-Hydroxypyruvic	(*24*)	162	Ethyl acetate	56–63; 70–73	48–62	70–76; 81–84	48–53; 61–65	Streak	Serine[c], alanine[c]
α-Keto-β-hydroxybutyric	(*24*)	157–8	Ethyl acetate	63–72	55–69	73–78	52–61	77–82	Threonine[c], α-aminobutyric acid[c]
β-Indolylpyruvic	(*15*)	169	Water	64–73	71–81	80–87	82–93	79–86	Tryptophan
p-Hydroxyphenylpyruvic	(*15*)	178	Water	66–70; 72–77	58–65	84–90	71–79	79–86	Tyrosine
α-Ketoisovaleric	(*15*)	196	Water	78–85	65–70	88–92	77–82	82–90	Valine

[a] Values given in table describe ranges of spots, and represent average values of three to five determinations; composition of solvents is given under Methods.
[b] Prepared by saponification of freshly distilled ethyl oxomalonate (obtained from Cohelfred Laboratories, Chicago, Ill.).
[c] Amino acids formed in amounts of approximately same order of magnitude.
[d] Darkened at 218°, but did not melt when heated to 250°; product washed with water.
[e] Formed in trace amounts.
[f] Obtained from Nutritional Biochemicals Corp.
[g] Contained about 5% of 2,4-dinitrophenylhydrazone of α-ketoglutaric acid.
[h] *l*-isomer, *d*-isomer, and racemic form exhibited identical behavior.
[i] Crystallization from water or alcohol gives m.p. of 192–7°; crystallization from ethyl acetate and petroleum ether gives m.p. of 162–4°. The two forms are interconvertible (*6*); their chromatographic behavior was identical.

acid spots were rendered visible by dipping the dried chromatograms into a solution of 0.25% ninhydrin in acetone (*2*).

RESULTS

The solvents employed effected separation of a number of the hydrazones (Table I). Thus, separation of the members of the homologous series from glyoxylic to α-ketoheptylic acids was achieved, and the hydrazones of phenylpyruvic and *p*-hydroxyphenylpyruvic acids were distinguished from those of the aliphatic α-keto acids. However, the degree of separation of the branched chain α-keto acids from each other and from the isomeric normal α-keto acids was not great. On the other hand, hydrazones of the dicarboxylic acids in general moved more slowly than did those of the monocarboxylic acids. The dicarboxylic keto acid hydrazones were readily separated from each other and the corresponding ω-amides. The hydrazones of β-cyclohexylpyruvic and phenylpyruvic acids did not exhibit significantly different values. The hydrazones of β-hydroxypyruvic and β-hydroxy-α-ketobutyric acids gave appreciably different values from those of the corresponding unsubstituted α-keto acids. The hydrazones of the α-keto analogs of ornithine and lysine were separated from each other as were those of methionine, ethionine, and methionine sulfone. Chromatography of the hydrazones of the α-keto analogs of histidine and arginine was, in general, unsatisfactory owing to the low solubility of these derivatives.

In most instances double spots were not observed. Double spots were frequently formed when the hydrazone solutions were permitted to stand for some time prior to chromatography, and when the hydrazones were dissolved in carbonate, acidified, and then extracted with ethyl acetate. When such double spots were separately eluted and hydrogenated, both spots yielded the same amino acid. This result is consistent with the concept that these spots represent different forms of the same α-keto acid hydrazone [see (*10, 20, 23*)]. The occurrence of double spots obviously places a distinct limitation on the value of chromatography of the hydrazones, although where two spots were observed, they were often relatively close together.

Hydrogenation of the hydrazones resulted in the formation of the analogous α-amino acid in all but one case. Alanine was the only amino acid product of the hydrogenation of β-mercaptopyruvic acid 2,4-dinitrophenylhydrazone. Some alanine (as well as the corresponding amino acids) was also formed from the hydrazones of oxalacetic, cysteic, and α-ketosuccinamic acids. Alanine and α-aminobutyric acids were found, respectively, after hydrogenation of the hydrazones of β-hydroxypyruvic and α-keto-β-hydroxybutyric acids; neither serine nor threonine was reduced under the conditions of the hydrogenation procedure. Hydrogenation of the hydrazones of the α-keto analogs of glutamine and asparagine resulted in the formation of the corresponding dicarboxylic amino acid, indicating that some deamidation had occurred. Partial reduction of nitroarginine and of α-aminophenylacetic acid was also observed. Both lysine and pipecolic acid were formed by hydrogenation of the hydrazone of α-keto-ε-aminocaproic acid; proline and ornithine were formed from α-keto-δ-aminovaleric acid hydrazone. These α-keto acids have been found to exist in equilibrium between open-chain and cyclic forms (*17*). Hydrogenation of the hydrazone of α-ketomalonic acid gave α-aminomalonic acid and glycine; the formation of glycine is consistent with the tendency of α-aminomalonic acid to undergo decarboxylation in aqueous solution at room temperature. Under the conditions employed, hydrogenation of α-ketoglutaric acid 2,4-dinitrophenylhydrazone gave only glutamic acid; however, when very large amounts of the hydrazone were hydrogenated, a very small quantity of γ-aminobutyric acid was found. Towers and others (*25*) have reported formation of γ-aminobutyric acid and β-alanine after hydrogenation of the hydrazones of α-ketoglutaric acid and oxalacetic acids, respectively. It is to be expected that some variation may occur in the amounts of certain amino acids formed depending on the conditions of hydrogenation.

DISCUSSION

It is apparent from the R_f values given in Table I, that paper chromatography alone is of somewhat limited value for the identification of α-keto acid 2,4-dinitrophenylhydrazones, although it is very useful in excluding the presence of certain α-keto acid hydrazones. The hydrogenation procedure appears to be of considerable value in identifying the α-keto acid analogs of the known amino acids, since paper chromatography of amino acids has become a highly developed and accurate procedure (*2*). Hydrogenation of the hydrazones of the α-keto analogs of all the amino acids studied (except cysteine) gave the corresponding amino acids. Although several hydrazones gave more than one amino acid, this complication, if recognized, would not be expected to provide undue difficulty. The present results emphasize the value of combining chromatography of the hydrazones with the hydrogenation procedure. Thus, after chromatography and tentative identification of the spots, the hydrazones may be separately eluted, hydrogenated, and the resulting amino acids chromatographed. The data given in Table I suggest that these procedures provide information useful for the identification of a wide variety of α-keto acids.

ACKNOWLEDGMENT

The authors thank Herbert A. Sober for his generous advice and valuable criticisms of this manuscript.

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RECEIVED for review June 16, 1955. Accepted November 2, 1955.

Photometric Determination of Copper in Aluminum and Lead-Tin Solder with Neocuproine

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Methods are described for the use of neocuproine (2,9-dimethyl-1,10-phenanthroline) in determining copper in aluminum and lead-tin solders. The methods are rapid and require no chemical separations except for the extraction of the copper complex, and the results compare favorably with other, more tedious, methods for determining copper. Although the procedure for determining copper in aluminum has been particularly useful for the analysis of high-purity aluminum (99.6 to 99.99% aluminum), it has been applied to high-silicon aluminum. The method is sensitive and accurate in the range of 0.002 to 1.00% of copper in aluminum. The procedure for determining copper in lead-tin solder has been applied in the range of 0.004 to 0.4% of copper. The copper-neocuproine complex has its absorbancy maximum at 454 mμ. Conformity to Beer's law is observed for both determinations. A calibration curve prepared with pure copper may be used for the calculations.

THE specificity of 2,9-dimethyl-1,10-phenanthroline (neocuproine) for cuprous copper has been described by Smith and McCurdy (*3*). Luke and Campbell (*2*) have applied the colored system to the determination of copper in germanium and reported no interference from 56 metals. Gahler (*1*) used the reagent for determining copper in iron and steels and reported on the effect of pH and some anions on the extraction of the colored complex.

The use of the reagent has been extended in this laboratory to the determination of copper in aluminum and lead-tin solder. Gahler's procedure for extracting the complex with an ethyl alcohol–chloroform mixture was followed.

APPARATUS AND REAGENTS

Most of the measurements of the absorbancies were made with a Beckman spectrophotometer, Model DU. The Klett-Summerson photometer was also found suitable for the work, though the sensitivity was not as good.

An ethyl alcohol–chloroform mixture (3 to 2) was used as a reference in 10-mm. cells, at 454 mμ in the Beckman spectrophotometer and with an S44 filter for the Klett-Summerson photometer. This mixture has the same proportions as the combined extracts and rinsings diluted to 50 ml.

All reagents were of A.C.S. specification grade if available.

Standard copper solution, 1.0001 grams of 99.999% pure copper dissolved in 15 ml. of nitric acid (1 to 3). The solution was boiled to eliminate oxides of nitrogen, cooled, and diluted to 1000 ml. with distilled water. A 100-ml. aliquot was diluted to 1000 ml. with distilled water to obtain 0.1 mg. of copper per ml. One hundred milliliters of this solution was diluted to 1000 ml. to obtain a solution containing 0.01 mg. of copper per ml.

Hydroxylamine hydrochloride solution, 10 grams of $NH_2OH.HCl$ dissolved in 100 ml. of distilled water. The solution was transferred to a separatory funnel and 10 ml. of neocuproine solution were added. It was extracted with chloroform until a colorless extract was obtained.

Neocuproine solution, 0.100 gram of 2,9-dimethyl-1,10-phenanthroline (neocuproine) dissolved in 100 ml. of ethyl alcohol. This solution is stable under ordinary storage conditions.

Sodium citrate solution, 75 grams of $Na_3C_6H_5O_7.2H_2O$ dissolved in 250 ml. of distilled water. This solution was scavenged, after treating with copper-free hydroxylamine hydrochloride solution, by neocuproine solution and subsequent extraction with chloroform.

Hydrobromic acid–bromine mixture, 20 ml. of bromine added to 180 ml. of hydrobromic acid, 48%.

CALIBRATION CURVE

A calibration curve was prepared by carrying through the procedure on samples containing varying amounts of the standard copper solution. The absorbancies, corrected for a blank of reagents, were plotted against the concentration of copper in milligrams per milliliter. The system obeys Beer's law between 0.001 and 0.004 mg. of copper per ml. when measured in a 10-mm. light path cell.

PROCEDURE FOR LEAD-TIN SOLDERS

Dissolve 0.500 gram of solder in 25 ml. of 48% hydrobromic acid. When the reaction ceases, add dropwise just enough hydrobromic acid–bromine mixture to clear the solution. Cool and dilute to 100 ml. in a volumetric flask. Transfer an aliquot containing 0.04 to 0.20 mg. of copper to a separatory funnel. Dilute to 50 ml. with water. Add 5 ml. of hydroxylamine hydrochloride solution (10%) and 10 ml. of sodium citrate solution. Adjust the pH of the solution to between 4 and 6 and add 10 ml. of neocuproine solution. Shake to mix thoroughly and extract by shaking with 10 ml. of chloroform for 30 seconds. Transfer the chloroform layer to a 50-ml. volumetric flask. Rinse the stem of the funnel with two 1-ml. portions of chloroform. Repeat the extraction with 6 ml. of chloroform and rinse the stem as before. Dilute to volume with ethyl alcohol and determine the absorbancy at 454 mμ. Make a correction for the absorbancy of a blank of the reagents using an ethyl alcohol–chloroform mixture (3 to 2) as a reference at 100% transmittance.

PROCEDURE FOR ALUMINUM

Transfer 0.500 gram of aluminum to a 150-ml. glass beaker and dissolve in 5 ml. of hydrochloric acid (1 to 1). Rinse the sides of the beaker with just enough concentrated nitric acid to aid solution and evaporate almost to dryness. Again rinse the sides of the beaker with a minimum of nitric acid, add 2 ml. of hydrofluoric acid, and heat until any silicon present dissolves and the solution is clear. Dilute to 25 ml. If necessary, filter through a medium fine paper collecting the filtrate in a 100-ml. flask. Wash any residue and paper thoroughly with hot water and hot hydrochloric acid (1 to 20). Make up to volume.

Transfer an aliquot containing 0.04 to 0.2 mg. of copper to a separatory funnel and extract the copper according to the procedure for lead-tin solders.

RESULTS

The results for the determination of copper in lead-tin solder are shown in Table I. Synthetic samples were prepared by adding known amounts of copper to NBS standards 127 and 53C. The provisional certificate value of NBS 127 is 0.014% copper. The average value of six results for this standard is 0.012 and none of the results were above 0.014%.

Table I. Determination of Copper in Lead-Tin Solders

Total Copper, Mg.	No. of Detns.	Av. Copper Found, Mg.	Range, Mg. Cu	Standard Deviation, Mg. Cu
0.07	5	0.057	0.013	0.006
0.57	3	0.585	0.094	0.051
1.07	5	1.075	0.078	0.037
2.07	5	2.076	0.016	0.006

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The results for the determination of copper in aluminum are shown in Table II. Two 1.000-gram samples each of the German standard and British chemical standard 198a were dissolved and extracted directly. For the BCS 198a, all results were higher than the certificate value 0.003.

The procedure for extracting the copper(I)–neocuproine complex is the same for solders and aluminum alloys. The procedures for dissolving the samples and keeping salts in solution prior to the extraction are specific for different alloys.

The method has been tested in the range 0.0017 to 0.3% for copper in aluminum standard sampled and 0.004 to 0.4% copper in solder standard samples.

A high concentration of aluminum was found to prevent the complete extraction of copper by neocuproine. The addition of hydrofluoric acid remedied this interference. When sodium fluoride was added instead of hydrofluoric acid, extraction of the copper complex was not complete.

DISCUSSION

The copper(I)–neocuproine complex is stable in several common organic solvents. Hexanol, isoamyl alcohol, and an ethyl alcohol–chloroform mixture have been used to extract the complex from an aqueous solution. Since the neocuproine is soluble in ethyl alcohol, the ethyl alcohol–chloroform extraction is most convenient. A minimum of 2 ml. of ethyl alcohol per 25 ml. of chloroform is required.

The copper must be in the cuprous state; hence, any excess of oxidizing agents is to be avoided. The hydrobromic acid–bromine mixture (9 to 1) is added dropwise until the solder is just dissolved. No attempt should be made to remove excess bromine unless sufficient additional hydrobromic acid is added to keep the solder in solution when diluted to 100 ml. About 25 ml. of hydrobromic acid (48%) are necessary to keep 0.5 gram of NBS sample 127 in solution.

Considerable trouble was encountered in keeping solder samples in solution during the copper neocuproine extraction. A saturated ammonium acetate solution buffered at pH 5 with acetic acid kept the solder in solution but was miscible with the chloroform, so that the extraction was not complete. β,β'-dichloroethyl ether showed promise of extracting the copper(I)–neocuproine complex from the ammonium acetate–acetic acid solution, but the extracts did not follow Beer's law above 0.003 mg. of copper per ml.

High-silicon aluminum alloys were dissolved with the aid of hydrofluoric acid. When NBS 87 was dissolved, there remained a siliceous residue which was filtered off and washed with hot water and hot hydrochloric acid (1 to 20). This silica was volatilized with hydrofluoric acid and the residue was fused and examined for copper. No copper was found in the silica residue and excellent copper recovery was made on the samples.

High purity aluminum dissolves very slowly in hydrochloric acid. The addition of copper ion in the preparation of the calibration curve noticeably hastened solution of the aluminum. Ferric ion produced the same desired effect; hence, for subsequent samples of pure aluminum 1 ml. of ferric sulfate solution was used to aid solution. The aluminum dissolved readily but reduced the iron which precipitated as a gray powder and was dissolved with a few drops of nitric acid. The nitric acid is kept to a minimum to prevent oxidation of copper.

The ethyl alcohol–chloroform solution (3 to 2) used as a reference at 100% transmittance has the same proportions as the combined extracts and rinsings diluted to 50 ml.

Table II. Determination of Copper in Aluminum

Sample	Recommended Value % Cu	No. of Detns.	Av. % Cu	Range, % Cu	Standard Deviation, % Cu
NBS 87 High-silicon aluminum	0.30	5	0.297	0.010	0.004
BCS 198a Super pure aluminum	0.003	5	0.0047	0.0005	0.0003
Reinaluminum German standard	0.0017	5	0.0017	0.0007	0.0002
Alcoa SA-1	0.008	5	0.0083	0.0007	0.0003

ACKNOWLEDGMENT

The authors are indebted to the Research Laboratory of the General Electric Co., Schenectady, N. Y., for the preparation of the reagent neocuproine for preliminary work before the material was available commercially.

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RECEIVED for review August 30, 1955. Accepted October 25, 1955.

Instrumentation and Principles of Flame Spectrometry

Multichannel Flame Spectrometer

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An instrument for the simultaneous photoelectric determination of several elements, using a flame source, is described. The instrument consists of an oxygen-hydrogen burner, a grating monochromator, photomultiplier detectors, and associated power supplies, amplifiers, and meters. Five elements—sodium, potassium, magnesium, calcium, and strontium—are determined simultaneously. The instrument detects as little as 0.002 γ of sodium in 1 ml. of water. The calibration curves cover a 1000-fold range of concentrations for each element. The precision of the instrument is within 1 to 2%.

ALTHOUGH photocells were used for the measurement of light emitted in the flame as early as 1934 (*7*) and 1937 (*11*), flame photometry was not widely accepted as an analytical technique until a practical instrument was introduced in 1945 by Barnes and others (*1*). Because of its speed, sensitivity, and precision, the method proved to be useful, particularly for the alkali metals, which are difficult to determine by gravimetric or volumetric techniques.

The flame is a comparatively stable source. A properly designed atomizer introduces the sample at a controlled, constant, and reproducible rate. If the burner is well constructed, the flame is steady, and the light emitted in the flame is constant

with time. For many elements, the intensity of radiation emitted in the flame is high. For these reasons, complex integrating circuits are not required with the flame source. In addition, spectra emitted in the flame are simpler than spectra from the arc or spark. It is therefore relatively easy to isolate the emission lines of the element to be determined from other radiation. However, improved resolution will increase the line to background ratio, improving the precision of analysis and decreasing interference from other radiating species in the flame (*8*).

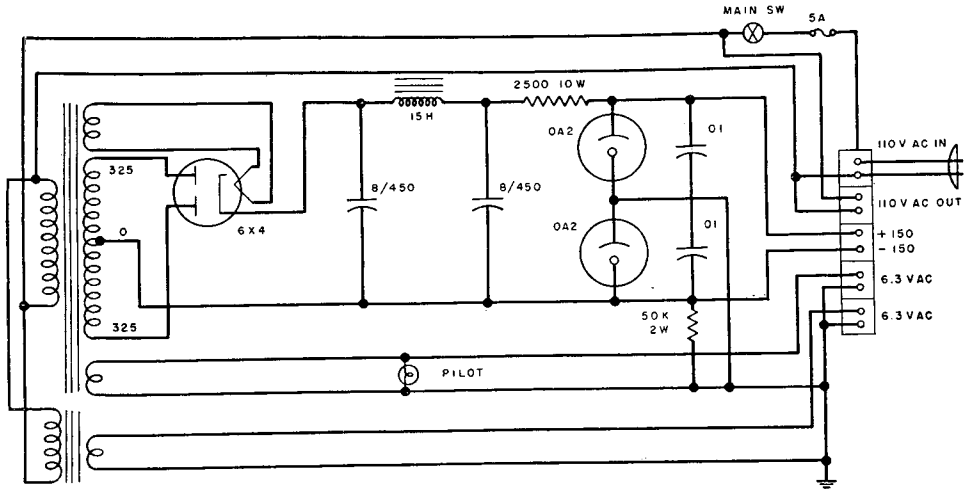

Figure 1. Low voltage power supply for multichannel flame spectrometer

The instruments at present available for flame analysis are commonly and accurately called "flame photometers." These instruments use a dispersing element or filter as a monochromator to isolate one region of the spectrum for measurement. The determination of each radiating species in a solution requires adjustment of the monochromator to isolate a different wavelength region, and, essentially, a separate sample.

Work in this laboratory has been directed toward the development of an instrument for the simultaneous determination of several elements at several wave lengths, using a flame source. Rather than using the dispersing element as a monochromator to isolate one wave-length region at a time, the authors have used a number of exit slits at the focal plane of a spectrograph to isolate several wave lengths of light simultaneously. As the instrument thus takes advantage of the spectral distribution of the dispersing element, in this case the grating, the authors have chosen to call it a "multichannel flame spectrometer."

The instrument described here was designed to have the following qualities: simultaneous detection of radiation emitted by different species, high dispersion and resolution to minimize interference, high sensitivity in the detection of radiation, and simplicity and ease of operation and maintenance.

An oxygen-hydrogen flame is used as the source. A grating spectrograph provides high dispersion and resolution. Five elements—sodium, potassium, calcium, magnesium, and strontium—are determined simultaneously. Photomultiplier detectors, chosen because of their marked response to low light levels, provide for sensitivities—in terms of limits of detection—to as low as 2 parts of sodium per billion parts of water. Integrating circuits are not required—the sensitivity being adequate without them—with a resultant simplification of the electronics. This instrument is therefore more nearly "direct reading" than others so designated (*3*, *4*, *6*, *10*), as the elapse of time between the

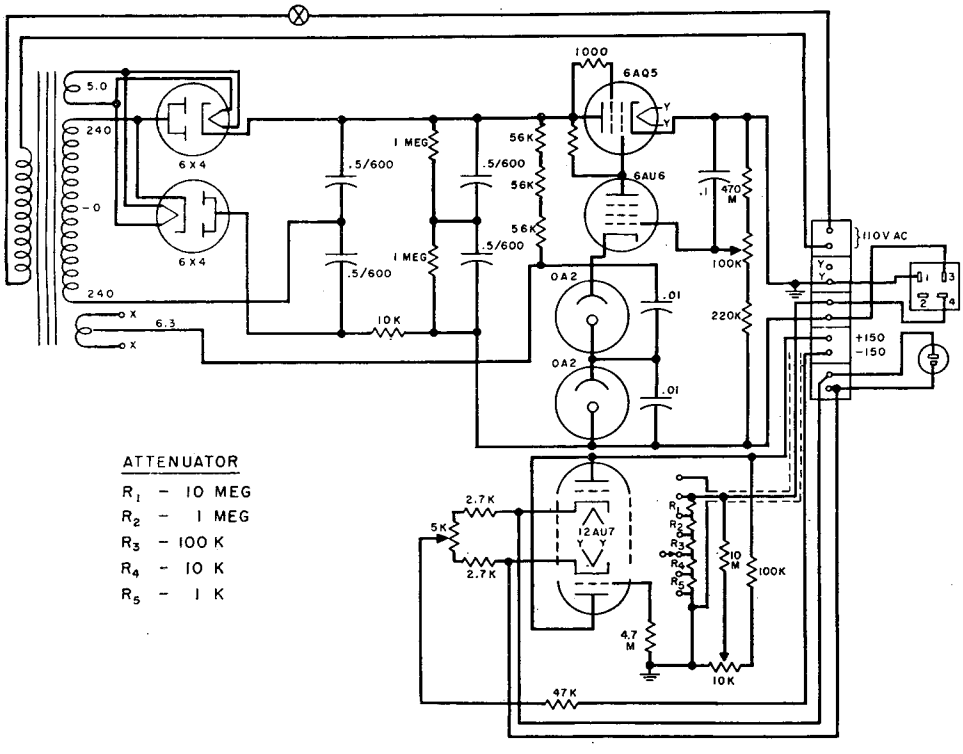

Figure 2. High voltage power supply and amplifier for multichannel flame spectrometer

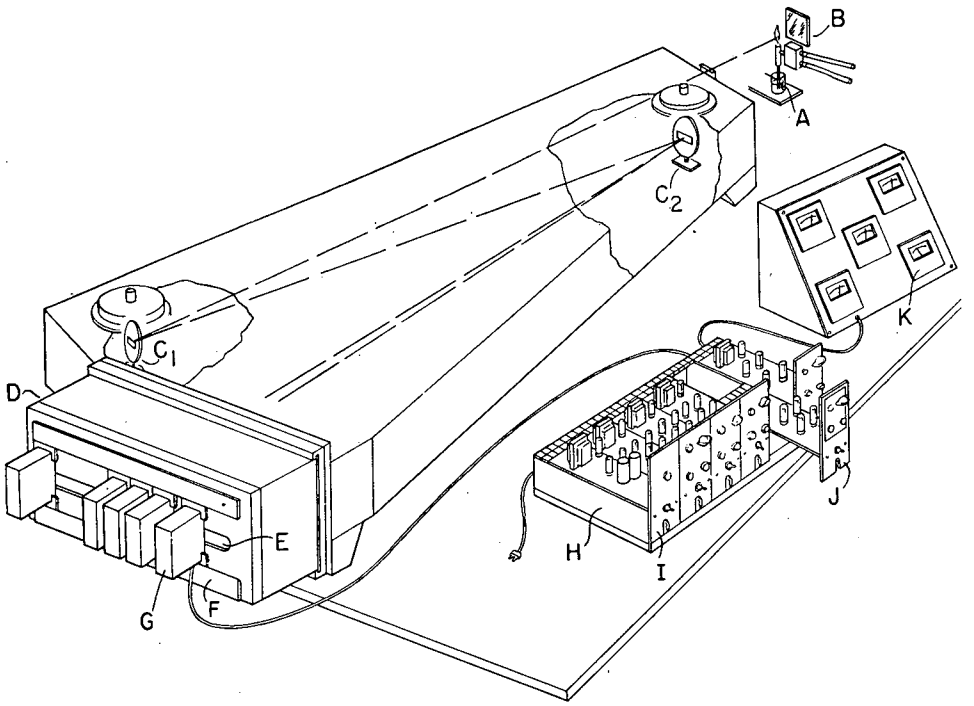

Figure 3. Multichannel flame spectrometer

A. Beckman atomizer burner
B. Plane front-surface mirror
C_1. Collimating mirror
C_2. Grating
D. Detector mount
E. Aperture in detector mount
F. Rails
G. Detector chassis
H. Bank of amplifiers
I. Low voltage power supply
J. High voltage power supplies and amplifiers
K. Microammeters

emission of light and its observation is minimized. The amplifiers are of the "plug-in" type to permit rapid interchange when repairs are needed. Internal standardization is not employed; adequate precision is obtained without it.

DESCRIPTION OF THE INSTRUMENT

Source. A Beckman atomizer burner (No. 4020, Beckman Instrument Co., Pasadena, Calif.) is used as the source. The fuel is commercial hydrogen at a pressure of 10 inches of water, in conjunction with oxygen at the pressure recommended by the manufacturer for the particular burner (usually 10 to 20 lb. per square inch). The gas pressures are regulated by a Beckman flame spectrophotometer attachment (No. 10300, Beckman Instrument Co.). The flow rate of the solution through the burner is approximately 1.5 ml. per minute. The burner is placed 6 inches from the entrance slit of the spectrograph. The height of the flame is adjusted to maximal meter deflections. Lenses are not used to focus light from the burner on the entrance slit or collimating mirror because of the comparatively large size of the source. A plane front surface mirror 3 inches behind the burner increases the amount of light from the burner entering the spectrograph and also prevents light from any object behind the burner from reaching the entrance slit.

Dispersing Element. A Jarrell-Ash 1.5-meter Wadsworth mount grating spectrograph (Jarrell-Ash Co., Newtonville, Mass.), with a 15,000-line per inch grating providing a reciprocal dispersion of 10.8 A. per mm. in the first order, was adapted to serve as the dispersing element. The effective grating aperture is 3.5 × 1.5 inches. The entrance slit width is 0.4 mm. With an exit slit of the same width, the resolution of the instrument is 8.6 A.

Detectors. 1P28 photomultiplier tubes (Radio Corp. of America, Harrison, N. J.) are used as the detectors for the emission of sodium (5890 A.), calcium (5498 A.), strontium (4607 A.), and magnesium (3705 A.), while a 1P22 photomultiplier tube (Radio Corp. of America) is employed for the potassium line (7664 A.). The dynode voltages are the same for all stages, and may be varied from 70 to 90 volts per stage.

The camera of the original spectrograph is replaced by facilities for the mounting of photomultiplier tubes. Two beveled rails are mounted on a box 21.5 × 5.5 × 9 inches at the focal plane. Small radio chassis, 2 × 4 × 4 inches, are mounted on these rails. Each chassis is provided with rails complementary to those of the holder, so that it may be readily moved to receive any part of the spectrum. The light reaches the photocell through a small oblong slot, 1 inch high by 0.5 inch wide, in each chassis, facing the grating. Razor blades taped across these chassis entrance holes form exit slits which are 0.4 mm. wide, except in the case of the photocell for sodium, where the extremely high intensity of the radiation necessitates the use of a narrower slit (0.2 mm.), in order to avoid saturation of the photocell.

The space between the radio chassis is covered with heavy black felt. The photocell mount is thus made light-tight.

Amplifiers. The instrument provides a separate high voltage power supply and amplifier for each photomultiplier tube. These are arranged on a common chassis in a group of five, with one low voltage power supply serving all of the amplifiers. The individual amplifiers can be removed readily for servicing, as they are of the plug-in type.

The low voltage power supply (Figure 1) provides voltages of 6.3 and 110 volts alternating current and +150 and −150 volts regulated direct current. The direct current voltage is regulated by two OA2 tubes in a full-wave rectifier with a pi-section filter.

The high voltage power supply (Figure 2) provides a regulated voltage variable from 700 to 900 volts direct current for the photomultiplier tubes. This circuit consists of a full-wave doubler rectifier and an r-c filter, followed by a degenerate voltage regulator. Output voltage is controlled by a 100,000-ohm variable resistor on the control grid of a 6AU6 tube.

The amplifier (Figure 2) is mounted on the same chassis as the high voltage power supply. The signal input is through an attenuator provided with precision resistors, so that the sensitiv-

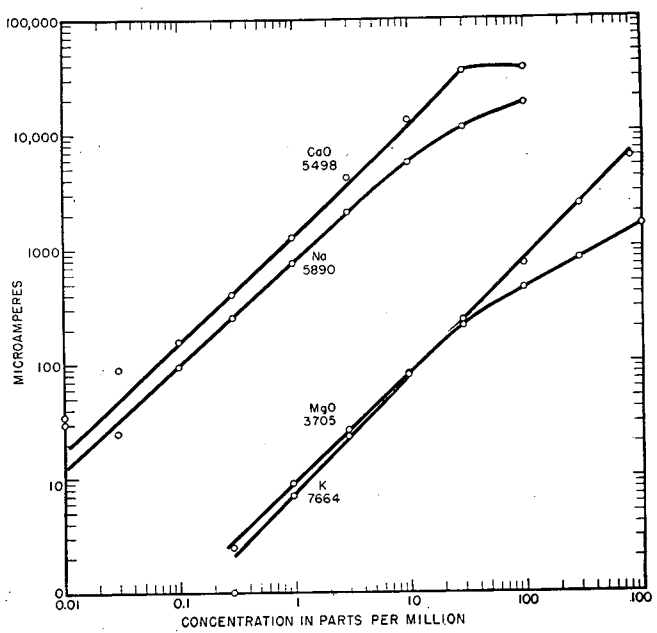

Figure 4. Typical calibration curves for multichannel flame spectrometer

Table I. Precision and Stability of Multichannel Flame Spectrometer

Solutions run 20 times on each date, at 2-minute intervals.
Solution 21–124a contained 3 p.p.m. of sodium, as chloride.
Solution 21–124b contained 3 p.p.m. of sodium, 3 p.p.m. of calcium, 10 p.p.m. of potassium as chlorides.

Solution	21–124a			21–124b					
Channel	Na			Na		Ca		K	
Date	12-2	12-3	12-6	12-3	12-6	12-3	12-6	12-3	12-6
Average meter reading, μa.	1021	998	1015	1044	1049	182.5	183.7	52.5	52.1
Standard deviation									
μa.	12	11	18	20	16	3.3	2.8	1.0	0.75
%	1.2	1.1	1.8	1.9	1.5	1.8	1.5	1.9	1.4

ity of the amplifier may be varied by a factor of 10 for each step of the attenuator switch. If the lowest sensitivity is 1, the next highest is 10, the next 100, and the highest sensitivity is 1000. This device effectively expands the scale of the microammeter used to record the output signal. Readings obtained at one setting of the sensitivity control can be converted to equivalent readings at another setting by multiplying or dividing by an appropriate power of 10. The attenuator circuit also includes a variable resistor which permits cancellation of the photomultiplier dark current or other undesired signal. After passing through the attenuator the signal is sent to one side of a 12AU7 tube in a bridge circuit, which also includes a variable resistor to permit setting of the amplifier balance.

Meters. The output signal of each amplifier is indicated on a direct current microammeter. Simpson Model 20 microammeters (Simpson Electric Co., Chicago, Ill.) with 4.5-inch scales are used, with a range of either 0 to 100 or 0 to 200 μa. These can be readily read to ±1 μa.

The appearance of the instrument is shown in Figure 3.

EXPERIMENTAL

The standards were solutions of sodium, potassium, calcium, magnesium, and strontium chlorides. Reagent grade chemicals were dried 18 hours or longer at 130° C., weighed out, and dissolved in water purified by a mixed bed ion exchange resin column. Solutions containing 1000 p.p.m. of the metal were prepared in this manner. The other standard solutions were prepared by dilution of these concentrated stock solutions. The concentrations used were 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, and 300 p.p.m. The solutions were stored in polyethylene bottles.

The instrument was usually allowed to warm up for at least 15 minutes before it was used. Although it can be used 2 or 3 minutes after being turned on, there is still some drift in the amplifier balance during this period. The flame does not have to be lit during this period.

The data were obtained by readings at the peak of each line or band being observed. With the sensitivity switch set to 0 (no input signal), the amplifier balance was adjusted so that there was not an output signal. Each amplifier was then set to a sensitivity of 1000 and, with water atomized into the flame, the dark current control was set to balance background from the flame and photomultiplier dark current. The settings were checked and reset if necessary when flushing the atomizer with water between samples. The samples or standard solutions were then flushed through the atomizer and the meter deflections were observed. The meter readings and amplifier sensitivity settings were recorded on each channel for each sample.

SENSITIVITY AND CONCENTRATION RANGE

Figure 4, a typical set of calibration curves for this instrument, shows the limits of detection and the range of concentrations which can be measured. Although the exact lower limits of sensitivity depend upon such factors as the particular burner used, gas pressures employed, slit widths, the quality of the grating, and the particular photomultipliers used (photomultiplier sensitivities are found to differ by a factor of three or more for different tubes) the lower limits of sensitivity for quantitative determination are normally 0.01 p.p.m. for sodium, 0.1 p.p.m. for calcium and strontium, 1 p.p.m. for potassium, and 3 p.p.m. for magnesium. These concentrations result in a signal of about 5 μa. Since a signal of 1 μa. can be observed, the limits of detection are lower by a factor of 5 than the limits for quantitative analysis. One milliliter of sample is adequate for the determination of five elements, and readings have been obtained with half this volume of solution, so that the concentrational sensitivity in parts per million is equivalent to the weight of metal, in micrograms, needed for the determination.

The concentration range covered by a single calibration curve is large, as shown in Figure 4. The highest concentration used is about 1000 times the lowest concentration. This is accomplished through the use of a stepwise sensitivity control with precision resistors. The sensitivity can thus be brought back to the same setting after being changed, and the readings made at different sensitivity settings can be plotted on the same calibration curve.

PRECISION AND STABILITY

The precision and stability of the instrument were determined by performing replicate determinations on the same solution over a period of 40 minutes. The procedure was repeated on three separate days with one solution and on two days with the other. Table I shows the results of the experiment. One of the solutions used (21–124a) contained 3 p.p.m. of sodium as the chloride; the other contained 3 p.p.m. of sodium, 3 p.p.m. of calcium, and 10 p.p.m. of potassium, all as chlorides. The solutions were analyzed 20 times at 2-minute intervals. The standard deviations were from 1 to 3 microammeters in all cases, or 1 to 2% of the average meter readings. (The readings on the sodium channel were made at a sensitivity of 100 and multiplied by 10 to convert them to a sensitivity of 1000. Thus a standard deviation given as 12 μa. in Table I is equivalent to a precision of 1.2 μa. in reading the meter.) There was no indication of a steady change of sensitivity during the experiment.

Table I also shows the stability of the instrument from day to day. The average ammeter readings for the same element in the same solution agree within 1% on different days, with the single exception of three sets of data for sodium in solution 21–124a, where the average meter reading on December 3 differed by 2% from the average readings of December 2 and 6. In other experiments the calibration curves were stable for periods of as long as 4 weeks, provided that changes were not made in the instrumental variables (burner position, slit width, gas pressures, dynode voltages, etc.). At other times larger differences have been observed for the same solution on successive days. It is possible that changes in room temperature affect the sensitivity of the instrument. The grating will expand and contract slightly with extreme changes in room temperature, resulting in changes of the spacing of the grooves and shifting the spectral lines at the focal curve.

At low ammeter readings the precision of the analyses depends chiefly on the precision with which the ammeters can be read. The error in reading the meters is about 1 μa., so that with a meter deflection of 10 μa. (corresponding, for example, to about 0.02 p.p.m. of sodium) the precision is ±10%. At higher concentrations, with higher ammeter readings, the precision

is increased, but other factors, such as detector and amplifier stabilities, become important.

DISCUSSION

Instruments have been developed previously for the direct, photoelectric measurement of light intensity at several wave lengths simultaneously (*3, 4, 6, 10*). These instruments, known as Quantometers or "direct readers," use the electric spark source and are capable of determining 20 or more elements in one sample. The intensity of light emitted by the spark is not constant from one moment to the next. The spark is, by nature, intermittent. In addition, when the material being analyzed is used as one of the electrodes, the spark alters the surface of the sample, changing the rate of introduction of the sample into the spark gap. The radiation intensity in the spark is also low. These factors necessitate the use of integrating circuits to achieve precision and sensitivity in direct reading spectrometry with a spark source. The use of such complex circuits makes the initial and maintenance costs of the instrument excessive for many applications. In addition, the sensitivities which can be achieved are poorer, at best, by several powers of 10 than can be achieved with the flame.

The multichannel flame spectrometer described here is at present being used for the simultaneous determination of five elements. Additional exit slits, photomultipliers, and amplifiers could increase the number of elements being determined. It is thus possible to measure several variables simultaneously in one sample. As it is not necessary to make a separate set of adjustments of the instrument for each element determined, the time required to analyze a sample for several elements is shortened considerably. Four or five elements may be determined in 25 or more solutions per hour. The volume of solution required for the determination of five elements is about 1 ml., and samples half this size have been handled. Most flame photometers require this volume of solution or more for each element determined.

The range of concentrations covered by this instrument on a single calibration curve is much greater than is usual for a flame photometer. This may be ascribed to a combination of several factors: (1) the wide range of intensity response of photomultiplier tubes; (2) use of a stepwise sensitivity control with precision resistors, so that it is possible to return the sensitivity to its original setting when it is changed and to compare readings on one sensitivity range with readings on another sensitivity range; and (3) the linearity of the amplifier. Changing the input signal does not change the amplification factor. The same input signal will be indicated as 150 μa. at a sensitivity of 1000 or 15 μa. at a sensitivity of 100.

Because of the wide concentration range covered, it is possible to perform analyses more quickly than with other flame instruments, particularly when a wide variety of samples is handled. When dealing with concentrated solutions, the correct dilution factor is found more readily than with instruments with narrower concentration ranges.

The precision of the instrument (1 to 2%) is about the same as for other instruments using the flame source. This includes all instrumental factors, but does not include variables in the sample (such as the effect of variable viscosity on the rate of atomization) and in sample preparation. Some of the variability in the readings is presumably due to noise generated in the flame or in the photomultipliers, or in both. Inclusion of a time constant of 1 or 2 seconds in the amplifier should reduce the effect of the noise considerably, increasing the precision of the instrument.

The long term (day to day) stability of the instrument appears to be exceptional, particularly for one employing direct current amplifiers. Some difficulty has been found in reproducing gas pressures each time the flame is turned on. This factor seems to be important in determining the stability of the calibration curves from day to day. However, with sufficient care in setting the gas pressures this source of instability can be minimized. The slight day to day variations in Table I include any changes in the flame conditions.

The stability of the calibration curves from day to day is particularly helpful when solutions are analyzed in small groups. Calibration for each group of samples is not necessary, resulting in a saving of time. In practice, the calibration curves may be quickly checked by running one standard solution containing each of the elements to be determined. If the readings are closely similar to those found when all of the standard solutions were run, the calibration curves may be used safely.

The very high analytical sensitivity possible with the instrument is an additional important advantage. The effect of such biological materials as urea and proteins on the readings obtained (*2, 5, 9*) may be minimized by diluting the sample. It is possible to determine sodium and potassium in a sample of normal urine diluted as much as 1000-fold. With such a high dilution factor, it is unlikely that the organic substances in the sample will have any measurable effect on the results.

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RECEIVED for review July 19, 1955. Accepted November 4, 1955. Presented in preliminary form at the Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., 1954. The instrumental design work was supported by grants-in-aid from the Kettering Foundation, Dayton, Ohio. The analytical work was supported by a contract between the Office of Naval Research, Contract No. Nr5ori-07660, and Harvard University. Marvin Margoshes is a research fellow of the Charles F. Kettering Foundation, Dayton, Ohio.

Instrumentation and Principles of Flame Spectrometry

Effect of Extraneous Ions in Simultaneous Determination of Five Elements

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The effects of extraneous ions on flame spectrometric or photometric analyses have been studied with the multichannel flame spectrometer. Extraneous cations produce heterochromatic background radiation and have no effect on the monochromatic emission intensity measured above background. A method of background correction is described. The precision of the correction depends upon the line-to-background ratio. Certain anions are shown to reduce the emission intensity of the alkaline earths. These anions form compounds with the alkaline earths with high melting and boiling points; their crystals, formed in the spray, do not evaporate during their passage through the flame. Methods of obviating anion interference are discussed briefly.

A MULTICHANNEL flame spectrometer for the simultaneous determination of five or more elements in one sample has been described (*22*). In its present form, the instrument registers the radiation emitted by five elements at five wave lengths when a sample is atomized into the flame. The photomultiplier receivers are placed at the intensity maxima of lines or bands emitted in the flame by the elements to be determined. Flame photometers, by contrast, permit the determination of only one element at a time.

Table I. Volatilization of Calcium Salts in Different Flames as Shown by Measurement of CaO Emission

Compound	Melting Point, ° C.	Meter Reading, Microamperes		
		Cool Bunsen flame	Hot Bunsen flame	Meker flame
$CaCl_2$	772	2500	4000	30,000
CaF_2	1360	20	30–40	2,000
$CaCO_3$	d. 825[a]	10	30	500
$Ca_3(PO_4)_2$	1670	60	80	350
$Ca(OH)_2$	d. 580[a]	5	20	400
CaC_2O_4	d.[b]	5–10	10	150
$CaSO_4$	1450	0	5	200

[a] Decomposes with loss of CO_2 or water to form CaO, melting point 2572° C.

[b] Decomposes with loss of CO and CO_2 to form CaO.

When several elements are to be determined simultaneously the effect of each species on all others present becomes an important consideration. This is particularly important when some elements are present in much higher concentrations than others. The determination of calcium and magnesium in biological fluids (such as urine and serum) is a typical example, as sodium and potassium are present in concentrations many times those of the alkaline earths.

Several workers have reported that one cation may affect the emission of light by another. Sodium and potassium have been variously reported to increase (*23*) and decrease (*2*) their respective emission and that of calcium (*9*, *18*). Bills and others (*3*) found that excess sodium led to either high or low results for potassium, depending on the potassium concentration. Others could find no effect of sodium on potassium when an instrument of adequate resolving power was employed (*12*).

The multichannel flame spectrometer is particularly useful for the study of interactions among the various cations in the flame. Readings can be made at several wave lengths simultaneously, when solutions containing one or several cations are atomized into the flame. Information can thus be obtained rapidly on the effect of each cation on the readings obtained for others in the solution. The high resolution of the instrument minimizes the possibility of "interferences" arising from optical components of the instrument. Though similar experiments could be performed with other instruments, data would be obtained with much greater difficulty.

The accurate analysis of biological fluids and many other substances requires that the presence of extraneous anions be considered. Various anions markedly affect the emission of the alkaline earths in the flame. Sulfate (*1*, *10*), phosphate (*1*), and aluminate (*16*), when present in concentrations of the same order of magnitude as those of the alkaline earths, cause a considerable and striking reduction of the intensity of the emission of magnesium, calcium, and strontium.

The effects of extraneous cations and anions on the emission of light in the flame have been reinvestigated with the multichannel flame spectrometer. As a result of these studies, mechanism of the observed effects is proposed and means of eliminating their consequences are suggested and have been developed.

Preliminary accounts of part of this work have been published (*20*, *21*).

EXPERIMENTAL

All solutions were prepared from reagent grade chemicals. Concentrated stock solutions were made by weighing chemicals dried overnight or longer at 130° C., and dissolving them in water purified by passage through a mixed bed ion exchange column. The column consists of 1 part of IR-120 and 2 parts of IRA-410 (Rohm & Haas), and the resultant water has a specific conductance of 1 micromho or less. When the reagents could not be dried conveniently without decomposition, as in the case of aluminum chloride, the stock solutions were prepared to have approximately the desired concentration and then analyzed by appropriate volumetric or gravimetric methods. The rest of the solutions were prepared by diluting the concentrated stock solutions.

All data were obtained with the multichannel flame spectrometer (*22*). The data of Figures 1 and 2 were obtained by readings made at the peak and at either side of the line or band being studied. The chassis with the exit slit and detector was moved along the rails to the point at which readings were to be made. A millimeter scale on one rail permitted the wave length to be set to within 0.2 mm. (1.7 A.). With the sensitivity switch of the amplifier set to 0 (no input signal), the amplifier balance was adjusted until there was no output signal. The amplifier was then set to its most sensitive position (sensitivity = 1000) and, with the entrance slit closed, the dark-current control was adjusted to balance any residual signal from the photomultiplier. The entrance slit was then opened and the solutions to be studied were aspirated successively and a reading was taken for each. This operation was repeated at several positions of the exit slit and detector along the spectrum. Data were thus obtained on both line-plus-background and background intensities.

The data of Table I were obtained by introduction of solid salts into the flame. A few crystals of the salt were picked up in a loop of tungsten wire and held in the flame, care being taken each time to introduce the wire into the same part of the flame, judged to be its hottest section. The reading at the 5498 A. calcium oxide bandhead was recorded during the period when the salt was vaporizing and coloring the flame. The Beckman burner could not be used for this experiment, because the wire distorted the flame unduly and the high temperature of the oxygen-hydrogen flame caused rapid erosion of the tungsten wire. A Bunsen

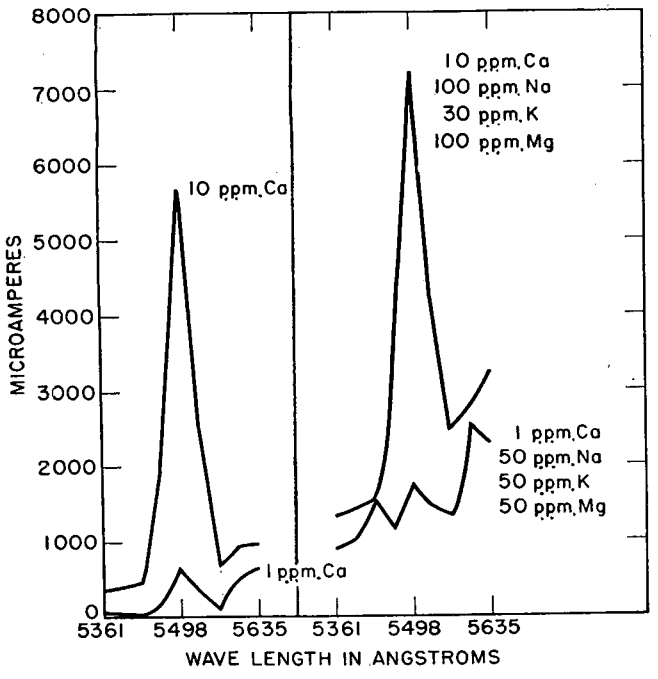

Figure 1. Flame emission intensities from 5361 to 5635 A., including CaO bandhead at 5498 A.

Solutions containing 1 and 10 p.p.m. of calcium alone and solutions containing indicated concentrations of sodium, potassium, and magnesium, as chlorides, in addition to calcium

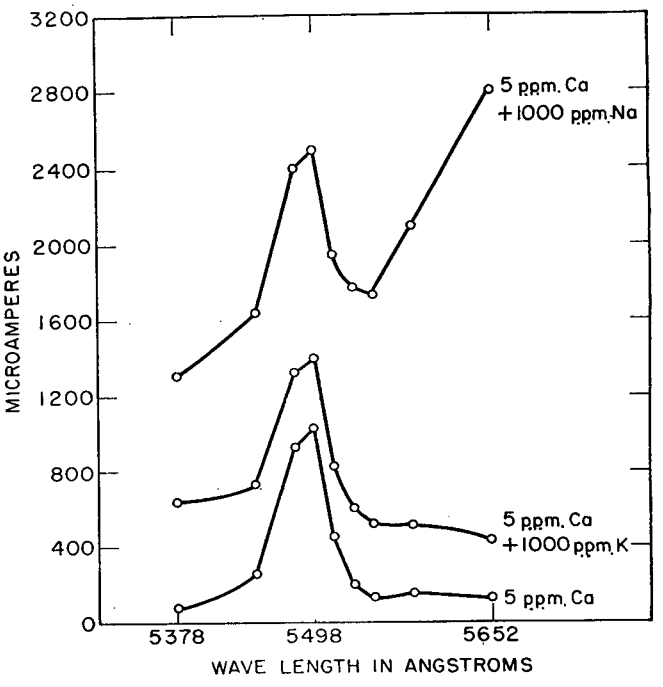

Figure 2. Flame emission intensities from 5361 to 5635 A., including CaO bandhead at 5498 A.

Solutions containing 5 p.p.m. of calcium alone and with indicated concentrations of sodium and potassium

burner and a Meker burner, both with natural gas–air flames, were used instead.

The balance of the data were obtained by readings at the peaks of the lines and bands, using the method described previously (*22*).

RESULTS

Effect of Cations. In order to assess the effect of extraneous cations, the intensities of various lines and bands and the background adjacent to them have been studied. Figure 1 shows the emission intensities obtained between 5360 and 5635 A., including the calcium oxide bandhead at 5498 A., when solutions containing 1 and 10 p.p.m. of calcium alone and in the presence of considerable excesses of sodium, potassium, and magnesium were atomized into the flame. Emission intensity increases over the entire wave-length region when the extraneous elements are present. This general increase of intensity of radiation is termed background. The intensity of the calcium oxide bandhead above background, however, is not affected by the presence of the extraneous cations. The signal above background is 500 μa. for both solutions containing 1 p.p.m. of calcium and is 5100 μa. for both solutions containing 10 p.p.m. of calcium.

The intensity of the calcium oxide bandhead above background is constant even in the presence of a 200-fold excess of sodium or potassium, as is seen in Figure 2, which shows the bandhead and background intensities for 5 p.p.m. of calcium alone and in the presence of 1000 p.p.m. of sodium and of potassium. The extraneous cation only causes background radiation, and it has no effect on the emission of monochromatic calcium radiation measured above background. Similar data have been obtained for magnesium in the presence of excess concentrations of sodium, potassium, and calcium. No changes in line intensity above background have been found which were larger than the experimental error in the determinations

The background produced by extraneous cations at the wave length of an analytical line or band may be found readily by atomizing solutions containing the extraneous elements (*21*).

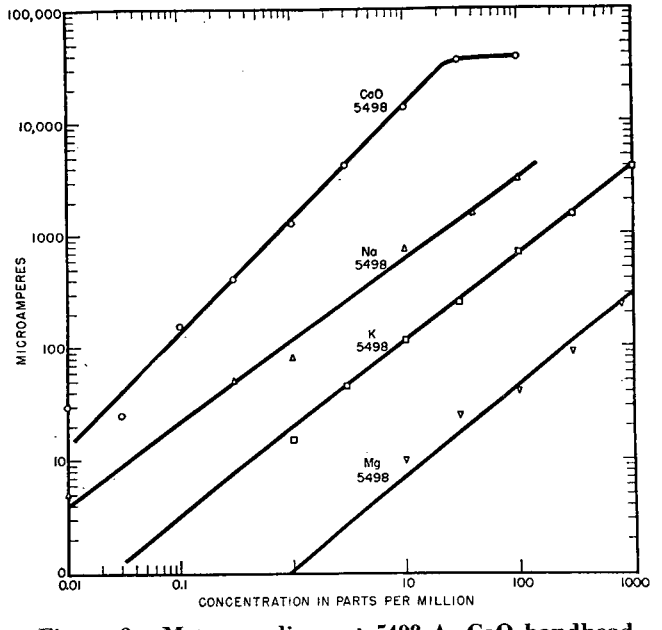

Figure 3. Meter readings at 5498 A. CaO bandhead

Solutions of calcium, sodium, potassium, and magnesium atomized into flame

A graph of meter reading versus concentration of each of the extraneous elements then constitutes the "background curves." Both the analytical curve and the background curves demonstrate similar proportionalities between meter reading and concentration. This is illustrated in Figure 3, which shows the response of a receiver at the 5498 A. calcium oxide bandhead to increasing concentrations of calcium, sodium, potassium, and magnesium. Sodium, potassium, and magnesium emit back-

ground radiation at this wave length. The absolute intensity of background differs for the three elements, but all three background curves are parallel to the calcium calibration curve.

It is possible to distinguish between scattered light in the monochromator and the emission of heterochromatic light in the source as causes of background radiation by placing between the source and the detector a filter absorbing the monochromatic light of the element, producing the background but transmitting at another wave length where observations may be made. For this experiment, a Corning No. 5970 filter was used in front of the entrance slit of the monochromator. Using a sodium vapor lamp as the source of monochromatic radiation, a reading of 140 μa. was obtained at 3700 A. without the filter. This reading was presumably due to light scattered in the monochromator. Insertion of the filter reduced the reading to 8 μa. Using a flame as a source of radiation, the signal at 3700 A. from 300 p.p.m. of sodium was 35 μa. without the filter and 21 μa. with the filter. This is equivalent to a 40% reduction in light intensity, compared with a 94% reduction when the monochromatic light source is used. Though part of the background is due to scattered light, it would appear that the major portion results from heterochromatic light emitted in the source.

Effect of Anions. Figure 4 shows the variation of calcium emission with increasing concentrations of aluminum (probably present as aluminate), oxalate, sulfate, phosphate, nitrate, and chloride. Phosphate, sulfate, oxalate, and aluminum, at concentrations below or approximately equal to the molar calcium concentration, sharply decrease the calcium oxide emission. Once a certain critical anion concentration is exceeded, no further changes in emission intensity take place with increasing anion concentration.

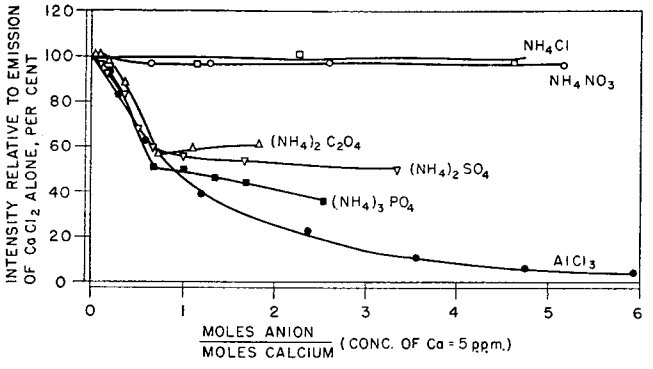

Figure 4. Effects of added aluminum, oxalate, sulfate, phosphate, nitrate, and chloride on emission intensity of 5498 A. CaO bandhead

Similar data for strontium in the presence of aluminum, phosphate, oxalate, and sulfate are shown in Figure 5. The effects are akin to those found with calcium, although oxalate appears to have no influence on strontium emission. Such decreases in emission intensity have also been found for magnesium in the presence of phosphate and sulfate. The lower intensity of radiation emitted by magnesium, as compared with calcium and strontium, makes it difficult to perform these experiments with magnesium at concentrations sufficiently low to prevent precipitation of insoluble salts prior to atomization.

The effect of phosphate on potassium (10 p.p.m. of potassium, 0 to 200 p.p.m. of phosphate) has been tested, as have the effects of 1000 p.p.m. of nitrate, chloride, oxalate, phosphate, sulfate, or aluminum on 10 p.p.m. of potassium. None of these anions are found to alter the emission of potassium. The effect of these substances on the emission of sodium has not been studied because of the difficulties involved in preparing reagents sufficiently free from sodium contamination to permit the detection of possible small changes in radiation intensity.

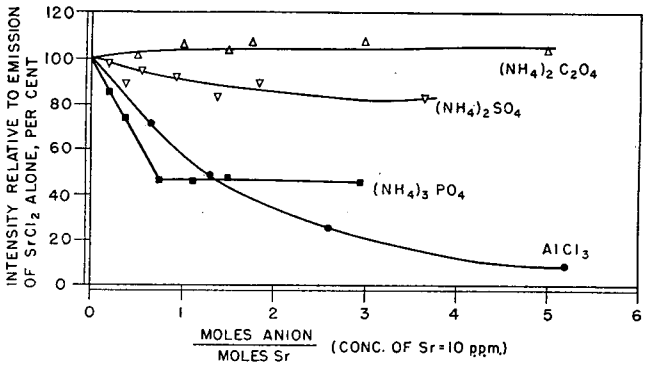

Figure 5. Effects of added aluminum, phosphate, oxalate, and sulfate on emission intensity of the 4607 A. strontium line

The effect of several anions on the emission of radiation by calcium has also been studied by the introduction of various solid calcium salts into the flame, by the method described in the experimental section. Table I lists the readings at the calcium oxide bandhead when various calcium salts were thus introduced into the flame. Three different flames were used: a cool Bunsen flame, without an inner cone; a hotter Bunsen flame, with an inner cone; and a Meker flame. In these three flames, different readings were obtained for the same salt, apparently corresponding to the temperature differences in the flames. With a hotter flame, the rate of evaporation of the salt is greater and a larger fraction of the metal atoms in the flame are excited, so that a higher ammeter reading is obtained. In each of the flames, however, higher ammeter readings are found with calcium chloride than with the other salts. The differences are much larger than the error involved in making the ammeter readings.

The rate of evaporation of the salts is another observable parameter, though one not easily quantified. Calcium chloride evaporated completely from the loop of wire within a few seconds. The other salts evaporated relatively slowly and, in the case of those compounds which evaporated very slowly, a bead of white hot material could be seen in the loop of tungsten wire while it was held in the flame. In this experiment, it was not possible to use calcium nitrate, because the rate of decomposition of the crystals was so rapid that the material disappeared from the tungsten loop before it was possible to take an ammeter reading. The crystals of calcium nitrate appeared to decompose explosively in the flame.

Correction for Background. When extraneous cations present even at large excess concentrations do not affect the intensities of lines or bands emitted in the flame, it is possible to perform accurate quantitative analyses by applying a correction for the background. The multichannel flame spectrometer (*22*) has been modified to correct for background automatically. A description of the modified instrument is in preparation for publication, and preliminary accounts have been rendered (*13, 15*).

Some flame photometers permit the observation of light intensity at a point to one side of the line or band being measured. With these instruments it is possible to correct for background by reading the emission intensity to one side of the line or band and subtracting this value from the line-plus-background reading made at the peak. Such methods can give excellent results (*5–8*), but require that two readings be made for each determination.

Many flame photometers, particularly those using filter monochromators, do not allow the direct observation of background at a wave length adjacent to the analytical line. A method of

indirect estimation of background has been developed which is applicable to such instruments.

To estimate the background at a particular wave length, data such as are shown in Figure 3 are obtained. At each analytical wave length, calibration curves and curves showing the background produced by each of the other elements in the solution are obtained.

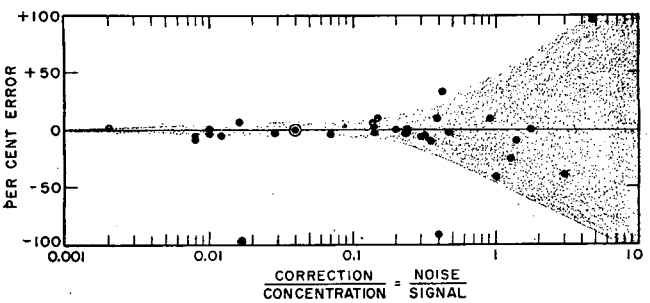

Figure 6. Percentage error in determination of a cation as a function of ratio of background to line intensities

The method of background correction is outlined in this paper

Uncorrected concentrations are obtained from the meter readings for a sample, through the calibration curves. The term "uncorrected concentration" is used to indicate that the background is neglected up to this point. The uncorrected concentrations are then used to determine the contribution of each element to the light intensity at the analytical wave lengths of the other elements, from the background curves. The sum of the contributions from all elements other than the one sought is equivalent to the background at a particular analytical wave length. It is subtracted from the meter reading at that wave length to determine the line intensity. The calculated value of the line intensity is then used to determine the corrected concentration from the analytical curve. Table II shows the sequence of calculations for a specific case.

This method of background correction has been tested on 19 solutions of known composition, each containing four cations, yielding 76 pairs of values of known and determined concentration. The results are shown in Figure 6, a plot of the relative error in the determination of a cation as a function of the ratio of background to line intensity. It is evident that the precision of the analytical data depends strongly on the relative magnitudes of the line and background intensities. When the background is small compared with the line intensity, the error in the determination is 1 to 2% (*22*). Errors as large as 10% are found when the meter readings are small. When the background becomes very large relative to the line intensity, the error in the determination increases rapidly.

A similar method of background correction has been described by Hinsvark, Wittwer, and Sell (*11*).

DISCUSSION

Origin of Anion Interferences. The formation of compounds with high boiling points appears to be a mechanism by which extraneous anions affect the emission intensity of the alkaline earths. When a solution is sprayed into the flame, the following events occur in rapid succession.

The water, or other solvent, evaporates, leaving minute particles of the salt or mixture of salts.

At the elevated temperature of the flame, decomposition or other chemical reactions may occur.

The salts, or their decomposition products, evaporate, and the compounds dissociate.

Only subsequent to these events can the metal atom be excited or form molecules such as calcium oxide, which are then excited. If the salts in solution or their decomposition products or reaction products have extremely high boiling points, the particles may not evaporate completely during their passage through the flame, so that the metal atoms will not be available for and subject to excitation. The formation of such compounds with high melting and boiling points appears to be the cause of the anion interferences in Figures 4 and 5.

The explanation may be illustrated by comparison of the melting points and, where known, the boiling points of the various calcium salts represented in Figure 4 and Table I. Calcium chloride melts at 772° C. and boils at a temperature greater than 1600° C. Calcium nitrate melts at 561° C. Both of these compounds give the same reading at the calcium oxide bandhead when the solution contains the same calcium concentration, and neither excess chloride nor nitrate affect the light emission when present at concentrations within the range covered in Figure 4.

Both phosphate and sulfate cause a marked decrease in the emission of light by calcium. Tertiary calcium phosphate melts at 1670° C.; secondary and primary calcium phosphates decompose at lower temperatures with the loss of water, forming calcium pyrophosphate (melting point, 1230° C.) and calcium metaphosphate (melting point, 975° C.). Calcium sulfate melts at 1450° C. These anions cause a reduction in the emission of light by calcium by forming compounds with that metal which have comparatively high melting points and presumably high boiling points, so that the calcium atoms cannot be made available for excitation.

The effect of oxalate on the emission of calcium constitutes an example of suppression of emission of radiation through formation of a decomposition product with a high boiling point. Calcium oxalate decomposes at the temperature of the flame with the loss of carbon monoxide and carbon dioxide, forming calcium oxide. The melting point of calcium oxide is 2572° C., the boiling point is 2850° C.

Strontium carbonate decomposes at a temperature of 1289° C., as compared with 900° for calcium carbonate (*19*). This may account for the lack of depression of strontium emission by oxalate.

Calcium oxide forms a series of compounds with aluminum oxide which may be considered to be calcium aluminates. These compounds also have high melting points. Calcium aluminate ($CaAl_2O_4$) melts at 1600° C., $Ca_3Al_2O_6$ at 1535° C. It appears that these compounds with high melting points (and presumably high boiling points) are responsible for the effect of aluminum on the emission intensity of calcium.

This interpretation is supported by the results of the experiments in which solid salts were introduced into the flame. In spite of the fact that a bead of white hot material could be seen in the loop of wire, such salts as calcium sulfate and calcium phosphate evaporated slowly and gave a much smaller reading at the calcium oxide bandhead than was found with the more volatile calcium chloride.

Keeping these considerations in mind, it is possible to understand the action of these anions at such low concentrations.

Table II. Sequence of Calculations for Determination of Concentrations by Application of Background Correction

Element	Na	Ca	K	Mg
Meter reading, μa.	430	12	180	32
Uncorrected concentration, p.p.m.	12	1.0	73	25
Background, μa.				
From background curves				
From 12 p.p.m. of Na	..	3	0	9
From 1 p.p.m. of Ca	0	...	0	0
From 73 p.p.m. of K	0	2	.	13
From 25 p.p.m. of Mg	0	1	3	..
Corrected meter readings, μa. (meter reading minus background)	430	6	177	10
Concentration determined, p.p.m.	12	0.44	71	7.6

Such phenomena would be improbable at extremely low concentrations if the effect depended upon collision processes in the flame. However, with the mechanism described, the degree of dilution of the original solution is of little consequence, since evaporation of the solvent leaves solid particles in any case.

The magnitude of the effect of a particular anion on the emission of light by a given metal is a function of the temperature of the flame. It has been reported (*17*), for example, that the presence of sulfate caused a 92% decrease in the emission of light by calcium in an air-hydrogen flame but only a 30% decrease in a hotter oxygen-hydrogen flame. "Interferences" are therefore functions of the particular system used for the analyses, and can be assessed better by consideration of the temperature of the flame and the boiling points of the compounds in the sample than by reliance on published data obtained with a different flame.

Background Correction. The method of background correction described above gives precise results as long as the background intensity is not excessively large compared with the line intensity (See Figure 6). As the background intensity becomes larger than the line intensity, the error in the determination increases rapidly. This is not unexpected. When the background intensity is large compared with the line intensity, it is necessary to subtract two large numbers (line-plus-background and background) to obtain a smaller number (line intensity). In this case, a relatively small error in determining one or both of the larger numbers is equivalent to a relatively large error in the small number.

The ratio of background to line intensity is dependent upon the resolution of the monochromator. The light to either side of a line or band is primarily heterochromatic background. Monochromators of low resolution permit more background light to reach the detector, increasing the background to line ratio. Although the method of background correction described here is applicable to all types of instruments, the precision of the data will be dependent on the resolution of the monochromator.

In using this method of background correction—or in using background intensities determined by readings made at a wave length adjacent to the line or band—it is necessary that the meter readings be proportional to the intensity of light reaching the detector, as only the light intensities are additive. If the readings are not proportional to the light intensity, the method is valid only if the readings are converted to values proportional to the light intensity. For most instruments, the light intensity is proportional to the concentration of the emitting species over a fairly wide range. Line-plus-background and background readings should then be converted to equivalent concentrations before subtraction.

Since flame photometric instruments and techniques have not made allowances for some of the phenomena discussed above, it has been necessary to devise procedures for their empirical management. Standard solutions containing the interfering ions in the same concentrations as are encountered in the sample are frequently employed to negate the effects of extraneous ions. The effectiveness and validity of this procedure is contingent upon a precise match of the compositions of the samples and standards. Differences will result in analytical errors. It is usually necessary to prepare, have available, and analyze daily a large number of such standards and to select the proper ones for each sample to be determined.

Careful matching of standard and sample compositions is particularly vital when this method is used to compensate for interfering anions. If the concentration of the interfering anion is less than that of the alkaline earth, the emission intensity will change considerably with a small change in anion concentration. This difficulty is sometimes overcome by the addition of an excess concentration of the interfering anion to both the samples and the standards. This technique involves a loss of sensitivity which may be serious, particularly if aluminum is present.

Internal standards are also often used to correct for variable sample composition. This technique is useful in compensating for factors which affect the rate of sample atomization, such as variable viscosity, density, and surface tension (*2*, *4*). However, the studies described above indicate that internal standards are not likely to be universally useful in compensating for the presence of extraneous ions. Background present at the wave length of both the element line and the internal standard line will alter the intensity ratio unless, by chance, the background intensities at the two wave lengths are in the same proportion as the line intensities. The presence of the internal standard, another radiating species, will, in fact, increase the background at the analytical wave length, further complicating the problem of background.

Compensation for the presence of interfering anions by the use of an internal standard is also likely to be a misleading procedure, unless it is established that the interfering anion affects the internal standard in precisely the same way as the element being determined. The chemical and physical characteristics of lithium are so different from those of the alkaline earths, for instance, that its response to the presence of extraneous ions can be expected to differ from that of calcium or magnesium. Even strontium would not always be an adequate internal standard for the determination of calcium, as is shown by inspection of Figures 4 and 5. If oxalate were present in the sample, for example, the calcium emission would be reduced while that of strontium would be unchanged.

As a result of the studies here described and experience with the multichannel flame spectrometer as modified for automatic background correction (*13*, *15*), it would seem best to compensate for the presence of interfering cations by the application of a correction for the background. Interfering anions are best separated from the sample before analysis. Methods for the performance of such separations have been discussed previously (*14*).

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RECEIVED for review July 19, 1955. Accepted November 4, 1955. Presented in preliminary form at the Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., 1955. This work was supported by a contract between the Office of Naval Research and Harvard University, Contract No. Nr5ori-07660, and a grant-in-aid from the National Institute of Health.

Spectrochemical Determination of Trace Quantities of Cobalt in Animal Tissues

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The accurate chemical determination of submicrogram quantities of cobalt in animal tissues has been rendered difficult in the past by the presence of varying amounts of interfering elements, notably iron, as well as by the use of methods of insufficient sensitivity. These difficulties have been overcome in this laboratory by the development and application of two highly sensitive spectrochemical methods. One method is applied directly to the ash of tissues of exposed animals containing at least 0.025 γ of cobalt per gram of fresh tissue, in excess of that present in normal tissue. The other method was developed for the determination of cobalt in normal tissues. It employs the principle of preliminary chemical concentration of the cobalt with an organic complexing reagent, followed by collection in a mixture of aluminum oxide, lithium chloride, and graphite as constant base material for direct current arc spectrography. The latter method has been used for the determination of millimicrogram quantities of cobalt in the tissues of small normal animals.

IN THE course of an experimental investigation of the toxicity of cobalt, the need arose for the determination of submicrogram quantities of this element in the tissues of animals exposed to cobalt metal fume at an air concentration approximating 1 mg. of cobalt per cubic meter. The purpose of the present paper is to present the two spectrochemical methods developed for the determination of cobalt (1) retained by the soft tissues of animals exposed to known concentrations of cobalt metal fume for specific periods of time or (2) deposited in the bones of exposed animals or present normally in the bone and soft tissues of unexposed animals. These methods have shown a high degree of sensitivity and accuracy on analyses of more than 1000 tissue samples from dogs, rabbits, guinea pigs, rats, and mice.

The first method is a direct spectrographic procedure which is applied to the ash of soft tissues from exposed animals only. The tissues should contain at least 0.025 γ of cobalt per gram of fresh tissue in excess of the quantity present in normal tissue. Under the conditions of this method, normal tissue ash does not yield a detectable line of cobalt.

The second method includes a cobalt concentration procedure and is designed for the determination of cobalt in the soft tissues of normal animals and in the bone samples of both exposed and normal animals. This method involves the extraction of cobalt with an organic complexing reagent and addition of a carrier element to provide bulk, followed by ashing and spectrographic analysis of the resulting oxide mixture. This procedure is based upon the principles set forth by other investigators (*3, 7, 8, 10*) for the determination of trace elements in plants, soils, and biological materials. Mitchell and Scott (*7, 8, 10*) employ a mixture of 8-quinolinol, tannic acid, and thionalide for the quantitative precipitation of a group of trace elements including cobalt, from a solution buffered at a pH of 5.2 and containing the unwanted alkali and alkaline earth elements and phosphate. However, the extremely large amount of phosphate in bone ash precluded the use of Mitchell and Scott's group separation procedure. Instead, it was decided to extract the cobalt with 1-nitroso-2-naphthol, in accordance with Saltzman's preliminary extraction procedure (*9*), to circumvent the difficulties imposed by a high concentration of phosphate. The efficiency of this extraction has been found to be very high and this method of isolating cobalt was applied successfully to all animal tissues encountered in this investigation.

Mitchell and Scott had reported aluminum to be a satisfactory carrier element for their group precipitation procedure and had found the aluminum oxide residue from the ashed precipitate to be a suitable spectroscopic matrix. Therefore, in the present method, aluminum nitrate is added to the chloroform solution containing the extracted cobalt complex. After ashing, however, a mixture of lithium chloride and graphite is added as a spectroscopic buffer to the aluminum oxide residue. This buffer was found by Keenan and White (*5*) to be effective in the suppression of cyanogen bands and background and also in the enhancement of line spectra. By the use of this buffer in both methods of the present paper, a sensitivity of 0.005 γ of cobalt on the electrode has been attained.

Cobalt present in the tissues of three species of normal animals has been determined by the second method.

DIRECT SPECTROGRAPHIC METHOD FOR COBALT IN TISSUES OF EXPOSED ANIMALS

Quantitative analysis by the direct spectrographic method may be applied to the tissue samples (other than bone) of cobalt-exposed animals. These tissues should contain at least 0.005 γ of cobalt per 2 mg. of tissue ash in excess of the amount of this element in the normal tissue ash used as base material for the preparation of the analytical curves. The 2-mg. quantity of ash, introduced in dilute hydrochloric acid solution to the electrode crater, has been found to be the optimal quantity for spectrographic exposure, as reported by others who have analyzed these materials (*2*). Bone tissue yields too large a quantity of ash to be amenable to analysis by this procedure and should be analyzed either chemically (*9*) or by the alternative spectrographic method, if the cobalt content is too low for the successful application of the chemical method.

REAGENTS

All reagents are analytical reagent grade, except where otherwise indicated. Double distilled water, from an all-glass borosilicate glass still, is used in the preparation of all reagent solutions.

Electrode Waterproofing Solution. Dissolve 20 grams of paraffin wax in C.P. benzene and dilute to 100 ml.

Standard Cobalt Solutions. Dissolve 0.1247 gram of cobalt oxalate [a specially purified salt containing 0.0005% nickel prepared by a procedure to be published (*1*)] or equivalent quantity of a soluble cobalt salt in 15 ml. of 6*N* hydrochloric acid and dilute to 1 liter with double distilled water. One milliliter of this solution contains 50 γ of cobalt. Make successive tenfold dilutions of this stock solution as needed to prepare the individual standard solutions.

Palladium Internal Standard Solution. Extensive experimentation with several metals led to the choice of palladium as internal standard. The volatilization rates of cobalt and palladium are similar, as shown by Vallee and Peattie (*12*). Moreover, palladium has not been detected in the animal tissues and the intensity of the 3460.8 A. line of palladium is not affected by a variation in the cobalt concentration. With an electrode content of 0.5 γ of palladium, the 3460 A. line falls in the middle portion of the densitometric range, as is desirable for the internal standard line.

Dissolve 20.0 mg. of palladium chloride dihydrate in water and dilute to 100 ml. Dilute 1 part of this stock solution with 9

parts of water to obtain an internal standard solution containing 0.5 γ of palladium per 0.05 ml.

Spectroscopic Buffer Mixture. Mix and grind thoroughly 1.000 gram of spectroscopically pure graphite and 0.400 gram of C.P. lithium chloride, using a mullite or agate mortar reserved for this purpose. Store over concentrated sulfuric acid in a desiccator. (Store ingredients of this mixture in same desiccator to facilitate weighing and grinding procedures.)

APPARATUS

Spectrograph. A Bausch & Lomb large Littrow spectrograph complete with quartz optics, a condensing lens system, and a 10-micron fixed slit. No sector was used during sample or standard exposures.

Excitation Source. A 220-volt direct current arc, operating at 9 amperes with a gap maintained at 10 mm.

Plates. Eastman III-O spectroscopic plates with antihalation backing.

Developing Equipment. Glass trays supported in a specially constructed galvanized metal tray maintained at 68° F. by means of water circulated from a constant temperature bath.

Densitometer. Jarrell-Ash Model 200 nonrecording microphotometer.

Micropipet Assembly. A micropipet assembly, constructed in the laboratory machine shop, was used to facilitate precise control over the addition of standard and sample solutions to the electrodes.

PROCEDURE

The tissue is transferred to a porcelain evaporating dish or crucible, dried overnight on a hot plate, and dry-ashed at 500° C. in a muffle furnace. The ash is extracted with 5 ml. of 6*N* hydrochloric acid and about 10 ml. of hot distilled water. The solution is filtered through Whatman No. 42 paper into a 125-ml. Phillips beaker (borosilicate glass). The filter is washed three times with hot distilled water and the washings are combined with the filtrate. The filter is transferred to the original dish, dried, and re-ashed in the furnace. The ash is re-extracted and washed as described previously, and the filtrate and washings are combined with the first filtrate. The resulting solution is evaporated to about 5 ml. and then transferred, with rinsing, to a 15-ml. centrifuge tube, graduated to 0.1 ml. The tube is placed in the drying oven at 105° C. and the sample solution is evaporated to a predetermined volume which will contain approximately 2 mg. of tissue ash per 0.05 ml. This volume is determined from the known fresh weight of the original tissue sample (weighed after surface blood is removed by rinsing with distilled water and blotting with filter paper) and the previously established ash percentage of each type of tissue.

Tissue portions which weigh less than 0.6 gram are ashed in porcelain crucibles. If ashing appears to proceed too slowly, a few drops of distilled water are added to the cooled crucible, the mixture is dried and ashing continued until a satisfactory white ash is obtained. Depending upon the estimated ash weight, 1, 2, or 3 drops of 6*N* hydrochloric acid are added to the cooled ash. The mixture is allowed to stand until solution is effected. The tissue ash solutions thus prepared are now ready for electrode loading.

Spectroscopically pure graphite electrodes, $^5/_{16}$ by 12 inches, are cut into 1.5-inch lengths. Uniform craters, 4.5 mm. wide and 3 mm. deep (including the cone produced by the tapered end of the drill), are drilled into each, using an electrode shaper. After the craters are waterproofed with the paraffin wax solution, the cone-shaped bottoms of the craters are slightly more than filled with about 10 mg. of the ground and desiccated lithium chloride spectroscopic buffer mixture.

All samples are run in triplicate when sufficient solution is available. A volume of 0.05 ml. of the sample solution is added to the electrode crater containing the buffer mixture, using a Pickard-Pierce blood pipet mounted in the micropipet assembly. The electrodes are dried at 85° C. for 15 to 20 minutes; after cooling, 0.05 ml. of the internal standard solution is added with a micropipet and the electrodes are dried at 105° C. for 1 hour, after which they are ready for spectrographic exposure.

The electrodes containing the samples are burned as anodes in a 220-volt direct current arc operating at 9 amperes, with the gap maintained at 10 mm. throughout each exposure. Clean, freshly pointed, spectroscopically pure graphite electrodes serve as the cathodes. The exposures are continued (usually 25 to 35 seconds) until burnout, as evidenced by increased wandering of the arc accompanied by the disappearance of the reddish radiation due to lithium in the arc. The central portion of the arc is focused on the slit of the spectrograph. The plates are developed and fixed at 68° F. After the plates have been air-dried, the per cent transmittance values of the cobalt 3453.5 and palladium 3460.8 A. lines and of the background adjacent to the cobalt line are determined with a nonrecording microphotometer. The intensity ratios of Co 3453.5/Pd 3460.8 (with background correction) are established by reference to an emulsion calibration curve obtained by the conventional step sector method, and the concentration of cobalt is estimated from the appropriate analytical curve. An average concentration value is calculated from the results of the triplicate determinations.

ANALYTICAL CURVES

Preparation. Separate analytical curves are usually prepared for each type of tissue to be analyzed. The tissues of normal animals provide the ash used as base material for each standard tissue solution. Each standard solution is prepared to contain 2 mg. of a particular tissue ash per 0.05 ml. These solutions are considered to be cobalt-free for the immediate purpose, since none of them contains a detectable quantity of cobalt by the direct spectrographic method as applied to their 0.05-ml. portions.

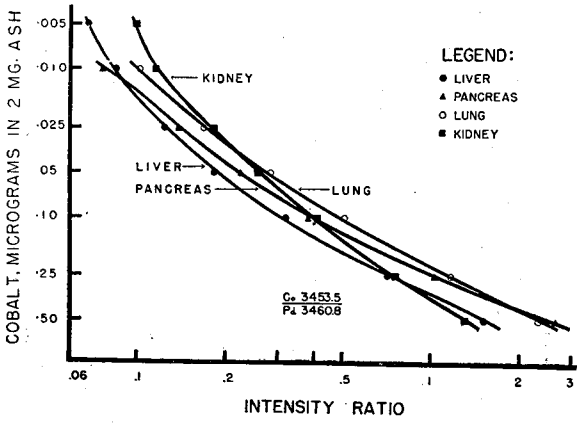

Figure 1. Analytical curves for cobalt added to normal tissue ash

In establishing each curve, the electrodes are loaded with the designated quantities of spectroscopic buffer and the appropriate tissue ash solution, then dried at 105° C. After drying, 0-, 0.005-, 0.010-, 0.025-, 0.050-, 0.125-, 0.250-, 0.500-, and 1.25-γ quantities of cobalt are added to triplicate sets of electrodes. After drying for 20 minutes, 0.5 γ of palladium is added and, after additional drying for 1 hour, the spectrographic exposures are conducted and the intensity ratios of Co 3453.5/Pd 3460.8 are determined as indicated in the procedure. The intensity ratios thus obtained are plotted individually for each corresponding electrode content of added cobalt and an average curve is drawn. The curves for cobalt added to normal liver, lung, kidney, and pancreas ash are shown in Figure 1.

Discussion. The curves shown in Figure 1 cover most of the usual working range of this method, which extends from 0.005 or 0.010 to 1.25 γ of cobalt in 2 mg. of tissue ash; these concentrations are in excess of the quantities present in normal tissue ash. In practice, the lung and pancreas curves have been extended to include increased quantities of cobalt up to 50 γ as the need arose for the analysis of larger quantities of this element. At these higher concentrations, the 3455.2 A. line of cobalt was used as the analysis line.

The variation of these curves with respect to each other is believed by the authors to be due to the varying inorganic composition of the ash of the different tissues. This effect could be minimized by the use of a larger quantity of buffer. However, it was feared that this might result in a sacrifice of sensitivity, as previous work with 20-mg. charges of this buffer limited the

sensitivity of lead, vanadium, titanium, and molybdenum determinations to 0.1 γ in the matrix (*5*). As maximal sensitivity was desired, the amount of buffer was maintained at 10 mg., a quantity which was sufficient to suppress background and act as an enhancing agent in the production of the cobalt spectrum.

Table I. Recovery of Cobalt and Deviation of Recovery from Expected Values for Indicated Amounts of Cobalt Added to 2 Mg. Portions of Liver Ash

No.	Co Added, γ	Co Recovered, γ	Deviation, γ		Recovery %
1	0.005	0.0048[a]	−0.0002		96.0
2	0.010	0.0080[a]	−0.0020		80.0
3	0.025	0.0233[a]	−0.0017		93.2
4	0.050	0.0428[a]	−0.0072		85.6
				Mean	88.7
20A	0.10	0.110	+0.010		110.0
21A	0.10	0.107	+0.007		107.0
11D	0.10	0.098	−0.002		98.0
13D	0.10	0.100	0.000		100.0
20A	0.25	0.250	0.000		100.0
21A	0.25	0.252	+0.002		100.8
13D	0.25	0.243	−0.007		97.2
27D	0.25	0.231	−0.019		92.4
20A	0.35	0.322	−0.028		92.0
21A	0.35	0.383	+0.033		109.4
11D	0.35	0.335	−0.015		95.7
13D	0.35	0.363	+0.013		103.7
27D	0.35	0.335	−0.015		95.7
				Mean	100.1

[a] Results of individual analyses.

After the concentration method described below was developed and applied to the determination of cobalt in normal animal tissues, the analytical curves of the direct method were replotted, using as ordinates the total electrode content of cobalt—i.e., normal plus added cobalt. The same variation of the curves shown in Figure 1 resulted; the individual curves were displaced slightly but still exhibited their independent character. Hence, it appears to the authors that this independent nature of the separate curves is not due to the residual cobalt in the tissue ash, but to a variation in the composition of the ash.

ACCURACY

To test the accuracy of the method, a series of analyses was carried out to determine the per cent recovery. Normal liver and kidney tissues were used as the base materials to test the lower end of the working range, extending from 0.005 to 0.05 γ of added cobalt per 2 mg. of tissue ash. The working range from 0.1 to 0.4 γ of cobalt per 2 mg. of ash was tested by using previously analyzed liver and kidney samples as base materials. In these recovery determinations, known quantities of cobalt were added to electrodes containing the quantities of spectroscopic buffer and tissue ash designated in the quantitative procedure. All solutions, including the internal standard, were added as described previously. Finally, the spectrographic exposures and cobalt estimations were performed in triplicate, except where indicated otherwise, in conformance with the procedure. An indication of the accuracy of the procedure may be obtained from the results of the recovery of cobalt added to liver tissues, presented in Table I.

As reported in Table I, the average recovery of 0.005- to 0.050-γ quantities of cobalt added to liver ash was 89%, as determined by individual determinations. The mean recovery of 0.10- to 0.35-γ amounts of cobalt was 100% as a result of conducting triplicate determinations on 13 ashed samples. The error of these latter determinations lies within ±10%, showing the advantage of triplicate determinations. Similarly, recovery determinations using 11 ashed kidney samples as base material gave a mean value of 102%. Consequently, the degree of accuracy of the direct spectrographic method, indicated by these recovery data, was considered satisfactory for the purpose of determining the retention of cobalt in the soft tissues, in excess of the quantities present normally.

CONCENTRATION METHOD FOR DETERMINATION OF COBALT IN BONE AND NORMAL ANIMAL TISSUES

Prior to the application of this method, separate tissues are prepared as for the direct spectrographic method except that the acid solutions of the ashed samples are not adjusted to definite volumes. Instead, these solutions are transferred to individual separatory funnels and subjected to the cobalt separation procedure.

REAGENTS

All reagents are analytical reagent grade. Double distilled water, from an all-borosilicate glass still, is used in the preparation of all solutions. In addition to those listed under the direct method, the following are required:

Nitric Acid, Concentrated. Redistill the C.P. reagent in an all-borosilicate glass still.

Nitric Acid, 1 to 1, and **Hydrochloric Acid,** 1 to 99. Dilute the redistilled reagents with water.

Phosphoric Acid, 1 to 49. Dilute the C.P. reagent with water.

Methyl Orange Indicator Solution, 0.1% in water.

1-Nitroso-2-naphthol. Dissolve 2.5 grams of the reagent in 125 ml. of glacial acetic acid and dilute to 250 ml. with water.

Sodium Citrate Solution. Dissolve 423 grams of the dihydrate salt in almost a liter of water in a separatory funnel, adjust the pH to 9 with sodium hydroxide, and extract metallic impurities with a solution of 100 mg. per liter of dithizone in chloroform until a green colored extract is obtained. Adjust the aqueous solution to pH 7 with citric acid and remove excess dithizone by washing with several portions of chloroform. Separate the aqueous layer and dilute to 1 liter with water.

Aluminum Nitrate Solution. Dissolve 1.472 grams of the nonahydrate salt in 20 ml. of redistilled 95% ethyl alcohol. Two milliliters of this solution contains the equivalent of 20 mg. of aluminum oxide.

PROCEDURE

Separation of Cobalt. The separation of cobalt from the other inorganic constituents of tissue ash is conducted in accordance with Saltzman's detailed procedure (*9*) for the preliminary separation of cobalt, using 1-nitroso-2-naphthol and including the removal of impurities by shaking the extract with 25 ml. of 1 to 99 hydrochloric acid. The chloroform solution of the extract is then separated and transferred to a porcelain crucible containing 2 ml. of the alcoholic solution of aluminum nitrate. The mixture is evaporated to dryness on the steam bath and is ready for ashing.

Ashing. About 5 ml. of concentrated nitric acid is added to the crucible containing the 1-nitroso-2-naphthol residue and the mixture is evaporated to dryness on the steam bath. The residual material is then subjected to alternate dry ashing in a muffle furnace at 450° C. and wet ashing with concentrated nitric acid on a hot plate. During the latter process the crucible is covered with a watch glass, placed so that a small open space permits the slow escape of vapor. When the cycle has been repeated twice, the residue is ashed a third time in the furnace; at this point there is produced an easily crushed, grayish white powder and ashing is considered to be complete.

Preparation of Electrode Charge. The residue of aluminum and cobalt oxides from the ashing procedure is crushed with a clean glass nail, then weighed on glazed paper, and three or four separate 5.0-mg. portions are weighed out for analysis. (The absolute amount is not critical but must be known to calculate the aliquot portion on the electrode.) To each weighed portion is added approximately 5 mg. of the lithium chloride–graphite spectroscopic buffer mixture. The mixtures are ground separately in an agate mortar until thoroughly mixed and then they are added to the waterproofed craters of spectroscopically pure electrodes. The crater is the same size as that used in the direct spectrographic method. After the addition of 0.5 γ of palladium, the electrodes are dried for 20 minutes at 80° to 85° C. and then for 1.5 hours at 105° C.

Spectrographic and Densitometric Procedures. The exposure conditions, plates, and plate-processing procedure are the same as those of the direct method. The intensity ratios of Co 3453.5 to Pd 3460.8 are determined as described previously; the cobalt concentrations in the aliquot portions on the separate electrodes

are estimated from an analytical curve obtained from a series of standards.

PREPARATION OF CURVE

A series of known quantities of cobalt, ranging from 0.03 to 1.00 γ, is added to porcelain crucibles containing 2 ml. of the aluminum nitrate and 5 ml. of the 1-nitroso-2-naphthol solutions. The ashing, electrode loading, spectrographic and densitometric procedures are conducted as described above. The plot of $I_{\text{Co 3453.5}}/I_{\text{Pd 3460.8}}$ *vs.* the quantity of cobalt on the electrode is prepared as the analytical curve and is shown in Figure 2.

The values plotted in Figure 2 resulted from the densitometric treatment of the individual spectrograms prepared from 5.0-mg. portions of the ashed standard residues. The present working range of the method is from 0.006 to 0.25 γ of cobalt on the electrode.

ACCURACY

Known amounts of cobalt, varying from 0.03 to 0.4 γ, were carried through the entire extraction, ashing, and spectrographic procedure. The results of this experiment, obtained by averaging the quadruplicate determinations of cobalt concentrated in the aluminum oxide residue, are presented in Table II.

The recovery data presented in Table II indicate the accuracy of the method to lie between 90 and 98%, with a mean value of 93.7% obtained with this set of determinations. These recoveries are considered adequate for a working range extending from 0.006 to 0.09 γ cobalt.

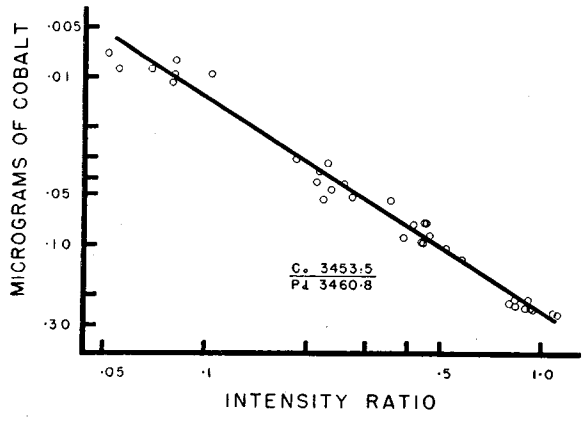

Figure 2. Analytical curve for cobalt in aluminum oxide, using lithium chloride graphite buffer

Table II. Recovery of Cobalt by Chemical Concentration Method

Co Taken for Extraction, γ	Al_2O_3 Residue, Mg. On total electrode		Co Detd. On Electrode, γ	Total Co Recovered, γ	Av. Co Recovery, γ	Recovery, %
0.03	26.4	5.3	0.0059	0.029		
		5.6	0.0057	0.027		
		5.2	0.0055	0.028		
		5.5	0.0066	0.032	0.029	96.7
0.04	27.3	5.4	0.0080	0.041		
		6.2	0.0071	0.031		
		5.9	0.0084	0.039		
		5.7	0.0069	0.033	0.036	90.0
0.10	28.0	6.5	0.0213	0.092		
		6.1	0.0208	0.095		
		4.9	0.0147[a]	...		
		5.8	0.0163[a]	...	0.0904	90.4
0.20	23.0	5.3	0.0418	0.189		
		6.0	0.0485	0.193		
		4.0	0.0248[a]	...		
		5.6	0.0410	0.175	0.186	93.0
0.40	26.0	5.1	0.0800	0.408		
		6.4	0.0870	0.354		
		5.1	0.0895	0.456		
		5.3	0.0725	0.356	0.394	98.5
					Mean	93.7

[a] Intensity ratio, Co 3453/Pd 3460, not in agreement with others in respective sets.

DETERMINATION OF COBALT CONTENT OF NORMAL TISSUES

The cobalt content of tissues from normal dogs, rabbits, and rats was determined in individual samples by the cobalt concentration method. The results are reported in Table III.

The data reported in Table III show cobalt to be widely distributed throughout the animal organism. In the dog, the small glandular tissues contain the highest concentrations of this element, whereas the liver, spleen, muscle, and bone contain cobalt at the lowest and at almost a constant concentration.

The small glandular tissues, with the exception of rabbit adrenals, were not taken from the rabbit and rat for cobalt analysis at the time these control animals were sacrificed. However, as in the case of the glandular tissues of the dog, the rabbit adrenals show the highest cobalt concentration. In the remaining tissues of both rabbits and rats, cobalt appears to be concentrated to a greater extent in the spleen, pancreas, and kidney than in the other tissues, although this relationship does not hold for the absolute quantities of cobalt in the entire organs. Upon investigating the latter relationship, repeated statements were noted in the literature that cobalt had been found concentrated in the liver, pancreas, and spleen. The present study bears this out partially on a total organ basis only; however, most data in the literature were obtained from ruminants whose metabolism of this element may differ from that of dogs, rabbits, and rats. The only data on any of the latter species appear to be those of Josland and McNaught (*4*), who analyzed certain tissues, composited from seven rats, by a chemical method. The present data were found to agree well with McNaught's corrected values (*6*). A comparison of both sets of determinations is presented in Table IV.

As reported in Table IV, there are some differences between the results obtained by the two methods. However, the agreement is believed to be satisfactory after consideration of the different sources of animals and the probable differences in their diets.

Of perhaps greater significance is the report in the present

Table III. Cobalt Content of Normal Animal Tissues Analyzed Individually

(Expressed in micrograms of cobalt per gram of fresh tissue)

Tissue	Dog			Rabbit			Rat		
	No. of animals	Concn. range, γ Co/g.	Av. concn., γ Co/g.	No. of animals	Concn. range, γ Co/g.	Av. concn., γ Co/g.	No. of animals	Concn. range, γ Co/g.	Av. concn., γ Co/g.
Lung	2	0.008–0.015	0.012	8	0.010–0.063	0.032	9	0.030–0.071	0.050
Liver	2	0.007–0.008	0.008	9	0.027–0.086	0.054	9	0.017–0.030	0.026
Kidney	2	0.010–0.014	0.012	9	0.047–0.163	0.103	9	0.038–0.088	0.069
Spleen	2	0.005–0.006	0.006	7	0.046–0.602	0.131	9	0.044–0.189	0.116
Pancreas	2	0.012–0.022	0.017	3	0.071–0.134	0.101	9	0.052–0.246	0.109
Muscle	2	0.006–0.007	0.007	4	0.015–0.024	0.021	9	0.015–0.104	0.049
Bone	2	0.002–0.007	0.005	10	0.007–0.048	0.030	8	0.028–0.103	0.069
Adrenals	2	0.074–0.167	0.121	5	0.069–0.316	0.170			
Pul. lymph nodes	2	0.047–0.125	0.086						
Thyroid	2	0.156–0.360	0.258						
Pituitary	1	0.112							
Brain	2	0.010–0.013	0.012						

paper of the quantity of cobalt occurring in bone and muscle tissues, a fact which may have escaped earlier detection because of the lack of a method as specific and sensitive as the one reported here.

SUMMARY

Two spectrochemical methods have been developed for the determination of trace quantities of cobalt in animal tissues. One, applied directly to the ash of tissues from exposed animals, is used for those samples containing 0.025 γ of cobalt per gram of fresh tissue in excess of that present in normal tissue. The analysis error is within $\pm 10\%$, as shown by triplicate recovery determinations of cobalt.

The other method, referred to as the cobalt concentration method and developed for the analysis of cobalt in normal tissues, employs the principle of preliminary chemical concentration of the cobalt. Aluminum is added as the carrier element to the isolated cobalt and provides (as the oxide after ashing) with lithium chloride and graphite the constant base material for spectrographic analysis. This method possesses a degree of sensitivity of about 0.001 p.p.m. for a 30-gram sample of fresh tissue. The average analysis error over the 0.006- to 0.1-γ portion of the working range is about -6%. This method provides greater accuracy in individual sample analysis than does the direct method.

The cobalt present in normal tissues and undetectable by the direct method has been determined in the tissues of the dog, rabbit, and rat by the concentration method. These data comprise the first published report of highly accurate individual determinations of the millimicrogram quantities of cobalt present in the organs of small normal animals.

The high degree of sensitivity realized with both spectrochemical methods is due in part to the lithium chloride–graphite buffer system which suppresses background and exerts an enhancing effect on the line spectra of trace elements.

OTHER APPLICATIONS

The principles of the cobalt spectrochemical methods described in this paper are being applied to similar problems in this laboratory. These include determination of vanadium in animal tissues, trace elements in animal diets, and lead in hair.

Table IV. Comparative Cobalt Content of Normal Rat Tissues Determined Chemically and Spectrochemically

Tissue	McNaught, γ Co/G.	Keenan-Kopp, γ Co/G.	McNaught, γ Co/Organ	Keenan-Kopp, γ Co/Organ
Lung	...	0.050	..	0.11
Liver	0.040	0.026	0.48	0.30
Spleen	0.128	0.116	0.14	0.13
Kidneys	0.043	0.069	0.11	0.16
Pancreas[a]	0.066	0.109	0.13	0.22
Muscle[b]	...	0.049	..	6.97
Bone[b]	...	0.069	..	2.42

[a] Assuming an organ weight of 2 grams for pancreas.
[b] Estimated weights of these tissues in individual animals are based on percentage weight data of rat tissues in relation to body weight given by Skelton (*11*).

The sensitivity and accuracy of either procedure may prove to be useful to those engaged in the determination of cobalt in plants, soils, rocks, or other materials.

ACKNOWLEDGMENT

The authors are indebted to Herbert E. Stokinger, under whose direction this work was performed, for his constructive criticism and guidance in the preparation of this paper.

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RECEIVED for review April 15, 1955. Accepted October 31, 1955.

Spectrophotometric Determination of Total Hemoglobin in Plasma

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A simple method has been developed for the spectrophotometric determination of total hemoglobin in plasma. All hemoglobin is first converted to methemoglobin; the absorbance of this solution is then evaluated before and after a small amount of cyanide is added to convert all methemoglobin to cyanmethemoglobin. The change in absorbance observed is directly proportional to the total hemoglobin and is calculated directly. The method offers the obvious advantages of simplicity, stable reagents, and the production of stable colors with an acceptable degree of precision and accuracy, which are not affected by varied dilutions of the test plasma.

THE determination of hemoglobin in irradiated liquid plasma has been a difficult problem because of the inherent color of all lots of plasma and the fact that glucose is added to stabilize the proteins which are present. Of the many methods which have been proposed, such as that of Karr and Chornock (*3*) and Creditor (*1*), none have offered the combined advantages of stable reagents, stable colors, and high accuracy and precision.

The purpose of this investigation was to develop an analytical procedure that has these characteristics.

THEORY

Since solutions of methemoglobin and cyanmethemoglobin follow Beer's law very well, the total hemoglobin concentration in plasma may be evaluated directly by the difference between the absorbance, A, of cyanmethemoglobin, A^{CMHb}, and the methemoglobin, A^{MHb}, in the plasma at the same wave length (540- to 550-mμ range) using a constant cell thickness (Figure 1). This principle has been applied by Michel and Harris (*4*) to the determination of methemoglobin in whole blood at 635 mμ.

The absorptivity, a^{MHb} and a^{CMHb}, for methemoglobin and for

cyanmethemoglobin, is evaluated with a standard material, in a 1-cm. cell, at a particular wave length, using the following equation:

$$a^{MHb} = \frac{A^{MHb}}{\text{concentration}} \quad (1)$$

The author has employed samples of known hemoglobin content in appropriate dilutions in distilled water or in plasma and has obtained identical absorbance differences with either medium. Hemoglobin is converted to methemoglobin by the use of ferricyanide; the corresponding cyanmethemoglobin solutions are prepared from the methemoglobin solutions by adding sodium cyanide.

ANALYTICAL METHOD

Apparatus. A Beckman Model DU spectrophotometer was employed for all measurements of absorbance.

Reagents. Phosphate Buffer (pH 6.6; 0.50*M*). This was prepared by dissolving 26.7 grams of anhydrous disodium phosphate and 42.7 grams of anhydrous monopotassium phosphate in distilled water and diluting to 1 liter. This solution is important in preventing changes in clarity of the plasma solutions which additions of sodium cyanide otherwise would cause.

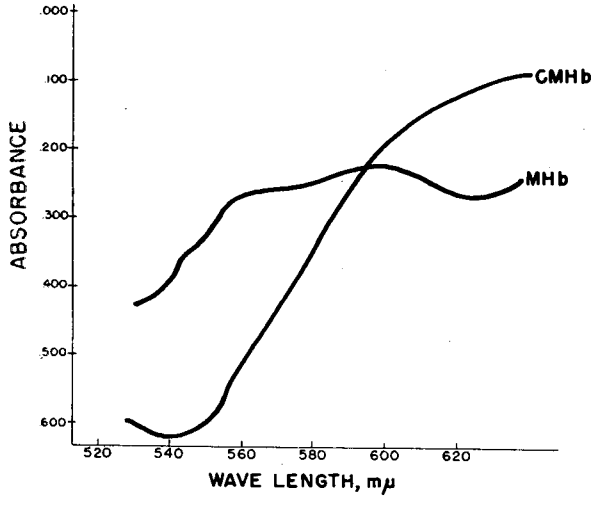

Figure 1. Absorption curves for methemoglobin (MHb) and cyanmethemoglobin (CMHb)

Phosphate Buffer (pH 6.6; 0.25*M*), prepared by dilution of the 0.50*M* buffer above with an equal volume of distilled water.

Potassium Ferricyanide Solution (20% w./v.). A reagent grade chemical was employed. As employed in the method described below, final solutions contain 0.3% (w./v.) ferricyanide. This concentration of ferricyanide has no measurable absorption between 500 and 700 mμ.

Sodium Cyanide Solution (10% w./v.). A reagent grade chemical was employed.

Calibration. A sample of whole blood of known hemoglobin content is satisfactory as the standard material. The hemoglobin content of the whole blood used may be determined by the oxygen capacity (manometric) method of Van Slyke (*2*), as was done by the author, or by one of the methods based on the iron content. This standard hemoglobin solution may then be employed using dilutions of it in distilled water (1:125 to 1:100 are suitable) or in liquid plasma.

To 10-ml. samples of the diluted standard hemoglobin (10 to 100 mg. % hemoglobin in water is appropriate for a 1-cm. cell) are added exactly 2 ml. of 0.25*M* phosphate buffer. Two drops of ferricyanide solution are next added, and the contents of the vessel are mixed well. After about 2 minutes the absorbance of each of the resulting standard methemoglobin solutions is evaluated at a specific wave length (540- to 550-mμ range) employing the reagent blank in which distilled water is used in place of diluted blood.

The corresponding standard cyanmethemoglobin solutions are then prepared by adding one drop of the sodium cyanide solution to each of the above solutions and mixing the contents thoroughly. The absorbance of each of these solutions is then determined at the same wave length selected for the methemoglobin solutions.

The data for a typical standardization are presented in Table I. In calculating the absorptivity, one may wish to report the hemoglobin concentration as milligram per cent, so that the use of the final constant gives directly the hemoglobin concentration as milligram per cent in the plasma sample. It is desirable for the analyst using this method to establish his own calibrations.

One may prefer to evaluate the final constant by adding standard amounts of hemoglobin to plasma. A useful procedure consists of adding 1 ml. of a diluted standard hemoglobin solution to 10 ml. of plasma, followed by adding 1 ml. of 0.50*M* phosphate buffer, and continuing as in the procedure above.

The hemoglobin content of plasma is determined by a procedure comparable to the standardization by substituting a 10-ml.

Table I. Determination of Constants[a]

Absorptivity = $a = \frac{\log_{10} 1/T}{bc} = \frac{A}{bc}$

b = 1 cm.
c = mg. of hemoglobin, as shown

Hemoglobin, Mg.	a^{MHb} × 10^{-3}	a^{CMHb} × 10^{-3}	$a^{CMHb} - a^{MHb}$ × 10^{-3}
1.24	3.06	5.80	2.72
	3.06	5.80	2.72
	3.06	5.80	2.72
2.48	3.10	5.72	2.62
	3.04	5.80	2.76
	3.06	5.72	2.66
	3.06	5.76	2.70
4.96	3.05	5.64	2.59
	3.03	5.58	2.55
	3.08	5.64	2.56
	3.06	5.66	2.60
9.92	3.03	5.55	2.52
	3.03	5.50	2.47
	3.01	5.59	2.58
	3.03	5.57	2.54
		Mean	2.62

[a] 1-cm. cell, 540 mμ, 10.0 ml. of hemoglobin standard + 2.0 ml. of buffer (+ ferricyanide, then cyanide). See text.

Table II. Recovery of Added Hemoglobin[a]

Sample, Mg.	$A^{CMHb} - A^{MHb}$	Hemoglobin, Mg.	Recovery, %
Water	(0.021)	...	...
1.24	0.033	1.25	100.8
	0.031	1.18	95.2
	0.031	1.18	95.2
2.48	0.065	2.47	99.6
	0.064	2.43	98.0
	0.062	2.36	95.2
4.96	0.130	4.94	99.6
	0.133	5.05	101.7
	0.127	4.83	97.5
9.92	0.247	9.80	98.8
	0.248	9.85	99.3
	0.249	9.89	99.7

[a] 5 ml. of plasma No. 2093 + 5 ml. of hemoglobin standard as shown, 1-cm. cell, 540 mμ.

Table III. Recovery of Added Hemoglobin[a]

Sample, Mg.	$A^{CMHb} - A^{MHb}$	Hemoglobin, Mg.	Recovery, %
Water	(0.005)	...	...
1.24	0.035	1.33	107.2
	0.030	1.14	92.1
	0.033	1.25	101.2
2.48	0.062	2.36	95.2
	0.063	2.40	96.8
	0.058	2.21	89.2
4.96	0.123	4.68	94.5
	0.120	4.57	92.3
	0.128	4.87	98.3

[a] 5 ml. of plasma No. 1492 + 5 ml. of hemoglobin standard as shown, 1-cm. cell, 540 mμ. Plasma 1492 was highly colored, but gave a negative benzidine test and zero hemoglobin by present method.

aliquot of the plasma for the standard hemoglobin solution. The hemoglobin concentration is then determined by a calculation employing Equation 2:

$$\text{Hemoglobin concentration} = \frac{A^{\text{CMHb}} - A^{\text{MHb}}}{a^{\text{CMHb}} - a^{\text{MHb}}} \qquad (2)$$

RESULTS OBTAINED AND DISCUSSION

The method has been used on many samples of plasma. In Table II are shown the results obtained from a study typical of the recovery of added hemoglobin. Plasma No. 2093 contained about 14 mg. % hemoglobin as evaluated by the present method. The absorbance differences shown in the table for the samples containing added hemoglobin were calculated by subtracting the observed absorbance of methemoglobin from that for cyanmethemoglobin, followed by subtraction of the absorbance difference of the plasma, in this case, 0.021. In the concentration range studied, the observed absorbance was from 0.125 to 0.658. On this basis, one would judge that the present method is about one tenth as sensitive as the benzidine method. Recoveries ranged from 95.2 to 101.7%; these are significantly greater than those reported by Creditor (*1*), particularly for the lesser dilutions of plasma. Greater precision and accuracy are noted in the higher concentrations of hemoglobin.

In Table III are shown the results of a study of the recovery of hemoglobin added to a particular lot of plasma, which was highly colored and caused some question to be raised by others in this laboratory as to the accuracy of this method. However, the plasma gave a negative test with benzidine and essentially zero hemoglobin concentration by the present method. This same problem has not occurred since, although the method has been used on hundreds of samples of plasma. Reference was made to it, simply to indicate that even in this unusual case recoveries of added hemoglobin were better than with other methods. A qualitative test for bile pigments was found to be strongly positive, and this may account for the lowered recovery and loss in precision of the method.

The only other trouble encountered with this method was associated with the aging of irradiated liquid plasma during a storage period at a specified temperature of 26° to 30° C. Over a period of 2 to 3 months the plasma gradually changes in color, and these changes are associated with a gradual lowering of the hemoglobin as determined by this method. Values obtained by a benzidine method [Karr and Chornock (*3*), modified], although varied, show no significant change with aging of the sample. This disadvantage is of no great concern, as the hemoglobin test is performed at the time of the preparation of this plasma product, and these conditions are peculiar only to this product.

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RECEIVED for review February 3, 1955. Accepted October 21, 1955. Division of Biological Chemistry, 126th Meeting, ACS, New York, September 1954.

Spectrophotometric Studies of Some 2,4-Dinitrophenylhydrazones

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A spectrophotometric study of forty 2,4-dinitrophenylhydrazones was initiated to determine if sufficient information was available from the infrared and ultraviolet spectra to classify the parent carbonyl compound. The ultraviolet and visible spectra of these derivatives in neutral and in basic solution provided information as to the aliphatic, aromatic, or olefinic character of the parent carbonyl compound. It was possible to determine whether the original compound was an aliphatic aldehyde or ketone by a time study of the deterioration of the color formed by the derivative in alcoholic base. The infrared spectra of these same derivatives as potassium bromide disks revealed that the position of the N—H stretching band indicated whether the parent compound was an aldehyde or ketone. The aliphatic or aromatic character could be determined with reasonable certainty by examination of the C—H stretching region. Olefinic and furanic derivatives were found to have characteristic bands which facilitated their identification.

THE use of 2,4-dinitrophenylhydrazine as a specific reagent for carbonyl compounds has been known for some time (*3*). Recently the problem of separation and identification of the 2,4-dinitrophenylhydrazones has received considerable attention. Chromatography (*2, 12, 19*) has been used for the separation of these derivatives, and ultraviolet studies in neutral solutions (*4, 9, 11, 18*) and in basic solutions (*11, 15*) have been used with some degree of success for identification. Recently, Mendelowitz and Riley (*10*) have reported that the location of the absorption maximum in a basic solution of fatty acid ketone 2,4-dinitrophenylhydrazones is influenced by the structure of the parent compound. Infrared studies (*13*) have offered further refinement in the problem of identification.

This paper reports observations obtained during a systematic spectrophotometric study of a series of aliphatic, olefinic, aromatic, and heterocyclic aldehyde and ketone 2,4-dinitrophenylhydrazones.

It was found that the information afforded by the ultraviolet and visible spectra of the 2,4-dinitrophenylhydrazones in neutral solution and in basic solution presented a means of differentiating the type of parent carbonyl compound. A time study of the deterioration of the color of the 2,4-dinitrophenylhydrazone in alcoholic base provided further information as to the structure of the parent carbonyl compound.

During the investigation of these derivatives by infrared spectroscopy, the relatively new potassium bromide technique (*14, 17*) was found to offer several distinct advantages over the Nujol mull technique. The absorption bands were sharper and the usual C—H bands attributed to Nujol were eliminated. This facilitated the location and evaluation of the relative intensities of such bands as the N—H stretching at 3.0 to 3.15 microns (3333 to 3279 cm.$^{-1}$), the phenyl C—H stretching at 3.25 microns (3076 cm.$^{-1}$), and the aliphatic C—H stretching at 3.45 to 3.55 microns (2899 to 2817 cm.$^{-1}$). Bands in the 6.85- and 7.25-micron (1460 and 1379 cm.$^{-1}$) region were readily apparent.

REAGENTS

2,4-Dinitrophenylhydrazine solution, 0.25*M*, was prepared according to the method of Johnson (*8*).

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The physical constants of the parent carbonyl compound were compared with those reported in the literature. If the agreement was poor, recrystallization or distillation was employed until the constants agreed with the reported values.

Chloroform, Baker's analytical reagent grade.

Alcoholic sodium hydroxide was prepared by dissolving 10 grams of reagent grade sodium hydroxide in 100 ml. of distilled water. The solution was made up to 1 liter with 95% ethanol, allowed to stand overnight, and filtered to remove any insoluble carbonate.

EXPERIMENTAL

Preparation of Derivatives. Two equivalents of the carbonyl compound were added dropwise to one equivalent of the 2,4-dinitrophenylhydrazine reagent with stirring at room temperature. If the carbonyl compound was a solid, it was dissolved in a minimum quantity of reagent grade methanol or 95% ethanol and added as described. After stirring for 1 hour, the mixture was filtered and the precipitate washed thoroughly with hot water to remove any excess phosphoric acid (*8*). The precipitate was recrystallized twice from a suitable solvent, and the product examined by paper chromatography (*16*) to determine the purity of the derivative. If two compounds were present, recrystallization was continued until the 2,4-dinitrophenylhydrazone was chromatographically pure. The derivative was then recrystallized to a constant melting point using several different solvents.

The melting points of the derivatives used for this investigation are included in Table I.

ULTRAVIOLET AND VISIBLE SPECTRA

Measurement of Spectra. The 2,4-dinitrophenylhydrazone was weighed on an analytical balance and sufficient chloroform was added to give a concentration of approximately 2×10^{-4} gram per ml. An aliquot of this solution was diluted so that the final concentration was approximately 10 γ per ml. An ultraviolet spectrum was obtained from this solution using 1-cm. silica cells in a Cary Model 11 recording spectrophotometer.

To obtain the basic solution spectrum, an aliquot of the original chloroform solution was diluted with 0.25N ethanolic sodium hydroxide. The final concentration of this solution was approximately 10 γ per ml. The solution was shaken for 10 seconds and the spectrum obtained immediately using the same proportions of chloroform and 0.25N ethanolic sodium hydroxide as reference. Chloroform in concentrations of 10% or less had little or no effect on the basic solution spectra.

To study the diminution of color intensity, the derivative was mixed with alcoholic base as previously described and placed in a 1-cm. silica cell with a ground-glass top, and a spectrum from 350 to 800 mμ was obtained every 15 minutes.

DISCUSSION OF RESULTS

Neutral Solutions. In general, the aliphatic ketone dinitrophenylhydrazones had absorption maxima that appeared at 364 to 367 mμ and aliphatic aldehyde derivatives had peaks at 344 to 358 mμ. Figure 1 illustrates the spectra obtained from the derivatives of hexanal, 2-undecanone, and 4-(2-furyl)-3-butene-2-one. No gross difference between the spectra within any one class could be observed, as shown by hexanal and 2-undecanone 2,4-dinitrophenylhydrazones, where lengthening of

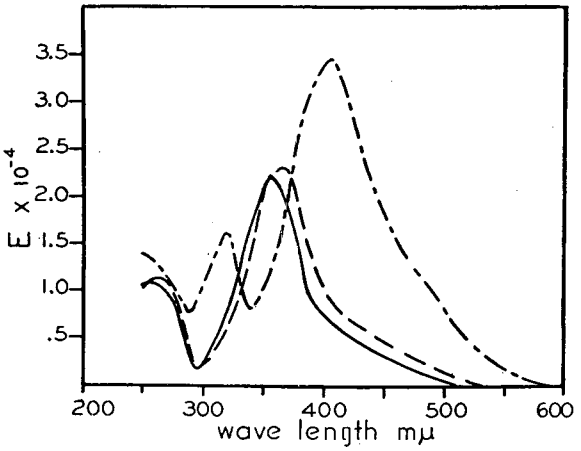


Figure 1. Absorption spectra of 2,4-dinitrophenylhydrazones in chloroform

—— Hexanal
--- 2-Undecanone
—·—·— 4-(2-Furyl)-3-buten-2-one

Table I. Physical Constants of Derivatives

Compound	M.P., °C. (Uncorr.)	Neutral[a] λMax, mμ	Neutral $E \times 10^{-4}$	Basic[a] λMax	Basic $E \times 10^{-4}$ at "0 Time"
Aliphatic Ketone Dinitrophenylhydrazones					
Acetone	126	364	2.24	431, 530	2.00
2-Butanone	113	365	2.16	430, 532	2.17
3-Pentanone	155	365	2.18	430, 530	2.16
4-Heptanone	75	367	2.25	432, 530	2.26
3-Methyl-2-butanone	124	363	2.27	430, 535	2.21
2-Nonanone[b]	54	365	2.25	432, 530	2.37
2-Undecanone	63	365	2.27	431, 534	2.22
2-Tridecanone	69	365	2.27	434, 535	2.39
2-Heptadecanone[c]	69	365	2.29	431, 535	2.40
2-Nonadecanone[d]	82	365	2.25	432, 535	2.36
1,3-Diphenyl-2-propanone	108	364	2.40	440, 526	2.54
Aliphatic Aldehyde Dinitrophenylhydrazones					
Formaldehyde	163	344, 260	1.90	430, 520, 264	1.50
Acetaldehyde	167	354, 260	2.22	430, 520, 270	2.25
Propionaldehyde	147	356	2.25	438, 520, 272	2.25
Butyraldehyde	114	358	2.10	426, 520, 274	2.04
Isobutyraldehyde	181	357, 262	2.18	428, 523, 270	2.51
Hexanal	104	358	2.21	430, 520, 258	2.30
Hydrocinnamaldehyde	156	358	2.28	435, 520	2.50
Phenylacetaldehyde	122	355	2.25	435, 515	2.43
Olefinic Aldehyde and Ketone Dinitrophenylhydrazones					
Acrolein	166	368	2.72	459	2.86
Tiglaldehyde	215	376	3.00	458, 258	2.89
Crotonaldehyde	188	373	2.72	452	3.20
Mesityl oxide	198	385	2.43	452, 250, 530[e]	2.62
4-Hydroxy-4-methyl-2-pentanone[f]	198	385	2.39	452, 250, 525[e]	2.65
Aromatic Aldehyde Dinitrophenylhydrazones					
Cinnamaldehyde	248	390, 308	3.88	486	4.26
Benzaldehyde	234	378	2.83	462	3.33
p-Dimethylamino-benzaldehyde	237	434, 322	3.02	478, 342	3.88
o-Hydroxy-benzaldehyde	252	381	2.96	475	3.53
p-Methoxy-benzaldehyde	249	390	3.09	460, 270	3.33
Aromatic Ketone Dinitrophenylhydrazones					
Benzophenone	235	386	3.06	490, 300	2.32
Chalcone	248	399, 310	3.74	508, 290	2.97
p-Methylacetophenone	256	382	2.73	458, 525[e]	2.27
2,4-Dimethyl-acetophenone	175	376	2.54	458, 530[e]	2.63
4-Phenyl-3-buten-2-one	220	392, 306	3.62	488, 288	3.82
Furan Aldehyde and Ketone Dinitrophenylhydrazones					
2-Furaldehyde (yellow form)	212	379, 298, 264	2.77	475, 270	2.74
2-Furaldehyde (red form)	225	386, 302, 261	2.65	468, 270	2.62
5-Methyl-2-furaldehyde	211	389, 302, 267	2.62	478, 278	2.72
2-Furanacrylaldehyde[g]	215	400, 322	3.79	490, 310	4.10
4-(2-Furyl)-3-buten-2-one[h]	236	405, 319	3.53	490, 300	3.54
β-2-Furylacrylophenone[i]	167	404, 326, 266	4.35	512, 300	3.94

[a] Underlined wave lengths denote major maxima.

	N Calcd., %	N Found, %
[b]	17.34	17.53
[c]	12.87	12.09
[d]	12.18	12.33
[h]	17.42	17.64
[i]	14.81	14.70

[e] Inflection.

[f] Connolly reports that this compound dehydrates to the mesityl oxide derivative (*7*). Infrared and ultraviolet spectra confirm this.

[g] Found, %			Calcd., %		
N	H	C	N	H	C
17.43	3.73	51.72	18.53	3.32	51.60

the carbon chain did not measurably affect the range of absorption maxima or the extinction coefficient.

Compared to their saturated counterparts, the spectra of α, β-unsaturated ketone and aldehyde derivatives showed bathochromic shifts. The molar extinction coefficients did not show any appreciable change, which is in accord with the results reported by Braude and Jones (*4*) and Johnson (*9*).

As previously reported (*9*), the aromatic ketone derivatives had λ_{max} at 382 to 392 mμ, while the derivatives of aromatic aldehydes exhibited maximum absorption between 378 and 390 mμ.

The 2,4-dinitrophenylhydrazones of unsaturated side chain aromatic carbonyls and para-substituted aromatic compounds had molecular extinction coefficients above 3.0×10^4. Aromatic carbonyl derivatives with saturated side chains were found to have values below 3.0×10^4.

The proximity of the carbonyl group to the aromatic nucleus appeared to influence the location of the absorption maxima—for example, benzophenone dinitrophenylhydrazone showed peak absorption at 387 mμ. However, the derivatives of 1,3-diphenyl-2-propanone and phenylacetaldehyde showed maximum absorption at 363 and 355 mμ, respectively. Thus, those aromatic carbonyls in which the aromatic nucleus was separated from the carbonyl group by a saturated side chain formed derivatives that exhibited spectral characteristics generally associated with aliphatic dinitrophenylhydrazones. For this reason, these compounds have been classed as "substituted aliphatics." These compounds also follow the general behavior of aliphatic carbonyl derivatives in basic solution.

A bathochromic shift was found to occur with substitution on the aromatic nucleus in the para position. A study of the data reported here and in the literature (*4*, *9*) showed that this shift occurred in the following order: halogen < hydroxy < methoxy < amino < dialkylamino. Para-substitution of the dialkylamino group on the aromatic nucleus causes such a large bathochromic shift (*9*) that these derivatives were readily recognized from their spectra. With the exception of this particular type of compound, however, para-substitution on the aromatic nucleus caused a bathochromic shift in λ_{max} of the same order of magnitude as did unsaturation in the carbonyl side chain.

The 2,4-dinitrophenylhydrazones of carbonyl compounds containing a furan nucleus were found to have absorption maxima within the limits of 380 to 404 mμ. Unsaturation in the side chain caused a bathochromic shift, as did substitution on the furan nucleus. This was similar to the observations obtained for aromatic carbonyl derivatives. However, as shown in Figure 1, there was a second well defined maximum which appeared at wave lengths 78 to 87 mμ shorter than the principal maximum. This second maximum is not present in the spectra of other derivatives and appeared to be characteristic of the furan 2,4-dinitrophenylhydrazones.

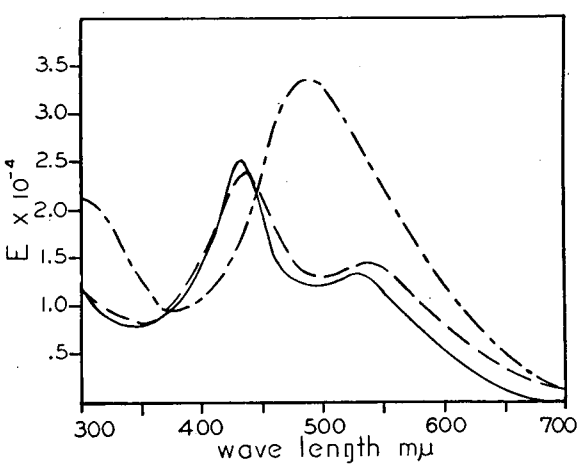


Figure 2. Absorption spectra of 2,4-dinitrophenylhydrazones measured immediately after mixing with 0.25*N* sodium hydroxide

—— Hexanal
— - — - 4-(2-Furyl)-3-buten-2-one
— — — 2-Undecanone

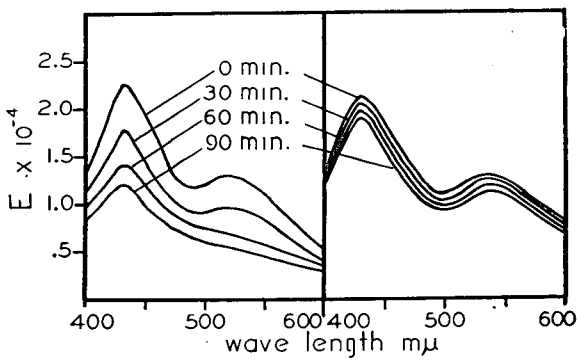


Figure 3. Decrease of *E* with time of a representative aldehyde and ketone 2,4-dinitrophenylhydrazone

Left. Hexanal *Right.* 2-Undecanone

Basic Solutions. In a solution of 0.25*N* ethanolic sodium hydroxide, the spectra of the 2,4-dinitrophenylhydrazones were radically different from those obtained in neutral solutions. Figure 2 illustrates the large bathochromic shifts that occurred in basic solutions. In addition it was noted that the color produced by the addition of base to the derivative faded with time.

A time study of the derivatives investigated revealed that the color produced with aliphatic aldehyde 2,4-dinitrophenylhydrazones faded more rapidly than the colors exhibited by other derivatives. Formaldehyde 2,4-dinitrophenylhydrazone exhibited the most rapid diminution of color, decreasing 50% in 15 minutes. The phenylacetaldehyde derivative produced the most stable color but still lost 30% of its original color at the end of 1 hour. The results of this time study offered further proof that the derivatives of phenylacetaldehyde and hydrocinnamaldehyde should be considered "substituted" aliphatic derivatives.

The aliphatic and substituted aliphatic aldehyde 2,4-dinitrophenylhydrazones showed maximum absorption between 426 and 438 mμ. In addition to this band, there was a small discrete maximum located at 520 to 535 mμ, which disappeared with the passage of time. As illustrated in Figure 3 with hexanal 2,4-dinitrophenylhydrazone, the position of λ_{max} did not change with time.

The small discrete maximum at 520 to 535 mμ disappeared within 60 to 90 minutes in the aliphatic aldehyde derivatives examined.

The color produced by the addition of base to the aliphatic ketone derivatives was more stable than that observed for the aldehyde derivatives. Further, the small discrete maximum found at 520 to 535 mμ did not disappear with time, as illustrated by the derivative of 2-undecanone in Figure 3. The derivative of the substituted aliphatic ketone, 1,3-diphenyl-2-propanone, deteriorated the fastest in base and showed a 10% decrease in color intensity in 90 minutes. The derivative of 2-pentanone produced the most stable color of the aliphatic ketone class, showing a decrease of only 2% in 90 minutes.

The aliphatic ketone dinitrophenylhydrazones in basic solutions were found to have their absorption maxima between 431 and 444 mμ in addition to the smaller maxima located at 520 to 535 mμ. However, the stability of the 520- to 535-mμ band has been found to be the deciding factor in differentiating between aliphatic aldehyde and ketone derivatives.

The most stable color was produced by the reaction of base and those derivatives in which the parent compound contained

a conjugated carbonyl group. Such derivatives as benzaldehyde, acrolein, and 2-furaldehyde 2,4-dinitrophenylhydrazones exhibited only a slight decrease in color even at the end of 3 hours and are illustrated in Figure 4 as "unsaturated DNPH." However, the position of λ_{max} was found to be of value in classifying these derivatives.

Aromatic aldehyde derivatives were found to have λ_{max} from 462 to 486 mμ. Aromatic ketones had λ_{max} at 452 to 508 mμ with an inflection appearing at ca. 520 to 530 mμ. In the compounds investigated this inflection did not appear if the maximum absorption occurred above 460 mμ. Aromatic ketone hydrazones which exhibited maxima beyond 460 mμ contained unsaturated side chains and it is possible that the region 520 to 530 mμ was obscured, since the absorption band is broad.

Furan-containing 2,4-dinitrophenylhydrazones had a major absorption band at 468 to 490 mμ and had secondary maxima at wave lengths that were 190 to 212 mμ shorter than λ_{max}, as illustrated by the derivative of 4-(2-furyl)-3-buten-2-one in Figure 2.

The 2,4-dinitrophenylhydrazone of 2-furaldehyde has been reported to exist as a mixture of the so-called yellow (cis) and red (trans) forms (*5*, *6*). The two isomers were separated by recrystallizing the derivative from xylene. As the red form was more insoluble in hot xylene than the yellow, separation was easily accomplished.

The two isomers of the 2-furaldehyde derivative gave spectra which exhibited a reversal in the position of λ_{max} from the values obtained in neutral spectra. In basic solution the red form and the yellow form had absorption maxima at 468 and 476 mμ, respectively (in neutral solution λ_{max} appeared at 386 and 381 mμ, respectively).

Certain general trends noted in the spectra of neutral solutions are followed in basic solution spectra. As the degree of unsaturation increased, a bathochromic shift was apparent. The number of carbon atoms in the chain affected λ_{max} only slightly in the aliphatic aldehyde and ketone derivatives.

The results of the time study carried out in the course of this investigation are summarized in Figure 4 as a ratio of E_{max} at time t to E_{max} at zero time. The 2,4-dinitrophenylhydrazone of formaldehyde has been omitted from the "aliphatic aldehyde DNPH" group, because the diminution of color is so rapid as to be positive identification in itself.

The absorption maxima and molecular extinction coefficients of the various derivatives examined are listed in Table I. The extinction coefficients indicated for the basic solution spectra are calculated from the absorption values obtained at "0 time."

Table II. Corrected N—H Absorption Bands for Dinitrophenylhydrazones

Ketones	Microns	Cm.$^{-1}$	Aldehydes	Microns	Cm.$^{-1}$
3-Methyl-2-butanone	3.00	3333	Formaldehyde	3.04	3289
Acetone	3.01	3322	Butyraldehyde	3.04	3289
2-Butanone	3.01	3322	Hexanal	3.04	3289
3-Pentanone	3.01	3322	Acetaldehyde	3.05	3279
4-Heptanone	3.01	3322	Propionaldehyde	3.05	3279
2-Nonanone	3.01	3322	Isobutyraldehyde	3.05	3279
2-Undecanone	3.01	3322	Hydrocinnamaldehyde	3.05	3279
2-Tridecanone	3.01	3322	Phenylacetaldehyde	3.05	3279
2,4-Dimethyl acetophenone	3.01	3322	Acrolein	3.05	3279
1,3-Diphenyl-2-propanone	3.01	3322	Benzaldehyde	3.05	3279
2-Heptadecanone	3.02	3311	2-Furaldehyde (red)	3.05	3279
2-Nonadecanone	3.02	3311	5-Methyl-2-furaldehyde	3.05	3279
Mesityl Oxide	3.03	3300	Crotonaldehyde	3.06	3270
4-Phenyl-3-buten-2-one	3.03	3300	Cinnamaldehyde	3.06	3270
4-(2-Furyl)-3-buten-2-one	3.03	3300	*p*-Dimethylaminobenzaldehyde	3.06	3270
β-2-Furylacrylophenone	3.03	3300	*o*-Hydroxybenzaldehyde	3.06	3270
4-Hydroxy-4-methyl-2-pentanone	3.03	3289	*p*-Methoxybenzaldehyde	3.06	3270
Chalcone	3.04	3289	2-Furanacrylaldehyde	3.06	3270
p-Methyl acetophenone	3.04	3289	2-Furaldehyde (yellow)	3.07	3257
Benzophenone	3.05	3279	Tiglaldehyde	3.07	3257

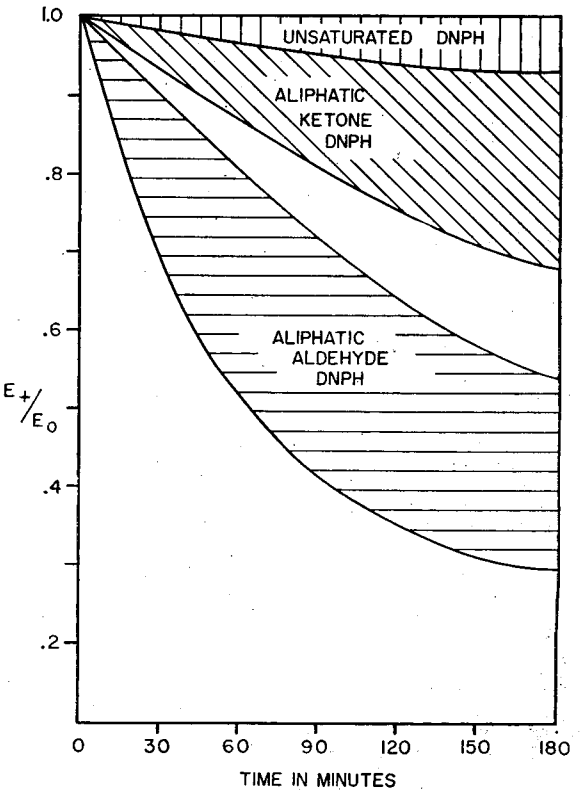


Figure 4. Change of E_{max} with time for indicated classes of 2,4-dinitrophenylhydrazones

INFRARED

Measurement of Spectra. The spectra of the 2,4-dinitrophenylhydrazones from 2 to 15 microns (5000 to 666 cm.$^{-1}$) were obtained with a Perkin-Elmer Model 21 infrared spectrophotometer equipped with sodium chloride optics.

The potassium bromide was of reagent grade and obtained from the Fisher Scientific Co. It was used without further purification and stored in a vacuum desiccator over silica gel when not in use.

The disks were prepared by grinding approximately 15 mg. of the sample in 200 mg. of potassium bromide for 10 minutes. The resulting fine powder was placed in a simple die consisting of a Lucite shell and two stainless steel plungers. This was then pressed in a Carver laboratory press to a gage pressure of 23,000 pounds. After pressing for 5 minutes, the disk was removed and a spectrum obtained using a 10-mesh wire screen in the reference beam.

The instrument was calibrated using atmospheric water vapor as a standard as prescribed by the manufacturer. The values in this paper have been corrected accordingly. The reproducibility of the instrument was found to be ±0.01 micron.

The band positions of the 2,4-dinitrophenylhydrazones are shown in Figure 5.

DISCUSSION OF RESULTS

Examination of the spectra revealed that considerable information could be obtained from the short-wave-length region from 3 to 4 microns (3333 to 2500 cm.$^{-1}$). The N—H stretching band was usually found near 3.05 microns (3279 cm.$^{-1}$) in the 2,4-dinitrophenylhydrazones. The exact position of this band appeared to be dependent on the structure of the parent carbonyl component. For example, the N—H absorption for 3-pentanone 2,4-dinitrophenylhydrazone appeared at 3.01 microns (3322 cm.$^{-1}$), while for the hexanal derivative it was located at 3.04

microns (3289 cm.$^{-1}$). In general, the N—H absorption for ketone 2,4-dinitrophenylhydrazones appeared at shorter wave lengths than 3.04 microns (3289 cm.$^{-1}$). Aldehyde 2,4-dinitrophenylhydrazones exhibited this band at longer wave lengths. However, when the parent carbonyl compound contained a phenyl or furan nucleus, the N—H absorption band of ketone derivatives approached 3.04 microns (3289 cm.$^{-1}$) and differentiation of these aldehydes and ketones became more difficult. The position of the N—H stretching band of the 2,4-dinitrophenylhydrazones investigated is listed in Table II.

The C—H stretching absorption found in the 3.25- to 3.50-micron region (3077 to 2857 cm.$^{-1}$) gave information as to the aliphatic or aromatic character of the parent carbonyl compound. The phenyl C—H stretching found around 3.20 microns (3125 cm.$^{-1}$) was present in all the derivatives investigated and could be attributed to the 2,4-dinitrophenyl portion of the hydrazone. There did not appear to be any increase in the intensity of this band as the number of phenyl rings in the derivative increased.

The aliphatic C—H stretching found near 3.50 microns (2857 cm.$^{-1}$) could be used with some degree of certainty to classify the type of parent carbonyl compound. Aliphatic 2,4-dinitrophenylhydrazones containing more than four carbons in the parent compound showed a definite aliphatic C—H stretching absorption as shown in Figure 6. As the number of aliphatic carbons increased, the intensity of this band also increased.

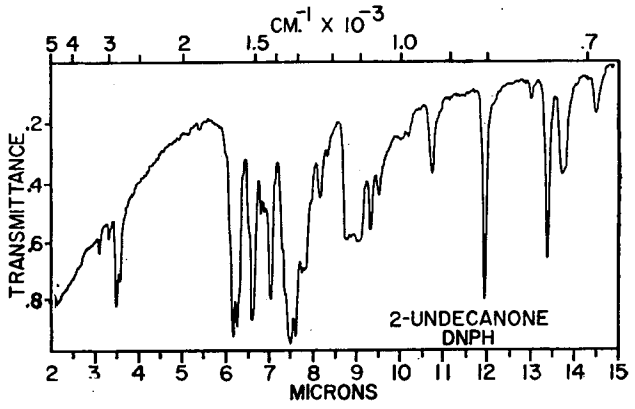


Figure 6. Infrared spectrum of 2-undecanone 2,4-dinitrophenylhydrazone as potassium bromide disk

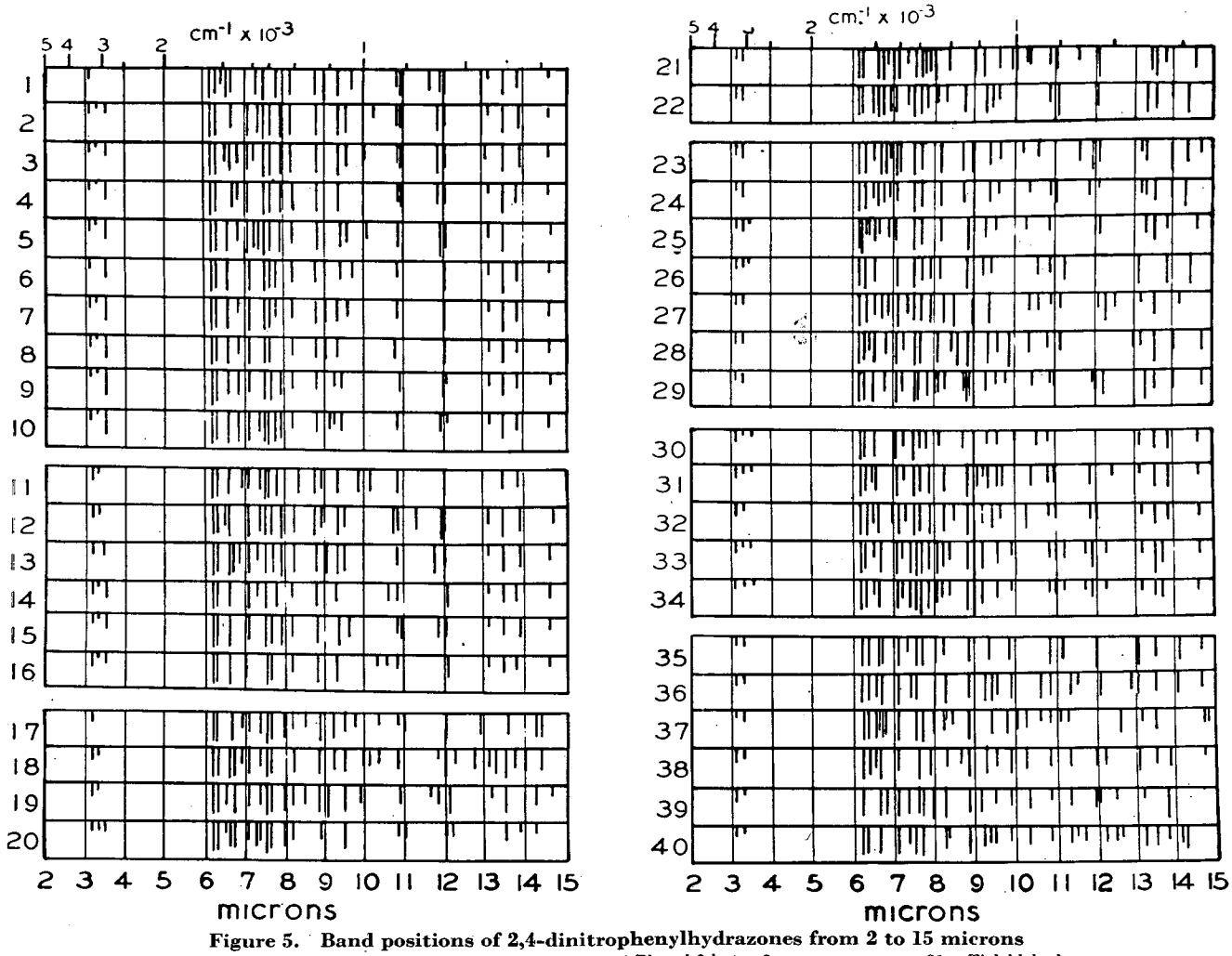


Figure 5. Band positions of 2,4-dinitrophenylhydrazones from 2 to 15 microns

1. Acetone
2. 2-Butanone
3. 3-Pentanone
4. 4-Heptanone
5. 3-Methyl-2-butanone
6. 2-Nonanone
7. 2-Undecanone
8. 2-Tridecanone
9. 2-Heptadecanone
10. 2-Nonadecanone
11. Formaldehyde
12. Acetaldehyde
13. Propionaldehyde
14. Butyraldehyde
15. Isobutyraldehyde
16. *n*-Hexanal
17. Benzophenone
18. Chalcone
19. *p*-Methylacetophenone
20. 2,4-Dimethylacetophenone
21. 4-Phenyl-3-buten-2-one
22. 1,3-Diphenyl-2-propanone
23. Hydrocinnamaldehyde
24. Phenylacetaldehyde
25. Cinnamaldehyde
26. Benzaldehyde
27. *p*-Dimethylaminobenzaldehyde
28. *o*-Hydroxybenzaldehyde
29. *p*-Methoxybenzaldehyde
30. Acrolein
31. Tiglaldehyde
32. Crotonaldehyde
33. Mesityl oxide
34. 4-Hydroxy-4-methyl-2-pentanone
35. 2-Furaldehyde (yellow form)
36. 2-Furaldehyde (red form)
37. 5-Methyl-2-furaldehyde
38. 3-(2-furyl)-acrylaldehyde
39. 4-(2-furyl)-3-buten-2-one
40. β-2-Furylacrylophenone

The region from 3.5 to 6.0 microns (2857 to 1667 cm.$^{-1}$) offered little or no information. Occasionally there appeared a series of small bands of low intensity between 5 and 6 microns (2000 and 1667 cm.$^{-1}$). These bands are generally associated with the phenyl ring overtones (*20*) and did not lend themselves to any characterization of the derivatives.

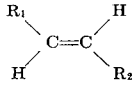
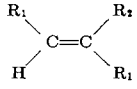
From 6 to 15 microns (1667 to 666 cm.$^{-1}$) the spectra of all the derivatives examined became more complex and characteristic. However, certain bands were found to be common to all spectra. The phenyl C = C skeletal in-plane stretching vibrations were found at 6.17 microns (1621 cm.$^{-1}$), 6.27 microns (1595 cm.$^{-1}$), and 6.57 microns (1522 cm.$^{-1}$). The 6.27-micron band (1595 cm.$^{-1}$) has been reported by Bellamy (*1*) to exist as a relatively weak band in aromatic compounds. A possible explanation for the strong intensity of this band in the spectra of these derivatives is the addition of the C = N stretching absorption to the normal C = C band. The band located at 6.57 microns (1522 cm.$^{-1}$) was broader than the other bands attributed to the phenyl C = C stretching vibrations. There is evidence (*1*) that the nitro groups substituted on the phenyl nucleus absorb in this region. This might account for the relatively broad band observed which occasionally split to give a doublet.

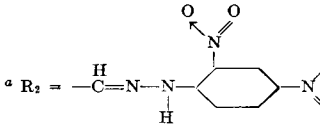
It has been reported that hydrogen bonding will occur readily in compounds containing strong electronegative groups such as nitro groups (*1*). Such bonding causes a broadening of the band associated with the electronegative group as well as shifting of the band to a longer wave length.

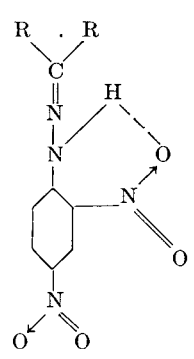
Evidence for hydrogen bonding of the inter- and intramolecular type was found in the 7.50- to 7.75-micron region (1333 to 1290 cm.$^{-1}$). Nitro groups absorb in this region and two prominent bands were attributed to the nitro valence vibrations. The band located at 7.50 microns (1333 cm.$^{-1}$) did not shift in position with a change in the class of the parent carbonyl. Intramolecular hydrogen bonding of the type illustrated in Form I would readily explain such a constancy of position independent of the parent carbonyl structure.

However, the second nitro band located at 7.64 microns (1309 cm.$^{-1}$) did shift with a change in the class of parent carbonyl. Aldehydes were found to cause a shift of this band to longer wave lengths, while ketones had little effect on the band position. It appeared possible that intermolecular hydrogen bonding as shown in Form II could cause a shift of this band in aldehyde 2,4-dinitrophenylhydrazones. Such bonding might also account for the shift of N—H frequency observed in aldehydes.

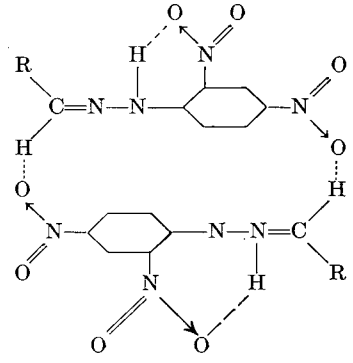
Table III[a]. Characteristic Absorption Bands of Olefinic 2,4-Dinitrophenylhydrazones

Type	Compound Dinitrophenylhydrazone	Band I	Band II
	1. Crotonaldehyde	6.10 μ (1639 cm.$^{-1}$)	10.20 μ (981 cm.$^{-1}$)
	2. Cinnamaldehyde	6.15 μ (1626 cm.$^{-1}$)	10.25 μ (975 cm.$^{-1}$)
	3. 4-Phenyl-3-buten-2-one	None	10.30 μ (971 cm.$^{-1}$)
	4. Chalcone	None	10.35 μ (966 cm.$^{-1}$)
	5. 3-(2-Furyl)-acrylaldehyde	None	10.20 μ (981 cm.$^{-1}$)
	6. 4-(2-Furyl)-3-buten-2-one	None	10.30 μ (981 cm.$^{-1}$)
	7. β-2-Furylacrylophenone	None	10.35 μ (966 cm.$^{-1}$)
	Tiglaldehyde	6.12 μ (1637 cm.$^{-1}$)	10.35 μ (966 cm.$^{-1}$)

[a] R_2 =  ; R_1 = substitution other than H.



Form I



Form II

The 1,2,4-trisubstitution in the dinitrophenyl portion of the 2,4-dinitrophenylhydrazone was characterized by four bands at 8.22 microns (1217 cm.$^{-1}$), 8.80 microns (1136 cm.$^{-1}$), 910 microns (1099 cm.$^{-1}$), and 9.40 microns (1064 cm.$^{-1}$). The latter two bands were shifted somewhat with a change in the structure of the parent carbonyl compound, but no correlation was immediately apparent. Ross (*13*) has reported the strong single or double band located near 12.0 microns to be characteristic of this type of substitution.

The band located in the 11-micron region (909 cm.$^{-1}$) is probably due to the bending vibration of the isolated hydrogen atom in the 3-position. The absorption located at 13.50 microns (740 cm.$^{-1}$) has been reported to be due to the nitro groups (*1*).

In addition to the information that could be obtained from the short-wave-length region, other characteristic bands were found which gave an indication as to the structure of the parent carbonyl compound.

The olefinic character of the parent compound was generally indicated by two bands which appeared near 6.05 microns (1653 cm.$^{-1}$) and 10.25 microns (985 cm.$^{-1}$). The first band sometimes appeared as a "shoulder" or a small well-defined peak on the side of the intense phenyl C=C absorption at 6.17 microns (1621 cm.$^{-1}$). However, further and more definite confirmation was found in the long-wave-length region as shown in Figure 7 by the spectrum of 4-(2-furyl)-3-buten-2-one dinitrophenylhydrazone. In general, if the derivative could exist in the trans form, a band of medium intensity appeared between 10.20 and 10.35 microns (980 and 966 cm.$^{-1}$) (Table III).

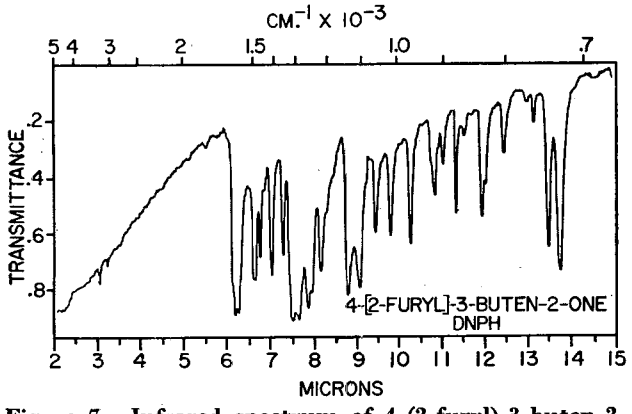


Figure 7. Infrared spectrum of 4-(2-furyl)-3-buten-2-one 2,4-dinitrophenylhydrazone as potassium bromide disk

For aliphatic carbonyl 2,4-dinitrophenylhydrazones, the region from 6 to 7 microns (1667 to 1429 cm.$^{-1}$) was relatively simple. Aromatic and furan-containing compounds gave spectra which were complex in this region, as illustrated by Figures 7 and 8. This complexity aided in the identification of the parent carbonyl compound.

The region from 8 to 15 microns (1250 to 666 cm.$^{-1}$) was more characteristic of the derivatives. The complexity observed for aromatic and furan derivatives in the 6- to 7-micron region (1667 to 1429 cm.$^{-1}$) extended through this region also. Aromatic dinitrophenylhydrazones usually exhibited four strong bands in the 13- to 15-micron region (769 to 666 cm.$^{-1}$), which is in agreement with the results obtained by Ross (*13*) and illustrated by the derivative of phenylacetaldehyde in Figure 8. Aliphatic derivatives were relatively free of absorption bands between 10 and 12 microns (1000 and 833 cm.$^{-1}$), with the exception of the strong band near 11 microns (909 cm.$^{-1}$) previously described. Aromatic and furan-containing derivatives had other bands in this region.

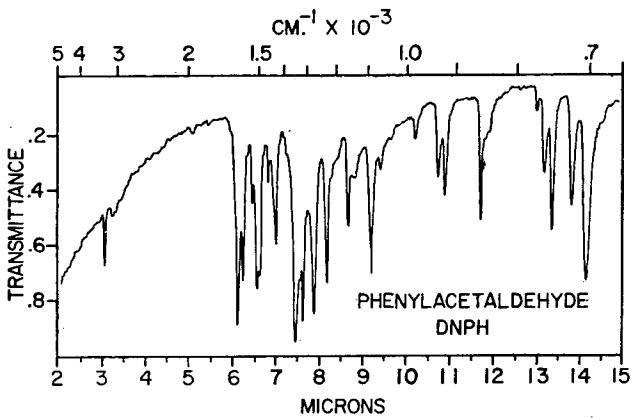


Figure 8. Infrared spectrum of phenylacetaldehyde 2,4-dinitrophenylhydrazone as potassium bromide disk

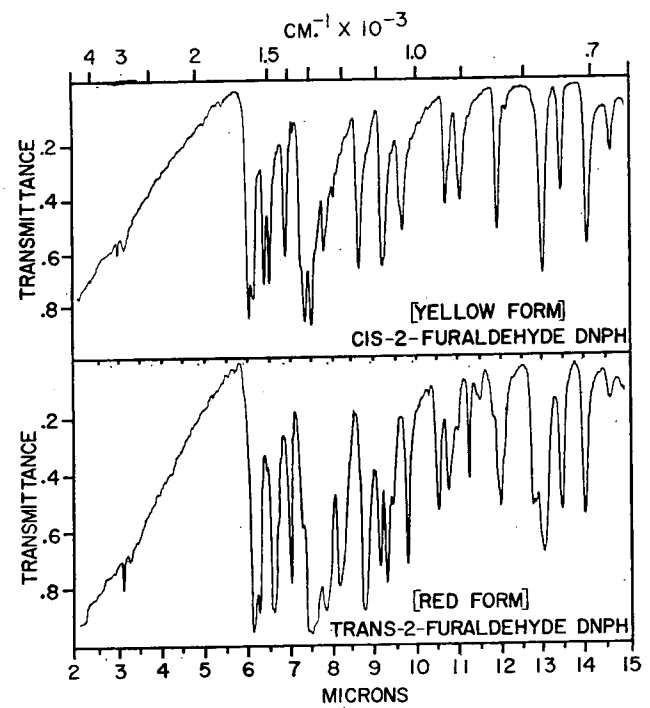


Figure 9. Infrared spectrum of cis- and trans- forms of 2-furaldehyde 2,4-dinitrophenylhydrazone as potassium bromide disks

Of particular interest was the fact that furan-containing derivatives were found to have two unique sharp bands, one at 9.75 to 9.85 microns (1026 to 1015 cm.$^{-1}$) and the other at 11.30 to 11.40 microns (885 to 877 cm.$^{-1}$). These absorptions could probably be attributed to the hydrogen bending vibrations on the furan ring. The two bands permitted easy characterization and, other than these bands, the compounds resembled the spectra of aromatic derivatives.

The infrared spectra of the *cis*- and *trans*-2-furaldehyde 2,4-dinitrophenylhydrazones are presented in Figure 9. The red and yellow forms both exhibit the 9.75- to 9.85-micron (1026 to 1015 cm.$^{-1}$) band, but the red form shows three bands from 10.50 to 11.50 microns (952 to 870 cm.$^{-1}$), while the yellow form has only two bands in the same region.

CONCLUSIONS

By examination of the ultraviolet and visible spectra of both neutral and basic solutions of dinitrophenylhydrazones, it has been found possible to differentiate carbonyl compounds of different structures.

Figure 10 illustrates that the following distinctions can be made when the basic spectra are obtained 90 minutes after mixing the base and derivative.

Aliphatic aldehydes from all other classes studied.
Aliphatic ketones from all other classes studied.
Aromatic aldehydes from all other classes studied, with the exception of olefinic aldehydes.
Aromatic ketones from all other classes studied when the major maximum falls below 460 mμ in basic solution.
Carbonyls containing the furan nucleus from all other classes studied.
Olefinic aldehydes from all other classes studied, with the exception of aromatic aldehydes.

Further qualitative classification can be found in the infrared spectra from the following distinctions.

Most aldehydes can be differentiated from ketones by examination of the N—H stretching absorption from 3.0 to 3.10 microns (3333 to 3225 cm.$^{-1}$).

Aliphatic derivatives can be distinguished from aromatic and furan-containing dinitrophenylhydrazones by examination of the aliphatic C—H stretching band at 3.47 microns (2881 cm.$^{-1}$) and by the lack of complexity in the spectra.

Aromatic dinitrophenylhydrazones can be detected by examination of the 13- to 15-micron region (769 to 666 cm.$^{-1}$) and the lack of aliphatic absorption at 3.47 microns.

Furan-containing derivatives can be differentiated from all others by the presence of two bands, one at 9.75 to 9.86 microns (1026 to 1015 cm.$^{-1}$) and the other at 11.30 to 11.40 microns (885 to 877 cm.$^{-1}$).

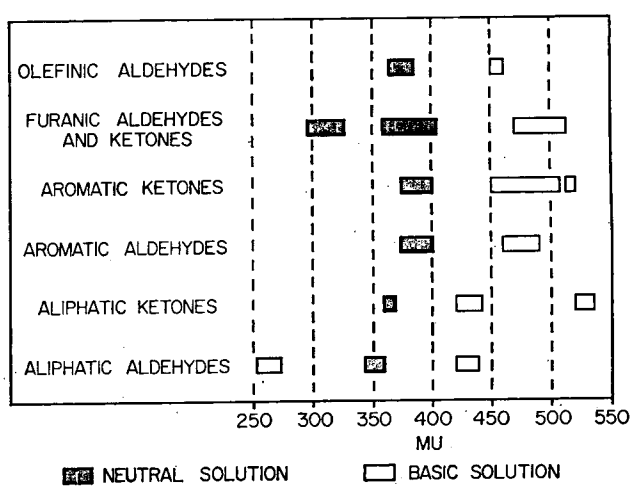


Figure 10. Absorption areas of different classes of 2,4-dinitrophenylhydrazones in neutral and basic solution

Trans-olefinic derivatives can be detected by the presence of a band in the 10.20- to 10.35-micron region (980 to 966 cm.$^{-1}$).

The combined use of ultraviolet, visible, and infrared spectra will permit the assignment of the class to which the parent carbonyl compound belongs. The assignment of this class greatly diminishes the number of infrared spectra which must be compared for positive identification.

ACKNOWLEDGMENT

The authors wish to thank E. K. Chavis, M. D. Edmonds, and F. L. Greene for preparing the solutions and obtaining the spectra and paper chromatograms. Thanks are also due to the members of the Philip Morris Research and Development Department for their many helpful suggestions during the course of this investigation.

The authors wish to express their appreciation to Philip Morris, Inc., for permission to publish this work.

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RECEIVED for review December 17, 1954. Accepted December 8, 1955. Presented in part at the Tobacco Chemists' Research Conference, Richmond, Va., November 11 and 12, 1954.

Spectrophotometric Determination of Iron with 5-Sulfoanthranilic Acid

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Although anthranilic acid has been used for many years as a precipitating agent in quantitative analysis, the present work represents the first use of the sulfonic acid derivative as an analytical reagent. A new method for the spectrophotometric determination of iron has been developed which involves the addition of ferric iron to a large excess of 5-sulfoanthranilic acid at pH 4.0. The absorbance is measured at 455 mμ.

ANTHRANILIC acid forms insoluble complexes with many ions and has been used for the quantitative precipitation and determination of cadmium, zinc, lead, mercury, manganese, cobalt, nickel, and copper (1–4).

Anthranilic acid was sulfonated in order to make the metal complexes soluble in water. This modified anthranilic acid was studied as a reagent for spectrophotometric analysis. It was found that ferric iron forms an intense red complex with the reagent and has an absorbance maximum at 455 mμ (Figure 1). This provided the basis for a convenient spectrophotometric determination of iron that was relatively free of interference by other ions.

Harris and Sweet (*5*) have determined the formation constants of the cobalt, nickel, copper, zinc, and cadmium complexes of 5-sulfoanthranilic acid. These are small, varying from a K_{av} value of 1.19×10^3 for copper to a K_{av} value of 3.76×10^2 for cobalt.

REAGENTS

5-Sulfoanthranilic Acid Solution. The 5-sulfoanthranilic acid was prepared according to the directions that were given in a previous communication (*5*). A 0.1*M* aqueous solution was prepared and was adjusted to pH 4.0 with 0.5*N* sodium hydroxide.

Standard Iron Solution. Primary standard grade iron wire (0.3040 gram) was dissolved in a minimum volume of 1 to 1 nitric acid. The solution was heated on a steam plate to remove excess acid and oxides of nitrogen. Several 10-ml. portions of water were added to prevent the mixture from going to dryness. The solution was diluted to 1 liter with water.

APPARATUS

All pH measurements were made with a Beckman Model G pH meter equipped with microelectrodes.

The absorption measurements were made with a Beckman Model DU quartz spectrophotometer equipped with 1-cm. Corex cells. A slit width of 0.04 was maintained during all the measurements.

PROCEDURE

Add 20 ml. of the sample (containing from 5 to 150 p. p.m. of iron) to 25 ml. of the 5-sulfoanthranilic acid solution and dilute to 50 ml. with water. The pH of the resulting solution should be 4.0. (If the sample solution is highly acidic, it may be necessary to add a larger quantity of the reagent solution or to adjust the pH of the 20-ml. sample to approximately 1.5 to 2 with sodium hydroxide before the addition of the reagent solution.) Measure the absorbance, defined as the function $\log_{10} \frac{I_{blank}}{I_{soln.}}$, at 455 mμ. Use a 0.05*M* reagent solution at pH 4.0 as the blank. Prepare a standard curve in the same manner by using various known aliquots of the standard iron solution.

DISCUSSION

As shown in Figure 2, the absorbance is dependent on the pH and has a maximum value at pH 4.0. The absorbance is nearly constant over the pH range 3.6 to 4.4.

Figure 3 shows the effect of reagent concentration on the absorbance. A final reagent concentration of 0.05*M* was used in the suggested procedure. This concentration was selected for two reasons. The change in absorbance with reagent concentration is very small and the excess 5-sulfoanthranilic acid [with a pH value of 4.70 (*5*)] is a good buffer at pH 4.0, thus eliminating the need of finding another suitable buffer system that does not complex with the iron.

A time study was made on the reagent solution. At measured time intervals, aliquots from the same 5-sulfoanthranilic acid solution were used in the determination of a 38.0 p.p.m. iron

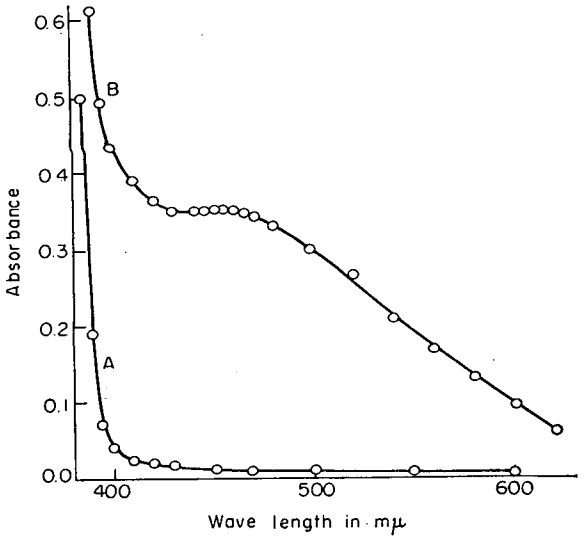

Figure 1. Absorption curves

A. **Solution 0.05*M* with respect to 5-sulfoanthranilic acid at pH 4.0**
B. **Solution $2.72 \times 10^{-4}M$ with respect to iron and 0.05*M* with respect to 5-sulfoanthranilic acid at pH 4.0**

solution. Less than ±1% error was observed over a period of one week.

A time study was made on the complex that formed. A solution was prepared by mixing 20 ml. of a 38.0 p.p.m. iron solution and 25 ml. of the freshly prepared reagent solution and diluting to 50 ml. with water. The absorbance of this solution was measured at intervals. One per cent fading was observed during the period from 10 minutes to 3 days after mixing and an additional 2% fading was noted during the next 4 days.

Table I. Limiting Concentrations of Interfering Ions

Ion	Limiting Concentration, P.P.M.
Chromium	20
Aluminum	250
Nickel	1500
Cobalt	100
Copper	75
Chloride	5000
Sulfate	7500

Standard iron solutions were measured according to the suggested procedure. Beer's law was obeyed over the concentration range 5 to 150 p.p.m. of iron.

Interferences were studied by the addition of known concentrations of various ions to a 38.0 p.p.m. iron solution. The iron solution was then analyzed according to the suggested procedure. Table I shows the limiting concentrations of several interfering ions. This limiting concentration is the concentration of interfering ion that must be present in the iron solution in order to cause a 1% relative error.

Aluminum decreases the absorbance, probably by forming a colorless complex with the reagent. Sulfate ions also decrease the absorbance. All other interfering ions increase the absorbance. The interference of chromium, cobalt, and nickel is caused by the color of these ions. Copper forms a colored complex with the reagent that absorbs to a small extent at 455 mμ, although its absorption maximum is at 700 mμ. Cadmium, zinc, and manganese do not show any interference up to 12,500 p.p.m., the highest concentration that was investigated. Lead shows

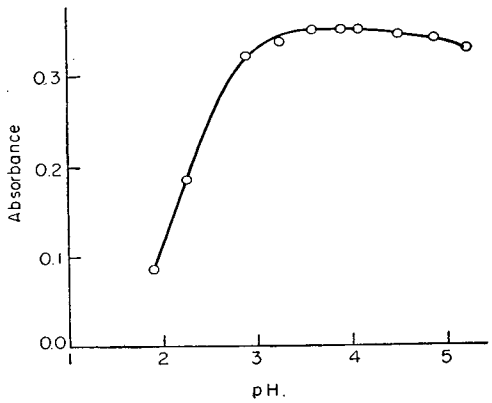

Figure 2. Variation in absorbance with pH

Solutions were $2.72 \times 10^{-4}M$ with respect to iron and 0.05*M* with respect to 5-sulfoanthranilic acid

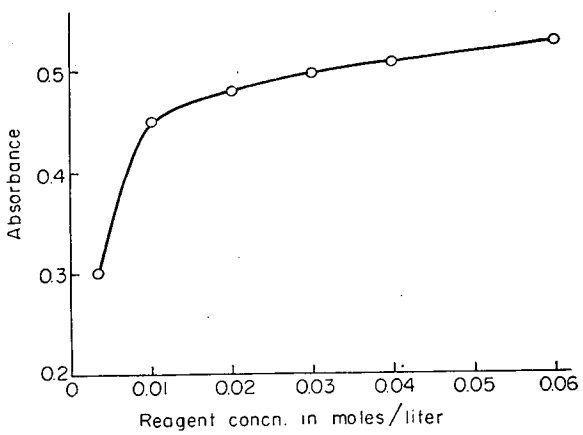

Figure 3. Variation in absorbance with reagent concentration

Iron concentration maintained constant at $2.72 \times 10^{-4}M$

no interference until it forms a precipitate at approximately 2250 p.p.m. which is probably caused by reaction with the sulfonic acid group.

Three samples were prepared in such a way as to contain known concentrations of iron, aluminum, chromium, zinc, cadmium, copper, nickel, and cobalt. These were analyzed for iron according to the suggested procedure. The results are shown in Table II.

The proposed method is not as sensitive as some spectrophotometric determinations of iron, but its wide range of at least 5 to 150 p.p.m. is a distinct advantage and should be useful for many

Table II. Analysis of Known Samples

Metals Present	Concn., P.P.M. Sample 1	Sample 2	Sample 3
Aluminum	4	8	44
Chromium	10	16	2
Zinc	65	130	195
Cadmium	224	112	112
Copper	12	6	31
Nickel	60	60	36
Cobalt	30	30	30
Iron	30.4	30.4	30.4
Iron, experimental value	30.5	30.6	30.5

purposes. Since the concentration limits are given for the iron that is in the 20 ml. of unknown iron sample used in the procedure, the lower limit could be extended by increasing the concentration of 5-sulfoanthranilic acid in the reagent solution and using a volume of unknown iron solution that is larger than 20 ml. By this means it is reasonable to expect that a concentration range limit of 2 to 60 p.p.m. could be approached. In addition, the lower limit probably could be extended significantly by the use of longer cells. This is especially promising, as the absorption of the reagent itself is so small.

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RECEIVED for review October 5, 1955. Accepted November 14, 1955. Taken in part from a thesis presented to the Graduate School of The Ohio State University by James M. Zehner in partial fulfillment of the requirements for the degree of master of science, August 26, 1955.

Infrared Analysis of Paint Vehicles Based on Alkyd-Nitrogen Resin Blends

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No satisfactory chemical method is available for estimating the urea formaldehyde–melamine resin ratio in a paint vehicle comprising both of these in admixture with an alkyd resin. A method based on infrared absorbance measurements at 5.8, 6.1, and 12.25 microns on thin vehicle films has provided a solution of this problem and affords a rapid means for estimating each individual resin component in typical two- and three-component alkyd-nitrogen resin blends. Results obtained on synthetic mixtures of known composition indicate a degree of accuracy and precision sufficient for many practical applications.

COATING resin combinations comprising oil-modified alkyd resins blended with "nitrogen" resins are widely used in the formulation of industrial baking enamels. Classes of nitrogen resins available for such use include butylated urea-formaldehyde (UF) and butylated melamine-formaldehyde (MF). A solution of one or both of these resins in butyl alcohol or butyl alcohol–hydrocarbon solvent is blended with a hydrocarbon solution of the alkyd and appropriate pigments are incorporated. The resulting enamel, when applied as a thin film and baked, undergoes further condensation and cross linking to produce a cured insoluble finish.

The complete analytical characterization of a "wet" sample of such an enamel involves estimation of total pigment, total nonvolatile resin content, total solvent (by difference), and component analysis of each of these three fractions. The present discussion, however, is confined to the problem of quantitative estimation of the individual resinous film-forming components in the vehicle fraction.

The first step in such an analysis involves separation of a sample of the resinous vehicle solution from the dispersed pigment phase by high speed centrifuging. In the case of a two-component resin system (urea-formaldehyde–alkyd or melamine-formaldehyde–alkyd), the nitrogen resin content can be estimated from total nitrogen as determined by ASTM designation D 1013 (*1*) if the nitrogen content of the nitrogen resin present is known or can be assumed. The total alkyd content can then be estimated by difference. Alternatively, if the phthalate content of the alkyd present is known or can be assumed, the total phthalate content of the blend can usually be estimated by an ultraviolet spectrophotometric modification (*5*) of the well-known Kappelmeier procedure (*2–4*) and total alkyd calculated from this value.

In the case of the three-component system (urea-formaldehyde–melamine-formaldehyde–alkyd), total alkyd may again be estimated from total phthalate as determined by the spectrophotometric modification of the Kappelmeier method. Total nitrogen resin content may then be estimated by difference or approximated from nitrogen content. Estimation of the relative amounts of the two nitrogen resins in such a system, however, poses a special problem. Total nitrogen does not afford a satisfactory basis for such an estimate, as the individual resins do not differ sufficiently in nitrogen content. The authors are aware of no previously published method suitable for this purpose.

Infrared spectrophotometry provides a solution to this problem and makes possible the rapid quantitative estimation of each resin component in typical two- and three-component blends. The method is based on infrared absorbance measurements at selected wave lengths where the various resins exhibit unique absorption bands. Because butyl alcohol and hydrocarbon solvents interfere and removal of solvent followed by re-solution in

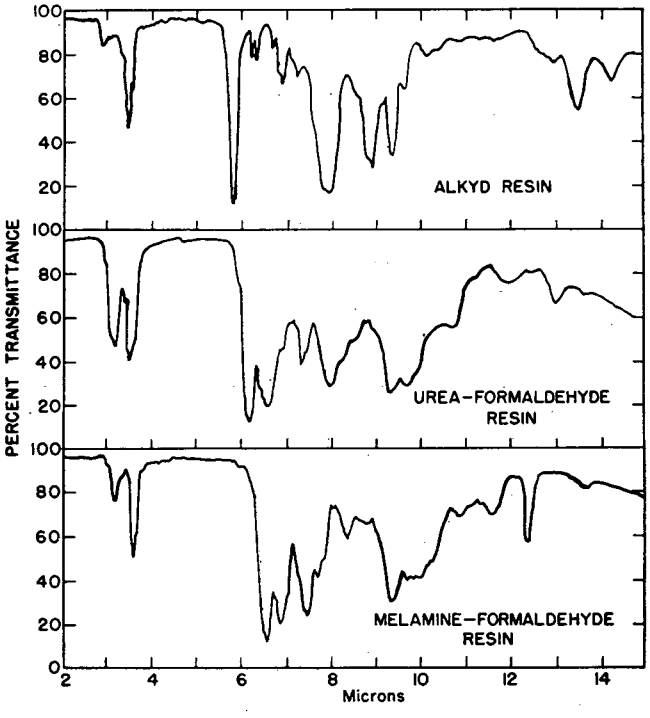

Figure 1. Infrared spectra of resins

Table I. Absorptivity Ratios for Binary Systems

System	Ratio of Absorptivity Values
UF/alkyd	0.88
MF/UF	0.32
MF/alkyd	0.27
	0.28 (calcd.)

Table II. Relative Absorptivity Values for Individual Components

Component	Absorptivity Value
Alkyd (I)	3.65
UF (II)	3.13
MF (III)	1.00

Table III. Analysis of Known Blends

% Composition					
Present			Found		
Alkyd	MF	UF	Alkyd	MF	UF
52.0	37.4	10.6	50.4	39.5	10.1
			51.2	39.2	9.6
59.4	0	40.6	59.8	0	40.2
			60.4	0	39.6
58.5	41.5	0	57.2	42.8	0
			57.2	42.8	0
59.1	21.6	19.3	59.0	21.1	19.9
			58.9	22.3	18.8
35.0	41.3	23.7	33.9	46.4	19.7
			33.4	45.3	20.9
47.7	27.0	25.3	46.3	27.2	26.3
			47.2	27.0	25.7
39.2	60.8	0	40.5	59.5	0
			39.9	60.1	0

Table IV. Analysis of Unknown Enamel Vehicles

Sample	Infrared Analysis	Chemical Analysis
1	71% alkyd 20% UF 9% MF	68% alkyd 32% mixed nitrogen resins
2	83% alkyd 17% UF	85% alkyd 15% nitrogen resin

infrared transparent solvents is not feasible, the spectral measurements are made on thin films prepared by spreading the resin solution on rock salt and drying in vacuum at 60° C. for 1 hour. As calculations are based on absorbance ratios, the problem of exact control of film thickness is obviated.

Figure 1 shows spectra of thin films of each of the three resins involved. The ester carbonyl absorption at 5.8 microns rather than a characteristic aromatic band was chosen as the analytical wave length for the alkyd because of its intensity and sensitivity to the ester linkages in the oil modifier as well as those in the phthalate ester. The 6.1-micron amide carbonyl band in the urea-formaldehyde spectrum and the 12.25-micron triazine ring band in the melamine-formaldehyde spectrum were selected for estimation of the two nitrogen resin components. The factors required to convert absorbance measurements to percentage composition were obtained from spectra run on films cast from solutions of resin mixtures of known composition. If absorbance ratio is plotted against concentration ratio for any two components and if the absorption laws are applicable, it follows directly that the slope of the line so obtained is the ratio of the appropriate absorptivity values. Figure 2 shows plots of these values which indicate that Beer's law holds reasonably well in these condensed resinous systems. As each resin occurs twice in this set of curves, the slope of any one line may be verified by calculation from the other two slopes, as shown in Table I.

If the absorptivity value for any one of these bands is arbitrarily set at some convenient value, corresponding values for the remaining bands are readily obtained from the ratios of Table I.

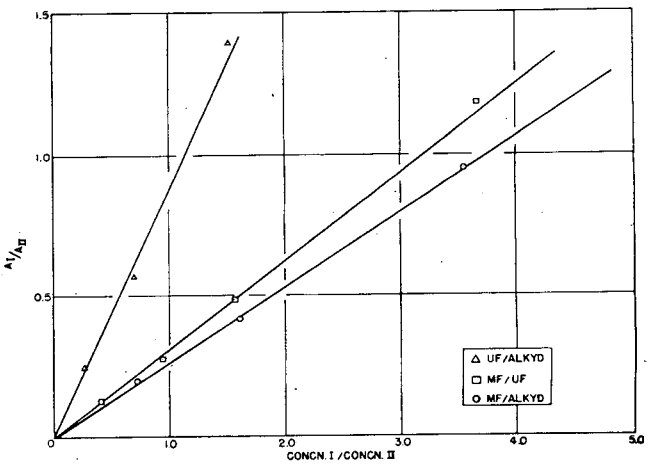

Figure 2. Absorbance ratios *vs.* concentration ratios for binary mixtures

In this case, the absorptivity value for melamine-formaldehyde was chosen as 1.00 and the others evaluated as shown in Table II.

ANALYTICAL PROCEDURE

After calibration data of the type shown in Table II are obtained for the particular instrument and set of conditions to be employed, a sample of unknown quantitative composition is analyzed as follows.

A polished rock salt plate is uniformly coated with a thin continuous film of the resin solution or centrifuged paint vehicle. The solvent is removed by vacuum oven drying at 60° C. and 1 mm. of mercury for 1 hour. The spectrum from 2 to 15 microns is first scanned qualitatively to determine the resin types present. If no extraneous material is observed, the spectrum is scanned from 13.5 to 11.5 microns and from 7 to 5 microns at a film thickness such that the measured absorbance at each analytical wave length falls in an accurately measurable range.

Absorbance values A_I, A_{II}, and A_{III} for alkyd (5.8 microns), urea-formaldehyde (6.1 microns), and melamine-formaldehyde (12.25 microns), respectively, are measured by the baseline method and composition is calculated as follows:

$$\% \text{ alkyd} = \frac{100\,\frac{A_I}{a_I}}{\frac{A_I}{a_I} + \frac{A_{II}}{a_{II}} \quad \frac{A_{III}}{a_{III}}}$$

where a_I, a_{II}, and a_{III} are the previously determined absorptivity values. The percentage of urea-formaldehyde and melamine-formaldehyde are calculated from analogous formulas.

The method was evaluated by analyzing a number of synthetic blends of known composition. Results are given in Table III. Table IV compares results obtained on two commercial enamel vehicles of unknown composition with the corresponding values obtained by chemical methods.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of R. M. McNamara in obtaining the spectral data discussed herein.

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RECEIVED for review August 27, 1955. Accepted October 28, 1955. Division of Paint, Plastics, and Printing Ink Chemistry, 128th Meeting ACS, Minneapolis, September 1955.

Spectrophotometric Determination of Magnesium with Sodium 1-Azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate)

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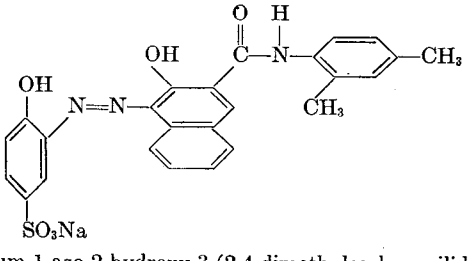
A new colorimetric method for the determination of trace concentrations of magnesium is described. The reagent is sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate). The method has a spectrophotometric sensitivity of 0.0008 γ of magnesium per sq. cm., and a practical sensitivity of 1 part of magnesium in 50,000,000 parts of solution. It is applicable to the determination of 0.5 to 10 γ of magnesium. Interferences by diverse ions have been studied and methods of separation, by extraction and by ion exchange fractionation, have been developed for use in the determination. Magnesium in "synthetic blood ash" solutions and in limestones has been determined with good precision and accuracy.

COLORIMETRIC methods in general use for small quantities of magnesium are based on indirect determinations after the formation of insoluble precipitates and on the formation of colored lakes. In one indirect method magnesium ammonium phosphate is precipitated, followed by the colorimetric determination of phosphate (*7*). A similar procedure involves the precipitation of magnesium 8-quinolinolate, with solution of the precipitate and colorimetric estimation of the oxine (*2*). A number of direct colorimetric methods utilizing the formation of color lakes have been advanced, Titan Yellow having received the most attention (*6*). Recently, procedures involving the formation of a colored complex of magnesium with Eriochrome Black T have been described (*3, 5, 10*)

This paper describes a method for the determination of trace quantities of magnesium based on the formation of a colored complex of magnesium with sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate).



Sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate)

The color reaction has been studied in detail to determine optimum conditions for its use. Data on the sensitivity, precision, accuracy, and interferences by diverse ions are presented.

APPARATUS AND REAGENTS

Spectrophotometer. A Beckman spectrophotometer, Model DU, with matched 1-cm. Corex cells was used.

pH Meter. pH measurements were made with a Beckman Model G pH meter, using shielded glass electrodes for external operation.

Ion Exchange Column. A column consisting of a tube 20 cm. × 6 mm. in inside diameter with a reservoir and ball and socket connections for application of pressure was used for the ion exchange separation. A reservoir for backwashing was attached to the bottom by a two-way stopcock.

Standard Magnesium Solution. The standard magnesium solution was prepared from reagent grade magnesium oxide. A sample of the ignited oxide (800° to 820°) weighing 1.658 grams was dissolved in 1 liter of 0.1*N* hydrochloric acid to give a solution containing 1 mg. of magnesium ion per ml. This solution was standardized gravimetrically by the oxine method and was stored in a polyethylene bottle.

Buffer Solution. The buffer was prepared by dissolving reagent grade borax in water to give an 0.08*M* solution.

Reagent Solution. The reagent solution was prepared by dissolving sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate) in 95% ethyl alcohol to give a solution containing 0.15 mg. of reagent per ml. The compound may be obtained from the LaMotte Chemical Products Co., Towson, Baltimore, Md.

Resin. Dowex 50-X12 (200 to 400 mesh) was used after washing it repeatedly with dilute hydrochloric acid (1 + 1) until the washings were colorless.

Oxine Solution. The oxine solution was prepared by dissolving 5 grams of Eastman White Label 8-quinolinol in 100 ml. of chloroform. A fresh solution must be prepared each day.

Other Reagents. All other reagents were analytical grade and were used without further purification.

PROCEDURE

Transfer a 5-ml. aliquot of the reagent solution to a 25-ml. volumetric flask; add to this an aliquot containing 0.5 to 10 γ of magnesium in not more than 5 ml. of solution which has been adjusted so as to be just acid to phenolphthalein. Add 0.5 ml. of 0.08*M* borax solution, make up to the mark with 95% ethyl alcohol, and mix. After at least 30 minutes, measure the absorbance of the solution at 510 mμ, using a distilled water blank. Determine the weight or concentration in the unknown from a working curve prepared by this procedure using known amounts of magnesium.

For best results, it is desirable to prepare a new working curve with each freshly prepared reagent solution. Maximum precision will not be obtained at the lower extremity of the working curve; hence, it is desirable to adjust the size of the magnesium aliquot to give 1 to 10 γ of magnesium for each determination.

DISCUSSION

Color Reaction. Sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate) is a brilliant red solid that is soluble both in water and in 95% ethyl alcohol to the extent of approximately 0.4 mg. per ml. The compound is stable indefinitely in the solid state, and for at least 4 months when dissolved in 95% ethyl alcohol. It is an acid-base indicator with a transition from red to blue-violet at pH 7 to 8 and another transition from blue-violet to pink at pH 11 to 12. In the pH range 8 to 11, magnesium ion causes a color change from blue-violet to red, yielding solutions with absorbance peaks of 555 (reagent) and 540 (complex) mμ. However, when solutions are prepared in 95% ethyl alcohol instead of water, the color of the reagent is blue instead of violet; that of the complex is salmon pink, rather than red. Absorption spectra of solutions prepared under these conditions are shown in Figure 1. The absorbance peak of the reagent is shifted from 555 to 615 mμ; the complex then shows two peaks, one at 510 mμ and another at 540 mμ. Application of the method of continuous variations to this system indicates the presence of reagent-magnesium ratios of 1 to 1 and 2 to 1.

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Optimum Conditions. The addition of ethyl alcohol to the system causes a desirable divergent shift of the absorbance maxima of the reagent and complex. Experiments involving a change of the alcohol concentration indicate that it should be maintained at about 80% or higher.

Several buffer solutions were tried; a borax buffer (0.08*M*), pH 8.95, was found to be satisfactory. For accurate measurements, it is necessary to hold the pH fairly constant. This may be done by making the magnesium solutions, from which aliquots are to be taken, just acid to phenolphthalein (which does not interfere with the determination) and then following the above procedure.

The reagent concentration may vary from 20 to 40 p.p.m., but it is necessary to use the same concentration for standards and unknowns.

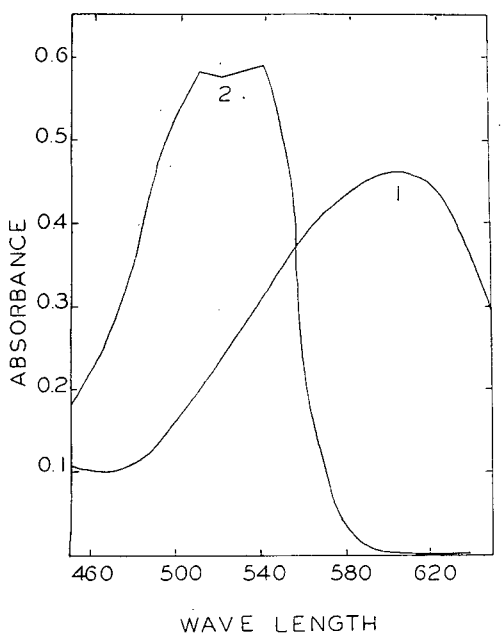

Figure 1. Absorption spectra of reagent and its magnesium complex

1. 14 p.p.m. of reagent
2. 14 p.p.m. of reagent plus 0.4 p.p.m. of magnesium

As seen in Figure 1, the reagent absorbs at the wave lengths of maximum complex absorption (510 and 540 mμ); however, the complex peaks do not overlap the reagent peak. As the reagent absorbs much less at 510 mμ than at 540, the former is better for analytical use. Obviously, the absorbance at 510 mμ is the sum of the absorbances of the complex and the unreacted reagent. The absorbance due to unreacted reagent may be calculated and deducted from the total, giving the absorbance due to the magnesium complex. This may be done because a proportional relationship exists between the reagent absorbance at 510 and at 615 mμ. Since the absorbance of the complex at 615 mμ is almost nil, the total absorbance of the solution at that wave length may be taken as a measure of the amount of unreacted reagent. Hence, the portion of the absorbance at 510 mμ due to unreacted reagent may be calculated from this measurement. In practice, it has been found convenient to prepare a calibration curve by measuring the absorbances of solutions of varying reagent concentration at the two wave lengths (510 and 615 mμ) and plotting one against the other (*4*). The absorbance at 510 mμ which is due to unreacted reagent may then be read from the calibration curve. Although equally precise results are obtained by either method of preparing working curves, when the correction is made, a working curve is obtained which passes through the origin. Also, it is then not necessary to prepare a new working curve for each reagent solution as suggested above because changes of reagent concentration within the range of 20 to 40 p.p.m. have no effect.

The absorbance of the complex does not attain its maximum value instantly; it increases about 1% during the first 30 minutes after preparation of the colored solutions. Then it remains constant for more than an hour. It is necessary, therefore, to allow at least 30 minutes for the full development of the color. Absorbance measurements of similar solutions that were placed in thermostats at 15° and 30°, respectively, showed no variation in absorbance. Hence, normal temperature changes in the laboratory introduce no error.

The reagent was tested with 71 ions on the spot plate to determine the effect of diverse ions. Thirty ions were found to cause interference. For quantitative evaluation, an ion concentration that causes a deviation of more than 0.010 unit in the absorbance of 0.12 p.p.m. of magnesium as the complex was arbitrarily taken as an interference. Table I summarizes the tolerances of some of the more common cations. In addition to the ions listed in Table I, the following ions are also known to interfere: As^{+++}, Be^{++}, Ce^{+++}, Cr^{+++}, Dy^{+++}, Eu^{+++}, Gd^{+++}, Au^{+++}, MoO_4^{--}, Nd^{+++}, Nb^{++}, Pd^{++}, Pr^{+++}, Sm^{+++}, Tl^{+++}, Tm^{+++}, and Yb^{+++}. It is necessary, therefore, either to remove or to mask most of the metal ions before a magnesium determination can be made. The common anions, halides, nitrate, sulfate, phosphate, acetate, and silicate, interfere only when they form salts that are insoluble in ethyl alcohol. This depends on the type and concentration of cation present. The limiting concentration of the alkalies is approximately 0.01*N*.

Table I. Interfering Ions

Ion	Added as	Limiting Concentration, P.P.M.
Al^{+++}	$Al(NO_3)_3$	4
Ba^{++}	$BaCl_2$	2
Cd^{++}	$CdCl_2$	0.01
Ca^{++}	$CaCl_2$	0.01
Co^{++}	$CoCl_2$	0.01
Cu^{++}	$CuSO_4$	0.03
Fe^{++}	$Fe(NH_4)_2(SO_4)_2$	0.08
Fe^{+++}	$FeNH_4(SO_4)_2$	0.02
Pb^{++}	$Pb(NO_3)_2$	0.1
Mn^{++}	$MnCl_2$	0.01
Ni^{++}	$Ni(NO_3)_2$	0.01
$C_2O_4^{--}$	$(NH_4)_2C_2O_4$	1000
Sr^{++}	$Sr(NO_3)_2$	1
Zn^{++}	$Zn(C_2H_2O_2)_2$	0.03

Precision, Sensitivity, and Range. In order to measure the precision, five series of 11 to 13 replicate determinations were made. The precision, expressed as the standard deviation from the mean for each of the five series, is shown in Table II.

Table II. Precision of Determination

Magnesium Concentration, P.P.M.	Absorbance[a], 510 mμ	Standard Deviation from Mean: Absorbance unit	Standard Deviation from Mean: % of magnesium present
0.02	0.320	0.008	40
0.04	0.342	0.003	13
0.12	0.470	0.009	7.5
0.24	0.523	0.006	2.5
0.40	0.635	0.010	3.5

[a] These values are the averages of 11 to 13 replicate determinations.

The spectrophotometric sensitivity is 0.0008 γ of magnesium per sq. cm. For comparison the recorded sensitivities of other methods are: Titan Yellow, 0.006 γ; quinalizarin, 0.017 γ; and

Brilliant Yellow, 0.02 γ of magnesium per sq. cm. (*8*, p. 649). The practical sensitivity of the new reagent may be taken as 1 part of magnesium in 50,000,000 parts of solution. Solutions containing this concentration will have absorbances that are about 0.015 unit greater than those of solutions which contain no magnesium.

Beer's law is obeyed up to 0.2 p.p.m. of magnesium; in addition, absorbances are sufficiently reproducible to allow the range of the determination to be extended to 0.4 p.p.m. The concentration range for optimum precision is 0.12 to 0.4 p.p.m. of magnesium.

Compensation for Calcium Interference. Interference by calcium is of special importance because it frequently occurs with magnesium and because of the difficulty in separating small quantities of these ions. In the determination of magnesium in the presence of calcium, the absorbance at 510 mμ is greater than when the calcium is absent. Immediately on preparation of the colored solutions, a red color forms, but it fades almost completely within an hour, leaving a solution with an absorbance only slightly higher than if calcium had been absent. The enhancement of color due to the presence of calcium is not entirely a function of the calcium concentration. When the concentration of calcium is increased, the absorbance increases up to 0.4 p.p.m. and remains constant up to 12 p.p.m., above which a red precipitate is formed. Hence, if the calcium concentration in the colored solution is between 0.4 and 12 p.p.m., or is adjusted to this concentration range, it is possible to compensate for the interference.

The procedure for the determination of magnesium in the presence of calcium is identical with that already given, except that it is necessary to make certain that the calcium concentration will fall within the specified limits (0.4 to 12 p.p.m.) when a determination is performed and when the working curve is prepared. In addition, it is necessary to allow sufficient time for the initial color due to calcium to fade. After 1 hour this fading is nearly complete; the average change in absorbance during the second and third hours is about 3%. Because the fading of solutions that contain varying amounts of magnesium proceeds at about the same rate, it is possible to achieve good precision by using a waiting period of ± 5 minutes. The precision and sensitivity of the method modified to compensate for the interference by calcium are about the same as those of the unmodified method. A series of 14 replicate determinations that contained 0.08 p.p.m. of magnesium and 8 p.p.m. of calcium showed a standard deviation of 5.2% of the magnesium present. The spectrophotometric sensitivity of the modified method is 0.0005 γ of magnesium per sq. cm., compared with 0.0008 γ of magnesium per sq. cm. for the unmodified method.

SEPARATION OF INTERFERING METAL IONS

Extraction with 8-Quinolinol. 8-Quinolinol (8-hydroxyquinoline) reacts with 23 metal ions in neutral or weakly acid solution to give quinolinates which are soluble in chloroform (*8*, p. 115); under these conditions it does not react with magnesium. To show that the proposed method of separation is compatible with the determination, experiments were performed involving the separation of the following metal ions from magnesium: aluminum, cadmium, cobalt, copper(II), iron(III), manganese(II), nickel, and zinc. For this purpose, solutions containing 50 γ of magnesium and 200 γ of each of the other ions in approximately 10 ml. of dilute hydrochloric acid (1 + 9) were used. The following procedure gives satisfactory separations.

The pH of the solution is adjusted to approximately 7 with 5% sodium bicarbonate solution. The solution is then extracted five times with 5-ml. portions of 5% 8-quinolinol in chloroform and twice with 5-ml. portions of chloroform. Magnesium may then be determined in an aliquot of the aqueous phase without further treatment. When iron is present in quantities exceeding 100 γ, it is desirable to adjust the pH initially to approximately 3, then extract with two or three portions of 8-quinolinol solution, neutralize the solution, and proceed as above. In this way, excessive hydrolysis of iron salts is avoided; otherwise incomplete separation might result.

When only 1 or 2 mg. of metal ions are present it is not necessary to use buffer solutions to control the pH, because the amount of hydrogen ion liberated is not sufficient to affect the acidity appreciably at pH 3; and the buffer capacity of the system produced by the addition of the bicarbonate is sufficient to prevent large changes at pH 7. When more than 1 or 2 mg. of metal ions are present, as in the precipitation of major constituents of the sample, it is necessary to add a buffer solution.

Table III. Recovery of Magnesium from Resin Column after Separation of 100 γ of Magnesium from 1 Mg. of Calcium

	Per Cent
	104.8
	91.4
	88.2
	94.0
	97.4
Av.	95.2

Table IV. Concentration of Metals in Whole Human Blood

Constituent	Concentration, P.P.M.
Aluminum	0.1
Boron	1.0
Calcium	50
Chromium	1
Copper	1.2
Iron	400
Lead	0.6
Magnesium	50
Manganese	0.1
Nickel	0.1
Phosphorus	100
Potassium	2000
Sodium	2000
Zinc	15

Separation of Calcium and Magnesium. When small quantities of calcium and magnesium are to be separated, the usual methods (precipitation of calcium oxalate or calcium sulfate) are unsatisfactory, owing to solubility and coprecipitation losses. A procedure has been devised, based on an ion exchange fractionation, which permits the separation of microgram quantities of magnesium from calcium. During the development of a procedure for the separation of milligram amounts of the alkalies, Sutton and Almy (*9*) noted that calcium and magnesium were also fractionated. A similar procedure has proved effective for the present purpose.

PROCEDURE. Wash the resin column with 25 ml. of 2.0*N* hydrochloric acid and use the washings as a blank. Then place on the column the sample containing magnesium in a weakly acid solution, the volume of solution corresponding to the sample volume being discarded. Pass 30-ml. portion of the 2.0*N* hydrochloric acid elutriant through the column at a flow rate of 1 ml. per minute (20 to 50 cm. of mercury pressure), discarding the first 5 ml. and reserving the remainder for the magnesium determination.

Evaporate both the blank and the solution containing magnesium to dryness in silica dishes and take up in 10 to 15 ml. of 0.002*N* hydrochloric acid. Dilute this solution to a volume which permits withdrawal of 1 to 10 γ of magnesium in a 3- to 5-ml. aliquot. Analyze identical aliquots of the solution containing magnesium and of the blank by the procedure for the determination when calcium is not present.

Wash the column with 20 ml. of dilute hydrochloric acid (1 + 1) to remove calcium and then back-wash with approximately 50 ml. of water. The column is then ready for use again.

DISCUSSION. The new procedure has been used in the separation of 25 to 100 γ of magnesium from 1 to 4 mg. of calcium. The data in Table III illustrate the efficiency of the separation.

The ion exchange fractionation can be combined with the 8-quinolinol extraction by placing the aqueous phase remaining after the extraction on the column and eluting it according to the recommended procedure. Two dilute hydrochloric acid solutions that contained 50 γ of magnesium, 200 γ each of aluminum, cadmium, cobalt, copper, iron, manganese, nickel, and zinc, and 1 mg. of calcium were subjected to the combined separation procedure. Determination of magnesium in aliquots of the resulting solutions showed 100% recovery from each. The improvements in these recoveries over those listed in Table III may be due to compensating errors. Starting with a sample in solution, the combined separation and determination requires from 3 to 4 hours.

APPLICATION TO COMPLEX MATERIALS

For the purpose of demonstrating the applicability of these procedures to the separation and determination of magnesium, two types of samples were chosen. The first was a "synthetic blood ash" solution containing many of the metallic elements found in human blood. This was analyzed using the extraction and ion exchange techniques to remove interfering ions. The second type of sample was a group of low-magnesium limestones, chosen because their high calcium content permitted a test of the modified procedure.

Preparation of "Synthetic Blood Ash" Solution. Values for the metals present in whole human blood given by Albritton (*1*), together with values made available by the Pratt Trace Analysis Laboratory, were used in the preparation of the solutions for this part of the work. These values are given in Table IV.

Table V. Analysis of Synthetic Blood Ash Solutions

Sample No.	Magnesium Added, P.P.M.	Magnesium Found, P.P.M.
1	25.0	23
		28
		Av. 26
2	50.0	57
		47
		49
		59
		47
		48
		49
		47
		52
		47
		55
		Av. 50
3	100	93
		107
		Av. 100

Standard deviation from mean magnesium concentration, 7.6%.

Upon ashing a sample of dried blood in a muffle furnace, the organic matter is burned and driven off, leaving an ash which consists largely of metallic carbonates and oxides. When this ash is dissolved in hydrochloric or nitric acid, a solution of chlorides or nitrates, is obtained. The synthetic blood ash solution, therefore, was prepared from the chlorides or nitrates of the elements listed in Table IV, except in the case of zinc, which was added as the acetate, and of phosphorus, which was added as sodium dihydrogen phosphate. The weights of the salts used yield the concentrations of the metals listed, except for sodium, potassium, and boron; the latter elements were not considered in the preparation of the solution, because the amounts that would be introduced by the reagents used in the analysis were large, compared with the quantities originally present in the aliquot of blood ash solution.

For the analysis of blood ash, a working curve should be prepared by carrying solutions of known magnesium concentration through the combined separation and determination procedure. Analyses may then be carried out under the conditions used to obtain the data for the working curve, and corrections for ion exchange column blank and for failure to obtain complete recovery from the separation need not be made. The working curve should cover a range of 25 to 100 p.p.m. of magnesium in the initial blood sample. Table V shows results of analyses of synthetic blood ash solutions made in this manner.

Analysis of Limestone. The analysis of limestone for magnesium was carried out, in part, by the usual procedure. The sample was decomposed with hydrochloric acid and the silica dehydrated by baking on a low temperature hot plate. The hydrous oxides were precipitated with ammonium hydroxide, using bromine water to oxidize iron and any manganese present.

Table VI. Determination of Magnesium in Limestone

Sample No.	Magnesium Oxide Present[a], %	Magnesium Oxide Found, %	Difference, %
1		0.75	
		0.69	
		1.04	
	0.85	Av. 0.83	−0.02
2		0.79	
		0.70	
		0.69	
		0.73	
	0.81	Av. 0.73	−0.08
3		0.96	
		1.06	
		1.15	
		1.21	
	1.10	Av. 1.10	±0.00
4		1.76	
		1.87	
		1.71	
		2.07	
	1.96	Av. 1.85	−0.11

Standard deviation from certified value 0.12

[a] Standard Sample Co., Ames, Iowa.

Ammonium salts were destroyed by evaporation with concentrated nitric acid and the residue was taken up in dilute hydrochloric acid. Magnesium was determined in an aliquot of this solution by the authors' procedure modified for the determination of magnesium in the presence of high concentrations of calcium. The ratio of calcium to magnesium in limestone is sufficiently high to permit the determination to be made without the addition of calcium to the final solution. The results of analyses of four standard samples of low-magnesia limestones are shown in Table VI.

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RECEIVED for review July 27, 1955. Accepted October 27, 1955.

X-Ray Diffraction Powder Data for Some 1,3,4-Oxadiazoles

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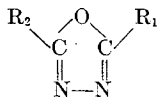
X-ray diffraction powder data were obtained as part of an effort to characterize a number of substituted 1,3,4-oxadiazoles physically. The diffraction data for fourteen of these are presented for the benefit of others interested in the compounds and in a convenient and specific means of their identification.

A RECENT publication (*1*) from this laboratory described the preparation of a number of 2-aryl- and 2-alkyl-5-aryl-1,3,4-oxadiazoles:



Most of the reported products are crystalline solids and can be conveniently identified by x-ray powder diffraction; therefore, these data have been assembled (Table I) for the benefit of others interested in the synthesis of the compounds. For convenience in indexing, the three strongest lines and innermost line of each pattern are listed (Table II).

Table I. Principal Powder Diffraction Lines and Relative Intensities

2-Phenyl-1,3,4-oxadiazole[a], $C_8H_6N_2O$

d, A.	I/I_1
8.65	0.40
5.77	0.15
5.26	1.00
4.65	0.65
4.30	0.65
3.96	0.15
3.75	0.40
3.15	0.25
3.09	0.25
2.48	0.15
2.03	0.15

2-(3-Pyridyl)-1,3,4-oxadiazole, $C_7H_5N_3O$

10.4	0.02
9.67	0.30
6.93	0.05
5.00	0.40
4.84	0.40
4.63	0.05
4.44	0.10
4.34	0.15
4.20	0.02
3.71	0.30
3.58	0.10
3.47	0.05
3.36	0.05
3.29	1.00
3.20	0.10
3.09	0.05
3.03	0.02
2.96	0.05
2.82	0.05
2.75	0.05
2.70	0.05
2.52	0.05
2.48	0.05
2.41	0.05
2.36	0.05
2.32	0.05
2.27	0.05
2.09	0.05
1.897	0.05

2-Ethyl-5-(4-pyridyl)-1,3,4-oxadiazole, $C_9H_9N_3O$

13.3	0.02
11.8	0.60
8.01	0.10
6.05	0.10
5.85	0.10
5.38	0.05
5.05	0.10
4.77	0.15
4.33	1.00
4.13	0.20
3.96	0.02
3.85	0.02
3.70	0.05
3.55	0.10
3.38	0.30
3.29	0.40
3.07	0.02
3.00	0.10
2.92	0.15

2-Ethyl-5-(4-pyridyl)-1,3,4-oxadiazole, $C_9H_9N_3O$ (*contd.*)

2.81	0.10
2.71	0.05
2.65	0.05
2.52	0.02
2.36	0.02
2.32	0.02
2.24	0.02
2.19	0.02
2.16	0.02
2.10	0.02
2.05	0.05
2.01	0.02
1.983	0.02
1.840	0.02
1.794	0.05

2-(*p*-Chlorophenyl)-1,3,4-oxadiazole, $C_8H_5ClN_2O$

8.28	0.05
5.87	0.15
5.62	0.05
4.89	0.65
4.49	0.40
3.76	0.25
3.64	0.40
3.51	0.25
3.46	0.15
3.24	1.00
3.20	0.15
3.13	0.15
2.97	0.15

2-(2-Quinolyl)-1,3,4-oxadiazole, $C_{11}H_7N_3O$

9.92	0.15
7.74	0.40
6.02	0.10
5.85	0.05
5.60	0.10
5.34	1.00
5.15	0.05
4.96	0.05
4.82	0.10
4.40	0.05
3.99	0.10
3.85	0.15
3.78	0.05
3.62	0.30
3.59	0.40
3.51	0.05
3.44	0.40
3.24	1.00
3.12	0.05
3.01	0.10
2.77	0.05
2.72	0.05
2.67	0.05
2.59	0.05
2.54	0.05
2.39	0.05
2.26	0.05
2.19	0.05
2.15	0.05
2.07	0.05

2-(2-Quinolyl)-1,3,4-oxadiazole, $C_{11}H_7N_3O$ (*contd.*)

2.05	0.05
1.994	0.05
1.894	0.05
1.786	0.05
1.755	0.05
1.673	0.05
1.619	0.05

2-(2-Indolyl)-1,3,4-oxadiazole, $C_{10}H_7N_3O$

8.98	0.65
6.15	0.35
5.20	0.65
4.98	0.35
4.79	0.35
4.49	0.35
3.69	0.20
3.48	1.00
3.36	0.35
3.08	0.35
2.74	0.35

2-(*o*-Methoxyphenyl)-1,3,4-oxadiazole, $C_9H_8N_2O_2$

10.0	0.10
9.26	0.10
8.41	0.30
7.26	0.20
6.65	1.00
5.87	0.05
5.30	0.10
4.42	0.10
4.18	0.15
3.92	0.05
3.81	0.05
3.70	0.15
3.61	0.15
3.46	0.05
3.36	1.00
3.15	0.20
3.08	0.15
2.92	0.05
2.76	0.05
2.55	0.05
2.23	0.05
2.10	0.05
1.986	0.05
1.944	0.05
1.925	0.05
1.718	0.05

2-Methyl 5-(4-pyridyl)-1,3,4-oxadiazole, $C_8H_7N_3O$

9.92	0.40
6.90	0.02
5.80	0.02
5.58	0.01
5.26	1.00
5.08	0.65
4.89	0.20
4.64	0.15
4.21	0.02

2-Methyl 5-(4-pyridyl)-1,3,4-oxadiazole, $C_8H_7N_3O$ (*contd.*)

4.10	0.05
3.98	0.02
3.62	1.00
3.45	0.02
3.37	0.02
3.29	1.00
3.26	0.65
3.14	0.02
2.96	0.02
2.78	0.02
2.60	0.02
2.52	0.02
2.44	0.05
2.33	0.05
2.28	0.02
2.21	0.02
2.17	0.02
2.04	0.02
1.983	0.02
1.901	0.02
1.835	0.10
1.651	0.05

2-(*p*-Nitrophenyl)-1,3,4-oxadiazole, $C_8H_5N_3O_3$

8.65	0.05
6.32	0.05
5.67	0.05
5.54	0.20
5.31	0.30
4.80	0.10
4.37	0.10
4.26	0.15
4.17	0.15
4.05	0.10
3.80	0.10
3.71	0.10
3.60	0.10
3.39	0.05
3.30	0.05
3.24	1.00
3.17	0.15
3.08	0.05
3.01	0.05
2.82	0.05
2.77	0.05

2-Methyl-5-phenyl-1,3,4-oxadiazole, $C_9H_8N_2O$

10.3	0.65
7.17	0.05
6.43	0.35
6.09	0.35
5.31	0.15
5.15	0.15
4.80	0.35
4.46	1.00
4.05	0.65
3.36	0.35
3.51	0.35
3.25	0.20
3.17	0.35
3.10	0.15
2.88	0.20

2-Methyl-5-phenyl-1,3,4-oxadiazole, $C_9H_8N_2O$ (*contd.*)

2.52	0.05
2.48	0.05
2.45	0.05
2.40	0.05
2.35	0.05
2.12	0.05
2.07	0.05
1.914	0.05
1.862	0.05
1.817	0.05
1.688	0.05

2-(4-Pyridyl)-1,3,4-oxadiazole, $C_7H_5N_3O$

9.19	0.15
7.95	0.05
7.14	0.02
6.51	0.60
5.30	0.40
5.07	0.05
4.79	0.60
4.58	1.00
3.98	0.15
3.81	0.10
3.70	0.15
3.44	0.15
3.34	0.05
3.25	0.40
3.18	0.05
3.09	0.05
3.01	0.05
2.84	0.02
2.77	0.02
2.66	0.05
2.58	0.05
2.54	0.02
2.45	0.02
2.42	0.05
2.30	0.05
2.22	0.02
2.19	0.05
2.11	0.02
2.00	0.02
1.920	0.02
1.771	0.02
1.736	0.02

2-Ethyl-5-(*p*-chlorophenyl)-1,3,4-oxadiazole, $C_{10}H_9ClN_2O$

12.9	0.50
8.33	0.15
6.40	0.15
5.69	0.50
5.16	0.15
4.87	0.15
4.39	1.00
4.26	0.65
4.04	0.15
3.74	0.15
3.54	0.40
3.37	0.50
3.30	0.40
3.15	0.15
2.99	0.50

2-Ethyl-5-(*p*-chlorophenyl)-1,3,4-oxadiazole, $C_{10}H_9ClN_2O$ (*contd.*)

2.84	0.15
2.48	0.15

2-(1-Naphthyl)-1,3,4-oxadiazole, $C_{12}H_8N_2O$

19.6	0.85
12.1	0.10
10.9	0.10
9.63	0.30
8.25	0.55
7.06	0.35
6.59	0.10
6.00	1.00
5.50	1.00
4.80	0.65
4.31	0.20
4.13	0.15
3.90	0.55
3.76	1.00
3.66	0.55
3.60	0.55
3.49	0.20
3.43	0.25
3.35	0.65
3.28	0.15
3.21	1.00
3.17	0.35
3.07	0.15
2.98	0.15
2.85	0.10
2.75	0.10
2.69	0.10
2.64	0.10
2.58	0.10
2.40	0.05
2.33	0.05
2.30	0.05
2.17	0.10
2.07	0.10
1.967	0.10
1.905	0.10
1.837	0.15

2-Ethyl-5-(*p*-nitrophenyl)-1,3,4-oxadiazole, $C_{10}H_9N_3O_3$

10.5	0.65
8.40	0.10
6.99	0.15
5.58	0.20
5.20	1.00
4.80	0.55
4.59	0.35
4.22	0.35
3.85	0.55
3.45	0.55
3.29	1.00
3.11	0.55
2.93	0.15
2.90	0.15
2.33	0.05
2.29	0.05
2.11	0.05
1.80	0.05

[a] Hygroscopic.

Table II. Index Lines from Diffraction Patterns

1,3,4-Oxadiazole Substituent(s)	Strongest Lines 1st	2nd	3rd	Innermost Line
2-Phenyl	5.26 (1.00)	4.65 (0.65)	4.30 (0.65)	8.65 (0.40)
2-(*o*-Methoxyphenyl)	6.65 (1.00)	3.36 (1.00)	8.41 (0.30)	10.0 (0.10)
2-(*p*-Chlorophenyl)	3.24 (1.00)	4.89 (0.65)	4.49 (0.40)	8.28 (0.05)
2-(*p*-Nitrophenyl)	3.24 (1.00)	5.31 (0.30)	5.54 (0.20)	8.65 (0.05)
2-(1-Naphthyl)	6.00 (1.00)	5.50 (1.00)	3.76 (1.00)	19.6 (0.85)
2-(4-Pyridyl)	4.58 (1.00)	6.51 (0.60)	4.79 (0.60)	9.19 (0.15)
2-(3-Pyridyl)	3.29 (1.00)	5.00 (0.40)	4.84 (0.40)	10.4 (0.02)
2-(2-Quinolyl)	5.34 (1.00)	3.24 (1.00)	3.44 (0.40)	9.92 (0.15)
2-Methyl 5-phenyl	4.46 (1.00)	10.3 (0.65)	4.05 (0.65)	10.3 (0.65)
2-Methyl 5-(4-pyridyl)	5.26 (1.00)	3.62 (1.00)	3.29 (1.00)	9.92 (0.40)
2-Ethyl 5-(*p*-chlorophenyl)	4.39 (1.00)	4.26 (0.65)	12.9 (0.50)	12.9 (0.50)
2-Ethyl 5-(*p*-nitrophenyl)	5.20 (1.00)	3.29 (1.00)	10.5 (0.65)	10.5 (0.65)
2-Ethyl 5-(4-pyridyl)	4.33 (1.00)	11.8 (0.60)	3.29 (0.40)	13.3 (0.02)
2-(2-Indolyl)	3.48 (1.00)	8.89 (0.65)	5.20 (0.65)	8.98 (0.65)

The samples were finely ground and packed into thin-walled Parlodion capillaries of about 0.5-mm. diameter. These were rotated during exposure in a camera with a 114.6-mm. diameter, using vanadium-filtered chromium radiation (CrK_α = 2.2909 A. used in calculations). Relative intensities were determined by visual comparison with a standard series of photographic densities. All samples were those used for the analyses reported in the reference cited.

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RECEIVED for review August 17, 1955. Accepted November 14, 1955.

Determination of Equivalent Weights by Gamma Ray Counting of Iodine-131–Labeled Derivatives

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A semimicromethod for the determination of equivalent weights of compounds possessing certain functional groups involves the formation of derivatives of the unknown and of a known compound with a single sample of a reagent homogeneously labeled with iodine-131. The specific activities of the unknown and the known derivatives are determined by gamma ray counting of the crystalline derivatives. Equivalent weights of derivatives of the alcohols, amine, and acids studied agreed with theory within 2%.

THE development of high sensitivity gamma ray Geiger-Müller counters of remarkable stability permits measurements of considerable precision to be made on small samples labeled at the tracer level with iodine-131. The successful use of gamma ray counting for the quantitative estimation of components of identical iodine-131 molar labeling on a chromatographic column (*13*) suggested the possibility of determining equivalent weights of iodine-131–labeled derivatives by comparing the specific activity (counts per minute per milligram) of the derivative of an unknown compound with that of a known derivative prepared from the same homogeneous sample of iodine-131–labeled reagent.

APPARATUS AND PROCEDURE

A Model G. R. Welch-Allyn Texaco type Geiger-Müller gamma ray detector was enclosed on sides and top, except for an access hole to the well, by a shield of 0.5 inch of steel and 1.5 inches of lead. A removable lead plug was used to close the access hole during counting. In this shield the counter had a background of approximately 600 counts per minute. In order to maintain constant source geometry, a 1.5 × 4.75 inch polystyrene cylinder was machined to fit snugly into the counter well. A 12-mm. axial hole was drilled down 67 mm. from the top and extended another 13 mm. at 6-mm. diameter. The cylinder was sawed along its axis from the top to a point 53 mm. from the bottom. At this level a transverse cut was made to the diameter, removing a section to allow the small (6 × 20 mm.) glass-stoppered sample vials to be conveniently inserted or removed from the holder. This arrangement placed the samples on the center line of the well and within the vertical limits of constant counting efficiency. An Atomic Instrument Co. No. 1050 scaler was used. It was fed from an electronically voltage-regulated source. Preliminary tests showed that the specific activity of a given sample at relatively low counting rate, where coincidence was not a factor, was independent of the amount of material present (from 5 to 50 mg.), and of the type and weight of glass used in the sample vials (2 to 4 grams). The specific activity of the samples measured was between 2500 and 250 counts per minute per mg. in the counter described.

When possible, several samples were counted in succession and the series was repeated until approximately 10^6 total counts had been accumulated for each sample. Though not essential, in one series the sample weights were adjusted so that the counting rate was approximately the same for all, thus eliminating the need for coincidence correction. The half life of iodine-131 was taken as 8.07 days (*10*). A table was prepared of the values of the decay factor for each 5-minute interval from zero time. This is given by the expression $D = N_0/N_t$ = antilog $2.5904 \times 10^{-5}\ t$ (minutes) where N_0 and N_t are the total net counts per minute at the start of the series and at the mid-point of the counting period for each sample, respectively. Equivalent weights were calculated from the expression $E_u = E_s\,(A_s/A_u)$ where E_u and E_s refer to the equivalent weights of the unknown and standard compounds, respectively, and A_u and A_s refer to the corresponding specific activities in counts per minute per milligram at the start of the series, as calculated from $A = (N - N_b)\ D/m$ where N and N_b are the total counts per minute of the sample and background, respectively, D is the decay factor referred to above, and m is the weight of the sample in milligrams. Samples were dried at 60° C. in a vacuum oven for weighing. Specific activities were then calculated. As the equivalent weight is inversely proportional to the specific activity for samples derived from the same homogeneously iodine-131–labeled reagent, the equivalent weights of unknowns can be calculated if one substance in the series is known.

REAGENTS

p-Iodobenzoyl chloride–iodine-131 was prepared for another experiment as previously described (*13*). One week later 98.52 mg. of this *p*-iodobenzoyl chloride–iodine-131 were added to

411.20 mg. of inactive *p*-iodobenzoic acid. The mixture was refluxed with thionyl chloride until a clear brown solution resulted. The thionyl chloride was distilled off under vacuum and the diluted *p*-iodobenzoyl chloride–iodine-131 was sublimed to a cold finger under vacuum. Melting points are given in Table I.

p-Iodobenzoic acid–iodine-131. Two hundred and fifteen milligrams of *p*-iodobenzoyl chloride–iodine-131 were added to a small flask with a few milliliters of water. The water was brought to a boil and the flask was allowed to stand overnight at 60° C. The contents were transferred to a separatory funnel with ether and the ethereal solution was washed in succession with water, 2% hydrochloric acid, and twice more with water, filtered, the ether was removed by an air jet, and the product was recrystallized from acetone.

Cholesteryl *p*-iodobenzoate–iodine-131, *p*-nitrobenzyl *p*-iodobenzoate–iodine-131, and *N*-*p*-iodobenzoylaniline–iodine-131 were made by adding a 20% excess of the *p*-iodobenzoyl chloride–iodine-131 to the corresponding alcohol or amine, dissolving the mixture in hot dry pyridine, and allowing the reaction flasks to remain overnight at 60° C. The mixtures were transferred to separatory funnels with ether and the solutions were washed with water, 2% hydrochloric acid, water, 5% sodium carbonate, and water twice. The ether was removed by an air stream and the products recrystallized; cholesteryl *p*-iodobenzoate from Skellysolve C, *p*-nitrobenzyl *p*-iodobenzoate and *N*-*p*-iodobenzoylaniline from acetone-water.

Methyl *p*-iodobenzoate–iodine-131 was prepared according to the method described by Cheronis (*4*) for ethyl 3,5-dinitrobenzoate. The product was recrystallized from methanol.

Cholesteryl *p*-iodobenzoate dibromide–iodine-131 was prepared from cholesteryl *p*-iodobenzoate–iodine-131 according to the method of Bretschneider and others (*1*), and *p*-iodoaniline–iodine-131 was prepared according to the method of Brewster (*2*) as modified by Stokes and others (*11*).

Propion-, acet- and diacet-*p*-iodoanilides–iodine-131. The *p*-iodoaniline–iodine-131 was treated directly with the respective anhydrides (*8*). The acet- and diacet-*p*-iodoanilides, which formed in the same reaction, were separated by radiochromatography (*11*) on silicic acid–Celite with a 2.5% solution of 1-propanol in Skellysolve C. The zones were sectioned and the components eluted with a 1 to 1 mixture of 1-propanol and Skellysolve C and crystallized from ethyl alcohol–water.

Table I. Experimental Results

Sample	Substance	Weight, Mg.	M.P., ° C.	Deriv. Eq. Wt. Detd.	Deriv. Eq. Wt. Calcd.	% Error
			Series I			
Std.	Cholesteryl *p*-iodobenzoate	39.962 40.230	182[a] (*13*)		616.65	..
1	*p*-Iodobenzoic acid	16.304 17.190	267–268 (*14*)	255.11 249.86		
	Mean			252.49	248.03	+1.8
2	*p*-Nitrobenzyl *p*-Iodobenzoate	26.077 17.966	143.8–145.0 (*7*)	385.91 381.47		
	Mean			383.69	383.14	+0.14
3	*N*-*p*-Iodobenzoylaniline	19.794 21.128	211.8 (*6*)	319.59 324.06		
	Mean			321.83	323.13	−0.40
4	Methyl *p*-iodobenzoate	20.686 21.730	114.4 (*9*)	265.36 267.27		
	Mean			266.32	262.05	+1.63
5	Cholesteryl *p*-iodobenzoate dibromide	47.200 55.134	134.8 (*12*)	770.92 776.84		
	Mean			773.88	776.48	−0.33
			Series II			
Std.	Propion-*p*-iodoanilide	10.00	168.0 (*11*)		275.10	...
1	Acet-*p*-iodoanilide	10.03	185.0 (*3*)	264.29	261.07	+1.23
2	Diacet-*p*-iodoanilide	6.15	165.0 (*11*)	297.33	303.10	−1.91

[a] Change to iridescent cholesteric melt.

Cholesteryl *p*-iodobenzoate was chosen as the standard for the first series of compounds (alcohols and amine), because cholesterol purified through the dibromide was available and previous work had indicated that the ester was easily purified. The physical properties of the propion-*p*-iodoanilide suggested its use as the standard for the second series.

In the main series, all errors, except those of the *N*-*p*-iodobenzoylaniline and cholesteryl *p*-iodobenzoate dibromide, are positive. Positive errors are most easily accounted for by the assumption of corresponding amounts of unlabeled impurity in the sample counted. Here such errors are to be expected, as no extraordinary purification steps were taken in the preparation of the derivatives. The negative error in the case of the *N*-*p*-iodobenzoylaniline might suggest the presence of a small amount of the doubly tagged compound—i.e., *N*-di-*p*-iodobenzoylaniline. The corresponding *N*-dibenzoylaniline is formed under similar conditions (*5*). The positive error in the case of the cholesteryl *p*-iodobenzoate dibromide suggests incomplete bromination. The large error in the equivalent weight of the diacet-*p*-iodoanilide was not unexpected, as only a single small sample, too small for recrystallization, was at hand.

The average deviation between successive counts made on the same sample during a single day was 0.40%, while the average deviation between duplicate samples of the same compound was 0.39%. These values would seem to be indicative of the precision of which the method is capable if very pure compounds are used. While the average error in the equivalent weights of the iodine-131–labeled derivatives was only 1.08%, the errors in the equivalent weights of the original compounds vary from 0.35 to 6.89%, depending on the magnitude of the equivalent weight in question, the purity of the derivative, and the amount of sample on hand for counting.

ACKNOWLEDGMENT

This work was supported by grants-in-aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, from the American Heart Association, from the Damon Runyon Memorial Fund for Cancer Research, and from the Rhode Island Cancer Society. The authors wish to express their appreciation to Gordon L. Brownell of the Massachusetts General Hospital and H. R. Seelen of the Radio Corp. of America for advice and loan of apparatus that made this work possible.

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RECEIVED for review August 1, 1955. Accepted October 26, 1955.

Polarography of Potassium Tetraphenylborate(III) in *N,N'*-Dimethylformamide

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A recording polarograph was used to obtain polarograms of potassium tetraphenylborate(III) dissolved in *N,N'*-dimethylformamide with tetrabutylammonium iodide as the supporting electrolyte. The wave heights were proportional to concentration over the range 0.0002 to 0.0075*M* which corresponds to 0.08 to 3.0 mg. of potassium in the polarograph cell. The half-wave potential was −1.55 volts with a mercury pool as nonpolarized electrode.

IN RECENT years sodium tetraphenylborate has been used as a reagent for the determination of potassium. Wittig and others (*8–10*) first found that the tetraphenylborate ion has the unusual property of forming lithium and sodium salts which are readily soluble in water while the potassium, ammonium, rubidium, and cesium salts are very insoluble in water. This research was initiated in order to determine if potassium tetraphenylborate could be determined by a polarographic method. Acetone readily dissolves potassium tetraphenylborate but has undesirable properties for polarographic applications. Arthur and Lyons (*1*) have reported their failure to record cationic waves in acetone due to the erratic nature of the diffusion current. This was confirmed in this laboratory. Methanol, ethanol, formamide, and acetone-water mixtures were tried as solvents without success because of the insolubility of the potassium salt or a slow rate of dissolution.

N,N'-dimethylformamide has been used extensively as a solvent for potentiometric titrations and to a limited extent for polarography in this laboratory. It readily dissolves potassium tetraphenylborate and is suitable as a solvent for obtaining polarographic waves of the potassium ion. Kemula and Kornacki (*3*) have determined potassium in the 0.1- to 1.0-mg. range by precipitation with excess sodium tetraphenylborate and back-titrating with 0.005*M* thallous nitrate, determining the end point amperometrically.

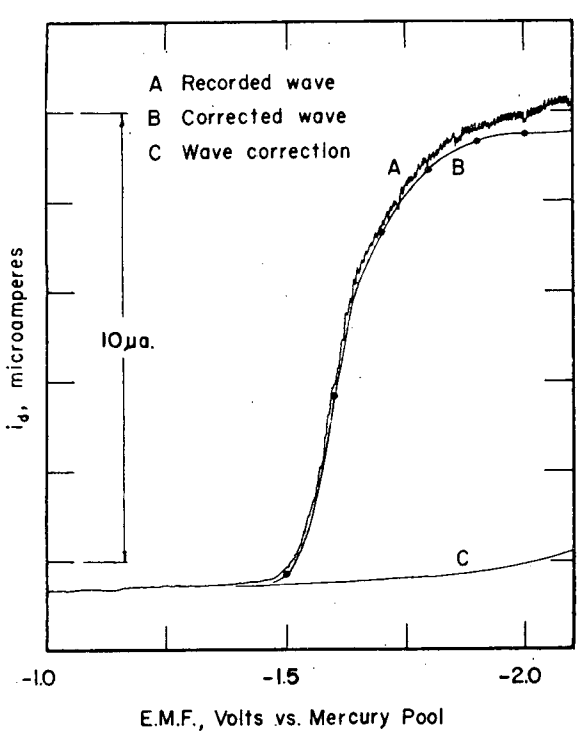

Figure 1. Polarogram

APPARATUS AND MATERIALS

Apparatus. All polarograms were obtained using a Leeds & Northrup Electro-Chemograph, Type E. A mercury pool cell was constructed out of an 8-mm. test tube, with a side arm at the bottom to degas the sample. The cell was designed in such a way as to contain 10 ml. of solution when about half full and it was immersed in a water bath with the temperature controlled at 25° ± 0.1° C.

The capillary used for the dropping mercury electrode was prepared from Corning marine barometer tubing of 28.4-micron radius and 10-cm. length. $m^{2/3}t^{1/6}$ was determined at −2.0 volts, which corresponds to the plateau of the potassium wave, and found to have the constant value 1.376, even though m and t were not constant. The cell resistance was 920 ohms and the dropping mercury electrode was kept at 20 mm. above the surface of the pool. The mercury column was maintained at 35 cm. above the top of the capillary. Oxygen was removed by degassing for 20 minutes with tank nitrogen which was bubbled through a vanadous sulfate train for removal of oxygen impurity (*5*).

Materials. The vanadous sulfate solution, which is violet when properly prepared, was made by reducing with zinc a solution of vanadium pentoxide (3 grams) in sulfuric acid (500 ml. of 1.0*M*). The solution was evaporated to about 150 ml. in the presence of mossy zinc and while still hot immediately placed in a washing bottle over lightly amalgamated zinc. Nitrogen gas was bubbled through the solution at once; after an hour the solution was colored a deep violet and ready for use. This solution was easily regenerated when it turned brown by the addition of 1 ml. of concentrated sulfuric acid. The supporting electrolyte was 0.1*M* tetrabutylammonium iodide in dimethylformamide.

N,N'-Dimethylformamide obtained from Du Pont was used as a solvent. No measurable difference was noted between polarograms obtained with solvent which was dried by distillation over calcium hydride and the technical grade solvent. Tetrabutylammonium iodide, prepared and purified according to the directions of Laitinen and Wawzonek (*4*), was used as a supporting electrolyte. Sodium tetraphenylborate (J. T. Baker Chemical Co.) was used without further purification. Potassium tetraphenylborate was prepared by precipitation from a potassium chloride solution and was recrystallized twice from acetone.

STANDARDIZATION AND RESULTS

A stock solution of 0.25*M* tetrabutylammonium iodide in dimethylformamide was prepared for use as supporting electrolyte. A stock solution of 0.125*M* potassium tetraphenylborate in dimethylformamide was used for preparing the standard samples. Ten milliliters of the supporting electrolyte stock solution was transferred to a 25-ml. calibrated flask by a calibrated transfer pipet, and a calculated volume of the potassium tetraphenylborate stock solution was added to the flask. The solution was then diluted to exactly 25 ml. This gave a solution 0.1*M* in supporting electrolyte. In this manner a range of concentrations from 0.0002*M* to 0.0075*M* was studied.

The polarographic cell was rinsed several times with small portions of the sample; approximately 10 ml. of the sample was added to the cell and then degassed for 15 to 20 minutes with oxygen-free tank nitrogen. This was adequate for oxygen removal, even though dimethylformamide dissolves huge quantities of many gases. The polarogram was obtained by polarizing from −1.0 to −2.4 volts, which is the decomposition potential of the supporting electrolyte. The wave for potassium has a half-wave potential of −1.55 ± 0.02 volts, which value was obtained

Table I. Correction Values for Residual Current

Applied Potential	Microamperes Solution 1	Solution 2
−1.0	0.00	0.00
−1.1	0.00	0.00
−1.2	0.05	0.03
−1.3	0.10	0.05
−1.4	0.18	0.10
−1.5	0.28	0.14
−1.6	0.40	0.15
−1.7	0.50	0.18
−1.8	0.62	0.23
−1.9	0.75	0.35
−2.0	0.95	0.50
−2.1	1.20	0.65
−2.2	1.42	0.85
−2.3	1.67	1.00

Table II. Variation of Diffusion Current with Concentration

$M \times 10^4$	i_d (Obsd.), μa.	i_d (Calcd.), Best Straight Line
2	0.43	0.425
6	1.21	1.27
10	2.35	2.13
12.5	2.60	2.66
25	5.32	5.31
37.5	8.00	7.96
50	10.4	10.62
62.5	13.1	13.3
75	16.6	15.92

from manually plotted waves in order to avoid the lag in galvanometer response inherent in automatically recorded waves using a highly damped galvanometer. A half-wave potential of −1.34 volts versus a mercury pool has been reported for potassium iodide in acetonitrile plus 0.1M tetrabutylammonium iodide by Wawzonek and Runner (*7*). All waves were corrected for impurity current of the supporting electrolyte which has an appreciable effect on the wave height as shown in Figure 1. The impurity current correction was obtained by averaging values from a number of polarograms of the supporting electrolyte under the same conditions as the sample.

This impurity current increased slowly with time because of decomposition of the supporting electrolyte, but it was sufficiently constant over a period of 3 or 4 weeks. The impurity current was shown to result from the reduction of *n*-butyl iodide resulting from the equilibrium in dimethylformamide between the quaternary salt and its tertiary amine and *n*-butyl iodide components.

$$(C_4H_9)_4NI \rightleftharpoons (C_4H_9)_3N + n\text{-}C_4H_9I$$

The supporting electrolyte is sensitive to light, and decomposition was retarded by keeping the solution in dark bottles. The presence of traces of acids in the solvent also had a deleterious effect on the nature of the residual current, because the solvent readily decomposes to formic acid and dimethylamine. Table I shows the average corrections for the impurity current obtained in this research for two different stock solutions of the supporting electrolyte. This correction was especially important for polarograms of low potassium concentration, as the uncorrected waves were ill defined and the true wave height could be evaluated only after the correction was applied.

A linear relation was found to hold for the corrected wave height when plotted versus concentration and these could be reproduced within 2% in the middle portion of the calibration curve. A summary of the results is tabulated in Table II. A plot of log $i/(i_d - i)$ versus potential corrected for iR drop gave values of 0.062 to 0.069 from the slope of the straight line, compared with a theoretical 0.059.

Effect of Water on the System. Since the method was to be applied to precipitated samples of potassium tetraphenylborate, information was desired about the effect of the presence of water on the waves. A series of polarograms was obtained for the supporting electrolyte solution alone in which the water concentration was varied from 0.0 to 10.0 ml. per 25 ml. of solution and with the electrolyte concentration 0.1M. The presence of water in the dimethylformamide caused a shift of the decomposition potential of the supporting electrolyte to more positive values. The potentials given in Table III are for those points on the curves where the current value was 3 μa. greater than the values at −1.00 volt. It is seen that the shift did not take place until more than 3 ml. of water was present in 25 ml. of the supporting electrolyte; and since the water in the precipitated sample could be removed far below this level, its effect may be neglected. It may be presumed that the shift is due to the decreased activity of mercurous iodide at the mercury pool caused by the addition of water.

Degassing Procedure and the Mercury Pool. Arthur and Lyons (*1*) report that in nonaqueous polarography the solution must be degassed before the mercury pool is added, and subjected to a final minute of degassing to remove oxygen inadvertently admitted during the addition of the pool. Fortunately, this was not found to be necessary in this research. It seemed to make no difference whether the pool of mercury was added before or after degassing the sample. No spurious waves were noted when the pool was added to the sample before degassing in this solvent system. Arthur and Lyons also report that the electrode spacing is critical; however, with the low cell resistance of 920 ohms encountered in this solvent system the electrode spacing is not critical as long as the spacing remains the same for all polarograms. No erratic polarograms were obtained.

TEMPERATURE EFFECT ON WAVE HEIGHT

Polarograms recorded at elevated temperatures showed a proportionate increase in wave height larger than that found in aqueous systems. The following ratios were obtained for $i_d(t°)/i_d(25°)$: 1.29 at 30°, 1.51 at 35°, 1.72 at 40°, 1.88 at 45°, 2.01 at 50°, and 2.14 at 55° C. Because this ratio is so large, temperature control was critical in obtaining reproducible wave heights.

PROCEDURE FOR PRECIPITATED SAMPLES

Since a linear relation was found to hold for wave height versus concentration and a calibration curve was established, the method as applied to precipitated samples was studied.

A standard solution containing 0.2237 gram of potassium chloride per 100 ml. of water was prepared. A solution of sodium tetraphenylborate in water was prepared by dissolving 1.54 grams of the salt in 80 ml. of water; 0.5 gram of aluminum chloride hexahydrate was added to clarify the slightly turbid tetraphenylborate solution and after standing for about 30 minutes the solution was filtered and diluted to 100 ml. This solution had a pH of 2 to 3 as determined by Hydrion paper. According to Schwaibold and Kohler (*6*) the sodium tetraphenylborate solution should be stable for some time. A 50% excess of the reagent is used when potassium ion is precipitated.

From 0.50 to 5.00 ml. of the potassium chloride stock solution was placed in a 15-ml. centrifuge tube and 5.0 ml. of the precipitant added. The precipitate was centrifuged for 5 minutes and the supernatant liquid removed with a syringe. It was found that the presence of the aluminum chloride in the precipitant was beneficial; otherwise, the precipitate may not be centrifuged adequately even over a period of 1 hour. The precipitate was

Table III. Shift of Decomposition Potential with Water Addition in a 0.1M Tetrabutylammonium Iodide Solution

Ml. H_2O per 25 Ml. Solution	Decomposition Potential
0	2.40
1	2.40
2	2.38
3	2.39
4	2.33
5	2.20
6	2.17
7	2.04
8	1.93
9	1.71
10	1.69

Table IV. Experimental Results

Taken, Mg.	Found, Mg.	Average	Standard Deviation
0.585	0.513, 0.541, 0.553, 0.562 0.566, 0.568, 0.583	0.555	0.024
2.34	2.32, 2.34, 2.35, 2.39, 2.40, 2.43, 2.46, 2.52	2.40	0.06
4.68	4.54, 4.60, 4.63, 4.68, 4.73, 4.87, 4.87, 4.87	4.72	0.13
5.85	5.68, 5.81, 5.91, 5.91	5.83	0.11

then washed with 5 to 7 ml. of a saturated solution of potassium tetraphenylborate and centrifuged; again the supernatant liquid was removed with a syringe. The precipitate was then dissolved in 10 ml. of dimethylformamide and washed quantitatively into a 25-ml. calibrated flask. Ten milliliters of 0.25*M* tetrabutylammonium iodide in dimethylformamide was added to the flask and the solution diluted to exactly 25 ml. A polarogram was obtained according to the directions given for standardization. The wave height was measured and the concentration calculated from the calibration curve. Data for the results of analysis by this method are shown in Table IV.

DISCUSSION OF THE METHOD

Although the polarographic method is not as precise or accurate as gravimetric and titrimetric procedures reported in the literature (*2*), it involves less manipulation and time. Its precision is within 3% in precipitated samples. Some of this deviation appears to come from mechanical difficulties in removing the last of the supernatant liquid from the precipitate. The error could be minimized by running replicate samples; in many cases the time consumed is less for three samples than for one by other methods. Interferences are ammonia, rubidium, and cesium. Ammonia may be removed easily, and rubidium and cesium present no real difficulty because the amount present is small in the majority of samples. This method should prove applicable for routine analysis, as the simplest modification of a polarograph may be used.

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RECEIVED for review July 5, 1955. Accepted October 26, 1955. Division of Analytical Chemisty, 128th Meeting, ACS, Minneapolis, Minn., September 1955. The work was supported by the Atomic Energy Commission under Contract No. AT(11-1)-163.

Some Components of Gas Phase of Cigarette Smoke

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The smoke produced when a cigarette is puffed consists essentially of a gas phase and a particulate phase. Reliable analytical techniques for determining the chemical composition of both of these phases, in as near their unchanged state as practical, are of primary importance, as such analyses are preliminary to determining what processes are involved during smoking. By application of an infrared compensation absorption procedure and mass spectral studies to samples of the gas phase previously fractionated at low temperature, it has been possible to determine the composition of the gas phase quantitatively as regards all of its major components and a considerable number of minor constituents. Substances determined include air, excess nitrogen, excess argon, hydrogen, carbon monoxide, carbon dioxide, methane, ethane, propane, ethylene, propylene, acetylene, isoprene, butadiene, acetaldehyde, acetone, methyl ethyl ketone, methanol, hydrogen cyanide, diacetyl, carbonyl sulfide, methyl chloride, and water.

CIGARETTE smoke, composed as it is of atmospheric gases and of a complex mixture of substances derived from the combustion and pyrolysis of the cigarette, poses a considerable analysis problem. The smoke exists, after formation, as an aerosol composed of a gaseous and a particulate phase. For purposes of analysis, it is convenient to separate these phases initially by filtration, removing the particulate phase and thus obtaining a residual gas phase.

Numerous investigations of the components of cigarette smoke have included work on the gas phase. Among the constituents reported as being present are: carbon dioxide (*9, 14, 38*), carbon monoxide (*2, 8, 9, 13, 15–17, 21, 28, 34, 35*), hydrogen sulfide (*14, 33, 37*), hydrocyanic acid (*20, 30, 31, 36*), ammonia (*6, 7, 9, 10, 19, 23, 24–26, 29, 37*), acetylene (*14*), unspecified saturated and unsaturated hydrocarbons (*14*), and cyanogen (*32*). The methods employed have been adaptations of conventional chemical absorption, colorimetric, or titrimetric methods. The results reported by the investigators cited above generally agree qualitatively, but show considerable quantitative differences.

In an effort to obtain reliable quantitative values for the concentrations of many of the constituents of the gas phase, a method was developed which involved standardization of the smoking procedures, adequate removal of particulate matter by filtration, low temperature fractionation of the gases in a closed all-glass system, and quantitative analysis of the fractions by means of an infrared spectrophotometer with check analyses performed by a mass spectrometer. This report deals only with the smoke drawn through the cigarette and not with the so-called "side stream" smoke.

APPARATUS AND EXPERIMENTAL PROCEDURES

Procedure for Smoking Cigarettes and Collecting Gas Phase. The cigarettes used were 70 mm. in length and were made of burley, cased burley (10% dextrose added), bright, and 50 to 50 blends of bright with burley and with cased burley tobaccos, conditioned in an atmosphere adjusted to a relative humidity of 60% at room temperature. Prior to smoking, the cigarettes were selected in groups of three, so that the maximum weight deviation within each group was less than 0.1 gram. This precaution minimized somewhat differences in air flow through the three cigarettes of each group during smoking. No attempt was made to measure the resistance to flow of air in each cigarette, however, the deviations among average butt lengths at the conclusion of smoking were generally not more than 2 mm.

The puffing of the three cigarette groups was accomplished by collecting the gas phase in a syringe (Figure 1) that was calibrated to give a total volume of 105 ml., or an average puff volume of 35 ml. per cigarette. The particulate phase of the smoke was removed almost quantitatively on an alpha-cellulose filter (*F*) located between the syringe and the cigarette holder. Small amounts of some of the gas phase constituents may have been retained by the filter medium, however, this possible source of error was not regarded as serious as it was found practicable to smoke a large number of cigarettes through the same filter, and the reproducibility of the results obtained was satisfactory. The duration of each puff and the interval between puffs were 2

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and 30 seconds, respectively. The cigarettes were smoked to butt lengths of approximately 2 cm. in a room at approximately 25° C. and 50% relative humidity.

By appropriate manipulation of stopcocks 37 and 38 shown in Figure 1, the gas collected in the syringe after each puff was transferred to an evacuated collection chamber, *CC*-1, and cooled to liquid air temperature (ca. −185° C.). Periodically, the noncondensable gas collected in *CC*-1 was permitted to expand into the previously evacuated reservoir, *MC*. When the noncondensable materials attained pressures approaching atmospheric, the pressure and volume were determined and the gas pumped out through liquid air–cooled trap *T*6. The flow of the noncondensable gas through *T*6 was adjusted to a sufficiently slow rate to allow the condensation of entrained condensable materials. Such condensed materials were finally returned to chamber *CC*-1. The collection chamber, *CC*-1, was not subjected to direct pumping during the noncondensable voiding process. A sample obtained from 99 cigarettes was used for the analysis of the condensed gas phase of bright and burley tobaccos and a sample from 45 cigarettes was used for cased burley and bright-burley blend cigarettes.

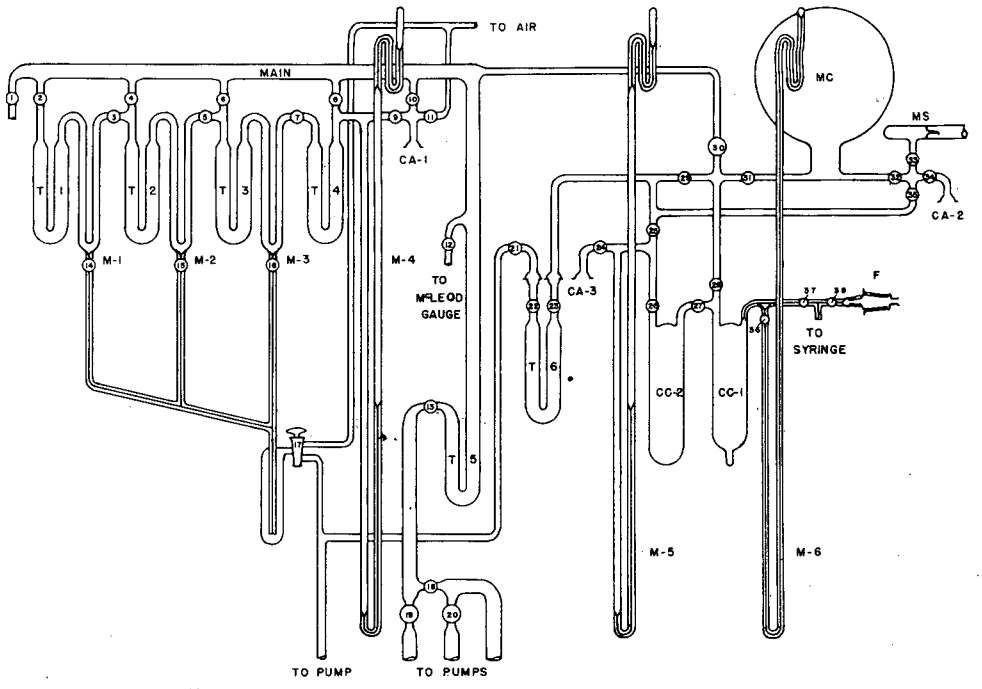

Figure 1. Apparatus for collection and fractionation

In the analysis of only the noncondensable portion of the gas phase, a modified procedure was adopted. One cigarette was smoked at a time, and the gas phase collected in *CC*-1 cooled with liquid air. After sufficient time, approximately 5 minutes, had elapsed to assure condensation of the condensable fraction in *CC*-1, the stopcock to *CC*-2 was opened and the noncondensable portion of the collected smoke distributed between *CC*-1 and *CC*-2, the latter being maintained at liquid air temperatures. This process was repeated for a total of three cigarettes, after which the liquid air was alternately removed and replaced on *CC*-2 to effect mixing of the noncondensable portion of the sample. After mixing, stopcock 27 was closed with liquid air surrounding both *CC*-1 and *CC*-2. The liquid air level was then slowly lowered to the constricted portion at the bottom of *CC*-1 to collect there all of the condensable fraction. Stopcock 27 was again opened and with the noncondensable portion of the sample at room temperature its pressure was measured. From the calibrated volume of the system the number of moles of gas was determined assuming the perfect gas laws applied. Following this, an aliquot was withdrawn through *CA*-3 into an infrared sample cell.

Fractionation of Liquid Air Condensables. The fractionation of the condensable gas phase was based on the relative vapor pressures exerted by the various constituents present in the mixture. In general, the following procedure was adopted.

The condensed mixture in *CC*-1 was transferred quantitatively to trap *T*5 by evaporation and recondensation at liquid air temperature. Traps *T*1 and *T*2 were set at a selected temperature, based in part on the vapor pressure data of possible constituents determined to be present from previous qualitative experiments. Trap *T*3 was cooled with liquid air and served as the collection vessel for the materials that were not condensable in traps *T*1 and *T*2. The volumes of the fractions thus collected at various temperatures were determined in calibrated trap *T*4 and corrected to standard temperature and pressure. To increase the efficiency, all transfer and fractionation procedures were made in systems evacuated to high vacuum ($<10^{-3}$ mm. of mercury).

The necessary temperature control of the fractionation train was provided by a liquid air cooling of a heat transfer fluid in which the traps were immersed. The heat transfer fluids were mixtures of isomeric heptanes at the lowest temperatures and alcohol at higher temperatures. The cooling system consisted of a coil of ¼-inch copper tubing wound around and soldered to two blocks of solid copper in such manner as to allow immersion of the coil and blocks into a pint-sized Dewar flask containing either heptanes or alcohol. The spacing between the copper blocks was such as to allow immersion of the trap between the blocks. Sections of the copper tubing exposed to the atmosphere were insulated with cork. Liquid air, from storage Dewars, was forced through the coils by means of dried air under pressure. Copper-constantan thermocouples were used to measure the temperatures. The thermoregulator controlling the trap temperatures consisted of a closed glass gas reservoir bounded on one side by an inclined arm filled with mercury. Solenoids located in the air lines to the liquid air storage Dewars were actuated by contacts in the mercury leg of the regulator, thus causing an increase of air pressure in the storage Dewars sufficient to force liquid air through the cooling coils around the traps when the temperature rose slightly. The system was capable of maintaining the bath at any given temperature between −170° C. and room temperature with a deviation of less than a degree at −150° C. In carrying out fractionation of the condensable portion of the gas phase, details as to trap design, relative vapor pressures, rates of transfer, etc., such as are discussed by Sanderson (*27*), were followed as carefully as practicable.

Analysis of the Gas Phase. Analysis of the individual constituents in a gas mixture was based on a compensation technique using a Perkin-Elmer double beam Model 21 recording infrared spectrophotometer equipped with 10-cm. gas cells. In the ideal case, the method required the identification of the constituents present in the sample cell, followed by the introduction of the pure constituent into the reference or compensating cell, until a selected characteristic absorption band due to the constituent in question was nullified. Such a procedure is greatly simplified if the specific wave length selected for the constituent is not subject to interference from the adsorption bands of other components present in the mixture and if there is no change in absorption of the constituent in the presence of other molecules.

The first condition can be met largely by selection of appropriate fractionation temperatures and close control of the fractionation procedure adopted prior to the analysis. In a complex mixture, the constituents determined to have unique absorption bands were compensated first. The greatly simplified spectrum thus obtained permitted the identification and further compensation of the remaining constituents. In this connection compensation by a single constituent of a band that is composed of contributions from several constituents can be immediately recognized by overcompensation that appears at other wave lengths.

The appearance of overcompensation thus provides a check on the validity of the procedure adopted. In the present work, information as to what substances were present in the sample was obtained from mass spectral results from previous exploratory experiments, combined with vapor pressure data, to indicate what compounds should be expected; absorption spectra of pure reference compounds; and simplified spectra of mixtures already partly compensated by the addition to the compensating cell of initially recognized constituents.

Low molecular weight substances frequently exhibit the phenomenon of changing their absorbancy with total pressure and with addition of foreign substances. Examples of this effect have been observed with methane, carbon monoxide, carbon dioxide, acetylene, ethylene, sulfur dioxide, nitrous oxide, hydrogen chloride, and other compounds (*1*, *3–5*, *11*, *12*, *18*, *22*). Pressure broadening effects are negligible, or are even absent, in the case of larger molecular weight constituents (*22*). The latter observation was verified in the case of acetaldehyde, hydrogen cyanide, isoprene, methanol, and acetone in the present work.

In the case of small molecules, it is apparent that the total pressures, as well as the partial pressures of each constituent must be essentially identical in both the sample and reference cells if a true measure of composition is to be obtained. To achieve this condition, a procedure involving a system of successive approximations may be applied. An apparatus suitably adapted for this purpose is shown in Figure 2. Essentially, it consists of a differential oil manometer connected between the sample and reference cell assemblies, a source of vacuum and dry nitrogen, a mercury manometer for measuring the total pressure in the reference cell, and a rotating fan device for mixing the constituents in the reference cell assembly.

There are several procedures that may be adopted for the synthesis of a mixture in the reference cell that corresponds to the partial and total pressure of the smoke mixture in the sample cell. The following general procedure was found to be satisfactory.

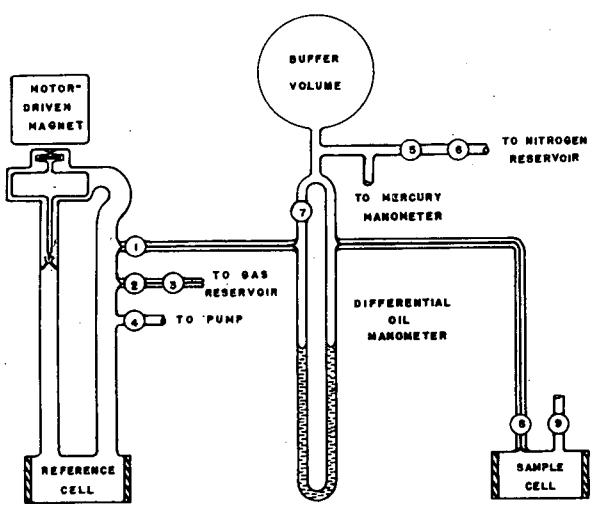

Figure 2. Apparatus for compensation analysis

The entire system excluding the sample cell was initially evacuated. Dry nitrogen was introduced into the sample and reference cell assemblies, including the reference cell itself, to an arbitrary pressure greater than the known total pressure of the gas sample in the sample cell. The pressures between the sample cell and the reference cell were then essentially equalized by appropriate additions of nitrogen, and the stopcock to the oil manometer was closed to permit a careful determination of any pressure differences between the two cells. When a suitable band had been selected for compensation, the pure reference gas was introduced into a reservoir and permitted to enter the reference cell assembly very slowly through two capillary lock stopcocks (Figure 2, stopcocks 2 and 3). The gas, which was rapidly distributed throughout the closed reference cell system by means of a magnetically controlled stirrer, was introduced in this manner until the absorption band being observed was completely compensated or nulled. The partial pressure of the added gas was noted by means of the sensitive differential octoil manometer. For a constituent that was subject to pressure effects, this partial pressure afforded a first approximation to the partial pressure of the same constituent in the sample cell. Equalization of the pressures in the two cells by the introduction of additional dry nitrogen into the sample cell again produced some uncompensation. By successive additions of the reference gas to compensate the band in question and equalization of pressures in the two cells by introduction of nitrogen in the sample cell, a means was obtained for arriving at a true compensation where both the partial and total pressures were essentially equal in both cells.

In principle, therefore, it is possible to synthesize a mixture in the reference cell in this stepwise fashion that is identical to the mixture in the sample cell, provided that the components present in the smoke mixture are known or can be identified and that unique absorption bands are obtained for each component as the compensation procedure progresses. The absorption spectrum, if the analysis is complete, should resemble the cell blank spectrum obtained before introduction of absorbing gases into either cell.

From the partial pressure data of the known added constituents, the total pressure of the gas phase sample mixture added initially to the sample cell, and application of the gas laws with appropriate temperature corrections, the required quantitative data at normal temperature and pressure are calculated readily. Since only relatively low pressures are dealt with, the ideal gas laws are generally applicable.

Table I. Analysis of Known Noncondensable Mixture by Infrared Methods[a]

Total Pressure, Mm. of Hg	Known Compn., Mole %		Compn. Detd. by Infrared Technique, Mole %	
	CH_4	CO	CH_4	CO
55			26.5	73.5
32	26.4	73.6	27.2	72.8
27.8			26.2	

[a] Constituents were added to reference cell with pressure equalization using dry nitrogen as a diluent.

Gases of high purity were obtained from the Matheson Co. Vapors were obtained from carefully purified samples of the less volatile compounds such as hydrogen cyanide, methanol, etc. Reference infrared curves were used in determining the purity of various samples.

RESULTS AND DISCUSSIONS

Analysis of Known Mixtures. A check on the validity of the infrared methods outlined was made by preparing and analyzing a synthetic mixture of known composition. The constituents chosen were those normally found in the noncondensable fraction, and in another case those representing a typical group of constituents found in the condensable gas fraction collected at −80° C. The results are summarized in Tables I and II.

The absorption spectra of both acetaldehyde and isoprene were obscured owing to interference from the other absorbing constituents in the mixture shown in Table II. Hydrogen cyanide, methanol, and acetone showed unique bands and were, consequently, compensated first. The removal of absorption bands attributable to methanol and acetone provided a unique acetaldehyde absorption band. The subsequent compensation of acetaldehyde in turn permitted the compensation of isoprene.

Table II. Analysis of Known Mixture of Condensable Gases by Infrared Methods

Component	Known Compn., Mole %	Compn. Measured Infrared Technique, Mole % Analysis 1[a]	Analysis 2[b]
Acetaldehyde	29.6	29.0	28.2
Acetone	23.9	23.3	24.0
Isoprene	18.5	...	18.2
Hydrogen cyanide	16.3	16.1	15.3
Methanol	11.7	12.6	12.2

[a] Constituents added to reference cell to compensation without pressure equalization with nitrogen.
Order of compensation: hydrogen cyanide (14μ), acetone (8.24μ), acetaldehyde (5.7μ), isoprene (6.24μ), methanol (9.7μ).
[b] Constituents added to reference cell with pressure equalization using nitrogen as a diluent.
Order of compensation: hydrogen cyanide (14μ), methanol (9.7μ), acetone (8.24μ), acetaldehyde (9μ), isoprene (11.2μ).

Table III. Results Noncondensable Fraction

Constituent, Mole %[a]	Burley	Cased Burley	Bright	Blend of 50% Burley 50% Bright	Blend of 50% Cased Burley 50% Bright
CO	6.02	3.98	2.84	4.50	3.94
	5.35	4.98	3.76	3.38	4.40
		4.34		5.58	
Av.	5.7	4.4	3.3	4.5	4.2
CH_4	0.61	0.53	0.54	0.79	0.62
	0.64	0.72	0.65	0.56	0.66
		0.75		0.63	
Av.	0.63	0.67	0.60	0.66	0.64

[a] Mole % values are calculated on basis of total gas phase including both condensed and noncondensed fractions.

Also an equally good analysis of materials listed in Table II was obtained when no attempt was made to equalize the total pressures in the sample and reference cells during the compensation procedure. It is apparent, at least in the case of the compounds listed in Table II, that there is virtually no "pressure broadening" effect involved. An experiment made in this connection showed conclusively that the absorbancy of a constant partial pressure of 22 mm. of acetaldehyde remained constant as nitrogen was added to bring the total pressure up to atmospheric pressure. The procedure that may be adopted for the analysis of larger molecular weight compounds is, therefore, considerably simplified.

Table IV. Analysis of Noncondensed Fraction from 50-50 Blend of Bright and Burley Tobaccos

Constituent	Analysis Mass spectral, mole %[a]	Infrared, mole %[a]
Air	63.4	
Excess nitrogen	26.6	
Carbon monoxide	3.94	3.98
Hydrogen	1.75	
Methane	0.66	0.67
Condensable materials[b]	3.33	
Total	99.7	

[a] Mole % values are calculated on basis of noncondensable fraction only.
[b] Some condensable constituents were present in noncondensable fraction involved in these analyses.

Analysis of Gas Phase of Cigarette Smoke. Table III summarizes the results of the infrared analysis of the noncondensable fraction of the gas phase of smoke from the cigarettes made from the various tobaccos. In preliminary experiments a considerable variation in carbon monoxide content was effected by varying the rate of smoking. This may account for some of the variation shown in Table III. A comparison of results of the mass spectral analysis and infrared analysis of a noncondensable fraction obtained from a blend of 50% burley and 50% bright tobaccos is recorded in Table IV. The reproducibility based on the two different methods lends support to the validity of the infrared analytical procedure described.

The analysis of the condensable fractions from bright, burley, cased burley, and bright-burley blend tobaccos was completed to a fractionation temperature of −50° C. The fractions obtained from bright tobacco at temperatures of −172°, −130°, and −101° C. were analyzed by both mass spectral and infrared methods. Mass spectral analyses were performed by the Consolidated Engineering Corp., Pasadena, Calif. The results are summarized in Table V. In general, reproducibility of duplicate infrared analyses was satisfactory, and the infrared results were generally substantiated by the mass spectral data.

Table V. Analysis of Condensable Phase Fractions by Mass Spectral and Infrared Methods

(Bright tobacco cigarettes)

Fractionation Temp., ° C.	Constituent	Analysis[a] Mass spectral	Infrared av. values
−172	Ethane	56.2	61.2
	Ethylene	34.5	31.6
	Acetylene	2.2	2.0
	Carbonyl sulfide	0.1	Trace
	Miscellaneous[b]	7.0	
	Total analyzed	100.0	94.8
−130	Carbon dioxide	93.0	95.3
	Methyl chloride	1.4	0.8
	Propane	0.7	0.9
	Propylene	0.2	0.4
	Acetylene	Trace	0.1
	Carbonyl sulfide	None	0.1
	Miscellaneous[c]	4.8	
	Total analyzed	100.1	97.6
−101	Carbon dioxide	63.8	70.4
	Methyl chloride	11.9	11.0
	Acetaldehyde	8.6	5.9
	Isoprene	4.5	5.5
	Hydrogen cyanide	0.2	2.4
	Miscellaneous[d]	11.1	
	Total analyzed	100.1	95.2

[a] Mole % calculated on basis of fraction volume only.
[b] Includes: carbon dioxide, propane, propylene, hydrogen cyanide, water, hydrogen sulfide, air.
[c] Includes: ethane, butanes, butenes, methane, air, other minor constituents.
[d] Includes: butanes, butenes, propane, methyl mercaptan, propylene, C_5 acetylenes, water, methane, other minor constituents.

A complete summary of the constituent analysis by infrared methods is given in Table VI. Generally the same constituents were present in all the smoke samples. The relative amounts of the individual constituents, on the other hand, varied considerably. The most significant differences were the appreciably smaller concentrations of the carbonyl compounds, methanol, hydrogen cyanide, carbon monoxide, carbon dioxide, and the higher molecular weight hydrocarbons in the smoke from samples of bright tobacco cigarettes. Combined with the over-all decrease of the condensable fraction concentration, these observations reflect the slower burning rate of bright tobacco, compared to burley and burley-bright blend. An average of 7 puffs was required to burn a burley cigarette to a standard butt length, 8 puffs in the case of burley-bright blend, and 10 puffs for a bright tobacco cigarette. Low molecular weight hydrocarbons, in particular methane, did not vary significantly among the tobacco types.

CONCLUSION

The results obtained indicate that a reasonably complete analysis of the individual components of the gas phase of cigarette smoke can be obtained by the methods set forth above.

ACKNOWLEDGMENT

The cooperation of the Research Laboratories of the American Tobacco Co. and of the Liggett and Myers Tobacco Co. is grate-

Table VI. Summary of Analyses by Infrared Methods of Gas Phase of Cigarette Smoke

Component	Bright	Burley	Cased Burley	Blend of 50% Bright 50% Burley	Blend of 50% Bright 50% Cased Burley
			Volume, Cc./Puff at NPT		
CO_2	2.5	3.9	3.5	2.8	
CO	1.0	1.8	1.5	1.4	1.3
CH_4	19.0×10^{-2}	20.0×10^{-2}	20.6×10^{-2}	20.4×10^{-2}	19.8×10^{-2}
Ethane	3.1×10^{-2}	3.6×10^{-2}	4.3×10^{-2}	3.8×10^{-2}	
Propane	2.5×10^{-2}	3.8×10^{-2}	4.6×10^{-2}	1.9×10^{-2}	
Ethylene	1.6×10^{-2}	2.6×10^{-2}	2.4×10^{-2}	2.4×10^{-2}	
Propylene	1.1×10^{-2}	2.3×10^{-2}	4.0×10^{-2}	1.3×10^{-2}	
Acetylene	0.40×10^{-2}	0.30×10^{-2}	0.90×10^{-2}	0.30×10^{-2}	
Isoprene	2.9×10^{-2}	7.7×10^{-2}	4.5×10^{-2}	14.0×10^{-2}	
Butadiene	0.30×10^{-2}	0.10×10^{-2}	Present	Present	
Acetaldehyde	3.4×10^{-2}	16.0×10^{-2}	12.0×10^{-2}	17.0×10^{-2}	
Acetone	1.7×10^{-2}	6.9×10^{-2}	5.7×10^{-2}	5.0×10^{-2}	
Methyl ethyl ketone	0.50×10^{-2}	1.8×10^{-2}	2.3×10^{-2}	2.2×10^{-2}	
Methanol	1.4×10^{-2}	2.5×10^{-2}	2.5×10^{-2}	3.7×10^{-2}	
HCN	3.4×10^{-2}	3.0×10^{-2}	6.0×10^{-2}	9.6×10^{-2}	
Diacetyl	Present	Present	0.40×10^{-2}	0.50×10^{-2}	
COS	2.4×10^{-2}	0.80×10^{-2}	5.1×10^{-2}	1.1×10^{-2}	
Methyl chloride	3.3×10^{-2}	3.3×10^{-2}	?	4.2×10^{-2}	
Ethylamine	Indicated				
Condensed phase analyzed	2.8	4.4	4.0	3.5	
Total condensables	3.3	5.1	4.7	4.4	
Total noncondensables	28.4	26.5	27.4	26.5	27.6
Total volume (NPT)	31.7	31.6	32.2	30.9	
			Mole % (of Total Volume)		
CO_2	8.0	12.2	10.8	9.2	
CO	3.3	5.7	4.4	4.5	4.2
CH_4	60. $\times 10^{-2}$	63. $\times 10^{-2}$	67. $\times 10^{-2}$	66. $\times 10^{-2}$	64. $\times 10^{-2}$
Ethane	9.7×10^{-2}	12.0×10^{-2}	13.5×10^{-2}	12.2×10^{-2}	
Propane	7.7×10^{-2}	11.8×10^{-2}	14.2×10^{-2}	6.3×10^{-2}	
Ethylene	5.0×10^{-2}	8.1×10^{-2}	7.6×10^{-2}	7.8×10^{-2}	
Propylene	3.6×10^{-2}	7.2×10^{-2}	12.4×10^{-2}	4.1×10^{-2}	
Acetylene	1.28×10^{-2}	0.93×10^{-2}	2.80×10^{-2}	1.10×10^{-2}	
Isoprene	9.0×10^{-2}	24.0×10^{-2}	13.9×10^{-2}	44.0×10^{-2}	
Butadiene	0.95×10^{-2}	0.45×10^{-2}	Present	Present	
Acetaldehyde	11.0×10^{-2}	49. $\times 10^{-2}$	37. $\times 10^{-2}$	55. $\times 10^{-2}$	
Acetone	5.3×10^{-2}	21.2×10^{-2}	17.7×10^{-2}	16.2×10^{-2}	
Methyl ethyl ketone	1.5×10^{-2}	5.6×10^{-2}	7.1×10^{-2}	7.3×10^{-2}	
Methanol	4.5×10^{-2}	7.9×10^{-2}	7.8×10^{-2}	11.9×10^{-2}	
HCN	10.8×10^{-2}	9.5×10^{-2}	18.6×10^{-2}	31.0×10^{-2}	
Diacetyl	Present	Present	1.3×10^{-2}	1.5×10^{-2}	
COS	0.76×10^{-2}	0.24×10^{-2}	1.60×10^{-2}	0.34×10^{-2}	
Methyl chloride	10.0×10^{-2}	10.0×10^{-2}	?	14.0×10^{-2}	
Ethylamine	Indicated	?	?	?	
Condensed phase analyzed[a]	8.8	13.8	12.4	11.4	
Condensed phase not analyzed[b]	1.53	2.3	2.2	3.0	
Total condensables	10.3	16.2	14.6	14.3	
Total noncondensables	89.7	83.8	85.4	85.7	

Average of Mass Spectral Analyses of Infrared Inactive Constituents.

Air	~57
Nitrogen (excess)	~24
Hydrogen	~ 1.6
Argon	~ 0.3

[a] Sum of mole % of condensables quantitatively accounted for.
[b] Based on mass spectral analysis, this material consists of approximately 80 mole % water and 20 mole % of other constituents, including some methanol and acetone.

fully acknowledged. The work was supported in part by grants from the Damon Runyon Memorial Fund.

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RECEIVED for review August 19, 1955. Accepted October 28, 1955.

Leaching of Some Fission Products from Soil

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A simple procedure permits the radiochemical determination of radionuclides of cesium, strontium, yttrium, cerium, ruthenium, zirconium, and niobium adsorbed on soil. The procedure consists of leaching the radionuclides with appropriate volumes and concentrations of boiling nitric, oxalic, or sulfuric acid; separation of the radionuclides from nonradioactive ions leached from the soil; and standard radiochemical separations. To determine the leaching efficiency of various solutions, radioactive tracers were adsorbed on Conasauga shale from aqueous solutions and then leached from the shale. It was found that 99% of the adsorbed radionuclides could be removed from the soil by the leach solutions. By modifying existing radiochemical procedures, it was possible to remove the contaminating nonradioactive ions leached from the soil and thus obtain the radionuclides free of ions which could interfere with their radiochemical determination.

SMALL amounts of radioactive wastes have been discharged to surface streams. As the wastes flow past the soil of the river, some of these radioactive substances are adsorbed on soils in contact with the stream. Under certain conditions a hazardous amount of radionuclides may be concentrated in this manner. Hence, it was desired to determine the radionuclides adsorbed on soil in rivers into which wastes from the Oak Ridge National Laboratory had been discharged.

In determining the radioactivity in soil, standard soil analysis procedures, consisting of treatment with strong acids followed by carbonate fusion of the insoluble residue, have been used (*1, 8–10*). This method is most applicable to soils containing large concentrations of radionuclides (so that only a small aliquot of the dissolved soil need be used in the analysis). No alternative to this method is available if the radionuclides are part of the soil structure, or if no knowledge concerning the identity of the radionuclides on the soil is obtainable. However, if large soil samples are used, as may be necessary if the radionuclides are present in low concentration, the large amounts of nonradioactive ions obtained by dissolving the soil interfere in the determination of the relatively minute amounts of radionuclides.

This problem may be reduced by simply washing the soil with solutions of salts or dilute acids, and removing from the soil the adsorbed radionuclides and only a small fraction of the nonradioactive ions present (*5–7*). This discussion is concerned with the choice of suitable leaching solutions and of favorable leaching conditions.

The radionuclides which are most important, both as health hazards and because they constitute a large fraction of the radioactive waste, are those of cesium, strontium, yttrium, the lanthanum rare earths, ruthenium, zirconium, and niobium. Hence, radionuclides of these elements were adsorbed from aqueous solutions on local Conasauga shale. The shale containing the adsorbed radioactive tracers was then brought into contact with various acid and salt solutions, and the fraction of the tracer removed from the soil was determined. Leaching solutions and conditions were selected which would remove from soil, with a minimum of time and solution volume, approximately 99% of the radioactive tracers, and which would be most suitable for subsequent radiochemical analysis.

PROCEDURE

The carrier-free radioactive tracers, obtained from the Operations Division, Oak Ridge National Laboratory, included cesium-137, ruthenium-106, cerium-144, zirconium-95, niobium-95, and strontium-yttrium-90. (Cesium, ruthenium, cerium, and strontium-yttrium were obtained as the chlorides; zirconium and niobium as oxalate complexes.) The first three were counted as liquids in a well-type sodium iodide scintillation counter at radioactive equilibrium, so that a count rate proportional to the activity of the parent radionuclides was obtained. Niobium-95, which has no radioactive daughter, was also counted with the scintillation counter. Zirconium-95 samples were evaporated on watch glasses 1 inch in diameter, under heat lamps, and counted with aluminum absorbers on end-window Geiger-Müller counters in such a manner that the count rate of the zirconium-95 beta particles only was obtained. To accomplish the latter, the contribution of the daughter niobium-95 beta particles and of the zirconium and niobium gamma rays was subtracted from the total count rate. In order to distinguish strontium-90 beta particles from those of yttrium-90, the same procedure was used for counting strontium-yttrium-90.

To fix the tracers on the soil, 5 grams of Conasauga shale, ground to pass a 270-mesh and be retained on a 324-mesh sieve and dried at 110° C., were added to 20 ml. of tap water. Approximately 10 microcuries of one of the tracers were added, and the slurry was mixed for 5 minutes. In order to remove most of the water from the soil, the slurry was centrifuged and the supernatant water was decanted three times. The soil, now containing the adsorbed radioactive tracer, was washed twice with 20 ml. of tap water, and dried by centrifuging. More than 99% of the cesium, yttrium, cerium, zirconium, and niobium tracers and approximately 95% of the strontium and ruthenium tracers added to the shale remained on the shale at this point.

To the soil was then added 20 ml. of a leach solution (such as one of those listed in Table I), and soil and solution were boiled and stirred for 3 to 4 minutes. The liquid and solid phases were separated by centrifuging. Using fresh portions of the same leach solution, leaching and phase separation were repeated three times, the volume of the supernates and the weight of the residue were determined, and aliquots of each were taken for counting. The removal efficiencies given in Table I are the ratios of the activity in the leach solution to the sum of the activity found in the residue and the activity in the leach solution. The activity adsorbed on the shale was compared with that leached and remaining and found to agree within 1% when gamma counting was used, and within 10% with beta counting.

Table I. Leaching of Fission Products from Soil

(Weight of soil, 5 grams; volume of solution, 20 ml.)

Radionuclide	Leach Solution	Removal per Leach, % (Av. of 8 Values)	Removal per 3 Leaches, %
Cs^{137}	9*M* H_2SO_4	80.0 ± 0.6	98.8
Sr^{90}	1*M* HNO_3	86.2 ± 1.1	99.5
Y^{90}	1*M* HNO_3	83.0 ± 2.6	99.1
Ce^{144}	1*M* HNO_3	84.7 ± 2.6	99.3
Ru^{106}	9*M* H_2SO_4 + 0.5 g. $KMnO_4$ (RuO_4 separated as vapor)	99.5 ± 0.1	...
Zr^{95}	0.5*M* $H_2C_2O_4$ and 1*M* HCl	88.7 ± 1.0	99.7
Nb^{95}	0.5*M* $H_2C_2O_4$ and 1*M* HCl	87.2 ± 1.2	99.6

The three leach solutions were combined; soluble cesium, strontium, yttrium; cerium, ruthenium, zirconium, or niobium salts (approximately 30 mg. in terms of the final precipitate of each) were added; and the respective isotopic carrier and radioisotope were precipitated. The precipitates were separated from the leach solution by centrifuging, dissolved, and analyzed by standard radiochemical procedures (*2*). As distillation was found to be more effective for removing ruthenium from the soil and for purifying it than the above method, that tracer was distilled from the leach solution in equilibrium with the soil.

EXPERIMENTAL RESULTS

Of the solutions evaluated, those found to be most satisfactory are listed in Table I. A leach solution was considered unsatis-

factory if less than 80% of a radioactive tracer was removed after one leaching, or less than 99% after three, or if the solution introduced difficulties in the radiochemical procedure. The removal efficiencies listed in Table I are the averages of eight observations, obtained from the first two of the three consecutive leaches in four series of leaching experiments (two with soil which had been dried in air for 1 month after the radioactive tracer had been added and two using moist soil immediately after addition of the tracer). There was no significant difference in the removal of tracers from moist and from dry soil, except in the case of cerium and yttrium, where removal from the dry soil was 5% less effective for both elements. The third consecutive leach removed a smaller fraction of the tracers from the soil than the two previous leaches. This may possibly be ascribed to the presence of radioactive impurities in the tracers, of which as much as 1% may have gone undetected.

In addition to the procedure described above, the effects of longer and shorter periods of stirring and of temperature were studied. It was found that boiling solutions were more effective for removing radioactive tracers from soil than were the same solutions at 20° C., and that removals from the soil increased with increasing stirring time up to approximately 2 minutes, after which no improvements in removal could be obtained by extending the time of stirring.

Both the solutions were found to be effective in leaching, and the more ineffective ones are described below; the subsequent chemical procedures are described cursorily, being given in detail elsewhere (*3*).

Cesium. Very little cesium-137 tracer was removed from soil by leaching with 20 ml. of saturated ammonium acetate, saturated barium chloride, or dilute mineral acids. The fraction removed increased with the concentration of hydrochloric acid, but even hot concentrated hydrochloric acid removed less than 70% of the tracer from the soil. Milligram quantities of cesium ion, on the other hand, were readily removed by ammonium salts and dilute acids, possibly indicating that a minute amount of cesium is incorporated in the soil structure, instead of being adsorbed as an ion.

The only leaching solution found satisfactory for cesium was boiling sulfuric acid in concentrations of 6 to 13*M*. After leaching with 9*M* sulfuric acid, the solution containing the cesium tracer was diluted to 6*M*, cesium carrier was added, and the cesium was precipitated by the addition of silicotungstic acid. The cesium silicotungstate was dissolved in sodium hydroxide, and the cesium was precipitated as the perchlorate in absolute alcohol.

Strontium. Strontium tracer was found to be readily removable from soil with dilute mineral acids, the removal efficiency increasing with acid concentration. The 1*M* nitric acid solution listed in Table I was used to obtain simultaneous removal of strontium and yttrium ions. A greater fraction of the strontium than of the yttrium could be leached by using, for example, a 0.1*M* nitric acid solution. To the leach solution containing strontium tracer, strontium carrier was added and the strontium was precipitated as the carbonate from a basic solution. After dissolving the carbonate in nitric acid, strontium was purified of calcium leached from the soil by precipitation of strontium nitrate with fuming nitric acid, and by washing the precipitate with acetone. The precipitate was dissolved in water, and strontium oxalate was precipitated from a basic solution.

Cerium and Yttrium. Radionuclides of these elements, whose behavior is representative of that of the lanthanum rare earths, were leached with dilute mineral acids such as 1*M* nitric acid. Cerium tracer was adsorbed on the soil both in the oxidized state from a sodium bromate solution, and in its reduced form in the presence of hydrogen peroxide, without any difference in removal efficiency being observed. After the addition of cerium or yttrium carrier to the leach solution, their fluorides were precipitated with hydrofluoric acid. The precipitate, which also contained calcium from the soil, was dissolved in fuming nitric acid to precipitate calcium nitrate. After the removal of the calcium, tetravalent cerium was precipitated with hydriodic acid and dissolved in hydrochloric acid. The separated cerium and yttrium were finally precipitated as oxalates from 0.2*M* hydrochloric acid.

Ruthenium. Removal of ruthenium-106 tracer from soil was found to be ineffective with dilute nitric acid (1*M*), and unsatisfactory with either dilute or concentrated hydrochloric acid, though the latter was more effective than nitric acid. If hydroxylamine hydrochloride was added to 1*M* hydrochloric acid, satisfactory removals were obtained in the first leach solution, but subsequent leaching removed almost none of the remaining ruthenium ions.

The satisfactory removal of ruthenium tracer from soil was attained by adding ruthenium carrier to a 9*M* sulfuric acid leach solution, oxidizing both carrier and tracer with potassium permanganate, and distilling the ruthenium tetroxide into a sodium hydroxide solution. In this manner, it was possible to leach 99%, and distill 98%, of the ruthenium on the soil with 20 ml. of leach solution. The ruthenium tetroxide in the basic solution was reduced with ethyl alcohol and precipitated as the dioxide, the dioxide was dissolved in hydrochloric acid, and the ruthenium was reduced to the metal with magnesium powder.

Zirconium and Niobium. Only a small fraction of niobium tracer was removed from soil with 1*M* hydrochloric acid; the fraction of zirconium removed was not much greater. As much as 80% of the zirconium and niobium could be leached with saturated oxalic acid, but results were not reproducible. It is probable that both zirconium and niobium are not adsorbed as ions, but as radiocolloids, and hence are not replaced by hydrogen ions, but are converted to soluble oxalate complexes by oxalic acid.

Satisfactory removals of zirconium and niobium tracers were obtained with 9*M* sulfuric acid, and with 0.5*M* oxalic acid solution, which was 1*M* in hydrochloric acid. The oxalic acid solution was preferred, because niobium pentoxide can be precipitated from it directly upon addition of nitric acid and potassium chlorate to the warm solution. The niobium pentoxide was dissolved in hydrofluoric acid, precipitated with ammonium hydroxide, dissolved in oxalic acid, and again precipitated with potassium chlorate. Upon addition of zirconium carrier to the leach solution, zirconium was precipitated in turn as the hydroxide, as the phenylarsonate, as barium fluozirconate, and as the cupferrate, before being ignited to the oxide.

DISCUSSION AND CONCLUSIONS

As indicated in Table I, 99% of the fission products studied were leached from Conasauga shale. Comparison of the leaching of 5 grams of soil with 20 ml. of solution, and of 10 grams of soil with 25 ml. of solution, indicated that the ratio of count rate per milliliter of solution to count rate per gram of soil is essentially constant for a given tracer. This implies that the fraction of radionuclide leached from any weight of soil with any volume of solution can be calculated, and, therefore, the amount of soil and leach solution used can be adjusted to obtain satisfactory count rates for the separated radionuclides. That the ratio of radionuclide per milliliter of solution to radionuclide per gram of soil is only between 1.0 and 2.0 for all the tracers indicates that either large solution volumes or several consecutive leaches are required for quantitative removal of the radionuclides from the soil. The amounts of soil and solution were chosen to permit use of the same 50-ml. centrifuge tube for leaching and for radiochemical separation, thus eliminating error caused by transfer of soil and solutions between centrifuge tubes or beakers. However, as indicated above, larger samples may be used.

Samples of river mud containing very little adsorbed radioactivity (5×10^{-11} curie per gram of dried mud) and samples of mud from a retention lake having a higher concentration of adsorbed radioactivity (8×10^{-8} curie per gram of dried mud)

were subjected to the leaching and the radiochemical separations described (*4*). In the more radioactive samples, the sum of the beta count rates of the separated radionuclides equaled the total beta count rate of the samples, indicating that all of the adsorbed radioactive nuclides were recovered. For the river mud samples, no such comparison could be made because of the low count rate of the mud samples; however, the individual radionuclides (mainly cerium and cesium) were readily identified and their concentration was determined by using large soil samples.

Errors would be introduced into the radiochemical determinations if nonradioactive isotopes of the fission products to be determined were in the soil—since the fraction of carrier recovered at the end of the procedure would then be too high—or if some of the natural activity found in soil were recovered with the fission products. Both possibilities were investigated: the former, by adding no carrier to the leach solutions; the latter, by adding carrier but no radioactive tracer. In both cases, negative results were obtained, eliminating these sources of error.

Hence, the leaching procedures developed for removing radionuclides of cesium, strontium, yttrium, cerium, ruthenium, zirconium, and niobium from Conasauga shale were found to be quantitative, and suitable for subsequent radiochemical analysis. The procedures are simple, rapid, and applicable to many other radionuclides. It is probable that the leach solutions can be used effectively with other soils, though, in view of the variation in soils encountered, it is suggested that the procedure be tested for removal efficiency, for contamination by natural radioactivity, and for the possibility of leaching interfering nonradioactive ions, before being applied to other soils.

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RECEIVED for review June 3, 1955. Accepted November 3, 1955. Author is at Robert A. Taft Sanitary Engineering Center, Public Health Service, assigned to Health Physics Division, Oak Ridge National Laboratory.

Colorimetric Determination of *p*-Acetophenetidide

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A new colorimetric method for the determination of *p*-acetophenetidide (acetophenetidin) involves acid hydrolysis of *p*-acetophenetidide, diazotization of the resulting *p*-phenetidine, and subsequent coupling with 2-naphthol in alkaline solution to give an orange-red dye. This color, with a maximum absorbance at 470 mμ, obeys Beer's law and may be quantitatively applied by comparing with suitable standards in a photoelectric colorimeter. The reaction is highly sensitive, making possible the determination of very small quantities of *p*-acetophenetidide. The simplicity of the procedure permits rapid analysis suitable for routine control for pharmaceutical preparations. Antihistamine compounds, caffeine, and salicylates commonly used in combination with *p*-acetophenetidide do not interfere.

ACETOPHENETIDIDE (*p*-acetophenetidin), commonly called phenacetin, is a well established analgesic and antipyretic drug which has been in widespread use for many years. Although the drug is official in the United States Pharmacopoeia, the British Pharmacopoeia, and the International Pharmacopoeia of the World Health Organization, no chemical assay is included in the monographs for *p*-acetophenetidide in these compendia. Identification of the drug is usually limited to a melting range and a color reaction. Determination of *p*-acetophenetidide in mixtures with other drugs in tablet or capsule form is accomplished either by extraction with an appropriate solvent or by the method of the Association of Official Agricultural Chemists (*2*), which consists of iodination and determination as the periodide either gravimetrically or titrimetrically through calculation of the amount of standard iodine solution consumed in the iodination. The former method lacks even limited specificity, while the latter is time-consuming, arduous, and often yields erratic results.

Although several colorimetric methods for *p*-acetophenetidide have appeared, these generally lack specificity. The methods of Miller (*7*) and of Horn (*6*) are based on the reaction of *p*-acetophenetidide with nitric acid to form a yellow color in solution. This color deepens to brown and lacks stability. Degner and Johnson (*5*) utilized the color developed by treating hydrolyzed *p*-acetophenetidide with chromic acid. This method is exceedingly exacting in its requirements of time and temperature during color development. A recent method of Afanas'ev (*1*) involves the reaction of *p*-acetophenetidide with chloramine T in ethyl alcohol on heating. These methods are rather susceptible to interference by other organic compounds.

p-Acetophenetidide on hydrolysis yields *p*-phenetidine or *p*-ethoxyaniline. This primary aromatic amine may be diazotized and coupled to yield a dye, thus offering a means of developing a sensitive and reasonably specific colorimetric method for the quantitative determination of the compound. It is this reaction in which diazotized *p*-phenetidine is coupled with 2-naphthol to yield an orange-red color which forms the basis of the method here reported.

REAGENTS AND SOLUTIONS

p-Acetophenetidide, recrystallized.
p-Phenetidine hydrochloride, freshly sublimed.
Sulfuric acid, 10%.
Hydrochloric acid, 10%.
Sodium nitrite solution, 1%.
Ammonium sulfamate solution, 5%.
Sodium bicarbonate–sodium hydroxide buffer. Prepared by dissolving 5 grams of sodium bicarbonate and 2 grams of sodium hydroxide in 100 ml. of water.

DEVELOPMENT OF STANDARD CURVE FOR *p*-PHENETIDINE

The colorimetric determination of *p*-acetophenetidide depends on its complete hydrolysis to *p*-phenetidine and subsequent dye formation by diazotization and coupling with 2-naphthol.

For the development of a standard curve, freshly sublimed *p*-phenetidine hydrochloride was used. For this purpose a solution containing 0.1 gram of the *p*-phenetidine hydrochloride dissolved in 1 liter of water containing 1 ml. of glacial acetic acid was used as a primary standard. Aliquots ranging from 2 to 10 ml., increasing in 2-ml. increments, were pipetted into each of five 100-ml. volumetric flasks and diluted to 25 ml. with water.

To each flask were added 2 ml. of 10% hydrochloric acid and 1 ml. of 1% sodium nitrite solution. The flasks were shaken and allowed to stand for 3 minutes.

At the end of this time 1 ml. of 5% ammonium sulfamate was added to each flask and these were allowed to stand for 1 minute longer.

The solutions were then made approximately neutral by the addition of 20 ml. of the sodium bicarbonate–sodium hydroxide buffer solution and shaken cautiously, and the color was developed by the addition of 1 ml. of 1% 2-naphthol in acetone. Solutions were then vigorously shaken and allowed to stand for 15 minutes. The colored precipitate was dissolved by the addition of 40 ml. of acetone, and sufficient water was then added to make exactly 100 ml.

A reagent blank was simultaneously prepared using 25 ml. of water instead of phenetidine solution. Colorimetric determinations were made in Corex cells with a Beckman DU spectrophotometer, using the reagent blank and reading the absorbance of the solutions at 470 mμ.

Absorbance values obtained plotted as the abscissa against the concentration of *p*-phenetidine as the ordinate resulted in a straight line which passed through the point of origin. The extinction coefficient $E^{1\%}_{1\ cm.}$ for *p*-phenetidine at 470 mμ was found to be 845 (Figure 1).

DISCUSSION OF COLOR REACTION

To determine the effect of concentration and the volume of the aliquot of solution used to develop color, aliquots of from 5 ml. to 50 ml., increasing by 5-ml. increments but containing a constant amount of *p*-phenetidine, were used to develop the color by the procedure given. All solutions developed color of maximum and uniform intensity within the 15-minute time period.

The 1 ml. of sodium nitrite solution was found to be adequate for full color development; smaller amounts gave erratic results and larger quantities were of no benefit.

As practiced in the Bratten and Marshall method (*3*), the use of five times more ammonium sulfamate than sodium nitrite was adequate to destroy the excess nitrous acid.

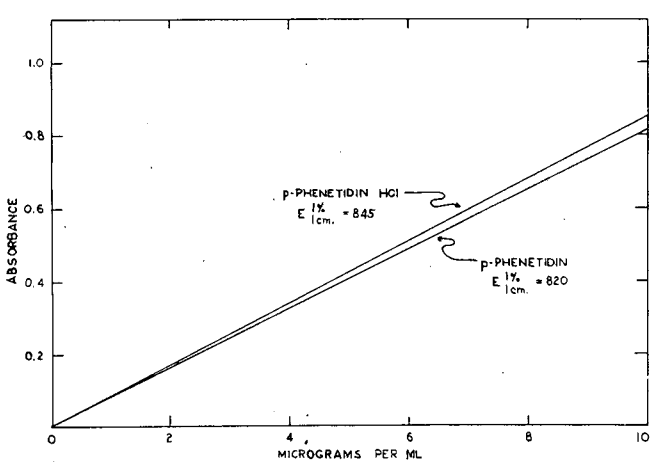

Figure 1. Standard curves for *p*-phenetidine dye solutions

2-Naphthol requires an alkaline solution for coupling with the diazonium compound. To determine the optimum pH for this reaction, solutions buffered from pH 5 to 11 were employed. Maximum and uniform color development was attained from pH 7 to 9. The buffer of sodium bicarbonate with sodium hydroxide added to neutralize the acid required for diazotization resulted in a final pH of approximately 8.0 (Figure 2).

A convenient method of adding the 2-naphthol was to dissolve it in acetone. Amounts of 2-naphthol varying from 2 to 20 mg. per ml. of acetone were used in color development without change or variation of color. The 1 ml. of 1% solution employed gives adequate color development without producing turbidity caused by larger quantities.

The time allowed the coupling reaction was varied from 5 minutes to 2 hours without measurable effect on the absorbance. Fifteen minutes was arbitrarily chosen as a convenient and adequate time.

Solutions containing 40% of acetone and 60% of water produced stable solutions of the dye compound. The sodium bicarbonate and 2-naphthol did not produce opalescence or turbidity in this mixture.

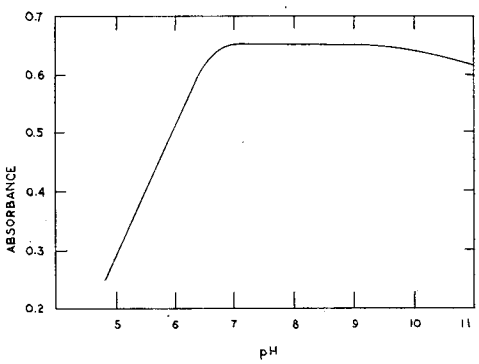

Figure 2. Effect of pH on color development of *p*-phenetidine dye

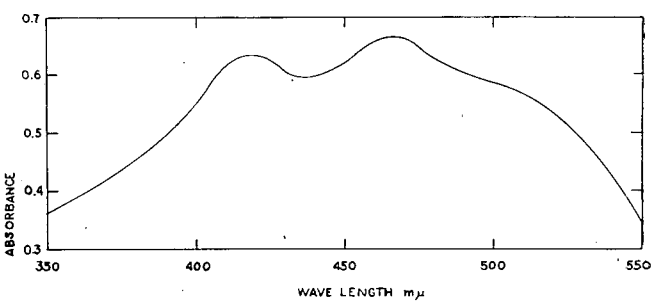

Figure 3. Absorbance curve for *p*-phenetidine–diazonaphthol derivative

0.00005*M* Solution

Of the two absorption maxima found when plotting the absorbance, the peak at 470 mμ was somewhat higher than the one at 415 mμ and was selected for this work (Figure 3).

The color formed is stable for a number of hours, but begins to fade after a day.

The addition of the small quantity of acetic acid to the *p*-phenetidine solution stabilizes it and prevents decomposition and precipitation as melanins.

PROCEDURE FOR HYDROLYSIS OF *p*-ACETOPHENETIDIDE

Accurately weigh 0.1 gram of *p*-acetophenetidide into a 100-ml. volumetric flask. Add 20 ml. of 10% sulfuric acid, warm on a steam bath for 3 hours, cool, and dilute to volume with water. Dilute a 50-ml. aliquot to exactly 1 liter. Transfer exactly 10 ml. of this solution to a 100-ml. volumetric flask and proceed to develop the color as previously described.

To assure that complete hydrolysis was attained and that the *p*-phenetidine resulting from hydrolysis of *p*-acetophenetidide produced data consistent with those obtained with pure *p*-phenetidine, a series of samples of acetophenetidin ranging from 0.025 to 0.3 gram was weighed into 100-ml. volumetric flasks and color was developed as previously described. The resulting absorbances, when plotted, resulted in a straight line which conformed to Beer's law and superimposed itself upon the line derived from pure *p*-phenetidine. With samples weighing more than 0.1 gram it is necessary to add a few drops of glacial acetic acid to prevent decomposition.

CALCULATIONS

In any given solution of hydrolyzed *p*-acetophenetidide, the concentration may be determined and calculated by the following expression:

$$\frac{\text{Absorbance of } p\text{-phenetidine dye solution}}{E^{1\%}_{1\text{ cm.}}\ p\text{-phenetidine dye solution}} \times \frac{\text{mol. wt. } p\text{-acetophenetidide}}{\text{mol. wt. } p\text{-phenetidine HCl}} \times \text{dilution factor} = \text{grams of } p\text{-acetophenetidide per sample}$$

Applying this to the examples listed in Table I the following working expression is derived:

$$\frac{\text{Absorbance of } p\text{-phenetidine dye solution}}{845} \times 1.032 \times 2000 = \text{grams of } p\text{-acetophenetidide per gram of sample}$$

DISCUSSION

Results given in Table I indicate variation of less than 1% and accuracy and reproducibility consistent with results usually considered satisfactory for colorimetric methods.

Table I. Reproducibility of Results in a Series of Analyses

Absorbance of *p*-Phenetidine Dye Solution Equivalent to 5 γ *p*-Acetophenetidide per Ml.		% of Theory
0.411		100.39
0.409		99.90
0.410		100.14
0.408		99.66
0.407		99.42
0.409		99.90
0.411		100.39
0.409		99.90
0.410		100.14
0.409		99.90
0.407		99.42
0.408		99.66
0.409		99.90
0.410		100.14
0.410		100.14
0.409		99.90
0.408		99.66
0.409		99.90
0.409		99.90
0.409		99.90
0.408		99.66
0.411		100.39
0.408		99.66
0.409		99.90
	Mean	99.87
	Max. dev.	0.52
	Stand. dev.	0.28

The use of hydrochloric acid in the hydrolysis procedure led to an unexpected difficulty. When concentrated acid was used, only about 70% of the *p*-acetophenetidide could be accounted for as *p*-phenetidine after coupling. This seemed due to incomplete hydrolysis rather than to partial destruction, as on heating for prolonged periods the result remained constant. This corroborates the observation of Casini (*4*), who found that even after 6 hours of boiling in hydrochloric acid only 74% of the *p*-acetophenetidide was hydrolyzed. Complete hydrolysis could be attained with lower concentrations of acid. Ten per cent sulfuric acid gave satisfactory and reproducible results and showed no evidence of decomposition of *p*-phenetidine when heated on a steam bath for periods up to 8 hours. Five per cent sulfuric acid required a somewhat longer time to complete the hydrolysis but was satisfactory. Higher concentrations of sulfuric acid showed evidence of decomposition of *p*-phenetidine (Figure 4).

The colored *p*-ethoxybenzenediazonium 2-naphthol is but slightly soluble in water. It was therefore found necessary to use a water-miscible solvent to keep it in solution. Of the solvents tried, 40% acetone alone was satisfactory. This not only maintains the dye in solution but also the unreacted 2-naphthol, thereby preventing it from producing turbidity.

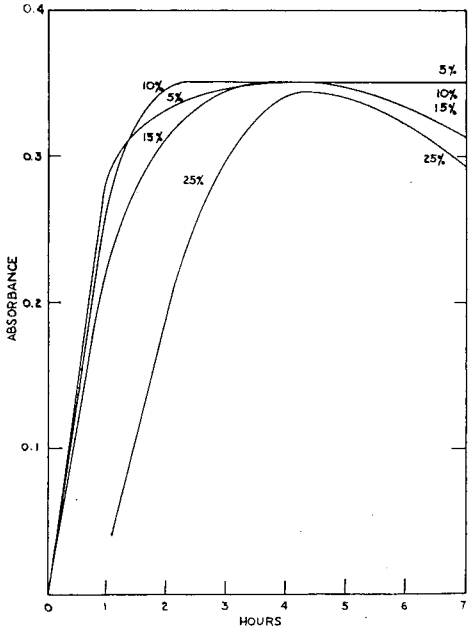

Figure 4. Effect of acid concentration on hydrolysis of *p*-acetophenetidide

The intensity of the color produced by the *p*-phenetidine in the reaction is directly proportional to the quantity of *p*-acetophenetidide from which it is derived and these quantities are related as the inverse proportion of their molecular weights. The fact that identical color intensities are given by equal molecular concentrations of *p*-phenetidine derived by hydrolysis from *p*-acetophenetidide and pure *p*-phenetidine hydrochloride, attests the complete hydrolysis of *p*-acetophenetidide and the authenticity of the method.

The extinction coefficient, $E^{1\%}_{1\text{ cm.}}$, of 820 for *p*-phenetidine (or 845 for *p*-phenetidine hydrochloride), is sufficiently high to obtain reliable readings on as little as 25 γ of *p*-acetophenetidide.

Table II. Assay of *p*-Acetophenetidide in Capsules of Aspirin, *p*-Acetophenetidide and Caffeine

Composition per Capsule, Grains		Grains *p*-Acetophenetidide per Capsule Found	% of Theory
Aspirin	3		
p-Acetophenetidide	2	2.00	100.0
Caffeine	0.5	1.99	99.5
		2.00	100.0
		2.03	101.5
		2.00	100.0
		1.98	99.0
		1.98	99.0
		1.98	99.0
		2.00	100.0
		1.99	99.5

Even smaller amounts may be determined by scaling down the volume of dilution.

A series of determinations carried out on a number of aliquots from a hydrolysis reaction indicated satisfactory reproducibility with a deviation expectation of 0.5% (Table I).

Certain diazotizable primary aromatic amines, such as the sulfonamides, *p*-aminophenol, and acetanilide interfere in the method, but many of these may be separated through preliminary extraction procedures. Medicaments commonly used in conjunction with *p*-acetophenetidide such as aspirin, caffeine, codeine, and antihistaminic compounds do not interfere.

When applied to the assay of *p*-acetophenetidide in tablets or capsules of aspirin, *p*-acetophenetidide, and caffeine, the method may be used directly on the mixture without separation of any of the components. Results of a series of assays on such a combination are given in Table II.

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RECEIVED for review May 23, 1955. Accepted November 7, 1955.

Standard Potential of Iodine–Iodine Monocyanide Half Cell

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The standard potential of the half cell $I_{2(s)} + 2HCN = 2ICN + 2H^+ + 2e^-$ has been measured in cells with negligible liquid junction potentials. The value calculated at 25° C. in solutions 0.2 to 4.0 volume formal in perchloric acid is −0.711 volt; the value for a hypothetical half cell 1 molal in iodine is −0.625 volt. The constant for the reaction $I_2 + HCN = ICN + I^- + H^+$ was calculated from solubility measurements to be 0.87.

TITRATIONS are in use which involve the oxidation of iodine to iodine monocyanide in acid cyanide solutions (*10*). These titrations do not require such high acid concentrations as do the corresponding iodine monochloride titrations (*12*). The study of the iodine–iodine monocyanide half-cell potential was made because of the use that this potential would have in the calculation of equilibria and end point conditions during iodine monocyanide titrations.

Earlier measurements have been made of the iodine–iodine monocyanide half-cell potential, but the lack of consistency of the results leaves the data in question. In 1912 Kovach (*5*) studied the iodine–iodine monocyanide system and from her data the value −0.60 volt is calculated for the half-cell reaction

$$I_2(1M) + 2HCN = 2ICN + 2H^+ + 2e^- \qquad (1)$$

However, from Kovach's data the iodine–iodide half-cell potential is calculated, with corrections for complex ion formation (*13*), to be −0.520 volt, 0.016 volt different from the value given by Latimer (*6*). More recently Gaugin (*2*) reported an average value of −0.640 volt for the half cell of Equation 1. In his study, deviations from the mean were large, becoming 0.180 volt at pH 6.

In the present investigation potential measurements were made in solutions which ranged from 0.2 to 4*VF* in perchloric acid and from 0.06 to 0.3*VF* in total cyanide, and the value −0.625 ± 0.003 volt was obtained for this half cell. In the course of the potential measurements a re-evaluation of the equilibrium constant for the disproportionation reaction

$$I_2 + HCN = ICN + I^- + H^+ \qquad (2)$$

was made by a study of the solubility of iodine in hydrocyanic acid solutions, and the value 0.870 ± 0.009 mole per liter was obtained.

EXPERIMENTAL

Reagents. Reagent grade chemicals were used in all preparations. Volumetric apparatus was calibrated prior to its use.

A 6*VF* perchloric acid solution was prepared from the 60% acid and was standardized against sodium hydroxide solution which had been standardized against potassium hydrogen phthalate.

Standard solutions of potassium iodate, approximately 0.1 and 0.002*VF*, and of silver nitrate, 0.1*VF*, were prepared by weight from the salts.

Sodium cyanide solutions, 0.6*VF*, were standardized by a modified Liebig titration just before each experiment. A 10.00-ml. portion of the cyanide solution was mixed with 2 ml. of 6*VF* sodium hydroxide solution, 2 ml. of 6*VF* ammonium hydroxide, and 1 ml. of 1.0*VF* potassium iodide solution. This solution was diluted to 50 ml. and titrated with silver nitrate solution to the appearance of the silver iodide precipitate.

The required quantity of resublimed iodine was ground in an agate mortar immediately before each experiment.

Commercial tank hydrogen was passed through a washing chain similar to that described by Kolthoff and Laitinen (*4*). In early experiments the potassium permanganate solution specified by these workers was troublesome because of the formation of large quantities of manganese dioxide, and was replaced by a solution 0.5*VF* in chromic trioxide, 4*VF* in phosphoric acid, and 9*VF* in sulfuric acid.

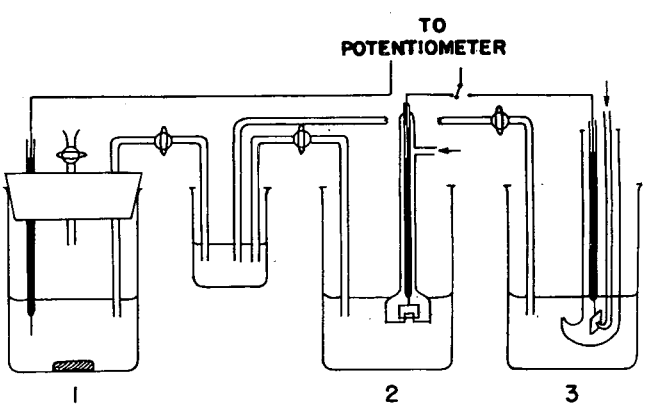

Figure 1. Cell construction

1. Iodine–iodine monocyanide half-cell compartment
2, 3. Hydrogen electrode compartments

Apparatus. The cell assembly, shown in Figure 1, consisted of three 200-ml. lipless beakers connected by glass tubing bridges to a single intermediate vessel. The bridges were prepared from 6-mm. outside diameter glass tubing and were fitted with ground-glass stopcocks. One of the 200-ml. beakers contained the iodine–iodine monocyanide half-cell solution, and the other two,

contained hydrogen electrode assemblies. The temperature of the half-cell solutions was maintained at 25.0° ± 0.5° C. by means of water baths.

The beaker which contained the iodine–iodine monocyanide half-cell solution had a tightly fitting stopper through which passed the bridge, a bright platinum wire electrode, mounted in 6-mm. glass tubing, and an inlet tube which was flared and ground to fit the tip of the pipets used for adding the half-cell solution components, thus permitting their addition without loss of hydrocyanic acid. A magnetic stirrer provided constant stirring throughout the measurements.

Two different types of hydrogen electrodes were used (*3, 9*); these are shown in Figure 1. No significant advantage was found in one type over the other for the purposes of this study. The electrodes were platinized by the procedure of Lorch (*8*).

The potential measurements were made with a Gray Instrument Co. Model E potentiometer with a Leeds & Northrup enclosed lamp and scale galvanometer.

Procedure. In experiments made to determine the equilibrium constant for the disproportionation of iodine in the presence of hydrocyanic acid, two sets of solutions were prepared in glass-stoppered flasks. One set was prepared by the addition of weighed portions of sodium perchlorate to standard perchloric acid; the other was prepared by mixing standard solutions of sodium cyanide and perchloric acid. The final perchloric acid concentration, sodium perchlorate concentration, and ionic strength were the same in both sets of solutions. Finely ground iodine was added in excess to all solutions and the mixtures were maintained at 25° ± 0.2° C. for periods of time up to 7 days. Samples were transferred by a 25-ml. pipet, using rubber bulbs for drawing the solution into the pipet, to 300-ml. iodine flasks which contained 5 ml. of carbon tetrachloride and sufficient hydrochloric acid to make its final concentration $4VF$. The partial pressure of the hydrogen cyanide above the pipetted solutions was less than that of water, therefore such solutions could be pipetted without significant change in concentration. The solutions were then titrated with $0.002VF$ potassium iodate by the procedure described by Swift (*12*). Titrations were repeated on successive days until the reproducibility of the results indicated that equilibrium had been achieved.

In making the potential measurements, calculated volumes of water, potassium iodate solution, and perchloric acid were added to an excess of freshly ground iodine in the iodine–iodine monocyanide half-cell beaker. The beaker was stoppered and standard sodium cyanide solution was added from a pipet through the inlet tube. The bridges, intermediate vessel, and hydrogen electrode chambers were filled with perchloric acid of the same concentration as the final molal concentration of perchloric acid in the iodine–iodine monocyanide half cell. Potential measurements were made at 5- to 10-minute intervals until the readings remained constant within 0.1 mv. for at least 30 minutes. In preliminary experiments it was found that the cell potential remained constant to 0.1 mv. for 24 hours.

DISCUSSION

Disproportionation of Iodine in Hydrocyanic Acid Solutions. The equilibrium constant for the disproportionation reaction (Equation 2) must be known if the concentrations of the species present in the iodine monocyanide half cell are to be calculated. Earlier calculations of the equilibrium constant for this equation by Kovach (*5*) and by Lewis and Keyes (*7*) depended upon conductivity and vapor pressure measurements, respectively. In each case the acid concentrations at which the measurements were made were appreciably lower than those which were used in the present potential measurements. Therefore, it was concluded that a determination of the equilibrium constant should be made by an independent method under conditions comparable to those used in the potential measurements.

The solubility of iodine at 25° C. in solutions $1.00VF$ in perchloric acid and $0.089VF$ in sodium perchlorate was found from a series of eight experiments to be $11.95 \pm 0.12 \times 10^{-4}VF$. The sodium perchlorate was added in order to keep the ionic strengths in these and subsequent experiments constant.

In Table I are data and calculations from experiments made to determine the value of the disproportionation constant. The concentrations of iodine and triiodide ions were calculated from the solubility of iodine determined above, the volume of potassium iodide required for the titration of the sample, and the dissociation constant for the triiodide ion, $K = 1.3 \times 10^{-3}$ at 25° C. (*1*). The iodine monocyanide concentration is equal to the sum of the iodide ion and triiodide ion concentrations, while the hydrocyanic acid concentration is the difference between the initial formal sodium cyanide concentration and the iodine monocyanide concentration. The value obtained for the disproportionation constant is 0.870 ± 0.009.

Table I. Determination of Disproportionation Constant

$$K = \frac{(ICN)\ (I^-)\ (H^+)}{(I_2)\ (HCN)} \text{ at } 25° \text{ C.}$$

(Solutions were $1.00VF$ in perchloric acid, $0.089VF$ in hydrocyanic acid, and $0.089VF$ in sodium perchlorate)

Expt.	KIO_3 Required, Mmole	I^-, VM	I_3^-, VM	ICN, VM	HCN, VM	K
1	0.02105	0.00700	0.00643	0.0134	0.0756	0.854
2	0.02100	0.00698	0.00641	0.0134	0.0756	0.853
3	0.02136	0.00711	0.00653	0.0136	0.0754	0.884
4	0.02112	0.00703	0.00645	0.0135	0.0755	0.866
5	0.02141	0.00713	0.00654	0.0137	0.0753	0.894
					Av.	0.870 ± 0.009

The values obtained for the constant by other methods are near this value. Calculations from the data of Lewis and Keyes (*7*) give values ranging from 0.91 to 1.67, with a "weighted mean" of 1.4. Kovach's values for the constant range from 1.17 to 1.50, with 1.38 as the average. Yost and Stone (*13*) have applied their formation constants for the iodine dicyanide and diiodocyanide complexes to Kovach's data and obtained a corrected average value of 1.50.

In the present calculations of the standard iodine–iodine monocyanide half-cell potential the value 0.87 was used for the disproportionation constant. It was found that the use of 1.50 for the constant would change the calculated standard potential by only approximately 1 mv.

Complexes of Iodine Monocyanide. Yost and Stone (*13*) obtained the values 1.17 and 2.50 for the association constants of the iodine dicyanide and diiodocyanide complexes, $K_1 = [I(CN)_2^-]/(ICN)\ (CN^-)$ and $K_2 = (I_2CN^-)/(ICN)\ (I^-)$, respectively. Hence, as the solutions used in the present investigation were acid and the iodide concentrations were low, these complexes were present only in small concentration. For example, in a solution approximately $0.5VF$ in perchloric acid, $0.05VF$ in hydrocyanic acid, $0.05VF$ in iodine monocyanide, and which is saturated with iodine, the calculated molal concentrations of diiodocyanide and iodine dicyanide are 2×10^{-4} and 1×10^{-11}, respectively. Less than 0.5% of the iodine monocyanide is complexed even to diiodocyanide.

Reference Electrodes. Hydrogen electrodes had the major advantage over other common reference electrodes of permitting virtual elimination of liquid junctions from the cell. The electrodes were found to cause no difficulty either in their construction or operation. The special precaution was made of providing an intermediate vessel between half cells and ungreased stopcocks in the bridges. These stopcocks were opened briefly at the time of potential measurements. These steps were taken to minimize diffusion of components of the iodine–iodine monocyanide half cell into the hydrogen electrodes.

In general, about 20 potential measurements could be made with the two hydrogen electrodes before the potential readings obtained began to differ by more than 0.1 mv. When this occurred, the electrodes were replatinized.

Perchloric acid was chosen for use in this study because data for its activity are available (*11*) and it evidently has only very slight tendency to form complexes with the half-cell constituents.

Iodine–Iodine Monocyanide Half Cell. Analyses made over a 24-hour period of solutions at 25° C. and at the acid concentrations used showed no significant change in cyanide concentration. Therefore the half-cell reaction shown in Equation 1 was assumed to be the potential controlling reaction.

Table II. Standard Potential, $E°$, of Half Cell
$I_2(1M) + 2HCN = 2ICN + 2H^+ + 2e^-$

$HClO_4$, VF	ICN, VM	HCN, VM	$-E_{cell}$, Volt	$-E°$, Volt
4	0.050	0.150	0.6871	0.6288
			0.6840	0.6257
2	0.053	0.247	0.6695	0.6225
			0.6707	0.6237
	0.0515	0.148	0.6838	0.6223
			0.6838	0.6223
	0.050	0.050	0.7114	0.6249
			0.7126	0.6261
	0.0501	0.0099	0.7609	0.6327[a]
			0.7565	0.6293[a]
			0.7567	0.6295[a]
			0.7651	0.6368[a]
1	0.0539	0.252	0.6730	0.6263
			0.6710	0.6243
	0.0540	0.156	0.6820	0.6227
			0.6842	0.6249
	0.0513	0.0487	0.7119	0.6240
			0.7111	0.6232
	0.0481	0.0488	0.7103	0.6232
	0.0494	0.0126	0.7411	0.6196[a]
			0.7502	0.6287[a]
0.5	0.062	0.254	0.6625	0.6124[a]
	0.062	0.259	0.6740	0.6247
	0.061	0.239	0.6769	0.6255
			0.6762	0.6248
	0.058	0.152	0.6895	0.6278
			0.6870	0.6253
	0.0526	0.0474	0.7125	0.6235
			0.7124	0.6234
	0.0506	0.0094	0.7475	0.6178[a]
			0.7556	0.6259[a]
0.2	0.0725	0.2275	0.6840	0.6269
			0.6833	0.6262
	0.065	0.135	0.6945	0.6268
			0.6925	0.6248
			0.6950	0.6273
			0.7000	0.6323
	0.0558	0.0442	0.7195	0.6271
			0.7167	0.6243
	0.0513	0.0087	0.7614	0.6293[a]
			0.7610	0.6289[a]
			0.7627	0.6306[a]
			0.7600	0.6279[a]
			Av. 0.6248 ± 0.0026 volt	

[a] Not included in calculation of average.

In preliminary experiments attempts were made to determine analytically the concentrations of iodide ion, iodine, and iodine monocyanide present in the cell solutions at the time of the potential measurements. However, the sampling technique did not entirely eliminate all the small particles of iodine that were present in the system; consequently this method did not yield satisfactory results. Therefore, the concentrations of species present at equilibrium were calculated in the following manner.

The concentration of iodine was determined from the solubility measurements discussed above. The concentration of iodine monocyanide was calculated from the volume of standard sodium iodate solution added, with the assumption that the reduction of iodate by iodine was quantitative. The difference between the total cyanide added and that present as iodine monocyanide gave the concentration of hydrocyanic acid. The hydrogen ion concentration was calculated from the quantities of perchloric acid and sodium iodate added, with correction for the hydrocyanic acid formed. Further refinement of the calculated concentration values was made by application of the disproportionation constant.

CALCULATIONS AND RESULTS

In Table II are shown the cell concentrations, measured cell potentials (E_{cell}), and calculated standard half-cell potentials ($E°$) for Equation 1. The calculations of the standard half-cell potential were made with the assumption that the volume molal concentrations of iodine monocyanide and hydrocyanic acid are equal to their activities at the concentrations involved. The reference state for iodine is taken as 1.0 molal in these calculations of the $E°$ value in order to simplify calculations involving unsaturated solutions. The standard potential was calculated from the expression

$$E° = E_{ref} + E_{cell} + \frac{RT}{F} \ln \frac{(H^+)\,(ICN)}{(I_2)^{1/2}(HCN)} \tag{3}$$

or, since

$$E_{ref} = \frac{RT}{F} \ln \frac{(P_{H_2})^{1/2}}{(H^+)}$$

$$E° = E_{cell} + \frac{RT}{F} \ln \frac{(ICN)}{(I_2)^{1/2}\,(HCN)} \tag{4}$$

The addition of $\frac{RT}{F} \ln (s_{I_2})^{1/2}$, where s_{I_2} is the molal solubility of iodine, to the value of $E°$ gives the half-cell potential for Equation 5

$$I_2(s) + 2HCN = 2ICN + 2H^+ + 2e^- \tag{5}$$

in which solid iodine is taken as the reference state.

The calculated values of $E°$ are reproducible with a standard deviation of ±0.0026 volt through the range of concentrations studied, except at approximately $0.01VM$ hydrocyanic acid, the lowest concentration of this species considered. A probable cause for the relatively large random errors at $0.01VM$ hydrocyanic acid is that $E°$ is dependent upon the ratio (ICN)/(HCN). The iodine monocyanide concentration is fixed by the quantity of iodate added, is approximately constant, and is subject to errors of the same relative magnitude in every experiment. The concentration of hydrocyanic acid, however, depends upon the difference between the sodium cyanide added and the iodine monocyanide produced. Therefore, the absolute errors in the concentration of hydrocyanic acid remain about constant, but become much larger relatively as the concentration of hydrocyanic acid decreases. The effect of this is seen by the consideration of two cases:

(A) HCN = $0.15VM$; ICN = $0.05VM$
(B) HCN = $0.01VM$; ICN = $0.05VM$

If the concentration of hydrocyanic acid is changed by 0.003 mole per liter, the change in the calculated $E°$ for case A is 0.0005 volt while that for case B is 0.0068 volt. Thus, the same absolute error in the concentration of hydrocyanic acid causes negligible error in the potential of case A and causes serious error in case B. Because of the large random deviations of the potential values in solutions $0.01VM$ in hydrocyanic acid, these potential values were not used in calculation of the average value.

A check upon the consistency of the data obtained can be made by the calculation of the iodine-iodide potential from the iodine–iodine monocyanide potential and the disproportionation constant for iodine in hydrocyanic acid. The following tabulation of equations indicates the calculation:

	ΔF
$I_2(1M) + 2HCN = 2ICN + 2H^+ + 2e^-$	28846
$2I_2(1M) + 2HCN = 2ICN + 2H^+ + 2I^-$	165
$I_2(s) = I_2(1M)$	3918
$2I^- = I_2(s) + 2e^-$	24763

or

$$E° = -0.5366 \text{ volt}$$

This value for the iodine-iodide potential differs by just 0.0011 volt from the value given by Latimer (*6*). Thus, the results of this study are found to be consistent with independent potential determinations of related systems.

ANALYTICAL APPLICATION

By making use of this half-cell potential of −0.63 volt, one can calculate the potential required for the oxidation of any given quantity of iodine (or iodide) to iodine monocyanide under specified conditions. For example, assume that the solution is

1.0 molal in hydrogen ion, 0.1 molal in hydrocyanic acid, that the iodine monocyanide formed is 0.01 molal, and that the iodine at the end point is 10^{-6} molal; the iodine–iodine monocyanide potential in such a solution would be −0.74 volt. Considering only equilibrium conditions, this potential value indicates that the oxidation could be effected by electrolytically generated bromine, chlorine, or tetrapositive cerium; in addition, under similar conditions, quantitative oxidation of tripositive arsenic and antimony should be obtained. Qualitative experiments have indicated that these predictions can be realized and quantitative studies are in progress.

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RECEIVED for review August 8, 1955. Accepted November 18, 1955. Contribution No. 2025 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology.

Determination of Sulfuric and Sulfonic Acids in Sour Oil

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In the sulfonation of lubricating oils a sour oil layer is obtained which contains sulfonic acids and a small amount of sulfuric acid. Methods of analysis were needed for both acids in order to follow the processing of the sour oil to a finished sulfonate. The aniline precipitation method, used by others on acid sludges where sulfuric acid contents were 20 to 90%, has proved to work satisfactorily on sour oils where the concentration is 0.01 to 0.25%. This method has been extended so that sulfonic acids can be determined on the same sample. The presence of sulfur dioxide in the sour oil does not appear to interfere in either analysis. At least 10 moles of water for every mole of sulfuric acid plus sulfonic acid should be present to obtain quantitative results. For sour oils the repeatability standard deviation for sulfuric acid is 0.007% in the range 0.01 to 0.25% and for sulfonic acid it is 0.097% in the range of 3 to 8%.

THE sulfonation of lubricating oils produces commercially valuable oil-soluble sulfonates, usually called mahogany sulfonates. The sour oil layer contains not only the mahogany sulfonic acids but also small amounts of sulfuric acid and sulfur dioxide which are salt formers. Methods were needed to follow the removal of the salt formers, to predict yields of the finished product, and to determine losses of sulfonic acid during processing.

Methods have been published for the determination of sulfuric acid in acid sludges or spent acids (*2, 6, 10*); however, the sulfuric acid concentrations were very high, usually 20 to 90%. The sour oils the authors are concerned with contain 0.01 to 0.30% of sulfuric acid.

Bacon (*2*) investigated a number of methods for determining sulfuric acid and acid sludges. One of these methods used aniline in chloroform to precipitate the sulfuric acid. The aniline sulfate was dissolved and titrated with potassium bromate. Parke and Davis (*8*) studied this same method on sulfuric acid in the presence of organic acids and mineral acids. Quantities as small as 1 mg. of sulfuric acid were analyzed with an accuracy of 0.2 mg. These workers improved the method of Bacon (*2*) by dissolving the precipitate in hot water and titrating the sulfuric acid released with base to a phenolphthalein end point.

More recently, Weiss and others (*10*) used this technique to determine sulfuric acid in spent acids and acid sludges. Holzman and Suknarowski (*6*) have determined sulfuric acid in acid sludges by making use of the partition of sulfuric acid and sulfonic acid between water and amyl alcohol. The sulfuric acid in the water layer is precipitated as barium sulfate and weighed.

There is little or no information in the literature on the direct determination of sulfonic acids in sour oils. However, methods are available for the indirect determination of sulfonic acids as the neutralized product. Sodium sulfonates have been analyzed by Marron and Schifferli (*7*) using the *p*-toluidine hydrochloride method. Epton (*5*) and Barr, Oliver, and Stubbings (*3*) have titrated the sodium sulfonates with cetyl pyridinium bromide. Brooks, Peters, and Lykken (*4*) have proposed a scheme for analyzing petroleum sulfonates, but this is a rather lengthy method. The ASTM procedure (*1*) can also be used on oil-soluble sodium sulfonates. Weiss and others (*11*) have suggested an improved ASTM method, but both of these methods are very time-consuming.

Sulfonic acids have been determined in spent acids and acid sludges where concentrations of from a few per cent to more than 50% are involved. Holzman and Suknarowski (*6*) have determined the sulfonic acids in the amyl alcohol extract by removing the alcohol under reduced pressure and weighing the dried acids. This method has the disadvantage of being time-consuming. Von Pilat and Starkel (*9*) determined total acidity and corrected for the sulfuric acid present to give a measure of the sulfonic acids plus other acidic materials. Any sulfur dioxide present would cause an appreciable error in the results. Weiss and others (*10*) determined sulfonic acids in acid sludges by correcting total acidity for sulfuric acid (determined by the aniline precipitation method) and sulfur dioxide (determined by iodine-thiosulfate titration). Their results, however, were not too satisfactory because of uncertainty in equivalent weights. They recommend the use of the ASTM analysis for determination of the sulfonic acids.

This work extends the aniline precipitation procedure to the analysis of sour oils for sulfuric acid concentrations of 0.01 to 1.0%. In addition, the same sample is used to determine sulfonic acids in the range of 3 to 8%. The method has the advantage of simplicity and it requires no special equipment.

Sulfur dioxide in acid sludges and spent acids has been de-

termined by the iodine-thiosulfate method (*10*). The authors have found no trouble in adapting this titration to the determination of sulfur dioxide in sour oils.

APPARATUS AND REAGENTS

Erlenmeyer flask, 125-ml.
Suction flask, 250-ml.
Graduated cylinder, 50-ml.
Beaker, 250-ml.
Pipet, 1-ml., graduated to 0.1 ml.
Buret, 25-ml., graduated to 0.05 ml.
Büchner funnel with fritted-disk filter, medium porosity.
pH Meter.
Chloroform.
Aniline in chloroform, 10% by volume.
Distilled water.
Isopropyl alcohol.
Phenolphthalein indicator.
Potassium hydroxide, 0.2*N* in isopropyl alcohol.
Sodium hydroxide, 0.05*N* in water.

PROCEDURE

Weigh accurately about 10 grams of sour oil sample (20 grams if sulfuric acid content is less than 0.1%) into a 250-ml. beaker. Add 15 ml. of chloroform from a graduated cylinder and 0.4 ml. of distilled water from a 1-ml. pipet. Stir until homogeneous. With constant stirring add from a graduated cylinder 50 ml. of 10% aniline in chloroform solution that has been heated to boiling on a steam bath. Break up any large lumps of aniline sulfate with the flattened end of a stirring rod. After allowing the mixture to cool to room temperature, filter the mixture through a Büchner funnel fitted with a medium porosity fritted-glass disk. Apply vacuum to the suction flask and filter off the liquid. Wash the reaction beaker with three 10- to 15-ml. portions of chloroform, adding each washing to the funnel. Continue washing the precipitate while breaking up the precipitate into fine particles until at least 100 ml. of chloroform have been used. Attach the funnel to a clean 250-ml. suction flask.

Save the filtrate for the determination of sulfonic acid.

Wash the precipitate with 2 to 3 ml. of acetone and then apply suction until the precipitate is dry. Add 10 to 15 ml. of hot water to the reaction beaker to dissolve any precipitate which was not previously washed out, and pour into the funnel. Wash a second time if necessary. Dissolve the remaining precipitate in the funnel by adding four 25-ml. portions of hot distilled water, sucking each portion into the flask. If lumps of the precipitate are still present, wash with a little acetone to free the oil, and continue washing with hot water. Titrate the contents of the flask containing the dissolved precipitate with 0.05*N* sodium hydroxide to the phenolphthalein end point, recording the volume (*A*) and normality (*N*) of the base.

Table I. Analysis of Known Blends of Sulfuric and Toluenesulfonic Acids in Oil

Wt. % Sulfuric Acid			Wt. % Toluenesulfonic Acid		
Added	Found	Devn.	Added	Found	Devn.
0.063	0.064	+0.001	1.92	1.94	+0.02
0.062	0.072	+0.010	1.94	1.98	+0.04
0.212	0.205	−0.007	1.93	1.92	−0.01
0.225	0.219	−0.006	1.95	1.96	+0.01
0.022	0.025	−0.003			
0.020	0.024	+0.004	2.04	2.03	−0.01
0.097	0.107	+0.010	2.10	2.06	−0.04
0.096	0.106	+0.010	2.09	1.98	−0.11
Standard deviation		0.007			0.048
95% confidence limits of duplicates		0.023			0.16

Place the filtrate containing the sulfonic acids on a steam bath and evaporate to approximately 20 ml. or until all of the chloroform has been evolved.

Transfer the residue quantitatively to a 250-ml. beaker, washing the flask alternately with approximately equal increments of distilled water and isopropyl alcohol. The total volume of washings plus sample should be about 150 ml. Titrate the contents of the beaker potentiometrically with 0.2*N* alcoholic potassium hydroxide using glass-calomel electrodes. When approaching the end point the increments of titrant should be 0.10 ml. Determine the end point at the greatest e.m.f. change for a 0.10-ml. increment of titrant. The normality (N_1) and the milliliters required (B) for the sample are recorded.

CALCULATIONS

$$\text{Weight \% sulfuric acid} = \frac{A \times N \times 49}{\text{sample weight} \times 10}$$

where 49 is the equivalent weight of sulfuric acid.

$$\text{Weight \% sulfonic acid} = \frac{B \times N_1 \times \text{equivalent weight}}{\text{sample weight} \times 10}$$

where equivalent weight is determined on the sodium mahogany sulfonate by ASTM D 855–52T (*1*) and corrected for the sodium. If good accuracy is not needed, a fair approximation of equivalent weight can be made by adding 80 (for the SO_3 group) to the average molecular weight of the aromatics of the charge to the sulfonation.

RESULTS

Accuracy on Blends of Sulfuric and Toluenesulfonic Acids. Weiss and others (*10*) report that the sulfonic acids of lower molecular weight cause the most trouble in their sulfuric acid determinations. Therefore, it was felt that if the proposed method were applicable for determination of sulfonic acid of low molecular weight—i.e., toluenesulfonic acid—less interference would be encountered from the mahogany sulfonic acids of higher molecular weight.

Known blends of sulfuric acid and toluenesulfonic acid (TSA) were made in a typical sulfonation charge oil as follows: Weighed quantities of 60% sulfuric acid were added to a 150-ml. beaker. A weighed amount of about 20% toluenesulfonic acid in isopropyl alcohol was placed in the same beaker. It was necessary to dissolve the toluenesulfonic acid in isopropyl alcohol because of the low solubility of the acid in the charge oil. To this was added known amounts of sulfonation charge stock oil and the blend was thoroughly mixed.

Table II. Addition of Known Quantities of Sulfuric Acid to Sour Oils

Wt. % Sulfuric Acid				
Sour Oil	Added	Expected	Found	Devn.
0.02	0.17	0.19	0.18	−0.01
0.02	0.34	0.36	0.34	−0.02
0.13	0.28	0.41	0.42	+0.01
0.02	0.51	0.53	0.52	−0.01
0.13	0.40	0.53	0.53	0.00
0.13	0.76	0.89	0.90	+0.01
0.13	1.11	1.24	1.27	+0.03
			Standard deviation	0.016

The blended samples contained 2% of sulfonic and 0.02 to 0.2% of sulfuric acid. The data obtained on these synthetic blends are given in Table I. For sulfuric acid the standard deviation is 0.007% over the range of 0.02 to 0.22%. For toluenesulfonic acid it is 0.048% at the 2% concentration level. These data indicate that the aniline precipitation method works well on mixtures of sulfuric and toluenesulfonic acids and that there should be little difficulty in applying the method to sour oils.

Accuracy of Sulfuric and Sulfonic Acid Values in Sour Oils. The accuracy of sulfuric acid values in sour oils was determined by adding known quantities of this acid to previously analyzed sour oils. The data obtained are given in Table II. The amounts of sulfuric acid added cover the range of 0.17 to 1.1 weight %. The standard deviation from known values is 0.016%.

It is possible to predict the salt value due to sulfuric acid in the final product to about ±0.1%, assuming that no desalting step is performed.

Mahogany sulfonate acids in sour oil are reasonably stable, but in pure form they become somewhat unstable. Therefore, they cannot be used for determining the accuracy of the method. However, based on the authors' analyses of toluenesulfonic acid in sulfuric acid–toluenesulfonic acid mixtures, the accuracy for the mahogany sulfonic acid should be within ±3% relative. It has been possible to predict actual plant yields to within 2% on 60% sodium mahogany sulfonates from data obtained by this procedure.

Table III. Repeatability of Sulfuric and Sulfonic Acid Determinations on Plant Sour Oils

Sample No.	Wt. % Sulfuric Acid			Wt. % Sulfonic Acid		
	1	2	Diff.	1	2	Diff.
1	0.01	0.01	0.00	5.15	5.11	0.04
2	0.05	0.04	0.01	5.08	4.90	0.18
3	0.09	0.11	0.02	7.71	7.55	0.16
4	0.17	0.17	0.00	6.82	6.76	0.06
5	0.20	0.19	0.01	8.20	8.40	0.20
6	0.18	0.18	0.00	..	..	...
7	0.05	0.05	0.00	3.00	3.10	0.10
8	0.15	0.14	0.01	..	..	...
9	0.18	0.19	0.01	..	..	...
10	0.22	0.22	0.00	..	..	...
Standard deviation			0.007			0.097
95% confidence limits of duplicates			0.02			0.34

Repeatability Data on Sour Oils. Table III shows duplicate results obtained on actual plant samples, taken at various stages of the processes and on different days. The charge oil is the same in all cases.

Table IV. Sulfur Dioxide Interference in Sulfuric and Sulfonic Acid Determinations

	Sample before Purging with Nitrogen		Sample after Purging with Nitrogen	
	A	B	A	B
SO_2[a], wt. %	0.45	0.40	0.001	0.033
H_2SO_4, wt. %	0.20	0.23	0.19	0.20
Sulfonic acid, wt. %	7.17	6.94	7.07	6.97

[a] By iodine-thiosulfate titration.

The repeatability standard deviation for sulfuric acid is 0.007% in the range 0.01 to 0.25%. For mahogany sulfonic acid it is 0.097% in the range 3 to 8%. The good repeatability for both determinations indicates that other acidic materials, such as sulfur dioxide, do not interfere. Sulfuric acid ester content is probably negligible in the sour oils. In many instances sulfur dioxide was not determined, but from previous experience it is known that some samples contain as much as 0.5% of sulfur dioxide.

Interference Studies. Weiss and others (*10*) state that sulfur dioxide does not interfere in the aniline precipitation method for sulfuric acid, but give no supporting data. Parke and Davis (*8*) indicate that 0.4 ml. of saturated sulfurous acid gives a precipitate with aniline. A study was made to determine whether or not sulfur dioxide would interfere in either the sulfuric or the sulfonic acid determinations.

Sour oil samples from plant production were analyzed for sulfur dioxide and sulfuric and sulfonic acids before and after purging with dry nitrogen for approximately 2 hours. The data obtained are given in Table IV. The sulfur dioxide content decreases in one case more than one hundredfold, yet the sulfuric and sulfonic acid values are within the established repeatability limits at the 95% confidence level. In the second case the sulfur dioxide content decreases about tenfold; however, here again the sulfuric and sulfonic acid determinations agree rather well.

In order to firm the authors' conclusion as to the extent of interference by sulfur dioxide, 50 ml. of the aniline-chloroform precipitating solution were bubbled with sulfur dioxide and diluted to 100 ml. A 25-ml. aliquot was acidified with hydrochloric acid and titrated by the iodine-thiosulfate method. This aliquot contained 0.18 meq. of sulfur dioxide. For a 10-gram sample of sour oil this would be equivalent to 0.23% of sulfur dioxide. Another 25 ml. aliquot showed 0.19% of sulfur dioxide by acid-base titration. Two other aliquots were evaporated on the steam bath to a low volume. The sulfur dioxide content was less than 0.01% on both of these samples as determined by iodine-thiosulfate on one aliquot and acid-base titration on the other.

Thus, evaporating the aniline-chloroform sample to a low volume drives off any sulfur dioxide which might otherwise result in high sulfonic acid values.

Minimum Water Required for Quantitative Results. Weiss and others (*10*) report that if less than 1 mole of water is present for every mole of sulfuric acid, poor results are obtained. Parke and Davis (*8*) show data that indicate that a much larger ratio of water to sulfuric acid should be used. The maximum recovery for a 0.004-gram sample of sulfuric acid was obtained at a mole ratio of about 350% of water to 1 of sulfuric acid. In the present work, water requirement was studied on mixtures containing xylenesulfonic and sulfuric acids. These mixtures were analyzed by the proposed procedure, except that the amount of water added was varied.

The data of Table V show that, contrary to expectation, poor results are obtained even when the ratio of water to sulfuric acid is much greater than one. In fact, in some cases, results become worse as this ratio increases. The data indicate that sulfonic acid plays a predominant role in water requirement. Probably the most important factor to be considered is the mole ratio of water to sulfonic acid plus sulfuric acid. This ratio should be at least 10 to 1 in order to obtain good results.

The exact reasons for the need of water is not clear. One reason may be that a partially soluble aniline–sulfonic acid complex is formed which is broken up only by the presence of a

Table V. Minimum Water Requirements

Mole Present			Mole Ratio		H_2SO_4, %		XSA, %	
H_2O[a]	H_2SO_4	XSA	H_2O / H_2SO_4	H_2O / (H_2SO_4 + XSA)	Calcd.	Found	Calcd.	Found
0.0104	0.0028	0.0006	3.7	3.1	47.0	47.5	19.6	17.9
0.0055	0.0012	0.0011	4.6	2.4	28.3	31.6	48.0	35.7
0.0037	0.0006	0.0015	6.2	1.8	14.4	25.4	69.2	27.1
0.0206	0.0025	0.0005	8.2	6.9	48.0	48.6	18.2	16.0
0.0193	0.0019	0.0013	10.2	6.0	31.7	32.9	42.9	37.6
0.0181	0.0015	0.0015	12.1	6.0	27.0	28.7	49.9	43.1
0.0326	0.0027	0.0005	12.1	10.2	48.9	49.1	16.8	17.7
0.0301	0.0018	0.0010	16.7	10.7	35.1	35.2	37.7	38.6
0.0287	0.0014	0.0015	20.5	9.9	25.1	26.0	52.9	53.6

[a] Includes water present in sulfuric and xylenesulfonic acids used in making blends plus water added.

large excess of water. Another reason may be that the aniline–sulfonic acid complex is stable but it is made more soluble in the aniline-chloroform medium with increased amounts of water.

When analyzing plant sour oil samples, the amounts of sulfuric and sulfonic acid present in a sample are much less than those used in these experiments. For plant samples, the 0.4 ml. of water recommended in the procedure gives a mole ratio of water to total acids of about 20 to 1.

CONCLUSIONS

The data obtained have shown that the determination of sulfuric and sulfonic acids in sour oils is possible. Sulfur dioxide does not interfere in the determinations. Minimum water requirement studies have shown that controlling the mole ratio of water to sulfuric acid, as previously reported (*10*), is not the determining factor in obtaining good results. The water requirements are higher, mainly because of the presence of sulfonic acids. More work is needed to determine the exact role the amount of water plays in this determination.

It is believed that the aniline precipitation procedure described here can be used to determine not only sulfuric acid in acid sludges, as suggested by Weiss and others (*10*), but for sulfonic acids as well. If the equivalent weight of the sulfonic acid is known and there is negligible sulfuric acid ester present, this method should be applicable to both acid sludges and spent acids. It should be particularly useful on alkylarenesulfonic acids. The authors have successfully analyzed a large number of "green acids"—i.e., the bottom layer produced during the sulfonation of lubricating oils—for both of these constituents. For this type sample about 1 gram is taken. Typical analyses would be 15% sulfuric and 60% sulfonic acids.

About eight samples can be analyzed in 1 man-day; the ease and rapidity of determination make this method a desirable one for plant control. The estimation of plant yields of finished product based on sulfonic acid values agrees well with yields actually obtained. The determination of the small amounts of sulfuric acid in the sour oil layer has made it possible to follow desalting operations.

ACKNOWLEDGMENT

The authors wish to express their thanks to the Sun Oil Co. for the privilege of publishing this paper and to acknowledge the help of Paul Congdon.

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RECEIVED for review July 13, 1955. Accepted November 1, 1955. Division of Petroleum Chemistry, 128th meeting, ACS, Minneapolis, September 1955.

Separation of Rare Earths from Thorium Nitrate

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The spectrographic determination of part-per-million quantities of rare earths in thorium nitrate requires the prior separation of the rare earths. A rapid method of separation is presented in which the bulk of the thorium is extracted with dibutoxy tetraethylene glycol (pentaether), and the residual thorium and other impurities are removed by 8-quinolinol-chloroform. The rare earths are collected by precipitation of the oxinates with lanthanum as a carrier. Recovery tests with samarium, europium, gadolinium, dysprosium, and erbium, the rare earths with the highest thermal neutron cross section, indicate that at least 90% recovery is achieved. The separation requires about 4 hours.

THE need exists for a rapid analytical method of separating trace amounts of the rare earths from thorium nitrate prior to spectrographic determination of the rare earths. A variety of separation methods has been applied. Precipitation (*8, 12*) and volatilization (*12*) methods of removing thorium may either result in rare earth losses or become tedious when the amount of thorium exceeds milligram quantities. A cellulose column procedure has been used (*2, 9*), but the elutions and subsequent precipitation steps are time-consuming.

Solvent extraction, a technique offering advantages of rapidity and ease of manipulation, has been applied to the problem (*1*). The distribution coefficients of thorium and some of the rare earths in nitric acid–tributyl phosphate systems have been investigated (*1, 3–5, 10*) and appear favorable for the adaptation of such an extraction system for an analytical separation. In the present work, however, dibutoxy tetraethylene glycol, commonly known as pentaether, was selected as the organic solvent because of its high selectivity (*11*) for thorium in a saturated aluminum nitrate solution, 0.4*M* in nitric acid, containing thorium and the rare earths.

Preliminary experiments showed that nearly complete separation of thorium from the rare earths could be achieved in this system, but subsequent isolation of the rare earths proved difficult because of the high concentration of aluminum nitrate. An 8*M* nitric acid–pentaether system was, therefore, investigated and was found to be satisfactory. Phase separation, slow in this system, was found to be helped by the addition of diethyl ether to the pentaether (*11*). An analytical separation was devised consisting primarily of an extraction with a pentaether–diethyl ether mixture to remove the bulk of the thorium and an oxine-chloroform extraction to remove the residual thorium and other impurities such as iron and titanium.

REAGENTS AND APPARATUS

Pentaether, dibutoxy tetraethylene glycol, Carbide and Carbon Chemicals Co.

Oxine, 8-quinolinol, Eastman Organic Chemicals Co.

Europium$^{152-154}$ tracer, not carrier-free, prepared at Oak Ridge National Laboratory by neutron irradiation of europium oxalate. Dysprosium, gadolinium, samarium, erbium, and europium oxides of at least 95% purity. Didymium oxide, commercial grade.

Other reagents, analytical grade.

High-pressure ionization chamber–vibrating reed electrometer apparatus.

EXPERIMENTAL

Distribution Coefficients. About 6 grams of thorium nitrate tetrahydrate were dissolved in 50 ml. of 8*M* nitric acid and extracted with 25 ml. of pentaether. The aqueous phase, containing some thorium and most of the gamma-emitting decay products which interfered with the europium tracer determinations, was discarded. This step also served to pre-equilibrate the pentaether with nitric acid. The organic phase was then shaken with 50 ml. of 8*M* nitric acid to which europium$^{152-154}$ tracer had been added. After equilibration, thorium determinations and europium$^{152-154}$ activity measurements were made on both phases. The thorium concentration in the aqueous phase was determined by precipitating the thorium with ammonium hydroxide and igniting the precipitate to the oxide, in the organic phase by extracting with water before precipitating and igniting. The distribution coefficients, D_o^a, defined as the ratio of the total concentrations of the elements in the organic phase (o) and the aqueous phase (a), were found to be 4.0 and 0.01 for thorium and europium, respectively.

Gamma Activity Measurements. The gamma activity was measured in a high-pressure ionization chamber–vibrating reed electrometer apparatus. The activity of the europium$^{152-154}$ added in each experiment was in the order of 5×10^5 disintegrations per second and the weight level of total europium was about 25 γ.

After the removal of most of the gamma-emitting decay products from 6 grams of thorium nitrate tetrahydrate, the residual gamma activity was less than 1% of this europium activity and could be ignored. When 50 grams of thorium nitrate tetrahydrate were taken, the residual activity was slightly higher but still far below the added europium activity. All of the separations were completed within 2 hours after the removal of the decay products. The activity of the daughters of thorium-228, therefore, did not increase significantly the blank activity which was assumed to be constant for the recovery calculations.

Pentaether Extraction. The distribution coefficients for thorium and europium indicated that practically all of the thorium could be separated from the rare earths in this system.

The separation was checked with 50-gram samples of thorium nitrate tetrahydrate. Gamma-emitting thorium decay products were removed by equilibrating the sample with 200 ml. of pentaether (previously equilibrated with an equal volume of 8*M* nitric acid) and 10 ml. of 8*M* nitric acid. The aqueous phase was discarded. The organic phase was washed again with 10 ml. of 8*M* nitric acid and the aqueous phase was discarded. To the pentaether, containing most of the thorium, was then added europium$^{152-154}$ and 15 ml. of 8*M* nitric acid. After equilibration, the aqueous phase was drawn off and the pentaether was washed with 15 ml. of 8*M* nitric acid. The aqueous phases were combined and extracted with 125 ml. of pentaether. The aqueous phase was separated and the pentaether was washed with 10 ml. of 8*M* nitric acid. The aqueous phases were combined and analyzed for thorium and europium$^{152-154}$. In triplicate tests, 98% of the europium$^{152-154}$ was recovered. The amount of thorium never exceeded 0.2 gram as the oxide.

Oxine Extraction. The residual thorium remaining after the pentaether extraction was removed by extraction with oxine-chloroform. A detailed study of the variables involved in this step was not made. The conditions of the final procedure are based upon some exploratory tests, the results of which are shown in Table I. In these tests, 10 ml. of glacial acetic acid were added to 600 mg. of thorium nitrate tetrahydrate and europium$^{152-154}$ in 100 ml. of 2*M* nitric acid. The acetic acid served as a solvent for the oxine and as a subsequent buffer and complexing agent. Concentrated ammonium hydroxide was added until the pH was about 1, the solution was cooled in an ice bath and 3 grams of oxine were added. The pH was then adjusted with a pH meter to the desired value. The solution was extracted with five 10-ml. portions of oxine in chloroform and washed with two 25-ml. portions of chloroform. In the extraction with the first 10-ml. portion of oxine-chloroform, any solid oxinate separating was redissolved by the addition of 5 to 10 ml. of chloroform.

The gamma activity of the aqueous phase was determined. Any thorium oxinate in the aqueous phase was then precipitated by the addition of sufficient concentrated ammonium hydroxide to make the solution alkaline. The oxinate was filtered and ignited to the oxide.

A single test was made with only 5 ml. of added acetic acid. In this test, the thorium was completely extracted by three 10-ml. portions of oxine-chloroform, 10 grams per 50 ml., but the europium$^{152-154}$ recovery was only 75%. The larger amount of acetate ion apparently masks the europium in much the same manner as very concentrated acetate solutions mask thorium in oxine extractions (*7*). Moreover, this complexing action of acetate ion is undoubtedly responsible for the large amount of oxine necessary for complete extraction of the thorium.

From the results shown in Table I, a concentration of oxine of 10 grams per 50 ml. and a pH of 4.2 were selected. Above a pH of 4.2, the europium loss begins to increase, while a pH of 3.0 or below results in some thorium contamination. The choice of pH 4.2 was also based upon another consideration. At a pH of 4 and above, the distribution of oxine greatly favors the chloroform phase (*13*). Accordingly, when the extracted solution is washed with chloroform at pH 4.2, much of the excess oxine is removed and the subsequent precipitate is not too bulky with precipitated oxine.

Complete removal of iron and titanium also occurred under the conditions of extraction. While other elements are extracted, their removal was not studied.

Table I. Effect of pH and Oxine Concentration on Separation of Thorium and Europium

pH	Oxine, Grams/ 50 Ml.	ThO_2 Recovered, Mg.	Eu Tracer Recovered, %
3.0	5	73	99
3.8	5	18	...
4.2	5	8	98
3.8	7.5	4	98
2.0	10	4	99
3.0	10	2	99
4.2	10	0	97
4.5	10	0	92
5.0	10	0	82

Phase Separation during Pentaether Extraction. After the first equilibration of a 50-gram sample with pentaether, the phase separation is slow. Even after 15 minutes, a small volume of aqueous phase separates. The addition of diethyl ether to the pentaether materially assists in speeding up the phase disengagement. The diethyl ether has no significant effect on either the europium or thorium distribution.

In the second extraction with the fresh batch of pentaether, the phase separation is ordinarily fairly rapid. The addition of diethyl ether here gives a cleaner aqueous phase containing little residual pentaether.

The 8*M* nitric acid is appreciably soluble in the diethyl ether as in the pentaether. A quantity of pentaether–diethyl ether mixture sufficient for a 50-gram thorium nitrate tetrahydrate sample is pre-equilibrated by adding 140 ml. of diethyl ether and 250 ml. of 8*M* nitric acid to 280 ml. of pentaether in a 2-liter separatory funnel. After the mixture is shaken, the phases are allowed to separate and the aqueous phase is discarded. Only a slight initial pressure develops in the separatory funnel when the contents are shaken.

Precipitation of Rare Earths. The solution remaining after the oxine-chloroform extraction contains some residual oxine. The europium$^{152-154}$ is completely precipitated by adding 1.0 mg. of lanthanum carrier as a solution of the nitrate, adding about 10% by volume of concentrated ammonium hydroxide, and heating until the oxinates flocculate.

Standard Separation Procedure. To 300 ml. of pre-equilibrated pentaether–diethyl ether mixture in a 500-ml. separatory funnel, add 50 grams of thorium nitrate tetrahydrate and 7.0 ml. of concentrated nitric acid. (This quantity of acid will yield, with the water of hydration, an aqueous phase about 8*M* in nitric acid.) Shake the contents until the salt is completely dissolved.

Drain the aqueous phase into a 250-ml. separatory funnel. Wash the organic phase with 15 ml. of 8*M* nitric acid and combine the aqueous phases. Discard the organic phase. Extract the combined aqueous phase with 200 ml. of fresh pentaether–diethyl ether mixture. Drain the aqueous phase into a 150-ml. beaker. Wash the organic phase with 10 ml. of nitric acid and combine the aqueous phases. Discard the organic phase.

Dilute the solution to about 100 ml., add 10 ml. of glacial acetic acid and adjust the pH to about 1.5 with concentrated ammonium hydroxide. Cool the solution in an ice bath, add 3 grams of oxine, and adjust the pH to 4.2, cooling again if necessary to avoid oxidation of the oxine. Transfer the contents of the beaker to a 250-ml. separatory funnel. Extract the solution with five 10-ml. portions of oxine in chloroform, 10 grams per 50 ml., and wash with two 25-ml. portions of chloroform.

Transfer the solution to a 250-ml. beaker. Add 1.0 mg. of lanthanum carrier and 50 γ of titanium as a solution of the sulfate to serve as a spectrographic internal standard. Add about 10% by volume of concentrated ammonium hydroxide and heat until the precipitate flocculates. Filter the hot solution through Whatman No. 42 paper and ignite the precipitate in platinum to the oxides for the spectrographic analysis.

Further Separation When Required. When the calcium and magnesium content of the residue is so high as to interfere with the spectrographic determination, the residue after ignition is dissolved in 5 ml. of hot 6*N* hydrochloric or nitric acid. The solution is diluted to about 25 ml. and, after the addition of 2 grams of ammonium chloride, the rare earths are reprecipitated with concentrated ammonium hydroxide.

Beryllium and aluminum, when high in certain samples, are reduced by a precipitation with an excess of 3*M* sodium hydroxide immediately following the pentaether extraction. The separation is incomplete owing to coprecipitation but the dilution effect of these metals leading to decreased spectrographic sensitivity is materially lessened. The sodium hydroxide precipitate is filtered immediately and washed with 0.1*M* sodium hydroxide before dissolution in 25 ml. of warm 4*M* nitric acid for the oxine extraction.

When it is desired to remove both large amounts of cerium and the last traces of thorium in the final residue, the addition of titanium is omitted before the precipitation of the rare earth oxinates. After the ignition step, the residue of rare earth and lanthanum oxides is dissolved in 50 ml. of 4*M* nitric acid. About 100 mg. of solid sodium bromate are added and the solution is warmed until the salt dissolves. Ten milligrams of mercurous ion as a solution of the nitrate and 5.0 ml. of a saturated solution of potassium iodate in 4*M* nitric acid are added (*6*), and the solution is stirred until the iodates precipitate and become flocculent. The precipitate is filtered through a sintered-glass Büchner funnel and washed with about 10 ml. of the potassium iodate–4*M* nitric acid solution. The titanium is next added to the filtrate and the hydroxides are precipitated with concentrated ammonium hydroxide.

Recovery Tests. The over-all recovery with the standard procedure outlined above was determined by the use of europium$^{152-154}$ added to 50 grams of thorium nitrate from which the gamma-emitting decay products (and rare earths) were removed as described. In duplicate tests, a mean recovery of 93% was obtained.

Table II. Mean Recoveries of Individual Rare Earths, %

Dy	Gd	Sm	Eu	Er
93	90	96	95	94

Similar tests were made with 5.0-mg. quantities of dysprosium, gadolinium, samarium, europium, and erbium added separately to 50 grams of purified thorium nitrate. After removal of the rare earths by washing the pentaether–diethyl ether solution of the thorium nitrate with three 10-ml. portions of 8*M* nitric acid, an additional 10 ml. of 8*M* nitric acid solution as the initial aqueous phase was carried through the standard procedure plus the final iodate separation. The lanthanum carrier addition, however, was omitted. No rare earth residue was obtained. The recoveries obtained after the addition of the individual rare earths could, therefore, be determined gravimetrically. Here, also, the standard procedure plus the iodate separation was used. The results shown in Table II show recoveries of 90 to 96% as predicted by the recovery figures for the pentaether–diethyl ether and oxine extraction steps.

Some final recovery tests were made by adding a mixture of 100 γ each of the five rare earths to 50 grams of purified thorium nitrate. The internal-standard titanium was added as described after the iodate precipitation step. Individual spectrographic results varied by at least 20% from the recovery values obtained in Table II. The mean values of six different tests, however, showed that the recoveries of each of the five rare earths was at least 90%.

These rare earths tested possess the highest thermal neutron cross section and, therefore, were of primary interest. Semiquantitative spectrographic determinations, however, on the residues obtained in recovery tests with didymium oxide added to thorium nitrate showed that recoveries of other rare earths—i.e., praseodymium, neodymium, and ytterbium—were either quantitative or nearly so.

DISCUSSION

The method has given excellent results in the analysis of thorium nitrate from a variety of sources. Emulsion formation during the pentaether or oxine extraction has sometimes occurred during the analysis of fairly crude samples. In these cases, the use of smaller samples, from 10 to 25 grams, has helped eliminate this difficulty.

In the analyses of samples other than the nitrate, conversion to the nitrate is necessary. When hydrofluoric acid is used in conjunction with nitric acid—for example, in the dissolution of the metal or oxide (*12*)—the smallest possible quantity should be used; otherwise some thorium fluoride precipitates with accompanying rare earth loss during the oxine extraction.

The 90 to 96% recovery of the rare earths is considered to be satisfactory for a plant control method. The separation takes about 4 hours, whereas the commonly used cellulose column–hexamine separation procedure requires a considerably longer time.

ACKNOWLEDGMENT

Acknowledgment is given to the New Brunswick Laboratory Spectrographic Analysis Section for the various spectrographic determinations made.

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RECEIVED for review August 31, 1955. Accepted November 10, 1955.

Determination of Cadmium and Zinc

Separation from Other Elements and Each Other by Anion Exchange

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Zinc and cadmium form negatively charged chloride complexes which are adsorbed by the strongly basic anion exchange resin Dowex 1. Maximum adsorption of zinc and cadmium is obtained in 0.12*N* hydrochloric acid containing 100 grams of sodium chloride per liter. In this medium most elements with which zinc and cadmium are associated in metals and minerals are not adsorbed by the resin. A 2*N* sodium hydroxide solution, containing 20 grams of sodium chloride per liter, quantitatively elutes the zinc while the cadmium is retained by the resin. Finally, cadmium is eluted with 1*N* nitric acid.

BRYSON and Lenzer recently summarized the complexity, caused by the lack of specific reactions, of the analytical chemistry of zinc and cadmium (*2*). Miller and Hunter cited recent developments in analytical methods for zinc, including two (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid) procedures, two cation exchange procedures, a polarographic method, and a procedure based on cellulose chromatography (*10*).

Kraus and Moore showed that the transition elements manganese, cobalt, copper, iron, and zinc form negatively charged chloride complexes which can be adsorbed and selectively eluted from a column of strongly basic anion exchange resin, while nickel and chromium remain in solution as cations. The formation of the various chloro anions depends on the individual element and on the concentration of the chloride ions. Manganese, for instance, is eluted with 9*N* hydrochloric acid and zinc with 0.005*N* or weaker hydrochloric acid (*8*).

Although the exact mechanism of the adsorption of the various chloro anions has not yet been established, several practical and efficient techniques have already been suggested. Hague, Maezkowske, and Bright, using Dowex 1 resin, separated and determined varying amounts of nickel, cobalt, manganese, and iron found in high temperature alloys (*4*). Miller and Hunter, using the strongly basic anion resin Amberlite IRA-400, chloride form, separated 50 mg. of zinc, in the absence of cadmium, from 100 mg. of various elements, forming no chloro anions or complexes which are less stable than zinc in 2*N* hydrochloric acid. Part of the bismuth, antimony, tin, and indium and a small amount of copper and iron are adsorbed on the column, but a subsequent 8-quinolinol procedure is described which obviates their interference (*10*). Baggott and Willcocks, taking advantage of the difference in stability between the complex iodides of cadmium and zinc in a mixed sulfate–iodide solution (CdI_4^{--} is more stable than ZnI_4^{--}), separated microgram amounts of zinc from as much as 1 gram of cadmium using the strongly basic anion exchanger De-Acidite FF (*1*). The adsorption of cadmium from chloride solutions by anion exchange resins was also observed recently by Jentżsch and Frotscher (*7*) and Kraus, Nelson, and Smith (*9*). However, no anion exchange procedures are described in the literature providing a separation of cadmium from zinc.

Because published anion exchange techniques for determining zinc and cadmium are of limited scope, a method was developed to provide a quantitative separation of cadmium and zinc from other elements and each other.

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APPARATUS AND REAGENTS

The apparatus used in the anion separations described below is similar to that used by Hague for his ion exchange work on high-temperature alloys (*4*).

Glass Columns. Columns 1 inch in inside diameter and long enough to hold a settled resin bed 10 inches deep are used when gram portions of elements are to be separated. These columns hold approximately 90 grams of resin and have a liquid capacity of 40 ml. Columns 1 cm. in inside diameter are used for milligram to decigram quantities; they should hold 15 grams of resin and have a 7-ml. liquid capacity.

A multiple arrangement of columns is used to carry out a number of simultaneous separations. The solutions used for elution are pumped from 5-gallon stock bottles into 2½-gallon delivery bottles (placed on a top stand to provide a 6-foot head of liquid), from which they flow by gravity through a system of stopcocks and glass tubes to the columns.

The following reagents are needed:

Dowex 1, a strongly basic anion exchange resin, 200 to 400 mesh, with 8 to 10% cross linkage. The resin is settled in 0.5*N* hydrochloric acid and the finest and coarsest material removed by decantation. A slurry of the main portion of the resin is used to fill the columns, which have a plug of glass wool in the bottom to retain the resin.

The liquid capacity of the resin columns is determined by washing with a 10% ammonium chloride solution until the resin darkens, then measuring the amount of 1*N* hydrochloric acid required to lighten the color of the resin (*4*). To extract organic substances which tend to slow down the flow rate, several hundred milliliters of 1*N* nitric acid should be passed through the resin before the column is used for the first time.

Hydrochloric acid, 0.12*N* containing 100 grams of sodium chloride per liter (Reagent I).

Sodium hydroxide, 2*N* containing 20 grams of sodium chloride per liter (Reagent II).

Nitric acid, 1*N*.

Benzotriazole. Dissolve 2 grams in 100 ml. of water.

PROCEDURE

Prepare the resin by transferring two 30-ml. portions of Reagent I to a 1-inch column and drain the solution to about 0.5 cm. above the top of the resin. If a 1-cm. column is used, two 10-ml. portions of the reagent are sufficient.

Dissolve the dry chloride or sulfate of the sample in a minimum amount of Reagent I. Quantitatively transfer small portions of the concentrated solution of the sample to the column with the bottom stopcock open, keeping the resin covered with solution to prevent formation of air pockets, and using Reagent I for washing. Finally, wash down the sides of the column with Reagent I.

Cap the column tightly to prevent air leakage and begin to deliver Reagent I from the reservoir bottle, adjusting the stopcock on the column to deliver approximately 3 to 4 ml. per minute. When a 1-inch column is used, 400 ml. of eluent is sufficient to ensure removal of 1-gram portions of elements not adsorbed on the column. Much less eluent is required when milligram or decigram amounts of elements are separated on a 1-cm. column.

When sufficient solution has been collected, adjust the stopcocks to deliver Reagent II to the column (if a sample is known to contain no cadmium, the zinc may be eluted with 1*N* nitric acid). As soon as the sodium hydroxide is about halfway down the column (indicated by the change in color of the resin), replace the beaker containing the elements eluted with Reagent I with a beaker to receive the zinc.

Continue the elution of the zinc at the full flow rate of the column. Check the completion of the zinc elution by acidifying a fresh portion of the eluent, then making it slightly ammoniacal and adding benzotriazole solution. If a precipitate forms, indicating incomplete elution of the zinc, and the determination of zinc is required, filter off the benzotriazole precipitate. Dissolve it by fuming with nitric and sulfuric acids and add to the main zinc solution.

After the elution of zinc is completed, change beakers and adjust the stopcocks to deliver distilled water. Collect about 75 ml. of water (1-inch column) to remove most of the sodium hydroxide solution, then adjust stopcocks to deliver 1*N* nitric acid. After 20 to 30 minutes, the acid will be about halfway down the column, indicated by the lightening of the resin color. Then replace the beaker containing the water and eluted sodium hydroxide with a clean beaker. Continue the flow of nitric acid until the color of the resin indicates complete removal of sodium hydroxide, then collect an additional 350 ml. of eluent.

Determination of Cadmium in the Eluent. To the nitric acid solution containing the cadmium, add 10 ml. of sulfuric acid and 2 ml. of perchloric acid and evaporate to dryness. At the fuming stage add a few drops of nitric acid to ensure complete destruction of organic compounds. Alternatively, cadmium can be precipitated with benzotriazole in slightly ammoniacal solution and the filtered precipitate fumed with nitric and sulfuric acids. If sufficient cadmium is present, determine it electrolytically (*5*).

Determination of Zinc. The final determination of zinc can be performed by a number of procedures.

Zinc > 100 mg., potassium ferrocyanide method (*6*)
Zinc < 100 mg., electrolytic determination (*3*)
Zinc < 10 mg., dithizone method (*11*)

Other procedures could be applied.

Ignition of the sulfide to oxide (*6*)
Determination as zinc pyrophosphate (*6*)
Precipitation with benzotriazole (*13*)

Regeneration of Resin. After cadmium is removed with 1*N* nitric acid, regenerate the resin by passing Reagent I through the column, which is now ready to receive the next sample.

DISCUSSION

Adsorption of Zinc and Cadmium. Previous work with chloro anions indicates that the separation of the individual complexes depends largely on the hydrochloric acid concentration. For instance, manganese can be eluted with 9*N* hydrochloric acid, thus providing a separation from cobalt, copper, iron, and zinc; but it is more efficiently eluted with 4*N* hydrochloric acid which also removes cobalt. Manganese is eluted even more rapidly with 1*N* hydrochloric acid which elutes cobalt, iron, and copper in addition. It is therefore not surprising that Miller and Hunter, by using a rather high acidity to adsorb zinc, encountered difficulties in removing all of the copper and iron with a 2*N* hydrochloric acid eluent (*10*).

It was found early in this investigation that, in the presence of considerable sodium chloride, the hydrochloric acid concentration can be lowered from 2*N* to 0.12*N*, without affecting adversely the adsorption of the zinc by the resin. When using Reagent I, maximum adsorption of both zinc and cadmium was obtained (Figure 1).

The stability of zinc and cadmium chlorides in a sodium chloride medium is demonstrated in Figure 1. The relative adsorption of zinc and cadmium by the resin approximates the ratio of their atomic weights. To obtain these data, 3-gram portions of zinc and cadmium were dissolved in hydrochloric acid and hydrogen peroxide, and these solutions were evaporated to dryness. The salts were dissolved in 75-ml. portions of dilute hydrochloric acid of varying strength containing from 0 to 15 grams of sodium chloride per 100 ml. of solvent. The solutions were transferred to 1-cm. columns which had been previously washed with the solvent used in each particular test. Elution of the excess zinc and cadmium was then continued with the same solvents until exactly 600 ml. of eluate was obtained. The adsorbed zinc or cadmium was then eluted by passing 400 ml. of 1*N* nitric acid through the columns. Zinc was determined by titration with potassium ferrocyanide (*6*) after evaporating the solution several times to dryness with intermittent additions of hydrochloric acid. Cadmium was determined electrolytically (*5*).

Cadmium is held preferentially by the resin when Reagent I is used. When solutions containing 5 grams of zinc and 50 mg. of cadmium were passed through columns having a capacity to adsorb 1.5 grams of zinc, 75 to 95% of the cadmium was held by the resin. However, attempts were unsuccessful to find conditions for the quantitative adsorption of cadmium in the presence of amounts of zinc exceeding the capacity of the columns.

Separation of Zinc from Cadmium. Of many reagents tried, only sodium hydroxide solution was found to provide a quantitative separation of zinc from cadmium. The elution of zinc as sodium zincate is quantitative, but the exact mechanism of the retention of cadmium in a sodium hydroxide medium has not yet been established. It was noted that the addition of sodium chloride to the sodium hydroxide solution not only speeds up the zinc elution considerably, but also helps to retain the cadmium. The cadmium band, which looks gray in a sodium hydroxide–sodium chloride medium, does not noticeably move, even when 5000 ml. of 2*N* sodium hydroxide followed by 500 ml. of water is passed through the columns. Neutralized portions of the sodium hydroxide eluates (usually 25 ml.) from each 100-ml. fraction gave no positive test for cadmium with dithizone. The tenacity with which the cadmium is held may be partly attributed to the presence of the sodium chloride.

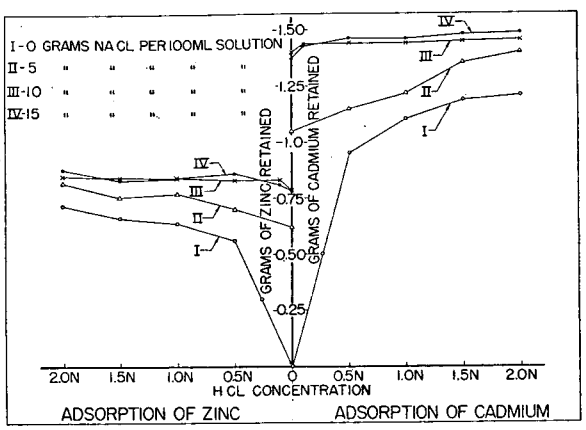

Figure 1. Adsorption characteristics of zinc and cadmium

Tests indicated that the elution of zinc is independent of the amount of cadmium present. When the 1-inch columns are used, approximately 350 ml. of the sodium hydroxide–sodium chloride solution was sufficient to elute 1 gram of zinc. With a 1-cm. column, only 200 ml. was required to elute the same amount of zinc.

Elution of Cadmium. Early in this investigation, dilute sulfuric acid was used for the elution of cadmium and of zinc in the absence of cadmium. After the chlorides from the sodium chloride–hydrochloric acid medium were adsorbed, the acidity of the sulfuric acid had to be raised to 15*N* to elute cadmium and zinc. However, it was found that cadmium can be readily eluted with 1*N* nitric acid without adversely affecting the resin. To avoid the formation of unnecessary sodium nitrate in the eluate, water is passed through the column for a few minutes prior to the application of the nitric acid. When the 1-inch column is used, 400 ml. of 1*N* nitric acid is sufficient to elute 5 grams of cadmium. The 1-cm. column requires only 150 ml. to elute 1 gram of cadmium.

INTERFERENCES

Sulfates, Tin, Arsenic, and Antimony. The adsorption of zinc and cadmium is performed in a chloride medium. The sulfates remaining after evaporation of sulfuric acid do not interfere with the proposed method; therefore, decomposition of metals and minerals with nitric and sulfuric acids is possible. Antimony,

Table I. Determination of Zinc and Cadmium in Synthetic Mixtures

Zinc, Grams		Cadmium, Grams		Other Element		Decomposition Procedure	Method for Final Determination[a]	
Used	Found	Used	Found	Name	Gram		Zn	Cd
0.0500	0.0506	0.1000	0.0992	..	..	HCl + H_2O_2[b]	b	e
1.0000	0.9982	0.1000	0.0991	..	..	HCl + H_2O_2	a	e
1.0000	1.0003	0.2500	0.2507	..	..	HNO_3 + H_2SO_4	a	e
0.5000	0.5009	0.5000	0.5011	..	..	HNO_3 + H_2SO_4	a	e
0.2500	0.2492	1.0000	1.0004	..	..	HNO_3 + H_2SO_4	a	e
0.0500	0.0498	1.0000	1.0001	..	..	HNO_3; HCl	b	e
0.0005	0.00042	2.0000	...	..	..	HNO_3; HCl	c	..
3.0000	...	0.0005	0.00048	..	..	HCl + H_2O_2	..	g
3.0000	...	0.0015	0.00156	..	..	HCl + H_2O_2	..	g
3.0000	...	0.0250	0.0247	..	..	HCl + H_2O_2	..	e
3.0000	...	0.1000	0.1003	..	..	HCl + H_2O_2	..	f
3.0000	...	0.5000	0.5014	..	..	HNO_3; HCl	..	e
0.2500	0.2502	0.2500	0.2504	Ca	0.3	HNO_3; HCl	a	e
				Mg	0.3			
				Al	0.3			
0.5000	0.5006	0.2500	0.2488	Fe	0.5	HCl + HNO_3	a	e
				Cr	0.2			
				Cu	0.5			
0.5000	0.5001	0.2500	0.2504	Co	0.2	HCl; HNO_3	a	e
				Ni	0.2			
				Mn	0.3			
				Ti				
0.2500	0.2518	0.2500	0.2482	Zr	0.2		a	e
				Th	0.2			
				Be	0.2			
0.5000	0.5014	0.2500	0.2516	Sb	0.2	HNO_3 + H_2SO_4; HBr	a	e
				Sn	0.3			
				As	0.3			
0.5000	0.5011	0.2500	0.2499	Pb	0.3	HNO_3 + H_2SO_4 (filtered $PbSO_4$)	a	e
0.5000	0.5000	0.2500	0.2503	Pb	0.1	HNO_3 + H_2SO_4 (Pb not filtered off)	a	e
2.0000	...	0.2500	0.2500	Pb	0.5	HNO_3 + H_2SO_4 to dryness (excess Pb filtered off)	..	e
0.1000	0.1004	0.2500	0.2487	Bi	0.3	HNO_3 + H_2SO_4	d	f
0.1000	0.1014	0.1000	0.0990	Bi	0.3	HNO_3; Zn + Cd collected with benzotriazole	a	e

[a] Zinc
a. Potassium ferrocyanide titration.
b. Electrolytically.
c. Dithizone color method.
d. Collected with benzotriazole in ammoniacal tartrate solution, finally determined electrolytically.

Cadmium
e. Electrolytically.
f. Collected with benzotriazole in ammoniacal tartrate solution, finally determined as sulfate.
g. Dithizone color method.

[b] + indicates reagents added at the same time; ; indicates reagents added one after the other.

tin, and arsenic are adsorbed to some extent by the resin, but can be expelled with hydrobromic acid from a sulfuric acid medium.

Elements with which zinc and cadmium are often associated in metals and minerals, such as trivalent iron, manganese, aluminum, beryllium, nickel, cobalt, chromium, copper, titanium, the rare earths, and the alkaline earths, are not adsorbed on the resin in the hydrochloric acid–sodium chloride medium; they can be quantitatively determined in the eluent by standard procedures. Many quantitative recoveries of the above elements were carried out in the course of this investigation, but results are not included in this report.

Table II. Determination of Zinc and Cadmium in Concentration Products

Material	Zinc, %		Cd, %		Other Elements Present above 0.5%
	Present	Found	Present	Found	
Zinc concentrate	47.32	47.49	2.20	2.20	Fe, Cu, Mn, Pb, As, Al, S
	64.98	64.81	...	...	Fe, Cu, Mn, Pb, Cd, Al, Si, S
Zinc skimming	84.32	84.49	...	...	Al, Fe
	87.68	87.55	...	...	Al, Fe
Cadmium fume	22.00	...	11.15	11.22	Pb, As, Fe
Cottrell dust	34.12	...	14.04	14.14	Zn, As, Pb, Fe
Zinc concentrate	65.04	...	0.23	0.24	Fe, Cu, Mn, Pb, Ca, Al, S
	57.13	...	0.47	0.47	Fe, Cu, Mn, Pb, Al, S
	55.85	...	0.97	0.98	Fe, Cu, Mn, Pb, Ca, Al, S

Lead. Lead chloride is adsorbed on the resin, but is quantitatively eluted with the zinc by 2*N* sodium hydroxide. Hence, lead chloride soluble in Reagent I does not interfere with the determination of cadmium. Amounts of lead exceeding the solubility of lead chloride in Reagent I (lead > 100 mg.) should be removed as lead sulfate or chloride before transferring the solution to the columns.

Bismuth. Bismuth chloride is very soluble in Reagent I and is quantitatively adsorbed on the resin. Tests indicate that it is partially extracted by the 2*N* sodium hydroxide, which dissolves about 10 mg. of bismuth per 100 ml.; the remainder is eluted by the 1*N* nitric acid solution. In the determination of cadmium, small amounts of bismuth can therefore be disregarded. Larger amounts of bismuth can be eliminated by precipitating the cadmium, or both cadmium and zinc, with benzotriazole before transferring the solution to the column. Bismuth may also be separated from zinc and cadmium by its precipitation as the sulfide from a strongly acid solution.

Possible interference of other elements was not investigated. Some, forming insoluble compounds in acid solution (silicon, tungsten, tantalum, and columbium), could be filtered off before transferring the chlorides to the columns; others, which are adsorbed by the resin in a chloride medium (indium and germanium), usually occur in zinc-cadmium material in such minor quantities that their possible effect on the suggested method did not justify further investigation.

ACCURACY

Experimental data are presented in Table I for determinations of zinc and cadmium by the proposed procedure in synthetic mixtures of a number of elements. The method was also applied to the determination of zinc and/or cadmium in various concen-

tration products which had been analyzed by the standard procedures given by Scott (*12*). These data are given in Table II.

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RECEIVED for review May 17, 1955. Accepted November 10, 1955.

Continuous Analysis of Trace Amounts of Oxygen in Gases

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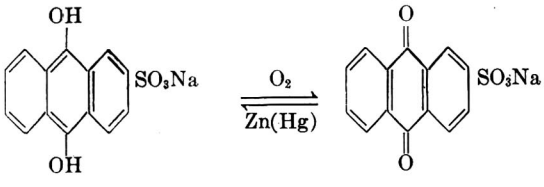
In many chemical processes, a knowledge of the oxygen content of gases is necessary. Although laboratory methods of adequate accuracy for determination of trace amounts of oxygen exist, the changing characteristics of plant processes make a continuous measurement desirable. A continuous oxygen analyzer of the colorimetric–differential photometer type suitable for plant service has been developed. Oxygen in the sample gas continuously oxidizes a reagent pumped in a closed cycle, producing a color change proportional to the amount of oxygen present. The oxygen-sensitive reagent, an alkaline solution of sodium anthraquinone-2-sulfonate, is continuously reduced to a deep red color by passing it over a zinc-mercury amalgam. Barrier-layer photocells in a direct current bridge circuit measure the differential absorptivity between the completely reduced, red reagent and the partially oxidized reagent of lighter color. The bridge is continuously balanced by a motor-driven potentiometer in a null-balance type servosystem. Angular position of this potentiometer is telemetered to a standard 10-mv. recorder. The instrument may be employed to monitor trace amounts of oxygen in any gas which does not rapidly destroy the reagent. The instrument is contained in a housing designed to be explosion-resistant in Class I, Group C, locations. The full scale sensitivity of the analyzer is adjustable from 0–50 to 0–500 p.p.m. by volume of oxygen.

IN CARRYING out many chemical reactions, especially those involving polymerizations, an accurate knowledge of oxygen concentration is desired. In continuous plant processes the results of laboratory control analysis are often obtained long after the required operating corrections should have been made. An instrument which provides continuous information would facilitate control of the plant process. Several laboratory methods for the analyses of trace quantities of oxygen in gases (*1, 2, 5–11*) were studied as the basis for design of a continuous instrument. A chemical system first studied by Feiser (*4*) and later used by Brady (*3*) showed the most promise. This system, based on the reversible reactions



has been employed in a continuous oxygen analyzer suitable for plant use in ranges below 1000 p.p.m.

PRINCIPLE OF OPERATION

Operation of the analyzer is based on the quantitative change in color of the reduced alkaline reagent solution when it is brought in contact with oxygen-bearing sample gas. The red, reduced dihydroxy form is oxidized by molecular oxygen to the colorless diketo form. The oxidation reaction is rapid; however, the gas and liquid must be brought intimately in contact under conditions such that all of the oxygen is reacted. The reaction is reversed by the reducing action of zinc amalgam so that the reagent returns to the original red, dihydroxy form (*3*).

Operation of the analyzer is illustrated schematically in Figure 1.

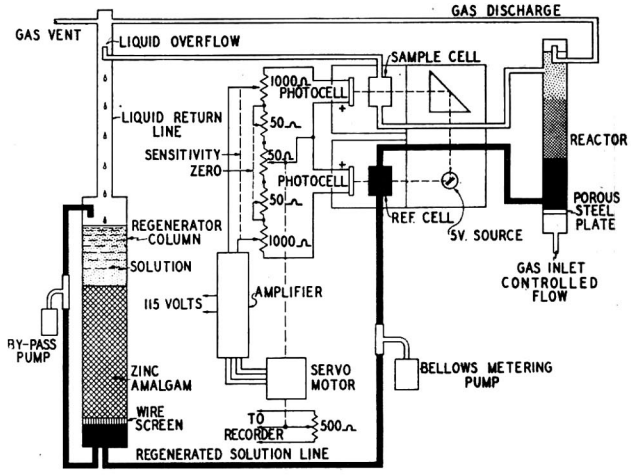


Figure 1. Schematic diagram of oxygen analyzer

Starting at the top of the regenerator, the reagent passes over the amalgam, becoming completely reduced to a deep red color. From the bottom of the regenerator, the reagent is accurately metered by a bellows-type positive-displacement pump equipped with check valves. At a flow rate around 30 ml. per minute, the solution passes through the reference cell, where the optical transmittance of the completely reduced reagent is measured by the reference photocell. In the reactor, the reagent comes in contact

with sample gas accurately metered by a flow controller and becomes lighter in color by an amount proportional to the oxygen content of the sample. The partially oxidized reagent is then passed into the sample cell, where its optical transmittance is measured by the sample photocell. The reagent is then returned to the top of the regenerator, where the cycle is repeated. A bypass pump on the regenerator column serves the dual purpose of assisting in the reduction of the reagent and removing gas which accumulates below the wire screen supporting the amalgam.

Two barrier-layer photocells, bridge circuit, amplifier, and servo motor comprise a null-balance type servosystem. The amplifier produces power of the correct phase and amplitude to the servo motor to drive the indicator potentiometer, so that the net direct current voltage across the bridge is zero. Thus, the relative angular position of the indicator potentiometer is related to the oxygen content of the sample gas.

Each potentiometer in the bridge is a three-turn dual helipot. The second section of the indicator potentiometer is utilized to telemeter its angular position to a standard 10-mv. recorder.

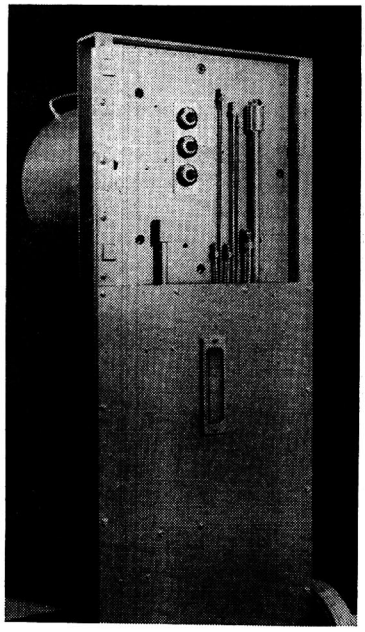


Figure 2. Front view of oxygen analyzer

The position of the span potentiometer establishes the sensitivity of the bridge by determining the ratio of the indicator potentiometer resistance to that of the remainder of the bridge. Adjustment of the zero potentiometer changes the balance point of the indicator by introducing resistance in one bridge arm and by removing resistance from the other arm. The employment of a differential type photometer minimizes the effects of changes in source intensity, temperature, and reagent strength as the solution ages.

DESCRIPTION OF THE PLANT INSTRUMENT

The system described has been embodied in a continuously operating instrument suitable for plant use. (It is now available from Consolidated Engineering Corp., Pasadena, Calif.) In Figure 2 is shown a front view of the plant instrument. All electrical components are enclosed by the steel bell and front panel which were designed to be explosion-resistant in Class I, Group C, locations. The zero, indicator, and span potentiometers are equipped with multiturn dials mounted on the front panel, which is protected by a removable door not shown in the illustration. A gas flow rotameter is mounted on the lower panel, where it is readable from the front of the instrument.

In Figure 3, the instrument is viewed from the left rear, with the explosion-resistant bell removed. The right side of the vertical mounting plate supports the electronic components including the amplifier, bridge potentiometers, and source power transformer. On the left side of the mounting plate may be seen the servo motor, photometer, and reactor column. All gas, liquid, and electrical connections to the mounting plate components are made through the front panel. On the bottom panel are mounted the sample gas flow controller and rotameter, regenerator, bellows pumps, vent gas condensate trap, and associated valves.

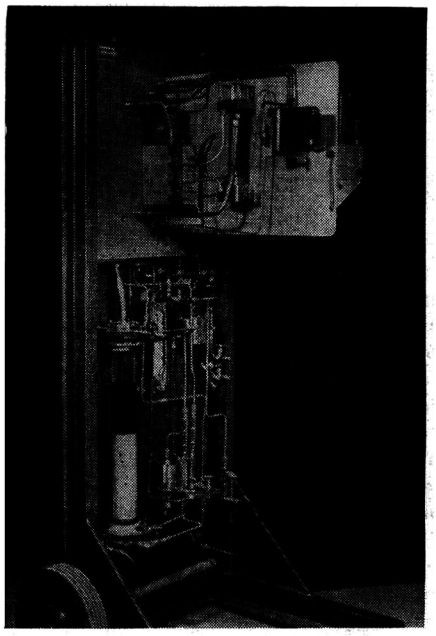


Figure 3. Exposed view of oxygen analyzer

Use of a glass regenerator chamber permits visual inspection of the zinc amalgam and reagent solution without the necessity of dismantling the instrument.

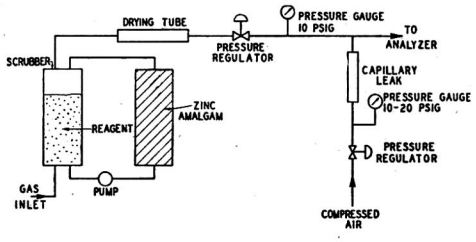


Figure 4. Schematic diagram of calibration device

The sample gas from the process stream is scrubbed of any interfering components and reduced to a pressure of from 8 to 15 pounds per square inch gage before entering the analyzer at the flow controller. Gas flow rates between 200 and 300 cc. a minute are used. The gas passes through the controller and rotameter and into the reactor column, where it comes in contact with reagent. In the regeneration process, hydrogen is released from the surface of the amalgamated zinc. To remove this hydrogen without allowing air to come in contact with the fully regenerated liquid, oxygen-free effluent gas from the reactor column flows through the top portion of the regenerator before being passed to vent.

REAGENTS

Indicator reagent. Dissolve 0.125 ± 0.005 gram of sodium anthraquinone-2-sulfonate in 1.5 liters of distilled water.

After the sulfonate has completely dissolved, add 25 ml. of 5% sodium hydroxide and make up to a volume of 2.00 liters with distilled water. The reagent deteriorates under the influence of light, especially direct sunlight, and should be stored in painted (black or aluminum) bottles.

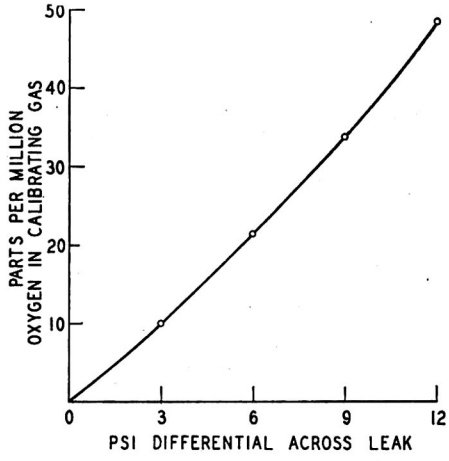


Figure 5. Capillary leak calibration curve

Zinc amalgam. Stir the required amount of 20- to 30-mesh granular zinc with a saturated solution of mercuric chloride for 4 minutes until the zinc has become uniformly dark gray in color. Pour off the excess mercuric chloride solution and wash with 15% hydrochloric acid until the amalgam is uniformly bright. Drain off the hydrochloric acid and wash with distilled water until a silver nitrate test indicates complete removal of chlorides.

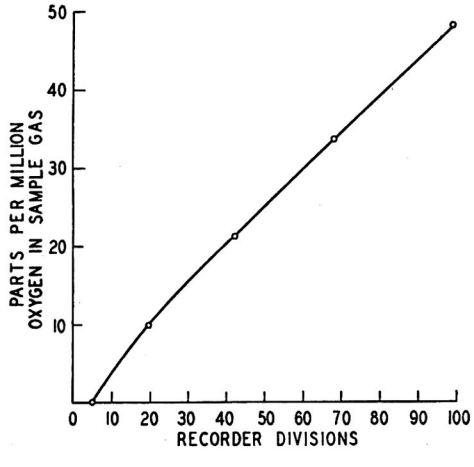


Figure 6. Analyzer calibration curve

When the amalgam coating has become exhausted, it may be reactivated with 15% hydrochloric acid as indicated above.

Scrubber reagent for removal of oxygen from calibrating gas. Make up in same manner as indicator reagent, except for the use of 0.625 gram of sodium anthraquinone-2-sulfonate.

CALIBRATION TECHNIQUE

The analyzer is calibrated with a stream of the gas containing known concentrations of oxygen.

In order to obtain known and variable concentrations of oxygen, a device was developed in which a calculated amount of air is bled through a capillary leak into a flowing oxygen-free gas stream. The leak is a Vecco standard capillary giving 7.7×10^{-4} standard cc. per second flow. It is manufactured by Vacuum-Electronics Engineering Co., New Hyde Park, Long Island, N. Y. This apparatus is shown schematically in Figure 4. The oxygen-free stream is obtained by passing a gas through a scrubber column containing fairly concentrated sodium anthraquinone-2-sulfonate solution which is continuously reduced by being pumped over zinc amalgam. It is convenient to use the process gas one intends to analyze as a source of calibrating gas during calibration in the plant. Prepurified nitrogen is suitable for use in laboratory checks of the instrument. Both these gases usually have oxygen contents below 100 p.p.m., which ensures complete oxygen removal in the scrubber. The maximum allowable concentration of oxygen in a suitable calibrating gas depends upon scrubber reagent strength and rate of gas flow. The flowing calibrating gas is next dried in a Drierite tube and reduced to a pressure of 10 pounds per square inch gage by means of a pressure regulator. Compressed air, regulated to various pressures above 10 pounds per square inch gage, is applied to the opposite end of the capillary leak. The amount of air which bleeds through the leak into the flowing gas will depend upon the pressure differential across the leak.

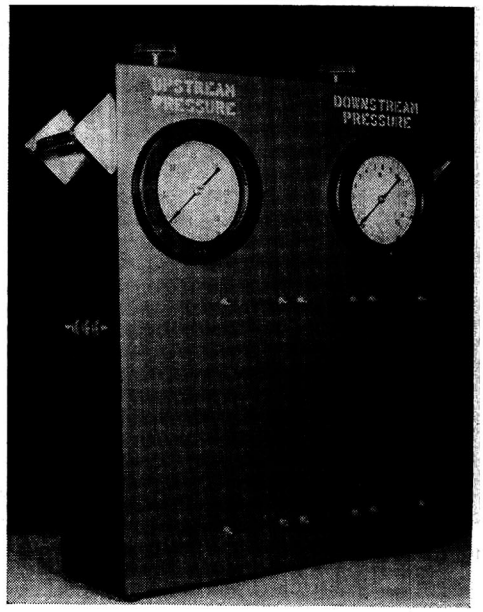


Figure 7. Front view of calibration device

Calibration of the capillary leak is accomplished by liquid displacement. A graduated cylinder of small diameter is partially immersed in a saturated solution of sodium chloride contained in a closed Lucite jacket. A fixed air pressure of 10 pounds per square inch gage is maintained above the outer surface of the brine by a dead-end service pressure regulator. Compressed air, regulated to various pressures, is applied to the capillary leak, and the air which bleeds through the leak at each pressure differential is collected over a period of several hours and measured in the graduated tube.

A typical leak calibration curve is shown in Figure 5. Unless it becomes clogged with foreign material, a leak will hold its calibration for very long periods. Calibrations have been reproduced after a leak has been in use for a year. Reproducibility of calibration is within ±2%.

The gas from the calibrator is passed into the analyzer at a constant flow rate of from 100 to 300 cc. per minute, depending upon the oxygen content of the process stream to be analyzed. The zero point for the analyzer is set with oxygen-free gas obtained as indicated above. The data present in the leak calibration curve are then used to set the instrument span adjustment to the desired full-scale recorder reading. For example, to have a 0 to 50 p.p.m. calibration, a differential of 12 pounds per square inch air pressure is placed across the capillary and then the elec-

trical sensitivity of the instrument is adjusted to provide 100 recorder divisions reading. Additional calibration points are obtained from the recorder readings corresponding to varying pressure differentials across the capillary leak. A typical calibration curve appears in Figure 6.

Calibration of the instrument may be changed by any of three variables: gas flow rate, reagent flow rate, and electrical sensitivity. Each has its particular limitations. The maximum gas flow rate is dependent upon producing complete reaction of oxygen with the reagent in the reactor column; reagent flow rate is limited by desired response time; and electrical sensitivity is limited by signal to noise ratio.

In Figure 7 is shown a front view of the calibrating device. Gas and air connections are made at the left side of the calibrator. The controls for the pressure regulators are above the corresponding pressure gages. Figure 8 is a rear view of the calibrator with the door removed. The three glass chambers contain, from left to right, Drierite, regenerator, and scrubber solution.

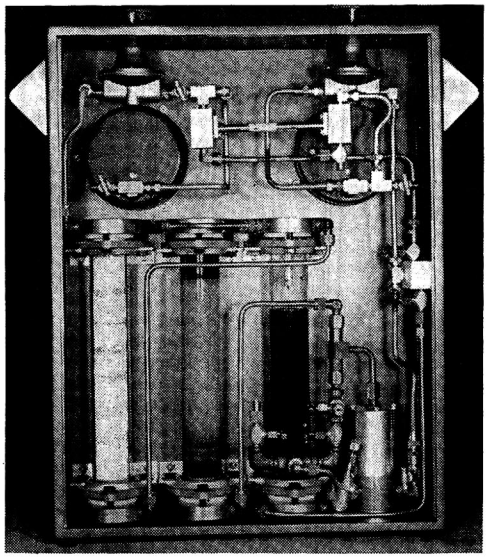


Figure 8. Exposed view of calibration device

The pump which circulates the reagent is powered by compressed air and is located in the lower right hand corner of the device. The capillary leak is at the top of the unit between the two pressure regulators.

DISCUSSION

The analyzer has given satisfactory service in plant installations for the continuous determination of oxygen in nitrogen, in ethylene, and in butadiene.

Instrument maintenance consists primarily of periodic replacement of reagent and reactivation of the zinc amalgam. The useful life of the reagent has been found to vary from 2 weeks to 6 months, depending upon the oxygen content of the gas and the other impurities present. The volume of gas which can be analyzed with one charge of amalgam depends upon the oxygen content of the gas.

The gas purification train necessary for each installation is determined by the interfering components present in the gas stream. Acidic contaminants such as carbon dioxide and hydrogen sulfide, which have been found to react with the reagent solution, are effectively removed with an Ascarite scrubber.

This analyzer was developed originally for the purpose of continuously monitoring the oxygen content of the butadiene charged to synthetic rubber polymerization processes. In these catalytic processes, oxygen contents of 0 to 100 p.p.m. cause considerable difficulty through acceleration of the formation of rubbery deposits and through interference with normal action of the polymerization catalysts. The main portion of the oxygen contamination was found to occur in the butadiene charged to the process and therefore the sample for the oxygen analyzer was taken from the butadiene charge line.

Pressure of the butadiene sample was dropped from 100 to 10 pounds per square inch gage by means of a vaporizer regulator. A trap was installed in the sample line to remove entrained liquids, mainly styrene, and the acidic gases and styrene vapor were removed by an Ascarite scrubber column.

In the early operation of the analyzer in this installation, the reagent was found to deteriorate after about 24 hours of continuous operation. This situation, first suspected of being due to a Diels-Alder condensation of the oxidized reagent and butadiene, was later traced to the presence of carbon disulfide in the sample. The reaction of carbon disulfide with the sodium hydroxide of the reagent solution results in the formation of sodium carbonate and sodium thiocarbonate. Yellow thiocarbonate interferes with the photometric measurements and also reacts to liberate hydrogen sulfide. A basic Carbitol (diethylene glycol monoethyl ether) scrubber was found to remove the carbon disulfide and results in an extension of the useful life of the reagent of at least 2 to 3 weeks.

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RECEIVED for review April 4, 1955. Accepted November 10, 1955. Presented in part before the 10th Regional Southwest Meeting, ACS, December 1954, and in part before the Division of Industrial and Engineering Chemistry, 127th Meeting, ACS, Cincinnati, Ohio, March 1955. Other papers in the Symposium on Process and Engineering Aspects of High Polymers were printed in the December 1955 issue of *Industrial and Engineering Chemistry*.

Corrections

Determination of Zinc in Presence of Iron and Nickel

The article on "Determination of Zinc in Presence of Iron and Nickel" [Lowen, Jack, and Carney, A. L., ANAL. CHEM. **27**, 1965 (1955)] was unfortunately omitted from both the author and subject indexes published in the December issue of ANALYTICAL CHEMISTRY.

Microscopic Identification of Microgram Quantities of L-Arabinose and L-Fucose

In the article on "Microscopic Identification of Microgram Quantities of L-Arabinose and L-Fucose" [ANAL. CHEM., **27**, 1998 (1955)] D-lyxose should be added to the list of sugars mentioned in footnote [a].

L. M. WHITE

In the subject index, page 2047, the 10th line under Sugars should read L-fucose instead of L-fructose.

Neutron Activation Analysis with the van de Graaff Accelerator

Application to the Halogens

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A rapid physical method of analysis for milligram quantities of fluorine and microgram quantities of the other halogens is based on neutron activation. The neutron source is the nuclear reaction $Be^9(d,n)B^{10}$ using deuterons accelerated at 2 m.e.v. by a van de Graaff accelerator. The available neutron flux is measured by the activation of metal foils. An apparatus for automatic irradiation and measurement of radioactivities having half lives on the order of 10 seconds is described.

ACTIVATION analysis offers many possibilities for rapid and sensitive methods of determining many of the elements in the periodic table. It has been applied to a number of analytical determinations in the past 10 years by investigators, usually connected with the American or British atomic energy programs, who have access to neutron sources. The technique has been used previously by this laboratory for the determination of trace impurities in high purity magnesium metal (*1*). To do this it was necessary to send samples to Oak Ridge, Tenn., or Chalk River, Canada, for neutron irradiation requiring Atomic Energy Commission authorization. Because of the transit time of the irradiated samples, work was limited to those elements with isotopes of more than 12-hour half life. The van de Graaff accelerator provides a means of obtaining a neutron source with a neutron flux of useful magnitude for activation analysis. Neutrons are produced by bombarding a beryllium target with deuterons accelerated to 2 m.e.v. by the accelerator.

The sensitivity of analysis for a specific element can be computed from the neutron activation cross section, isotope abundance, and half life. The complication of Senftle and Leavitt (*4*) has been put into a form which, using the neutron flux obtainable from the authors' source, gives the analytical sensitivity for a particular element under various bombardment times and counting efficiencies.

The review article of Boyd (*2*) has explained the method of activation analysis. Subsequent papers, such as those of Leddicotte and Reynolds (*3*), have described the use of the nuclear reactor for this method. The general procedure is to irradiate the analytical sample along with pure standards of each element to be determined as flux monitors. The several samples are then given the same chemical treatment, and measurements are made of the radioactivity induced in each separate element. Using the accelerator neutron source the neutron flux is established by measurement with standard detector foils, and then changes in flux are determined by relative neutron measurements made with counters. Employing short-lived activities is advantageous, since the required irradiation time is short and the use of differential counting methods can often permit determining a specific element without chemical separations. This is dependent upon the elements present in the sample and the interferences obtained.

This report describes the method of producing thermal neutrons with the van de Graaff accelerator, the results of neutron flux measurements, and the methods and results obtained for the determination of chlorine, bromine, iodine, and fluorine by neutron activation using the van de Graaff as a neutron source.

NEUTRON SOURCE

Reaction for Neutron Production. The nuclear reaction used in this work to produce neutrons with the van de Graaff accelerator is:

$$Be^9 + D^2 \longrightarrow B^{10} + n^1 + 4.3 \text{ m.e.v.}$$

A thick beryllium target was bombarded with deuterons accelerated to 2 m.e.v. by the van de Graaff accelerator.

Beryllium Target. The beryllium target was obtained in the form of a disk $^1/_8$ inch thick and 1 inch in diameter (Machlett Laboratories, Springdale, Conn.). It was mounted by the manufacturer in a stainless steel ring. The mounting ring was soft-soldered into a stainless steel retaining ring which had been silver-soldered to the end of the titanium target tube. With a beam of 30 μa. of deuterons at 2 m.e.v. bombarding the target, 60 watts are dissipated in the target. This makes it necessary to cool the target. Cooling water was passed through a polystyrene water jacket and across the outer face of the beryllium target. The first beryllium target used was coated with Glyptal varnish on the water-cooled face to protect the beryllium from corrosion. After 5 months the target corroded to the point where it began to leak. A second target was installed. This target was coated with an evaporated film of titanium 3.9 microns thick and an evaporated film of aluminum 2.3 microns thick. Thicker coatings are desirable, but with the equipment available the time necessary to secure a thick film becomes excessive. This target showed pitting after 3 months of operation and methods of obtaining heavier coatings of titanium or stainless steel are needed.

Paraffin Moderator. Neutrons produced by 2-m.e.v. deuterons by the nuclear reaction $Be^9(d,n)B^{10}$ have an energy spectrum extending up to 6 m.e.v. For activation work with thermal neutrons it is necessary to reduce the energy of the neutrons to energies of the order of 0.002 to 0.5 m.e.v. This was accomplished in the apparatus by surrounding the target with a cube of paraffin (Quaker State 165 M wax cream). The high energy neutrons lose energy by elastic collisions with the hydrogen atoms of the paraffin. On the average after 20 to 25 collisions the neutrons have reached an equilibrium state with the energy of the paraffin atoms. The moderator for the neutron source is in the form of a cube 2 feet on a face. Samples to be irradiated are placed at various positions in the mass of paraffin by a polyethylene stringer. The polyethylene stringer or slider has a machined cavity into which the sample containers are placed. A photograph of the moderator showing the polyethylene slider partially withdrawn is shown in Figure 1.

Equation Relating Neutron Flux and Induced Radioactivity. The thermal neutron flux was measured by irradiating known weights of metal foils. The induced radioactivity of the foils was measured with a thin mica-window (1.4 mg. per sq. cm.) Geiger tube. The neutron flux was calculated from the relationship:

$$N^*\lambda = N\sigma fk\,(1 - e^{-\lambda t})$$

where

$N^*\lambda$ = activity, disintegrations per second at zero decay time
σ = isotopic activation cross section
N = number of atoms in the foil
f = flux in neutrons per sq. cm. per second
k = isotopic abundance of target nuclide
λ = decay constant of radioactive nuclide formed
t = time of irradiation, seconds

The foils were irradiated both with and without a $^1/_8$-inch cadmium filter to correct for activation by neutrons of resonance energy.

Foils for Absolute Measurements. The foils were circular evaporated silver and indium films mounted on 1-mil polystyrene film in polystyrene rings. The rings were of 1-inch inner diameter, so they could be slipped over a National Bureau of Standards reference source blank. The samples could then be compared directly with a National Bureau of Standards Ra D-E β standard of identical area (1.13 sq. cm.), backing, and nearly the same weight. Three different counting efficiencies were used at various times in the thermal neutron flux measurements. The counting efficiency values were 3.92, 5.51, and 18.81%.

Disintegration rates at zero decay time were determined by counting irradiated foils over a period of time and plotting the results on semilog paper. A straight line of proper slope was then drawn through the experimental points and extrapolated to zero decay time.

Table I. Thermal Neutron Flux, at Optimum Distance from Target

Sample Activated	Thermal Neutron Flux, Neutrons/Sq. Cm./Sec./μa. ($\times 10^{-6}$)	
	Target 1	Target 2
Ag No. 2	11.5	10.2
Ag No. 3	8.4	7.4
Ag No. 4	9.1	..
In No. 10	8.1	7.8
Average	8.9	8.5

Methods for Relative Measurements. In addition to the foil method of absolute flux measurement, two methods of relative flux measurement were used. One method used a commercial boron trifluoride neutron counter (Radiation Counter Laboratories, Mark 2, Model 3-12E6C) as a detector. The pulses from the counter were fed to an amplifier (Nuclear Instrument and Chemical Corp., Model 1061, plus one additional stage) and then through a cable from the accelerator room to a rate meter (Nuclear Instrument and Chemical Corp., Model 1615-B) in the control room. A Brown circular chart recorder was used to record the counting rate from the rate meter.

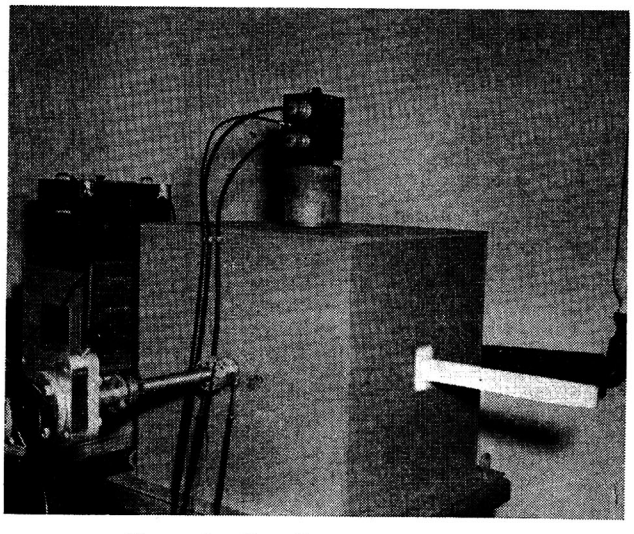

Figure 1. Paraffin moderator cube

The second relative method used to measure neutron flux used a 5819 photomultiplier tube with a mixture of Du Pont Q28-2349 phosphor (essentially zinc, cadmium sulfide) and boron oxide coated on the phototube face. The current output from the phototube was passed to ground through a high resistance. A vacuum tube voltmeter was used to measure the voltage across the resistor. The voltage was read on a meter at the accelerator control console. The photomultiplier detector is shown on top of the moderator in Figure 1.

Thermal Neutron Flux at Optimum Distance from Target. The results of thermal neutron flux measurements for the two beryllium targets are given in Table I. These data were obtained with silver and indium foils at the optimum distance from the target along the target tube axis. Deuterons of 2-m.e.v. energy were used to bombard the beryllium targets.

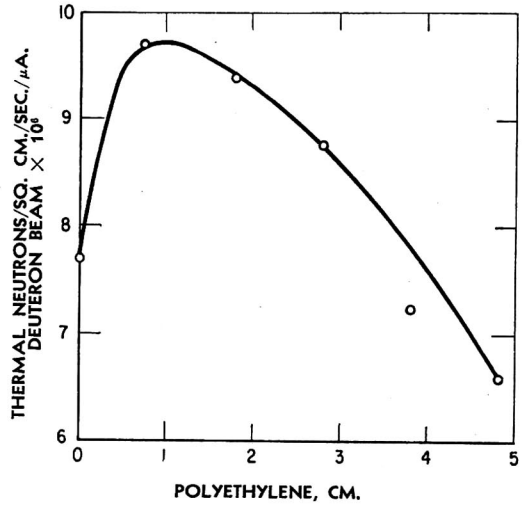

Figure 2. Thermal neutron flux for various distances along target axis

These results are in agreement with the value of 5.8×10^6 thermal neutrons per sq. cm. per second per μa. determined earlier in experiments at the High Voltage Engineering Corp. laboratory.

Flux Distribution in Moderator. Flux distribution in a moderator is generally shown by a curve of activity xr^2 *vs.* r, where r is the distance from the source to the point of measurement in the moderator. For this purpose the plot of thermal neutron induced activity *vs.* r is more useful. The point of maximum thermal neutron flux was determined by measuring the flux distribution along an extension of the target tube axis with varying thicknesses of polyethylene between the target and the test foil. Results obtained with silver foils are tabulated in Table II and plotted in Figure 2.

With 0.75 cm. of polyethylene between the target and the sample holder, the effect of displacing the center of the sample

Table II. Thermal Neutron Flux Distribution in Moderator Along Target Axis

Distance between Polyethylene Target and Foil, Cm.	Thermal Neutron Flux, Neutrons/Sq. Cm./Sec./μa. ($\times 10^{-6}$)				
	Foil 1	Foil 2	Foil 3	Foil 4	Average
0.0	6.3	9.5	6.2	9.0	7.7
0.75	..	11.5	8.4	9.1	9.7
1.8	7.8	10.7	8.8	10.3	9.4
2.8	..	10.0	7.9	8.1	8.7
3.8	..	8.2	6.0	7.3	7.2
4.8	..	7.3	5.3	6.8	6.5

Table III. Thermal Neutron Flux Distribution in Moderator Normal to Target Axis

Displacement from Target Axis, Cm.	Thermal Neutron Flux, Neutrons/Sq. Cm./Sec./μa. ($\times 10^{-6}$)		
	Foil 3	Foil 4	Average
0	8.4	9.1	8.8
2	8.1	9.7	8.9
4	7.2	8.1	7.6
6	5.3	5.9	5.6

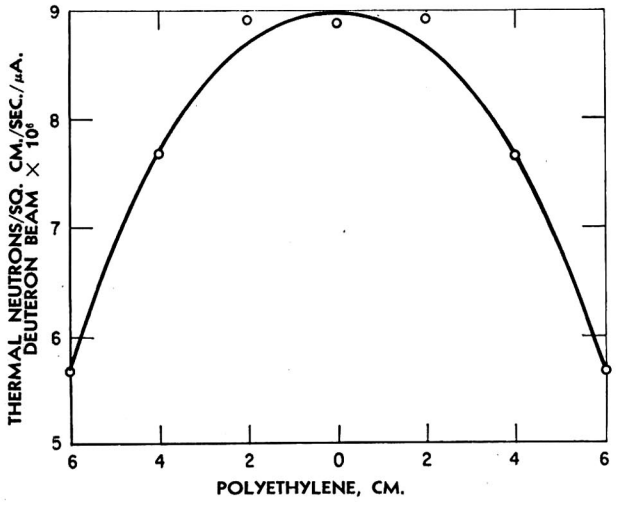

Figure 3. Thermal neutron flux for various distances normal to target axis

laterally from the target tube axis was investigated. Results obtained with silver foils are tabulated in Table III and plotted in Figure 3.

Effect of Deuteron Energy. The voltmeter of the accelerator was calibrated by accelerating protons onto a lithium fluoride target. The nuclear reaction $Li^7(p,n)Be^7$ has a reaction threshold at 1.88 m.e.v. By slowly increasing the accelerator voltage while bombarding the lithium fluoride target with protons, the voltmeter was set at 1.88 m.e.v. at the point where neutron production began. The effect of deuteron energy on neutron production with a thick beryllium target was investigated over the energy range 1.8 to 2.2 m.e.v. Results obtained with silver foils are tabulated in Table IV and plotted in Figure 4.

Table IV. Voltage Effect on Thermal Neutron Flux

Voltage, Millions of Volts	Thermal Neutron Flux, Neutrons/Sq. Cm./Sec./μa. (× 10^{-6})		
	Foil 3	Foil 4	Average
1.8	6.3	7.7	7.0
1.9	8.0	9.4	8.7
2.0	8.4	9.1	8.8
2.1	9.1	10.9	10.0
2.2	9.9	11.1	10.5

Estimation of Errors in Thermal Neutron Flux Measurement. The absolute thermal neutron flux values are believed to be good to about 30%. Errors arise from the following causes:

Not all the measured ion current is expended in the target. This does not produce an error in the neutron measurement, but could make an error in the neutrons per microampere of as much as 25%.

The Ra D-E β has an $E_{max.}$ of 1.2 m.e.v.; for indium it is 0.8 and for silver it is 2.0. The flux calculated from the silver data is high. That from the indium data is low. An average will be off by not more than ±10%. This error could be eliminated by extrapolating aluminum absorption curves for the standard and test foils to zero absorber.

The back-scattering of the samples is not so large as for the standard, because of the thin polystyrene layer between the samples and the silver disk. This will make the measured flux lower than the true value by 10 to 15%.

The preparation of the evaporated metal foils involve handling and weighing foils of 0.4 to 0.8 mg. mass. The measured weight of the foils used in this work may be in error by ±5%.

The sum of these errors in one direction could make the measured value low by as much as 55%. A better estimate would be 30%.

ACTIVATION ANALYSIS OF HALOGENS

Determination of Chlorine. Nuclear Reaction to Produce Radioactive Chlorine. The nuclear reaction Cl^{37} $(n,\gamma)Cl^{38}$ was used for a method of determining chlorine by activation. Chlorine-38 decays with a half life of 37.29 minutes. The radiation emitted in the decay process consists of three β particles and two γ rays. The energies of the β particles are as follows: 53% 4.81 m.e.v., 16% 2.77 m.e.v., 31% 1.11 m.e.v.; 47% of the disintegrations result in γ rays of 2.15-m.e.v. energy, and 31% result in γ rays of 1.60-m.e.v. energy.

Irradiation and Counting Procedure. Aqueous samples were placed in a 1-ml. polyethylene cup to be irradiated. The cup was made to fit a two-piece polystyrene irradiation cell. A piece of 0.001-inch polyethylene film was placed over the polyethylene cup and held in place by pushing the ring portion of the polystyrene irradiation cell down over the film, cup, and bottom part of the irradiation cell. The cup is 2.5 cm. in diameter and 0.2 cm. thick.

Samples were irradiated for 30 minutes at a thermal neutron flux of 2.5×10^8 neutrons per sq. cm. per second. This was obtained by using a deuteron beam of 30 μa. at 2 m.e.v. At the end of the irradiation period the sample solution was transferred from the irradiation cup to a polyethylene counting dish. A 1-ml. hypodermic syringe was used to make the transfer. The needle was pushed through the thin polyethylene film, and the contents of the irradiation cell were drawn into the syringe and then expelled into the counting dish. The radioactivity was measured by placing the open counting dish directly under the window of a thin mica-window (1.4 mg. per sq. cm.) Geiger counter. The total number of counts above background observed during the interval of 3 to 18 minutes after the end of the irradiation was determined.

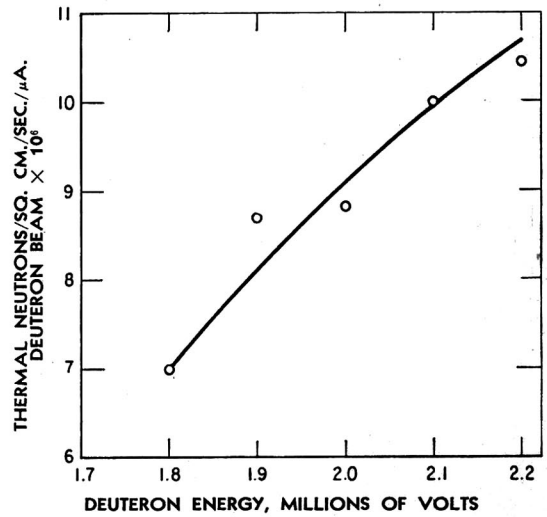

Figure 4. Thermal neutron flux for various deuteron voltages

The sample transfer to a nonirradiated counting dish was found to be necessary in order to reduce the background to a low and constant value. Twenty-two polymers and copolymers were irradiated in an effort to find a material which could serve as sample cell but which would not become radioactive when irradiated with neutrons. No such material was found so the sample transfer procedure was used.

Results. A calibration curve was prepared by activating and counting ammonium chloride solutions of known chlorine content. A typical calibration curve in the range 0 to 5.0 mg.

of chlorine irradiated is a straight line. The slope of the plot of total counts collected from 3 to 18 minutes after the end of the irradiation minus the background is 8600 counts per mg. Known samples prepared from 0.1000*N* hydrochloric acid were analyzed, with the results shown in Table V.

The minimum amount of chlorine that can be detected by this procedure is 0.02 mg. with an uncertainty of ±15%.

Table V. Results of Chlorine Analysis

Cl, Mg.		
Sample	Found by activation analysis	Error, %
3.545	3.555	+ 0.3
0.355	0.400	+12.7
0.035	0.030	−14.3

Determination of Bromine. NUCLEAR REACTION TO PRODUCE RADIOACTIVE BROMINE. The nuclear reaction Br^{79} $(n,\gamma)Br^{80}$ was used to produce radioactive bromine. The half life of bromine-80 is 18.0 minutes. The radiation emitted in the decay process consists of two β particles and one γ ray. The energies of the β particles are as follows: 85% 1.99 m.e.v. and 15% 1.1 m.e.v. A γ ray of about 0.9-m.e.v. energy occurs in 15% of the disintegrations. Neutron capture by bromine-79 also results in the formation of a metastable state of bromine-80 which decays by isomeric transition with a half life of 4.4 hours. The decay of the activity observed in this work was followed for 60 minutes. The activity decayed with a half life of 18.5 minutes.

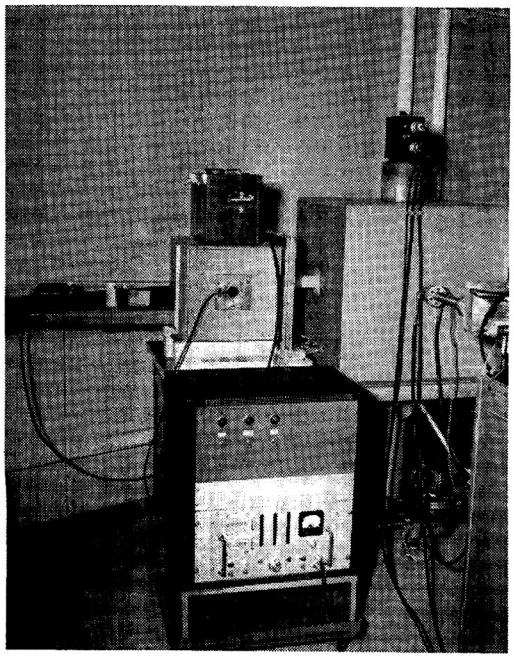

Figure 5. Automatic neutron irradiation equipment

IRRADIATION AND COUNTING PROCEDURE. The irradiation and counting procedures were the same as those used for chlorine determinations.

RESULTS. A calibration curve was prepared by activating and counting lithium bromide solutions of known bromine content. The plot of counts collected *vs.* weight of bromine irradiated in the range zero to 100 γ is a straight line of slope 218 counts per γ. Aqueous solutions of lithium bromide were checked against the calibration curve, with the results listed in Table VI.

Unknown samples of dilute aqueous solutions of a brominated polymer were analyzed for bromine by this method. The results obtained are tabulated in Table VII.

Table VI. Results of Bromine Analysis

Br, γ		
Sample	Found by activation analysis	Error, %
40.2	40.2	0.0
20.1	19.5	−3.0
20.1	19.5	−3.0
20.1	20.0	−0.5

Table VII. Results of Brominated Polymer Analysis

Sample	Bromine, P.P.M.
47A	13.6, 13.5, 11.3
47B	0
47C	4.0, 3.5
47D	2.8, 3.0
1-1	4.4, 4.5
1-2	5.2, 4.7
1-3	5.0, 5.0
1-4	3.6, 3.6
1-5	8.2, 7.9
1-6	5.4, 5.8

The minimum amount of bromine detectable by this method is 1 γ with an uncertainty of ±15%.

Determination of Iodine. NUCLEAR REACTION TO PRODUCE RADIOACTIVE IODINE. The nuclear reaction $I^{127}(n,\gamma)I^{128}$ was used to produce radioactive iodine. The half life of iodine-128 is 24.99 minutes. The radiation emitted in the decay process consists of a β particle of 2.02-m.e.v. energy plus about 7% of the disintegrations resulting in the production of a γ ray of 0.43 m.e.v.

IRRADIATION AND COUNTING PROCEDURE. The irradiation and counting procedures were the same as those used for chlorine determinations.

RESULTS. A calibration curve was prepared by activating and counting lithium iodide solutions of known iodine content. The calibration curve is a straight line over a weight range of 0 to 100 γ of iodine irradiated. The slope is 307 counts per γ. Aqueous solutions of ammonium iodide and calcium iodide were checked against the calibration curve with the results tabulated in Table VIII.

Table VIII. Results of Iodine Analysis

	I, γ		
Sample	Sample	Found by activation analysis	Error, %
NH_4I	65.1	66.2	+1.7
NH_4I	65.1	64.2	−1.4
CaI_2	52.3	51.8	−1.0
CaI_2	52.3	50.2	−4.0

The minimum amount of iodine detectable by this method is 0.7 γ with an uncertainty of ±15%.

Determination of Fluorine. NUCLEAR REACTION TO PRODUCE RADIOACTIVE FLUORINE. The nuclear reactions are $F^{19}(n,\gamma)F^{20}$ and $F^{19}(n,\alpha)N^{16}$. The radioactive fluorine decays with a 10.7-second half life to give a β ray of maximum energy of 5.41 m.e.v. followed by a 1.63-m.e.v. γ ray. The nitrogen-16 has a 7.35-second half life and β rays of maximum energy 3.8, 4.3, and 10.3 m.e.v.

IRRADIATION AND COUNTING PROCEDURE. The short half life necessitates only a short irradiation time to bring about saturation activity and only a short counting time is available. An automatic timing device was constructed to sequence the opera-

tions of the short irradiation and counting and to allow accurate measurement of the time of irradiation, the time at which the irradiation is stopped, and the time at which the counting starts.

Figure 5 shows the moderator cube on the right and the counter in its shield in the center. The slider with the hole for the sample cup is shown between the two. At the left is the sample drive motor which pulls the slider from the moderator to the counter. Also shown are the recorder, the sequence timer, and the scaler.

The circuit of the sequence timer is given in Figure 6. The sample is placed in the sample slider and positioned in front of the target. The beam is turned on by pressing the "on" button on the console energizing $RL103$ (through contacts $R1$) which, by contacts not shown, turns the beam on and lights the indicating pilot light, G. The timing motor, TM, is also activated. The timing motor controls a series of cam operated switches, $TM1$, $TM2$, and $TM3$. After 68 seconds have elapsed $TM2$ closes, starting the Sanborn recorder chart drive. At 75 seconds, elapsed time $TM3$ closes for 0.5 second, energizing $R1$ and cutting off the accelerator beam. At the same time $R1_2$ closes, supplying the "radiation off" timing pulse from the 22.5-volt battery to the recorder. Also $R1_3$ closes, supplying current to the sample drive motor through $R2_2$.

The sample drive motor pulls the sample slider from the irradiation position in the moderator to the counting position. At the end of its travel the slider opens the limit switch and stops against a metal plate. The single-pole, double-throw limit switch supplies current to relay $R3$ applying 6 volts to the recorder galvanometer. After 0.5-second delay for the positioning of the sample in front of the counter by the tension on the slider chain, time delay relay RTD opens $RTD1$. This drops the 6 volts on the recorder, and this drop is used as the "start count" time marker. $RT2$ opens at the same time and the Berkeley scaler starts counting the pulses from the sample. The recorder then receives pulses from the scaler at a scaling factor set by the "predetermined-count" switch on the scaler—i.e., every 20th, 40th, 100th, etc., pulse received by the scaler is recorded. The chart tape on the recorder is run at a known speed (25 mm. per second) by a synchronous motor and thus forms the time base of the measurements. The decay of the radioactivity can be plotted using the data from the recorder chart.

At the end of 3 minutes the timing motor has turned one revolution and shuts off by contact $TM1$.

The timing pulses are applied with the speed of action of the relays, on the order of 0.03 second. The recorder chart can be read to about 0.01 second. A typical recorder chart shows the "radiation-off" pulse as a sharp pip. During the next 4 seconds the sample is moved. When the slider trips the limit switch the recorder line is displaced, and when counting begins the line falls to normal position marking the start of counting. Then successive pips appear on the chart marking the counts collected per time interval. These pips become less frequent as the sample decays, and after 80 seconds can be used to measure background.

The counting was done by a thin-window Geiger counter housed in a shield consisting of a 1-inch-thick lead cylinder with a 0.5-inch layer of 50% boric oxide–50% paraffin surrounded by ordinary paraffin. This shielding prevents the Geiger tube from becoming radioactive during the bombardment and also decreases the background due to annihilation radiation from nitrogen-13 activity at the target. This nitrogen-13 is made from the (d,n) reaction on carbon, which is deposited on the target from the vacuum system. A correction for background was made by measuring the radioactivity of the cell and sample after the fluorine-20 activity had decayed to a negligible value. To do this the sample was measured for 50 seconds, and then at the end of 1.5 minutes another measurement was made for 50 seconds. The second value, which was a measure of the long-lived activity in the cell, and the sample was subtracted from the first result to give the short-lived activity for the cell and sample. This result was then corrected by subtracting the short-lived activity found for blank runs with the cell. The resulting value represented the radioactivity due to short-lived isotopes in the sample.

Results. A calibration curve was prepared by activating and counting aqueous potassium fluoride solutions of known fluorine content. A typical calibration curve in the range 0 to 24.0 mg. of fluorine irradiated is a straight line of slope 256 counts per mg. Reproducibility is shown in the data of Table IX.

Aqueous solutions of C.P. ammonium fluoride were prepared and the fluorine contents of aliquots of the solutions were determined by activation analysis. Two aliquots of each sample solution were used. Multiple determinations were carried out on the aliquots by allowing the short-lived activities to decay and then reirradiating the aliquot. The results obtained are tabulated in Table X along with chemical analyses. The chemical method used is the titration of fluoride with thorium

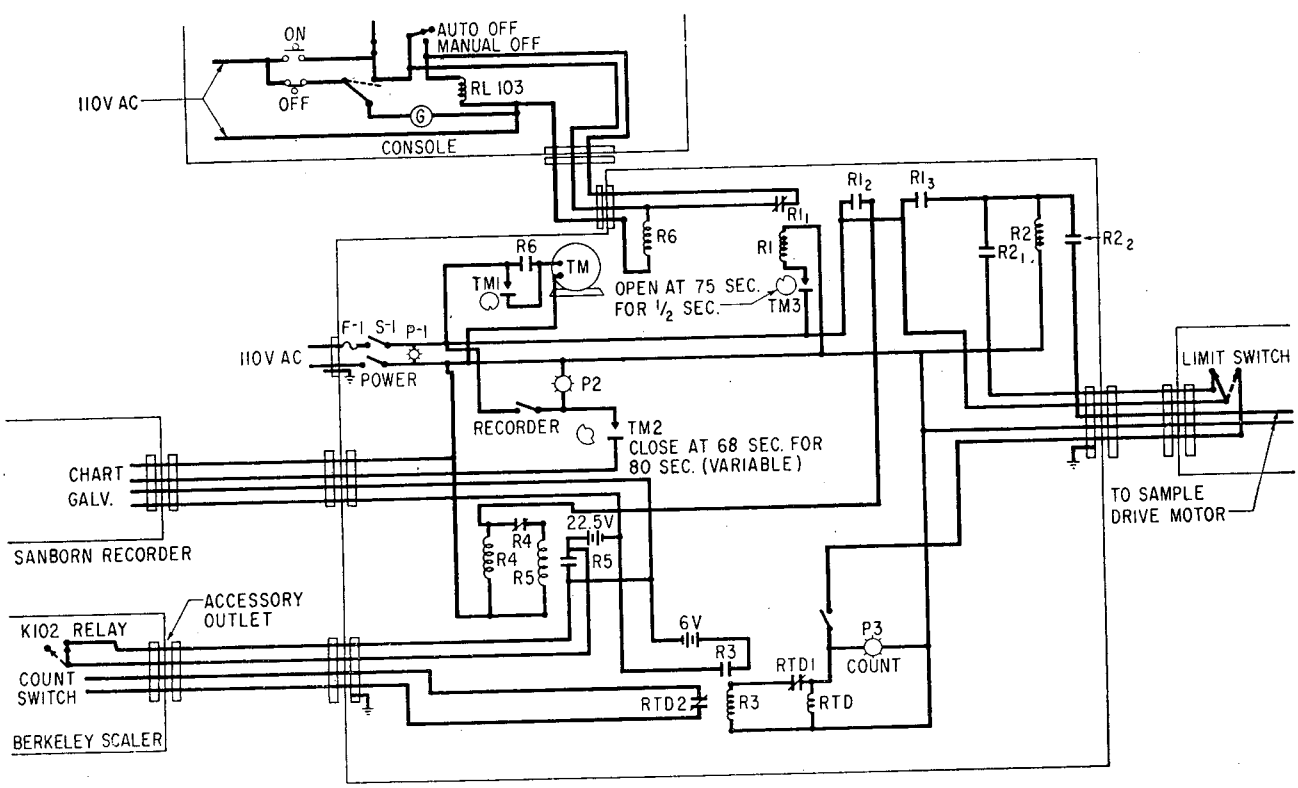

Figure 6. Timing Circuit

nitrate with alizarin S as the indicator. Three chemical laboratories designated A, B, and C contributed chemical analyses on fluorine.

The aqueous solutions were prepared from C.P. sodium fluoride, and the fluorine content of aliquots of these solutions were determined. In this case it was necessary to correct for the activity of fluorine-20 formed from sodium-23. This is discussed below in the section on interferences. The results obtained are given in Table XI.

A water solution of low and unknown fluorine content was prepared using lithium fluoride. Aliquots of the unknown solution were activated and the fluorine content of the solution was determined. The results are tabulated in Table XII.

The method was tested with organically bound fluorine by preparing sample solutions of organic fluorine-containing compounds in suitable solvents. Aliquots of the sample solutions were then irradiated and the fluorine content was determined.

Table IX. Determination of Fluorine in Standard Potassium Fluoride Solution Used to Prepare Fluorine Calibration Curve

	F, Mg.	
Date	In sample for irradiation	Found by activation analysis
4-18-55	5.94	6.2
4-28-55	2.41	2.3
5-12-55	16.39	15.6
5-13-55	11.86	11.5
5-17-55	16.40	16.2
5-17-55	11.75	11.5
5-17-55	5.92	5.9

Table X. Determination of Fluorine in Ammonium Fluoride Solution

	NH_4F, Mg.		F Found by Activation, %		F Found by Titration (Lab. C), %
Sample	In sample solution	In aliquot irradiated		Average	
Solution 1	486.02	21.17	56.6 54.8 53.7 55.4 56.8 53.7	55.2	48.9
		20.10	54.7 55.4 55.2 54.7 52.3 52.1	54.1	..
		20.69	54.2 57.0 53.6	54.9	..
Solution 2	411.67	43.66	55.0 52.2 53.8 52.7 53.1 52.8 51.8	53.1	49.7
		41.77	54.2 52.0 54.3 52.8 55.1 51.5 53.0	53.3	..
		40.60	53.5 52.8 53.2	53.2	..
Solution 3	262.85	27.02	53.4 52.6 51.0 52.9 54.6 54.1 53.2	53.1	52.4
		26.79	52.0 53.1 54.7 54.2 51.3	53.1	..
		26.27	54.4 54.4	54.4	..

Table XI. Determination of Fluorine in Sodium Fluoride Solutions[a]

	F, Mg.	
Sample	Found by activation analysis	Found by titration (Lab. A)
2.05[b]	2.15 2.45 2.00 2.10	..
2.49	2.6	2.67
2.49	2.5 2.7	2.74
6.70	7.0	6.46
6.70	6.8 6.9	6.47
2.65	2.8 2.7 2.9	..
7.13	7.4 7.2 7.3	..

[a] Approximately 1-gram samples.
[b] Standard sodium fluoride solution from Laboratory B.

Table XII. Determination of Fluorine in Lithium Fluoride Solution

	F, %	
Sample No.	Found by activation analysis	Found by titration
1	0.057 0.043 0.052	...
2	0.065 0.060 0.046	...
3	0.042 0.056 0.061	...
Chemical analysis		
Lab. A	...	0.051 0.060
Lab. C	...	0.085 0.094 0.080

Table XIII. Determination of Fluorine in Organic Compounds

			F Found by Activation Analysis, %			F Found by Chemical Analysis (Lab. C), %
Compound	Solution No.	Amount of Compound in Aliquot, Mg.		Average	Theory	
C_6H_5F	1	82.53	20.8 19.4	19.6	19.8	19.80
	2	92.69	20.7 19.0 19.5 18.5 18.5	..	..	...
$CF_3CO_2C_2H_5$	1	24.34	42.4	42.6	40.1	38.61
	2	23.81	42.8	..	..	...
$CClF_2$-CCl_2F	1	30.88	35.6	36.2	30.4	30.78
	2	33.41	36.8			
CF_3CONH_2	1	27.12	53.2 55.4 50.3 50.9	52.6	50.4	47.12
	2	25.70	49.8 53.3 52.5 52.5	..	..	...
	3	17.70	54.3 57.1 55.8 54.7	..	..	...
	4	36.76	50.6 51.9 51.9 51.2	..	..	...
	5	31.59	49.8 53.3 52.5 52.5	..	..	...

The results obtained for four different compounds are given in Table XIII. The chemical analyses were done by potassium fusion of the organic compound, distillation of fluosilicic acid, and titration with thorium nitrate.

Quantities of fluorine between 0.5 and 25.0 mg. can be determined by this procedure. The uncertainty of results ranged between ±20% for low amounts of fluorine to ±3.5% for higher amounts. When run successively, the method takes 5 to 10 minutes per sample.

INTERFERENCES. No attempt was made in this investigation to work out methods of avoiding interferences. Chlorine, bromine, and iodine all interfere in the determination of any one of the three elements. Chlorine could be determined in the presence of the other two by using a γ-ray spectrometer to measure the activity of the 2.1- and 1.6-m.e.v. γ-rays from chlorine-38. The use of this technique with bromine and iodine however would result in loss in sensitivity since the percentages of disintegrations resulting in γ-ray production from both bromine-80 and iodine-128 are low. Because of the short half life, fluorine-20 does not interfere in the determination of the other three halogens.

Fluorine determinations can be made in the presence of chlorine by applying a background correction for the chlorine-38 activity. This does not work in the cases of bromine-80 and iodine-128, because the activity levels from these isotopes are too high. The use of suitable absorbers or a scintillation counter with pulse height discrimination offers possibilities for eliminating interference from these elements.

Sodium interferes in the determination of fluorine because of the nuclear reaction $Na^{23}(n,\alpha)F^{20}$. If the sodium content of the sample is known a suitable correction can be applied. Determinations with C.P. sodium benzoate gave a value of 37 counts in 50 seconds per milligram of sodium for the conditions of irradiation and counting used for the fluorine method. Other reactions which might produce a short-lived activity and thus interfere in the fluorine determination were checked. These reactions, all of which produced negligible activity under the irradiation conditions for the fluorine method, were $N^{15}(n,\gamma)N^{16}$ $O^{16}(n,p)N^{16}$, and $Cl^{37}(n,\alpha)P^{34}$.

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RECEIVED for review July 21, 1955. Accepted November 21, 1955.

Amperometric Titrations of Micromolar Solutions

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Amperometric titrations of dilute solutions of metal ions with disodium ethylenediamine tetraacetate have been carried out using a mercury pool indicator electrode. Some of the problems of mercury pool polarography, such as the reproducibility of the electrode area and the constancy of stirring, have been eliminated by this technique. The area of the electrode used was 1 to 2 sq. cm. and stirring was accomplished by bubbling nitrogen through the solution. Cadmium, copper, lead, nickel, and zinc have been titrated at concentrations well below the range of conventional amperometric methods which employ the dropping mercury electrode. In favorable cases, titrations were feasible at dilutions approaching $10^{-7}M$. For example, solutions containing 0.02 γ of cadmium per ml. can be titrated with an average error of 0.0001 γ. It was also found possible to titrate solutions of ions which could be polarographically determined with a mercury pool cathode.

THE advantages of amperometric titrations over conventional polarographic procedures have been discussed by various authors (*2, 3*). Such titrations are more precise, and have a somewhat greater sensitivity than polarographic methods, but these advantages are offset by the necessity of using burets and standard solutions. The choice as to which of the two procedures to apply is determined by the requirements of the particular problem confronting the analyst.

The increased precision arises from the fact that the electrode is not used to translate current directly into concentration, as is the case in polarographic procedures. The error in the measurement of the titration volume is much smaller than the error in the translation of current into concentration. Thus, many of the factors that limit the precision in polarography are no longer critical. The electrode is used only to indicate the appearance or the disappearance of a particular species during the course of a titration. Both dropping mercury and rotating platinum electrodes have been used to indicate the end point of various titrations, but because of the high overvoltage of hydrogen on mercury, the former electrode covers an important range of potentials and has been extensively applied to amperometric methods. The purpose of this research was to evaluate a mercury pool electrode in such titrations. This electrode offers a greater sensitivity than the dropping electrode in some polarographic applications (*3*) and it was hoped that the titration of more dilute solutions could be accomplished.

In amperometric titrations of dilute solutions involving precipitation reactions, the limit of sensitivity is often determined by the solubility of the product formed. The titration curves in such cases deviate from straight lines over a large portion of the titration and precision, as well as sensitivity, is decreased. Even when the solubility of the salt formed is sufficiently small, the slow rate of formation of the precipitate becomes a problem with dilute solutions ($<10^{-5}M$). For the above reasons it was felt that amperometric titrations of solutions more dilute than $10^{-5}M$ would be limited to processes involving strong complexing agents or redox reactions.

Ethylenediaminetetraacetic acid [(ethylenedinitrilo) tetraacetate] has been widely used for the titration of a variety of metal ions (*5*). In some cases the end point of these titrations has been detected amperometrically (*1*). Because of the smallness of the instability constants and the rapid rate of complex formation, the titration curves should be useful even in the micromolar range of concentrations. However, the dropping mercury electrode is inadequate for indicating the end point in the titration of such dilute solutions. This low sensitivity is caused by the high charging current associated with the renewal of the mercury drop, which is superimposed upon the diffusion current. Below $10^{-5}M$ the magnitude of this background current becomes

larger than the diffusion current of the ion being determined. A mercury pool cathode at a constant voltage has no charging current, and is sensitive to much lower concentrations of reducible materials. It was found possible to use the mercury pool as a useful indicator electrode to considerably extend the sensitivity of amperometric titration procedures.

EXPERIMENTAL

The titration cell used in this work is illustrated in Figure 1. The mercury pool electrode at the bottom of the vessel was prepared before each titration by the addition of about 2 ml. of fresh mercury. The salt bridge of a saturated calomel electrode, a coarse gas dispersion frit, and the tip of a buret entered the cell through small ground-glass joints as shown. Oxygen was removed from the solutions by bubbling high purity nitrogen through the solution for about 10 minutes before beginning a titration. Burets of 1- and 5-ml. capacity were used to dispense the standard solution of disodium ethylenediamine tetraacetate. Because of the high sensitivity of the mercury pool electrode it was found necessary to remove oxygen from the titrant when solutions of $10^{-6}M$ or less were being titrated. The solution of the titrating agent was therefore deaerated with nitrogen before use. The standard solution was about 100 times more concentrated than the solution being titrated.

A Fisher Elecdropode was used as a source of the polarizing voltage and the diffusion currents were calculated by measuring the IR drop across a standard resistance with a potentiometer (galvanometer sensitivity 0.5 μv. per mm.). Because large current fluctuations were encountered, a damping capacitor of 10,000 mfd. was connected across the standard resistance.

Samples of the metal ions were diluted from $0.1M$ stock solutions which had been standardized by conventional procedures. The stock solution of disodium ethylenediamine tetraacetate was titrated against dried calcium carbonate using chemical indicators.

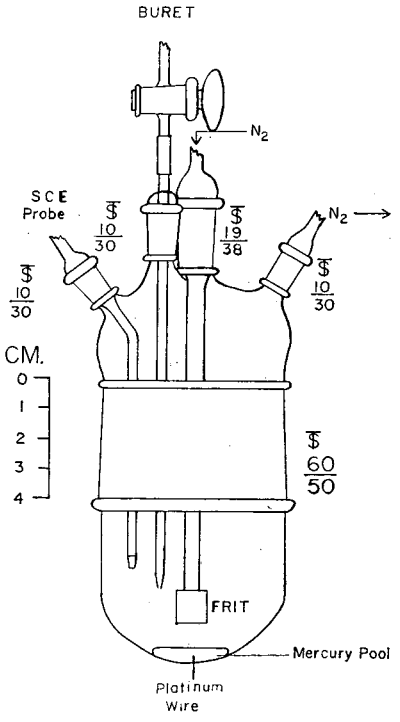

Figure 1. Amperometric titration cell

In order to obtain greater sensitivity, the solution being titrated was agitated to decrease the thickness of the diffusion layer. As the reproducibility of the agitation from one run to another is unimportant in amperometric titrations, stirring was simply accomplished by forcing nitrogen through the gas dispersion frit during the titration. The maximum flow of nitrogen which could be used was limited by the formation of spray which caused a loss of some of the solution being titrated. It was found that a convenient rate of flow was one that would give approximately a fourfold increase in the diffusion current above that obtained in a quiet solution.

To check the feasibility of the proposed method, polarograms of a cadmium solution at various stages of titration were obtained using the apparatus described above. These polarograms, reproduced in Figure 2, show that the uncomplexed cadmium ion is reduced at a substantially more positive applied potential than the ethylenediamine tetraacetate complex. Therefore an amperometric titration seemed to be possible.

Because of the nature of an amperometric titration, the exact size of the mercury pool indicator electrode is unimportant. However, certain experimental limitations must be considered in choosing the approximate area of the cathode. If the area is too large, an appreciable portion of the sample would be removed from the solution in the time necessary to carry out the titration. On the other hand, the electrode should be large enough to produce diffusion currents which can be conveniently measured. It was found that the diffusion currents were more easily measured when the potential was applied continuously, rather than intermittently between additions of reagent.

To ascertain the rate of loss of an ion by deposition, 100 ml. of a $10^{-6}M$ cadmium solution were electrolyzed using a 3.0-sq. cm. electrode. The solution was agitated with nitrogen as previously described. The rate of decrease in diffusion current was found to be 0.1% per minute. Under these conditions the error involved in a titration which requires 10 minutes would amount to 0.5%. This assumes a uniform rate of addition of reagent, so that the average concentration during the titration is one half the initial concentration. For this reason, a smaller electrode, 1 to 2 sq. cm., was used. The diffusion currents obtained with an electrode of this size were of a reasonable magnitude for measurement with simple equipment. If the volume of the solution to be titrated was smaller than 100 ml. as described above, the rate of depletion of the sample would be proportionally higher. Thus, a smaller electrode would be necessary as well as a more sensitive current-measuring device.

Table I. Summary of Amperometric Determinations with Mercury Pool Electrode

Metal Ion	Cadmium			Lead	Nickel
Concentration, M	1.22×10^{-5}	2.31×10^{-6}	2.31×10^{-7}	2.5×10^{-6}	8.51×10^{-6}
Amount present, γ	137.0	26.0	2.60	51.8	49.9
Amount found, γ	139.1	25.2	2.62	53.6	47.5
	137.6	25.9	2.67	53.3	49.4
	137.7	25.9	2.59	49.7	51.2
	137.7	26.2	2.55	49.3	
Mean	138.0	25.8	2.61	51.5	49.4
Average deviation from mean, %	±0.4	±1.1	±1.2	±3.9	±2.4
Average error, %	+0.7	−0.8	+0.4	−0.6	−1.0

Metal Ion	Copper			Zinc	
Concentration, M	5.70×10^{-5}	5.70×10^{-6}	5.70×10^{-7}	1.00×10^{-5}	2×10^{-6}
Amount present, γ	362	36.2	3.62	65.4	13.08
Amount found, γ	367	36.8	3.65	66.0	12.94
	365	36.2	3.70	66.8	12.94
	356	35.6	3.61	65.1	13.08
	...	36.1	3.59	63.2	13.35
Mean	363	36.1	3.64	65.3	13.08
Average deviation from mean, %	±1.0	±0.8	±1.1	±1.7	±1.0
Average error, %	+0.3	−0.3	+0.5	−0.2	0.0

Supporting electrolytes were chosen, so that the half-wave potentials of the ions being titrated would be as positive as possible. This was done because the pool electrode is more sensitive to the discharge of hydrogen than the dropping mercury electrode and the useful voltage range is decreased (*4*). A 0.1 or 0.01*M* solution of potassium nitrate was used for the titration of copper, cadmium, lead, and zinc. If necessary, the pH of the solution was adjusted to a value between 6 and 9 with nitric acid or sodium hydroxide. For the titration of nickelous ion a buffer of 0.1*M* ammonium chloride–0.1*M* ammonium hydroxide was found to be satisfactory.

PROCEDURE

Fifty to 100 ml. of the solution to be analyzed were placed in the titration cell and deaerated for 5 minutes. A quantity of mercury sufficient to form an electrode of 1 to 2 sq. cm. was then added and deaeration was continued for an additional 5 to 10 minutes. The desired potential was applied, and the rate of flow of nitrogen was adjusted so that the diffusion current did not fluctuate as a result of nonuniform stirring. The standard solution of disodium ethylenediamine tetraacetate was added in appropriate increments and the diffusion currents were measured. It was generally necessary to wait about 1 minute for the diffusion current to reach an equilibrium value. This was probably caused either by the high capacity condensers in the circuit or a slow rate of complex formation.

RESULTS

Titration curves for two different levels of concentration are shown in Figure 3. In the lower titration curve the solution is 100 times more concentrated than in the upper curve, but the corresponding diffusion currents are relatively higher because of a larger electrode and more vigorous agitation. The results of the proposed method applied to various ions are summarized in Table I. The average error of all determinations was 1.5%, with some variation with concentration and the particular ion being titrated.

The cadmium and copper ion titrations were the most satisfactory and no difficulty was experienced in determining concentrations in the range of $10^{-7}M$. Lead, nickel, and zinc were chosen as examples which would be expected to be more troublesome because of the difficulties experienced with these ions in mercury pool polarography (*4*). Lead ion in a noncomplexing supporting electrolyte such as potassium nitrate gave results which were less reproducible than other ions studied (*4*). This

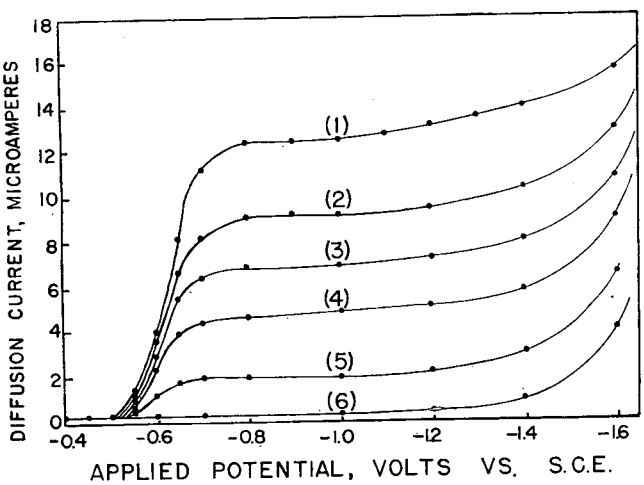

Figure 2. Polarograms of cadmium (II) at various stages of titration

Amounts titrated:

1. 0%
2. 20%
3. 40%
4. 60%
5. 80%
6. 100%

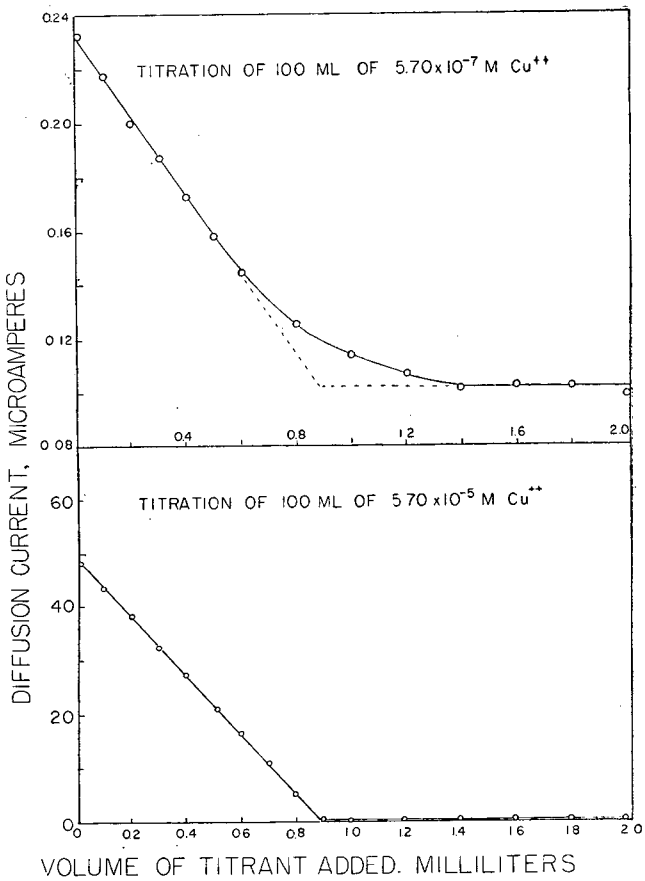

Figure 3. Titration curves of copper (II) solutions at two different levels of concentration

was also found to be the case with the amperometric titrations, as evidenced by the fact that the precision of titrations of $5 \times 10^{-7}M$ solutions was about 10%. Nickel and zinc were also difficult to determine with the mercury pool electrode because the half-wave potentials of both of these ions were close to the hydrogen discharge wave (*4*). Extrapolation of the residual current base line was uncertain which made quantitative polarography difficult. However, it was found that these ions could be titrated amperometrically, but titrations at dilutions greater than $10^{-6}M$ are not recommended because of the higher background current due to the decomposition of the supporting electrolyte. The method is undoubtedly not limited to the above ions, which were chosen merely to illustrate the principle involved in applying the mercury pool electrode to amperometric titrations.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of this research by the United States Air Force, through the Office of Scientific Research of the Air Research and Development Command.

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RECEIVED for review June 22, 1955. Accepted October 24, 1955.

Apparatus for Molecular Filtration

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A new type of molecular filter consists of a long tubular cellulosic membrane surrounded throughout its entire length by a flexible braid. This filter element is coiled and encased in a nylon bag. Closures are made at the entrant and the exit ends of the tube. The main advantage of this design is the high filtration rate, up to 500 ml. per hour at 100-pound pressure for the laboratory sizes. It may be operated continuously.

MOLECULAR filtration is mainly a sieving operation whereby large molecules are separated from smaller ones. For this purpose membranes with pores of molecular dimensions and larger are used.

Although this process is old (*1–3*), its application to filtration of large volumes of solutions is not common. This is due to a combination of such factors as complexity of technique, low rate of throughput, and apparatus cost.

This article describes the construction and operation of a laboratory tubular filter which does not suffer from the above limitations. The filter element is prepared in the form of an easily replaceable cartridge, capable of being reactivated when blocked and sterilized for aseptic operation, and so designed that filtrate and residue may be continuously or intermittently removed.

The problems of producing a molecular filter are primarily those of attaining a high rate of throughput while maintaining a low holdup volume. As the filtration rate is proportional to the pressure drop across the filter element and inversely proportional to its thickness, it is desirable to work with the thinnest membranes at the highest pressures possible. Therefore, adequate support of and an adequate closure with the delicate membranous element must be achieved. These requirements suggest that a flexible tubular filter element be used. With such an element, the solution is forced into the tube at one end, while residue is continuously or intermittently removed at the other end. The ultrafiltrate passes through the walls of the filter tube.

The tubular elements have been found to be most satisfactory not only in laboratory and plant practice but also from the standpoint of apparatus design. The advantages of this type of filter element are several. In the first place, it is easier to make a leak-tight closure on a tube than on a sheet of filtration membrane, because with a filter press, closure pressure must be applied at the perimeter of the sheet. As the number of sheets and their area increase, the chance for leakage increases. With a tube, however, closure pressure is applied only at the two ends and hence the chance of leakage is determined by the diameter of the tube, but not by its area. Thus by making the tubes long enough, any desired filtering area may be secured without increasing the difficulty of closure. Secondly, because a tubular membrane may be supported by a light-gage cloth, plastic, or metallic braid woven about its periphery, there is no need for heavy supporting elements. Indeed, using cloth supports, the filter element and its braided support become so light and cheap that the entire assembly may be discarded upon failure. Thirdly, the use of a flexible tube supported by a flexible support allows the whole element to be assembled into a volume equal to but preferably less than the expanded volume of the tube. By making the final volume small, the hold up volume may be kept to any reasonable value which still allows the passage of solution throughout the entire tube length. Finally, a tubular filter permits either intermittent or continuous withdrawal of residue. This advantage is probably not important for laboratory filtrations but is a very nice feature when solutions are to be concentrated or when high molecular weight fractions are to be thoroughly purified from low molecular weight fractions by passage through filter units in series.

The unit designed for laboratory operation is sketched in Figure 1. (The apparatus described here may be purchased from the Manostat Corp., 20–26 North Moore St., New York 13, N. Y. Patent applications have been made covering the novel features of the device.) In this sketch the right half is presented in cross section. To avoid metal pickup by the solutions being processed, the unit is made of stainless steel, borosilicate glass, and natural saran parts. It consists of a stainless steel filter head, *C*, to which the filter cartridge is attached. The glass filter cell, *L*, is clamped to the head with an anodized aluminum clamping ring, *K*. On the upper surface of the lip of the cell a pure gum rubber gasket, *D*, is inserted to make a vacuum and bacterial seal. To prevent chipping or cracking of the glass cell by the clamping ring, an aluminum slip ring, *F*, and a rubber O ring, *E*, are used on the underside of the lip.

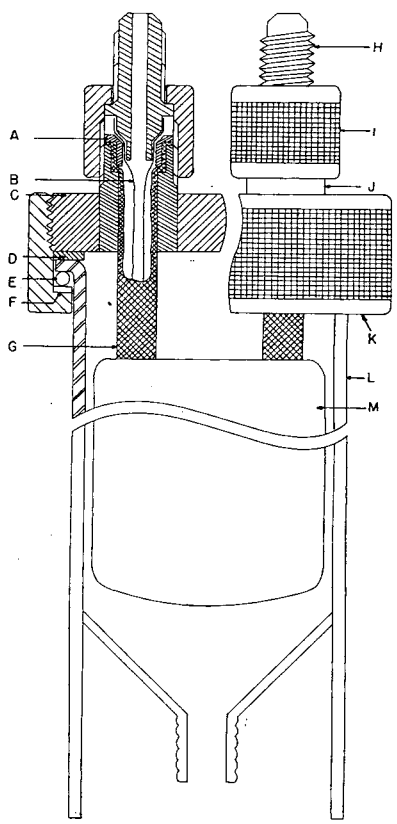

Figure 1. Filtration cell with cartridge installed

The ends of the tubular filter cartridge are connected to the head at the two threaded posts, *J*, one end going to each post. It will be seen from an inspection of the cross-sectional detail that the supporting braid, *G*, is separated from the membrane, *B*, and held securely by pressure of a small collar, *A*, against the top of the threaded post, *J*. The flared connector, *H*, pressed

down on the collar by a knurled nut, *I*, makes a leak-tight contact with the membrane at the tapered section.

The filter cartridge is composed of a calibrated tubular membrane, from 10 to 15 feet long with a 0.25-inch internal diameter, supported throughout its entire length by cotton braid. Except for the ends, most of the braided membrane is coiled into a small bundle and encased in a snugly fitting nylon bag, *M*. After assembly the entire unit may be autoclaved for aseptic operation.

The solution to be filtered is stored in a stainless reservoir fitted at the bottom with a valve and at the top with 0.25-inch flare fittings. For convenience of piping, all fittings were standardized with 0.25-inch flare fittings. This has the advantage that when saran tubing and saran coupling nuts are used, all piping operations can be made by hand, without any need for hand tools. This plastic will also stand repeated autoclaving, provided that it is not kept under high internal pressure.

The loading of the filter is effected by connecting the reservoir to one of the flared connectors. To the other connector, a valve is connected to provide easy venting of air or residues. In operation, a small pressure is applied to the surface of the liquid in the tank. With both valves open, liquid is allowed to flow from the tank through the tubular filter until it emerges at the exit valve. When this loading operation is completed, the exit valve is closed and the pressure on the surface of the liquid is brought up to about 100 pounds per square inch for the duration of the filtration.

With intermittent withdrawal, this unit has been operated with cartridges having filtration speeds up to a 0.5 liter per hour and with a holdup volume of as little as 150 ml. The membrane in this instance had a pore volume of 50% and an average pore diameter of 5 mμ. Smaller cartridges with lesser speeds and holdup volumes were made, as well as cartridges for larger units operating in the gallons per hour range. These cartridges may be prepared in advance and stored for protracted periods prior to use.

During operation, the filtration rate diminishes as the pores of the membrane become plugged or matted over with precipitate. Reactivation is effected by washing through the tubular element with water. The reactivated cartridge does not always recover its initial rate and may plug more rapidly with successive reactivations. Ultimately, it must be discarded because its flow rate has been drastically reduced or because of mechanical failure.

The unit described above has been applied satisfactorily to a large number of laboratory operations. Larger units constructed along similar lines and operating with rates of gallons per hour have also been experimented with. The problems for which the filter has been particularly successful are:

The quantitative removal of proteins from many kinds of vegetable and animal extracts, beers, etc.

The removal of bacterial pyrogens from solutions intended for parenteral and intravenous use. In this application the filter retains the pyrogens by a sieving action. Consequently no break through occurs, as it does when filter pads are used.

The preliminary concentration of dilute solutions of proteins, hormones, viruses, etc. The use of large quantities of solvents and electrolytes is avoided by substituting filtration in the first step.

The direct removal of many hydrophobic species from aqueous solutions including colloidal micelles and constituents solubilized therein.

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RECEIVED for review May 17, 1955. Accepted October 22, 1955.

Electrometric Titration of Compounds Containing Positive Chlorine or Bromine

With Sulfur Dioxide in Pyridine

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In a new application of the basic Karl Fischer reaction sulfur dioxide in pyridine-methanol solution is used to titrate liberated iodine or bromine quantitatively. Either a visual or electrometric end point is employed. The reagent is stable and is standardized by conventional iodometric methods. The method is applied specifically to the titration of *n*-chloro or *n*-bromo compounds. However, it promises to be applicable to a wide range of compounds in both aqueous and nonaqueous solution.

SULFUR dioxide in solution reduces iodine (*1*, *2*) and bromine (*3*). In the Karl Fischer reaction (*4*), sulfur dioxide reacts with iodine in pyridine-methanol solution with the consumption of water. The over-all reaction is expressed as follows:

$$SO_2 \cdot C_5H_5N + I_2 \cdot C_5H_5N + C_5H_5N + H_2O + CH_3OH \rightarrow C_5H_5N(H)SO_4CH_3 + 2C_5H_5N \cdot HI$$

Excess pyridine, iodine, and methanol are required for quantitative consumption of water.

In the present investigation it was found that, in the presence of excess water, sulfur dioxide reduces iodine or bromine quantitatively resulting in a colorless solution. Very sharp end points are obtained visually or electrometrically.

Iodine or bromine liberated from *N*-halogeno compounds by the addition of potassium iodide or bromide and acid is titrated with sulfur dioxide in pyridine-methanol solution. This is done either in aqueous or alcoholic solution (containing a twofold or larger excess of water).

The sulfur dioxide solution is reasonably stable having been stored for several months with little change in titer (Table II). Standardization is similar to that of potassium thiosulfate. Iodometric titration using standard potassium dichromate and employing an electrometric end point is effective for this purpose.

APPARATUS

The circuit is of a conventional type used for dead-stop titrations. The unusually high applied voltage (ca., 200 mv.) makes it possible to use a rugged inexpensive meter. The residual current which flows after all iodine or bromine has been reduced is too small to be of consequence. The platinum electrodes are silver-soldered to copper leads, and then cast in a glass tube (Figure 1) using a cold-setting epoxy resin. Araldite Resin CN 502 (Ciba) is very satisfactory for this purpose. The microammeter is mounted in a meter case and wired internally (Figure 1). A switch or disconnecting jack, *S*, is placed in the circuit to prevent drain on the battery when the apparatus is not in use. The size of the platinum wire and distance between the electrodes are not critical. The protruding electrodes are bent at right angles to prevent interference with the stirring bar. If a full scale reading is not obtained on shorting the electrodes, either of the resist-

ances is trimmed with parallel resistors of high value in order to obtain the proper potentiometric balance.

REAGENTS

Potassium dichromate, 0.1*N*, prepared as a primary standard from C.P. crystals dried at 110° C.

Table I. Titration of *N*-Halogeno Compounds with Sulfur Dioxide Reagent

Compound	Solvent	% Available Chlorine or Bromine: Theoretical	% Available Chlorine or Bromine: Found
N-Chlorosuccinimide[a]	Methanol	53.2	52.9
N-Bromosuccinimide[b]	Chloroform and methanol	89.9	89.6
N-Chloroacetamide[c]	Methanol	75.8	74.8
Chloramine T[d]	Water	25.2	25.0
Dichlorodimethylhydantoin[e]	Methanol	71.5	72.0

[a] Recrystallized from carbon tetrachloride (5), m.p. 149.5–150° (reported 150°).
[b] Reagent grade.
[c] Recrystallized from chloroform, m.p. 110° (reported 109°, 110°, and 111°).
[d] Reagent grade.
[e] Commercial grade, Wyandotte, recrystallized from benzene, m.p. 132° (reported 133°).

Table II. Effect of Atmospheric Moisture on Stability of Sulfur Dioxide in Pyridine-Methanol Solution

Solution	Time, Days	Normality
No. 1 (exposed to air)	0	0.538
	3	0.502
	44	0.407
	66	0.339
No. 2 (protected by Drierite)	0	0.305
	13	0.303
	36	0.287

Table III. Estimate of Precision Obtained from Duplicate Analyses[a]

Compound		% Available Chlorine	$\lvert d \rvert$ ($\times 10^4$)	$\lvert d \rvert^2$ ($\times 10^8$)
N-Chloroacetamide		74.07	25	625
		74.12	25	625
	Av.	74.095		
N-Chlorosuccinimide		50.66	750	562,500
		50.51	750	562,500
	Av.	50.585		
Chloramine T		25.20	1250	1,562,500
		24.95	1250	1,562,500
	Av.	25.075		4,251,300

Standard deviation (S.D.) $= \sqrt{\frac{d^2}{D.F.}} = \sqrt{\frac{0.04251}{3}} = 0.12\%$

[a] The batch of compound selected for each duplicate set was not necessarily pure.

Sulfur dioxide solution, prepared by diluting Karl Fischer reagent B (Matheson, Coleman and Bell) with 9 volumes of absolute methanol. This reagent can also be prepared by the addition of liquid sulfur dioxide from an inverted cylinder to well stirred pyridine kept at 0° to 10°. The solution should be 3 to 5*N* in sulfur dioxide. It is diluted to ca. 0.5*N* with methanol.

Methanol, absolute, reagent grade.

Pyridine, reagent grade.

Potassium iodide, 15% aqueous solution.

Acetic acid, glacial.

PROCEDURE

About 3 meq. of sample are dissolved in 50 ml. of methanol. Water-soluble salts such as chloramine-T (sodium *p*-chlorotoluenesulfonamide) are dissolved in 50 ml. of water instead. Compounds which are not very soluble in methanol are stirred thoroughly with 20 ml. of chloroform and 50 ml. of methanol are added.

The dissolved sample is treated with 10 ml. of potassium iodide solution and 5 ml. of acetic acid. The mixture is titrated to a colorless end point or to a microammeter reading of 0 to 5 μa.

The initial reading of the instrument in the presence of free iodine is high or full scale. It will start to drop rapidly about 3 to 4 drops before the end point. The end point is reached when a single drop causes no further decrease in current.

The electrometric end point is used for standardization, because the green color of chromium(III) interferes with the visual end point. A 25.00-ml. portion of standard 0.1*N* potassium dichromate is treated with 25 ml. of water, 10 ml. of potassium iodide solution, and 5 ml. of concentrated hydrochloric acid in a 125-ml. Erlenmeyer flask. The mixture is titrated with sulfur dioxide as described above.

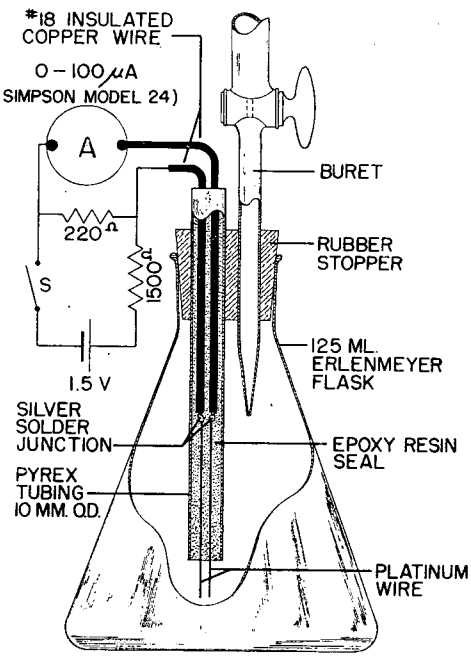

Figure 1. Electrometric titration apparatus

The results of titration of known compounds are presented in Table I. The amount of positive halogen present is expressed as "available chlorine" (6) or in an analogous manner as "available bromine."

METHOD OF CALCULATION

The normality of the sulfur dioxide solution is calculated by the following expression:

$$\frac{25.00 \text{ (normality of dichromate)}}{\text{Volume of sulfur dioxide, ml.}}$$

The theoretical per cent of available chlorine or bromine is obtained from:

$$\%\ X = \frac{2\,[\text{atoms} \times (\text{valence} + 1)\ \text{per molecule}]\ (\text{at. wt. } X)\ 100}{\text{mol. wt. of compound}}$$

For example, for an *N,N'*-dichloroimide, molecular weight 200:

$$\%\ \text{available chlorine} = \frac{2\ (2)\ (35.5)\ (100)}{200} = 71.0$$

The experimental per cent of available chlorine or bromine is obtained from the following equation:

$$\% X = \frac{\text{Ml. } SO_2 \text{ (N.}SO_2\text{) (at. wt. } X\text{) (0.1)}}{\text{wt. of sample, grams}}$$

DISCUSSION

The *N*-halogeno compounds chosen to demonstrate the method are readily available or easily prepared and purified. The method has been shown to be accurate by close agreement between experimental and theoretical values for "available chlorine" (or "available bromine"). The precision of the method was estimated from data obtained by duplicate analysis of three *N*-chloro compounds. A standard deviation of 0.12% was obtained from the data presented in Table III.

Sulfur dioxide titration has several advantages over thiosulfate iodometry. Its use in nonaqueous media is advantageous for water-insoluble compounds. Another advantage is the flexibility inherent in the permissible range of concentrations. The reagent can vary in strength from 5*N* downward, depending on the scale of the titration and size of the apparatus. Sulfur dioxide is preferred to thiosulfate for use in the direct titration of bromine. Liberated or excess bromine is titrated using the electrometric end point..

ACKNOWLEDGMENT

The author wishes to express his thanks to Seymour Schmukler of the Colgate Research and Development Department for the preparation and purification of *N*-chloroacetamide. The recrystallization of dichlorodimethylhydantoin by Meyer Malakoff of these laboratories is also appreciated.

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RECEIVED for review March 31, 1955. Accepted September 21, 1955.

Paper Chromatography of Nitrophenylhydrazones of Mono- and Dicarbonyl Compounds

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A solvent system of dibutyl ether–dimethylformamide–tetrahydrofuran is used for paper chromatographic separation of 2,4-dinitrophenylhydrazones and *p*-nitrophenylhydrazones of mono- and dicarbonyl compounds.

VARIOUS workers have described the separation of 2,4-dinitrophenylhydrazones by column chromatography on a variety of adsorbants (*1*, *6*, *9–12*). Only recently has this been extended to include the insoluble dicarbonyl derivatives (*4*).

The use of strips of a mixture of silica gel and poly(vinyl alcohol) deposited on glass has been reported for the separation of the 2,4-dinitrophenylhydrazones of aliphatic aldehydes. The R_f values in this system are very sensitive to moisture content (*7*).

The paper chromatography of monocarbonyl-2,4-dinitrophenylhydrazones has been reported (*5*, *8*). It is further claimed that treated paper will give better separation of R_f values (*2*).

The only method that is applicable to both mono- and dicarbonyl compounds requires acetylated filter paper (*3*). Therefore, a solvent system was developed that separates the nitrophenylhydrazones of mono- and dicarbonyl compounds on ordinary filter paper. This system is the only one of 78 systems tried that gave favorable results in separating both types of compounds. Table I lists the compounds tested with their R_f values and other means of identification.

Table I. Separation Data for Nitrophenylhydrazones

Compound	R_f	U.V. Light	NaOEt Spray
2,4-Dinitrophenylhydrazine	0.32	Absorbs	Brown
2,4-Dinitrophenylhydrazones			
Diphenyl-2,2′-dialdehyde (bis)	0.00	Absorbs	Violet
Methyl glyoxal (bis)[a]	0.20	Absorbs	Violet
Glyoxal (bis)	0.23	Absorbs	Violet
Formaldehyde	0.60	Absorbs	Brown
Acetaldehyde[a]	0.67	Absorbs	Brown
Furfuraldehyde	0.70	Absorbs	Red-brown
Benzaldehyde	0.81	Absorbs	Red-brown
Crotonaldehyde[a]	0.90	Absorbs	Brown
Propionaldehyde	0.91	Absorbs	Brown
p-Nitrophenylhydrazones			
Crotonaldehyde	0.08	Absorbs	Red-brown
Glyoxal (bis)[a]	0.24	Orange	Indigo blue
Methyl glyoxal (bis)	0.30	Orange	Indigo blue
Biphenyl-2,2′-dialdehyde (bis)	0.49	Yellow	Purple
Acetaldehyde[a]	0.54	Absorbs	Brown, then rose
Furfuraldehyde	0.55	Orange	Purple
Benzaldehyde	0.72	Golden	Purple

[a] In mixtures, the boundaries of this spot overlap the boundaries of the spot with the next highest R_f value.

EXPERIMENTAL

Materials. The solvents were Kodak White Label products. All were carefully dried by the usual methods; failure to do so resulted in lower R_f values. Whatman No. 1 paper was used.

Method. The solvent system was dibutyl ether–dimethylformamide–tetrahydrofuran in the ratio of 85 to 15 to 4. The nitrophenylhydrazones were dissolved in tetrahydrofuran and applied to the paper in amounts of 2 to 5 γ. The capillary ascent method was used and each run was continued about 12 hours. Location of the spots was aided by the use of ultraviolet light and a spray of sodium ethoxide solution, which was prepared by dissolving 0.3 gram of sodium in 100 ml. of ethyl alcohol. Both the sodium ethoxide solution and the chromatographic solvent system had to be freshly prepared for reproducible results.

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RECEIVED for review June 24, 1955. Accepted November 16, 1955. Abstracted from a portion of a thesis submitted by William J. Schmitt, S.J., to the Graduate School of Fordham University in partial fulfillment of the requirements for the degree of doctor of philosophy.

Determination of Silicones in Textile Materials

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An estimation of the amount of silicone in textile materials is required for the control of this process for the durable waterproofing of textile materials. In a method which requires no unusual or specialized equipment, the textile material, treated with silicone, is wet-ashed, using concentrated sulfuric and nitric acids and, eventually, concentrated perchloric acid. The silica is determined by conventional methods. Attempts to extract cured silicones from textile materials by solvents gave unsatisfactory results.

SILICONE oils, also known as low molecular weight polysiloxanes, are extensively used in processing textile materials in order to impart a water-repellent finish which is resistant both to washing and to dry cleaning. The silicone oil is dispersed in water with an emulsifying agent or dissolved in a suitable solvent, and is mixed with a catalyst which promotes polymerization. The catalyst is usually an organometallic compound. It is then applied to the textile material in equipment which is found in most textile mills. The silicone oil thus deposited, is then cured (polymerized) by heating the impregnated textile material briefly at a high temperature. For instance, heating at 160° C. for 5 to 8 minutes is recommended for silicone oils on cotton (*1*).

In order to develop a water-repellent finish which has good durability to laundering and to dry cleaning, it is usually necessary to have 0.75 to 1.25% of silicone present in the fabric, depending on the type of fiber and the construction of the fabric. Accordingly, the analysis of fabrics treated with silicone assumes considerable importance in connection with this specified silicone content.

The silicone oils which have been most extensively used for this purpose are methyl hydrogen silicone, $\left(\begin{array}{l} H \\ | \\ -Si-O- \\ | \\ CH_3 \end{array}\right)_n$, and dimethylsilicone, $\left(\begin{array}{l} CH_3 \\ | \\ -Si-O- \\ | \\ CH_3 \end{array}\right)_n$, where the siloxane unit is repeated several times per molecule (n varies from about 12 to about 70; $n = 20$ may be considered representative).

McHard, Servais, and Clark (*2*) have proposed colorimetric, volumetric, and gravimetric methods for determining the silicon content of organosilicon compounds. In each case, the organosilicon compound is converted by peroxide bomb or by wet oxidation with various acids to sodium silicate or to silicic acid. After the destruction of the organosilicon molecule, further treatment depends on the method selected for the final determination of silicon.

QUALITATIVE TEST FOR SILICONES

When silicone oils, alone or in mixtures, are burned in a crucible, the smoke contains white flakes of silica, and a white collar of silica forms inside the crucible at the rim. This test holds for silicone oils in textile materials, prior to the heat treatment during which the silicone oils are polymerized. However, after polymerization this qualitative test usually fails.

SOLVENT EXTRACTION OF SILICONES

Before the silicone has been cured, solvent extraction with petroleum ether (boiling point, 30° to 60° C.), isopropyl alcohol (about 99.8%), benzene, or solvent naphtha usually recovered 95% or more of the silicone. After curing, prolonged extraction with benzene or solvent naphtha in a Soxhlet apparatus gave nonreproducible recoveries which were generally in the range of 30 to 60%.

QUANTITATIVE DETERMINATION OF SILICONES

Reagents. Sulfuric acid, 95%, reagent grade.
Nitric acid, 70%, reagent grade.
Perchloric acid, 60%, reagent grade.
Hydrofluoric acid, 48%, reagent grade.
Hydrochloric acid, 37%, reagent grade.

Apparatus. Borosilicate glass beakers, watch glasses, and funnels, and platinum crucibles were used throughout.

Sampling. Sampling in a textile finishing plant is usually limited to the ends of runs. As samples so taken are less representative of the entire run than samples taken at regular intervals through the run, larger samples are needed for analysis than in the latter case. As the peroxide bomb procedure is limited to samples generally less than 1 gram, the gravimetric procedure described below is preferred.

Procedure. For the quantitative determination of silicones in textile fabrics, weigh 5 to 10 grams of the material to 0.01 gram. Place the sample in a 600-ml. borosilicate glass beaker which is covered with a borosilicate watch glass, add 30 ml. of concentrated sulfuric acid, and, in small portions, add about 35 ml. of concentrated nitric acid. Heat moderately ("medium" heat on an electric heater) for 20 minutes to 2 hours. Use a medicine dropper to add 1 to 5 ml. of concentrated perchloric acid, and heat the sample vigorously. The earlier addition of perchloric acid may be hazardous. If the liquid remains opaque, cool it slightly, add 5 to 10 ml. of concentrated nitric acid and, after further moderate heating, add 1 to 5 ml. of concentrated perchloric acid, followed by vigorous heating. Continue this cycle until the liquid is clear. It is usually water-white or only slightly tinted. Heat the sample vigorously until the volume of liquid is reduced to about 20 to 25 ml. Cool to room temperature, dilute cautiously with 100 to 150 ml. of distilled water, and add 1 to 2 ml. of concentrated hydrochloric acid. Boil and filter the solution while hot, on a Whatman No. 40 filter paper or its equivalent. Police the beaker vigorously, as the silica is a very adherent precipitate. Wash the precipitate several times with hot water, and then ash in a platinum crucible. Weigh the crucible and contents to 0.0001 gram. Add a few drops of concentrated sulfuric acid and 2 to 15 ml. hydrofluoric acid, and volatilize the acids, together with the silica, at a temperature low enough so that there is no spattering. Heat the crucible strongly, cool, and weigh again. The difference in the two weights is silica (SiO_2). $SiO_2 \times 1.00$ = methyl hydrogen silicone; $SiO_2 \times 1.23$ = dimethyl silicone.

ANALYTICAL RESULTS

Some strips of acetate-viscose fabric, approximately 9 × 60 inches, which weighed about 65 grams, were treated with methyl hydrogen silicone emulsions of various concentrations on a Butterworth laboratory padder. Two sets of analyses were made, one on samples taken from the middle of each strip, the other on samples taken from the trailing end of the strip. The results were:

Number	Middle Sample $(MeHSiO)_n$, %	End Sample $(MeHSiO)_n$, %	Decrease, %
B264-9	0.42	0.34	19
B264-10	0.65	0.53	18
B264-11	1.00	0.81	19
B264-12	1.66	1.34	19

Only insignificant differences have been found in samples taken from mill runs. In small scale laboratory runs, however, in order to obtain significant results, samples for analysis must be taken from the same position on each piece of fabric.

DISCUSSION

Analyses made by this method agreed within 0.02% with those made in another laboratory by a colorimetric method in which the sodium silicate from the peroxide bomb is made to react with ammonium molybdate, and the intensity of the resulting blue color is measured at 715 mμ. The agreement was very poor with analyses made in still another laboratory by dry oxidation in crucibles, as would be expected from the partial volatility of silicone oils at high temperatures. The average difference between duplicate samples is 0.007%.

Silica is a normal constituent of soil. If the textile fabric is not clean, or if silicon-containing compositions such as clay, talc, or colloidal silica have been used in processing, all the silicon present will be reported as silicone when determined by this method. The usual silica content of textile materials finished without the use of silicon-containing baths, and subsequently protected from soil, is 0.00 to 0.01%; occasionally amounts up to 0.03% are found. The silicon content of textile materials which have been processed with silicon-containing compositions will be much higher than where silicon compounds are accidentally present. In any case, it is always well to run an analysis for silica on a sample of the fabric before it has been treated with a silicone water-repellent, if such untreated material is available.

ACKNOWLEDGMENT

The author wishes to express his appreciation for the substantial encouragement and advice given by Alton A. Cook, technical director of The Arkansas Co., Inc.

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RECEIVED for review April 8, 1955. Accepted November 5, 1955. Presented in part before the Analytical Group, North Jersey Section, ACS, Meeting-in-Miniature, January 1955, Newark, N. J.

Titrimetric Determination of Zirconium in Magnesium Alloys

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The addition of zirconium to magnesium alloys intended for use at high temperatures requires rapid methods for determining zirconium in such alloys. The titrimetric measurement of zirconium by standard cupferron solution, employing amperometric detection of the equivalence point, has been applied to the direct determination of zirconium in commercial magnesium alloys following acid dissolution. The method is rapid, simple, and accurate, and should supplement the colorimetric alizarin S and gravimetric *p*-halomandelic acid procedures.

THE need for accurate and rapid methods for the determination of small amounts of zirconium in magnesium and magnesium alloys was cited by Wengert (*5*) in describing the colorimetric determination of zirconium in magnesium alloys by the alizarin red S method, and Papucci and Klingenberg (*4*) in describing a similar gravimetric procedure using *p*-bromo- or *p*-chloromandelic acid. The addition of zirconium to such alloys improves the operating temperatures and grain structure without adversely affecting the machinability and creep resistance when used for high temperature purposes as in jet engines.

As zirconium can be rapidly and accurately determined gravimetrically and titrimetrically, employing cupferron as precipitant in 10% (*ca.* 2*M*) sulfuric acid solution (*3*), it seemed that the titrimetric method employing amperometric equivalence-point detection might be advantageous as a supplement to the colorimetric (*5*) and gravimetric (*4*) methods. The present study describes the application of a titrimetric zirconium procedure to two magnesium alloys which were being used in a cooperative evaluation of the colorimetric alizarin red S procedure.

Commercial magnesium alloys containing zirconium fall into two groups; one contains up to 6% of zinc and 0.8% zirconium; the other about 3% of rare earths and 0.4% of zirconium. The zirconium occurs in both acid-soluble and acid-insoluble forms; the amount of the latter is usually small in comparison with the acid-soluble zirconium, and in currently available alloys is usually kept to within 0.02 to 0.05%, since large percentages may be harmful. The dissolution procedures used in the present study are based on those proposed by Wengert (*5*), which separate acid-soluble and acid-insoluble zirconium by treating the sample with dilute (1 to 4) hydrochloric acid; the insoluble fraction is changed to a soluble form by fusion with potassium hydrogen sulfate. Essentially the same dissolution procedure was used by Papucci and Klingenberg (*4*).

EXPERIMENTAL

Reagent grade sulfuric acid (specific gravity 1.84) was diluted 1 to 10 by volume with distilled water. Cupferron was purified and stored as previously described (*3*); an approximately 0.01 to 0.02*M* standard solution was prepared daily by dissolving a carefully weighed portion in air-free water. Commercial water-pumped nitrogen was used for deoxygenating without further purification; other chemicals used were of reagent or C.P. grade.

Titration was performed in a 150-ml. beaker fitted with a four-hole rubber stopper to accommodate the electrodes, buret (5- or 10-ml. capacity, graduated to 0.02 or 0.05 ml.) and nitrogen inlet; the beaker lip served as gas outlet. A Sargent Model XXI polarograph or Fisher Electropode was used in conjunction with a dropping mercury electrode and a reference saturated calomel electrode (S.C.E.).

PROCEDURES

Dissolution (Soluble and Insoluble Zirconium). Weigh 3.0 grams (0.3% Zr) or 2.0 grams (0.6% Zr) of sample into a 400-ml. beaker. Cover with a watch-glass, cool in an ice bath, then add cautiously 150 ml. of cold (1 + 1) hydrochloric acid. After dissolution is complete as indicated by the cessation of gas bubbles, filter through No. 42 Whatman paper into a 250-ml. volumetric flask, wash the beaker and residue with hot water which is transferred to the flask via the filter paper, transfer the residue to the filter paper, and dilute the flask contents to volume; this solution contains the acid-soluble zirconium.

Place the filter paper and residue in a porcelain crucible, char slowly, and heat at 950° C. for 30 minutes. Add 1 gram of potassium hydrogen sulfate and fuse. Cool and dissolve the melt in a 150-ml. beaker with about 50 ml. of 10% sulfuric acid; this solution contains the acid-insoluble zirconium.

Dissolution (Total Zirconium). Weigh about 0.5 gram of sample into a 150-ml. beaker. Cover the beaker and dissolve the alloy at room temperature with the minimum amount of 10% sulfuric acid (about 10 ml.). Add 5 ml. of concentrated sulfuric acid and evaporate to the appearance of sulfur trioxide fumes. Cool, dilute to 50 ml. with distilled water, and proceed with the titration.

Amperometric Titration. Transfer a 50-ml. aliquot from the acid-soluble zirconium solution to a 150-ml. beaker or use all of the acid-insoluble or total zirconium solution. Cover the beaker with the four-hole stopper. Remove oxygen by flushing with nitrogen (about 10 minutes). Add standard cupferron solution in 1-ml. increments (if the titration is expected to consume less than 5 ml. of standard solution, 0.5-ml. increments are advisable) 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 between −0.84 and −1.00 volt *vs.* S.C.E. (no gelatin need be added to the sample solution for this potential range). Plot the points so obtained in the conventional manner; read the equivalence point volume from the intersection of the two straight lines; calculate per cent zirconium in each fraction by the following expression and report as soluble, insoluble, or total zirconium:

$$\% \text{ Zr} = \frac{V \times T \times D}{10 \times S}$$

where V equals milliliters of cupferron solution used; T is the cupferron solution titer—i.e., milligrams of zirconium equivalent to each milliliter of cupferron solution; D is the dilution factor—i.e., reciprocal of the fraction of the prepared solution taken for titration; and S is the number of grams of sample taken.

Table I. Determination of Zirconium in Magnesium Alloys, Employing Amperometric Titration with Cupferron

Sample			Hydrochloric Acid Dissolution					Sulfuric Acid Dissolution
No.	Insoluble[a] Zr, %	Soluble[a] Zr, %	Run No.	Insoluble Zr, %	Soluble[b] Zr, %	Soluble[c] Zr, %	Total[d] Zr, %	Total[e] Zr, %
ZK 60	0.05	0.59	1	0.034	0.564	0.574	0.608	
			2	0.025	0.571	0.580	0.605	
			3					0.606
EK 30	0.06	0.33	4	0.029	0.311	0.317	0.346	
			5	0.031	0.308	0.313	0.344	
			6					0.349

[a] Based on 1:4 hydrochloric acid dissolution for separation and colorimetric alizarin red S method for measurement.
[b] Dissolved in 1:1 hydrochloric acid and aliquot of cloudy solution titrated.
[c] Aliquot of solution mentioned in [b] evaporated with sulfuric acid to sulfur trioxide fumes and then diluted to 10% sulfuric acid before titration.
[d] Sum of insoluble and soluble Zr (first and third columns).
[e] Sulfuric acid dissolution of original sample.

RESULTS AND DISCUSSION

The basis for the amperometric titration of zirconium with cupferron in 10% sulfuric acid solution has been discussed (*3*); the following reaction is moderately rapid as well as essentially complete and stoichiometric.

$$Zr(SO_4)_3^{--} + 4\ Cup^- = ZrCup_4 + 3SO_4^{--}$$

The procedures described were applied to two representative magnesium alloys (Table I). Sample ZK 60 contained 6% of zinc and Sample EK 30, 3% of rare earths [these are the same as samples 67333 and 67365 in Wengert's Table II (*5*)]; the zirconium percentages, furnished by the sample supplier, were obtained by the alizarin red colorimetric method.

The hydrochloric acid (1+1) dissolution used to separate acid-soluble and -insoluble zirconium results in sufficient heat to warm the solution to near the boiling point; this is believed to be the cause of the somewhat discordant results for insoluble zirconium. The use of 1 to 4 hydrochloric acid results in rapid dissolution but some zirconium may hydrolyze and be found in the insoluble fraction; perhaps hydrochloric acid (1+2) would be a more suitable concentration for separating the two forms of zirconium (*2*).

The behavior of the alloys on treatment with sulfuric acid indicated that it might be possible to separate acid-soluble and -insoluble zirconium using dilute sulfuric acid—e.g., 1 to 5%—as dissolution agent. It has been the authors' experience that sulfuric acid and perchloric acid solutions of zirconium do not show the hydrolytic behavior observed in hydrochloric acid solution. In addition, the dissolution of magnesium alloy is a smoother process in sulfuric acid as compared with that in hydrochloric acid.

The final zirconium solution prepared by dilution to volume of the hydrochloric acid solution apparently is unstable in reference to precipitation of zirconium salts, since a haze appeared in the volumetric flask shortly after dilution. Titration with cupferron would probably not pick up all of the zirconium in such a suspension. The presence of minute amounts of zirconium in the suspensions was confirmed by taking an aliquot of each solution after shaking to render the suspension homogeneous, evaporating with 5 ml. of concentrated sulfuric acid to sulfur trioxide fumes, diluting with 50 ml. of water, and titrating.

As a further check, the total zirconium content was directly determined by sulfuric acid dissolution of single samples and titration (Table I). The agreement between the results obtained by the latter procedure and those based on the corrected sum of acid-soluble and -insoluble zirconium, when considered on the basis of the known precision of the cupferron procedure for zirconium, may mean that the present results are more accurate than those obtained by the colorimetric procedure as given for the sample analysis in Table I. The actual discrepancy is in the data for insoluble zirconium, where results by the present procedure differ from the given values by 0.02 or 0.03%.

Based on previous studies (*1*, *3*), iron(III), vanadium(V), niobium(V), uranium(IV), and large amounts of tin(IV) are the only commonly interfering metals in the amperometric titration; hafnium and titanium show behavior similar to zirconium (*1*). Iron is usually extremely low in magnesium alloys containing zirconium and will tend to concentrate in the acid-insoluble fraction, since iron and zirconium form an acid-insoluble intermetallic compound.

As compared with the colorimetric alizarin red S procedure, anions such as fluoride, phosphate, and sulfate do not interfere in the cupferron titration.

Obviously, the zirconium cupferrate precipitate could be filtered, washed, and ignited to the oxide for a gravimetric measurement if it were so desired.

ACKNOWLEDGMENT

The authors wish to thank M. F. Loucks of The Dow Chemical Co. for furnishing the analyzed magnesium samples and much helpful information concerning the determination of zirconium in magnesium alloys, and the Atomic Energy Commission for support of an investigation of the polarography of organic compounds of which the work described is an application.

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RECEIVED for review August 25, 1955. Accepted October 26, 1955.

Chromatographic Separation of Alcohols as Xanthates

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The separation of C_1 to C_5 alcohols as the potassium xanthates on cellulose columns is satisfactory for most of the alcohols. Identification of the separated xanthates was made by conversion to insoluble thallous salts having characteristic melting points. Recoveries of more than 95% of the xanthates could be obtained.

THE separation and identification of milligram quantities of the alcohols contained in fusel oil were required for an investigation, by radioactive tracers, of the conversion of leucine to isoamyl alcohol by fermentation. Although several chromatographic methods for alcohol separations have been reported, none have worked well for the separation of isoamyl alcohol.

Table I. Chromatography as Potassium Xanthates of Pairs of Alcohols

Excellent Separation	Good Separation	No Separation
Methyl-ethyl	Ethyl-propyl	Butyl-isobutyl
Methyl-*n*-propyl	Ethyl-isopropyl	Isobutyl-*sec*-butyl
Methyl-isopropyl	Isopropyl-isobutyl	Isobutyl-amyl
Propyl-isopropyl	Butyl-*sec*-butyl	*sec*-Butyl-amyl
Propyl-butyl	Butyl-amyl	
Propyl-isobutyl	Isobutyl-isoamyl	
Isopropyl-butyl	*sec*-Butyl-isoamyl	
Isopropyl-isobutyl		
Butyl-isoamyl		
Amyl-isoamyl		

Chromatography on a silicic acid column using carbon tetrachloride to elute the fractions did not give a good separation of *n*- from isoamyl alcohol and required the collection of an inconvenient number of fractions. Failure to obtain any separation of these alcohols by this method has been reported (*3*). Furthermore, isoamyl alcohol after passage through the silicic acid showed a carbonyl impurity, as ascertained by infrared spectrum, that was not in the original alcohol. The source of this impurity is unknown.

Chromatographing the 3,5-dinitrobenzoates of the alcohols on a silicic acid–Celite column (*7*) was also tried, again without good separation of the isoamyl ester.

Since paper chromatography of alcohols as the potassium xanthates has been recommended (*4, 5*), the adaptation of this method to cellulose columns was examined. The potassium xanthates were easily prepared in 90% yields and were readily distinguished on the column by their fluorescence in ultraviolet light. Conversion of the separated xanthates to the thallous salts affords a positive identification by melting points.

REAGENTS AND APPARATUS

Column. Two-foot lengths of 12-mm. glass tubing tapered at one end were used. The tapered end was plugged with cotton and the column was prepared by tamping Whatman cellulose powder, standard chromatographic grade, into it 1 cm. at a time until a height of 30 cm. was reached.

Solvents. Water-saturated *n*-butyl alcohol containing 0.5% of potassium hydroxide was used in most of this work; better quantitative recoveries, although not better separations, were obtained from a water-saturated 4- to 7-*n*-butyl alcohol–*n*-amyl alcohol mixture and this was used for the results of Table II.

Light Source. A Sylvania Blacklite, 15 watts, was used to determine fluorescence in the columns. All work with the potassium xanthates was carried out in subdued light to minimize photochemical decomposition.

Preparation of Potassium Xanthates. The procedure given by Welcher was followed (*6*). After washing with ether, the xanthates were dried and stored in the dark.

PROCEDURE

The column was first washed with 30 ml. of water-saturated butyl alcohol, and then 1 ml. of potassium xanthate solution, containing approximately 10 mg. of the solid xanthates, was pipetted on the column, forced in by air pressure, and followed by solvent to fill the tube. Pressure was adjusted to give a flow rate of 6 to 10 drops per minute. After sufficient separation (50 to 75 ml. of solvent used) the bands were marked and the column was cut into sections. The individual bands were dispersed in water and filtered and washed to remove cellulose. The filtrate was made neutral and excess thallous sulfate solution (8 grams per liter) was added and the solution was heated to boiling just long enough to produce coagulation. After filtration of the precipitated thallous xanthate the precipitate was dried at 80° C. and the melting points were obtained [values in parentheses are from the literature (*1*)]: methyl, 134–5°; ethyl, 140–1° (140°); *n*-propyl, 153–4°; isopropyl, 172–3° (171°); *n*-butyl, 156–7° (161°); isobutyl, 185–7°; *sec*-butyl, 145° (143°); *n*-amyl, 138° (131°); isoamyl, 163–4°; and benzyl, 128–9°.

RESULTS AND DISCUSSION

Chromatography of 21 pairs of alcohol xanthates (Table I) showed good results with few exceptions, all of which contained either isobutyl or *sec*-butyl alcohol. A minimum distance of 12 mm. between bands was considered an excellent separation, while separation of two distinct bands not separated by as much as 12 mm. was called "good." A satisfactory isolation of isoamyl alcohol from all the pairs in which it was a component was obtained.

Separation of a mixture of C_1 to C_5 normal alcohols gave a spacing between bands of 15,7,3, and 6 mm. in order of increasing carbon content. A mixture of isopropyl, isobutyl, and isoamyl alcohols gave 20- and 3-mm. separation of bands.

Table II. Recovery of Xanthates from Cellulose Column

Xanthate	Thallous Salt, Mg. Calcd.	Thallous Salt, Mg. Found	Recovery, %
Ethyl	7.3	7.0	96
n-Propyl	7.2	7.0	97
n-Butyl	7.1	7.0	99
n-Amyl	6.45	6.4	99

A synthetic mixture of potassium xanthates of ethyl, *n*-propyl, *n*-butyl, and *n*-amyl alcohols with the purity of each xanthate established by iodine titration (*2*) was chromatographed and the recovery of each component was determined by weighing the thallous salt. Better than 95% of each xanthate could be recovered (Table II).

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RECEIVED for review September 6, 1955. Accepted November 14, 1955.

Colorimetric Determination of Aliphatic Alpha-Nitrohydroxy Compounds

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A colorimetric procedure for the determination of α-nitrohydroxy compounds has been adapted to the determination of small amounts of nitro alcohols in amino alcohols. It is specific for the nitro alcohols in the absence of formaldehyde or other compounds that hydrolyze to formaldehyde in alkaline solution. The proposed method will quantitatively determine nitro alcohols in the range of 1 to 100 γ with an accuracy within ±2% and a precision within ±1%.

BOTH primary and secondary nitroparaffins condense with formaldehyde to form α-nitrohydroxy compounds. Catalytic reduction of a nitro alcohol produces the corresponding α-aminohydroxy compound. Completeness of reduction could be determined by a specific method for trace amounts of nitro alcohol at this step.

An aliphatic nitro alcohol determination per se is not reported in the literature. The methods reported by Grebber and Karabinos (*8*), Jones and Riddick (*10*), and Turba, Haul, and Uhlen (*14*) for secondary and tertiary nitro compounds are unsatisfactory for the determination of nitro alcohols.

Nitro alcohols decompose in the presence of excess alkali into the starting materials: formaldehyde and nitroparaffin (*9*). Amino alcohols do not undergo this reaction. Chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) forms a violet-colored complex with formaldehyde in a sulfuric acid medium (*7*). This method utilizes the color intensity of the formaldehyde complex for the quantitative determination of the nitro alcohol content. The method may be used to determine 1 part of nitro alcohol in 1000 parts of an amino alcohol sample as well as both crude and purified samples of nitrohydroxy compounds.

APPARATUS

The following is needed: a Beckman Model DU spectrophotometer, equipped with 1-cm. Corex cells; Lewis-Benedict tubes (Corning No. 7860), graduated at 12.5 and 25.0 ml.; a cooling bath at 25° C.; a steam bath; and refluxing apparatus.

REAGENTS

2-Nitro-2-methyl-1-propanol, melting point and elemental analysis to indicate a purity of 99.9% or better. Prepare an aqueous standard to contain 1 mg. per ml. of solution.

Sulfuric Acid. Specific gravity 1.84, Mallinckrodt, low nitrogen.

Chromotropic Acid Reagent. Prepare a 2% aqueous solution from the sodium salt of 1,8-dihydroxynaphthalene-3,6-disulfonic acid.

Sodium Hydroxide Solution. Prepare aqueous 0.50*N* and 5.00*N* solutions.

Sodium Bisulfite Crystals. Merck, dried.

PROCEDURE

Preparation of Calibration Curve. Transfer 0, 1.0, 3.0, 5.0, 7.0, and 10.0 ml. of the 2-nitro-2-methyl-1-propanol standard solution to six 100-ml. volumetric flasks and dilute to volume with water. These dilutions contain 0, 10, 30, 50, 70, and 100 γ, respectively, of 2-nitro-2-methyl-1-propanol per ml. of solution.

Transfer 1 ml. of a diluted standard to a Lewis-Benedict tube. Add 1 ml. of 0.50*N* sodium hydroxide solution, mix, and place in the 25° C. cooling bath for 5 minutes. Add 1 ml. of chromotropic acid reagent, dilute to 12.5 ml. with sulfuric acid, and mix well. Immerse the tube in the steam bath for 10 minutes. Remove and cool to 25° C. in the cooling bath. More acid may be added to bring the volume to 12.5 ml. Transfer the solution to a 1-cm. Corex cell.

Set the spectrophotometer at a wave length of 580 mμ. Adjust the slit width to give an absorbance reading of zero for the dilution containing no sample. Read the absorbance of each sample in a 1-cm. cell. Plot absorbance against concentration using rectangular coordinate paper.

Determination of α-Nitrohydroxy Compound Impurity in an α-Aminohydroxy Compound. Weigh about 10 grams of the α-aminohydroxy compound into a 100-ml. volumetric flask. Dilute to volume with water and mix well. Transfer a 10-ml. aliquot to a 250-ml. round-bottomed flask. Add 10 grams of sodium bisulfite crystals, 10 ml. of 5.00*N* sodium hydroxide solution, and 55 to 60 ml. of water. Reflux for 30 minutes, then cool to 25° C. Transfer the contents quantitatively to a 100-ml. volumetric flask and dilute to volume with water. Prepare a blank by treating 10 ml. of water in the round-bottomed flask with the same reagents as the sample.

Transfer a 2.0-ml. aliquot to a Lewis-Benedict tube and treat in the same manner as the calibration standards.

EXPERIMENTAL

The method consists of the decomposition of the nitrohydroxy compound with alkali to formaldehyde, and the formation of a colored complex with the formaldehyde and chromotropic acid in strong sulfuric acid.

The effect of variables upon the degradation of 2-nitro-2-methyl-1-propanol to formaldehyde was investigated. These included the stoichiometry of the reaction, the strength of alkali required for decomposition, and the time and temperature needed for the release of formaldehyde.

It was found that the normality of the alkali had no effect on the amount of formaldehyde released. Any excess of alkali was adequate to decompose the nitro alcohol almost instantly at room temperature. A 1.0-ml. aliquot of a 0.50*N* solution of sodium hydroxide and a 5-minute reaction time were chosen to ensure complete degradation.

Stoichiometry of Reaction. Samples of each nitro alcohol were analyzed by the method and calculated as formaldehyde, using formaldehyde as a standard. In all cases the expected molecular ratio of formaldehyde was found.

2-Nitro-1-butanol and 2-nitro-2-methyl-1-propanol decompose to yield one molecule of formaldehyde each. 2-Nitro-2-methyl-1,3-propanediol, 2-nitro-2-ethyl-1,3-propanediol, and tris(hydroxymethyl)nitromethane decompose to yield two molecules of formaldehyde per one molecule of nitro alcohol.

Formation of Color Complex. The variables encountered during the sulfuric acid condensation of formaldehyde and chromotropic acid have been adequately examined and reported (*1–7, 11–13*).

APPLICATIONS

The five commercially available nitrohydroxy compounds mentioned above were quantitatively determined by the described method. It was possible to determine small amounts of each in the presence of the corresponding amino compound.

The aminohydroxy compounds prepared by the reduction of the above nitro compounds are stable in alkali and do not release formaldehyde. However, the presence of aminohydroxy compounds does inhibit the color formation between chromotropic acid and the formaldehyde released by the nitrohydroxy impurity. The results obtained by the direct analysis of a sample of 2-nitro-2-methyl-1-propanol (NMP) in the presence of large amounts of 2-amino-2-methyl-1-propanol (AMP) are illustrated in Table I.

The amine present apparently either combined with the formaldehyde to form a Schiff's base, combined with chromotropic acid to the exclusion of the formaldehyde, or buffered the reaction

Table I. Effect of Amine on Determination

AMP Added, γ/Ml.	NMP Added, γ/Ml.	NMP Found, γ/Ml.
5018.8	50	5
5018.8	100	14
1003.0	50	35
1003.0	100	77

Table II. Determination of 2-Nitro-2-methyl-1-propanol in 2-Amino-2-methyl-1-propanol Mixtures

AMP Added, G.	NMP Added, Mg.	NMP Found, Mg.
0.5045	5.0	4.950, 5.005
0.5045	1.0	1.005, 1.000
0.5045	0.5	0.500, 0.495
0.5045	10.0	9.960, 9.980

mixture to such an extent that all of the formaldehyde was not released.

The formaldehyde may be conveniently separated from the interfering aminohydroxy compounds by refluxing in alkaline bisulfite solution. The formaldehyde released from the nitrohydroxy compounds complexes with bisulfite and the complex reacts quantitatively with the chromotropic acid.

Replicate results of the determination of small amounts of 2-nitro-2-methyl-1-propanol in 2-amino-2-methyl-1-propanol, using the reflux modification, are given in Table II.

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RECEIVED for review September 29, 1955. Accepted November 14, 1955.

Determination of Total Nitrogen in Reformer Charge Stock

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A method is described for the determination of traces of combined nitrogen in reformer feed stocks that is particularly suitable for the concentration range from 1 to 100 p.p.m. It is a modification of a procedure originally described by ter Meulen, and involves conversion of nitrogen compounds to ammonia by catalytic hydrogenation and colorimetric determination of ammonia with Nessler's reagent. Application is limited to petroleum stocks having end points lower than 450° F. The sensitivity is about 1 p.p.m. of nitrogen. Repeatability and accuracy are of the same order of magnitude as the sensitivity in the range below 20 p.p.m. The equipment is relatively inexpensive and simple to operate. An analysis may be completed in a little less than 2 hours.

DURING the past decade the petroleum industry has become increasingly aware of the serious problems caused by nitrogen-containing compounds. The presence of even trace quantities of these materials may affect adversely the processing, storage, and quality of petroleum products. The presence of nitrogen compounds reduces the activity of cracking catalysts. It has been demonstrated that in catalytic charge stocks they seriously decrease the conversion to gasoline (*7*, *8*). Other catalysts, such as those used in reforming, polymerization, and isomerization, are susceptible to poisoning by nitrogen compounds (*2*).

The increased commercial use of platinum catalysts for reforming straight-run naphthas has made necessary the determination of total nitrogen in such materials. The nitrogen content is generally in the range below 20 or 30 p.p.m. and therefore lies below the reliable detectability limits of the conventional Kjeldahl and Dumas methods (*4*). The low nitrogen content of these stocks makes a negligible reagent blank essential. This requirement may be most conveniently met by a catalytic hydrogenation technique in which organic nitrogen is quantitatively converted to ammonia. A method embodying this principle has recently been described by Wankat and Gatsis (*10*). A sample up to 1 liter in volume is hydrogenated at high pressure using a nickel catalyst. The resulting ammonia is adsorbed on alumina pellets, the alumina neutralized, and the ammonia distilled into boric acid and titrated. They reported excellent results on a number of naphthas using this technique. However, the method is time-consuming and requires the use of a large capacity autoclave at pressures up to 200 atm. with the attendant dangers of operation.

In 1924 ter Meulen (*6*) described a semimicroprocedure for the determination of nitrogen by destructive hydrogenation. The method is based on the fact that when 10 to 200 mg. of an organic compound containing nitrogen are pyrolyzed in a stream of hydrogen and the products are passed over a heated nickel catalyst, the nitrogen is quantitatively converted to ammonia, which may be absorbed and determined by customary procedures. The ter Meulen method has been generally accepted in Germany, but has received little attention in this country because of the short life of the catalyst. Recently Holowchak, Wear, and Baldeschwieler (*3*) reported on the application of this method to petroleum fractions. These authors were able to develop an improved catalyst which was resistant to poisoning by sulfur or halogens. They showed that the lower limit of detection was about 100 p.p.m., but suggested that by increasing the sensitivity of determining ammonia by spectrophotometry it might be possible to analyze accurately samples containing as little as 10 p.p.m. of nitrogen.

It seemed desirable to attempt to extend the ter Meulen method to enable detection and estimation of concentrations as low as 1 p.p.m., as it is a more rapid and convenient method than a high pressure hydrogenation technique. The idea of developing more sensitive means of detecting the ammonia produced was abandoned in favor of one which would allow larger quantities of sample to be analyzed than in the conventional procedure. In addition to increasing the sensitivity by producing larger quantities of ammonia, such an approach would eliminate the rather tedious handling of semimicro quantities of volatile materials

If one attempts to pyrolyze samples much larger than about 300

mg. in the equipment described by Holowchak, Wear, and Baldeschwieler (*3*), the catalyst rapidly becomes inactive, owing to excessive accumulation of carbon. An apparatus design which would prevent or minimize such carbon deposition, and at the same time allow the pyrolysis of low boiling samples with a minimum of operator attention, seemed necessary. Equipment was ultimately developed employing a vertical reaction tube with separate pyrolysis and catalyst zones which permitted up to 3 grams of sample to be analyzed. The apparatus and procedure are well suited for routine work, and are readily adaptable to multiple determinations.

APPARATUS

Gas Flow System. The general arrangement of the apparatus is shown in Figure 1. Hydrogen is supplied by a conventional pressure cylinder and reducing valve (*A*, Figure 1). Gas flow is controlled by a needle valve, *B*, and microrotameter, *C*. The mercury gage, *D*, permits an estimation of the back pressure existing within the system. The hydrogen stream enters the reaction tube, *G*, through the side arm in the tube cap. This side arm is fitted with a bypass line, *E*, to equalize pressure and allow the sample to be admitted at a uniform rate from the sample pipet, *F*. During an analysis 1 to 3 grams of sample are allowed to drip from the pipet into the pyrolysis zone of the tube. This section contains a roll of nickel gauze, *J*, and is maintained at 900° to 950° C. by the small furnace, *K*. As the droplets come in contact with the hot gauze pyrolysis takes place. Carbonaceous material is deposited on the gauze and the gaseous products are swept by hydrogen over the nickel-magnesium catalyst, *M*. The catalyst is maintained at 350° to 360° C. by the furnace, *L*. The products of the catalytic reaction are swept into the absorber, *P*, where ammonia is scrubbed out and retained. The ammonia is then determined colorimetrically with Nessler's reagent (*1*, *9*).

Pyrolysis Furnace. The pyrolysis furnace consists of a 3.25-inch length of 1-inch inside diameter Alundum tubing held in place within a cylindrical brass shell by end plates of 0.25-inch Transite. The Alundum tube is wound with a helical coil of 15 feet of No. 24 B and S gage Nichrome V, and the winding is completely covered with a layer of RA 1162 Alundum cement. The annular space between the tube and the brass housing is filled with vermiculite as insulation. Such a unit will attain a temperature of 950° C. in 10 minutes. The temperature of the pyrolysis furnace is measured with a Chromel-Alumel thermocouple and controlled by means of a variable autotransformer.

Catalyst Furnace. A large furnace capable of maintaining a temperature of 350° to 360° C. is necessary for heating the catalyst zone. A Hevi-Duty furnace, Type FD 303, has proved suitable. The temperature is measured with a Chromel-Alumel thermocouple and controlled by means of a variable autotransformer.

Quartz Reaction Tube and Cap. The quartz reaction tube is shown in detail in Figure 2. It is 550 mm. long and consists of an 80-mm. pyrolysis section, a 270-mm. catalyst zone, and an exit section 5 mm. in diameter and 80 mm. long. The pyrolysis and catalyst sections are 20 mm. in inside diameter. The upper end of the reaction tube is fitted with a quartz standard-taper 24/40 joint, and the exit end is provided with a 12/5 quartz spherical joint for connection to the absorber system. Quartz joints are obtainable from the Cleveland Quartz Works, General Electric Co.

A borosilicate glass standard-taper 24/40 joint fitted with a side arm and standard-taper 10/30 joint placed concentric with the reaction tube serves as a cap.

The reaction tube is supported in the vertical furnace by means of a conventional laboratory clamp which grips the tube just below the top ground joint.

Weighing Pipet. A weighing pipet fitted with standard-taper 10/30 joints to fit the cap and bypass is used to introduce the sample. Details of its construction are shown in Figure 2.

Absorber. The absorber is constructed of borosilicate glass as shown in Figure 3.

REAGENTS

Hydrogen.

Magnesium oxide. Merck reagent grade, low in sulfate, is suitable.

Nickel nitrate hexahydrate, Merck reagent grade.

Hydrochloric acid, approximately 0.02*N*, prepared with ammonia-free water.

Nessler's Reagent (*1*, *9*). Dissolve 50 grams of potassium iodide in a minimum volume of cold distilled water (approximately 35 ml.). Slowly add a saturated solution of mercuric chloride until the first slight precipitate of red mercuric iodide persists. Add 400 ml. of a clarified 9*N* solution of sodium hydroxide. Dilute to 1 liter with ammonia-free water and allow to clarify. The clear supernatant liquid is decanted and used.

Standard ammonium chloride solution, 10 mg. of nitrogen per liter.

Standard Nitrogen Blend. Prepare a blend of Eastman Kodak Co. white label quinoline and Knock Test Grade iso-octane to contain 20 to 30 p.p.m. of nitrogen. This blend is used to check catalyst activity periodically.

Ammonia-Free Water. This may be conveniently prepared by redistilling laboratory-distilled water from a solution containing 1 or 2 ml. of concentrated sulfuric acid.

PROCEDURE

Preparation of Calibration Curve for Colorimetric Determination of Ammonia. Pipet 1-, 2-, 4-, 6-, 8-, and 10-ml. aliquots of

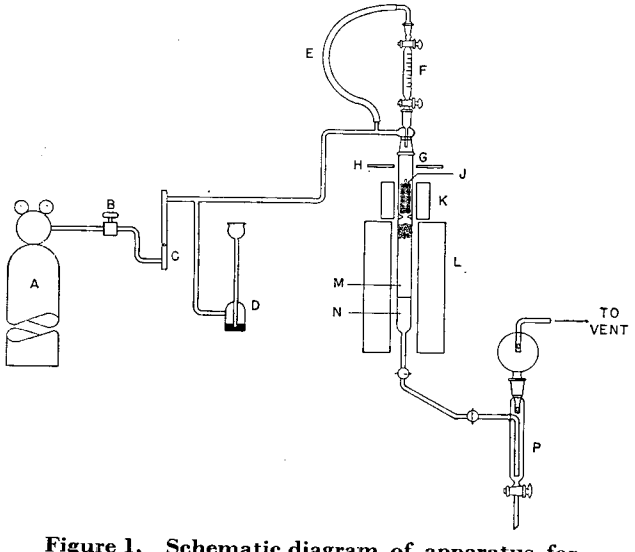

Figure 1. Schematic diagram of apparatus for determining trace quantities of nitrogen

A. Hydrogen cylinder
B. Needle valve
C. Rotameter
D. Mercury pressure gage
E. Bypass line
F. Sample pipet
G. Reaction tube
H. Transite shield
J. Nickel gauze
K. Pyrolysis furnace
L. Catalyst furnace
M. Nickel-magnesium catalyst
N. Glass wool
P. Absorber

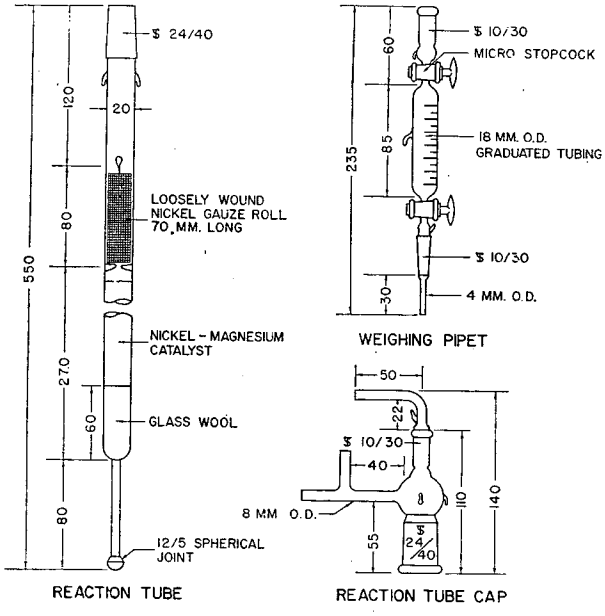

Figure 2. Details of reaction tube, cap, and weighing pipet

All dimensions in millimeters

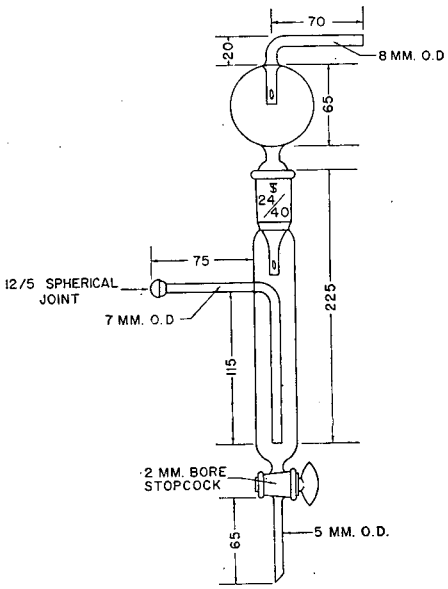

Figure 3. Details of absorber, all dimensions in millimeters

the standard ammonium chloride solution into 50-ml. volumetric flasks. Dilute almost to the necks with ammonia-free water, and pipet into each flask 1 ml. of Nessler's reagent. Swirl the flask during the addition of the reagent to prevent precipitation. Fill the flasks to the mark with ammonia-free water and shake until the solutions are thoroughly mixed. Allow to stand for 5 minutes, and measure the absorbance on a spectrophotometer at 400 mμ. Compensate for the blank color of the reagent by using a solution of 1 ml. of reagent and 49 ml. of ammonia-free water in the reference cell.

Prepare a calibration curve by plotting the concentrations of ammonia nitrogen in micrograms per 50 ml. against the corresponding absorbances. A new calibration should be prepared whenever a new quantity of Nessler's reagent is made up.

The successful use of Nessler's reagent depends upon cleanliness and attention to detail. Glassware should be cleaned with chromic acid and rinsed with ammonia-free water just prior to use. Absorption cells may be dried after cleaning by rinsing with ethyl alcohol and blowing with a stream of filtered air. As the complex formed is colloidal in nature, it is necessary to standardize the procedure used for production of the color. Nessler's reagent is best added in a squirt, as from an automatic pipet with a bulb; solutions should be at a uniform and reasonably constant temperature, and the time interval allowed for color development should be adhered to rigidly.

Catalyst Preparation. The nickel-magnesium catalyst has been described by Holowchak, Wear, and Baldeschwieler (*3*).

Prepare a slurry of 125 grams of magnesium oxide in 1.25 liters of distilled water at 50° C. Dense crystalline forms of magnesia are not suitable. The material used should be a finely divided, fluffy solid. Dissolve 400 grams of nickel nitrate hexahydrate in 4 liters of distilled water at 50° C. Add the nickel nitrate solution slowly to the magnesia slurry with constant stirring. Adequate mixing at this point is essential to the preparation of a successful catalyst and a mechanical stirrer is desirable. Allow the precipitate to settle and decant the mother liquor. Transfer the precipitate to a Büchner funnel, filter, and wash with distilled water until the washings show no more than a trace of nitrate ion. Score the precipitate in 0.25-inch squares, dry in air, and then at 100° C. in vacuum. After vacuum drying, a suitable catalyst is generally very pale green in color. Screen the dried precipitate through 4- and 8-mesh screens, breaking into smaller pieces, if necessary. The catalyst retained on the 8-mesh sieve is used for filling the reaction tube. Follow instructions for preparing and reducing the catalyst closely, in order to obtain a reagent of high activity.

Operation of Apparatus. Fill the catalyst zone of the reaction tube and assemble the apparatus. The sample pipet may be omitted and the bypass line connected directly to the reaction tube cap. Ignite the roll of nickel gauze and allow to cool. Remove the cap and drop the gauze into place in the pyrolysis zone. Sweep out the air with a stream of hydrogen and reduce the catalyst by raising the temperature of the large furnace to 340° to 380° C. while maintaining a hydrogen flow of 30 to 40 ml. per minute. When reduction is complete (disappearance of condensation in the exit end of the tube) adjust the temperature of the furnace to 350° to 360° C. and maintain it at this temperature during the course of the determinations. The furnace may be turned off over week ends, but a slow rate of flow of hydrogen should be maintained continuously during nonoperating periods.

Make a blank determination by performing all operations as described in the following paragraph, but momentarily disconnecting the bypass instead of inserting the sample pipet. If the blank value is greater than 1γ of nitrogen, repeat the determination. With a new reaction tube filling the first blank is generally high, but will drop to a negligible value after an additional determination. It is necessary to run a blank whenever fresh catalyst is added to the reaction tube or fresh batches of reagents are used.

Fill the sample pipet and weigh. With the catalyst furnace at operating temperature add 20 ml. of ammonia-free water to the absorber. Add 1 ml. of 0.02*N* hydrochloric acid to the absorber contents. Remove the bypass from the reaction tube cap, place the pipet in the cap, and place the bypass in the joint on the top of the pipet. Adjust the flow of hydrogen to a rate of 60 to 70 ml. per minute and raise the temperature of the pyrolysis furnace to 900° to 950° C. Introduce from 1 to 3 grams of sample at a rate of 1 drop every 12 to 18 seconds. After addition of the sample, raise the temperature of the pyrolysis furnace to 980° to 990° C. for 10 minutes. Turn off and allow to cool while purging the system for an additional 25 minutes with hydrogen.

The operations described above are not hazardous, provided all air is swept out of the system and reasonable care is taken to prevent entry of air during addition of the sample. If the operations pertaining to introduction of the weighing pipet are performed quickly, and some sample is always retained in the pipet, there need be no concern. As a precaution the operator should wear safety goggles during addition of the sample.

Without discontinuing the flow of hydrogen, drain the absorber contents into a 50-ml. volumetric flask. Rinse the absorber with several small portions of ammonia-free water, adding the rinsings to the contents of the flask. Remove the pipet and weigh. Add 1 ml. of Nessler's reagent to the contents of the flask, dilute to the mark with ammonia-free water, mix well, and read the color developed on a spectrophotometer at 400 mμ, 5 minutes after addition of the reagent. Determine the number of micrograms of ammonia nitrogen present by comparison to the calibration curve. Calculate the nitrogen content as follows:

$$\text{Nitrogen, p.p.m.} = \frac{\text{ammonia nitrogen, micrograms}}{\text{sample weight, grams}}$$

As the method depends upon catalysis, the activity of the catalyst must be checked when freshly prepared and periodically thereafter using the synthetic blend of known nitrogen content. Nitrogen recovery should be at least 95%.

RESULTS AND DISCUSSION

Although the nickel gauze causes the major part of the carbon to be deposited in the pyrolysis zone, it was observed that after about a week's operation, excessive back pressure developed in the apparatus. Upon inspection of the reaction tube it was found that approximately the top 2 inches of the catalyst were covered with soft carbon. This portion was removed and replaced with fresh catalyst, and the apparatus was reassembled. This operation is now performed routinely whenever back pressure becomes greater than 1.5 to 2 inches of mercury. The tendency for carbon to deposit on the catalyst can be alleviated by periodically removing the nickel gauze and burning off the carbon.

In the preparation of synthetic samples, organic compounds which were representative of the type of material that might be expected to occur in petroleum were used. In addition, the applicability of the method for naturally occurring nitrogen compounds was examined by analyzing blends of a shale naphtha whose nitrogen content had been well established by the Kjeldahl method (*5*). As conventional procedures can be applied above 100 p.p.m., the test blends were prepared so as to cover the region

below this amount. Emphasis was placed on the region 0 to 20 p.p.m., as it was anticipated that the nitrogen content of most virgin naphthas would fall within this range. Results on these synthetic naphtha samples are shown in Table I. The data show that the technique developed is entirely satisfactory for the determination of nitrogen in virgin naphthas even at concentrations as low as 1 or 2 p.p.m. The sensitivity is about 1 p.p.m. The accuracy is of the same order of magnitude in the low range.

Although the catalyst preparation used has been reported to be resistant to poisoning, a few experiments to determine the effect of the presence of organic sulfur compounds were performed. The results are summarized in Table II. Thiophene and benzyl mercaptan were used to simulate naturally occurring sulfur. The synthetic samples were purposely made up to contain large amounts of sulfur to give a severe test. In the majority of petroleum distillates, the sulfur content would rarely be so high. The data show that the method gives acceptable results when considerable quantities of sulfur are present.

A few virgin naphthas of widely differing crude source have been analyzed. Some typical results are shown in Table III. These naphthas show considerable variation in nitrogen content, from less than 1 to as high as 7 p.p.m. Such data may be helpful in crude evaluation studies. As expected, the nitrogen contents of naphthas derived from asphaltic crudes are considerably higher than those from other sources. A California naphtha of relatively high nitrogen content was fractionated in a small laboratory distillation column packed with glass helices. The fractions were then analyzed to check the distribution of nitrogen with boiling range. Approximately 85% of the nitrogen was found in the two fractions boiling above 375° F. These fractions constituted roughly 30% of the original naphtha. The fractions comprising the initial 70% of the sample could be combined to give a naphtha containing about 1.5 p.p.m. of nitrogen.

Table I. Analysis of Synthetic Blends

Sample	Boiling Range, ° F.	Nitrogen, P.P.M. Added	Nitrogen, P.P.M. Found	Deviation, P.P.M.
Iso-octane + quinoline	...	44.6	40.3	−4.3
			45.3	+0.7
			42.5	−2.1
Iso-octane + quinoline	...	29.5	29.5	0.0
			31.6	+2.1
Iso-octane + quinoline	...	19.9	21.3	+1.4
			22.0	+2.1
Iso-octane + quinoline	...	10.0	10.4	+0.4
			9.4	−0.6
			10.2	+0.2
Iso-octane + quinoline	...	5.4	5.9	+0.5
			5.5	+0.1
			5.3	−0.1
Straight-run naphtha	260–405	..	<1	...
			<1	...
+ quinoline + indole	260–405	82.0	80.6	−1.4
+ quinoline	260–405	3.4	3.1	−0.3
			4.3	+0.9
+ shale naphtha[a]	250–405	22.0	22.0	0.0
			22.7	+0.7
+ shale naphtha	250–405	10.0	10.0	0.0
			9.5	−0.5
+ shale naphtha	250–405	6.0	5.4	−0.6
			6.0	0.0
+ shale naphtha	250–405	5.0	4.9	−0.1
			5.2	+0.2
+ shale naphtha	250–405	2.5	2.9	+0.4
			3.1	+0.6
			3.0	+0.5

[a] Contains 0.80 wt. % nitrogen by Kjeldahl analysis.

Table II. Analysis of Synthetic Blends Containing Sulfur

Sample	Boiling Range, ° F.	Thiophenic Sulfur, Wt. %[a]	Nonthiophenic Sulfur, Wt. %[b]	Total Sulfur, Wt. %	Nitrogen, P.P.M. Added	Nitrogen, P.P.M. Found
Straight-run naphtha	250–405	0.90	0.37	1.27	81.8	74.7
						78.1
+ shale naphtha						80.0
	250–405	0.27	0.08	0.35	80.8	78.2
	250–405	0.22	0.09	0.31	20.1	19.5
						18.1

[a] As thiophene.
[b] As benzyl mercaptan.

Table III. Analysis of Typical Virgin Naphthas

Boiling Range, ° F.	Origin of Crude	Total Nitrogen, P.P.M.
250–410	Mississippi	<1
250–400	Illinois Basin	1
260–405	Gulf Coast I	<1
280–460	Gulf Coast II	1.3
250–400	Canada I	<1
250–400	Canada II	<1
250–410	Venezuela	2.9
		2.4
250–410	Middle East	<1
250–410	Saudi Arabia	<1
250–410	California I	3.8
		3.4
250–410	California II	6.8

Distillation of California Naphtha (6.8 P.P.M. of N)

Fraction, ° F.	Wt. % of naphtha	Total N, p.p.m.	Contribution to N in whole naphtha, p.p.m.
250–300	26.4	<1	<<1
300–350	31.0	1	0.3
350–375	15.5	5.4	0.8
375–400	13.5	11.4	1.5
Over 400	13.6	33.9	4.6
		Total	7.2

Thus, where it is economically practical to cut reformer stock to a lower end point, the refiner can reduce considerably the danger of catalyst poisoning by nitrogen compounds.

The method described has not been applied routinely to naphthas with end points higher than about 420° F. On the basis of a limited number of determinations on material of higher end point, it is believed that samples boiling as high as 450° F. could be satisfactorily analyzed. Above that temperature incomplete cracking of the sample would permit hydrocarbon material to distill through the catalyst zone, condense in the absorber, and interfere with the ammonia determination. It is probable that the sensitivity could be somewhat increased by using absorption cells of longer optical path for the spectrophotometric determination of ammonia. Although all the results reported here were obtained by analysis of a known weight of sample, some saving in time could be effected if the pipet were calibrated and the sample added by volume. A specific gravity, which is generally available on such samples, would then enable conversion to weight.

Holowchak, Wear, and Baldeschwieler (*3*) have reported that the conventional ter Meulen method is applicable to a wide variety of nitrogen linkages. In addition to amines, nitriles, and simple heterocyclic compounds, they found that hydrazine nitrogen or heterocyclic rings with nitrogen-to-nitrogen bonds could be successfully analyzed. Simple nitro compounds also gave good results (*3*). This modification of the ter Meulen method is undoubtedly applicable to the same nitrogen types. Therefore, it should be satisfactory for any nitrogen compounds which would occur in reformer charge stock.

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RECEIVED for review May 26, 1955. Accepted October 22, 1955. Division of Refining, American Petroleum Institute, St. Louis, Mo., 1955.

Concentration of Solutes for Paper Chromatography

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Extreme concentration of small volumes of solution (2 ml.) leads to inaccuracy in analysis because of losses by evaporation, wetting, transfer, chemical reactions, increasing ionic strength, and other factors. Paraffin dikes were devised to hold and concentrate the solutions of extracts from small amounts of plant tissue directly on the chromatographic paper. Aliquots of 50 to 100 μl. were deposited at once in the area normally given to 5 μl. Handling of solutions is simplified and more precision is obtained. The method is versatile and simple; reactions may be carried out directly on the paper.

FREQUENTLY, in the chromatographic assay of crude extracts, a special technique is required to get enough solute from dilute solutions onto the paper. The customary use of micropipets of small capacity (5 μl.) becomes time-consuming and tedious. Extensive evaporation of solvent may sometimes be impractical when the extract yields are limited, or when such treatment causes changes in the amounts of constituents because of chemical reactions. Three simple techniques have been devised which have proved useful in concentrating extracts.

Two of the techniques involve the use of paraffin dikes. For the first method the edge of a heated glass microscope slide is touched to a block of paraffin and then to the chromatographic paper to surround a square or rectangle with an area of 1 to 2 sq. cm. It is important to deposit just enough paraffin to permeate the paper completely, but this is easily accomplished and can be checked by looking at the underside of the paper. The solution to be analyzed is placed on the square area of paper surrounded by the dikes in successive aliquots of 50 to 200 μl., and each aliquot is evaporated by a stream of warm, dry air. This effect is illustrated in Figure 1, *A*. Sheets containing the solute material may be stored in a dry atmosphere or developed immediately. Prior to developing, a number of razor blade cuts are made through the paraffin dikes on two sides of the square to permit the solvent to move into and out of the solute-containing area (Figure 1, *B*). R_f values remain unaltered and no additional spots are contributed in the usual solvents by the paraffin. In case of doubt, controls may be run simultaneously.

In the second method the extract is placed in a rectangular area surrounded by paraffin dikes. The area is cut out after drying, as indicated by the dashed lines in Figure 1, *C*. Other shapes or subdivisions may be used, but each portion of paper should have one solute-free area attached. This arrangement is illustrated in Figure 1, *D*.

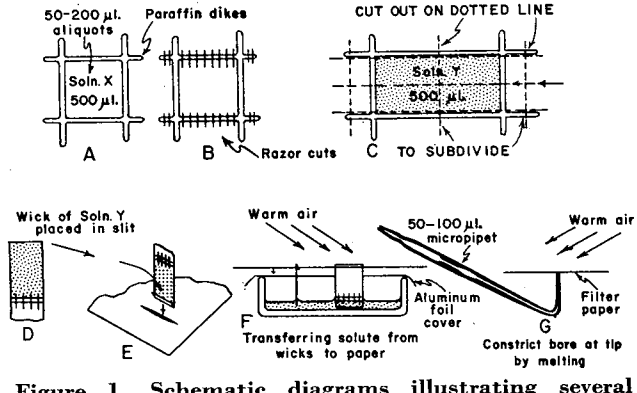

Figure 1. Schematic diagrams illustrating several methods for concentrating extracts for paper chromatography

These paper sections, with the indicated cuts through the dikes, are used as wicks; the solutes contained in them are transferred to the paper to be chromatographed as illustrated in Figure 1, *E* and *F*. A small fold (0.5 mm.) is made in the solute-containing end of the wick and pressed into a slit in the desired location on the chromatographic paper. The solute is transferred to the paper by elution. By immersing only the solute-free area of the wick in the solvent, loss of the solute is prevented and a quantitative transfer is obtained. Short wide wicks are eluted quickly; long narrow ones more slowly. This means that the spot size around the slit can be controlled.

To obtain the transfer conveniently, to prevent the saturation of the paper with vapor from the eluting solvent, and to provide adequate support for the paper and wick over the solvent, the dish containing the solvent should be covered with aluminum foil with appropriately spaced slots for the wicks (Figure 1, *F*). A current of dry air passed over the paper will evaporate the rising solution with negligible evaporation of the solvent in the dish.

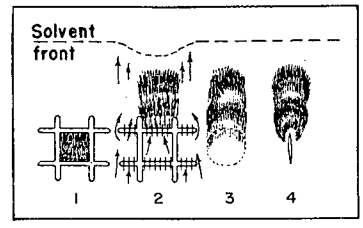

Figure 2. Diagram of chromatograms

1, 2. Solutes deposited in dikes
3. Solutes deposited from micropipets
4. Solutes deposited from paper wicks

This technique has been most useful in depositing solutes from very dilute solutions. The solutes are deposited in a narrow band around the slit so that there is a preliminary separation. Chromatograms made in this way show compact spots with little "tailing," and the method gives unusually good resolution. The proper choice of solvents will help to eliminate undesirable constituents of the extracts originally deposited.

These dike and wick techniques have been used in analysis of amino acids. It is also possible to carry out enzymatic reactions at low temperatures directly on the paper area surrounded by the dikes by keeping that portion of the paper in a moist atmosphere between two Petri dish tops.

The third method, which is represented in Figure 1, *G*, involves the transfer of the solution from a bent micropipet onto the paper by capillarity. The size of the spot is controlled by the rate of evaporation and by the rate of delivery, which is determined by the size of the orifice in the pipet tip. This is a simplification of a method previously reported (*1*).

Figure 2 shows the pattern of movement of the spots in each of the described methods. The solvent front may be depressed for a short distance above the spots because of the slower movement through the dikes.

ACKNOWLEDGMENT

This work was supported in part by the Atomic Energy Commission.

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RECEIVED for review September 26, 1955. Accepted November 14, 1955.

Determination of Methylol Groups in Phenolic Resins

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An accurate and simple method for the determination of methylol groups in phenol formaldehyde resins is described. Methylol groups and phenol condense in the presence of an acid catalyst to form water as one of the products. The water in the reaction mixture is titrated directly with Karl Fischer reagent and water produced in the reaction is calculated after correction for water in the sample and reagents. In contrast with previously available techniques, this method provides an accurate analysis using small quantities of sample, and thus facilitates examination of small scale laboratory preparations.

A NEED has existed for an accurate and simple method for determination of methylol groups in phenolic resins. Several techniques have been described in the recent literature but have not been found generally satisfactory. Halogenation procedures, involving the use of iodine (*11*) or bromine (*19, 22*), suffer from variable substitution reactions involving the aromatic rings. Lilley and Osmond (*12*) describe a method utilizing a reaction with hydrogen bromide and titration of the released water with Fischer reagent. The reaction is slow, requiring as long as 24 hours for completion.

Martin (*13*) developed a method for methylol determination based on a condensation reaction between methylol groups and phenol to form water as one of the products. The method involved reaction of the sample with 100 to 500 grams of phenol in benzene solution with *p*-toluenesulfonic acid as a catalyst. The water produced was continuously distilled from the mixture as an azeotrope with benzene, collected as a lower layer, and measured in a calibrated receiver. Good recoveries were obtained in the analysis of benzyl alcohol, methylol phenols, methylol ureas, and trimethylolnitromethane. The disadvantages inherent in the Martin method are the large quantities of reagent and sample required for accurate volume measurement of the water produced. In the analysis of small scale laboratory preparations, it is particularly imperative that samples be small. It appeared that the use of Karl Fischer reagent for measurement of the evolved water, as suggested by Martin, would result in a fiftyfold reduction in sample size and a considerable reduction in quantities of reagents required.

Although this investigation is concerned primarily with the quantitative determination of methylol as a functional group, it is of interest to note the work of Freeman, who reported the separation and identification of individual methylol phenols by paper chromatography (*7*), and used the latter technique in a kinetic study of the phenol-formaldehyde reactions (*8*).

PRELIMINARY STUDIES

Initial tests were made using the reagent specified by Martin, consisting of 100 grams of phenol and 3 grams of *p*-toluenesulfonic acid dissolved in 50 ml. of benzene. Water produced in the reaction was measured by azeotropic distillation with xylene and titration of the distillate with Fischer reagent or by direct titration of the reaction mixture with Fischer reagent. The results obtained for benzyl alcohol by the distillation technique showed considerable variation in the recovery of water. The method was cumbersome and required special distillation equipment and considerable operator's time. As subsequent tests showed the more convenient direct titration to be preferable, the distillation modification was abandoned.

The direct titration procedure was applied using several reaction temperatures and varying reaction times. Acidic catalysts tested were either toluenesulfonic acid or boron trifluoride. The latter catalyst was introduced as a 26% boron trifluoride—phenol complex, a form superior from the standpoint of safety and convenience to that of the gas. Xylene was also substituted for benzene to reduce the vapor pressure of the reagent and thus diminish its toxicity. The procedure tested was as follows.

Place 25 ml. of reagent in a dry 100-ml. volumetric flask. Add a quantity of sample containing a total of 5 to 10 millimoles of methylol plus water. Stopper the flask and heat the mixture in an oven at a fixed temperature for a measured length of time. Cool the mixture to room temperature, add 10 ml. of anhydrous 4 to 1 glycol-pyridine mixture and titrate with Fischer reagent to the visual end point. Run a blank in an identical manner and determine the water content of the sample by titration with Fischer reagent.

Data obtained under varying reaction conditions in the analysis of benzyl alcohol and several substituted benzyl alcohols are shown in Table I. Excellent accuracy was observed with the boron trifluoride reagent at 60° C., and variation of the reaction time between 1 and 3 hours had no effect upon the extent of reaction. Toluenesulfonic acid was the less active catalyst, as shown by a comparison of the values obtained with the two catalysts after 1 hour at 60° C. The extent of reaction, using toluenesulfonic acid catalyst, reached a maximum of approximately 95%, and increasing the temperature to 100° C. did not result in increased reaction.

Table I. Effect of Time, Temperature, and Catalyst on Reaction of Methylol Groups with Phenol

(Reagents. *A*. 70 wt. % phenol, 28 wt. % benzene, 2 wt. % *p*-toluenesulfonic acid. *B*. 70 wt. % phenol, 29 wt. % xylene, 1 wt. % boron trifluoride)

Compound	Reaction Conditions: Reagent	Temp., °C.	Time, hours	Reaction, %
Benzyl alcohol	A	25	1	25
		60	1	68
		60	3	97.5, 94.7, 96.1, 96.9, 95.8, 95.2
		67	2	94.9
		67	3	94.8
		100	2	95.9, 94.9
	B	60	1	99.6
		60	2	99.6
		60	3	99.0
2,6-*bis*(Hydroxymethyl)-4-methyl phenol	A	60	3	96.2, 94.5, 93.7, 94.5, 94.8
	B	60	3	99.6, 100.5
o-Hydroxybenzyl alcohol	B	60	3	99.2, 99.4
p-Hydroxybenzyl alcohol	B	60	3	100.2, 99.6
Benzhydrol	B	60	3	100.0, 99.6

The method using toluenesulfonic acid catalyst was applied to known amounts of water to test the possibility that low recoveries were due to physical loss of water. Low recoveries of the added water, 94 to 97%, were obtained under all conditions, including a test in which the heating period was omitted. As the values showed no consistent trend with severity of reaction conditions, the possibility of physical loss of water was excluded. The reason for the low recovery of water, corresponding to a loss of 1 to 5 mg., is not known. It did not appear profitable to pursue this question further, inasmuch as excellent recoveries were obtained with boron trifluoride.

The effect of phenol concentration in the boron trifluoride reagent was studied with a view toward the possibility that a less corrosive reagent might be used. The results of these tests,

shown in Table II, indicate that no reduction in accuracy was caused by reduction of the phenol concentration in the reagent from 74 to 17%. However, subsequent application of the method to phenolic resins showed that the higher phenol concentrations are necessary for solution of some samples.

APPARATUS AND CHEMICALS

Buret assembly. An all-glass buret system designed to exclude atmospheric moisture (*17*).

Safety oven, explosion-proof, capable of maintaining a temperature of 60° ± 1° C.

Ethylene glycol–pyridine mixture. Mix 1 volume of C.P. anhydrous pyridine with 4 volumes of C.P. anhydrous ethylene glycol.

Fischer reagent, prepared as described by Peters and Jungnickel (*17*).

Table II. Effect of Phenol Concentration on Extent of Reaction of Benzyl Alcohol

(Catalyst. Boron trifluoride, 1.0%. Reaction. 3 hours at 60° C.)

Solvent	Phenol Concentration in Reagent, Wt. %	Reaction of Benzyl Alcohol, %
Benzene	74[a]	100.5
Xylene	70	99.0
	54	100.8
	29	101.3
	17	99.7
	10	99.2, 96.9

[a] Gaseous boron trifluoride used in preparation of reagent. Reagent in all other determinations prepared with boron trifluoride–phenol complex.

Phenol–boron trifluoride reagent. Add 100 ml. of C.P. xylene and 10 ml. of 26% boron trifluoride–phenol complex to 200 grams of C.P. phenol in a 500-ml. glass-stoppered flask. The flask may be warmed on a hot plate to effect solution.

Benzyl alcohol, Eastman Kodak Co. Assay by acetyl chloride method (*20*), 99.4%.

2,6-Bis(hydroxymethyl)-4-methylphenol, laboratory preparation. Assay by acetic anhydride method (*23*), 97.4%. Melting point, 128° C.; melting point reported, 130° C. (*24*).

Benzyl ether, Eastman Kodak Co. Hydroxyl content, by lithium aluminum hydride, using method similar to that of Hochstein (*9*), less than 0.001 equivalent per 100 grams.

Benzyl ethyl ether. Hydroxyl content, by acetyl chloride method, 0.055 equivalent per 100 grams.

Benzhydrol, Eastman Kodak Co. Assay by lithium aluminum hydride (*9*), 100.5%.

o-Hydroxybenzyl alcohol, Eastman Kodak Co., recrystallized from water and benzene. Assay by lithium aluminum hydride (*9*), 98.4%.

Bis(2-hydroxybenzyl)ether, prepared by heating *o*-hydroxybenzyl alcohol at 100° C. for 8 hours (*13*). Melting point, 119–120° C.; reported, 120–121° C.

p-Hydroxybenzyl alcohol, prepared by hydrogenation of Eastman Kodak Co. *p*-hydroxybenzaldehyde. Recrystallized from water. Assay by lithium aluminum hydride (*9*), 99.5%. Melting point, 108° C.; reported, 110° C.

Bis(2-hydroxyphenyl)methane, obtained as one product from reaction of phenol and *o*-hydroxybenzyl alcohol. Infrared spectrum is consistent with that shown by Richards and Thompson (*18*) for the compound. Melting point, 113–114° C.; reported, 119° C. (*2*).

Bis(2-hydroxy-3,5-dimethylphenyl)methane, prepared by condensation of formaldehyde with 2,4-xylenol. Assay by acetic anhydride (*23*), 100.3%. Melting point, 148° C.; reported, 145–146° C. (*1*).

PROCEDURE

Pipet 25 ml. of the phenol–boron trifluoride reagent into a clean, dry 100-ml. glass-stoppered volumetric flask. Introduce into the flask a weighed sample containing 6 to 9 millimoles of methylol plus water. Swirl the flask to dissolve the sample and heat the mixture for 3 hours in a safety oven (or water bath) at 60° C. At the end of the reaction period, cool the mixture to room temperature and add 10 ml. of anhydrous glycolpyridine mixture from a protected buret. Titrate the mixture to the orange-red end point with Fischer reagent.

Make a blank determination on the reagents in an identical manner but omit the sample. Determine the water content of the original sample by titration with Fischer reagent (*4*) and the carbonyl content by the Fischer carbonyl method (*15*).

CALCULATION

Calculate the methylol content of the sample by means of the following equation:

$$\text{Methylol content, equivalent per 100 grams} = \frac{(A - B)(F)}{(W)(10)(18.02)} - C - \frac{D}{18.02}$$

where

A = volume of Fischer reagent required for methylol determination on sample, milliliters

B = volume of Fischer reagent required for reagent blank in methylol determination, milliliters

F = standardization factor for Fischer reagent in milligrams of water equivalent to 1 ml. of reagent

W = weight of sample taken for methylol determination, grams

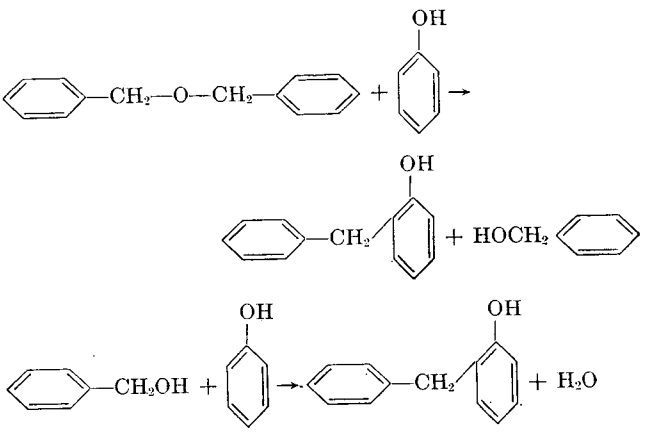
C = carbonyl content, equivalent per 100 grams

D = water content, weight %.

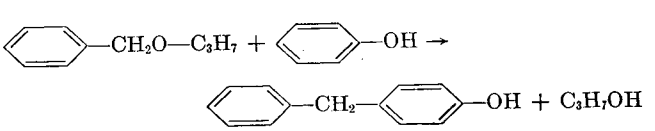
RESULTS AND DISCUSSION

Side Reactions. In view of the presence of considerable unreacted formaldehyde in the phenolic resins under consideration, the stoichiometry of the reaction of aldehydes in the method was tested. The reactions of ketones, benzyl ethers, and isopropyl alcohol were also studied. The results of these tests are shown in Table III. Formaldehyde, acetaldehyde, and benzaldehyde reacted to form 1 mole of water per mole of aldehyde, showing excellent agreement with theory. In view of the observed stoichiometry, the methylol content of phenolic resins can be obtained by application of a correction for carbonyl content. The ketones tested, acetone and methyl isobutyl ketone, reacted to the extent of 7.3 and 0.2%, respectively. The low reactivity of ketones is not a serious handicap, as ketones are not constituents of the phenolic resins under consideration. Isopropyl alcohol reacted to a negligible extent.

Dibenzyl ethers reacted to produce almost 1 mole of water per mole, possibly via a two-step reaction as follows:



Water is not produced in the similar reaction of alkyl benzyl ethers with phenol. Monacelli and Hennion (*16*) describe acid catalyzed reactions of alkyl benzyl ethers with aromatic compounds—for example, the reaction of benzyl-*n*-propyl ether with phenol in the presence of boron trifluoride:

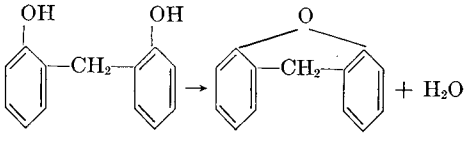


Ethers of this type should not interfere in the determination of methylol, as water is not produced in the reaction and the simple alcohol does not react further. A slight reaction observed with benzyl ethyl ether was probably due to the presence of benzyl alcohol as an impurity, since an independent hydroxyl determination showed the presence of alcohol.

Table III. Side Reactions in Methylol Method

Compound	Reaction, %
Formaldehyde	101.0, 101.0
Acetaldehyde	99.5, 100.5
Benzaldehyde	100.4, 100.4
Acetone	7.3
Methyl isobutyl ketone	0.2
Isopropyl alcohol	0.1
Benzyl ethyl ether	3.7, 3.6
Dibenzyl ether	96.3, 96.5
Bis(2-hydroxybenzyl)ether	97.2, 97.8
Bis(2-hydroxyphenyl)methane	0.5
Bis(2-hydroxy-3,5-dimethylphenyl)methane	0.5

Bis(2-hydroxyphenyl)methanes were tested because of the possibility that they might react to produce xanthenes and water:



Negligible reaction was observed with these materials, showing the absence of such a dehydration reaction, in agreement with Buehler, Cooper, and Scrudder (*3*), who showed a relatively high stability of bis(2-hydroxyphenyl)methane toward dehydrating agents.

Table IV. Functional Oxygen Balance Obtained for Phenolic Resins

Functional Group, Eq./100 Gram	Resin 1	Resin 2	Resin 3
Carbonyl	0.007	0.133	0.407
Water	0.109	0.243	0.048
Methylol plus dibenzyl ethers	0.345, 0.348, 0.348	0.691, 0.680	0.819, 0.820
Phenolic hydroxyl[a]	0.79	0.67	0.68
Total functional oxygen	1.26	1.81[b]	1.96
Total oxygen[c]	1.30, 1.30	1.76, 1.79	1.97
Total hydroxyl[d]	1.03	1.28	1.42
Phenolic hydroxyl	0.79	0.67	0.68
Alcoholic hydroxyl	0.24	0.61	0.74
Methylol plus dibenzy ethers[e]	0.35	0.69	0.82
Dibenzyl ethers[e]	0.11	0.08	0.08

[a] Potentiometric titration in ethylenediamine (*6*).
[b] Includes 0.07 eq./100 grams of ethoxyl by Zeisel method (*10*).
[c] Modified Unterzaucher (*5*).
[d] Lithium aluminum hydride gasometric determination of active hydrogen (*9*).
[e] Calculated as difference between above two values.

Analysis of Phenol-Formaldehyde Resins. The method was applied to three phenol-formaldehyde resins of low molecular weight of the resol type with results as shown in Table IV. These resins had been prepared with excess formaldehyde under alkaline conditions and were stored in a refrigerator at −15° C. to prevent further polymerization. Water was determined by titration with Fischer reagent after reaction of the interfering aldehyde groups with hydrocyanic acid (*4*). Carbonyl content was determined by a method involving reaction with hydroxylamine and measurement of the evolved water by titration with Fischer reagent (*15*). The methylol content was determined under the conditions outlined above, including corrections for water and carbonyl content, with good agreement in duplicate determinations.

In order to gain further information about the constitution of these materials and as a further check on the accuracy of these procedures, the resins were also analyzed for total hydroxyl, phenolic hydroxyl, and total oxygen. Total hydroxyl was determined by lithium aluminum hydride (*9*), and phenolic hydroxyl by potentiometric titration in ethylenediamine (*6*). Total oxygen was determined by a modified Unterzaucher method (*5*). In every case the Unterzaucher values agreed well with the total functional oxygen obtained by adding the values for carbonyl, water, methylol, and phenolic hydroxyl. The alcoholic hydroxyl is determined as the difference between the total hydroxyl and the phenolic hydroxyl. In the resins studied, the alcoholic hydroxyl should equal the methylol content. Thus an estimate of the dibenzyl ether content is obtained as the difference between the alcoholic hydroxyl values and the methylol plus dibenzyl ether values. It is recognized that this computation would be in serious error in preparations containing much water, owing to the variable reaction of water with lithium aluminum hydride (*9*).

The presence of bis(2-hydroxyphenyl)methane linkages would introduce an error in this calculation, as such compounds are hydrogen bonded internally and only partially titratable in ethylenediamine (*6, 21*). However, the concentration of this type of structure is expected to be low in resins based on phenol or phenols unsubstituted in the para position made under alkaline conditions. Megson (*14*), in a study of the acid-catalyzed reaction of phenol and formaldehyde, was unable to isolate bis(2-hydroxyphenyl)methane, although he did obtain 2,4′- and 4,4′-dihydroxydiphenylmethanes, isomers which behave normally upon titration. Freeman (*8*), in the kinetic study of the alkaline-catalyzed reaction, showed the formation of 3,3′,5,5′-tetramethylol-4,4′-dihydroxydiphenylmethane but did not report the 2,2′-dihydroxy isomer.

ACKNOWLEDGMENT

The authors express their appreciation to R. W. Martin, D. B. Luten, and C. G. Schwarzer for advice and for materials, and to R. J. Shreve and A. P. Poenisch, who carried out a major portion of the experimental work.

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RECEIVED for review November 22, 1954. Accepted November 7, 1955.

Periodate Test for the Catechol and Hydroquinone Structures

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Sodium periodate solution is used for the detection of the catechol structure in organic compounds. Results with selected structures are tabulated, the identity of the crystalline product from affirmative reactions is established, and a reason for the specificity postulated.

A NEED arose in a structural investigation for a method of determining the location of phenolic hydroxyl groups present in a complex molecule. Contradictory results had been obtained when various standard tests for the detection of vicinal dihydroxy groups were applied. The action of periodic acid on a molecule containing adjacent dihydric groups results in a fission at this point with the conversion of each carbinol group into a carbonyl group (*3*). In an extension of this procedure to aromatic dihydric phenols, a method was devised which would impart limited oxidation ability to the periodate but still enable a reduction of the periodate group to be detected readily.

Table I. Action of Sodium Metaperiodate on Selected Organic Compounds

Compound	Sample Wt., G.	Results	Color of Soln.
Rotenone	0.00985	−	Colorless
Osajin	0.01010	−	Pale yellow
Pomiferin	0.01050	+	Red
Pomiferin trimethyl ether	0.01155	−	Colorless
Quercitin	0.00846	+	Red, fading
Morin	0.00846	−	Did not dissolve
Catechol	0.00275	+	Pale yellow
Resorcinol	0.00275	−	Colorless
Hydroquinone	0.00275	+	Colorless
o-, *m*-, and *p*-cresol	0.00270	−	Colorless
Quinone	0.00272	−	Pale red
Quinhydrone	0.00546	+	Red-brown
p-Aminophenol	0.00273	+	Maroon
o- and *p*-phenylenediamine	0.00272	+	Red
m-Phenylenediamine	0.00272	−	Red
1-Naphthol	0.00360	−	Colorless
Benzoic acid	0.00305	−	Colorless
Gallic acid	0.00470	+	Tan
Blank	...	−	Colorless

The addition of a 0.25*M* aqueous solution of sodium metaperiodate to the substance dissolved in methanol resulted in the formation of a crystalline precipitate within 0.5 hour. This precipitate could have resulted from the reaction of the periodate with any adjacent hydroxyl groups present; therefore, the same procedure was applied to various other compounds known to contain this structure and to some which did not. Only those substances with a catechol or hydroquinone grouping in the molecule showed a similar reaction. Of especial interest are the results obtained with osajin and pomiferin (*5*), two complex isoflavones structurally similar, except that pomiferin possesses an additional hydroxyl adjacent to one already present in osajin. Pomiferin alone gave the characteristic positive test.

No obvious mechanistic analogy can be made to the Malaprade reaction (*3*) since a nonvicinal diphenol, hydroquinone, gives a positive test. Probably the reaction involves other types of oxidation such as quinone formation or oxidative coupling. The presence of iodine in an oxidized state in the crystalline precipitate was established by its liberation on addition of sodium iodide and hydrochloric acid. A negative periodate test (*2*) and a positive iodate test (*1*) were obtained together with a flame test for sodium. X-ray diffraction analysis gave identical patterns with the precipitate and an authentic sample of sodium iodate, recrystallized from water.

The specificity of this test apparently depends on the relative degree of solubility in methanol of the periodate and iodate salts; the less soluble iodate salts drop out of solution when formed. The lower oxidation potentials of *o*-dihydric phenols (*4*) may offer an explanation for their preferential susceptibility to attack by periodate under the experimental conditions.

EXPERIMENTAL

An amount equal to 0.000025 mole of each of the compounds in Table I was dissolved in 10 ml. of dry methanol, then 0.00025 mole of sodium metaperiodate was added (1 ml. of 0.25*M* aqueous solution). The crystalline material was deposited within 0.5 hour except with hydroquinone, where an additional time was required. Under the same conditions, ethylene glycol and glycerol immediately deposited a white amorphous precipitate; D-glucose formed needles when the mixture was allowed to stand overnight.

Occasionally, after the reagents had been mixed, a trace of amorphous material appeared in the solution. After removal by filtration the tests appeared normal. From a reaction with catechol 7.5 mg. of crystalline precipitate was collected. A theoretical yield of 9.7 mg. is indicated for an initial reaction between sodium periodate and catechol on a 2 to 1 basis.

CONCLUSION

A simple, rapid, and accurate method has been developed which can be used for the detection of the catechol or hydroquinone structures in molecules of relatively high complexity. The use of small amounts of sample makes its application suitable as a tool for work in the structure of natural products. Its true value, however, can be realized only when the test is used in conjunction with elemental analysis and functional group determination, because these procedures obviate consideration of similarly placed amino groups which give identical tests (see Table I).

ACKNOWLEDGMENT

This work was initiated at The Ohio State University. The author is indebted to M. L. Wolfrom for valuable suggestions and to Alva Thompson for the x-ray diffraction patterns.

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RECEIVED for review May 31, 1955. Accepted September 23, 1955.

Polarographic Determination of 2-Cyanopyridine

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A precise and accurate method is described for the polarographic determination of 2-cyanopyridine. The optimum pH range for the determination is between pH 5 and 6.

THE formation of 2-cyanopyridine by the addition of cyanogen to butadiene at moderately high temperatures has been reported by Janz and coworkers (*1–3*). Owing to the nonbasic nature of 2-cyanopyridine, the conventional aqueous and nonaqueous potentiometric titration methods were not applicable for determination of the product yields. The present note describes a study of the polarography of 2-cyanopyridine as a method for the quantitative determination of this compound.

EXPERIMENTAL

The 2-cyanopyridine (Aldrich Chemical Co., Inc.) was purified by fractionation in a Podbielniak semimicro distillation column. The fraction collected at 104° to 105° C. (10 mm.) was used for the polarography; (melting point, 27.0–27.50°; literature (*1*) 29°, 26° C.; n_D^{25} 1.5221).

Table I. Results of Polarography of 2-Cyanopyridine

Concentration of 2-Cyanopyridine, $M \times 10^4$	pH	$E_{1/2}$, Volts *vs.* S.C.E.	Wave Height, μa.	Wave Height, mM
12.4	5.65	−1.35	17.60	14.21
9.29	5.65	−1.35	12.84	13.82
6.2	5.65	−1.35	8.20	13.23
2.32	5.65	−1.35	3.28	14.14
12.4	6.2	−1.36	16.00	12.90
9.29	6.2	−1.36	11.52	12.40
4.65	6.2	−1.36	4.72	10.15
2.32	6.2	−1.36	2.76	11.90
9.29	7.1	−1.39	10.48	11.28
4.65	7.1	−1.39	5.44	11.70
2.32	7.1	−1.40	2.32	10.00
9.29	7.4	−1.41	10.48	11.28
4.65	7.4	−1.41	4.60	9.89
2.32	7.4	−1.42	1.68	7.24

The preparation of the polarographic solutions was carried out by making an approximately 10^{-2} solution of 2-cyanopyridine in ethyl alcohol. This solution was used as a bulk solution for the preparation of the solutions used in the polarography. Aliquots of this bulk solution were added to 50 ml. of a prepared aqueous buffer solution (0.2M potassium dihydrogen phosphate) and made up to 100 ml. with ethyl alcohol. Small amounts of concentrated sodium hydroxide solution were added to attain the desired pH.

The polarograph used was a Sargent pen recording instrument, Model XXI. The polarographic solution was thermostated at 25° ± 0.1° C. and the solution was deoxygenated by passing a stream of nitrogen through for 15 minutes. The characteristics of the capillary used were a drop time of 2.4 seconds and a mass of mercury of 3.06 mg. flowing per second into distilled water on open circuit at a mercury height of 56.2 cm. The applied potential is referred to the saturated calomel electrode as zero volts.

RESULTS AND DISCUSSION

The results for the polarography of 2-cyanopyridine obtained at various concentrations and at different pH values are summarized in Table I, and the three typical polarograms to illustrate the type of wave are shown in Figure 1. Inspection of these data shows that both the half-wave potential and the wave height are dependent on the pH of the solutions. Application of this method to the determination of 2-cyanopyridine requires a knowledge of the pH of the solution. The present work indicates that the optimum pH range for the purpose is between 5 and 6. At the higher pH, the 2-cyanopyridine wave coalesces with the buffer wave, making an accurate determination of the wave height more difficult.

Comparison of these results with the polarography of pyridine and related compounds is of interest. From the data for pyridine and nicotinamide reported by Knobloch (*5*), an extremely high value for the ratio of wave height to concentration is obtained (270 and 50, respectively). The value for picolinic acid, estimated from the work of Tompkins and Schmidt (*6*) is much lower (14). Reference to Table I shows that the ratio for 2-cyanopyridine is more nearly in accord with that for picolinic acid than for pyridine and nicotinamide. Interpretation of the exact electrode mechanism must await further investigation.

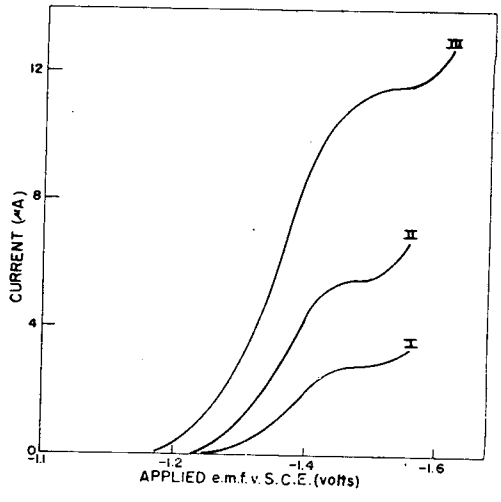

Figure 1. Typical Polarograms

An illustration of the precision and accuracy of the polarographic method for 2-cyanopyridine is given by the results summarized in Table II. In each case a weighed sample (0.2413 gram) of purified 2-cyanopyridine was analyzed as an unknown. The results leave little to be desired in this method for the quantitative determination of 2-cyanopyridine. This is of especial interest, since, owing to the nonbasic properties of 2-cyanopyridine, the conventional volumetric methods are not applicable. In the application of this method of 2-cyanopyridine determina-

Table II. Polarographic Determination of 2-Cyanopyridine

pH	Wave Height, μa.	2-Cyanopyridine Given, gram	2-Cyanopyridine Found, gram	Purity, %
5.65	16.40	0.2413	0.2408	99.8
7.40	12.95	0.2413	0.2423	100.4

tion of an unknown mixture, it is advisable to separate the 2-cyanopyridine fraction by distillation as described elsewhere (*4*).

ACKNOWLEDGMENT

Support of this work by a grant-in-aid from the National Science Foundation is gratefully acknowledged. The purchase of the Sargent pen recording polarograph was made possible through an apparatus grant to one of the authors (R.A.O.) by Trustees Research Committee, Rensselaer Polytechnic Institute. The authors wish to thank S. C. Waite, Jr., for assistance in some of the measurements.

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RECEIVED for review June 1, 1955. Accepted November 10, 1955.

Colorimetric Ninhydrin Reaction for Measurement of Alpha-Amino Nitrogen

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The photometric ninhydrin reaction of Cocking and Yemm can be applied safely to the measurement of free amino nitrogen in tungstic acid filtrates of plasma. Excess of ninhydrin or of cyanide reagent exerts a deleterious effect on color development, as does the presence of peroxides. Metal salts were not found to interfere when a citrate buffer was employed. Other protein precipitants tested were not found satisfactory. Arterial plasma from normal fasted dogs gave an average value of 4.91 mg. per 100 ml.

COLORIMETRIC measurement of α-amino nitrogen by means of the purple color formed on reaction with ninhydrin was originally proposed by Harding and MacLean (*2*), and first placed on a sound quantitative basis by Moore and Stein (*5*). This procedure has undergone numerous improvements in recent years. The use of reducing agents such as stannous chloride (*5*), ascorbic acid (*10*), potassium cyanide (*1, 9, 11*), or reduced ninhydrin (*6*), and addition of various organic solvents such as pyridine (*9, 10*) or methyl Cellosolve (*1, 5*) to the reaction mixture result in uniform yield of the same colored product for equimolecular amounts of almost all of the α-amino acids. An adequate buffer, generally a citrate buffer at pH 5 (*1, 4, 5*), permits use of the method with biological fluids or protein hydrolyzates. The method of Cocking and Yemm (*1, 11*), which incorporates all these features, has been employed in this laboratory because of its simplicity and accuracy.

INFLUENCE OF PEROXIDES

Yemm and Cocking (*11*) specify that the methyl Cellosolve employed should give a faint or negative peroxide test with 10% potassium iodide. The importance of this warning is shown in the following observations. After a long period of successful use of the method as described by the authors, it was found that the reference standard solutions of pure amino acids had begun to yield progressively less color. This decrease became increasingly rapid, until after a month the absorbance was only 15% of its usual value. At first, increasing the amount of ninhydrin reagent above the recommended quantity of 0.2 ml. restored the full color yield, but later, amounts of 1.5 or even 2.0 ml. failed to do so.

The methyl Cellosolve used in preparation of the ninhydrin and cyanide reagents had been originally redistilled and stored in a dark bottle. It was now found, however, to have developed a high content of peroxide as indicated by liberation of free iodine from acidified potassium iodide solution, decolorization of black oxide of nickel, Prussian blue test, and blackening of metallic mercury. Tests for reactive carbonyl groups were negative. After distillation from a zinc-copper couple the Cellosolve was free of peroxide, and reagent solutions made with it gave the usual full color yield in the ninhydrin reaction.

Hydroquinone added to the stock reagents in concentrations of 0.001 to 0.01% does not impair the color reaction, and may serve to prevent peroxide formation. Storage of the Cellosolve under nitrogen, or in small filled dark bottles, is probably also advisable.

INFLUENCE OF CYANIDE CONCENTRATION

In view of the harmful effect of peroxides, the influence of variation in the amount of cyanide reagent was determined. Using replicate samples of a standard amino acid solution, the reaction was carried out with 0.5, 1.0, 1.5, and 2.0 ml. of $2 \times 10^{-4}M$ cyanide-Cellosolve reagent. All reaction mixtures were made to the same volume with methyl Cellosolve before heating. When peroxide-free reagents were employed, maximal color yield was obtained with all but the smallest amount of cyanide reagent. When the reagents were made with Cellosolve contaminated with peroxides, use of increasing amounts of cyanide

Table I. Effect of Added Substances on Colorimetric Ninhydrin Reaction

Added Material	Absorbance
None	0.208
Cobalt nitrate	0.202
Copper sulfate	0.248
Ferrous sulfate	0.206
Versene (satd. solution in 0.2*M* citrate buffer, pH 5)	0.213

Each reaction mixture contained 5.4 γ of α-amino nitrogen. Absorbance of each was determined by comparison with a reagent blank containing the same added material.

reagent resulted in a progressive increase in color yield, approaching maximal values with 2.0 ml. of cyanide reagent.

The use of 1.0 ml. of $2 \times 10^{-3}M$ cyanide reagent, however, resulted in a deep orange-brown color in the reagent blank, which grew steadily more intense over a 20-minute period following the heating. Amino acid reaction mixtures prepared with the $2 \times 10^{-3}M$ cyanide reagent showed a color which appeared to be the resultant of this orange-brown color and Ruhemann's purple. When compared photometrically with the blank, using the Evelyn 575 filter, the reaction mixtures showed a rapid progressive decrease in absorbance which was really due to the increase in the blank reading. This suggests that the excess cyanide reacts with ninhydrin, producing the orange colored substance with measurable absorbance at 575 mμ. Since less ninhydrin

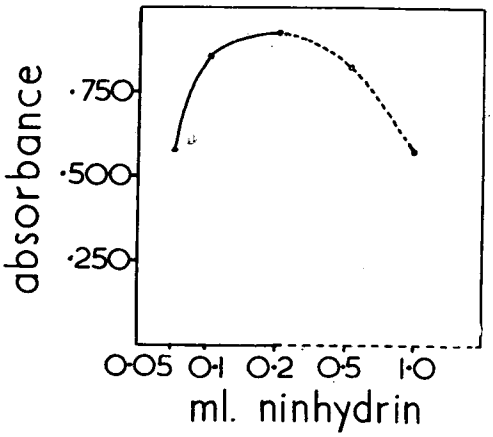

Figure 1. Effect of varying amounts of ninhydrin reagent on color development with 5 γ of α-amino nitrogen

remains in the test mixtures than in the reagent blank, this superimposed color is less, the larger the amount of amino nitrogen in the sample. Therefore, proportional readings cannot be obtained in the presence of such an excess of cyanide.

INFLUENCE OF METALLIC CONTAMINANTS

Meyer and Riklis (*4*) have suggested that various cations impair the ninhydrin reaction with amino acids. The influence of such contaminants was tested in the Cocking and Yemm method. Milligram quantities of several salts and of Versene [(ethylenedinitrilo) tetraacetic acid] were added to standard reaction mixtures and corresponding blanks, prior to heating. The results shown in Table I indicate that in the presence of citrate buffer the added salts were without inhibitory effect; if anything, copper sulfate appeared to improve color development slightly. Versene was also without effect. Meyer and Riklis added the metallic contaminants to unbuffered reaction mixtures; the Versene which they used was dissolved in citrate buffer. It is possible, therefore, that the beneficial effect noted by them was due to the citrate buffer rather than to the Versene.

INFLUENCE OF NINHYDRIN CONCENTRATION

Using reagents made with peroxide-free methyl Cellosolve, a series of equal samples of standard aspartic acid solution and corresponding blanks were subjected to reaction with various amounts of ninhydrin reagent. All were made to the same volume with methyl Cellosolve before heating, so that the same water-Cellosolve ratio obtained in all tubes. Figure 1 shows the correlation between color yield and amount of ninhydrin. Amounts larger than the recommended 0.2 ml. of 5% solution were found to inhibit color development. Lea and Rhodes (*3*) have called attention to the inhibitory effect of an increased proportion of Cellosolve in the reaction mixture. In the present case, however, as the same water-Cellosolve ratio was present in all tubes, the inhibitory effects observed with larger amounts of ninhydrin reagent may be attributed to the excess of ninhydrin itself. No ready explanation is at hand for this fact, but the finding is in conformity with the suggestion of Moubasher (*7*) that the amine must be present in excess of the reduction product of ninhydrin, for maximal color yield. The inhibitory effect of larger quantities was not observed with ninhydrin reagent contaminated with peroxides. The peroxides appear to have so reduced the effective concentration of ninhydrin, that the effective quantities of reactant used were all on the ascending limb of the curve in Figure 1.

APPLICATION TO DEPROTEINIZED PLASMA

The ninhydrin reaction of Cocking and Yemm was applied to samples of plasma which had been deproteinized by ultrafiltration under positive pressure through a cellulose membrane, and precipitation with several protein precipitants. Ultrafiltration was found to yield very variable results, due to small quantities of protein escaping through the filter. This is in agreement with the findings of Stein and Moore (*8*). Ethyl alcohol, 1% tungstic acid, and 5% trichloroacetic acid, when added in 0.2-ml. quantities to standard reaction mixtures and blanks, did not appear to affect color development; 5% sulfosalicylic acid in the same amount inhibited it completely. When the former three agents were used for deproteinizing plasma, however, only tungstic acid proved satisfactory. Ethyl alcohol did not give complete deproteinization at concentrations low enough to permit most of the amino acids to remain in solution. Use of trichloroacetic acid resulted in the development of a sherry color, rather than the purple color normally given by amino acids.

Recovery of added amino acids from plasma samples precipitated with nine volumes of 1% tungstic acid was 97 ± 4% (extremes of range). Nine samples of arterial plasma from normal dogs in the postabsorptive state showed amino nitrogen concentrations ranging from 4.11 to 5.48 mg. per 100 ml., with a mean and standard deviation of 4.91 ± 0.45 mg. per 100 ml. The corresponding venous levels ranged from 4.03 to 5.49, with a mean and standard deviation of 4.72 ± 0.46 mg. per 100 ml.

ACKNOWLEDGMENT

This work was carried out during tenure of a Senior Medical Research Fellowship of the National Research Council of Canada.

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RECEIVED for review July 20, 1955. Accepted October 26, 1955.

103. Isovanillin (3-Hydroxy-4-methoxybenzaldehyde)

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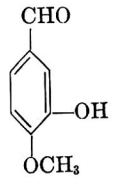

Structural Formula for Isovanillin

Good crystals of isovanillin consisting of 010 blades and 001 plates may be obtained by crystallization from water. Although the study of vanillin (*1*) showed several polymorphic forms, no polymorphism was found for isovanillin.

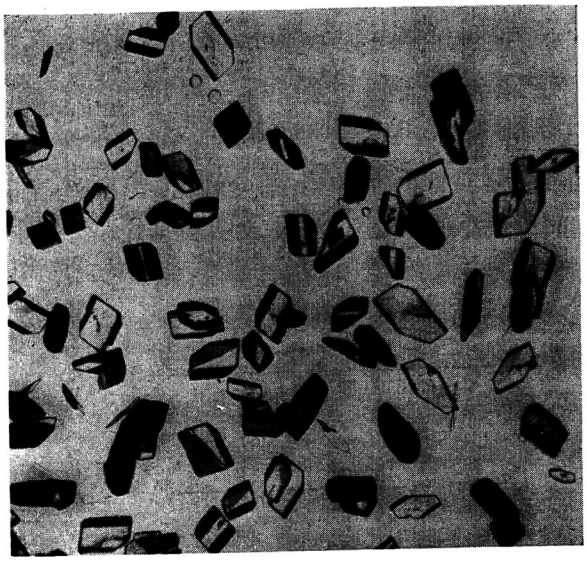

Figure 1. Sublimed crystals of isovanillin obtained on cover slip while heating a few milligrams of sample

The x-ray powder diffraction data were obtained using a camera 114.6 mm. in diameter with chromium radiation and vanadium filter. A wave-length value of 2.2896 A. was used in the calculations.

The determination of the space group as $P2_1/a$ was based on a and c axis rotation patterns and a zero layer Weissenberg pattern with b axis rotation. These patterns showed that $h0l$ was present only with h even and that $0k0$ was present only with k even.

Crystal Morphology

Crystal System. Monoclinic.

Form and Habit. Blades lying on 010 elongated parallel to a and plates lying on 001. The blades are terminated by the prism {110}.

Axial Ratio. $a:b:c = 0.6341:1:0.4791$.

Interfacial Angle (Polar). $110 \wedge 1\bar{1}0 = 64° 20'$ (x-ray).

Beta Angle. 97° 30′.

Cleavage. The crystals show very good cleavage parallel to the 201 plane.

Twinning. 101.

X-Ray Powder Diffraction Data

d	I/I_1	hkl	d(Calcd.)
7.12	0.20	$\bar{1}10$	7.14
6.71	1.00	020	6.71
6.37	0.20	001	6.37
5.26	0.33	120	5.25
5.02	0.07	$11\bar{1}$	5.04
4.62	0.03	021	4.62
4.22	0.03	200	4.22
4.04	0.03	210	4.03
3.91	0.27	130, 121	3.95, 3.90
3.66	0.07	031	3.66
3.46	0.07	$13\bar{1}$	3.45
3.33	0.20	201	3.32
3.24	0.67	211	3.23
3.12	0.13	$14\bar{0}$	3.12
3.03	0.03	$11\bar{2}$	3.04
2.817	0.03		
2.652	0.07		
2.511	0.03		
2.473	0.03		
2.226	0.07		
2.093	0.03		
2.012	0.03		
1.903	0.07		

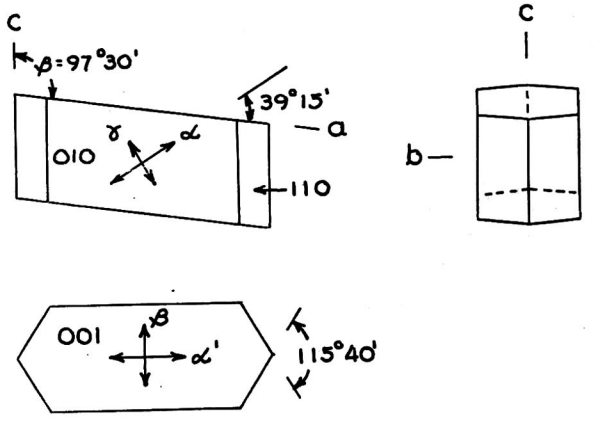

Figure 2. Orthographic projection of isovanillin

X-Ray Diffraction Data

Cell Dimensions. $a = 8.51$ A., $b = 13.42$ A., $c = 6.43$ A.

Formula Weights per Cell. 4.

Formula Weight. 152.1.

Density. 1.401 grams per cc. (displacement), 1.390 grams per cc. (x-ray).

Space Group. $C_2^5h - P2_1/a$.

OPTICAL PROPERTIES

Refractive Indices (5893 A., 25° C.). $\alpha = 1.450$, $\beta = 1.680$, $\gamma = 1.97$.

Optic Axial Angle. $2V = (+)83°$ (calculated from α, β, and γ).

Dispersion. Strong, $r > v$.

Optic Axial Plane. 010.

Acute Bisectrix. α.

Extinction. $\alpha \wedge a = 39° 15'$ in obtuse β.

FUSION BEHAVIOR. Isovanillin melts at 114–5° C. with sublimation but no decomposition. The sublimate is well formed (Figure 1) and shows plates lying on 010 with {110}, {101}, {100}, and {011}. The melt solidifies spontaneously with slight supercooling to give large blades with high birefringence and an occasional optic axis interference figure [$2V = (+)85°$, $r > v$].

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104. D-Fructose 2,4-Dinitrophenylhydrazone Dioxane Solvate

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THE preparation of D-fructose 2,4-dinitrophenylhydrazone dioxane solvate ($C_{12}H_{16}N_4O_9 \cdot C_4H_8O_2$) has been described by White and Secor (*1*, *2*). Their yellow crystalline preparations were used in this study. Fresh preparations were used in order to avoid a surface film of decomposed material. Fresh surfaces obtained by fracturing the crystals could be used for refractive index determinations. The tips appeared different from the blade in some cases.

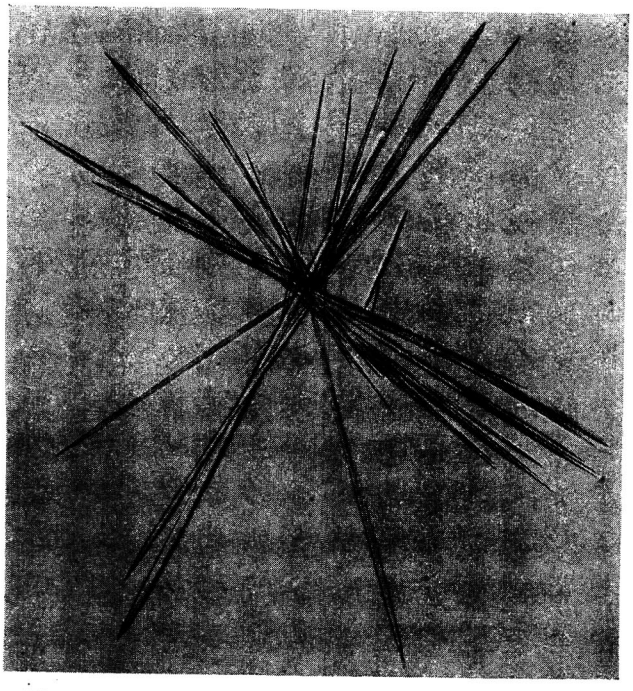

Figure 1. D-Fructose 2,4-dinitrophenylhydrazone dioxane solvate (200×)

A crystal mounted on a goniometer head was used for x-ray diffraction. The same mounted crystal was used for the observation of the relationship between crystallographic axes and optical properties. In spite of many attempts to grow large crystals, the crystal faces were always too small to give observable reflections on the optical goniometer.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic, Class 6 bisphenoidal, enantiomorphic.

Form and Habit. Loose clusters of slender blades or needles tapering to a sharp point, often striated and splintery. Figure 1 shows crystals grown from dilute solution on a slide (*1*). The elongation is parallel to *c*. Except for {010} the forms shown in Figure 2 are inconspicuous. Twinning was not observed.

Interfacial Angles. No angles could be measured, although the cross section shown in Figure 2 is approximately correct.

X-RAY DIFFRACTION DATA

Space Group. Probably $D_2^4 - P2_12_12_1$, but possibly $D_2^3 - P2_12_12$, since only the first four orders of $00l$ observed. Also observed first 14 orders of $h00$ and first 10 orders of $0k0$.

Cell Dimensions. $a = 20.669$ A., $b = 20.788$ A., $c = 4.788$ A.

Axial Ratio. $a:b:c = 0.9943:1:0.2303$.

Formula Weights per Unit Cell. 4(4.061 calculated).

Formula Weight. 448.38.

Density. 1.4693 grams per cc. (density gradient tube).

OPTICAL PROPERTIES

Refractive Indices (5893 A.; 27° C.). $\alpha = 1.593 \pm 0.001$. $\beta = 1.607 \pm 0.002$. $\gamma = 1.872 \pm 0.004$.

Optic Axial Angles (5893 A.; 27° C.). $2E = 42 \pm 2°$; $2V = 26°$ calculated from $2E$ and β; $2V = 29°$ calculated from $\alpha\beta\gamma$.

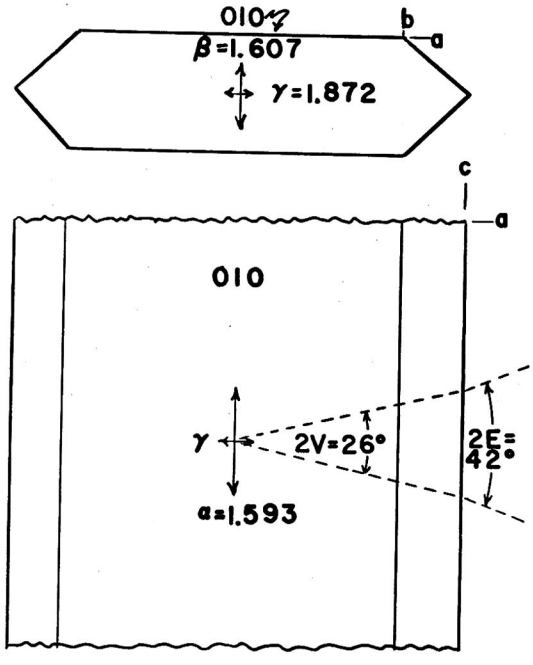

Figure 2. Orthographic projection of typical crystal of fructose 2,4-dinitrophenylhydrazone dioxane solvate

X-Ray Powder Diffraction Data

(The camera radius was 7.181 cm. λ for CuKα = 1.5418 A. and a nickel filter was used. The relative intensities were visually determined with a calibrated intensity scale)

d	I/I_1	d	I/I_1	d	I/I_1	d	I/I_1
14.66	0.09	4.14	0.08	2.924	0.05	2.030	0.02
10.38	0.35	4.04	0.20	2.849	0.09	1.988	0.02
7.31	0.06	3.94	0.07	2.779	0.09	1.956	0.02
6.54	0.10	3.87	1.00	2.687	0.08	1.919	0.01
5.73	0.18	3.68	0.30	2.589	0.06	1.862	0.02
5.21	0.04	3.55	0.10	2.506	0.06	1.795	0.02
5.03	0.04	3.46	0.20	2.447	0.02	1.729	0.01
4.88	0.23	3.33	0.08	2.370	0.04	1.695	0.01
4.67	0.20	3.23	0.02	2.320	0.02	1.659	0.01
4.54	0.20	3.14	0.25	2.261	0.08	1.617	0.01
4.36	0.20	3.08	0.11	2.163	0.06	1.572	0.01
4.25	0.35	2.997	0.11	2.070	0.06		

Dispersion. ($v>r$) strong.
Optical Character. (+).
Optic Axial Plane. (010). Fragments usually lie on (010) and show an optic normal interference figure.
Acute Bisectrix. γ.
Pleochroism. Slight, γ orange yellow, α greenish yellow.
FUSION. When crystals on a slide are warmed to about 150° C. they become opaque and orange in color. Continued heating with a cautery needle held close to the cover glass causes the crystals to melt with slight bubbling to an amber liquid. Overheating will cause darkening. If the melt is kept warm, unmelted crystal fragments will seed regrowth of hairs and needles. Unseeded drops of warm melt will develop "isotropic" spherulites which resemble the "gel" reported by White and Secor (*2*). These spherulites appear predominantly around the margins of the melt.

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CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Research, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

SCIENTIFIC COMMUNICATION

Analysis of Phosphorus Compounds

Use of Nuclear Magnetic Resonance Spectra in Differential Determination of the Oxyacids of Phosphorus

STUDIES are now under way on developing the nuclear magnetic resonance technique (*3*) as a qualitative and quantitative analytical tool in phosphorus chemistry. Nuclear magnetic resonance is a rapid and elegant method for carrying out chemical analyses; and, as this work on mixed oxyacids of phosphorus demonstrates, the nuclear magnetic resonance method offers a fresh approach to analytical problems for which wet-chemical procedures have not been satisfactory. This technique is unique as an analytical tool, in that there can be no interference from compounds of any element other than the one under study, as long as the substances being studied are not precipitated from solution.

It has been pointed out (*6*) that isolated PO_4 ions, end PO_4 groups, and middle PO_4 groups in the phosphates (*4, 5, 8*) give definite, resolved resonance peaks. This means that all mixtures of orthophosphates, chains, and rings (*7*) can be characterized in terms of the relative number of moles of phosphorus pentoxide present as orthophosphates, end groups, and middle groups (*4*). Nuclear magnetic resonance can be used equally well to identify anions of the phosphorus oxyacids of lower oxidation states. Three resonance peaks are found for hypophosphite, two for ortho- or pyrophosphite (which cannot be differentiated by this method), and one for hypophosphate, in accordance with their structures (*1, 4*).

In Table I, the anions of the various oxyacids of phosphorus are listed in the order of their chemical shift. The intensity factor shown indicates the relative area (and to a rough approximation, the height) of the resonance peak for equal numbers of phosphorus atoms. Thus, at equal molar concentrations the hypophosphite peak at 133 p.p.m. shift is only one quarter of the area of the orthophosphate peak with a shift of 100 p.p.m. under the same experimental conditions. (Practical measurements are conveniently made with respect to orthophosphoric acid, and this compound is therefore used as a reference for a 7140-gauss field. A value of +100 p.p.m. is arbitrarily added to the shifts measured with respect to orthophosphoric acid in order to convert all shifts to positive values. The chemical shifts between the various oxyacids of phosphorus are precise to ca. ±2 p.p.m. and can be made still more precise if the need arises.) Even though

Table I. Nuclear Magnetic Resonance Peaks for Oxyacids of Phosphorus

Anion	Chemical Shift[a], P.P.M. ± 2 P.P.M.	Relative Intensity 1 0.5 0	Scale[a], P.P.M.
Hypophosphite	133		140
Phosphate middle groups and ortho- and pyrophosphites	120		120[b]
Phosphate end groups	109		
Orthophosphate	100		100
Hypophosphate	91		
Hypophosphite	87		80
Ortho- and pyrophosphites	64		60
Hypophosphite	42		40

[a] Measured in p.p.m. of a 7140-gauss field with a probe resonating at 12.3 mc. Reference substance from which the shifts are measured is 85% orthophosphoric acid. The position of the orthophosphoric acid reference standard was arbitrarily defined as +100 on the p.p.m. scale of chemical shifts. Other chemical shifts are then defined by the relation $\delta = 100 + 10^6 \times (H - H_{H_3PO_4})/H_{H_3PO_4}$.
[b] Phosphate middles shown as black bar and phosphites as cross-hatched bar.

the peak for phosphate middle groups and one of the peaks for phosphite (ortho- or pyro-) exhibit the same chemical shift of 120 p.p.m., it is possible to determine both of these species in a mixture by using the second phosphite peak at 64 p.p.m. to determine the relative amount of phosphite and subtracting this value from the relative amount of the middle group plus phosphite as determined from the 120 peak. The other case where interference is possible is between the hypophosphate and hypophosphite, but here again another peak for the hypophosphite at either 42 or 133 p.p.m. can be used.

The relative concentrations of the different phosphorus-containing anions listed in Table I are determined by measuring the area under the respective peaks. So far we have measured relative amounts in various mixtures with an accuracy within 2 to 10%. These relative values can be converted to absolute quantities by adding a known amount of one of the phosphorus-containing anions to the solution being analyzed. However, the easiest procedure is to determine total phosphorus by one of the standard procedures (such as a molybdate titration following conversion of all of the phosphorus to the orthophosphate form). At the present stage of the art, analyses by nuclear magnetic resonance are applicable only to relatively concentrated solutions containing at least a few moles of phosphorus per liter. This restricts this method of analysis to the acids, alkali metal, and ammonium salts in most cases. However, cations causing precipitates can be removed prior to analysis by ion exchange resins (*2*); and concentration by evaporation has proved feasible. In some cases, it is convenient to make the spectral measurements on supersaturated solutions.

The spectrometer used in these studies was a high-resolution instrument manufactured by Varian Associates (*9*), equipped with a 12.3-mc. probe. By using a 17.2-mc. probe and a 10,000-Gauss field, the resolution and signal-to-noise ratio should improve substantially, and give much higher accuracy and less difficulty with interferences. Consecutive analyses can be carried out at the rate of approximately 12 samples per hour. The resonance measurement itself takes less than a minute.

A detailed paper on this subject will be published as soon as certain effects now under study are precisely evaluated. These effects include a slight, theoretically predicted, shift of the resonance peak with pH, and the interference between phosphate middles and pyrophosphite, as well as the interference between hypophosphate and hypophosphite. Substitution of nitrogen for oxygen in the inorganic acids seems to cause very little shift in the resonance peak. Thus, mono- and diamido-orthophosphates resonate very close to the regular orthophosphate ion.

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RECEIVED for review August 19, 1955. Accepted November 7, 1955.

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MEETING REPORT

Society for Analytical Chemistry

AT A joint meeting of the Scottish Section with the Stirlingshire Sections of the Royal Institute of Chemistry and the Society of Chemical Industry, held at Grangemouth on November 2, the following lecture was given.

Some Industrial Applications of Ion Exchange Materials. T. R. E. KRESSMAN, Permutit Co., Ltd., London.

Ion exchange resins are now widely used in industry. The major use is still in the field of water treatment, but the high capacity and stability of the modern resins based on polystyrene have enabled them to be used in many fields other than water treatment—in metal finishing, in glycerol and gelatin manufacture, and in the manufacture of antibiotics.

The conventional method of demineralizing water and solutions of nonelectrolytes is with a column of cation and a column of anion exchanged material in series. More recently, the mixed-bed technique has been applied, in which the liquid is flowed through a single column of an intimate mixture of the two exchangers.

Brackish waters, and solutions of high electrolyte content, cannot be economically treated by the column method. A very recent process whereby they can be desalted economically makes use of an electric current in a multicompartment electrodialysis cell in which cation and anion exchange membranes are arranged alternately between a pair of electrodes. Much development work still needs to be done on this, however, before it becomes a commercial possibility.

At a meeting of the Midlands Section held in Nottingham October 25 a lecture demonstration on ring-oven technique was given by H. Weisz, Technical University, Vienna, winner of the Feigel Prize, 1955.

The ring-oven technique is a simple method for separating ions, or groups of ions, in one single drop. An apparatus called the ring oven is designed to wash soluble materials out from a spot on a filter paper and to concentrate them in a sharply bounded circular ring zone, where they can be detected. Some other pieces of equipment have been developed for this purpose. With the aid of this method an analytical scheme for 14 commoner ions has been worked out; one drop of about 1.5 μl. is sufficient for the analysis. The method has also been employed for ring colorimetric analysis.

The Physical Methods Group met jointly with the Western Section at Bristol University on October 28. The following papers were presented and discussed.

X-Ray Analysis of the Structure of Vitamin B_{12}. DOROTHY CROWFOOT HODGKIN.

Since the isolation of crystalline vitamin B_{12} 7 years ago, we have used x-ray diffraction methods to assist in finding the chemical structure of the vitamin. Our examination has been closely interlocked with chemical investigations carried out in a number of laboratories. These showed that the vitamin had approximately the formula $C_{61-64}H_{83-92}N_{14}O_{13-14}PCo$, and contained a CN group, a nucleotide-like group built of benziminazole, ribose, and phosphate units, a

propanolamine residue, and probably six amide groups attached to a large remaining nucleus of unknown structure containing about 45 carbon atoms.

We have found it possible to discover the arrangement of the atoms in this nucleus through the calculation of the electron density distribution for four different crystals, air-dried and wet B_{12} crystals, B_{12}.SeCN, and a hexacarboxylic acid degradation product of B_{12} prepared by Cannon, Johnson, and Todd. In these calculations we have used phases for the terms in the Fourier series employed based on, first, the cobalt atoms, and then on gradually increasing known regions of the structure; the correct atomic positions can be selected from among the many spurious peaks that appear in the first approximate calculated distributions. The whole project has required very heavy computing which has been carried out partly in this country, partly in America.

We can now write a tentative complete structure for the vitamin. The inner nucleus is found to have interesting and unexpected structural relations with porphyrins. The molecule as a whole is very compact; a number of small chemical modifications can be made in its structure, which leave unchanged its ability to form crystals with essentially similar molecular arrangement.

X-Ray Fluorescent Quantitative Analysis as a Tool in Archeology. E. T. HALL.

During the past few years in America the use of x-rays for chemical analysis has been investigated, and in one instance a commercial apparatus developed for use in industrial applications. In this country, however, very little work has been done in this direction.

This paper described an apparatus which has been developed for use in archeology and kindred subjects. A knowledge of the composition of artifacts can help to elucidate problems concerning not only their genuine attribution, but also trade routes and methods of manufacture in the ancient world. The possibility of using this method for routine laboratory and industrial applications was discussed, giving some idea of the advantages and limitations of x-ray analysis.

X-Ray Diffraction Techniques in the Investigation of Crime. E. B. PARKES.

The employment of x-ray diffraction analyses—a further step towards actual identification in criminological investigations.

A MEETING organized by the Biological Methods Group was held November 22 in London to discuss "The Evaluation of Antifungals."

Laboratory Evaluation of Drugs for Clinical Trial against Dermatomycoses. H. O. J. COLLIER AND G. K. A. SMITH, Allen & Hanburys, Ltd., Ware, Herts.

Dermatophytes—fungi causing superficial infection of skin or its appendages—present an important clinical problem in man and domestic animals. The laboratory evaluation of potential remedies is at present handicapped by the scarcity of suitable experimental dermatomycoses. In the absence of in vivo therapeutic tests, evaluation requires a variety of methods, particularly for studying skin toxicity, keratin penetration, and in vitro antifungal activity. In toxicological studies methods include intradermal injection and repeated application to the unbroken skin; for the latter test hairless mice are found suitable. Evaluation of in vitro antifungal activity includes tests of fungistatic and fungicidal action and of possible antagonism by materials such as hair and serum, with which the potential remedy is likely to come in contact. Aspects of the above methods were illustrated from experience, using new bis-quaternary antifungals.

Some Factors in the Planning of Fungitoxicity Experiments in the Laboratory. R. J. W. BYRDE AND G. M. CLARKE, University of Bristol, Research Station, Long Ashton.

Controlled hydrogen ion concentration is important in all laboratory fungitoxicity experiments, particularly when testing weak acids or bases. In general, such compounds are most toxic in the undissociated form, owing to greater ease of penetration of the lipophilic cell membrane. For valid comparison of inherent toxicity, they should be tested at a pH level where they are predominantly undissociated. The buffer chosen should not react with the test fungicides, while the culture medium should be the simplest which permits normal growth of the fungus. The performance of a fungicide may be profoundly modified by its physical form, or by the presence of a mutual solvent. Possible modifications in technique when testing volatile fungicides are discussed.

Laboratory experiments are carried out in incubators which themselves introduce sources of variation in the fungal material used. These variations must be examined and experiments so designed as to eliminate them from treatment comparisons. The results of fungicide tests are now never exactly reproducible, in that a standard substance will induce a slightly different response under identical conditions on different occasions. Inherent variability differs from fungus to fungus, and may influence the choice of test organism. Comparisons of two fungi in the same test are usually difficult.

Cattle Ringworm. Problems in the Evaluation of Treatment. K. C. SELLERS, Animal Health Trust Livestock Research Station, Stock, Essex.

Recent work (Ainsworth, G. C., and Austwick, P. K. C., *Vet. Rec.* 1955, 67, 99; Sellers, K. C., La Touch, C. J., and Sinclair, W. B. V., in preparation) has shown that *Trichophyton verrucosum* Bodin, var. *discoides* Georg, is the prevalent cause of cattle ringworm in this country. In Yorkshire the infection was found on a high proportion of farms, more commonly in calves in their first year of life and during the winter. Humans contract the infection, but no accurate estimate of the incidence in this species has been made. As a preliminary to work on control and treatment of cattle ringworm in the field, experimental observations have been carried out with the following results.

Healthy calves were infected artificially by rubbing natural material or culture suspensions into the unabraded skin. Lesions were detectable within 30 days and spontaneous recovery occurred in about 4 months, when no indication of infection could be detected. Recovered calves resisted reinfection. Histological studies showed that in the early stages of infection there was a prolific distribution of fungal mycelium in the extra- and intrafollicular stratum corneum. Later the follicular space around the hair was invaded and a loose mycelial network developed round the hair shaft. The mycelium then penetrated all parts of intrafollicular portion of the hair except the living bulb and a spore sheath formed both on the surface and in the medulla of the hair shaft. No tissues other than those in which keratin could be demonstrated histologically were invaded. The tissue response of the host resulted in the eventual separation of the stratum corneum from the underlying tissues.

These surveys and experimental studies have shown that although satisfactory in vivo trials might be performed in the artificially infected calf, the final and accurate assessment of an antifungal under field conditions presents many problems. Nevertheless, cattle ringworm lends itself to well planned clinical trials; not only is it widespread, but ethical and cosmetic considerations do not bulk so largely as in man. Also, it is possible that the results so gained would be applicable to the ringworms of other species.

AT A MEETING of the Midlands Section on November 9 in Birmingham a discussion on spectrophotometric titrations was opened by R. A. Chalmers, Durham University, and S. J. Clark, British Nylon Spinners, Pontypool, Mon.

Mr. Chalmers. It is possible to classify spectrophotometric titrations according to the component of which the extinction is measured, and to predict from theory the conditions necessary for a satisfactory end point to be obtained. Spectrophotometric titrations seem to be particularly suitable for development as automatic or semi-automatic methods.

Mr. Clark. Some advantages of spectrophotometric detection of the end point of a titration were considered and practical examples were described. Milligram amounts of weak organic acids and bases may be titrated accurately and precisely by this technique. The method is in routine use for the determination of neutralisation equivalents of such compounds. The ultramicrodetermination of nitrogen may be achieved by Kjeldahl digestion followed by oxidimetric determination of the ammonia formed. Spectrophotometric titration allows the use of solutions as dilute as $10^{-4}M$. Turbidimetric and nephelometric titrations were considered; the nephelometric titration of potassium was described. The conversion of a commercial spectrophotometer was described, together with some of the difficulties which arise when this instrument is used for turbidimetric titration.

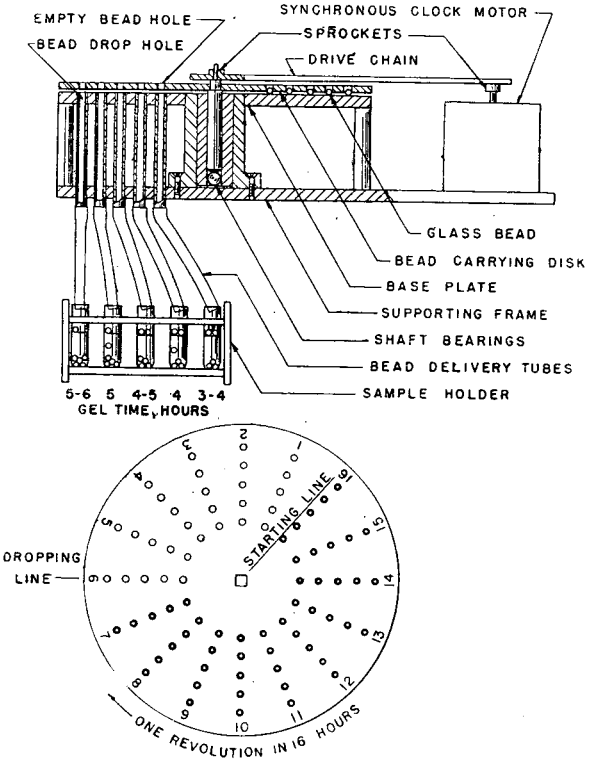
At a special meeting held November 29 in London, a lecture on the development of polarographic analysis was given by J. Heyrovský, Central Institute of Polarography, Prague.

The subject was treated under the following main headings: types of mercury capillary electrodes; direct and indirect polarographic determination; conversion of inactive substances into depolarizers; subtractive and derivative polarography; continuous recording of the mean current or the current-voltage curves with special electrodes; the use of nonaqueous solutions; polarometric titrations; chromatopolarography; cathode-ray polarographs; the electronic polaroscope based on potential-time curves produced with alternating current.

Apparatus for Automatic Determination of Gel Time

John S. Billheimer and Richard Parrette,
Aerojet-General Corp., Azusa, Calif.

In the course of development and control work on bulk polymerization in this laboratory, the need arose for automatic determination of the gel time of many samples. Although a practiced observer can duplicate gel times precisely by manually probing the polymerizing mixture from time to time, the end point is subjective and the method is inconvenient when the gel time is long. An automatic probing machine which drives a wire loop through the sample in a reciprocating motion (*4*) is suitable for polymerizations characterized by rapid formation of the gel structure, but with slowly reacting systems, where the gel attains strength only gradually, the reciprocating probe tears the gel structure as it forms. The probe then continues to move in this channel without indicating an end point. A torque-measuring probe or rotor operated in the polymerizing mixture likewise results in channeling, and is therefore of limited applicability.



The practice of determining gel point by measuring viscosities up to the point of maximum rate of increase in viscosity is general, and a wide variety of viscometers has been employed (*1–3, 5, 6*). Although the method serves to characterize the gelation thoroughly from a quantitative standpoint, the labor involved becomes prohibitive when applied to routine control determinations. Automatic recording adaptations of some of the viscosimeters for gel-time determinations are conceivable, but in general require expensive instrumentation.

A simple, inexpensive automatic gel-time apparatus has been developed which avoids many of the difficulties encountered in the methods discussed above. In principle, the apparatus periodically dispenses a glass bead to the surface of a polymerization sample contained in a vial suspended in a constant temperature bath. The dispensed beads sink to the bottom of the vial as long as the sample is fluid. After a gel has formed, subsequent beads are retained on the top surface of the sample. The gel time is thus indicated by the number of beads in the bottom of the gelled mass, each bead representing one time interval.

The apparatus, shown in the diagram, performs five gel-time determinations simultaneously. The upper or rotating disk is fabricated of $^1/_8$-inch aluminum sheet and contains five concentric rings of 16 or 24 equally spaced $^{13}/_{64}$-inch holes. The bed plate contains a single aperture for each of the five rings of holes in the rotary disk. The shaft for the rotary disk rotates within two Marlin-Rockwell Corp. K-5 ball bearings, mounted so that the rotary disk turns with a $^1/_{32}$-inch clearance from the stationary bed plate. A synchronous clock motor drives the rotary disk by means of suitable sprockets so as to bring a row of beads into dropping position every $^1/_3$, 1, or 3 hours as desired. The upper disk is loaded with 4-mm. glass beads in the holes. When the clock drive brings a radial row of five beads into coincidence with the apertures in the base plate, the beads fall through these apertures and are conducted by means of flexible tubing to the surface of the polymerization specimen. The flexible tubing permits positioning of the gel sample in a constant temperature bath or under an inert atmosphere as necessary for gelation of the resin under controlled conditions.

Typical gelation specimens into which beads were dropped at 1-hour intervals for 6 hours are shown in the diagram. If a bead is captured in fall, the gel time is reported as the time corresponding to the dispensing of the captured bead. In general, however, a bead is not captured in fall, and the gelation is known only to have occurred between the arrival of two sequential beads, so that the gel time is reported as the mean of the times for the last bead to sink through the resin and the first bead to be retained on the surface. With a suitable selection of sprockets and starting time of the apparatus, the gel time of slow polymerization processes can be measured to the nearest 10 minutes in 8 hours, the nearest 0.5 hour in 24 hours, or the nearest 1.5 hours in 72 hours, as the case may warrant. A limitation on the frequency of bead dispensing is the impracticability of counting a large number of beads within the gelled specimen. A preliminary run or general familiarity with the resin system will usually indicate the appropriate choice of bead interval and starting time of bead dropping.

One of the chief advantages of this instrument is the ease of disposing of the completed sample. More elaborate viscometers involve a difficult and impractical resin removal after each test. With the bead-dispensing apparatus, both the vial and the beads are inexpensive and expendable.

An example of the applicability of this apparatus to development studies in resin formulation is shown in Table I.

Table I. Effect of Inhibitor Concentration on the Gel Time of a Resin Copolymer

Concentration of Inhibitor, %	Gel Time, Hours
0.0075	3
0.020	12
0.040	28
0.080	64
0.150	100

Table II. Control Gel-Time Determinations on a Resin Copolymer

Bbl. No.	Gel Time, Hours		
	1st run	2nd run	Mean
A	6.0	6.0	6.0
B	5.5	5.3	5.4
C	4.1	3.9	4.0
D	4.8	5.0	4.9
E	3.5	3.5	3.5
F	6.2	6.2	6.2
G	3.5	3.5	3.5

It is evident that the apparatus is capable of measuring a broad range of gel times. Table II gives an example of a study of quality control, in which the gel times of resin from seven barrels were determined in duplicate.

The estimated standard deviation for a single determination from this series of duplicate determinations, calculated from the formula

$$\sigma_{est} = \sqrt{\frac{\Sigma(\Delta x)^2}{2m}}$$

where Δx is the difference between duplicate determinations, and m is the number of duplicate determinations, is 0.11 hour, or 2.3% of the average value of 4.8 hours.

ACKNOWLEDGMENT

This paper is based upon work performed for the Navy Department, Bureau of Aeronautics, under Contract NOa(s) 10146 with the Aerojet-General Corp.

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Multiple-Unit Fusion Rack

A. P. Marranzino and William H. Wood, U. S. Geological Survey, Denver Federal Center, Denver, Colo.

A MULTIPLE-unit fusion rack was developed in the U. S. Geological Survey to facilitate the fusion of approximately 20,000 soil and rock samples per year, preparatory to the determination of many trace constituents. Previously, a small sample was mixed with the appropriate flux in a borosilicate glass test tube, the fusion was made by heating in a rack over a Bunsen burner, and finally the tube was rotated manually as it cooled, to obtain a thin coating of the melt on the sides of the test tube to facilitate dissolving the product.

By means of the multiple-unit fusion rack, in use for the past year, 11 fusions can be made simultaneously. The equipment serves three purposes: It provides a uniform sample treatment to improve the precision of the analytical methods; it automatically rotates the cooling tubes so that the molten flux solidifies in a thin layer; and it saves much time and effort. The apparatus (shown in Figures 1 and 2) heats each test tube uniformly by constantly passing the tube, rotating on the axis of the unit, over a series of burners for a definite time. The rotation of each test tube keeps the sample in intimate contact with the molten flux to provide adequate sample treatment, and during the cooling cycle causes the melt to coat the wall of the tube with a thin layer of the fusion product as it solidifies. Neither of these procedures requires the attention of an operator; the operator is relieved of the manual procedure that was necessary prior to the development of the multiple-unit fusion rack.

CONSTRUCTION

A perspective dimensional drawing is shown in Figure 1. The entire unit is 23 inches long, 12 inches wide, and 16 inches high, and weighs approximately 45 pounds.

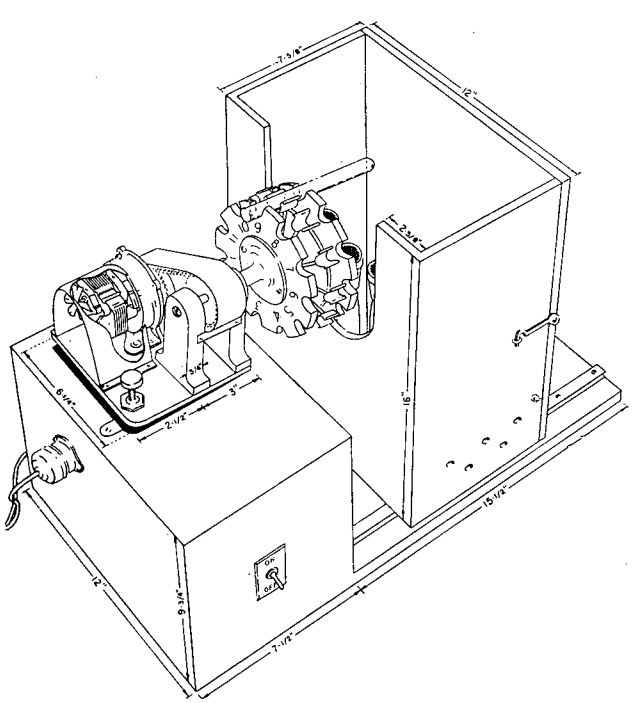

Figure 1. Drive mechanism

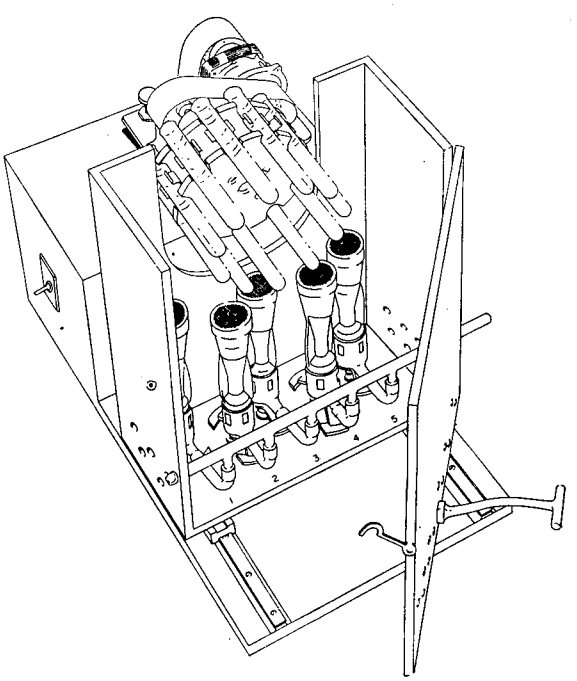

Figure 2. Burner assembly

The carriage assembly for holding the test tubes is rotated at 15 r.p.m. by a 1/75-h.p., 115-volt, alternating current, continuous-action, gear-reducing motor connected to the drive shaft of the carriage by a chain drive (a No. 1A steel ladder chain having a yield point of 37 pounds). A sprocket (20-teeth, 1.18-inch pitch diameter with a $^7/_8$-inch hub that has a $^5/_{16}$-inch hole) is mounted on the drive shaft of the motor and is connected by the chain to a sprocket of the same size between the aluminum bearing posts of the carriage. This arrangement gives the drive unit a 1 to 1 ratio; other ratios may be obtained by a suitable selection of sprockets and chains.

A fan is mounted on the motor and serves both to cool the

motor and to drive any corrosive fumes away from the unit. Safety covers are mounted over both the fan and the chain drive.

The carriage shaft is carried by two roller bearings. The carriage assembly is fastened to the end of the drive shaft by a brass flange and is made of two 3/8-inch asbestos cement disks, 5 7/8 inches in diameter, which are separated by a 1 1/2-inch brass insert 1 inch in diameter. Eleven semicircular sectors, 3/4 inch in diameter, have been cut out of the circumference of the disks at equal intervals. Eleven steel posts (1/8 × 1 3/4 inches) are fastened with silver solder on the brass insert in a line with the 11 semicircular sectors. A curved test tube clamp, 5/8 inch wide, fashioned from 1/32-inch sheet stainless steel is fastened to the end of the 11 posts with silver solder.

The motor, gears, and carriage are mounted on an aluminum plate (see Figure 1) that is fastened to the chassis by a piano-type hinge placed under the chain-drive assembly. A thumbscrew at the opposite end of the plate allows the entire unit to be rotated about the hinge to 20 degrees from the horizontal and thus permits the closed end of the test tube to be depressed into the furnace unit.

Two flat brass ways, 1/8 × 1 1/2 inches, on the underside of the furnace are fitted to two T-shaped aluminum ways fastened to the chassis, providing tracks on which the furnace moves.

The furnace is constructed from 0.5-inch asbestos cement board and is assembled with machine screws. Figure 2 illustrates interior design and Figure 1 gives dimensions. A curved slot is cut in the back wall of the furnace to permit the entry of the carriage when the furnace is moved toward the motor and gear assembly. Twenty holes, 1/2 inch in diameter, are drilled along the lower edges of the sides and front of the furnace to furnish sufficient ventilation for the gas burners. Five high-temperature natural gas-type burners, whose bases have been cut to permit close assemblage, are connected to a copper manifold by short lengths of rubber tubing. The burners can be regulated in the normal manner and can be removed easily for occasional cleaning. The front of the furnace is a door held by two piano-type hinges 1.5 inches in length and fastened by a hook and eye. A projecting handle is placed on the door to facilitate moving the furnace. The chassis is made of hard pine painted with heat-resistant metal paint.

OPERATION

The fusion is accomplished in the multiple-unit fusion rack as follows:

The position of the furnace and the carriage at the beginning of the fusion is shown in Figure 2. Eleven 16 × 150 mm. borosilicate glass test tubes containing the samples of soil or rock mixed with an appropriate flux are placed around the periphery of the carriage. The carriage is adjusted by the thumbscrew to lower the bottom end of the test tubes, and the motor is started. The burners are then lighted and the furnace is pushed under the revolving test tubes. After the fusion is completed, the furnace is withdrawn from the carriage, which is then adjusted to support the test tubes in a horizontal position. The unit is allowed to rotate until the melt has solidified on the sides of the test tubes in a thin layer.

While the fusion process is taking place, the operator is free to utilize his time on another operation, thus cutting down greatly the number of man-hours spent in making a large number of test tube fusions.

PUBLICATION authorized by the Director, U. S. Geological Survey.

Electrolytic Oxidation of Plutonium

Roy Ko, General Electric Co., Hanford Atomic Products Operation, Richland, Wash.

FOR quantitative electrodeposition of plutonium by the method of Miller and Brouns [Miller, H. W., and Brouns, R. J., ANAL. CHEM. 24, 536 (1952)], in which Pu(VI) is reduced cathodically in a potassium hydroxide solution and deposited as $Pu(OH)_4$, the metal must be in the sexivalent state. The most suitable oxidation method has been ozonation (Miller and Brouns), which oxidizes plutonium quantitatively without introducing ions which interfere in the electrodeposition. Ozonation, however, is slow—the oxidation of milligram amounts of plutonium takes at least 4 hours—and requires a special apparatus and equipment. This paper reports an electrolytic oxidation method for plutonium which is faster and simpler than ozonation and is still quantitative and exclusive of foreign ions.

APPARATUS

The electrolysis apparatus is similar to that described by Miller and Brouns, except that a polyethylene bottle and screw cap without gaskets are used for the electrodeposition cell. The cathode is a platinum wire coated with paraffin, except for an area of approximately 0.033 sq. cm. at the tip.

PROCEDURE

The plutonium solution is transferred to the assembled electrodeposition cell and made 0.5*M* in perchloric acid and 10 ml. in volume. A 20-sq. cm. platinum anode is rotated to produce vigorous stirring. The platinum wire cathode is placed in the cell, and a current of 60 ma. is passed through the solution for 15 minutes. The plutonium is 98% oxidized at the end of this time as determined by lanthanum fluoride carrying of plutonium(III) and (IV). If electrodeposition is desired, sodium hydroxide is added to adjust the hydroxide ion concentration and the deposition is performed as described by Miller and Brouns.

RESULTS

A perchloric acid concentration of 0.5*M* was found to be the optimum for the oxidation (Table I). A current of at least 30 ma. was required for efficient oxidation at a 5-sq. cm. anode. Decreasing the anodic current density increased the plutonium oxidation efficiency as shown in Table II. Plutonium was 98% oxidized at 3 ma. per sq. cm.

The oxidation is essentially complete in a matter of minutes. Microgram quantities were 98% oxidized in 10 minutes, milligram amounts in 15 (Table III).

Table I. Effect of Perchloric Acid Concentration on Anodic Oxidation of Plutonium

(65 ma., 5-sq. cm. Pt anode, 0.5 γ of plutonium, 1-hour electrolysis)

$HClO_4$, *M*	Pu Oxidized, %
0.1	55
0.2	88
0.25	88
0.5	92
1.0	78
2.0	72
4.0	50

Table II. Effect of Anodic Current Density on Electrolytic Oxidation of Plutonium

(60 ma., 0.5*M* $HClO_4$, 1-hour electrolysis)

Pu Taken for Oxidation	Anodic Current Density, Ma./Sq. Cm.	Pu Oxidized, %
1.5 γ	30	72.0
	15	91.3
	6	97.0
	3	97.8
	1.5	97.2
1.5 mg.	6	97.5
	3	97.8
	1.5	99.0

Table III. Effect of Time on Anodic Oxidation of Plutonium

(3 ma./sq. cm., 0.5*M* $HClO_4$)

Pu Taken for Oxidation	Time of Oxidation, Min.	Pu Oxidized, %
1.5 γ	5	85.6
	10	97.8
	15	96.9
	30	96.7
	60	97.8
1.5 mg.	10	95.4
	15	98.3
	30	98.6
	60	99.0

Multiple Exposure Film Method of X-Ray Diffraction Powder Analysis

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In the qualitative analysis of a powder material by x-ray diffraction, the practice of combining a standard substance of accurately known lattice spacing with a test sample to obtain convenient calibration lines has the disadvantage of possible overlap of lines of the standard with those of the sample. Furthermore, comparing lines between two diffraction patterns for the complete identification of compounds in a mixture can be rather tedious when numerous lines are involved. Although the desirable features of both these practices without their associated disadvantages may be obtained by the use of multiple-exposure cameras (*1*, *4*, *5*), it has been found that an adapter similar in function to that of Frevel (*2*, *3*) but of simpler construction, can produce equally effective results in a conventional camera.

EQUIPMENT AND TECHNIQUE

The adapter is a brass ring (Figure 1,*A*) with diametric cutouts which fit partly around the collimator and beam trap of an x-ray camera. Slotted adapter mounting brackets at the collimator and beam trap supports permit more or less concentric mounting of the ring. A small shielding tab added to the beam trap mounting bracket completely masks the x-rays diffracted at low angles. The diameter of the adapter is large enough to permit the use of an oscillating wedge sample mount. The adapter shown in Figure 1,*B*, suffices for small cylindrical-shaped samples. In use, the adapter is situated between the irradiated sample and the film (Figure 2). In this position it intercepts slightly less than half of the x-rays diffracted along the longitudinal film axis.

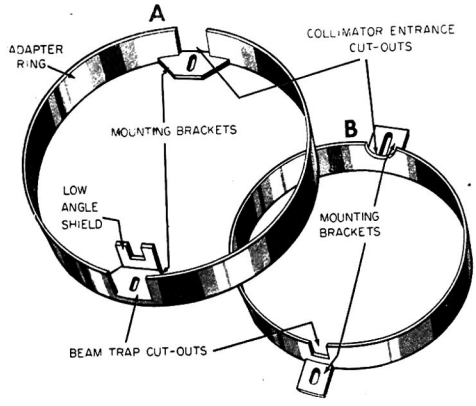

Figure 1. Adapter rings

A uniform technique of sample preparation and mounting is essential. Line displacements caused by x-ray absorption should be minimized by making the cylindrical samples small in diameter and relatively transparent to x-rays. A powder sample to be analyzed is first given a proper x-ray exposure, after which the adapter ring is removed and remounted on the opposite side of the camera without disturbing the partially exposed film. Then this powder is replaced with a standard material which is then given its proper exposure. At this stage, a series of standard compounds either singly or in combination may be exposed and recorded on this one edge of the film. An example of a completed film is shown in Figure 3.

RESULTS AND APPLICATIONS

It will be noted in Figure 3 that the film consists of three longitudinal sections. One edge section contains only the lines of the test mixture; the other, only those of the standard material. A composite of the two appears at the center where the background is slightly higher because of the multiple exposure. The composite pattern looks the same as that of an intimately mixed standard material and test sample.

When used for identifying lines, this method permits a direct elimination of the lines of compounds already identified, since the lines of the known standard are continuous with those of an identical constituent in the mixture. Therefore, if a combination of all known compounds is used as the standard, any discontinuous lines of the test sample on the film represent unidentified components. Equatorial 2Θ measurements of these lines are possible when the background is not excessive. This technique has been found particularly effective in identifying fine impurities in clays and soils.

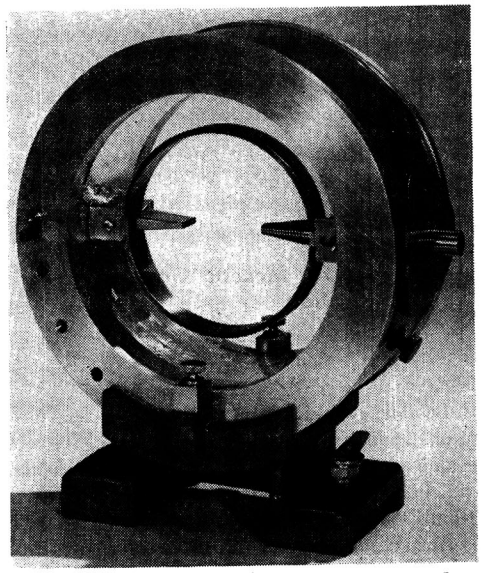

Figure 2. Mounted adapter rings for cylindrical samples with sample mount removed

In the study of effects of physicochemical processes (*2*) on various compounds, this method allows small changes in lattice spacings to be more easily detected Lines from each edge section of the photograph, though unresolved at the center section, are perceptible as lines of different d-spacings if they are fairly sharp, moderately intense, and if their centers are very slightly separated. A test pattern of 300-mesh quartz and halite, each exposed with filtered copper $K\alpha$ radiation for an equal period of time on opposite edges of a film in a 14.32-cm. diameter camera, showed that in the composite, the 220-line of halite, completely obscured the 201-line of quartz. However, a closer examination of each section of the film at the adjoining edges revealed that the centers of these two lines did not actually coincide.

ADVANTAGES AND DISADVANTAGES

There are two obvious drawbacks to the use of this technique: the longer time to register two patterns on one film and the increased background in the center section of the film.

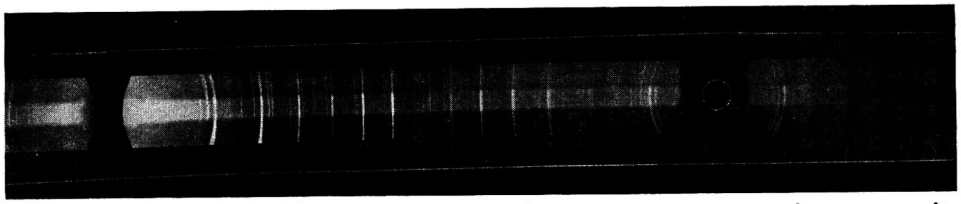

Figure 3. Fully exposed film showing overlap of exposures at center to give a composite

The inherent simplicity of the method is an advantage. Because no basic change is made in the existing camera design, the diffraction characteristics remain the same. The additional time spent in making a second exposure on the same film is compensated for, because the analyst is better able to resolve uncertainties that may exist in the relative line spacings of test sample and standard material. The problem of nonuniform shrinkage between two films is eliminated, and reference lines are readily available for calibration.

ACKNOWLEDGMENT

The author wishes to thank Jarvis Todd for assistance in the preparation of this paper, the Engineering Services Section for the fabrication of the device, and the Photographic Section and Illustrating Section for the preparation of photographs and drawings.

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Simplified Method for McLeod Gage Design and Calibration

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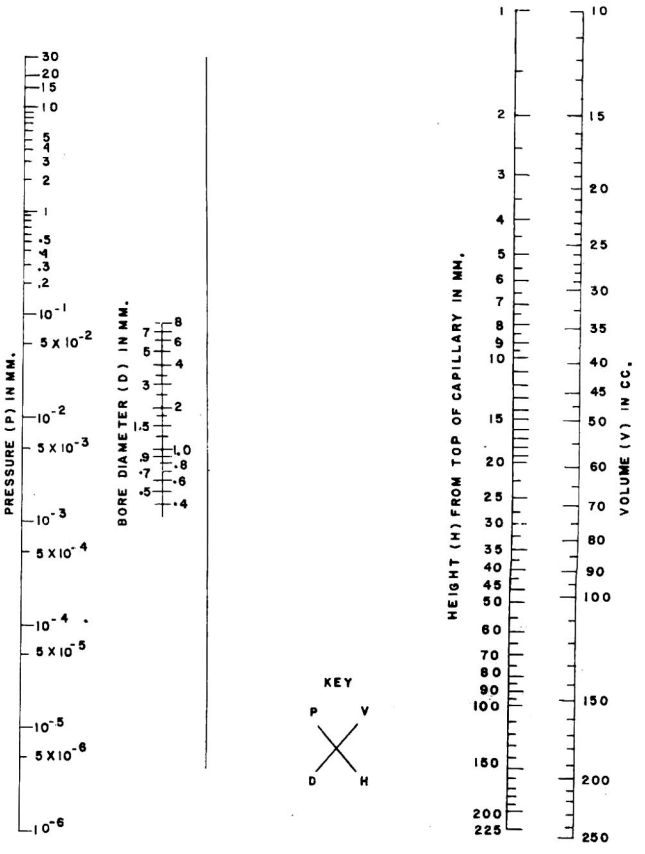
THE McLeod gage is the most commonly used low-pressure measurement instrument in the laboratory. Its construction is simple and adequately described in numerous sources (*1–4*), but it is difficult to find information as to the exact size of capillary bore and bulb volume for a desired pressure range. The nomograph described eliminates this difficulty, as it makes it possible to set specifications which can be followed by the individual or sent to a glass blower. It is usable for any McLeod gage where the mercury in the outside arm is brought level to the sealed top of the capillary tube fixed to the bulb of the McLeod gage and pressure readings are made by the length of the column of gas compressed in this capillary. This gage follows the formula $P = \frac{\pi D^2 H^2}{4000V}$ where P is the pressure in the system in millimeters of mercury, D is the diameter of the capillary tube in millimeters, H is the length of the air column in the capillary tube in millimeters, and V is the volume of the bulb in cubic centimeters (*1, 3, 5*). Tipping McLeod gages are also included in this category.

The nomograph is designed for pressures between 30 and 10^{-6} mm. and bulb volumes from 10 to 250 cc. As shown in the key on the nomograph, one pairs the P-H scales and the D-V scales.

Suppose it is desired to construct a McLeod gage to read pressures accurately from 0.01 to 5 mm. The first graduation (0.01 mm.) then should be about 3 mm. from the top of the capillary and, for convenience, the length of the sealed capillary which is attached to the bulb should be 100 mm. long. A ruler is then connected from the 5-mm. graduation on the P scale to the 100-mm. mark on the H scale. The intersection of the ruler with the index line (unmarked line in the center of the chart) is marked. Next the ruler is placed on the 0.01-mm. mark on the P scale and the previous mark on the index line to see whether the intersection on the H scale is about 3 mm. The actual intersection is 4.7 mm. on the H scale. This shows that a McLeod gage can be constructed for this pressure range with a capillary length of 100 mm. If the intersection were less than 3 mm., the length of the capillary would have to be increased or the pressure range reduced.

The ruler is now pivoted about the fixed index point to connect the D and V scales. All intersections of D and V with the pivot will satisfy the conditions for the desired pressure range, such as 4-mm. bore and 26-cc. volume; 7-mm. bore and 75-cc. volume; 3-mm. bore and 14-cc. volume, etc. The last condition would be suitable for a tipping McLeod gage, as the volume of the bulb is low and the bore diameter is large enough to prevent sticking of the mercury. Unless the gage is needed to read low pressures, it is desirable to keep the capillary bore diameter larger than 1.5 mm. to prevent mercury sticking in the capillary. Hence the gage would be constructed by sealing a length of 3-mm. bore capillary tubing somewhat longer than 100 mm. to a bulb of about 14 cc. volume.

To calibrate the gage, the procedure is reversed. The capillary bore and the volume of the bulb are determined in the usual manner. If the experimentally determined bore were 2.5 mm. and the actual bulb volume 12 cc., then these points on the D and V scales would be connected and a new index point marked. The ruler is pivoted about this new index point to connect the P and H scales. A pressure of 0.01 mm. would be 5.0 mm. from the top of the capillary; 5×10^{-3} mm. pressure at 3.6 mm.; and 5-mm. pressure at 110 mm. from the top of the capillary.



The calibration of the McLeod gage by use of the chart is approximate. The error due to inaccuracies in the ease of reading the chart up to a pressure of 5 mm. is about 5%. If precision calibration is necessary, it is recommended that the formula be used and one of these references be consulted (*1–3, 6*). Above 5-mm. pressures, it is necessary to calibrate by the following formula, $P = \pi D^2 H^2/4[1000V - (\pi D^2/4)H]$, which contains a correction term for the volume of mercury displaced from the total bulb volume by the gas in the capillary (*1, 3, 5*). This correction is not taken into account by the chart, as it will not affect the use of the chart for design purposes but calibration by the chart would be in error in the range above 5 mm.

ACKNOWLEDGMENT

The author wishes to thank Ernest R. Kline for his interest.

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Inexpensive Microtitrator

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CONVENTIONAL microtitration assemblies are both cumbersome and expensive. In addition, they generally employ standard medical syringes as burets, which add substantially to the cost and have the further disadvantages of rapid chemical attack by alkaline solutions and gradual wear between the ground glass plungers and barrels. Even when new, there is usually some leakage around the plungers, and while the latter are no doubt ground sufficiently accurately for medical purposes, variations of ±3% were found in the diameter of the plunger of a 0.2-cc. tuberculin syringe.

One method of overcoming some of these difficulties is to drive a precision-ground rod through a gasket into an oversize barrel. In the apparatus described by Kelley [Kelley, M. T., *Proc. Instr. Soc. Amer.* **7**, 63 (1952)] a platinum rod was used for this purpose. Glass would be preferable from the standpoint of expense, but in either case a chemically inert gasket must make a leakproof seal between two surfaces which move relative to one another, adding to the difficulties of construction. There is the slight additional objection that the rod is external to the barrel at least part of the time and thus exposed to accidental mechanical alteration.

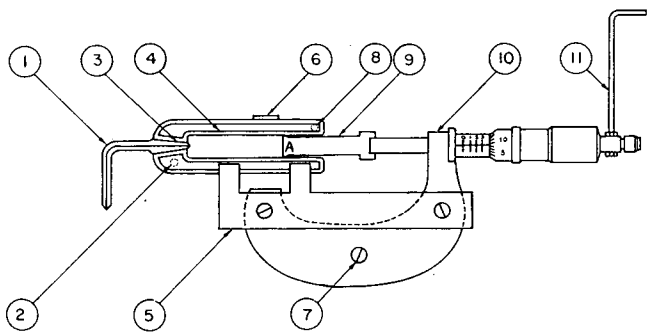
The parts necessary for constructing the device shown in the figure may be obtained for a few dollars. The possibility of chemical corrosion in the buret is reduced to a minimum, and the original model has shown no detectable leakage after several weeks of intensive use. Accuracy in the measurement of delivered volumes is controlled by the bore of the tubing used for the buret barrel. Standard precision-bore tubing is generally quoted as having a tolerance of ±0.01 mm.; the piece used here has an inside diameter of 3/8 inch (~10 mm.), which indicates possible variations in the cross-sectional area of 2 parts in a thousand. The results of actual titrations with this apparatus show average deviations which are in excellent agreement with this estimate.

Volumes corresponding to a piston travel of 0.1 inch (about 0.2 ml.) may be read to within 0.1% directly on the micrometer, and for titrations of this or larger amounts in a laboratory of reasonably constant temperature the water jacket may be eliminated. For smaller increments of the total buret volume a simple calculation based on the thermal expansion of the titrating liquid is necessary to ascertain the tolerable temperature fluctuations during any one titration.

The economy actually realizable from the construction of this device over the cost of conventional assemblies is, of course, a function also of the labor expenditure required for its fabrication. A good glass blower can turn out the buret in a few hours, however, and this together with the small amount of shop work necessary should still allow a real saving. The latter can be greatly increased if it is possible to dispense with the water jacket. At institutions such as colleges and universities the construction of such a device would be a worth-while project in the glass-blowing and shop courses usually given graduate students.

This device is readily adaptable to automatic operation; in the present installation a synchronous motor drives the micrometer at the rate of about 1 revolution per hour. Titrations requiring 10 μl. of reagent are completed in less than 15 minutes, and the delivered volumes are read to within 2 parts in a thousand on the chart of a Brown potentiometer which records potential changes in the sample solution. Although the total piston travel in such a run is only 0.006 inch, the results of a large number of standard oxidimetric titrations of 50-γ samples of iron with 0.1*M* ceric sulfate indicate an average deviation of less than 5 parts per thousand. Titrations involving one tenth of these amounts are good to within 5%. For such small fractions of the buret volume, temperature control to within ±0.05° C. is necessary; this requirement is at least partially offset, however, by the advantage of being able to run more than a hundred determinations without refilling the buret.

The microtitrator consists of a capillary tip, 1, 0.05 to 0.1 mm. in inside diameter attached to the buret with a 5/20 standard-taper joint, 3. An inlet, 2, and outlet, 8, are provided in the glass jacket for the circulation of constant temperature water.



The precision-bore tube, 4, of 3/8 inch inside diameter, is held rigidly in place on the micrometer by the plastic supports, 5, and the metal strap, 6. The butt of the micrometer, 10, is removed as shown. A handle, 11, allows accurate manipulation of the titrator, which may be conveniently attached to a standard laboratory support rod by the screw, 7. The Teflon piston, 9, is turned 0.010 to 0.020 inch oversize at point *A* in order to provide a liquid-tight seal in the buret.

Ethylene Carbonate as a Cryoscopic Solvent

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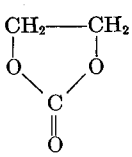
IN THE course of an investigation of methods for the characterization of isolated lignins, the use of the cyclic carbonate of ethylene glycol as a cryoscopic solvent has been studied. This



compound has a number of advantages which make it a generally useful solvent for determinations of molecular weight.

Ethylene carbonate is a white crystalline solid with a melting point of 37° C. This low freezing temperature permits molecular weight determinations of compounds which decompose at the melting point of camphor. Ethylene carbonate is nonhydroscopic; a dried sample did not change in weight on exposure to the moist laboratory air for 2 days. It is a good cryoscopic solvent for a variety of compounds, including hydrocarbons such as phenanthrene, polar compounds such as hydroquinone and vanillin, and even benzoic acid (0.02*M*), which is frequently associated in solution. The molar freezing point depression constant [$K = 7.03$] as determined herein compares favorably with both dioxane [$K = 4.63$] and benzene [$K = 5.7$] (Skau, E. L., and Wakeham, H., "Physical Methods of Organic Chemistry," A. Weissberger, ed., vol. I, p. 35 ff., Interscience,

Table I. Evaluation of Freezing Point Depression Constant of Ethylene Carbonate

Compound	Molecular Weight	Mole/1000 Grams of Solvent	K
Azobenzene	182	0.017	7.16
		0.0181	7.13
Benzoic acid	122	0.020	6.95
Benzophenone	182	0.041	6.93
		0.062	7.10
Hydroquinone	110	0.0229	6.95
		0.0427	7.01
Maleic anhydride	98	0.0268	7.15
		0.0566	6.89
p-Nitroaniline	138	0.0163	7.08
		0.0359	7.08
Phenanthrene	178	0.013	6.89
Vanillin	152	0.0234	7.09
		0.0393	6.92

New York, 1945). Although ethylene carbonate reacts with a variety of functional groups, it is stable under the conditions used.

EXPERIMENTAL

Ethylene carbonate (Jefferson Chemical Co., Inc.) was purified by fractional distillation at pressures of approximately 10 mm. of mercury through a column packed with single-turn helices. Fractions of freezing points within 0.02° C. were combined and allowed to solidify in small crystals. The solid material was well mixed and stored in fairly small, sealed bottles. The freezing points were determined in a standard Beckmann freezing point depression apparatus. No special precautions to ensure high accuracy were taken. After supercooling, a definite plateau in the cooling curve was reached. The temperature remained constant for approximately 4 minutes, thus giving a constant freezing point and making an extrapolation to the liquid cooling line unnecessary. The results are summarized in Table I. The cryoscopic constant was equal to 7.03 ± 0.14.

Spinning Band Still for Vacuum Operation

Ralph G. Nester, E. I. du Pont de Nemours & Co., Wilmington, Del.

DURING recent years there has been much interest in the development of laboratory stills having rotating members as packing in view of desirable performance characteristics such as low pressure drop, low operating holdup, and high efficiency. Provision of a trouble-free seal for the rotating shaft to permit operation under high vacuum has given considerable trouble in the past, although in 1947 Birch, Gripp, and Nathan (*1*) described a spinning band column operable at 0.01 mm. with a total pressure drop of only 0.04 mm. More recently Foster and Green (*2*) have reported a magnetic drive for spinning band columns that makes possible continuous distillations of 120 hours at pressures as low as 0.1 mm. Another variation of spinning band type columns is reported by Murray (*3*).

The spinning band still described (Figure 1) offers certain advantages over previous designs, especially from the standpoint of a simple, highly effective combination seal and suspension bearing for the rotating band, an improved reflux and take-off needle valve that requires no grease and is free from leaks, and a spinning spiral wire gauze band that is particularly efficient. Stills of this design are easy to fabricate, are sturdy, and have proved to be very practical pieces of laboratory research equipment that in almost all instances are far superior to other types of laboratory stills in ability to fractionate heat-sensitive or high-boiling, high-viscosity liquids.

Seal and Bearing. The unique combination seal and suspension bearing seat is made of a plug of a self-lubricating plastic that fits tightly the top of the still column. The plug has a passage through it for the shaft of the spinning band. This passage is wide at the top, narrows down to form an inverted conical or hemispherical bearing seat, and continues as a narrow passage to the bottom of the plug (Figure 2). Any self-lubricating plastic such as Teflon tetrafluoroethylene resin, nylon (polyhexamethyleneadipamide), polymonochlorotrifluoroethylene, or polyethylene may be used. The bearing is a glass or metal spherical or inverted conical bead mounted on the shaft of the spinning band and forms a very effective rotary seal. The assembly adjusts itself for slight wear or misalignment.

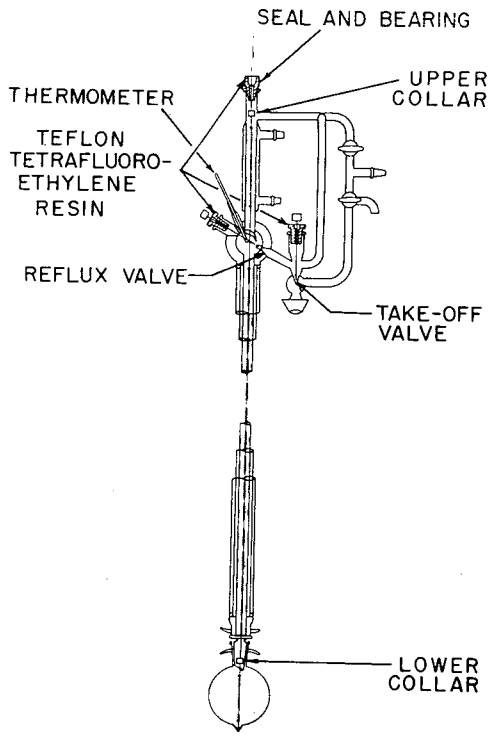


Figure 1. Spinning band still

Patented

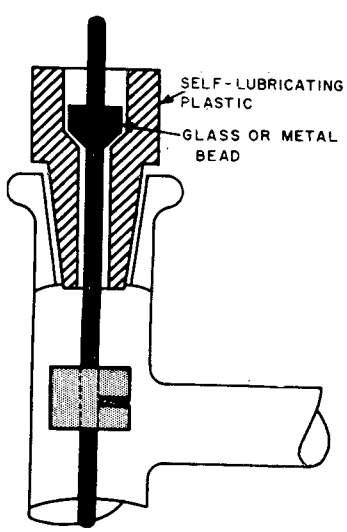


Figure 2. Detail of seal and suspension bearing seat

Spinning Band. The spinning band is formed by attaching 80- to 100-mesh wire gauze spirally to the shaft; usually there are about 3 to 16 spirals per foot of shaft length. There are three sections of spirally twisted wire gauze on the shaft, one in the condenser section of the column, one in the fractionating section, and the third in the still pot. The two outer longitudinal strands of wire are removed from the gauze, so that the transverse wires extend beyond the outer longitudinal wire and come in

contact with the inner column wall. This construction provides for violent agitation of the reflux liquid and for intimate contact of descending liquid and ascending vapors. This contact is enhanced by the spiral construction of the band which throws both liquid and vapor to the column wall. The pitch and direction of rotation of the spiral create a downward pumping action which accelerates the return of the descending liquid to the still pot. The section of band in the still pot ensures smooth boiling and uniform boil-up rates. All of these factors contribute to give a still of high flexibility and efficiency.

A guide bearing at the bottom of the still pot prevents vibration of the shaft. The construction whereby the transverse wires of the spiral band brush the column wall also helps keep the spinning band centered and free of vibration.

Take-Off Valve. Reflux ratio of the still is controlled very accurately by means of an adjustable needle valve (Figure 3). The valve is made by threading a stainless steel rod through a plug of Teflon tetrafluoroethylene resin fitted tightly into the top of a glass tube. The lower end of the rod has a Teflon tip which fits into the valve seat located between arms which connect with the still column and the take-off tube. Adjustment of the valve is made by means of a brass turn nut at the top of the rod.

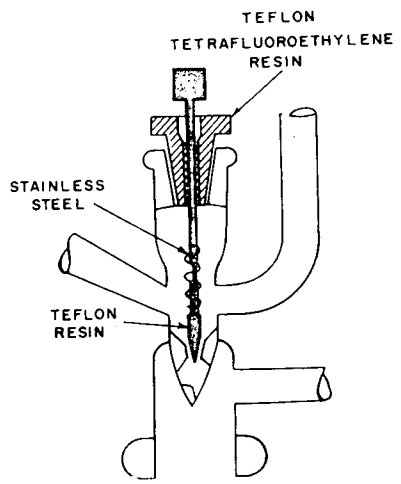


Figure 3. Detail of adjustable needle valve

Use of such a valve eliminates the possibility of air leaks or bubbling back such as may occur with a stopcock.

OPERATIONAL CHARACTERISTICS

Stills of this design have been made with interior column diameters ranging from 6 to 50 mm. and with column heights of 1 to 6 feet. Such stills have been operated readily at pressures as low as 10^{-4} mm. Dependent on still size and operating conditions, a height of equivalent theoretical plate as low as 0.74 inch and a pressure drop of 0.04 mm. have been obtained. A column measuring 23 mm. in diameter by 36 inches in height has been used for a large number of distillations at a through-put rate of 4 liters per hour using a 12-liter distillation flask. A 1-liter distillation flask may be used with this column, provided an adapter of correct length is used for connecting the column and flask.

Microstills with columns 6 mm. in diameter and height from 12 to 36 inches are characterized by small holdup, as low as 0.3 ml., and high boil-up rates. Such microcolumns can be operated with a few milliliters of starting material.

Table I. Operational Characteristics of Spinning Band Still at Different Boil-Up Rates

Boil-up rate, ml./hour	1622	333	205
Operating hold-up, ml.	4.3	0.8	0.7
Pressure drop, mm. Hg	1.09	0.23	0.23
H.E.T.P., inches	3.50	1.07	0.74

To give a more precise idea of the operational characteristics of a typical still, data are given in Table I. The still used had an interior column diameter of 10 mm., column band length of 29.75 inches with 12 spirals per foot, and condenser band length of 5.25 inches. The band was operated at 3300 r.p.m. using a mixture of 25% *n*-heptane and 75% methylcyclohexane by volume.

ACKNOWLEDGMENT

The author wishes to express his acknowledgment and appreciation to J. W. Robson for assistance in obtaining distillation data, and to T. J. Uhrig for help in fabrication of the still.

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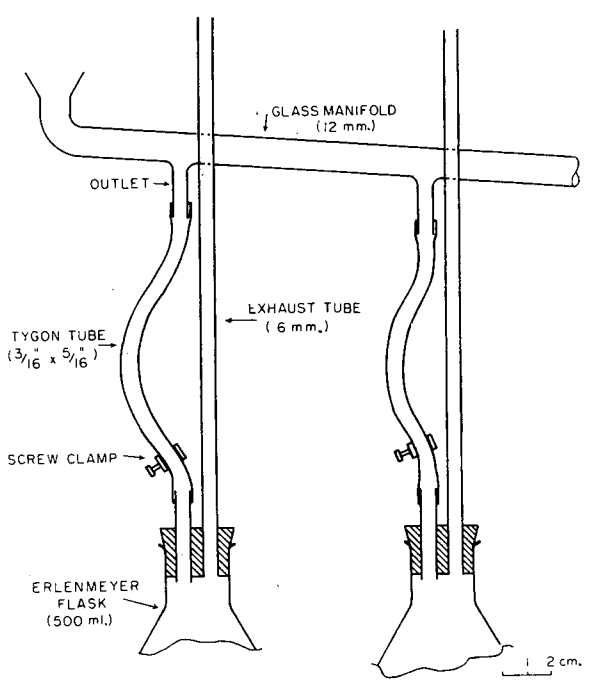
CONTRIBUTION 340, Chemical Department, Experimental Station, E. I. du Pont de Nemours & Co.

Simple Large Volume Fraction Collector for Column Chromatography

Victor Ginsburg, Department of Plant Biochemistry, University of California, Berkeley, Calif.

A SIMPLE inexpensive fraction collector has been devised for the automatic fractionation of the eluate from columns.

With the increasing use of column chromatography for chemical separations, many automatic fraction collectors have been described. The receivers in these collectors are usually changed by means of electric motors that are activated by impulses from timers or by the closing of circuits after certain volumes of eluate have been collected.



For the separation of nucleotides by column chromatography, it is often necessary to collect up to 100 liters of eluate in 500-ml. fractions or less. In this laboratory, a simple versatile apparatus

has been devised that is as satisfactory for this purpose as the more complicated machines.

APPARATUS

The eluate from the column is introduced into the flared upper end of a glass manifold equipped with outlet tubes at 4-inch intervals. The manifold is held at a slope of 1 in 12 inches so that the eluate flows down the manifold into the first outlet which is connected by tygon tubing to a 500-ml. Erlenmeyer flask. When the first flask is filled, eluate rises in its exhaust tube until the pressure head formed equals that of the incoming eluate. The eluate then bypasses the first outlet and flows into the second one until the second flask is filled. In this manner, as many fractions can be collected as there are outlets in the manifold. The volume of the fractions can be varied by using flasks of different capacities.

Stationary air bubbles that automatically form in the Tygon tubes after each flask is filled ensure the collection of clean fractions. The filled flasks are removed after the screw clamps have been tightened to avoid contamination of the fractions with the small amounts of eluate trapped above the air bubbles.

If contact between the eluate and the rubber stoppers is undesirable, the exhaust tubes may be extended into the flasks.

Adaptation of Existing Potassium Bromide Disk Press for Microdisk Pressing in Vacuo

Roger A. Pickering, Spectroscopy Laboratory, Argonne Cancer Research Hospital, Chicago 37, Ill.

THE recent commercial development of silver chloride infrared beam condensing optics (*1*) by the Eastman Kodak Co., Rochester, N. Y., led to an interest in potassium bromide microdisk pressing in this laboratory for possible use in infrared microanalysis.

Although some easily constructed microdies have been described (*2*, *3*), and a die based on the design of Anderson and Smith (*2*) is to be available from the Beckman Co., Fullerton, Calif. (*4*), no mention has been made apparently of adaptation of existing commercial 0.5-inch disk presses to do microdisk pressing. The simplicity of an adaptation devised in this laboratory for pressing microdisks permits a convenient conversion of a commercially distributed press supplied by the Research and Industrial Instruments Co., 30, Langton Road, Brixton, London, S.W. 9, England. The adaptation utilizes the existing die shell with comparable evacuation through the use of a sleeve holder suggested by Anderson and Smith's description of their microdie (*2*). In this method the disks are pressed directly into a steel sleeve, which then serves as a convenient ring holder for mounting and handling. A disk die 6 mm. in diameter is illustrated in Figure 1. The same principles have been used to construct a die pressing 3 × 10 mm. rectangular pellets.

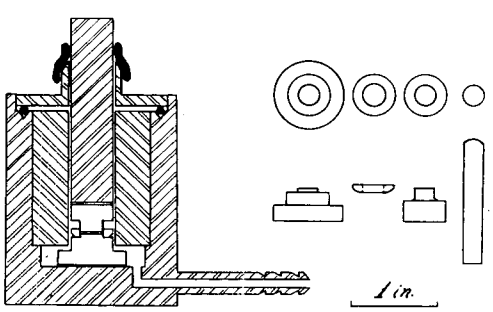


Figure 1. Adaptation for pressing 6-mm. potassium bromide disks in vacuo

From left. Cross-sectional view of press, showing position of microdie, lower die, sleeve, upper die, and tamper pin

All die parts are made of stainless steel tooled to fit the press, making slight allowances for evacuation, with the die surfaces properly hardened and polished to optical flatness. The sleeve is beveled on the lower edge for greater ease of removal after pressing. One procedure used is to put the powder mixture on the sleeved lower die, distributing the powder smoothly with a tamper pin before placing the bored cylinder over the die. Next the upper die and the plunger are inserted. The assembly is then carefully inverted while the dies are held in place (an operation made easier by the fact that the cylinder is magnetized) to put it in the outer shell. Variations are possible, such as using a loading tube to place the powder on the sleeved lower die after assembly in the cylinder and the outer shell, in which case the upper die and the plunger are then merely slid into place.

ACKNOWLEDGMENT

The services of John Stupka and Robert Henry of the machine shop in the construction of the microdie are gratefully acknowledged. Prepublication information from Don H. Anderson and Richard G. Smith facilitated the design described.

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Evaporator Feeder

W. O. Phillips, Laboratory Division, Goodyear Atomic Corp., Portsmouth, Ohio

IN THE operations of analytical and spectro chemistry, it is frequently necessary to evaporate solutions to dryness for examination of the residue. Large quantities of liquid samples must often be used to obtain an adequate residue for handling. However, the evaporating dish should be small to reduce contamination and to facilitate removal of the residue.

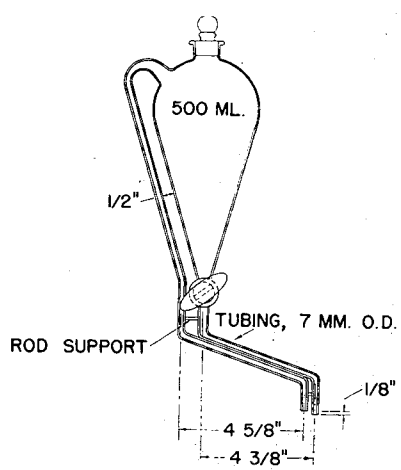

Figure 1. Automatically controlled evaporator feeder

The two aims can be reconciled by an automatically controlled feeder of sufficient reservoir capacity. A device providing these features has been described by Telang [Telang, M. S., IND. ENG. CHEM., ANAL. ED. **18**, 454 (1946)]. In the laboratories of the Goodyear Atomic Corp., Telang's design has been modified for ease of handling and operation as shown in Figure 1. The re-

designed separatory funnel is filled with a predetermined volume of solution, and supported so the outlet tips provide the desired liquid level in an evaporating dish when the stopcock is opened. (The side-arm level-regulator is not filled at the start to the same level as the liquid in the flask; however, the liquid levels are automatically equalized after evaporation commences.) The device is easily held by a 4-inch ring support from which a 1-inch section has been removed.

The feeder operates without attention until the evaporation is complete. The offset design permits the use of infrared lamps over the dish. With such lamps and dish holders fabricated from carbon block [Susano, C. D., ANAL. CHEM. 27, 1038 (1955)], very rapid evaporations can be made with little effort.

ACKNOWLEDGMENT

The problem and original approach were suggested to the author by L. E. Owen, Laboratory Division, Goodyear Atomic Corp., Portsmouth, Ohio.

Aluminum Melting Point Block

F. F. Anderson, Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

OVER the past several years, the organic chemists in these research laboratories have found the aluminum melting point block most convenient for routine measurement of melting points.

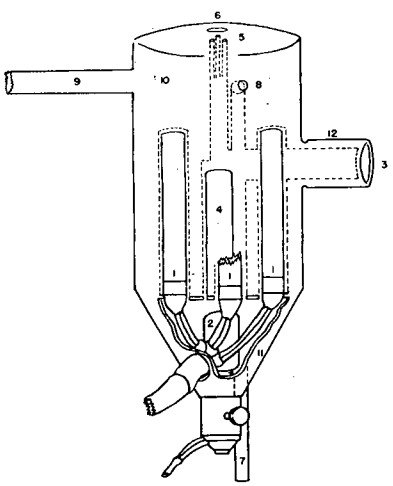

Figure 1. Aluminum melting block

1. Heaters, 2.5 × 3/8 inch, 75 watts
2. Light, 6 watts
3. Lens, 5/8-inch diameter
4. Glass rod, 2 × 5/16 inch
5. Capillary holes, 1/16-inch diameter
6. Thermometer hole, 9/32 × 1.75 inches
7. Air inlet, 0.75 × 3.75 inches
8. Air outlet, 3/16-inch diameter
9. Support, 3/8 × 8 inches
10. Aluminum block, 2 × 3/75 inches
11. Aluminum cup, 2 inches at top, 7/8 inch at bottom
12. Brass eyepiece, 0.75 × 0.5 × 1.5 inches

The design of the block has slowly changed as new features were incorporated. The apparatus described has been found to be most useful, permitting melting points to be measured with an ordinary yellow-back immersion thermometer, from room temperature to over 300° C., with an accuracy of about 1° C. (Table I).

Table I. Performance Data[a]

Standard[b]	Thermometers[c, d] I	II	III
43.5– 44.0	42.0– 43.5	43.0– 44.0	42.0– 43.0
81.5– 82.0	79.5– 81.0	80.0– 81.0	81.0– 82.0
135.8–136.3	134.0–135.5	136.0–137.0	136.0–137.0
149.5–150.0	147.5–148.5	148.0–149.0	150.0–151.0
174.0–174.5	173.5–174.5	174.5–177.0	174.0–175.0
215.5–216.5	214.0–215.5	216.0–217.0	215.0–216.0
243.5–244.0	243.0–244.0	244.0–246.5	242.0–244.0
305.5–306.0	304.5–306.5	305.5–306.5	305.0–306.0

[a] Readings are at heating rates of 2° C. per minute.
[b] Temperature at which crystal and liquid are in equilibrium (not whole range for complete melting).
[c] Ordinary 3-inch yellow-back thermometers.
[d] Columns I and II represent performance on same block; column III is for a different block.

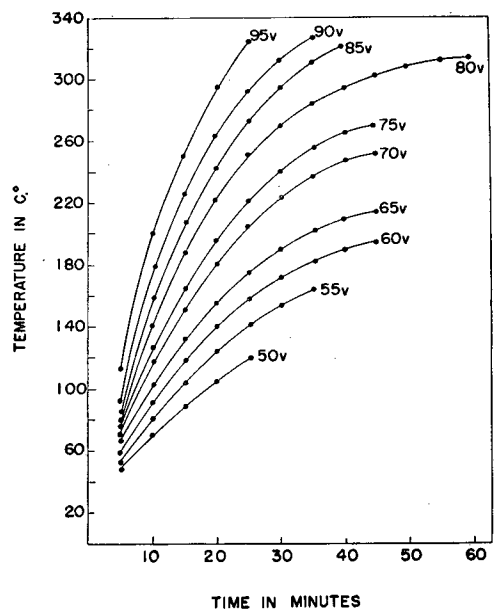

Figure 2. Calibration of melting point apparatus

Figure 1 shows the construction of the block. Three holes for capillaries and one thermometer hole can be seen at the top. The lens is mounted on the side for observation. Three 75-watt Chromolox heaters, controlled by the attached variable voltage transformer (Powerstat), are inserted from the bottom. Light from a 6-watt lamp in the hollow base passes through a borosilicate glass rod to the sample chamber. A compressed air hose may be connected to the tube at the bottom for rapid cooling of the block after a reading has been taken. Calibration curves for the apparatus are shown in Figure 2.

Improved Migration Cell

E. R. Nightingale, Jr., Department of Chemistry and Chemical Engineering, University of Nebraska, Lincoln 8, Neb.

A SIMPLE method for determining whether ionic aggregates in solution are positively or negatively charged is to observe the direction of migration of the species in an electric field. While a simple U-tube may serve adequately, it is desirable to use a cell in which the test solution and electrolyte are not subject to mixing and which is rapidly and conveniently filled and emptied. Figure 1 shows a cell designed to fulfill these requirements.

Two Corning No. 416750 tubes with 10-mm. coarse fritted-glass disks are joined to form a U-cell about 3 × 6 inches. A T-joint is made at the bottom of the cell using 13-mm. borosilicate glass tubing and bent as shown in Figure 1 (right). This delivery tube is fitted with a stopcock and reservoir of about 20-ml. capacity, through which the center compartment of the cell is filled and emptied. The cathode and anode compartments of the cell are joined with a 1-mm. capillary leveling tube with stopcock to prevent a difference in hydrostatic pressure.

The solution to be tested is poured into the reservoir and allowed to fill the center compartment of the U-cell. As the solution fills the center compartment, the glass disks become wetted and may hinder the further escape of air through the disks. Any remaining bubbles are easily removed by rotating the cell clockwise (Figure 1, right) to trap the bubbles in the delivery tube. After the center compartment is filled, the cathode and anode compartments may be washed and rinsed with electrolyte to remove traces of the solution to be tested. Temporary inversion of the cell does not permit leakage of the test solution through the fritted glass disks. The electrode compartments are then filled with electrolyte and leveled before electrolysis.

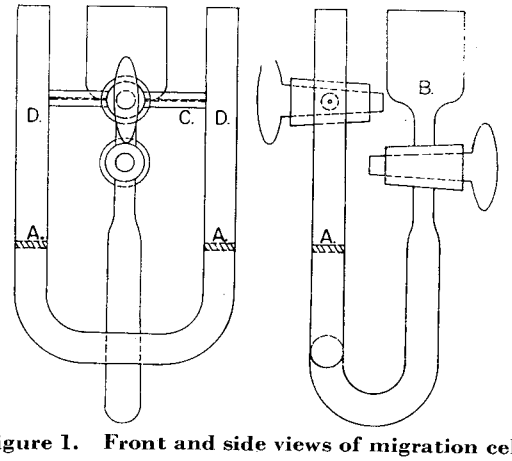

Figure 1. Front and side views of migration cell

A. Fritted-glass disks
B. Reservoir
C. Capillary leveling tube
D. Electrode compartments

After electrolysis, the electrode compartments are rapidly emptied without contamination by inverting the cell and draining the solutions simultaneously from the anode and cathode compartments into separate beakers placed beneath the cell. The migration of small amounts of slightly colored species is especially easy to determine by viewing vertically the color against the white background of the fritted-glass disks. If the reservoir is fitted with a 1-hole stopper, the contents of the center compartment are quickly removed using suction. The fritted disks may be dried by rinsing with alcohol and ether.

ACKNOWLEDGMENT

The assistance of Walter Sampson in fabricating these cells is gratefully acknowledged.

Universal Reactor-Crystallizer-Dryer for Unitized Handling of Several Operations

Ray Wendland
North Dakota State College, Fargo, N. D.[1]

When a chemist aims to purify a substance by crystallization, he is faced with three operations, at least: dissolving and crystallizing the substance from an appropriate solvent, filtering and washing the precipitate, and removing the last traces of solvent by evaporation or drying. Ordinarily, these unit operations are performed separately and involve three different devices with resultant losses of material by transfer and waste of time by frequent handling. There would be great advantages in a single device that combines all three operations in one and reduces transfer losses to zero. A fourth feature should provide for carrying out all operations under an atmosphere of inert gas, because many sensitive substances—e.g., polyunsaturated compounds and metal organic derivatives—react with oxygen or other gases and suffer deterioration during ordinary handling. A further modification of the appropriate apparatus could permit the operator to conduct numerous reactions in it and thereafter to proceed to purification of products without transfer or exposure to the atmosphere.

The design and construction of such a device as illustrated in Figure 1. The functional principles of the reactor-crystallizer-dryer are apparent in the procedure pertaining to its use for

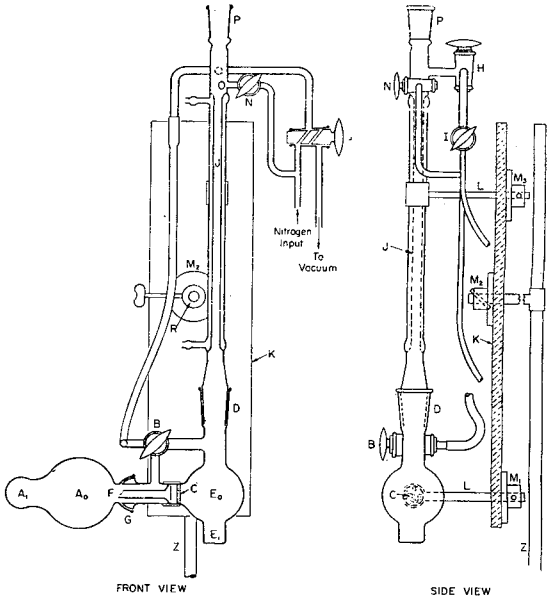

Figure 1. Universal reactor-crystallizer-dryer

	Item	Suggested Construction for Demonstration Model
A_0.	Receiver vessel for filtrate drawn from C	250-ml. flask
B.	Three-way stopcock	
C.	Filter tube joined to flask E_0 with filter disk very close to E_0	4-mm. opening in plug Filter disk 27–28 mm. in diameter
D.	Interchangeable joint for connecting condenser J to E_0	Standard taper 29/42
E_0.	Crystallization vessel, serving also as reactor when desired	200-ml. flask for central sphere
E_1.	Well in which crystallization is induced or bar magnet mixer operates during reaction (unit tilted to bring E_1 to lowest position)	Diameter 34–35 mm. to permit rotation of small bar magnet; depth 20–22 mm. (inside measure)
F.	Drip tube conveying filtrate into A_0	10-mm. inner diameter
G.	Ball joint ensuring airtight connection of A_0 to E_0. Clamp to be attached here to hold A_0 firmly in place (not shown)	Ball and socket joint, size 35/25
H.	Three-way stopcock linking suction pump to top of condenser J and to stopcock B	4-mm. opening in plug
I.	Three-way cock; plug with two channels as shown	3- to 4-mm. bore
J.	Condenser assuring return flow of solvent to E_0	Cold tube 250 to 300 mm. long
K.	Mounting frame to which are bolted three clamp holders M_1, M_2, and M_3	Plywood, Bakelite, light metal, etc., approximately 110 × 400 mm.
L.	Clamps for fastening model to frame	Support rod 0.5 inch in diameter
M.	Clamp holders for mounting clamp L to frame. M_2 has setscrew with wing nut to permit rotation at R	Flexaframe mounting feet (Fisher), 0.5-inch diameter
N.	Ordinary stopcock to regulate flow of inert gas into system	N and I joined compactly to minimize distances from stopcock H and condenser J
O.	Side arm for introducing inert gas into system	
P.	24/40 standard-taper joint	
R.	Rotation point permitting frame K to be rotated right or left to aid filtration, mixing, etc.	Shaft at R passes through holder M_2. Setscrew on M_2 controls rotation at R
Z.	Support rod to which clamp at R is attached	

[1] Present address, Mellon Institute, Pittsburgh 13, Pa.

purification of a solid compound mixed with impurities common to its origin.

PROCEDURE

By combination of evacuation and inflow of nitrogen through stopcocks *I*, *N*, and *B*, flush air out of the apparatus; dissolve the compound in a suitable volatile solvent and draw the solution into flask *E*. Close *P* with a stopper. Chill E_1 to induce crystallization in the well. Switch stopcock *I* to the vacuum position and *H* and *B* to the points where suction applies only to flask A_0. Rotate the apparatus (suspended on the shaft *R*) 90° counterclockwise, thus throwing the solution onto filter plate *C*. At the same time open cock *N* to admit nitrogen into top of condenser *J*, thus forcing fluid through *C*. Chill well A_1 in a refrigerant mixture to induce the filtrate to deposit additional solid. Rotate apparatus back to the vertical position, holding filtrate solids in A_1, while the clear solvent returns to A_0. Close *N*, turn *B* so that flask *A* communicates only with *E*, and turn *H* so that vacuum applies only to the top of condenser *J*. Again chill E_1 and apply gentle heat to A_0, so that the solvent will distill under reduced pressure through *B* back into E_0. (If some solid has accidentally plugged filter disk *C*, this may be cleared easily by momentarily closing stopcock *B* so that vapors from *A* are forced through *C* during redistillation.)

Restore apparatus to normal pressure with nitrogen, close the passage from *E* through *B* to *A*, and warm E_0 until refluxing solvent redissolves the precipitated solid. Chill to induce crystallization of the compound, rotate, filter again by suction, distill back the solvent, etc., and crystallize a third or fourth time if desired. Finally, introduce fresh cold solvent through *P* so that the compound may be washed and freed of solution by suction. Detach flask *A* containing filtrate solids and attach a closure cap or empty flask in its place. Turn *I* to the vacuum position, close *N*, and open *B* and *H* to the three-way positions. Bring up an infrared lamp or suitable heater and dry the compound under reduced pressure. (Because the unit centering around *E* is compact, it may be detached from *A* and *J* and put into a vacuum drying oven if desired.)

After drying, admit nitrogen, detach the condenser and closure cap, and weigh to determine the amount of pure, dry compound deposited in *E* (the unit having previously been weighed empty).

Modifications. To use *E* as a reactor vessel it is necessary only to detach *A*, put a cap over the joint, and introduce a Teflon-coated magnetic stirrer into well *E* to promote good mixing of the contents. Again, all operations can be conducted under the appropriate gaseous atmosphere. Reagents may be added through a dropping funnel attached to condenser joint *P*. If a substance is to be carbonated as in a Grignard process, chlorinated, or oxidized, the desired gas can be introduced by way of the cocks *I*, *H*, and *B* and bubbled into the medium through the filter disk while the unit is in the horizontal position. With stopcock *N* closed, excess gas passes out through *P* and may be metered, absorbed, or otherwise treated according to requirements.

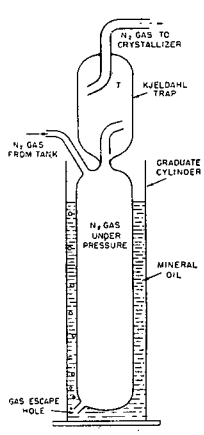

Figure 2. Gas reservoir for pressure control

One problem is the control of gas pressures when the apparatus under vacuum is restored to normal pressure (stopcock *N* closed, *I* in vacuum position, *I* is then switched to nitrogen inflow). A simple device that has proved satisfactory in this laboratory is shown in Figure 2.

The long reservoir tube provides a supply of gas under pressure greater than atmospheric as regulated by the height of liquid in the graduated cylinder, while excess gas bubbles out through *C*. By use of a viscous mineral oil in the cylinder, the surge of liquid when stopcock *I* (Figure 1) is switched from vacuum to nitrogen occurs at a moderate rate, and none can be forced out through the top because of the Kjeldahl trap. The operator, by slightly increasing the nitrogen flow from the tank valve, can bring the system quickly to equilibrium, for which purpose the rise or fall of liquid levels provides a convenient indicator.

An advantageous alteration of the unit in Figure 1 is attachment of a suction tube containing a filter disk like *C* to the side of flask *A*. Upon crystallization of filtrate solids in *A* (easily induced by partial evaporation of solvent through cock *B* or by chilling), a secondary purification can be accomplished by drawing out the remaining solution through the suction tube. Thereafter, it is necessary only to introduce fresh solvent into flask *E* and repeat the crystallization, gathering pure components in both flasks *E* and *A*.

The proposed design lends itself to compactness of construction around the central unit, *E*, so that a model could be constructed on a small scale to permit manipulation of quantities approaching micro dimensions. (For miniature scale operation a test tube could replace flask *E* and filter disk *C* be correspondingly reduced in size.) On the other hand, increase in size to handle large batches is no problem.

RESULTS

The apparatus described has proved valuable in the purification of sensitive compounds—e.g., drying-oil acids like eleostearic and licanic acids which must be handled under inert gas—and in the purification of precious substances where losses must be kept low—e.g., the resolution of *dl* mixtures by preparation of diastereo isomers where repeated crystallization is necessary.

ACKNOWLEDGMENT

The author is grateful to the Research Corp. of New York for support of a project during which this apparatus was perfected.

Chromatographic Chamber for Simultaneous Preparation of Many Paper Chromatograms

Irving R. Hunter, David F. Houston, and Harry S. Owens, Western Utilization Research Branch, U. S. Department of Agriculture, Albany 10, Calif.

It is frequently an advantage in preparing paper chromatograms, especially in quantitative work, to run a number of papers simultaneously under identical conditions. This operation requires a great deal of space and equipment if 18 × 22 inch papers are used. A trend to smaller papers has resulted, and several investigators (*1–3*) have found that amino acids can be nicely separated on smaller papers. In this laboratory, a convenient chromatographic tank has been devised by modification of commercial photographic developing tanks. It will accommodate as many as 60 papers 9 × 9 inches or smaller.

APPARATUS

The complete assembly, shown in Figure 1, consists of a large outer tank, into which one to three smaller ones can be inserted. These are the actual developing units. All construction material is stainless steel except the neoprene gaskets. The outer tank

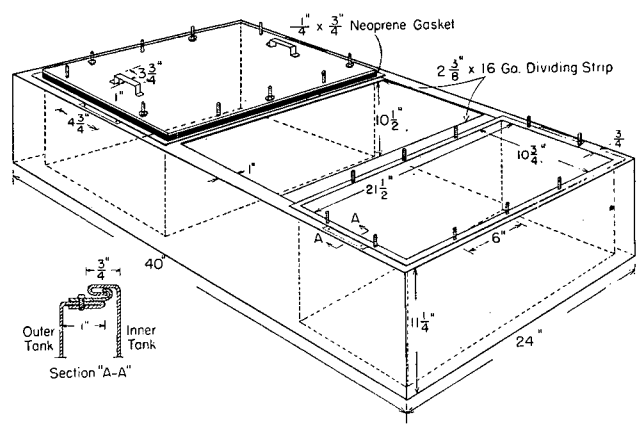

Figure 1. Stainless steel chromatographic tank

serves as an air or water bath to maintain constant temperature, which has been shown necessary by Rockland (*4*) for maximum reproducibility of R_f values. If the assembly is located in a constant-temperature room, three developing tanks can be inserted into the outer jacket. If a constant-temperature room is not available, the outer jacket can be used as a water bath. The center tank can be replaced by a thermostatically controlled heating and stirring unit.

The developing tanks are held in the outer jacket by stainless steel clips (*A*, Figure 1). These are shaped to engage the flange of the inner tank as shown, and are then screwed to the outer tank. Two clips are used, one on each end of the developing tank. When the outer jacket is filled with water, the buoyant effect can be utilized to level the inner tanks. Their height and position above the outer jacket are regulated simply by adjusting the screws holding the clips to the outer container. Where an air bath is used, the leveling adjustment can be made by inserting shims between the clip and the outer jacket.

The covers of the inner tanks are secured tightly by stainless steel bolts, washers, and wing nuts. The bolts are welded to the flange of the inner tank and are not removed when the cover is taken off. When frequent inspection of the papers is desired, a plate-glass cover can be used instead of a metal cover. Here a modified outer jacket is employed, whereby the glass cover is held against the gasket by clamps arranged on swivels attached to the outer tank (Figure 2).

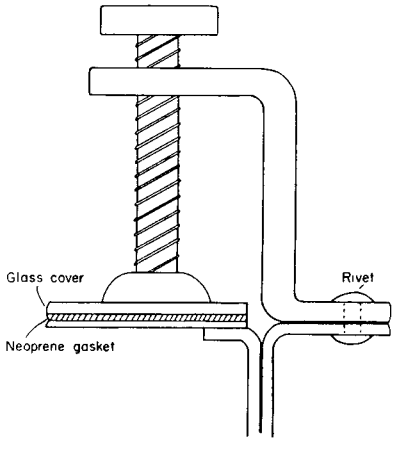

Figure 2. Clamp arrangement

The tanks are designed to accommodate 9 × 9 inch chromatographic papers, upon which adequate resolution of 18 amino acids or several sugars has been found practicable. Papers of this size were obtained ready cut (Schleicher and Schüll) in 500-sheet packages. They are fastened to the racks shown in Figure 3 with stainless steel spring clips.

Each rack will hold five papers, and four racks can be inserted in each developing chamber. Hence the total capacity of the apparatus is 40 to 60 papers. Normally there is sufficient space in the tank so that the end papers do not come in contact with the walls of the tank. Certain solvent systems cause excessive curling and bowing of the papers. In these solvents only four papers are placed on a rack. In this way contact between the papers or with the tank walls is prevented.

OPERATION

The mixture to be analyzed is applied from a micropipet as a spot in one corner 1 inch from each edge of the paper. The papers are then clipped to the frame with care that the spotted corners are all at the same lower corner of the frame, and that the papers are level and do not touch the sides of the frame. Wearing surgical gloves during manipulation of the papers avoids finger marks on the final chromatogram. The papers rest against the crossbars, *A* (Figure 3), which prevent contact between adjacent papers. This does not interfere with migration of the spots in any way.

The paper-filled racks are placed in the chromatographic tank containing about 3500 ml. of the appropriate solvent and left until the solvent front has reached the top of the paper. The racks are removed and the papers allowed to air-dry. In two-dimensional work, the papers are then rotated 90° and treated

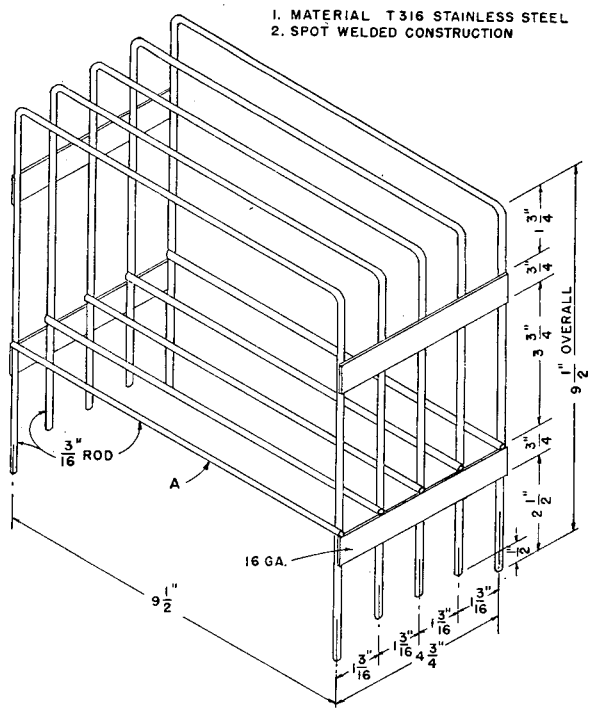

Figure 3. Stainless steel rack

T316 stainless steel; spot-welded construction

with a second solvent in the usual way. Colors of the resulting spots are subsequently developed by a suitable indicator, such as ninhydrin for amino acids or aniline-trichloroacetic acid for reducing sugars.

As many as 40 papers have been run simultaneously with this apparatus. This capacity is a decided advantage in quantitative paper chromatography, where several replicates of each determination are desired.

ACKNOWLEDGMENT

The authors wish to thank Earl L. Muller and John G. Gill of the plumbing shop for their aid and advice and B. C. Lovett and N. Floy Bracelin for preparing the illustrations.

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MENTION of specific products or equipment does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Convenient Starch Electrophoresis Apparatus

Kenneth Paigen[1], Virus Laboratory, University of California, Berkeley, Calif.

ZONE electrophoresis on a preparative scale has come into increasing use, and starch, first introduced by Kunkel and Slater (*2*), has proved a versatile supporting medium. Electrophoresis in starch has been used in this laboratory for some time, and a simple and convenient apparatus has been developed which possesses a number of advantages, particularly in construction

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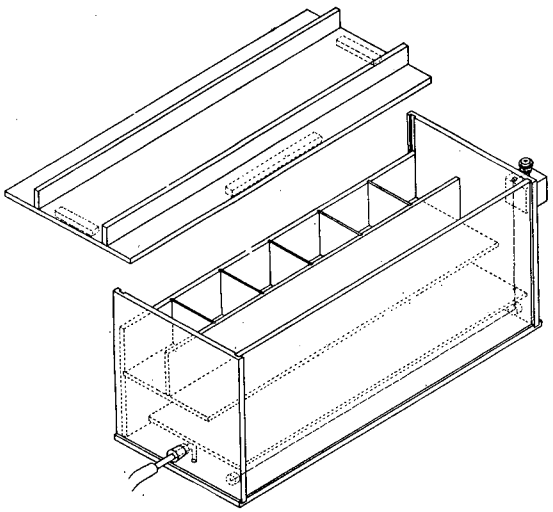

Figure 1. Electrode chamber

Over-all dimensions 35 × 14 × 13 cm. Subcompartments are 5.4 cm. wide to accommodate troughs used. No dimensions are critical

and operation, over the usual wax paper and glass arrangement (*1*, *2*).

APPARATUS

Electrode Vessel. An electrode vessel is shown in Figure 1. The baffle plates serve to isolate any pH changes occurring at the electrodes. For the anode chamber it is desirable to have a number of subcompartments, into which the troughs dip, which are connected to the main chamber by paper or agar bridges. These compartments serve two functions. They conserve material in cases where expensive or rare buffers are required, as it is not necessary to have the same buffer in both the main and subcompartments, and when a series of troughs at different pH's or ionic strengths are run they serve to isolate the troughs from any changes induced by the electro-osmotic flow of buffer into them. In the case of starch, electro-osmotic flow is toward the cathode, so the compartments are not required for the cathode chamber. Carbon rod may be substituted for the platinum wire as the cathode, but carbon anodes are not advisable, as they show a marked tendency to disintegrate, particularly at higher voltages. Lifting the filled apparatus, which contains about 3 liters, frequently causes fractures to develop at some of the joints, so a drainage outlet is provided. During operation this is clamped off. The outlet is provided by a hole in the front, which is plugged with a one-hole rubber stopper carrying a glass tube to which is attached a section of rubber tubing.

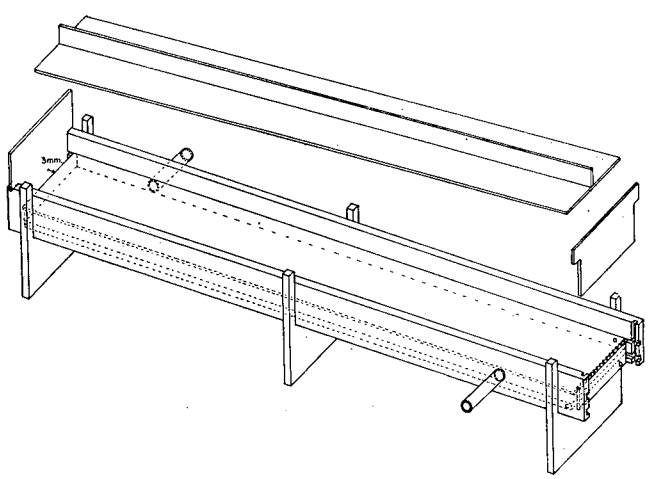

Figure 2. Trough

30 cm. long and 3.9 cm. wide (inner diameter)

Troughs. Two types of troughs have been used. In Figures 2 and 3 is shown a water-jacketed trough which is used when very high voltages are required. For operations at lower voltages a simpler trough may be constructed; partitions *a* and *b* and the hose nipples are omitted, and base plate *c* is reduced in thickness to $^1/_{16}$ inch.

Construction. In general $^1/_8$-inch Lucite was used in the construction of both the electrode chambers and troughs. However, surfaces which function in heat exchange, and the end gates, were made of $^1/_{16}$-inch Lucite. All joints were cemented, and wherever possible mortised construction was employed to obviate leakage problems. Construction of the troughs is simplified if a wooden jig is used, although this is not essential.

OPERATION

Preparation of Starch. The starch used was prepared from commercial potato starch by a simultaneous extraction of the water-soluble materials and removal of the smaller starch particles which would otherwise be difficult to remove from the final eluates. To 1500 grams of commercial potato starch in a 4-liter beaker sufficient water is added to bring the volume up to 3.5 liters, and the mixture is stirred thoroughly. The supernatant fluid is decanted after a settling time of 120 minutes. This procedure is repeated a total of eight times, or until the supernatant fluid has an absorbance of less than 0.02 against water at 260 mμ, with a gradual reduction of the settling time to 45 minutes. The final product is washed three times with 95% ethyl alcohol and air-dried. The yield is about 35% of a rapidly settling starch with a low content of water-extractible materials.

Preparation of Trough. For use, a 16-inch strip of 1.5 inches wide filter paper (Whatman No. 3 mm) is folded to produce an eight-layered pad measuring approximately 1.5 × 2 inches. One of these is placed against each end of the trough, and the end gates are inserted. The end papers are wetted with buffer, and a slurry of equal weights of potato starch (30 to 60 grams) and buffer is poured in. Excess fluid drains out through the end papers which subsequently serve as conducting bridges to make connection with the electrode vessels. The drainage time is about 1 hour. The bed thickness is approximately 1 mm. for each 15 grams of starch employed.

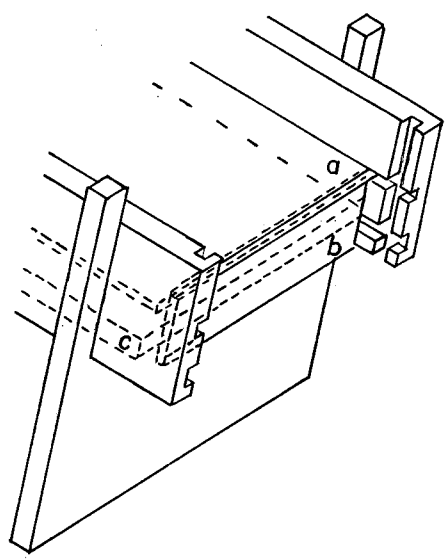

Figure 3

Introduction of Sample. Two methods have been used for the introduction of the sample. A rectangular hole, perpendicular to the long axis of the trough, is cut in the starch and a slurry of sample plus starch spooned in, or, more easily, the sample is taken up in a hypodermic syringe fitted with a blunt needle and injected into the trough to form a narrow zone extending across the starch. A 0.4-ml. sample introduced by the latter method into a 40-gram (dry weight of starch) bed, results in a starting zone of 0.5-cm. width.

Voltage. Potentials of from 90 to 1200 volts (3 to 40 volts per cm.) have been used. The maximum voltage permissible in any

given experiment is set by the conductance of the bed, which depends on the bed thickness, the conductivity of the buffer, and the rate of heat dissipation.

Temperature Control. The apparatus is normally used in a cold room. The jacketed troughs are cooled by circulating ice water through them, the unjacketed by directing a fan at them. The maximum wattage permissible depends on the thickness of the starch bed and the type of trough employed. With the air-cooled trough containing 40 grams of dry starch (a bed thickness of 3 mm.) a power input of 1 watt does not result in any appreciable temperature rise over an extended period of time. Over a 24-hour period 2 watts may be used without serious heating effects; for runs lasting only several hours 10 watts can be used. With thicker starch beds the problem of heating is more serious because of the reduced rate of heat dissipation. For applications in which high wattages over long periods are required, the water-jacketed trough is recommended. In this case as much as 10 watts can be applied over a 24-hour period.

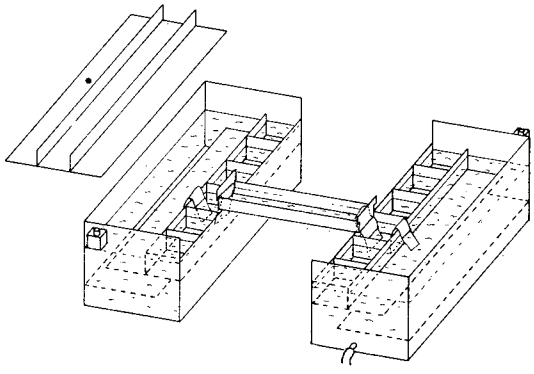

Figure 4. Schematic representation of apparatus in operation, showing arrangement of parts

Location of Material. Three methods have been used for the location of colorless samples. The trough may be sectioned and the individual sections analyzed. For sectioning, the bed is dried slightly by inserting a paper strip edgewise and then marked with a comb having teeth 0.5 cm. apart. After removal of the end gates and papers, the sections are easily sliced off and removed in serial order using a plastic strip 3.9 cm. wide, with a beveled edge. The individual sections are then mixed with an appropriate solvent and, if purified starch has been used, a clear supernatant can be removed after the starch has settled for 1 hour.

For substances which fluoresce or absorb in the ultraviolet, examination of the trough under an ultraviolet lamp will often show the location of the material. Finally, a strip of filter paper inserted edgewise into the starch bed soaks up solution, and tests with dye markers have shown that there is an excellent correspondence between the distribution of sample material on the paper strip and on the original starch bed. The strip may be stained or sprayed with an appropriate reagent to determine the positions of the materials under study.

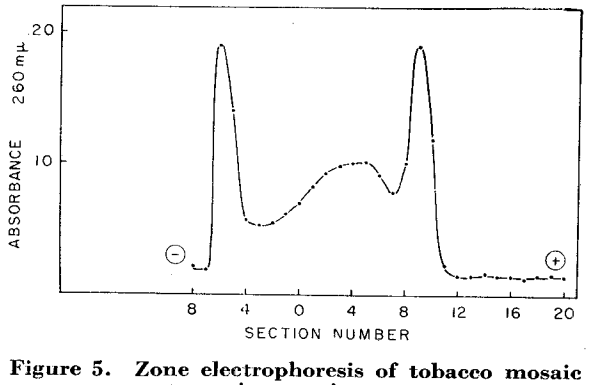

Figure 5. Zone electrophoresis of tobacco mosaic virus strains

Electrophoresis was carried out for 3.25 hours at 10 volts per cm. in 0.01*M* phosphate buffer of pH 7.1. An air cooled trough was employed. Following electrophoresis starch bed was cut into 0.5-cm. sections and each section eluted with 5 ml. of water. The absorbance at 260 mμ of each eluate was determined

APPLICATION

Figure 4 shows the arrangement of the parts during an electrophoresis experiment. An example of the type of result which can be obtained with this apparatus is shown in Figure 5. A mixture of two strains of tobacco mosaic virus [U_3 and U_5 (*3*)], which showed three components in the Tiselius electrophoresis apparatus, was run. The object of the experiment was to isolate each of the three components for further study. As examination of Figure 4 shows, each of the components could be eluted from the starch bed from a region essentially free of the other two components.

ACKNOWLEDGMENT

The author wishes to thank W. M. Stanley, for making the facilities of the Virus Laboratory available throughout this work, and Albert Siegel for the preparation of tobacco mosaic virus.

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WORK carried out during tenure of a Public Health Service Research Fellowship of the National Cancer Institute.

Needle Valve Stopcock

Theodore D. Perrine, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

BY INCORPORATING a needle valve in the plug of a laboratory stopcock, the author has developed a device which has proved useful in this laboratory, and which may be of value to others. Teflon has been used for the stopcock plug and Monel or stainless steel for the needle. Teflon has the advantage that the hole in the plug may be machined somewhat undersize, so that

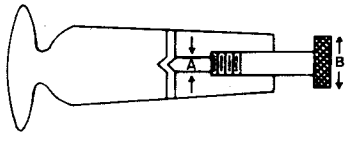


Figure 1

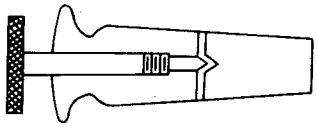


Figure 2

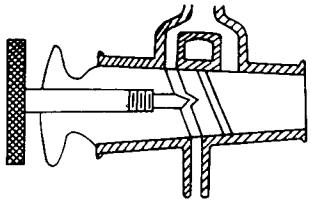


Figure 3

the walls hug the needle and obviate the necessity for a packing gland around the needle stem. This principle is also used to provide a positive seal in the bore of the plug when the needle valve is fully closed [the dimension *A* (Figure 1) being slightly greater than the bore of the plug].

Teflon has the disadvantage that it is difficult to get a completely leak-proof joint between the plug and the barrel of the stopcock. Usually this is not a serious matter, and the ordinary spring and nut holding device is sufficient to prevent excessive leakage. Where extreme tightness is essential, the plug should be carefully chosen to match the barrel, or the plug may be machined from Monel metal, or some other material which may be ground into the stopcock barrel. Where the plug is made of a material other than Teflon, the design in Figure 2 has the advantage that there is more room to construct a packing gland around the needle stem.

The ease of adjusting the flow rate depends upon the pitch of the threads and the diameter of the adjusting head (Figure 1,*B*). *B* should be small enough to pass through the barrel of the stopcock (for convenience in removal of the plug assembly, although this is not an essential feature). The design shown in Figure 2 permits the use of a big adjusting head, but it is not as convenient to adjust as is the design shown in Figure 1. If fine threads are cut in Teflon, care must be used not to screw the needle too tightly or the threads may be stripped.

One of the most useful applications for this device is in the slow addition of reagent by means of a dropping funnel. It is possible to set the addition rate very accurately, and to reproduce this rate time after time without altering the needle setting, as the flow can be stopped or started by turning the entire plug. Also if solids are present in the solution being metered, as in the addition of ethereal aluminum lithium hydride ($AlLiH_4$) to a reaction, plugging of the stopcock can be overcome by rotating the plug about its axis 180°. The flow through the bore is reversed and the plugging material is washed away, thus restoring the original flow rate. Another useful application is the control of flow in chromatograph and ion exchange columns. In hazardous reactions, such as the addition of sulfuric acid to water, this device could present the accidental discharge of a large, uncontrolled amount of the reagent.

The arrangement illustrated in Figure 3 is designed to permit alternate large and small flow rates—as, for example, in routine titrations. This is fabricated from a three-way stopcock such as Corning No. 7380 (Corning Glass Works, Corning, N. Y.). In this arrangement, however, it is not possible to overcome plugging of the bore by rotating the plug.

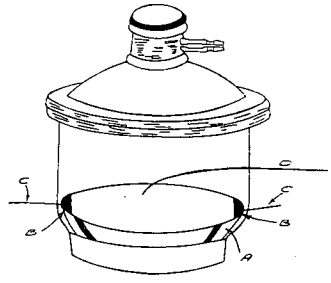
Heated Vacuum Desiccator

F. T. Wadsworth, American Oil Co., Texas City, Tex.

Frequently, occasions arise in which it is desirable to dry or remove trace quantities of a solvent from a product in a relatively short time. Although vacuum ovens can be employed, space limitations and availability of such equipment are frequent obstacles. Drying pistols can be used for small samples but are not convenient for many applications. Many of these obstacles can be overcome with a standard vacuum desiccator equipped with a low wattage, electric heating plate.

The essential features of this equipment are shown in the diagram. The porcelain plate supplied with the desiccator is replaced by a circular plate of Corning heating glass (Corning Glass Works, Corning, N. Y., Bull. B-89), rated at 500 watts per square foot at 110 volts. This plate can be easily cut to fit with a glass saw and should be of such size that a minimum of 0.25-inch clearance exists between the plate and the wall of the desiccator. The heating plate is supported by a glass frame, *A*, equipped with three equally spaced supporting points to minimize heat transfer to the desiccator.

Electrical contact is provided by silvering sectors of the plate as shown by *B*. Each sector is approximately one fifth the circumference of the plate. Clamps made of spring brass, which fit over the edge of the glass, provide electrical contact with the silvered surfaces. Electrical leads through the desiccator wall are provided by drilling two $^1/_{16}$-inch holes, *C*, through opposite sides of the desiccator approximately 0.25 inch below the bottom surface of the heating plate. These leads are soldered to the brass clamps. A third hole, *D*, is drilled about 1 inch above the upper surface of the plate to accommodate a thermocouple for measuring the temperature of either the plate or the substance being dried.



Vacuum-tight seals for these wires can be made with a porcelain cement. The temperature of the plate is controlled by adjusting the applied voltage with a variable transformer. Temperatures as high as 80° C. may be maintained for prolonged periods without heating the desiccator excessively.

Anhydrous Hydrogen Chloride Generator

Harry Taniguchi and George J. Janz, Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N. Y.

In the course of investigations on electrode potentials in nonaqueous media, an apparatus to meet a need for pure anhydrous hydrogen chloride was designed in this laboratory. Some of the elements of labor and hazard associated with the conventional generators (Maxson, R. N., "Inorganic Synthesis," Vol. I, p. 147, McGraw-Hill, New York, 1939) are effectively eliminated in the operation of this generator. In addition, an all-glass construction avoids contact of the hydrogen chloride with rubber.

The arrangement of the apparatus is shown schematically in Figure 1. The required amount of sodium chloride is charged through side arm *D* to the reaction chamber, *F*. The glass beads in *F* have been helpful in preventing caking of the chloride charge

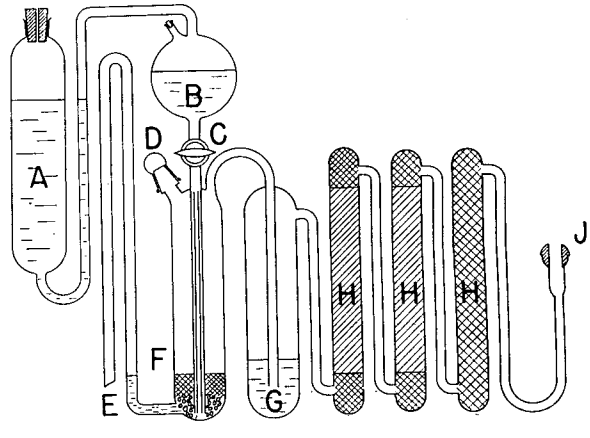


Figure 1. Anhydrous hydrogen chloride generator

once the sulfuric acid is added. In practice, reservoir *A* is filled with more than sufficient concentrated sulfuric acid for the experiment. By gentle suction, an aliquot volume of sulfuric acid can be transferred to the dropping funnel, *B*, from the storage reservoir. Stopcock *C* controls the rate of sulfuric acid addition to the chloride in the reaction vessel. The rate of hydrogen chloride evolution is indicated by the bubbler, *G*, containing concentrated sulfuric acid. The latter thus serves for the preliminary drying of the hydrogen chloride. The three towers, *H*, are packed with Drierite and glass wool. The stock solution receiving the anhydrous hydrogen chloride connects in at the exit of train *J*.

A novel feature is that the spent reaction mixture can be siphoned from chamber *F* through outlet *E* by applying gentle suction. Preparation for a new charge can thus be effected with very little labor and a minimum of hazard. In addition, by maintaining the bulk of the sulfuric acid in *A*, the danger of a serious acid spill through failure of stopcock *C* is effectively avoided. A safety tube, consisting of a mercury bubbler open to the atmosphere, is used in practice between *F* and *G*.

In this laboratory the apparatus was designed to handle sodium chloride charges of 5 to 50 grams. The apparatus was all borosilicate glass. Vessels *A*, *F*, and *G* were 45 mm. in diameter and 30 cm. in length, and *H* was 25 mm. in diameter and 60 cm. in length. Reservoir *B* was 100 ml., and side arm *D* was a 24/40 standard taper joint. All the other tubing was 6 mm. in diameter.

The generator operates equally well with concentrated hydrochloric acid as with a solid chloride for the reaction charge. From chamber *F* to exit *J*, escape of the hydrogen chloride to the atmosphere is impossible, for the apparatus is a continuous glass unit. Once the storage reservoir, *A*, has been charged, all transfers of the sulfuric acid, and the spent reaction mixture can be effected by siphoning action.

ACKNOWLEDGMENT

This apparatus was developed in the course of work supported by the Office of Scientific Research, Air Research and Development Command, under Contract AF 18(600)-333.

Trough for Use in Descending Paper Chromatography

John F. Thompson and Maurice V. Marion, U. S. Plant, Soil, and Nutrition Laboratory, Agricultural Research Service, Ithaca, N. Y.

ENAMELED iron troughs have been recommended (*6*) for use in descending two-directional paper chromatography. However, it has been found that in using these with acidic solvents—e.g., butanol and acetic acid (*5*)—the enamel chipped off, exposing the metal surface to the paper and solvent. This is undesirable in quantitative analysis of amino acids, because metals form complexes with amino acids which do not chromatograph well and do not react with ninhydrin (*3*). Metals also catalyze the oxidation of phenol, forming products which deaminate amino acids (*2*). Stainless steel troughs (*1*) are unsatisfactory for this reason. Another type of trough is made of glass tubing (*3*, *4*), but this is fragile and requires a rigid support over the entire length.

A more satisfactory trough is made from borosilicate glass pipe with a wall thickness of 4 mm., 42 mm. in outside diameter and 26 inches long (fabricated by Scientific Glass Apparatus Co., Bloomfield, N. J.). This trough is strong and rigid and requires no auxiliary support. There has been no breakage in two years of intensive use. In addition to resistance to acidic solvents, the glass troughs hold larger quantities of solvent than the enameled ones (*6*) (300 ml. *vs.* 200 ml. maximum capacity), and are less expensive.

The principal disadvantage of the glass trough lies in the fact that the glass supporting rods must be mounted separately. This problem has been solved by making one rack for all supporting rods in a cabinet. Glass rods are fastened in a paraffined wooden support at each end, just beyond the ends of the trough. The support is positioned with four wooden dowel pins which fit into holes in the rack, automatically aligning rods over the edges of the troughs. By clipping the developed papers to the glass rods, all chromatograms in a cabinet can be removed simultaneously by lifting out the whole rack. The solvents are then evaporated from the paper in this position.

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Packing Adsorbents in Chromatographic Columns

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UNIFORMITY of packing is a necessity for clear separation of constituents on a chromatographic column (*4*, *5*, *8*), but some recent references have been somewhat vague on the best method of packing a column (*1*, *2*, *6*, *7*). Engineers in the field of soil mechanics have shown that the densest mass of a given group of particles is obtained when those particles are compacted in a confined space in a "saturated" condition—i.e., there are no air voids present (*3*).

Maximum compaction of sand in a container of water can be obtained by tapping the sides of the container while the water is flowing slowly downward. Any upward movement of the water causes disturbance of the compacted sand. The following method of preparing a chromatographic column is therefore recommended. The adsorbent (Florisil, silica, etc.) should be slurried with the first solvent to be used in the separation. The column is half filled with the solvent, and the slurry is poured into the column; the clear solvent is drained from the bottom if necessary. After all of the adsorbent has been transferred to the column, the solvent is allowed to drain at some intermediate rate (0.1 to 1.0 ml. per minute), while the side of the tube is firmly tapped with a rubber mallet or a large rubber stopper. Some liquid should always be present above the top of the adsorbent to avoid entrapment of air.

By this method of column preparation, the maximum packing can be achieved with little lowering of the flow rate (provided adsorbents larger than 200-mesh size are used). Because no air is present, there is no air-liquid interfacial tension, and flow rates of 1 or 2 ml. per minute are easily attained. No pressure, suction, or pressing with a rod is necessary, and the maximum surface area of contact between adsorbent and solvent is achieved.

ACKNOWLEDGMENT

This work was conducted with the support of the Iowa Division of the American Cancer Society.

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