ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

What's in a Name?

BEGINNING in 1939 a few microchemists within the AMERICAN CHEMICAL SOCIETY gathered together informally at national meetings to discuss their problems and present a few papers. At that time those interested in analysis usually presented their papers before the Division of Physical and Inorganic Chemistry. This was logical, inasmuch as most papers on analysis dealt with inorganic systems.

In 1938 the Division of Microchemistry was authorized. This step crystallized the thinking of other analysts who felt their needs were not being served as part of another division, and so they asked to become part of the Division of Microchemistry. This officially took place in 1940, and the name was changed to the Division of Analytical and Micro Chemistry.

During the intervening years the scope and responsibility of the division have increased to the point where microchemistry and inorganic analysis are but a part of the interests which are, and should be, served by the division. The growth of the division has more than tripled this year. This growth, coupled with the developments of analytical chemistry, which now encompasses many new approaches and instruments not analytically developed ten years ago, has caused many to ask why it should not simply be called the Division of Analytical Chemistry. We believe, in keeping with the broad scope of analytical chemistry in which the division is now interested, that a change in name is desirable and that it in no way diminishes the importance of microchemistry in the activities of the division.

The division's Executive Committee, after careful thought and sampling of opinion, plans this month to submit a letter ballot requesting an official expression from division membership relative to the change in name. This action is timely, since at this time also new by-laws will be submitted for approval in accordance with the new constitution and regulations of the Society.

Under this proposed new and more appropriate name we believe the division can become one of the larger and more important of the A.C.S. divisional groups. Although some progress has been made in this direction during the past several years, there will be greater opportunity under the broader title to expand still further the division's scope and influence and thus better serve the needs and interests of all analytical chemists.

Successful Summer Symposium

IN THE COURSE of discharging our editorial duties we naturally find it necessary to be present at many scientific and professional gatherings. Occasionally one of these meetings stands out in our memory as particularly stimulating, fruitful, and pleasurable. Such an occasion was the recent Second Annual Summer Symposium on Analytical Chemistry. There on the lovely campus of Wesleyan University at Middletown, Conn., gathered the cream of the nation's analysts.

The formal program devoted to organic reagents brought forth a prominent cast of speakers. They came well prepared and made fine presentations. All the arrangements with regard to registration, housing, and other details were handled so smoothly that these frequently irksome chores became almost a pleasure. Although the technical subject was covered comprehensively in the formal program, the pace was unhurried and ample opportunity was available for discussion.

Even now as we write this, several weeks after the meeting, we have a clear mental picture of the pleasant atmosphere of the event. Between sessions many small groups took advantage of the opportunity to relax under the shade of Wesleyan's beautiful trees and discuss analytical problems or just engage in general conversation. From personal participation we know that these friendly and informal discussions are highly valuable, especially to the younger members, because of the information exchanged and lasting friendships that are made. A heart-warming aspect was the welcome given to the two distinguished guests, world-famous analyst Fritz Feigl from Brazil and Arthur B. Lamb, retiring editor of the *Journal of the American Chemical Society*.

No meeting operates smoothly without a lot of planning and hard work behind the scenes. We know there was plenty of both in connection with the Summer Symposium. All who were involved, the officers of the division and the various committees, may take pride in a job well done. S. E. Q. Ashley, general chairman, and M. G. Burford, chairman of the committee on local arrangements, especially are to be congratulated for their splendid handling of a most successful symposium.

Benzene, Toluene, Ethylbenzene, o-Xylene, m-Xylene, and p-Xylene

Determination by Ultraviolet Spectrophotometry

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An ultraviolet absorption method is presented for the determination of the C_6, C_7, and C_8 aromatic hydrocarbons. The accuracy of the determination of each component is about 1% of the total aromatic content, except for toluene and ethylbenzene which are determined to about 2% when both are present in the same sample. Tests to determine the presence of interfering components are included in the method. A chemical treatment is described which is effective in removing many interfering unsaturates and sulfur compounds.

THE determination of the individual C_6, C_7, and C_8 aromatics by chemical methods is difficult because of the great similarity in the chemical properties of these compounds. Fortunately, their ultraviolet absorption spectra differ sufficiently to permit an accurate determination of each component. Ultraviolet absorption is particularly useful for the determination of aromatics, for they are often associated with saturated hydrocarbons which have no absorption in the spectral region used in the analysis and, consequently, do not interfere. Unsaturates and sulfur compounds which are often present in low concentrations with aromatics usually can be removed by chemical treatment. Shaking the diluted sample with mercuric nitrate solution has been found effective for this purpose. The spectrophotometric method is also comparatively rapid and does not require a high degree of skill in routine operation.

The number of individual aromatics that can be determined simultaneously in a mixture is limited to the number of suitable spectral positions available for the analysis. The spectral positions must be taken at wave lengths that will result in a set of independent simultaneous linear equations relating the concentration of each component to the observed optical densities. As the equations approach dependency the errors in the analysis become very large. This condition is usually observed only when there is a marked similarity between the absorption spectra of two of the components. However, even when this does not occur a condition of linear dependence will exist if the absorption spectrum of one component is very similar to the absorption spectrum of a mixture of two or more of the other components. This condition can be detected by making a preliminary calculation of the expected errors as described in a later section.

In the analysis of complex mixtures of aromatics suitable spectral positions may not be available, so the mixture must first be separated by distillation into fractions containing only a limited number of aromatics. Methods are given below for determination of the individual aromatics present in the following mixtures: benzene and toluene; ethylbenzene, *o*-xylene, *m*-xylene, and *p*-xylene; and benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, and *p*-xylene. A simplified procedure is also included for the determination of benzene alone and for toluene alone. The ultraviolet absorption spectrum of each of these compounds is shown in Figures 1 and 2.

After this paper was written a method for the determination of ethylbenzene and the xylenes was outlined by Fulton and Heigl (*5*). To the best of the authors' knowledge, an ultraviolet absorption method for the determination of these components was developed independently and simultaneously by the Esso Laboratories, Standard Oil Company of New Jersey; the Research Laboratory, Shell Oil Company, Houston, Tex.; and Shell Development Company, Emeryville, Calif. A method for the analysis of benzene-toluene mixtures was distributed by Shell Development Company at the Rubber Reserve Company–Petroleum Administration for War meeting at Houston, Tex., in August 1943.

The absorption spectra of toluene and ethylbenzene are very similar, as would be expected from their similarity in structure. This fact materially decreases the accuracy which can be obtained in determining each of these components when both are present. Consequently, for the most accurate results, the C_6, C_7, and C_8 aromatics should be separated by distillation into two fractions, one containing the C_6 and C_7 aromatics, and the other the C_8 aromatics. Each component in the 2-component, the 4-component, and the 6-component systems can be determined to 1% of the total aromatic content except that, as indicated above,

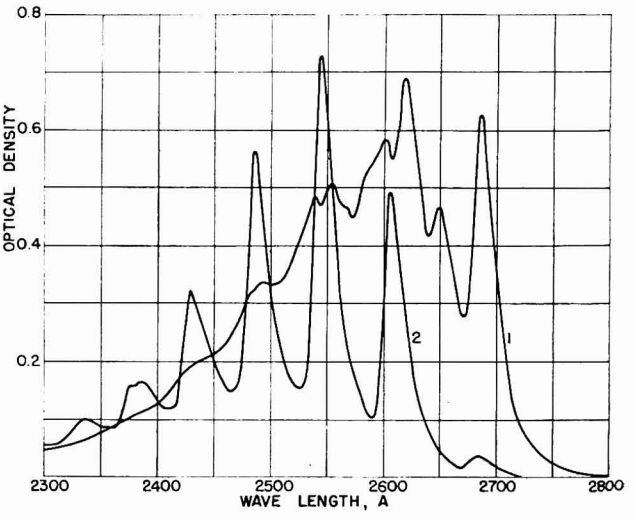

Figure 1. Absorption Spectra of Benzene and Toluene in Iso-octane
1. Toluene
2. Benzene
0.250 gram per liter
Cell length 1.00 cm.

[1] Present address, Tracerlab Inc., 2295 San Pablo Ave., Berkeley 2, Calif.

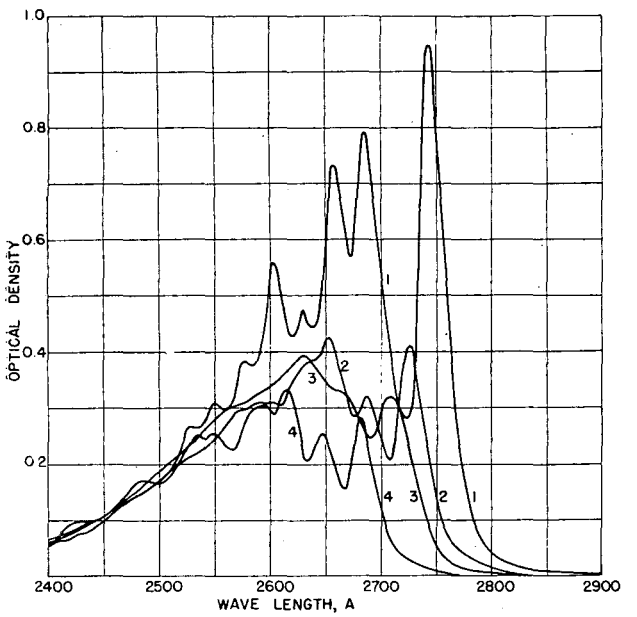

Figure 2. Absorption Spectra of Ethylbenzene and Xylenes in Iso-octane

1. *p*-Xylene
2. *m*-Xylene
3. *o*-Xylene
4. Ethylbenzene

0.160 gram per liter
Cell length 1.00 cm.

the toluene and ethylbenzene in the 6-component mixture can be determined to only 2 to 3%. The sum of the two, however, is accurate to 1%.

THEORY

The principles of multicomponent spectrophotometric analyses have been discussed previously (*1, 2, 5, 12*).

According to Beer's law for a single absorbing component in a cell of constant length and at a given wave length

$$D = Ec \qquad (1)$$

where

D = optical density = $\log_{10} \frac{1}{\text{transmission}}$

transmission = ratio of energy transmitted by the sample to energy incident on the sample

E = a constant (the extinction coefficient)

c = concentration of the absorbing component

E is, of course, a function of wave length. The concentration can be expressed in any desired units which are directly proportional to the actual moles of the absorbing compound per unit volume. These may be grams per liter, moles per liter, or milliliters per liter. In this paper it is assumed that the concentration is expressed in grams per liter and that the results of the analysis will be calculated in weight per cent.

The total optical density at wave length i for n absorbing components is given by:

$$D_i = E_{i1}c_1 + E_{i2}c_2 + E_{i3}c_3 + \ldots\ldots + E_{in}c_n \qquad (2)$$

The values of the extinction coefficients in Equations 2 are determined by calibrating with pure compounds. The concentration of each of the n components in an actual sample is determined by measuring the optical density at n suitable wave lengths and solving the resulting simultaneous equations. Considerable time can be saved in these calculations by the use of the inverted equations which are calculated by the method of Crout (*4*). These equations take the form:

$$c_n = k_{n1}D_1 + k_{n2}D_2 + k_{n3}D_3 + \ldots + k_{nn}D_n \qquad (3)$$

where k_{ij} = new constants calculated from the extinction coefficients—i.e., (k_{ij}) is the matrix inverse to (E_{ij}).

Deviations from the relation expressed in Equation 1—i.e., deviations from the linear relation between optical density and concentration—may occur for two possible reasons:

1. Molecular interaction between the molecules of the absorbing substance. A deviation from this source may be designated as a real deviation from Beer's law. Such deviations have not been observed for aromatics in the dilute solutions used in the method described below.
2. Spectrophotometers do not measure the absorption of only one wave length of light but of a small range of wave lengths. If the optical density varies considerably through this range of wave lengths, deviations from Equation 1 may be observed. This type of deviation may be designated as an apparent deviation from Beer's law because it is not a property of the absorbing substance but is due solely to the limitations of the spectrophotometer. Experience has shown that apparent deviations will not be significant if the nominal band width isolated by the spectrophotometer does not exceed 5 Å. as determined from the dispersion data furnished with the Beckman quartz spectrophotometer. If wider slits are used and such deviations are observed, the accuracy of the determination will be reduced unless suitable corrections are applied. The method of using these corrections has been described by Brattain, Rasmussen, and Cravath (*1*).

At still wider slits (nominal band width 20 Å.) deviations have been observed from the assumption that the total optical density of a mixture is equal to the sum of the optical densities of the individual components. The correction method mentioned above does not include correction for deviations of this type. Corrections for this type of deviation can be developed and applied, but the procedure is very laborious.

The use of narrow slits, thus avoiding all apparent deviations, is much to be desired, as the use of corrections entails considerable additional work both in the calibration and in the calculation of the results of the analysis, and also entails a possible loss of accuracy.

APPARATUS AND REAGENTS

Spectrophotometer. A Beckman quartz spectrophotometer (*3*) equipped with ultraviolet accessories was used in the development of this method.

It is recommended that this instrument be equipped with a special entrance-mirror-mounting block which has provision for the circulation of constant-temperature water. This part can be obtained from the manufacturers of the spectrophotometer. It maintains the cell compartment at a constant temperature and reduces temperature fluctuations of the monochromator. Otherwise, temperature changes in the instrument may be rather large, owing to changes in the room temperature and to heat from the hydrogen lamp.

Thermostating is desirable for two reasons: (1) The absorption spectra themselves are temperature-dependent. Thus, significant changes in the absorption spectra of aromatics have been reported for a temperature change of 5° C. (*10*). Even much greater effects are observed in other analyses—for example, the optical density of 1,3-butadiene in the vapor phase was found to change 1% of the value at 2410 Å. with a temperature change of only 0.5° C. (*9*). Similar changes have been observed for other dienes. (2) Variations in the temperature appreciably change the wave-length interval isolated by the monochromator. This is particularly important in the analysis of aromatics because readings are taken at times on the sides of steep absorption bands where small errors in the wave-length setting cause large errors in optical density.

The wave-length reproducibility can also be improved by always setting the wave-length scale from the same side and by setting directly on one of the index lines. Changes in the wavelength position can be caused by improper focus of the hydrogen

lamp which affects the distribution of light across the first slit of the monochromator. The spectrophotometers used in this laboratory are tilted forward 20° for convenience in operation. This tilting produced a small but nevertheless significant and reproducible shift in wave lengths. Consequently, it is essential that the calibration and the analysis be done with the instrument in the same position.

Shifts in the mean wave length of the energy passing through the second slit for a given setting of the wave-length scale can be detected by periodically measuring the optical density of a standard solution of *p*-xylene at 2760 and 2735 Å. Because these positions are on opposite sides of the *p*-xylene peak at 2745 Å., a wave-length shift will cause the optical density at one of the wave lengths to increase and the optical density at the other wave length to decrease. Small but significant wave-length shifts have been observed for each of the three Beckman spectrophotometers in use in this laboratory. Whenever this occurs the wave-length scale is reset by adjusting the collimating mirror (according to the instructions furnished with the spectrophotometer) so as to give the correct optical density for the *p*-xylene solution. After the wave-length scale has been reset in this manner the results obtained are as accurate as those obtained just after the calibration of the instrument. All the synthetic samples included in this paper were analyzed after resetting the wave-length scale and using a calibration made approximately 20 months previously. Although the wave-length scale was reset successfully for the ultraviolet region, no data were taken to show the accuracy with which it was reset in the visible.

The slits should be taken as narrow as is practical but not so narrow that a slight reduction in intensity of the hydrogen lamp will require an increase in the slit width. A satisfactory compromise is the use of slit widths at each wave length corresponding to an adjustment of the "sensitivity" control about two turns back from the position giving the minimum slit widths.

If narrower slits are desired, the 2000-megohm resistance in the grid circuit of the electrometer tube can be replaced by a 5000-megohm resistance. For best results only resistors supplied by the manufacturers of the spectrophotometer, or IRC metallized glass resistors, should be used for this purpose. This resistance is designated as R_{10} in Figure 5 of (*3*). The 5000-megohm resistance has been used in two spectrophotometers in this laboratory for some time. The only effect seems to be to increase slightly the time constant in the amplifying circuits and thus increase slightly the time required to balance the potentiometer.

Table I. Wave Lengths Used for Determination of Combinations of Aromatics

Analysis	Wave Lengths for Analysis, Å.	Wave Lengths for Interference Test, Å.
Benzene only	2605, 2545	2900, 2470
Toluene only	2685, 2620	2900, 2470
Benzene and toluene	2685, 2545	2900, 2470
C_8 aromatics	2475, 2725, 2710, 2545	2900, 2470
C_8, C_7, and C_8 aromatics	2745, 2725, 2710, 2685, 2590, 2545	2900, 2470

Table II. Analysis of Synthetic Benzene-Toluene Samples

Sample No.	Benzene			Toluene		
	Theory %	Found %	Error %	Theory %	Found %	Error %
1160-2A	0.0	0.1	+0.1	100.0	99.1	−0.9
1160-2B	0.0	0.2	+0.2	100.0	98.7	−1.3
1158-49A	10.3	10.7	+0.4	89.7	89.9	+0.2
1158-49B	10.2	10.7	+0.5	89.8	89.2	−0.6
1160-4B	29.2	29.7	+0.5	70.8	71.1	+0.3
1160-4C	29.7	30.0	+0.3	70.3	70.9	+0.6
1158-50G	50.8	51.1	+0.3	49.2	49.8	+0.6
1158-50H	49.0	48.8	−0.2	51.0	51.1	+0.1
1160-2G	70.8	70.6	−0.2	29.2	28.6	−0.6
1160-4A	70.7	71.4	+0.7	29.3	29.5	+0.2
1158-49C	88.4	89.2	+0.8	11.6	12.2	+0.6
1158-49D	90.2	90.2	0.0	9.8	10.7	+0.9
1160-2C	100.0	100.5	+0.5	0.0	0.1	+0.1
1160-2D	100.0	101.5	+1.5	0.0	0.8	+0.8
	Av. error		0.4			0.6
	Theoretical av. error		0.5			0.5

Table III. Analysis of Synthetic Ethylbenzene-Xylene Samples

Sample No.	Ethylbenzene			*o*-Xylene			*m*-Xylene			*p*-Xylene		
	Theory %	Found %	Error %	Theory %	Found %	Error %	Theory %	Found %	Error %	Theory %	Found %	Error %
1941-3A	100.0	101.0	+1.0	0.0	−0.4	−0.4	0.0	−0.2	−0.2	0.0	0.1	+0.1
1941-3B	100.0	100.7	+0.7	0.0	0.0	0.0	0.0	−0.1	−0.1	0.0	0.0	0.0
1941-3C	0.0	0.4	+0.4	100.0	101.3	+1.3	0.0	−0.9	−0.9	0.0	0.2	+0.2
1941-3D	0.0	0.9	+0.9	100.0	101.1	+1.1	0.0	−0.7	−0.7	0.0	0.1	+0.1
1941-4A	0.0	0.0	0.0	0.0	0.4	+0.4	100.0	99.0	−1.0	0.0	0.0	0.0
1941-4B	0.0	0.5	+0.5	0.0	−0.6	−0.6	100.0	100.3	+0.3	0.0	0.1	+0.1
1941-4C	0.0	−1.7	−1.7	0.0	2.7	+2.7	0.0	−2.0	−2.0	100.0	101.0	+1.0
1941-4D	0.0	−1.2	−1.2	0.0	2.5	+2.5	0.0	−1.1	−1.1	100.0	100.1	0.0
1941-8A	26.7	28.2	+1.5	24.9	24.0	−0.9	25.1	24.7	−0.4	23.4	23.3	−0.1
1941-8B	25.1	27.6	+2.5	26.0	25.6	−0.4	25.8	25.9	+0.1	23.1	22.9	−0.2
	Av. error		1.0			1.0			0.6			0.2
	Theoretical av. error		1.1			1.1			0.9			0.2

Table IV. Analysis of

Sample No.	Benzene			Toluene		
	Theory %	Found %	Error %	Theory %	Found %	Error %
1941-5A	100.0	100.2	+0.2	0.0	0.5	+0.5
1941-5B	100.0	101.4	+1.4	0.0	0.4	+0.4
1941-5C	0.0	0.4	+0.4	100.0	95.1	−4.9
1941-5D	0.0	0.6	+0.6	100.0	96.9	−3.1
1941-6A	63.0	64.3	+1.3	0.0	0.0	0.0
1941-6B	63.3	64.1	+0.8	0.0	−2.6	−2.6
1941-6C	52.5	52.8	+0.3	0.0	0.2	+0.2
1941-6D	48.9	49.7	+0.8	0.0	1.0	+1.0
1941-7A	0.0	0.2	+0.2	50.4	51.8	+1.4
1941-7B	0.0	0.0	0.0	49.7	53.3	+3.6
1941-7C	0.0	0.2	+0.2	50.2	49.5	−0.7
1941-7D	0.0	0.1	+0.1	53.1	52.9	−0.2
1941-8A	0.0	0.2	+0.2	0.0	−0.9	−0.9
1941-8B	0.0	0.6	+0.6	0.0	−2.4	−2.4
1941-10A	15.3	15.3	0.0	14.0	10.5	−3.5
1941-10B	17.1	17.2	+0.1	17.2	15.4	−1.8
	Av. error		0.5			1.7
	Theoretical av. error		0.6			2.6

Absorption Cells. Two fused silica absorption cells complete with covers are required. The method was developed using 1-cm. cells.

Spectroscopic Solvent. A transparent hydrocarbon solvent is required for diluting the sample. It may be prepared from isooctane or similar saturated hydrocarbon which is essentially free of aromatics and unsaturates. Traces of aromatics and unsaturates can be removed by percolating the solvent through silica gel as described by Graff, O'Connor, and Skau (*6*). The silica gel column shown in Figure 3 has been found to be both convenient and effective for the purpose. The lower end of the column fits into the ground-glass joint on a 3.785-liter (1-gallon), Pyrex, glass-stoppered bottle. The solvent is siphoned into the column through the Saran tube on top of the column, the siphon being started by applying suction to the side tube at the bottom of the column. The flow rate when filled with 453.6 grams (1 pound) of Davco 659528-2000C silica gel is approximately 2 gallons per hour.

The transmittance of the purified solvent in a 1-cm. cell as compared to distilled water should not be less than 95% in the spectral region between 2900 and 2450 Å. The solvent must be carefully stored in clean containers and must not come into contact with cork, rubber, or stopcock lubricants containing hydrocarbons. However, a lubricant consisting of starch, mannitol, and glycerol (*7*) can be used on stopcocks in a solvent dispenser.

Synthetic C_6, C_7, and C_8 Aromatic Samples

Ethylbenzene			*o*-Xylene			*m*-Xylene			*p*-Xylene		
Theory %	Found %	Error %	Theory %	Found %	Error %	Theory %	Found %	Error %	Theory %	Found %	Error %
0.0	−0.5	−0.5	0.0	0.2	+0.2	0.0	−0.3	−0.3	0.0	0.0	0.0
0.0	−1.2	−1.2	0.0	−0.3	−0.3	0.0	0.2	+0.2	0.0	0.1	+0.1
0.0	3.3	+3.3	0.0	3.8	+3.8	0.0	−1.4	−1.4	0.0	0.0	0.0
0.0	1.7	+1.7	0.0	2.4	+2.4	0.0	−1.2	−1.2	0.0	0.1	+0.1
0.0	0.3	+0.3	0.0	−0.2	−0.2	0.0	0.2	+0.2	27.0	37.4	+0.4
0.0	2.6	+2.6	0.0	0.8	+0.8	0.0	−0.3	−0.3	36.7	37.8	+1.1
0.0	−0.7	−0.7	47.5	48.6	+1.1	0.0	0.0	0.0	0.0	0.1	+0.1
0.0	−1.3	−1.3	51.1	52.1	+1.0	0.0	0.2	+0.2	0.0	−0.1	−0.1
0.0	−2.5	−2.5	0.0	1.0	+1.0	49.6	49.4	−0.2	0.0	0.4	+0.4
0.0	−4.5	−4.5	0.0	1.1	+1.1	50.3	49.8	−0.5	0.0	0.3	+0.3
49.8	50.2	+0.4	0.0	1.9	+1.9	0.0	−0.8	0.8	0.0	0.1	+0.1
46.9	46.2	−0.7	0.0	1.5	+1.5	0.0	−0.4	−0.4	0.0	0.1	+0.1
26.7	28.8	+2.1	24.9	24.3	−0.6	25.1	24.6	−0.5	23.4	23.3	−0.1
25.1	28.8	+3.7	26.0	26.3	+0.3	25.8	25.7	−0.1	23.1	23.0	−0.1
23.1	26.8	+3.7	17.2	18.3	+1.1	15.8	15.3	−0.5	14.6	15.1	+0.5
16.3	17.2	+0.9	17.0	18.1	+1.1	16.3	16.1	−0.2	16.1	16.2	+0.1
		1.9			1.2			0.4			0.2
		3.3			1.1			0.9			0.2

Pure Aromatics for Calibration. A pure sample of each of the aromatics that are to be included in the analysis is required for the calibration. These may be obtained from A.P.I. Project 46.

10% Mercuric Nitrate Reagent (for Removal of Interfering Materials). Ten milliliters of concentrated nitric acid are added to 100 grams of C.P. mercuric nitrate and diluted to 1 liter with water. The clear supernatant liquid is decanted off.

CALIBRATION OF SPECTROPHOTOMETER

The calibration of the spectrophotometer consists of determining the extinction coefficients of each aromatic at each wave length to be used in the analysis. Suitable wave lengths for the various combinations of aromatics are listed in Table I. They include 2900 and 2470 Å. which are used in the test for interfering absorption.

The calibration is made in the following steps:

1. Measuring the optical density of suitable dilutions of each of the pure aromatics at the prescribed wave lengths.
2. Choosing the extinction coefficients from a plot of the values calculated from Equation 1 against optical density.
3. Formation of the n simultaneous equations and inversion of these equations.

For each wave length standard slit widths are chosen as described above.

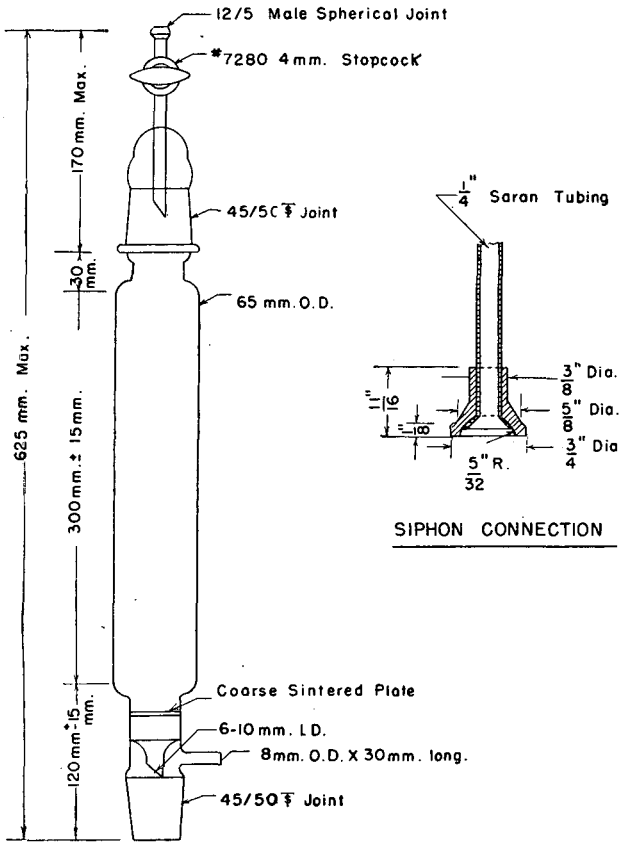

Figure 3. Silica Gel Column

Prepare suitable dilutions of each aromatic to be included in the analysis and, following the techniques given under analysis of sample, measure the optical density of each dilution at each of the wave lengths listed in Table I for the analysis and for the interference test. Choose the concentrations of aromatic in the dilutions to give at least four values distributed through the range in optical densities from 0.3 to 0.9 at the wave length with the maximum absorption. As none of these aromatics has significant absorption at 2900 Å., an optical density exceeding 0.003 at this wave length indicates either that the sample is impure or that the solution has become contaminated.

Using Equation 1, calculate from the observed optical density and the known aromatic concentration, expressed in grams per liter, the value of the extinction coefficient for each dilution and at each wave length except 2900 Å. Plot the calculated extinction coefficients for each aromatic at each wave length, against the observed optical density, and draw the best straight line through the points. If there are no deviations from Beer's law, this line will be parallel to the optical density axis and will give the value of the extinction coefficient. If there are deviations from Beer's law, the line will slope, and the value of the extinction coefficient will decrease at the higher optical densities. If such deviations are observed, proceed according to the method described in (*1*).

Substitute in Equation 2 the extinction coefficients for the wave lengths listed in Table I for the analysis of each combination of aromatics. This will give the same number of equations as there are components included in the analysis. Using the method of Crout (*4*), invert these equations to give Equation 3.

When only benzene or only toluene is to be determined—no other aromatic present—the results can be calculated either by the direct use of Equation 1 or from a suitable graph. If the latter method is preferred, take data from the plot of extinction coefficient against optical density and plot a curve of optical density against concentration in grams per liter. A graph is simpler than Equation 1 for calculating the concentration of a single absorbing component whenever there are deviations from Beer's law.

After completing the calibration and the calculation of Equation 3, check the method by the analysis of several synthetic samples, following the procedure described below.

ANALYSIS OF SAMPLE

Measuring Optical Density. Weigh 0.5 to 1.0 gram of sample into a glass-stoppered volumetric flask. If the sample contains high concentrations of benzene, add a few milliliters of the spectroscopic solvent to the flask before commencing the weighing, to avoid loss of benzene by evaporation and errors in the weight due to the buoyancy effect of the benzene vapor. Fill the flask to the mark with spectroscopic solvent, mix, and then prepare further volumetric dilutions as required until the optical density of the diluted sample at the wave length of maximum absorption falls in the range 0.3 to 0.9. The dilution of the sample that fulfills this condition is referred to as the "diluted sample" in the following discussion.

Measure the optical density of the diluted sample at each wave length listed in Table I for the particular type of analysis, including readings at 2900 and 2470 Å. Use the standard slit

widths employed in the calibration and use some of the spectroscopic solvent, used in preparing the dilutions, as a blank. Flush and dry the inside of the absorption cells, taking care not to contaminate the outside surfaces. Refill the cells with sample and solvent but place the sample in the cell used previously for solvent and again measure the optical density at each of the prescribed wave lengths. This interchange corrects for differences in transmission of the two cells and for imperfect cleaning of the outside surfaces, provided these surfaces are left unchanged during the measurements. Take the average of the two optical densities as the optical density of the sample for that wave length.

Cover both cells during the measurements to prevent loss by evaporation.

If it is suspected that the sample contains interfering unsaturates or sulfur compounds, or if the test described below shows the presence of interfering absorption, shake 15 to 20 ml. of the diluted sample with an equal volume of the mercuric nitrate reagent for 10 to 20 minutes. Proceeding as described above, determine the optical density of the treated sample, using as a blank, a portion of the solvent which has been similarly treated.

Calculations. BENZENE ONLY. Read from the calibration curves for 2605 and 2545 Å., or calculate using Equation 1 the concentration of benzene corresponding to the optical density observed at these wave lengths. From the average of these two values (see exception below) and from the dilution, calculate the percentage of benzene present in the sample.

TOLUENE ONLY. Determine the concentration of toluene from the optical densities at 2685 and 2620 Å. as with benzene only.

BENZENE AND TOLUENE. Substitute the optical densities observed at 2685 and 2545 Å. in the inverse equations (Equation 3, $n = 2$) obtained from the calibration and calculate the concentration of benzene and toluene. From these values calculate the percentage of benzene and toluene present in the sample.

ETHYLBENZENE, *o*-XYLENE, *m*-XYLENE, AND *p*-XYLENE. Substitute the optical densities observed at 2745, 2725, 2710, and 2545 Å. in the inverse equations (Equation 3, $n = 4$) and calculate the concentration of each component. From these values calculate the percentage of each aromatic present in the sample.

BENZENE, TOLUENE, ETHYLBENZENE, *o*-XYLENE, *m*-XYLENE, AND *p*-XYLENE. Substitute the optical densities found at 2745, 2725, 2710, 2685, 2590, and 2545 Å. in the inverse equations (Equation 3, $n = 6$) and calculate the concentration of each component. From these values calculate the percentage of each aromatic present in the sample.

CORRECTION FOR BENZENE LOST IN CHEMICAL TREATMENT. It was first reported by Powell and Rappoport (*8*) and later confirmed in this laboratory that part of this benzene in a dilute solution is lost when the solution is shaken with aqueous reagents. The nature of this loss is unknown; experiments have shown that only a negligible amount of the loss can be accounted for by solubility in the aqueous phase. Similar losses have not been observed for the other aromatics. A number of experiments have shown that the loss is dependent on the ratio of the volumes of the solution and the reagent. For equal volumes the loss was found to be approximately 2% of the benzene content of the solution. Consequently, the benzene content of treated samples should be increased by 2% of the value found.

Test for Interfering Absorption. Occasionally, samples contain unknown compounds which absorb in the spectral region used for the analysis and consequently the analysis gives incorrect results. Unless the samples are known to contain no interfering compounds, they should be tested by the two methods described below.

1. Determination of the optical density of the diluted sample at 2900 Å. Because none of these aromatics has appreciable absorption at this wave length, an optical density at 2900 Å. which exceeds 0.003 shows the presence of interference.

2. Using the concentrations determined from the analytical procedure described above calculate the optical density that should be observed at 2470 Å. if this optical density is due solely to the aromatics. This value is calculated by substituting in Equation 2 the calculated concentration of each of the aromatics and the corresponding extinction coefficients for 2470 Å. as determined during the calibration. The observed optical density should not exceed the calculated value by more than 0.010. As the interference usually increases at shorter wave lengths, this test may show the presence of interference when the first test does not.

The same tests can also be applied in the determination of a single component. In this case, the agreement between the concentrations determined at the two wave lengths is an additional test. Because the interference usually increases at the shorter wave length, the optical density determined at the longer of the two wave lengths will be more nearly correct and should be used for calculating the concentration if there is a significant difference between the two values which cannot be reduced by chemical treatment.

If any of the above tests indicates interference, the diluted sample is treated with mercuric nitrate as described above. If the interference persists, it may be possible to devise some other chemical treatment. An algebraic method of correcting for interfering absorption is presented in another paper (*11*).

DISCUSSION

The results obtained by the analysis of a number of synthetic samples are given in Tables II, III, and IV. The average error in these results agrees satisfactorily with the theoretical errors calculated from the expressions

$$\Delta c_i = \sqrt{\sum_{j=1}^{n} (k_{ij}\,\Delta D_j)^2} \qquad (4)$$

where Δc_i = average error in concentration of component i
k_{ij} = values of k from Equation 3 for component i and wave length j
ΔD_j = average error in optical density at wave length j

The theoretical average errors given in Tables II, III, and IV were calculated by assuming a value of ΔD_j of 0.003 at all wave lengths. Experience has shown this to be a conservative value. These errors were converted to percentage by assuming a total aromatic content of 0.25 gram per liter, which is in the range of aromatic content used in the determination.

These theoretical average errors have been found useful in predicting the errors of a new analysis before the actual work of the calibration and the analysis of synthetic samples is undertaken. Approximate extinction coefficients such as may be obtained directly from absorption spectra are usually sufficiently accurate for this purpose.

ACKNOWLEDGMENT

The authors wish to acknowledge the excellent work of M. Morse and R. Hatch in obtaining the necessary calibration data and in analyzing the synthetic samples listed in this paper.

LITERATURE CITED

(1) Brattain, R. R., Rasmussen, R. S., and Cravath, A. M., *J. Applied Phys.*, **14**, 418 (1943).
(2) Brode, W. R., Patterson, J. W., Brown, J. B., and Frankel, J., IND. ENG. CHEM., ANAL. ED., **16**, 77 (1944).
(3) Cary, H. H., and Beckman, A. O., *J. Optical Soc. Am.*, **31**, 682 (1941).
(4) Crout, P. D., *Trans. Am. Inst. Elec. Engrs.*, **60**, 1235 (1941).
(5) Fulton, S. C., and Heigl, J. J., *Instruments*, **20**, 35 (January 1947).
(6) Graff, M. M., O'Connor, R. I., and Skau, E. L., IND. ENG. CHEM., ANAL. ED., **16**, 556 (1944).
(7) Meloche, C. C., and Fredrick, W. G., *J. Am. Chem. Soc.*, **54**, 3264 (1932).
(8) Powell, J. S., and Rappoport, D. A., Southern California Gas Co., Los Angeles, Calif., private communication.
(9) Rasmussen, R. S., Tunnicliff, D. D., and Brattain, R. R., *J. Chem. Phys.*, **11**, 432 (1943).
(10) Tucker, E. B., Standard Oil Co. (Indiana), Whiting, Ind., private communication.
(11) Tunnicliff, D. D., Rasmussen, R. S., and Morse, M., ANAL. CHEM., **21**, 895 (1949).
(12) Weissler, A., IND. ENG. CHEM., ANAL. ED., **17**, 695 (1945).

RECEIVED August 10, 1948.

Correction for Interfering Absorption in Spectrophotometric Analyses

Application to Determination of C_6, C_7, and C_8 Aromatic Hydrocarbons and to Determination of Naphthalene by Ultraviolet Absorption

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An algebraic method for the correction of spectrophotometric data for the effect of interfering absorption is based on the assumption that the optical density of the interference can be represented by an analytical function of wave length, and that this function does not represent the optical density of any of the components being determined. The optical density is measured at a sufficient number of wave lengths to determine the constants of the function as well as the concentrations of the desired components. However, in practice, the calculation of the constants is omitted. The specific applications described are for the determination of benzene and toluene; ethylbenzene, *o*-xylene, *m*-xylene, and *p*-xylene; and naphthalene. The degree of correction for different types of interfering absorption is shown by the results of the analysis of a number of synthetic samples containing interference.

THE application of spectrophotometric analysis is often seriously limited by the presence of unknown substances which absorb at one or more of the wave lengths used in the analysis. This intereference can often be removed by chemical treatment (*5*). However, in cases where the interfering material resists chemical treatment, the interference may cause such large errors in the analysis that the results are valueless unless suitable corrections are applied. This condition is frequently encountered in the determination of aromatics by ultraviolet absorption. It is very difficult to ascertain definitely the nature of these interfering compounds or even to determine the exact shape of their absorption curves. Possible compounds include olefins, compounds containing sulfur, and peroxides. Although many compounds in the first two categories can be removed by chemical treatment, others are very resistant.

Experience has shown that the interfering absorption in the spectral region used for the analyses is usually a smooth function of the wave length; the absorption decreases, with various degrees of steepness, as wave lengths increase. Occasionally the absorption curve of the interference has a maximum or a minimum in the spectral region used for the analysis. Absorption curves of types believed to be typical—actual absorption curves of various pure compounds—are shown in Figures 1, 2, and 3. The compounds chosen for this purpose are not necessarily those that may be present in actual samples but were selected because their absorption curves simulate the absorption curves of the actual interfering substances.

Two methods, which are simpler than the algebraic method, are frequently useful. Figure 4, curve 1, shows the absorption curve of an interfering substance; curve 2 shows the absorption curve of a sample of benzene containing this substance. A very simple method which gives an approximate correction for the interference is based on a measurement of the optical density of the sample at a benzene peak and at the base of the peak—e.g., at 2545 and 2530 Å. The benzene content is then calculated from this difference (d_1 in Figure 4) and from calibration data obtained at the same wave lengths with pure benzene. This method assumes that the interference does not differ significantly between 2545 and 2530 Å.—i.e., Δd_1 is not significant. This assumption is valid only if the interfering optical density is changing very slowly with the wave length or the absorption peak is very steep on one side, so that two wave lengths can be chosen which lie very close together. Although the correction in this example is not very accurate, this method is often useful in favorable circumstances.

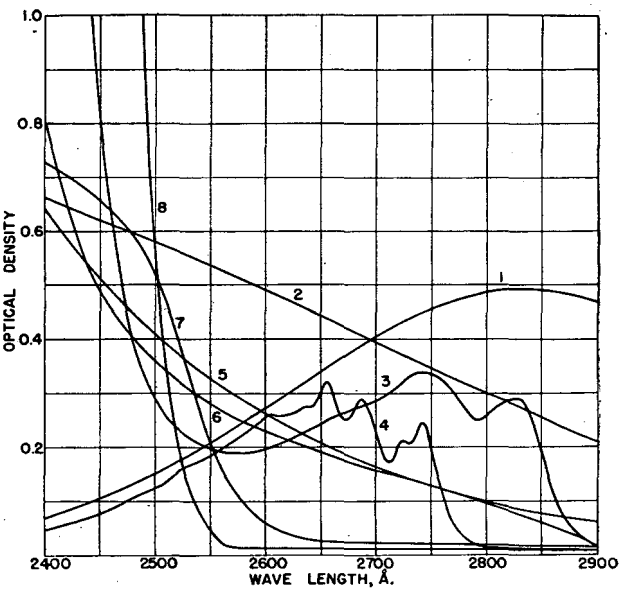

Figure 1. Absorption of Interfering Materials Added to Synthetic Samples Listed in Table I

A more accurate method (*1, 3, 4, 7*) consists of measuring the optical density on both sides of the absorption peak and calculating the correction to be applied at the peak by linear interpolation. This method is commonly referred to as the base-line method. Referring to the example in Figure 4, the benzene concentration would then be calculated from the difference, d_2. In this case it is assumed that the absorption is changing linearly through this short spectral interval—i.e., Δd_2 is not significant. This method usually gives good results for very sharp absorption peaks where the wave-length positions on opposite sides of the peak are close together, as is the case with benzene.

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Table I. Analysis of Synthetic Benzene-Toluene Samples Containing Interference

Sample No.	Interference	Theory		2685 and 2545 Å., No Correction				Quartic Correction[a]				Least-Squares Quartic Correction[b]			
				Benzene		Toluene		Benzene		Toluene		Benzene		Toluene	
		Benzene	Toluene	Found	Error	Found	Error	Found	Error	Found	Error	Found	Error	Found	Error
		%	%	%	%	%	%	%	%	%	%	%	%	%	%
4082-38A	None	100.0	0.0	100.6	+0.6	0.3	+0.3	101.1	+1.1	0.8	+0.8	100.4	+0.4	1.0	+1.0
4082-38B	None	100.0	0.0	101.7	+0.7	−0.1	−0.1	101.9	+1.9	0.5	+0.5	100.2	+0.2	1.0	+1.0
4082-36A	None	0.0	100.0	−0.1	−0.1	100.0	0.0	0.7	+0.7	100.0	0.0	−0.1	−0.1	100.1	+0.1
4082-36B	None	0.0	100.0	−0.5	−0.5	100.1	+0.1	1.1	+1.1	99.4	−0.6	0.1	−0.1	99.7	−0.3
4082-41A	None	52.0	48.0	52.6	+0.6	47.7	−0.3	53.3	+1.3	47.8	−0.2	52.3	+0.3	48.1	+0.1
4082-44A	None	48.1	51.9	48.3	+0.2	51.9	0.0	48.9	+0.8	52.4	+0.5	48.5	+0.4	52.5	+0.6
4082-39A	Figure 1, curve 7	100.0	0.0	131.3	+31.3	0.4	+0.4	110.0	+10.0	−2.4	−2.4	100.0	0.0	0.3	+0.3
4082-39B	Figure 1, curve 7	100.0	0.0	140.1	+40.1	0.8	+0.8	111.8	+11.8	−3.4	−3.4	99.3	−0.7	0.0	0.0
4082-37A	Figure 1, curve 7	0.0	100.0	37.2	+37.2	100.7	+0.7	11.4	+11.4	96.4	−3.6	−0.1	−0.1	99.3	−0.7
4082-37B	Figure 1, curve 7	0.0	100.0	38.2	+38.2	100.9	+0.9	12.6	+12.6	96.4	−3.6	0.2	+0.2	99.6	−0.4
4082-42A	Figure 1, curve 7	52.0	48.0	81.4	+29.4	48.1	+0.1	60.5	+8.5	44.4	−3.6	51.3	+0.7	46.8	−1.2
4082-43A	Figure 1, curve 7	48.1	51.9	77.7	+29.6	52.3	+0.4	56.2	+8.1	49.3	−2.6	47.0	−1.1	51.7	−0.2
4082-42B	Figure 1, curve 5	52.0	48.0	80.6	+28.6	72.6	+24.6	54.3	+2.3	47.7	−0.3	52.2	+0.2	48.4	+0.4
4082-43B	Figure 1, curve 5	48.1	51.9	77.1	+29.0	76.3	+24.4	49.8	+1.7	50.3	−1.6	48.0	−0.1	50.8	−1.1
4082-42C	Figure 1, curve 1	52.0	48.0	39.3	−12.7	108.2	+60.2	51.2	−0.8	48.7	+0.7	51.5	−0.5	48.6	−0.6
4082-43C	Figure 1, curve 1	48.1	51.9	36.1	−12.0	113.1	+61.2	47.2	−0.9	52.3	+0.4	48.5	−0.4	52.0	+0.1
4082-42D	Figure 1, curve 8	52.0	48.0	58.1	+6.1	47.9	−0.1	50.3	−1.7	48.9	+0.9	52.7	+0.7	48.9	+0.9
4082-43D	Figure 1, curve 8	48.1	51.9	54.2	+6.1	51.6	−0.3	47.4	−0.7	53.1	+1.2	48.8	+0.7	53.3	+1.4
4082-42F	Figure 1, curve 2	52.0	48.0	83.0	+31.0	108.2	+60.2	52.4	+0.4	47.0	−1.0	49.8	−2.2	47.7	−0.3
4082-43E	Figure 1, curve 2	48.1	51.9	80.9	+32.8	113.4	+61.5	48.8	+0.7	51.6	−0.3	47.2	−0.9	52.1	+0.2
4082-42E	Figure 1, curve 3	52.0	48.0	50.9	−1.1	90.0	+42.0	53.9	+1.9	46.3	−1.7	52.1	+0.1	46.8	−1.2
4082-43F	Figure 1, curve 3	48.1	51.9	47.1	−1.0	94.2	+42.3	49.3	+1.2	49.5	−2.4	48.2	+0.1	49.8	−2.1
4082-42G	Figure 1, curve 4	52.0	48.0	46.8	−5.2	93.0	+45.0	48.9	−3.1	57.3	+9.3	52.1	+0.1	56.5	+8.5
4082-43G	Figure 1, curve 4	48.1	51.9	42.4	−5.7	97.6	+45.7	44.0	−4.1	61.1	+9.2	47.8	−0.3	60.0	+8.1
4082-42H	Figure 1, curve 6	52.0	48.0	75.2	+23.2	71.5	+23.5	54.2	+2.2	46.1	−1.9	51.8	−0.2	46.8	−1.2
4082-43H	Figure 1, curve 6	48.1	51.9	71.3	+23.2	75.6	+23.7	49.6	+1.5	50.0	−1.9	47.5	−0.6	50.5	−1.4
	Theoretical av. error[c]				0.5		0.5		1.0		1.0		0.6		0.9

[a] Based on optical density measurements at 2745, 2685, 2670, 2620, 2590, 2545, and 2505 Å. Interference represented by equation $D_{int} = a_0 + a_1(\lambda - \lambda_0) + a_2(\lambda - \lambda_0)^2 + a_3(\lambda - \lambda_0)^3 + a_4(\lambda - \lambda_0)^4$.

[b] Based on optical density measurements at 2745, 2685, 2670, 2620, 2605, 2590, 2545, 2530, and 2505 Å. Interference represented by equation $D_{int} = a_0 + a_1(\lambda - \lambda_0) + a_2(\lambda - \lambda_0)^2 + a_3(\lambda - \lambda_0)^3 + a_4(\lambda - \lambda_0)^4$.

[c] Based on error in optical density of 0.003 and aromatic concentration of 0.25 gram per liter.

ALGEBRAIC CORRECTION METHOD

The algebraic correction method described in this paper is an extension of the base-line method. Instead of the assumption that the interference is linear over a small spectral interval, it is assumed that the optical density of the interference can be represented by some general analytic expression as a function of the wave length. A suitable function with adjustable parameters is chosen from a general knowledge of the absorption curves of the interfering materials found in the samples. The optical density of the sample is measured at a sufficient number of wave lengths to fix the numerical values of the parameters and the concentrations of each of the components being determined.

In practice the function must be expressed as a series with variable coefficients as the parameters:

$$D_{int}(\lambda) = \sum_{j=0}^{p} a_j f_j(\lambda) \quad (1)$$

where $D_{int}(\lambda)$ is the optical density of the interference as a function of the wave length, λ.

The function chosen to represent the optical density of the interference must satisfy two conditions: (1) It must accurately represent the optical density of the interference through the required spectral interval. (2) It must be impossible for the function to represent even approximately, at the wave lengths used in the analysis, the optical densities of any one component or any combination of the components to be determined.

The particular functions which have been found to be useful are the power series and a sum of descending exponentials. The general method followed in the use of these functions is illustrated below with the power series. The application of both types of functions to specific analyses is described in later sections.

In the case of the power series the optical density of the interference is represented by an equation of the following form:

$$D_{int}(\lambda) = a_0 + a_1(\lambda - \lambda_0) + a_2(\lambda - \lambda_0)^2 + \ldots\ldots + a_p(\lambda - \lambda_0)p \quad (2)$$

The choice of λ_0, the origin of the wave-length scale, is purely arbitrary; its purpose is solely to reduce the size of the numbers in the subsequent calculations.

The total optical density at wave length i of a sample containing interfering absorption in addition to n components to be determined is then given by the expression:

$$D_i = a_0 + a_1(\lambda_i - \lambda_0) + a_2(\lambda_i - \lambda_0)^2 + \ldots + a_p(\lambda_i - \lambda_0)p + E_{i1}c_1 + E_{i2}c_2 + \ldots + E_{in}c_n \quad (3)$$

Figure 2. Absorption of Interfering Materials Added to Synthetic Samples Listed in Table III

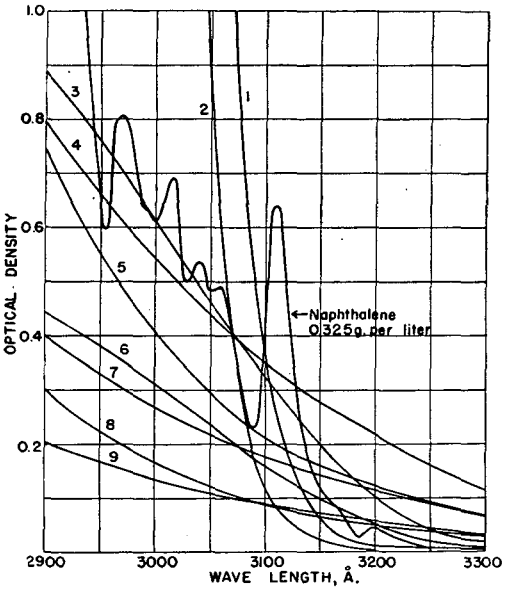

Figure 3. Absorption of Naphthalene with Interfering Materials Added to Synthetic Samples Listed in Table V

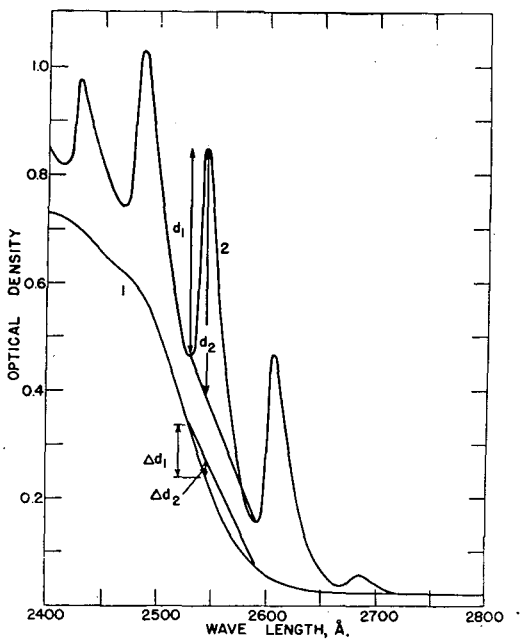

Figure 4. Absorption Curves

1. Interference
2. Benzene plus interference

where c_j = concentration of component j and E_{ij} = extinction coefficient of component j at wave length i.

In Equation 3 for a given wave length λ_i, the only unknown quantities are the values of the a's and the c's (the extinction coefficients can be determined by calibration with the pure components); consequently, this equation contains $p + 1 + n$ unknowns. By measuring the optical density at $p + 1 + n$ wave lengths, chosen so as to give a set of independent equations, it is possible to solve for all the a's and all the c's. The solution of this set of equations will have the form:

$$
\begin{aligned}
a_0 &= k_{11}D_1 + k_{12}D_2 + \ldots + k_{(1,p+1+n)}D_{(p+1+n)} \\
a_1 &= k_{21}D_1 + k_{22}D_2 + \ldots + k_{(2,p+1+n)}D_{(p+1+n)} \\
a_p &= k_{(p+1,1)}D_1 + k_{(p+1,2)}D_2 + \ldots \\
&\quad + k_{(p+1,p+1+n)}D_{(p+1+n)} \\
c_1 &= k_{(p+2,1)}D_1 + k_{(p+2,2)}D_2 + \ldots \\
&\quad + k_{(p+2,p+1+n)}D_{(p+1+n)} \\
c_2 &= k_{(p+3,1)}D_1 + k_{(p+3,2)}D_2 + \ldots \\
&\quad + k_{(p+3,p+1+n)}D_{(p+1+n)} \\
c_n &= k_{(p+1+n,1)}D_1 + k_{(p+1+n,2)}D_2 + \ldots \\
&\quad + k_{(p+1+n,p+1+n)}D_{(p+1+n)}
\end{aligned}
\qquad (4)
$$

The sequence of operations followed in the application of this method to the analysis of samples is as follows:

1. Choose a suitable function to represent the interference.
2. Choose n wave lengths that emphasize the differences between the absorption spectra of the n components. These will usually be the wave lengths used in a noninterference method for the same components.
3. Choose $p + 1$ wave lengths that will characterize the shape of the absorption curve of the interference throughout the spectral region containing the n wave lengths chosen above.
4. Determine the extinction coefficients of each of the n pure components at the $p + 1 + n$ wave lengths.
5. Substitute the extinction coefficients, the corresponding values of λ_i, and an arbitrary value of λ_0 in Equation 3 to give $p + 1 + n$ equations.
6. Invert these equations by the method described by Crout (*2*) to give equations of the form of Equations 4.
7. Measure the optical density of the sample to be analyzed at the $p + 1 + n$ wave lengths.
8. Substitute these optical densities in Equations 4 and solve for the concentrations of each of the n components. The values of the a's can be calculated also, if desired, but they are seldom of interest.

A similar procedure is followed when the interference is expressed in terms of a sum of descending exponentials. These exponentials will have the general form:

$$
D_{\text{int}}(\lambda) = a_0 + a_1 10^{-k_1(\lambda - \lambda_0)} + a_2 10^{-k_2(\lambda - \lambda_0)} + \ldots \qquad (5)
$$

The value of the k in each term is chosen in accordance with the nature of the interference expected in the samples. A value of k is chosen in one term so that this term can represent the steepest interference curve anticipated. Other values represent intermediate slopes. Figure 5 shows the curve obtained for various values of k.

Table II. Average Errors from Table I

Interference	Average Errors: No correction %	Quartic correction %	Least-squares quartic correction %
None	0.3	0.8	0.4
Figure 1, curve 7	17.4	6.8	0.5
Figure 1, curve 5	26.7	1.5	0.7
Figure 1, curve 1	36.5	0.8	0.5
Figure 1, curve 8	3.2	1.2	1.0
Figure 1, curve 2	46.3	0.7	0.9
Figure 1, curve 3	21.6	1.8	0.9
Figure 1, curve 4	25.5	6.5	4.3
Figure 1, curve 6	23.4	1.9	0.8

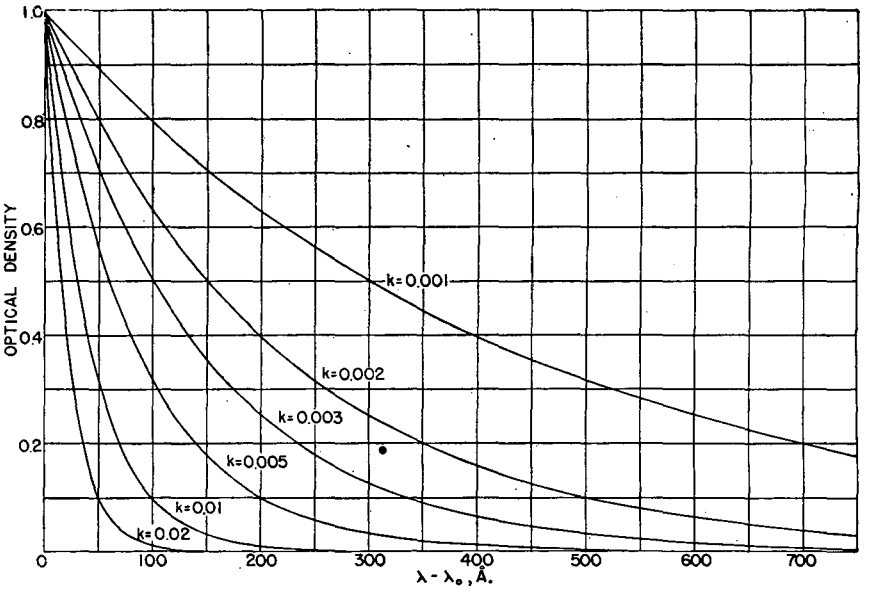

Figure 5. $D = 10^{-k(\lambda - \lambda_0)}$

Table III. Analysis of Synthetic Ethylbenzene-Xylene Samples Containing Interference

Sample No.	Interference	Theory				No Correction for Interference[a]						Algebraic Correction for Interference[b]									
		Ethyl-benzene	o-Xylene	m-Xylene	p-Xylene	Ethylbenzene		o-Xylene		m-Xylene		p-Xylene		Ethylbenzene		o-Xylene		m-Xylene		p-Xylene	
		%	%	%	%	Found %	Error %	Found %	Error %	Found %	Error %	Found %	Error %	Found %	Error %	Found %	Error %	Found %	Error %	Found %	Error %
1147-13A	None	26.2	24.7	25.0	24.1	25.8	−0.4	24.3	−0.4	25.3	+0.3	24.3	+0.2	26.1	−0.1	24.0	−0.7	25.1	+0.1	24.2	+0.1
1147-15A	None	25.5	24.3	23.4	26.8	24.8	−0.7	24.7	+0.4	23.4	0.0	26.9	+0.1	25.3	−0.2	24.8	+0.5	23.3	−0.1	26.8	0.0
1147-19A	None	32.6	38.5	16.9	12.0	32.2	−0.4	39.0	+0.5	17.0	+0.1	11.9	−0.1	31.9	−0.7	38.0	−0.5	16.8	−0.1	11.7	−0.3
1147-20A	None	34.0	40.0	15.3	10.7	34.0	0.0	40.3	+0.3	15.6	+0.3	10.5	−0.2	33.9	−0.1	39.1	−0.9	15.3	0.0	10.2	−0.5
1147-21A	None	12.2	17.0	28.3	42.5	11.4	−0.8	17.7	+0.7	28.7	+0.4	42.7	+0.2	13.4	+1.2	19.0	+2.0	28.7	+0.4	42.7	+0.2
1147-21B	None	12.5	15.7	29.2	42.6	12.4	−0.1	15.4	−0.3	29.9	+0.7	42.7	+0.1	13.1	+0.6	15.4	−0.3	29.7	+0.5	42.6	0.0
1147-22A	None	51.1	19.7	14.5	14.7	49.8	−1.3	19.8	+0.1	15.0	+0.5	14.8	+0.3	49.6	−0.8	19.6	−1.0	14.1	0.0	14.9	0.0
1147-22B	None	50.4	20.6	14.1	14.9	49.6	−0.8	20.7	+0.1	14.4	−0.2	15.2	+0.3	49.6	−0.8	19.6	−1.0	14.1	−0.4	15.7	−0.1
1147-23A	None	27.2	23.7	31.4	17.7	26.3	−0.9	24.7	+1.0	31.2	−0.2	17.8	+0.1	27.1	−0.1	24.6	+0.9	31.0	−0.4	17.6	−0.1
1147-23B	None	27.5	22.7	33.3	16.5	27.3	−0.2	23.7	+1.0	33.1	−0.2	16.6	+0.1	26.9	−0.6	23.0	+0.3	32.8	−0.5	16.4	−0.1
1147-2A	None	100.0	0.0	0.0	0.0	100.2	+0.2	−1.0	−1.0	0.5	+0.5	0.1	+0.1	99.3	−0.7	−2.4	−2.4	0.3	+0.3	−0.2	−0.2
4082-48A	None	100.0	0.0	0.0	0.0	98.6	−1.4	−0.7	−0.7	0.5	+0.5	0.2	+0.1	96.8	−3.2	−3.4	−3.4	0.0	0.0	−0.2	−0.2
4082-49A	None	100.0	0.0	0.0	0.0	99.3	−0.7	−0.4	−0.4	0.5	+0.5	0.1	+0.1	98.7	−1.3	−1.6	−1.6	0.3	+0.3	−0.1	−0.1
1147-18A	None	0.0	100.0	0.0	0.0	−1.6	−1.6	101.6	+1.6	−1.1	−1.1	0.0	0.0	−3.6	−3.6	99.0	−1.0	−1.5	−1.5	−0.4	−0.4
1147-3A	None	0.0	100.0	0.0	0.0	−0.1	−0.1	100.2	+0.2	−0.3	−0.3	0.1	+0.1	−0.3	−0.3	99.9	−0.1	−0.3	−0.3	−0.1	−0.1
1147-4A	None	0.0	100.0	0.0	0.0	−0.2	−0.2	99.9	−0.1	−0.1	−0.1	0.1	+0.1	−0.9	−0.9	98.8	−1.2	−0.3	−0.3	−0.1	−0.1
1147-18B	None	0.0	0.0	100.0	0.0	0.4	+0.4	1.0	+1.0	−0.1	−0.1	−0.1	−0.1	1.6	+1.6	1.4	+1.4	99.7	−0.3	−0.1	−0.1
1147-11A	None	0.0	0.0	100.0	0.0	0.4	+0.4	0.6	+0.6	99.8	−0.2	−0.1	−0.1	2.0	+2.0	1.7	+1.7	99.8	−0.2	−0.1	−0.1
1147-6A	None	0.0	0.0	0.0	100.0	−1.9	−1.9	0.1	+0.1	0.3	+0.3	100.7	+0.7	−3.0	−3.0	−2.3	−2.3	0.4	0.4	100.3	+0.3
1147-12A	None	0.0	0.0	0.0	100.0	−2.9	−2.9	0.9	+0.9	0.3	+0.3	101.5	+1.5	−1.1	−1.1	1.6	+1.6	0.2	+0.2	101.4	+1.4
1147-17A	None	0.0	0.0	0.0	100.0	−0.4	−0.4	−0.3	−0.3	1.0	+1.0	100.1	+0.1	0.3	+0.3	0.7	+0.7	1.2	+1.2	100.1	+0.1
1147-13B	Figure 2, curve 6	26.2	24.7	25.0	24.1	46.6	+20.4	35.8	+10.8	32.6	+8.5	27.9	+8.8	25.6	−0.1	33.0	+8.3	27.1	+2.1	25.3	+1.2
1147-15B	Figure 2, curve 6	25.5	24.3	23.4	26.8	44.8	+19.3	71.4	+47.1	34.9	+11.5	35.6	+8.8	25.6	−2.8	31.4	+7.1	25.8	+2.4	27.9	+1.1
1147-13B	Figure 2, curve 6	100.0	0.0	0.0	0.0	113.3	+13.3	36.4	+36.4	9.0	+9.0	7.0	+7.0	97.2	−2.8	4.1	+4.1	1.9	+1.9	1.0	+1.0
1147-2B	Figure 2, curve 6	100.0	0.0	0.0	0.0	114.9	+14.9	37.7	+37.7	9.1	+9.1	7.3	+7.3	98.9	−1.1	5.4	+5.4	1.9	+1.9	1.2	+1.2
4082-49B	Figure 2, curve 6	0.0	0.0	0.0	0.0	20.3	+20.3	148.1	+48.1	9.9	+9.9	8.8	+8.8	1.9	+1.9	109.0	+9.0	1.0	+1.0	1.3	+1.3
1147-3B	Figure 2, curve 6	0.0	0.0	0.0	0.0	16.2	+16.2	138.4	+38.4	8.6	+8.6	7.1	+7.1	−0.1	−0.1	104.5	+4.5	1.0	+1.0	0.8	+0.8
1147-4B	Figure 2, curve 6	0.0	100.0	0.0	0.0	21.6	+21.6	48.3	+48.3	111.3	+11.3	8.7	+8.7	4.4	+4.4	10.0	+10.0	102.3	+2.3	1.2	+1.2
1147-5B	Figure 2, curve 6	0.0	100.0	0.0	0.0	20.8	+20.8	47.0	+47.0	109.2	+9.2	8.5	+8.5	3.9	+3.9	9.4	+9.4	100.5	+0.5	1.2	+1.2
1147-11B	Figure 2, curve 6	0.0	0.0	0.0	100.0	41.8	+41.8	96.8	+96.8	24.0	+24.0	118.9	+18.9	4.2	+4.2	16.8	+16.8	5.6	+5.6	103.4	+3.4
1147-12B	Figure 2, curve 6	0.0	0.0	0.0	100.0	38.1	+38.1	94.3	+94.3	22.8	+22.8	117.0	+17.0	3.4	+3.4	18.6	+18.6	5.3	+5.3	102.2	+2.2
1147-17B	Figure 2, curve 6	0.0	0.0	0.0	100.0	36.0	+36.0	99.3	+99.3	22.0	+22.0	117.2	+17.2	3.3	+3.3	25.2	+25.2	4.4	+4.4	102.5	+2.5
1147-13C	Figure 2, curve 1	26.2	24.7	25.0	24.1	23.4	−2.8	56.8	+32.1	37.2	+12.2	35.1	+11.0	25.3	−0.9	27.5	+2.8	26.1	+1.1	25.5	+1.4
1147-15C	Figure 2, curve 1	25.5	24.3	23.4	26.8	23.2	−2.3	57.0	+32.7	35.7	+12.3	37.7	+10.9	26.5	+1.0	28.4	+4.1	24.6	+1.2	28.1	+3.8
1147-14A	Figure 2, curve 3	26.2	24.7	25.0	24.1	−2.5	−28.7	77.6	+52.9	50.1	+25.1	43.6	+19.5	27.1	+0.9	25.2	+0.5	25.8	+0.8	24.3	+0.2
1147-16A	Figure 2, curve 3	25.5	24.3	23.4	26.8	−4.1	−29.6	79.6	+55.3	48.0	+24.6	46.2	+19.4	27.6	+0.7	25.4	+0.7	23.1	+0.3	26.3	+0.5
1147-14B	Figure 2, curve 7	26.2	24.7	25.0	24.1	22.9	−3.3	36.9	+12.2	30.2	+5.2	28.1	+4.0	27.1	+0.9	25.6	+0.9	25.3	+0.3	24.2	+0.1
1147-16B	Figure 2, curve 7	25.5	24.3	23.4	26.8	21.5	−4.0	37.4	+13.1	29.0	+5.6	30.5	+3.7	24.1	−1.4	23.3	−1.0	23.6	−1.4	26.2	+0.6
1147-14C	Figure 2, curve 4	26.2	24.7	25.0	24.1	0.1	−25.1	86.8	+62.1	48.0	+23.0	43.4	+19.3	25.6	+0.6	26.1	+1.4	23.6	−1.4	24.2	+0.1
1147-16C	Figure 2, curve 4	25.5	24.3	23.4	26.8	−25.4	−65.5	85.5	+61.2	48.1	+24.7	46.0	+19.2	25.6	+0.5	25.2	+0.9	23.6	+0.2	26.7	+0.6
1147-14D	Figure 2, curve 5	26.2	24.7	25.0	24.1	−6.6	−62.9	39.1	+38.2	35.7	+14.1	35.7	+11.6	25.5	−0.7	23.6	−1.1	25.3	+0.2	24.7	+0.6
1147-16D	Figure 2, curve 5	25.5	24.3	23.4	26.8	−5.8	−64.1	+39.8	37.1	+13.7	37.6	+10.8	24.2	−1.3	21.8	−2.5	22.8	−0.6	26.1	+0.7
1147-23C	Figure 2, curve 2	27.2	23.7	31.4	17.7	−39.0	−66.2	90.2	+66.5	66.3	+34.9	45.3	+27.6	26.8	−0.4	18.3	−5.4	29.9	−1.6	16.7	−1.0
1147-23D	Figure 2, curve 2	27.5	22.7	33.3	16.5	−37.2	−64.7	87.1	+64.4	68.3	+35.0	43.6	+27.1	29.1	+1.6	18.3	−4.4	32.6	−0.7	15.7	−0.8
Theoretical av. error[c]						1.1		1.1		0.9		0.3		1.8		2.3		0.9		0.3	

[a] Based on optical density measurements at 2745, 2725, 2710, and 2685 Å.
[b] Based on optical density measurements at 2900, 2800, 2745, 2725, 2710, 2685, 2670, and 2620 Å. Interference represented by equation $D_{int} = a_0 + a_1 10^{-0.001(\lambda - \lambda_0)} + a_2 10^{-0.003(\lambda - \lambda_0)} + a_3 10^{-0.01(\lambda - \lambda_0)}$
[c] Based on error in optical density of 0.003 and aromatic concentration of 0.25 gram per liter.

The number of terms used in either the power series or the sum of descending exponentials is largely dependent on the particular analysis. Although increasing the number of terms will increase the ability of the function to represent the interfering absorption accurately, it will also greatly increase the possibility that it can also represent the optical densities of the components being determined. As the number of terms increases, the latter effect outweighs the former; consequently, it is advisable to use only a few terms. More terms can be used successfully when the absorption spectra of the components being determined contain a number of sharp absorption peaks rather than a single peak or broad peaks, as there is much less chance that the function will represent the optical density of a component.

METHOD FOR BENZENE AND TOLUENE

A quartic gives a satisfactory representation of the optical density of the interference usually encountered in the determination of benzene and toluene. This method requires optical density measurements at seven wave lengths ($p + 1 = 5$, $n = 2$): 2745, 2685, 2670, 2620, 2590, 2545, and 2505 Å. The accuracy of the method has been improved in some instances by measuring the optical density at nine wave lengths and making a least-squares solution. In this case it is not certain whether the gain in accuracy is due to the use of the nine wave lengths or to the use of a particular group of wave lengths. It is possible that seven wave lengths could be chosen which would give equally good results. The wave lengths used in the least-squares method are 2745, 2685, 2670, 2620, 2605, 2590, 2545, 2530, and 2505 Å. The least-squares solution of a set of equations containing more equations than unknowns is discussed by Whittaker and Robinson (*6*). The first step of the least-squares solution is the calculation of the "normal" equations. The normal equations are then inverted, using a straightforward extension of the method described by Crout (*2*).

Table IV. Average Errors from Table III

Interference	Average Errors: No correction %	Average Errors: Algebraic correction %
None	0.5	0.7
Figure 2, curve 6	26.7	4.4
Figure 2, curve 1	14.6	1.8
Figure 2, curve 3	32.0	0.7
Figure 2, curve 7	6.9	0.7
Figure 2, curve 4	31.2	0.7
Figure 2, curve 5	18.6	1.0
Figure 2, curve 2	48.3	2.0

Table I gives the results of the analysis of a number of synthetic samples containing interference. Figure 1 shows the absorption curves of the interfering substances used in preparing the samples. The results given in Table I include values calculated both with and without the least-squares treatment and include for comparison results calculated from optical densities at 2685 and 2545 Å. without any correction for the interference. Table II compares the average errors of the methods.

Although the interfering absorption in these samples is much greater than that normally encountered, the least-squares quartic method gave satisfactory results for all types of interference except that represented by curve 4 (Figure 1) which consists of a mixture of C_8 aromatics. Even in this case, where the absorption of the interference is not a smooth curve as assumed in the method, there is a considerable gain in accuracy over the results obtained by a noninterference method. Inasmuch as for a given interference the errors introduced in the analysis are directly proportional to the concentration of the interfering material, it is apparent that the method will give satisfactory correction for low concentrations of the C_8 aromatics in benzene-toluene samples.

METHOD FOR ETHYLBENZENE AND THE XYLENES

A power series to represent the interference was found to be unsatisfactory for the determination of ethylbenzene and the xylenes. Some of these components, particularly *o*-xylene, do not have sharp absorption peaks as do benzene and toluene; consequently, their optical density can be approximately represented by the power series, which leads to large errors in the analysis. The sum of descending exponentials given in Equation 6 was found to be more satisfactory for this analysis.

$$D_{int}(\lambda) = a_0 + a_1 10^{-0.001(\lambda - \lambda_0)} + a_2 10^{-0.003(\lambda - \lambda_0)} + a_3 10^{-0.01(\lambda - \lambda_0)} \quad (6)$$

The wave lengths used in this method are 2900, 2800, 2745, 2725, 2710, 2685, 2670, and 2620 Å. These positions are grouped together at the longer wave lengths as much as possible. This has two advantages: (1) The interference is usually much weaker at the longer wave lengths and consequently more easily handled. (2) A short spectral interval greatly increases the accuracy with which the function can represent the optical density of the interference.

Table III gives the results obtained from the analysis of a number of synthetic samples containing interference. The absorption curves of the interfering materials are shown in Figure 2. Table III also includes results calculated using data obtained at 2745, 2725, 2710, and 2685 Å. without correction for interference. These wave lengths are used rather than those recommended in the previous paper (*5*) because these are the wave lengths included in the interference method and consequently they give a more satisfactory comparison of the results obtained with and without correction for the interference. Table IV compares the average accuracy of the two methods.

Although the errors in the analysis of some of these synthetic samples are significant, they cannot be considered excessive in view of the magnitude of the interference in these samples.

METHOD FOR NAPHTHALENE

The method for the determination of naphthalene in the presence of interfering absorption is similar to the method described for the analysis of ethylbenzene and the xylenes. The optical density of the interfering absorption is represented by the following equation:

$$D_{int}(\lambda) = a_0 + a_1 10^{-0.003(\lambda - \lambda_0)} + a_2 10^{-0.02(\lambda - \lambda_0)} \quad (7)$$

The wave lengths used in this method are 3180, 3140, 3110, and 3090 Å. Table V gives the results obtained from the analysis of a number of synthetic samples. The absorption spectrum of naphthalene and the absorption curves of the interfering materials added to these samples are shown in Figure 3. Table V also includes results calculated from the total optical density at 3110 Å. without correction for interference and results calculated using a base-line correction method based on a correction for the interference by a linear interpolation between 3140 and 3090 Å. This shows that the algebraic correction method gives satisfactory results for naphthalene even in the presence of a large amount of interfering absorption.

DISCUSSION

The best wave lengths for the analysis and the best function to represent the interference usually have to be selected by trial and error. Errors are often due more to the tendency of the function to represent the optical densities of one or more of the components of interest than to the failure to represent the interference accurately. There are two ways of evaluating a method. Both tests require the calculation of the inverse equations for a particular set of wave lengths and a particular interference function. The extinction coefficients used for this purpose may either be approximate values from absorption curves or values determined by calibration.

1. Calculation of the theoretical errors to be expected when there is no interference. These errors are calculated from the inverse equations by the method described in the previous paper (*5*).

Table V. Analysis of Synthetic Naphthalene Samples Containing Interference

(Theory 100% naphthalene)

Sample No.	Interference	Per Cent Naphthalene: Measurement at 3110 Å. Found	Measurement at 3110 Å. Error	Linear Interpolation Method[a] Error	Algebraic Correction Method[b] Error	Algebraic Correction Method[b] Found	Algebraic Correction Method[b] Error
4115-4A	None	100.4	+0.4	99.2	−0.8	99.3	−0.7
4115-4B	None	100.6	+0.6	99.9	−0.1	99.9	−0.1
4115-8A	None	101.0	+1.0	99.6	−0.4	98.8	−1.2
4115-8B	None	101.3	+1.3	100.2	+0.2	100.3	+0.3
4115-5A	Figure 3, curve 2	117.4	+17.4	85.2	−14.8	98.8	−1.2
4115-8C	Figure 3, curve 2	117.7	+17.7	86.0	−14.0	99.3	−0.7
4115-8E	Figure 3, curve 1	154.0	+54.0	80.0	−20.0	100.7	+0.7
4115-13A	Figure 3, curve 9	122.0	+22.0	99.0	−1.0	98.8	−1.2
4115-13B	Figure 3, curve 7	142.3	+42.3	99.7	−0.3	100.0	0.0
4115-13C	Figure 3, curve 4	182.7	+82.7	98.5	−1.5	98.3	−1.7
4115-14A	Figure 3, curve 8	125.6	+25.6	97.5	−2.5	97.7	−2.3
4115-14B	Figure 3, curve 5	158.0	+58.0	96.6	−3.4	97.0	−3.0
4115-15A	Figure 3, curve 6	139.0	+39.0	98.5	−1.5	98.6	−1.4
4115-15B	Figure 3, curve 3	176.0	+76.0	96.9	−3.1	96.9	−3.1
Theoretical av. error[c]			0.6		1.1		1.5

[a] Based on optical density measurements at 3140, 3110, and 3090. Interference at 3110 Å. determined by linear interpolation between 3140 and 3090 Å.

[b] Based on optical density measurements at 3180, 3140, 3110, and 3090 Å. Interference represented by equation:

$$D_{int} = a_0 + a_1 10^{-0.02(\lambda - \lambda_0)} + a_2 10^{-0.003(\lambda - \lambda_0)}$$

[c] Based on error in optical density of 0.003 and naphthalene concentration of 0.25 gram per liter.

Large errors indicate either that the function used to represent the interference also approximately represents the optical densities of one or more of the components being determined or that the wave lengths chosen do not emphasize the differences between the absorption spectra of these components. A new function must be chosen, different wave lengths selected, or both.

2. Calculation of the accuracy with which the function can represent the absorption of various types of interference alone (the components to be determined are not present). For this test the apparent concentration of each component included in the analysis is calculated by substituting the optical densities of various interfering materials in the inverse equations. For an ideal method the calculated concentrations will, of course, all be zero. The magnitude of these concentrations gives the errors in the concentration of each component which would be observed in the analysis of a sample containing an equal amount of interference. Calculations of this type for a variety of types of interference clearly indicate the limitations of the method.

These tests will give a good evaluation of any prospective method without the analysis of check samples. However, analysis of a few synthetic samples by the method finally selected is advisable as a check on the calculations.

The theoretical errors expected in each interference method for the analysis of samples containing no interference are included with the results of the analysis of the synthetic samples listed in Tables I, III, and V. The average errors observed are usually slightly smaller than the theoretical errors, which is an indication that the assumed error of 0.003 in optical density is slightly larger than the actual error.

Duplicate readings in optical density with the cells reversed between sets as described in the preceding paper (*5*) may be omitted, if desired, with any of the interference methods described, for the method itself corrects for differences in the absorption cells. However, for the most accurate results, duplicate measurements are recommended. Duplicate readings were taken in the analysis of the synthetic samples listed in this paper.

ACKNOWLEDGMENT

The authors wish to acknowledge the excellent work of B. J. Scott and R. Hatch in obtaining the necessary calibration data, the analysis of the synthetic samples listed in the tables, and the calculation of the large number of inverse matrices required in the development of these methods.

LITERATURE CITED

(1) Banes, F. W., and Eby, L. T., IND. ENG. CHEM., ANAL. ED., **18**, 535 (1946).
(2) Crout, P. D., *Trans. Am. Inst. Elec. Engrs.*, **60**, 1235 (1941).
(3) Heigl, J. J., Bell, M. F., and White, J. U., ANAL. CHEM., **19**, 293 (1947).
(4) Seyfried, W. D., and Hastings, S. H., *Ibid.*, **19**, 298 (1947).
(5) Tunnicliff, D. D., Brattain, R. R., and Zumwalt, L. R., *Ibid.*, **21**, 890 (1949).
(6) Whittaker, E. T., and Robinson, G., "Calculus of Observations," 2nd ed., Chap. IX, London, Blackie and Sons, 1929.
(7) Wright, N., IND. ENG. CHEM., ANAL. ED., **13**, 1 (1941).

RECEIVED August 10, 1948.

Identification of Pennsylvania Lubricating Oils by Infrared Absorption

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AS PART of a study conducted for the Pennsylvania Grade Crude Oil Association, comparing certain properties of lubricating oils made from different types of crudes, it was observed that all oils made from Pennsylvania grade crudes show a characteristic infrared absorption band at 10.3 microns. Inasmuch as with minor exceptions, this band is not found in oils made from crudes from other parts of the country, its absence from an oil indicates that the oil does not originate in the Pennsylvania grade field. The band itself has been examined rather intensively for natural distribution and for interpretation in terms of the type of compound producing it, and the results are reported in this paper as due to a particular type of olefin. A simple chemical test based on the properties of the 10.3 band has been developed and is described below.

It has been recognized that "Pennsylvania Grade" oils, commonly known as Pennsylvania oils, are those oils refined from a grade of crude oil produced in a well-defined geographical region located in western Pennsylvania, southwestern New York, eastern Ohio, and West Virginia.

INFRARED MEASUREMENTS

For this study a number of lubricating oils of known origin and history were obtained from various sources. Properties of typical oils of each type are listed in Table I.* The number of non-Pennsylvania oils listed is not representative in terms of crude oil production; more examples were selected from crude types resembling Pennsylvania crudes than from markedly dissimilar crudes in order to emphasize the differentiation.

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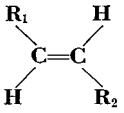
Infrared spectra of typical oils are shown in Figures 1, 2, and 3 as graphs of sample transmittance as a function of wave length from 2.5 to 15.5 microns, measured with a Perkin-Elmer Model 12-B spectrophotometer. These graphs were traced from the original records obtained with a recording potentiometer, using the attenuator furnished with the spectrophotometer for maintaining full-scale deflection for a blank sample. In this instrument the angle of refraction through the rock salt prism is decreased uniformly by a motor drive and consequently the wavelength scale is nonlinear because the dispersion of the prism varies with wave length. The wave-length scale has a discontinuity at 6 microns, below which the angular speed was halved to make the scale more uniform. In addition, the ordinate scale has been changed to absorbance, defined as the logarithm of the ratio of the transmittance of the blank to the transmittance of a sample, inasmuch as this quantity increases linearly with concentration. However, in infrared spectrophotometry the measurement of the transmittance through a blank cell is somewhat arbitrary and variable because of aging of the rock salt windows, and furthermore there is a certain amount of continuous absorption spectrum, especially for lubricating oils, which varies with the nature of the sample. Hence, it is customary to measure the intensity of an infrared absorption band as the difference between the absorbance at the peak and that at the neighboring background. Because the absorbance scale is nonlinear it is necessary to consider the actual absorbance values for peak and background in comparing intensities of bands in different samples because of the background variation.

Most of the figures show the spectra of a number of samples.

Evidence of the existence of olefins in certain sources of crude petroleum is presented. These olefins were identified by means of a characteristic infrared absorption band at 10.3 microns as due to the class



where R_1 and R_2 are methyls or groups that are aliphatic for at least the first two carbon atoms adjacent to the double bond. The wave length, width, and intensity of the band agree uniquely with literature data on this class. The abundance of double bonds is roughly constant (about 1% of the carbon-carbon bonds) as a function of boiling point from gasoline to heavy cylinder stock. Changes of intensity of the infrared band on chemical treatment such as bromination, catalytic hydrogenation, and silica gel adsorption, follow the behavior to be expected for olefins. These olefins have been found in all crudes examined from the Pennsylvania grade field and not in other crudes, with the exception of a few nearby fields of mixed crude type. They are easily observable in finished lubricating oil, and hence their absence from an oil is evidence that the oil did not originate in Pennsylvania grade crude. The observation may be made on the whole lubricating oil by infrared spectrophotometry, or by bromine number on fractions from which the aromatics have been removed by silica gel adsorption. Specifications for these tests are given.

Where indicated, individual curves have been successively displaced vertically 10% of full scale transmittance to reduce overlapping. The sample thickness used in this work was 0.1 mm., which is advantageous for weak absorption bands but too thick for the strong bands shown by all oil samples at 3.4, 6.8, and 7.2 microns, which show almost complete absorption. At these points, consequently, the figures are confusing. These strong bands, however, are of no interest in the present work.

NATURAL ABUNDANCE DISTRIBUTION OF 10.3-MICRON BAND BY CRUDE TYPE

Infrared absorption spectra of typical Pennsylvania neutrals are shown in Figure 1, while Figure 2 shows the spectra of some of the corresponding Pennsylvania bright stocks. A number of neutrals and bright stocks of oils not of Pennsylvania origin are shown in Figure 3. Code numbers on the individual curves refer to the listing in Table I.

The terms "neutral" and "bright stock" are used in the petroleum industry to designate commercially refined oils derived from the lubricating oil fraction of the crude. Neutral oils are distillates of relatively low viscosity, whereas bright stocks, are residues of relatively high viscosity.

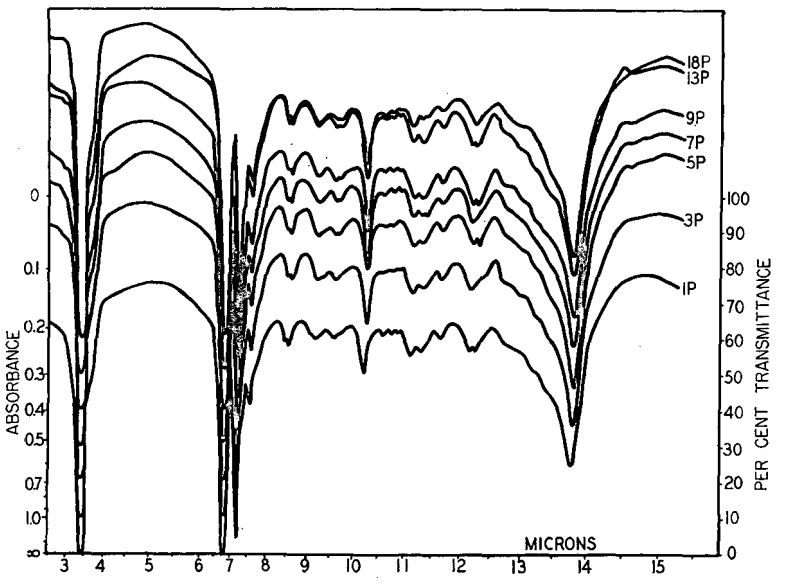


Figure 1. Infrared Absorption Spectra of Pennsylvania Neutrals

Ordinate at left, absorbance for bottom curve. Ordinate at right, % transmittance. Ordinates for curves of other samples can be obtained by successive 10% displacements of ordinate scale (equivalent to distance from ∞ to 1.0 on absorbance scale)

Some of the oils shown in Figure 3—samples 41, 42, 43, and 44—have an absorption band at 10.3 microns very similar to that in Pennsylvania oils but with reduced intensity. These samples were obtained from Ohio crude fields of small production immediately adjacent to the Pennsylvania grade field, and were propane deasphalted and drastically refined. These and other characteristics of these samples indicate that these crudes are a mixture of Pennsylvania and non-Pennsylvania types. Except for neighboring fields, however, no other lubricating oils have been found with the 10.3-micron "Pennsylvania" band. Some of the other oils shown in Figure 3 have spectra with an absorption band in the region of 10.3 microns, but close examination shows that the shapes of the bands and sometimes the wave length are different and in any case the intensities are very much less.

These characteristics are listed in Table I as wave length of maximum absorbance in the vicinity of 10.3 microns; magnitude of maximum difference between peak absorbance and estimated background absorbance at 10.3 for a cell thickness of 0.1 mm. and a spectrophotometer slit width of 0.145 mm. corresponding to a band width of 0.026 micron; and half-width of the band, defined as width in microns of the band at an absorbance level halfway between the absorbance of the peak and that of the background. The width of the band so defined has been found to be constant at 0.13 micron for silica gel fractions of Pennsylvania oil in which the intensity varies over a tenfold range, as reported in Table II. The width is not so independent of intensity when measured on a transmittance basis—i.e., at a transmittance level halfway between the transmittance of the peak and that of the background—although the variation is negligible for the intensities normally encountered. Hence, it is clear that both the wave length of the 10.3-micron band and its width must be of the proper values in order that the presence of the band may be used to designate an oil as originating in the Pennsylvania grade region. The large width of the weak 10.3-band in non-Pennsylvania oils indicates spurious absorption.

Although the number of different non-Pennsylvania grade crudes listed in Table I is not exhaustive, it is believed that all the

Table I. Properties of Lubricating Oils

Sample No.	Description	Crude Source	A.P.I. Gravity	Viscosity 100° F. S.U.S.	Viscosity 210° F. S.U.S.	Viscosity Index	Pour Point ° F.	Flash Point ° F.	Color[a]	10.3-Micron Wave-Length μ	10.3-Micron Half-Width μ	10.3-Micron Absorbance
1P	St. Mary's 180 neutral	Buckeye (Pa.)	31.0	176.7	45.1	101.3	20	420	2.5	10.34	0.13	0.115
3P	Wolf's Head 180 neutral	Middle District (Pa.)	31.6	185.1	45.8	103.1	25	420	D 2.5	10.34	0.13	0.122
5P	Freedom 180 neutral	Eureka No. 1 (Pa.)	31.3	184.5	45.8	103.7	10	425	D 2.5	10.34	0.13	0.100
7P	United 180 neutral	Bradford (Pa.)	30.3	182.9	45.6	101.8	25	425	D 2.5	10.34	0.13	0.175
9P	Allegany 180 neutral	Bolivar (Pa.)	29.8	181.03	45.05	95.2	20	425	3	10.34	0.13	0.155
13P	Elk 180 neutral	Eureka No. 2 (Pa.)	30.8	186.3	45.85	102.8	25	425	2	10.34	0.13	0.110
17P	Emlenton 150 neutral, extracted	Buckeye (Pa.)	32.9	148.3	43.5	108.9	25	410	L 2	10.34	0.13	0.123
18P	Emlenton 150 neutral, extracted	Bradford (Pa.)	33.0	141.8	43.2	112.5	20	415	D 2.5	10.34	0.13	0.179
69P	Sinclair 200 neutral	Allegany (Pa.)	29.4	180.0	44.88	93.8	10	475	D 2.5	10.34	0.13	0.173
85P	United long cut neutral	Bradford (Pa.)	30.5	184.0	45.67	102.4	30	425	D 2.5	10.34	0.13	0.173
2P	St. Mary's bright stock	Buckeye (Pa.)	27.1	2074.8	140.8	100.5	20	555	TR 1.75	10.34	0.13	0.123
4P	Wolf's Head bright stock	Middle District (Pa.)	26.6	2425.9	157.0	101.9	20	555	7.5	10.34	0.13	0.128
6P	Freedom bright stock	Eureka No. 1 (Pa.)	27.7	2102.8	144.8	103.0	20	560	8	10.34	0.13	0.114
8P	United bright stock	Bradford (Pa.)	26.0	2473.4	154.6	99.2	25	555	D 7	10.34	0.13	0.190
14P	Elk bright stock	Eureka No. 2 (Pa.)	26.0	2577.9	156.6	97.9	25	560	7.5	10.34	0.13	0.122
41	Experimental neutral	Corning (Ohio)	28.6	139.1	42.0	86.9	20	...	2	10.34	0.13	0.097
42	Experimental bright stock, deasphalted	Corning (Ohio)	24.4	2644.4	145.1	87.0	20	...	TR 1	10.34	0.13	0.096
43	Experimental bright stock, extracted	Corning (Ohio)	27.0	1241.1	100.5	97.2	20	...	TR 1	10.34	0.13	0.113
44	Experimental bright stock, deasphalted	Meigs Co. (Ohio)	24.7	2923.2	156.1	88.8	20	...	TR 1	10.34	0.13	0.113
10	Commercial neutral	Mid-continent	30.3	207.9	46.6	92.9	0	425	L 1.5	10.31	0.24	0.036
12	Commercial neutral	Mid-continent	31.6	174.8	44.8	98.5	5	420	D 1	10.33	0.20	0.041
39	Commercial neutral	Mid-continent	30.3	208.4	46.6	93.0	0	420	D 1	10.34	0.21	0.039
89	Experimental 100 neutral, dewaxed	West Edmond (Okla.)	28.9	105.2	39.1	68.1	...	...	...	10.34	0.17	0.047
T-9	Commercial SAE 10W motor oil	Mid-continent	31.6	169.8	44.3	95.2	−20	405	L 2	10.34	0.20	0.043
11	Commercial bright stock	Mid-continent	26.1	2553.3	145.7	90.3	0	580	L 6	10.34	0.23	0.031
117	Commercial bright stock	Mid-continent	22.2	4580	991	42.7	40	520	7–8	10.38	0.21	0.052
109	Experimental neutral	Lake Long (La.)	28.4	...	...	...	...	...	...	10.34	0.19	0.028
110	Experimental bright stock	Lake Long (La.)	22.7	...	...	...	...	...	...	10.34	0.18	0.013
40	Commercial neutral	Texas	33.6	150.5	44.0	114.7	0	405	D 1	10.34	0.20	0.033
45	Experimental neutral	Winkler Co. (Tex.)	32.3	130.1	42.2	110.5	15	...	1.5	10.34	0.13	0.029
83	Experimental neutral	Winkler Co. (Tex.)	31.4	158.8	44.4	110.8	...	...	...	10.34	0.18	0.036
46	Experimental bright stock	Winkler Co. (Tex.)	25.8	1686.9	119.1	96.0	20	...	TR 1	10.34	0.21	0.023
15	Commercial 150 neutral	Gulf Coast	24.2	164.8	42.3	46.7	−10	360	D 2.5	10.29	0.28	0.031
16	Commercial 275 neutral	Gulf Coast	23.3	286.6	47.7	44.7	−10	395	D 2.5	10.30	0.25	0.034
T-38	Experimental neutral	California	30.4	147.5	42.6	88.3	...	...	...	10.34	0.24	0.039
T-44	Experimental neutral	California	30.0	165.9	43.6	84.3	...	...	...	10.34	0.27	0.040
19	Synthetic motor oil	.....	10.4	303.2	62.53	140.9	−10	500	3	...	...	...

[a] N.P.A. color except as noted. TR indicates Tag Robinson.

important types from which appreciable amounts of lubricating oils are made are included. It is noteworthy in particular that none of the oils somewhat resembling Pennsylvania oils, such as the ones from Winkler County, Tex., shows sufficient 10.3 absorbance with the exception of those listed from Ohio.

To aid in understanding the sharp distinction obtained and to provide a fundamental basis for the empirical results, a number of chemical and physical fractionations of Pennsylvania oils were made. The effects on the intensity of the 10.3-micron band are described in the next sections.

Table II. Properties of Successive Silica Gel Fractions of Pennsylvania Neutral

Cut No.	Wt. %	n_D^{25}	d^{25}	Specific Dispersion[a]	Bromine Number[b]	10.3 Absorbance Observed	10.3 Absorbance Calculated[c]	Remarks
1	10.0	1.46230	0.8363	125.5	1.8	0.062	0.064	
2	6.5	1.46260	0.8370	125.3	2.4	0.070	0.077	
3	6.4	1.46280	0.8374	125.3	2.8	0.094	0.086	
4	5.4	1.46300	0.8378	125.3	3.5	0.102	0.101	
5	5.8	1.46350	0.8390	125.2	4.8	0.130	0.130	Paraffin-naphthene fractions
6	6.8	1.46390	0.8396	125.7	6.5	0.164	0.167	
7	8.1	1.46457	0.8409	126.0	8.6	0.204	0.212	
8	9.3	1.46535	0.8426	126.3	11.6	0.270	0.278	
9	6.6	1.46625	0.8444	127.3	14.1	0.314	0.332	
10	4.8	1.46775	0.8481	127.3	21.0	0.470	0.482	
11	3.9	1.47005	0.8518	128.5				
12	0.2	1.47250	0.856	134.5	32.3	0.710	0.729	.....
13	1.0	1.50920	0.9075	189.5	35.6	0.340	0.801	
14	2.2	1.52130	0.9288	202.5	29.7	0.230	0.682	
15	6.8	1.52797	0.9359	213.0	26.8	0.163	0.609	Aromatic fractions
16	7.4	1.53478	0.9451	226.6	22.5	0.163	0.515	
17	8.2	1.53473	0.9451	226.5	22.8	0.163	0.522	
	99.4							

[a] $10^4(n_7^{25} - n_D^{25})/d^{25}$.
[b] A.S.T.M. method D 875–46T.
[c] Calculated from Equation 1 from octene data and bromine number.

MOLECULAR WEIGHT DISTRIBUTION OF 10.3 BAND

Figures 1 and 2 show that the 10.3 band is approximately equally intense in neutrals and bright stocks, which cover a three- or fourfold range in molecular weight. It was of interest to determine the complete range of molecular weight in which the band could be found. Because all the Pennsylvania samples examined were essentially similar, in spite of having originated in various parts of the field and having been manufactured at different refineries, it appeared sufficient to ex-

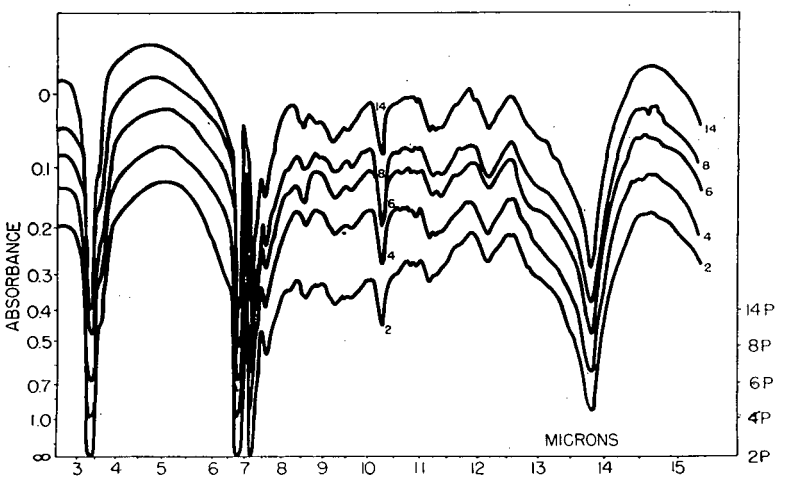


Figure 2. Infrared Absorption Spectra of Pennsylvania Bright Stocks

Curves displaced as in Figure 1 and position corresponding to complete absorption for each sample indicated at right

amine any one crude. Accordingly, a Bradford crude which had received no previous treatment except heating to 120° C. to remove volatile hydrocarbons was fractionated into boiling ranges conventionally designated as gasoline, kerosene, nonviscous neutral, neutral, bright stock, and cylinder stock (the nonviscous neutral, neutral, and bright stock fractions were also dewaxed).

Spectra of these samples, together with the original crude, are shown in Figure 4. It is clear from the great range in molecular weight in which the band is found that it must be due to a particular class of hydrocarbons and not a few particular compounds. Furthermore, from its presence in the original crude, it cannot have been produced during refining operations. The 10.3 band has been found also in other Pennsylvania crudes, in the same relative intensities as in the corresponding lubricating oils listed in Table I. These samples of crude received no treatment at all, and are listed in Table III.

SILICA GEL SEPARATION

From the comparatively uniform molecular weight distribution of the 10.3 band it is apparent that isolation of the compounds responsible must be attempted on the basis of differences in chemical or physical behavior of the various hydrocarbon classes. There are three such classes to be considered, according to the conventional description of natural petroleum: paraffins, naphthenes, and aromatics. The naphthenes are believed to consist of cyclopentane and cyclohexane derivatives only; cycloparaffins of other than five or six carbon atoms per ring are of negligible abundance. Various numbers of fused rings of all three types are to be expected, with paraffinic side chains enhancing the molecular weight to the lubricating oil range. Other classes of hydrocarbons, such as olefins and acetylenes, are believed absent according to the literature.

The most effective separation devised is the removal of aromatics by a silica gel adsorption procedure analogous to that in common practice in the gasoline range. The method developed is a modification of two procedures described by Mair and Forziati (*14, 15*) of the National Bureau of Standards. Lipkin and co-workers (*13*) have extended the original method to the lubricating oil range. The present method differs principally in the manner of elutriation.

The column used is similar to those described in the literature, such as the A.S.T.M. proposed method (*2*), except that the water jacket has been found unnecessary for this application. The column consists of a top reservoir of approximately 250-ml. capacity, an upper gel column 900 by 25 mm., and a lower gel column 350 by 12 mm. The column ends with the usual medium porosity sintered-glass plate and a stopcock. The gel capacity of the column when using mesh size through 200 is about 250 grams. For the separation of neutral lubricating oils the following materials are introduced in order: (1) 20 ml. of low boiling petroleum ether, (2) 50 grams of oil diluted with 50 grams of petroleum ether, (3) 20 ml. of petroleum ether, (4) 40 ml. of benzene, and (5) enough ethanol to fill the upper reservoir.

In each step all of a given material is allowed to enter the silica gel before the next is added. After the oil sample is introduced, air pressure is applied and gradually increased from an initial 5 pounds per square inch (3500 kg. per sq. meter) to a maximum of 20 pounds per square inch. The time required is 2.5 to 4 hours.

The boundary between the colorless petroleum ether fraction containing the paraffins and naphthenes and the brown benzene fraction containing the aromatics is sharp and is taken as the cut point for separation of the two fractions by visual indication alone, as this point coincides with the one obtained by using the refractometer. Similarly, the nonhydrocarbon components can be distinguished as a black ring just below the ethanol. After separation the diluents are stripped from the oil by heating in a vacuum Engler apparatus; the pressure is gradually reduced to 10 mm. to avoid heating the oil to a temperature higher than 100° to 150° C.

The procedure for bright stocks is similar except that the oil is diluted with twice its weight of petroleum ether. The paraffin-naphthene fraction in this case is not completely colorless. Hydrocarbon recovery for bright stock is about 95%, whereas the recovery for neutrals is 97 to 99%.

In order to provide larger quantities of fractions for analytical work a larger column of similar proportions, holding 1200 grams of adsorbent, was constructed. Using the previously described procedure 350 grams of oil were filtered through this column, a number of fractions were collected, and properties of interest were determined for each fraction.

In Figure 5 are plotted values of the refractive index, n_D^{25}, and the specific dispersion, $(n_\rho^{25} - n_D^{25})/d \times 10^4$, of a typical Pennsylvania neutral (7P) as a function of weight per cent through the column. The dispersion is practically constant throughout the

Table III. Characteristic Infrared Absorption Bands of Pennsylvania and Other Crudes

Sample No.	Crude Source	10.3-Micron Wave Length μ	10.3-Micron Half-Width μ	10.3-Micron Absorbance	10.3-Micron Absorbance of Neutral[a]
164P	Lee Co., Va. (Pa.)	10.34	0.13	0.084	...
165P	Weir (Pa.)	10.34	0.13	0.092	...
166P	Eureka (Pa.)	10.34	0.13	0.117	0.110
163P	Buckeye & W. Va. (Pa.)	10.34	0.13	0.125	0.115
146P	Tiona (Pa.)	10.34	0.13	0.137	...
162P	Middle District (Pa.)	10.34	0.13	0.141	0.122
143P	Bradford (Pa.)	10.34	0.13	0.160	0.175
167P	Allegany, N. Y. (Pa.)	10.34	0.13	0.170	0.173
90	Ellenburger (Tex.)	10.37	0.20	0.031	...
91	Posa Rica (Mexico)	10.35	0.21	0.032	...
92	Santa Barbara (Venezuela)	10.34	0.25	0.036	...
152	Greenwood Co. (Kan.)	10.34	0.20	0.060	...
154	Cleveland Co. (Okla.)	10.34	0.20	0.050	...
155	Sweetwater Co. (Wyo.)	10.34	0.25	0.032	...
157	Rusk Co. (Tex.)	10.34	0.19	0.048	...
158	Cooke Co. (Tex.)	10.34	0.21	0.039	...
159	San Patricio Co. (Texas Gulf Coast)	10.34	0.14	0.065	...
160	Duval Co. (Texas Gulf Coast)	10.26	0.14	0.057	...

[a] Samples of lubricating oil were not obtained from identical corresponding crude samples but should be representative.

paraffin-naphthene fractions, except for cut 12 which is the last 0.2% to emerge before the visual boundary normally taken as the separation point, and which shows a dispersion increase of only a few per cent of that of the aromatic fraction. Measurement of the ultraviolet absorption, of the total paraffin-naphthene fraction which is a more sensitive test than dispersion, shows it to be of the order of 0.1% of that of the aromatic fraction and as the spectra of the two fractions are otherwise similar, the distribution of condensed rings is similar. Hence, the aromatic content of each should be in approximately the same ratio as the ultraviolet absorbances. For practical purposes the removal of aromatics may be said to be complete.

Olefins, if present, would be expected to be found concentrated near the end of the paraffin-naphthene fraction by analogy with their behavior in gasoline (*14*).

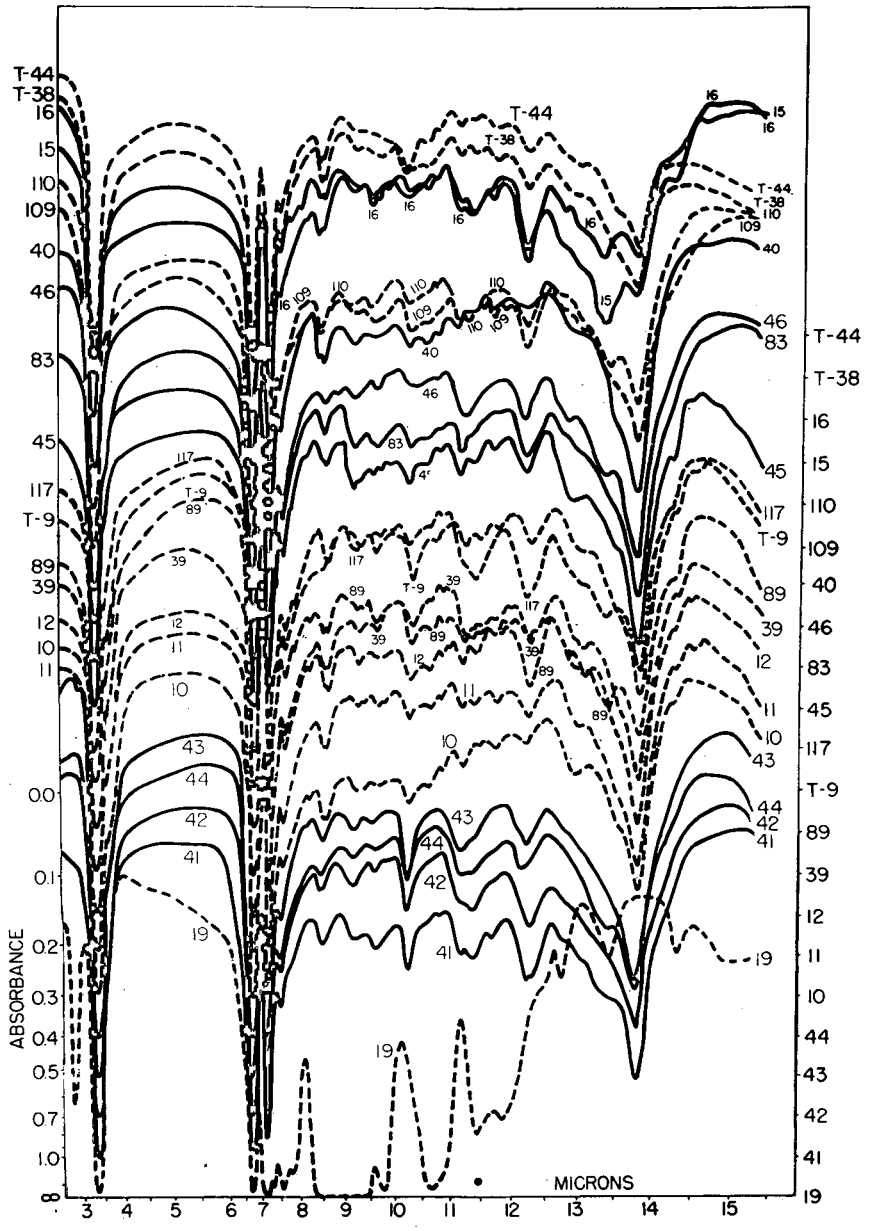


Figure 3. Infrared Absorption Spectra of Typical Neutrals and Bright Stocks Not of Pennsylvania Origin

Curves of given group of solid or dashed lines indicate samples from similar crudes. Curves displaced as in Figures 1 and 2

DISTRIBUTION OF 10.3 BAND BETWEEN SILICA GEL FRACTIONS

Using the silica gel separation procedure described, a number of Pennsylvania lubricating oils and some representative non-Pennsylvania oils were treated and the fractions were examined by infrared. Figure 6 shows some typical spectra. The 10.3 intensity in the Pennsylvania oils is approximately equal in both fractions. Inasmuch as it has been shown that the aromatic concentration of the paraffin-naphthene fraction is negligible, the 10.3 band shown by this fraction cannot be due to aromatics. Correspondingly, because the molecules constituting the aromatic fraction must contain a considerable proportion of carbon atoms in naphthenic rings and paraffinic side chains in order to be liquids at this high molecular weight, and it is very improbable that there are two classes of hydrocarbons peculiar to Pennsylvania oil with the same characteristic infrared band, it is reasonable to assume that the 10.3 band in the aromatic fraction is due to nonaromatic components. The aromatic fraction, incidentally, constitutes only about 25% of the total oil. Hence, it is concluded that the 10.3 band is not due to aromatics.

UNSATURATED NATURE OF 10.3 BAND

After aromatics were shown not to be responsible for the 10.3 band, attempts to isolate the various classes of saturated hydrocarbons were begun. It was suggested by V. I. Komarewsky of the Illinois Institute of Technology that the alkyl cyclohexanes could probably be separated by dehydrogenating them to aromatics, which could then be removed by silica gel adsorption, the original aromatics in the oil having previously been removed to avoid interference. Preliminary vapor phase dehydrogenation experiments on typical Pennsylvania and non-Pennsylvania oils using a platinum-alumina catalyst indicated appreciable conversion to aromatics. Further investigation of the effectiveness of dehydrogenation in isolating the cyclohexanes, however, was diverted by the observation that dehydrogenation also enhanced the intensity of the 10.3 band, indicating that the band is associated with unsaturation. This effect was unexpected, for aromatics were the only unsaturated class of hydrocarbons expected in petroleum crude. The reaction of the 10.3 absorber to other tests of unsaturation was therefore investigated.

It was found that the band could be removed almost completely by catalytic hydrogenation using a platinum catalyst at room temperature and atmospheric pressure, and that stirring the sample in contact with 95% sulfuric acid at ice temperature tended to remove the band. Oxidation, accomplished by bubbling air through the sample heated to 200° C. for 3 hours, also reduced the 10.3 intensity and produced the 5.9-micron carbonyl band, as shown in Figure 7. All these reactions are characteristic of olefins.

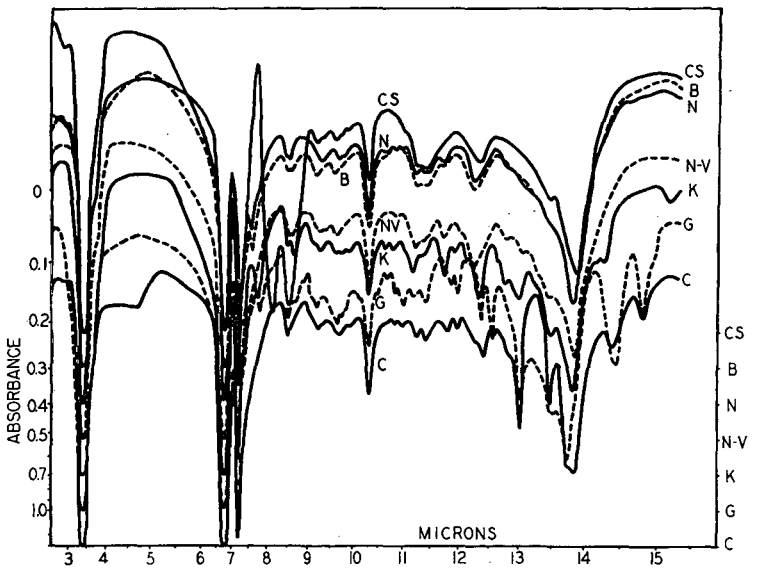


Figure 4. Infrared Absorption Spectra of Pennsylvania Crude and Rough Distillation Fractions Obtained from It

C. Crude
G. Gasoline
K. Kerosene
NV. Nonviscous neutral
N. Neutral
B. Bright stock
CS. Cylinder stock
Curves displaced

CHEMICAL EVIDENCE OF OLEFINS

Other treatments selective toward olefins were then carried out. A Pennsylvania oil sample containing no aromatics was brominated by A.S.T.M. method D 875–46T for bromine number determination. The infrared spectrum of the product (Figure 7) shows reduced 10.3 intensity and also shows a new band at 13 microns associated with the presence of carbon-bromine bonds, as it is also produced by the bromination of known olefins. The brominated sample was then debrominated by refluxing with ethanol and zinc dust, which is a reaction believed to be fairly specific for the type of dibromides obtained by brominating an olefin. It is seen from Figure 7 that debromination restores the 10.3 band. A precedent for a part of this experiment is found in the report by Gore and Johnson (*9*) that bromination eliminates absorption due to olefins in the 10.3 region.

Recent work on bromination of olefin concentrates from Pennsylvania oil indicates that the 13-micron band for brominated paraffin-naphthene fractions (and for pentene too) may have been due to carbon tetrachloride impurity. There are, however, bands at 5.8 and 8.2 microns, which it seems certain can be attributed to the carbon bromine band.

Further evidence that the 10.3 band is due to olefins was obtained by close silica gel fractionation. Olefins would be expected to be found concentrated toward the end of the paraffin-naphthene fraction, and the close fractions shown in Figure 5 were accordingly examined by infrared. The 10.3 bands of these fractions are shown in Figure 8. For comparison, bromine numbers were determined on each fraction and are listed with other data in Table II. The 10.3 intensities are plotted against bromine numbers in Figure 9.

There is no correlation with dispersion but there is a linear dependence of 10.3 intensity on bromine number, up to the point (cut 13) where aromatics start to come through the column. The bromine numbers of the aromatic fractions are higher than corresponding paraffin-naphthene fractions with the same 10.3 intensities; this indicates, if the olefin hypothesis be assumed for the moment, that lubricating oil aromatics interfere in the determination of olefins by bromine number. This agrees with the observations of the authors of the bromine number method (*10*), who found interference with anthracene but not with lower aromatics. The fact that the linear portion of Figure 9 does not go through the origin is probably due to a slight variation of background with wave length not accounted for by assuming that the background at 10.3 microns is an average of that at about 10.0 and 10.5.

It may be concluded on the basis of the chemical evidence that the unique infrared absorption band found in Pennsylvania lubricating oils is due to olefins present in the original crude.

Harold M. Smith of the Bureau of Mines states that the presence of olefins was strongly indicated in gasoline made from crude oil obtained directly from a well in the Bradford, Pa., field (*16*).

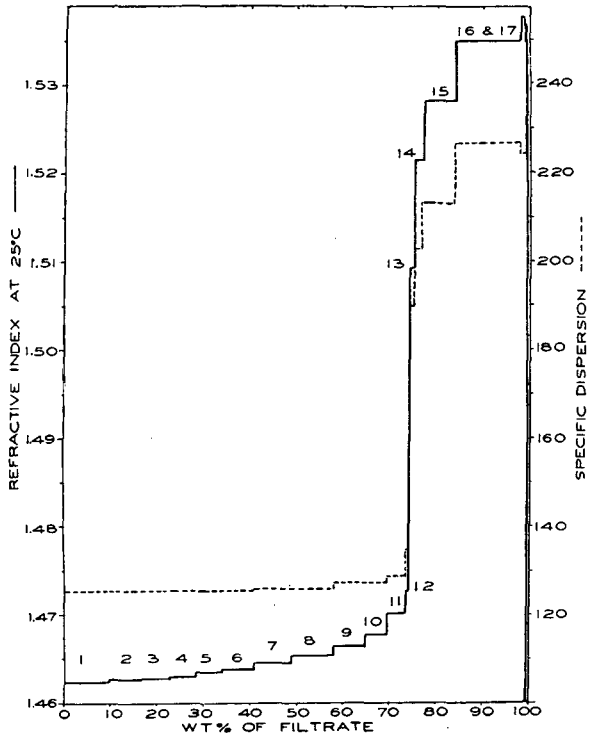


Figure 5. Refractive Index and Specific Dispersion of Successive Silica Gel Fractions of Pennsylvania Neutral

KNOWN 10.3-MICRON ABSORBERS

As it has been shown that the 10.3-micron absorption band in Pennsylvania oil is due to a particular class of compounds, it is of interest to examine the infrared literature to see whether this band is one of those commonly listed as characteristic of a given functional group. The most comprehensive literature source of hydrocarbon infrared spectra is the Catalog of Infrared Spectrograms (*1*). Inspection of these curves shows a number of individual compounds that have an absorption band in the vicinity of 10.3 microns, but in general the compounds are unrelated and the spread in wave length, width, and intensity of the bands is considerable.

As an example of a group of related compounds having a band in the neighborhood of 10.3 microns, but without the consistency of the Pennsylvania samples, curves are shown in Figure 10 for cyclohexane, methylcyclohexane, ethylcyclohexane, and 9-cyclohexylheptadecane, taken from the A.P.I. catalog. Methylcyclohexane shows a strong band at 10.3 microns similar to that found in the oil samples. This circumstance caused some

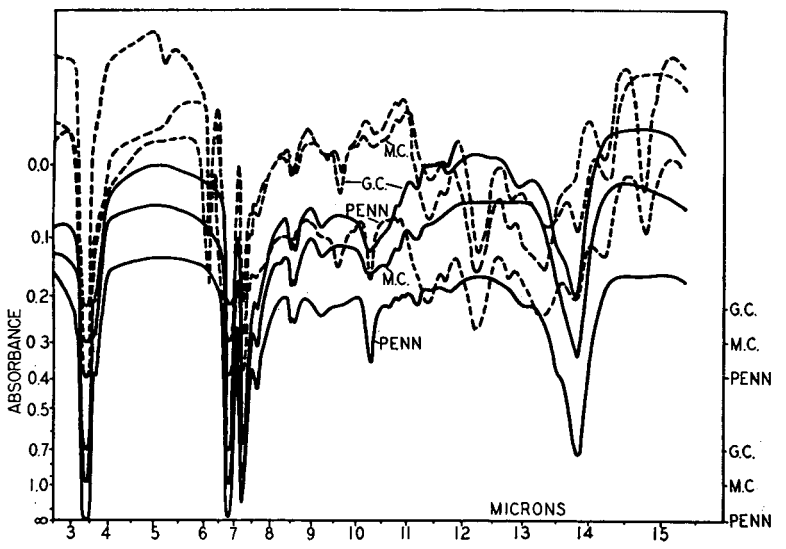


Figure 6. Infrared Absorption Spectra of Silica Gel Adsorption Fractions of Typical Pennsylvania, Mid-continent, and Gulf Coast Neutrals

Solid lines, paraffin-naphthene fractions
Dashed lines, aromatic fractions
Curves displaced

confusion in early work on this project, in which it had been reasoned that it would be of advantage to investigate the gasoline range in detail because the chemistry of hydrocarbons of lower molecular weight is much simpler and more familiar than that of heavier hydrocarbons. The chemical behavior of the 10.3 band in the gasoline range was different from that in the lubricating oil range for such reactions as acid extraction and hydrogenation. By examining gasoline distillation fractions and observing also the intensities of the subsidiary methylcyclohexane bands shown in Figure 10 at 11.0, 11.5, and 11.9 microns it was found that the 10.3 band in Bradford straight-run gasoline consists partly of methylcyclohexane and partly of a series of unsaturated compounds covering a broad range of boiling points. This was conveniently made evident by examining the aromatic silica gel fraction of Pennsylvania straight-run gasoline which was distilled on a Podbielniak 100-plate Hyper-cal column. Owing to an imperfect silica gel separation, small fractions were obtained boiling below benzene and between most of the individual aromatics. These small fractions showed considerable 10.3-band intensity, and the subsidiary methylcyclohexane bands were absent in these fractions.

Aside from the chemical behavior, Figure 10 indicates that the alkyl cyclohexanes cannot be responsible for the 10.3 band observed in lubricating oil, as the absorption bands of different compounds of this class vary in wave length over a range of more than 1 micron and the average effect of the many different bands produced in lubricating oil would be expected to be a broad, weak band such as that actually observed in non-Pennsylvania oils. Moreover, it would appear, in general, unlikely that Pennsylvania crude which contains fewer naphthenes than other crudes is the only one to contain a particular class of naphthenes. Finally, it can be calculated that the 10.3 absorbance of alkyl cyclohexanes is much too weak to account for the amount of 10.3 absorbance present in Pennsylvania oils. For example, pure neopentylcyclohexane has an absorbance in this region of 0.70 (for a cell path of 0.101 mm.), which is much more than most cycloparaffins of this molecular weight range. A concentration of 25% of even this naphthene would be required to give a sample with the same 10.3 absorbance as Bradford neutral. The smooth distillation curve for lubricating oils shows that no individual compounds can be present in oils to anywhere near this concentration. Because of the low average absorbance for naphthenes showing absorbance at 10.3 microns, an impossibly high naphthene content would be required to account for the observed 10.3 absorbance.

The A.P.I. catalog does contain one class of hydrocarbons with a sharp band at 10.3 microns of the same general appearance as that observed in Pennsylvania oil—namely, olefins with an internal, unsubstituted double bond. Figure 11 shows a few spectra of this class traced from the catalog. The similarity of the octene spectra to that of Pennsylvania oil is evident. As nearly as can be measured from the published curves, 2-, 3-, and 4-octene show wave lengths of maximum absorption of 10.31, 10.29, and

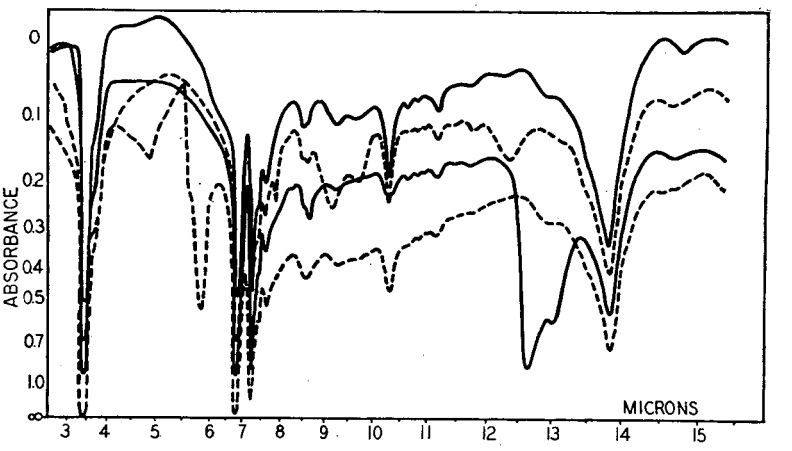


Figure 7. Infrared Absorption Spectra Obtained by Bromination and Oxidation of Paraffin-Naphthene Silica Gel Fraction of Pennsylvania Neutral

Top solid line, original sample
Lower solid line, brominated sample
Upper dashed line, debrominated sample
Lower dashed line, oxidized sample
Curves displaced vertically

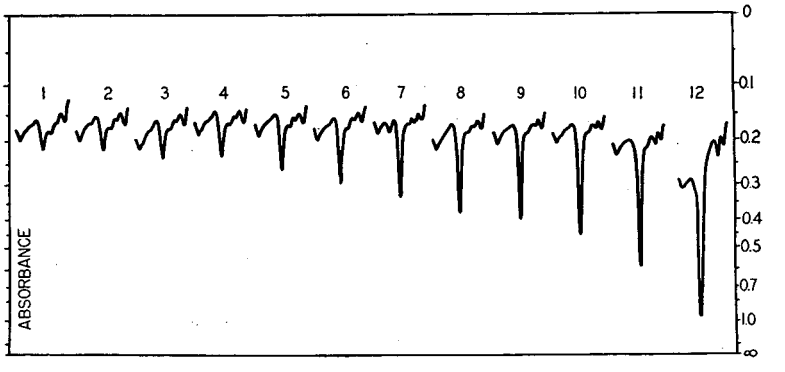


Figure 8. Infrared Absorption Spectra in Vicinity of 10 Microns of Successive Silica Gel Fractions of Pennsylvania Neutral

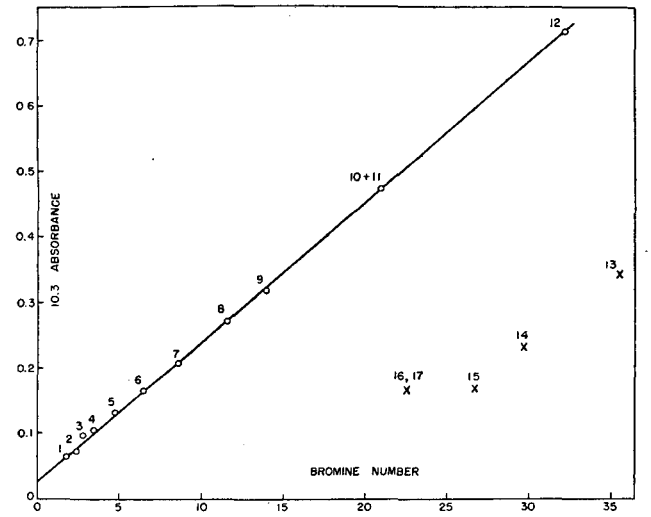


Figure 9. Relation between Bromine Number and 10.3 Intensity of Successive Silica Gel Fractions of Pennsylvania Neutral

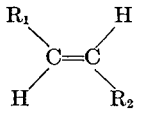
10.25 microns, respectively, and half-absorbance widths of 0.18, 0.17, and 0.23 micron. [The wave lengths of the bands of the three compounds may actually be closer together because of a shift in spectrophotometer calibration during measurements (*17*). In general, the results of different laboratories cannot be compared in the second decimal place unless standardized.] The widths are slightly greater than reported for the oils in Table I, which may be due to the somewhat greater slit widths than used in the present work. The band width for *trans*-2-pentene is about twice as great as for the octenes. The catalog also includes *trans*-2-butene, which has a 10.3 band of width comparable to that of the pentene, and *trans*-2- and *trans*-3-hexene. The widths of the 10.3 bands of the hexenes are not measurable from the published curves because the cell thickness used for these compounds was so large that complete absorption was obtained from 10.2 to 10.4 microns. The larger widths of the butene and pentene bands are evidently not due to measurement in the vapor phase, for vapor spectra of the octenes are identical in shape with the octene liquid spectra.

The A.P.I. catalog does not contain spectra of any olefins of this class of the molecular weight range of lubricating oil, nor do other sources that have been examined. However, a few examples have been found which are almost as heavy. The spectrum of a sample of 9-heptadecene prepared and measured by American Petroleum Institute Project 43C at the Massachusetts Institute of Technology (*4,5*) shows a band with maximum absorption at 10.37 microns, half width of 0.11 micron, and intensity about one third of the octene 10.3 bands. Samples of 6-tridecene and 7-pentadecene, synthesized by the method of Komarewsky (*11*) by condensation of *n*-heptanal and *n*-octanal, respectively, were measured at Armour Research Foundation and found to have bands at 10.35 microns with widths of 0.10 micron and intensities of the order of that of 8-heptadecene but not measurable exactly because of strong overlapping absorption due to oxidation. All the olefins of high molecular weight synthesized by American Petroleum Institute Research Project 42 at the Pennsylvania State College have the double bonds on the end of the molecule or adjacent to a side chain or ring, and consequently their infrared spectra would not be applicable to the present purpose. Two final examples may be cited which are not hydrocarbons but long-chain organic acids with an internal double bond far removed from the carbonyl group—namely, oleic acid and elaidic acid. These compounds are cis and trans isomers, respectively, with the formula $C_8H_{17}CH{=}CHC_7H_{14}COOH$, and their spectra are shown in Figure 12.

The fact that olefins with an internal, unsubstituted double bond have an absorption band at 10.3 microns was pointed out by Gallaway (*8*). Gore and Johnson also report this fact (*9*). Barnes *et al.* (*3*) list the 10.3 band as characteristic of the group $R_1CH{=}CHR_2$. This generalization has also been published by Thompson and Torkington (*18*), Field, Woodford, and Gehman (*7*), and Dinsmore and Smith (*6*), in interpreting the structure of synthetic rubber, where this group occurs especially in butadiene-styrene copolymers on 1,4- addition. Rasmussen, Brattain, and Zucco (*17*) have pointed out that the 10.3-micron band is due to the trans form of this group, which agrees with the spectra of *cis*- and *trans*-2-pentene shown in Figure 11 and the acids shown in Figure 12. Trans olefins are apparently more abundant in nature than cis olefins in accordance with their greater stability, and internal double bonds are less reactive than terminal double bonds.

Some insight into the reason for the consistency shown by various members of this class may be gained by considering the theoretical interpretation of the spectrum of the lightest member, *trans*-2-butene. Kilpatrick and Pitzer (*11*) have assigned the 10.3-micron band of this molecule to a vibration consisting primarily of a motion of the two hydrogens attached to the double bond out of the plane of the carbon skeleton, the carbon atoms moving very little because of their much greater mass. Hence, it appears reasonable that substitution of heavier alkyl groups for the methyls should hardly affect the frequency of the double bond hydrogens, particularly for compounds of higher molecular weight. However, a substitution that changes the force field at the double bond, as by conjugation, would be expected to have a noticeable effect, and substitution of an alkyl group for one of the double bond hydrogens would result in an entirely different band.

Although the wave length of the 10.3 band for different members of the class



is thus independent of the number of carbon atoms in R_1 and R_2, the intensity of absorption would be

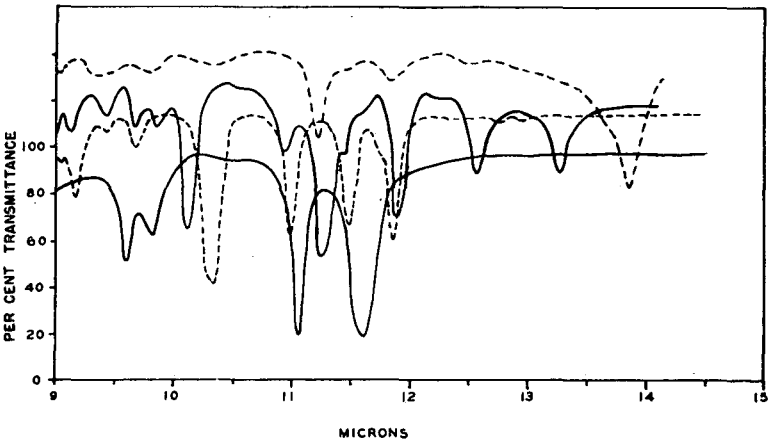


Figure 10. Infrared Absorption Spectra of Alkyl Cyclohexanes

Taken from (*1*). Ordinates, per cent transmittance. Successive curves displaced vertically 20% of full scale
Contributed by Radiometry Section, National Bureau of Standards, Washington, D. C.
Lower solid line, cyclohexane, Serial No. 368
Lower dashed line, methylcyclohexane, Serial No. 369
Upper solid line, ethylcyclohexane, Serial No. 384
Contributed by Naval Research Laboratory, Washington, D. C.
Upper dashed line, 9-cyclohexylheptadecane
Cell thickness 0.05 mm.

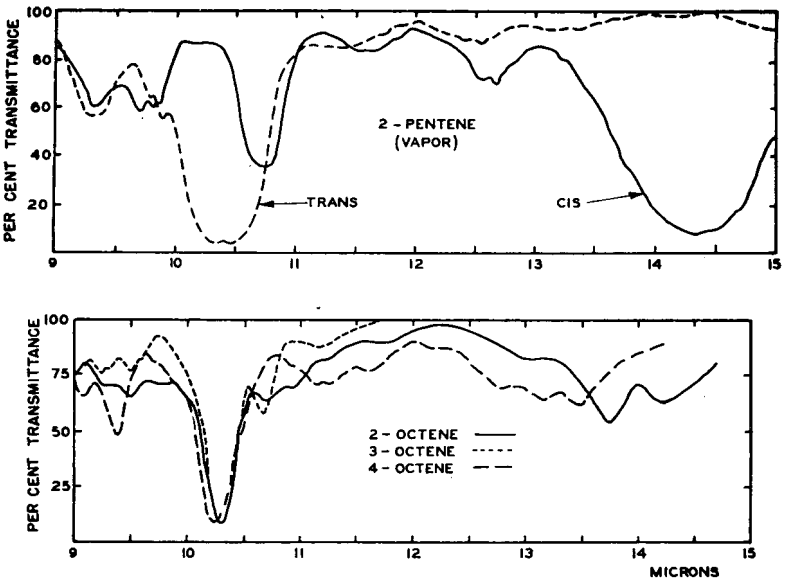


Figure 11. Infrared Absorption Spectra of Olefins with Internal Unsubstituted Double Bond (*1*)

Serial No. 357, *cis*-2-pentene, and Serial No. 358, *trans*-2-pentene, contributed by Socony-Vacuum Oil Co., Paulsboro, N. J. 10-cm. cell, 100-mm. Hg pressure
Serial No. 31, 2-octene, Serial No. 33, 3-octene, and Serial No. 35, 4-octene, contributed by Shell Development Co., Emeryville, Calif., 0.036-mm. cell
Successive curves not displaced

expected to vary approximately inversely as the molecular weight. This is because the intensity per double bond should be constant but the concentration of double bonds per unit volume (for liquids) is in effect diluted as the length of the saturated portion of the chain is increased. In a mixture of hydrocarbons of various types the 10.3 intensity should be proportional to the mole per cent olefins of the given class. Now, the data of Figure 9 indicate that the molar concentration of olefins in a lubricating oil sample can be determined by bromine number, if aromatics are absent. Hence, it should be possible to predict the 10.3 intensity to be expected for a sample from the observed bromine number, provided other types of olefins are absent, if a value for the intensity per bromine number is available. This may be obtained from the olefin spectra described above, of which the data for the octenes are probably the most reliable. Measurement of Figure 11 gives an absorbance for the 10.3 band in octene of about 0.95 for a 0.036-mm. cell, equivalent to 2.64 for a 0.1-mm. cell as used in the present work. As the bromine number of octene is 143, we obtain 0.0185 absorbance unit per bromine number. This factor must be increased by 18% to be applicable to paraffin-naphthene fractions of lubricating oils, because the latter have a physical density of 0.85, compared with 0.72 for octene; this leads to increased absorption per unit volume for the same number of double bonds. Finally, a correction of 0.025 absorbance unit should be added to the predicted intensities to take into account the background variation with wave length, given by the intercept of the linear portion of Figure 9 on the axis of zero bromine number.

Using the formula just derived,

$$\text{Absorbance} = (0.0218 \times \text{bromine number}) + 0.025 \quad (1)$$

intensities were calculated from the bromine numbers of the close silica gel fractions of Table II and are shown in the last column of that table. The agreement of observed and calculated intensities is very good for the paraffin-naphthene fractions; the excess intensity calculated for the aromatic fractions is undoubtedly due to the large bromine number contribution of the polycyclic aromatics (*10*).

Summarizing the literature information on known 10.3 absorbers, we find that olefins with internal, unsubstituted, trans double bonds are the only class of hydrocarbons with a characteristic 10.3 band. This class has bands that agree in wave length, width, and intensity with those observed in Pennsylvania lubricating oils and explains the behavior of the oil spectrum on chemical treatment.

In the average Pennsylvania neutral lubricating oil with a 10.3 absorbance of about 0.15, approximately 0.7% of the carbon-carbon bonds are olefinic or about 20 mole % of the compounds. The types of olefins found are the most stable of all olefins.

INTERFERENCES

In order to use the presence of a sharp absorption band at 10.3 microns as a property by which to characterize a whole lubricating oil sample as to Pennsylvania or non-Pennsylvania origin, account must be taken of the effect of additives frequently present in modern lubricating oils. Because the chemical compositions of lubricating oil additives are not readily available, a number of typical additives were examined in the infrared. The additives are listed in Table IV and the spectra of the whole additives, including carrier oil but without further treatment, are shown in Figure 13.

Because these additives would in practice be diluted by the oil by a factor of 20 or more, they would have to have a sharp band at 10.3 microns with an absorbance of about 3, corresponding to a transmission of 0.1%, in order to produce an effect in a non-Pennsylvania oil equivalent to that of the olefins in Pennsylvania oil. Only one of the additives shown has an absorption band near 10.3 microns approaching this intensity, and in this additive, as well as in another additive (not shown) of similar composition but different manufacturer, the wave length of maximum absorption is at 10.2 microns and the width 0.28 micron. Hence, no interference is to be expected from the additives in current use. (In the event that a Pennsylvania oil is used as a carrier to dissolve the additive, absorbance at 10.3 microns would be contributed but of very low intensity.) Finally, the possibility of interference from additives can be eliminated, if required, by a

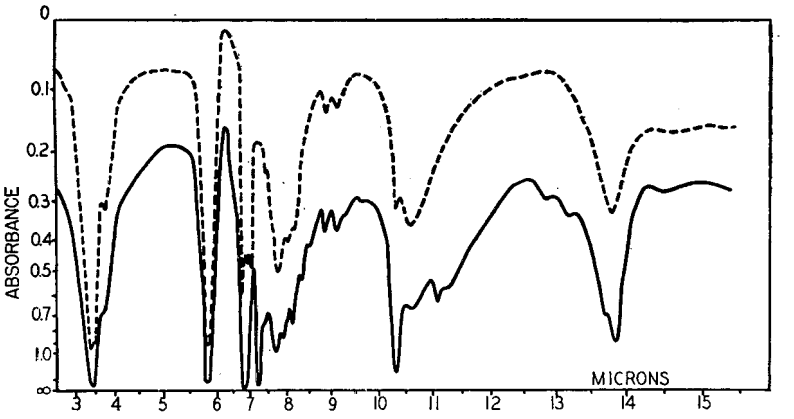


Figure 12. Infrared Absorption Spectra of Elaidic Acid and Oleic Acid

Solid line, elaidic acid, slurry in mineral oil, 0.1-mm. thickness
Dashed line, oleic acid, pure, 0.025-mm. thickness
Curves displaced

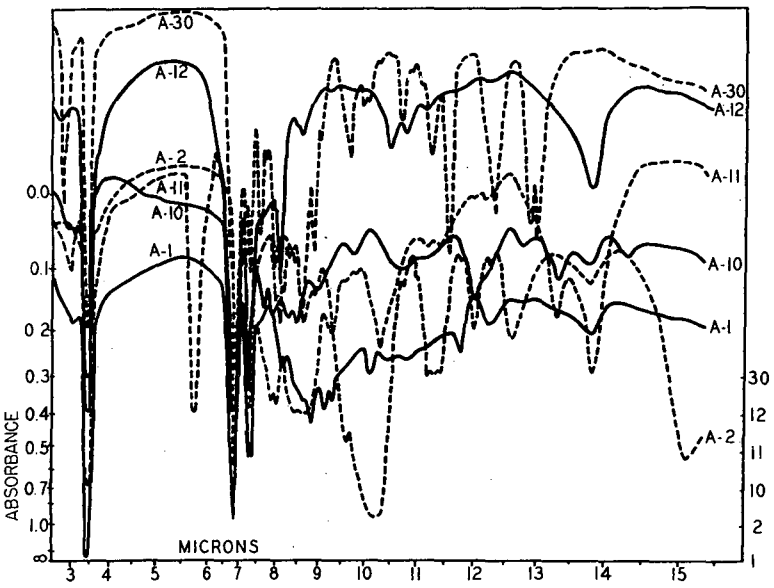


Figure 13. Infrared Absorption Spectra of Lubricating Oil Additives

Curves displaced

preliminary silica gel separation. The silica gel separation would be expected to remove new types of additives developed in the future, as an additive to be effective in relatively small concentrations must necessarily be more polar than "10.3" olefins, except possibly polymers used for pour point depressants and viscosity index improvers.

IDENTIFICATION OF PENNSYLVANIA OILS BY BROMINE NUMBER

From the data on the close silica gel fractions described above it is evident that most of the olefins in a sample of Pennsylvania lubricating oil can be determined by bromine number on the paraffin-naphthene silica gel fraction.

In order to verify the expectation that Pennsylvania oils can be distinguished in this way, a number of typical lubricating oils were treated by silica gel as described above and bromine numbers were determined on the paraffin-naphthene fractions. The results are listed in Tables V and VI for neutrals and bright stocks, respectively. The fractions were also examined by infrared and the relationship between 10.3 intensity and bromine number for the Pennsylvania samples is shown in Figure 14. Bromine numbers of all the samples are shown in Figures 15 and 16, plotted against density of the paraffin-naphthene fraction. The bromine numbers were determined by A.S.T.M. method D 875-46T, using 0.3 gram of oil and two to four times the specified amount of carbon tetrachloride. The refractive indexes were determined with a Valentine improved precision refractometer and the five-place densities were determined with a Becker density balance. Both instruments were calibrated with National Bureau of Standards hydrocarbons. The four-place densities were determined by pycnometer.

Table IV. Commercial Additives Examined

Sample No.	Additive Type	Comment
A-1	Inhibitor-detergent	Contains alkali metal, phosphorus, and sulfur
A-2	Inhibitor	Contains zinc, phosphorus, and sulfur
A-10	Inhibitor-detergent	Contains calcium, phosphorus, and sulfur
A-11	Pour point depressant	Contains polyester
A-12	Viscosity index improver	Contains polyolefin
A-30	Antioxidant	Trialkylphenol

Table V. Properties of Paraffin-Naphthene Fractions of Neutrals

Oil No.	Crude Source	P-N Wt. %	n_D^{25}	d^{25}	Bromine Number	10.3-Micron Wave Length μ	10.3-Micron Half-Width μ	10.3-Micron Absorbance
1P	Buckeye (Pa.)	74.5	1.46577	0.84286	4.7	10.34	0.13	0.106
3P	Middle District (Pa.)	76.1	1.46573	0.84256	4.3	10.34	0.13	0.117
5P	Eureka No. 1 (Pa.)	78.5	1.46575	0.84260	4.6	10.34	0.13	0.116
7P	Bradford (Pa.)	73.5	1.46622	0.84361	7.0	10.34	0.13	0.178
9P	Bolivar (Pa.)	72.6	1.46696	0.84560	7.3	10.34	0.13	0.167
13P	Eureka No. 2 (Pa.)	72.8	1.46573	0.84298	4.5	10.34	0.13	0.108
17P	Buckeye (Pa.)	79.0	1.46515	0.84165	4.8	10.34	0.13	0.116
18P	Bradford (Pa.)	77.5	1.46483	0.84070	6.1	10.34	0.13	0.167
69P	Allegany (Pa.)	70.0	1.46636	0.84440	6.2	10.34	0.13	0.164
85P	Bradford (Pa.)	71.7	1.46598	0.84314	7.0	10.34	0.13	0.158
41	Corning (Ohio)	72.8	1.46612	0.84513	3.3	10.34	0.13	0.108
10	Mid-continent	88.4	1.47244	0.86070	0.5	10.39	0.30	0.035
12	Mid-continent	86.3	1.46934	0.85228	0.7	10.30	0.25	0.040
39	Mid-continent	77.9	1.46944	0.85255	1.1	10.34	0.24	0.051
89	West Edmond (Okla.)	70.4	1.46523	0.84408	2.0	10.34	0.14	0.055
T-9	Mid-continent	79.5	1.46851	0.85137	1.1	10.34	0.21	0.052
40	Texas	88.7	1.46573	0.84404	0.4	10.34	0.24	0.041
45	Winkler Co. (Tex.)	78.6	1.46545	0.84362	0.4	10.34	0.21	0.036
83	Winkler Co. (Tex.)	74.5	1.46690	0.84644	0.6	10.34	0.21	0.032
15	Gulf Coast	69.5	1.47433	0.86792	1.9	10.34	0.21	0.051
16	Gulf Coast	63.6	1.47621	0.87115	1.0	10.34	0.28	0.054
T-38	California neutral	81.1	1.47034	0.85660	0.6	10.34	0.30	0.050
T-44	California neutral	80.8	1.47136	0.85906	0.2	10.34	0.28	0.047

Table VI. Properties of Paraffin-Naphthene Silica Gel Fractions of Bright Stocks

Oil No.	Crude Source	P-N Wt. %	n_D^{25}	d^{25}	Bromine Number	10.3-Micron Wave Length μ	10.3-Micron Half-Width μ	10.3-Micron Absorbance
2P	Buckeye (Pa.)	63	1.4760	0.8630	4.6	10.34	0.13	0.137
4P	Middle District (Pa.)	64	1.4761	0.8635	4.5	10.34	0.13	0.125
6P	Eureka No. 1 (Pa.)	75	1.4770	0.8634	5.0	10.34	0.13	0.115
8P	Bradford (Pa.)	58	1.4761	0.8624	7.2	10.34	0.13	0.189
14P	Eureka No. 2 (Pa.)	64	1.4765	0.8635	4.8	10.34	0.13	0.121
42	Corning (Ohio)	55	1.4758	0.8639	3.5	10.34	0.14	0.096
44	Meigs Co. (Ohio)	51	1.4765	0.8641	4.5	10.34	0.13	0.119
11	Mid-continent	75	1.4815	0.8782	0.8	10.34	0.27	0.031
117	Mid-continent	54	1.4837	0.8778	2.7	10.36	0.20	0.050
46	Winkler Co. (Tex.)	60	1.4775	0.8671	0.6	10.34	0.16	0.027

The bromine numbers of the Pennsylvania samples fall between 4.0 and 7.5. The densities of the paraffin-naphthene fractions of the neutrals are between 0.840 and 0.846, while the bright stock densities are between 0.861 and 0.865. It seems desirable to specify both bromine number and density in characterizing an oil sample, for the bromine number is a measure of a minor though characteristic constituent and density is essentially a measure of the abundance ratio of naphthenes to paraffins—i.e., a bulk property. The limits just described are shown in the figures by dotted lines. More extensive sampling may require that the limits be slightly broadened. The method is somewhat less accurate for bright stocks than neutrals, probably because the separation of aromatics is not so sharp as for neutrals. For both neutrals and bright stocks, however, the distinction between Pennsylvania and non-Pennsylvania oils is sharp except for the neighboring fields in Ohio. The bromine number method has

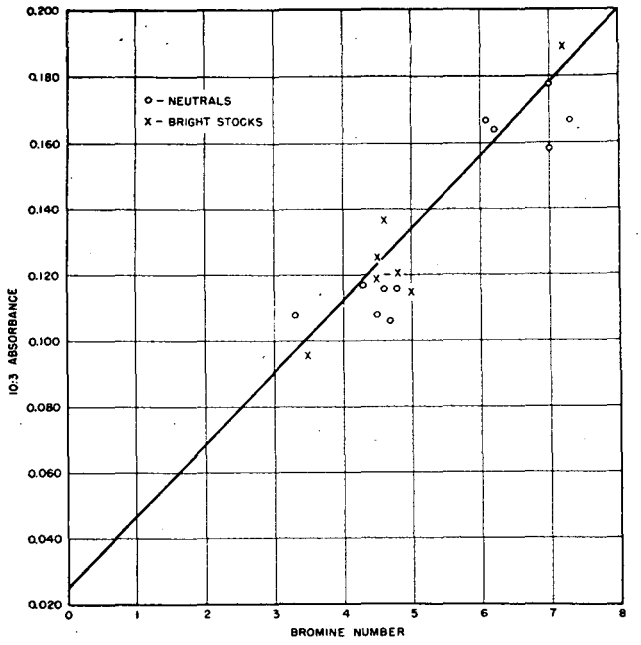


Figure 14. Relation between Bromine Number and 10.3 Intensity of Paraffin-Naphthene Silica Gel Fractions of Pennsylvania Neutrals and Bright Stocks

Line calculated by Formula 1 from literature data on octene

the obvious advantage over the infrared method of not requiring expensive specialized equipment; however, it is more difficult and costly per test.

CONCLUSION

Lubricating oils made from Pennsylvania grade crudes have a sharp infrared absorption band at 10.3 microns which is not present, with minor exceptions, in the spectra of oils of other origin available in this country. It is especially noteworthy that the band does not appear in certain Texas oils which have physical characteristics somewhat similar in other respects to oils from Pennsylvania. It is believed as a result of chemical treatment and literature survey that the 10.3 band appearing in Pennsylvania oils is due to olefins with internal, unsubstituted, trans double bonds, present in the original crude and distributed approximately equally over the whole molecular weight range of the crude.

Therefore, the 10.3 band furnishes a good basis for identification of Pennsylvania lubricating oils. Any lubricating oil that shows lack of absorption at 10.3 microns is not processed from Pennsylvania grade crude oil. This same information can be obtained with less precision by bromine number determination of the olefin concentration in the paraffin-naphthene silica gel fraction of the oil.

ACKNOWLEDGMENT

Most of the infrared measurements reported in this paper were performed by Lorna Patterson. V. I. Komarewsky of the Illinois Institute of Technology has been very helpful in discussions of this work and the authors are indebted to him for loaning the olefins of high molecular weight. They wish to thank W. S. Gallaway of Universal Oil Products Company for first pointing out that olefins with an internal, unsubstituted double bond have an absorption band at 10.3 microns. They would also like to thank Harold M. Smith of the Bureau of Mines for calling attention to (*16*) and for permission to include it. Finally, they would like to express appreciation to H. M. Randall and the Harrison M. Randall Laboratory of Physics of the University of Michigan for preliminary measurements of the infrared absorption spectra of a number of oil samples.

LITERATURE CITED

(1) Am. Petroleum Inst., Research Project 44, Natl. Bur. Standards, Catalog of Infrared Spectrograms.
(2) Am. Soc. Testing Materials, Philadelphia, A.S.T.M. Standards on Petroleum Products and Lubricants, Appendix III, "Proposed Method of Test for Aromatic Hydrocarbons in Mixtures with Naphthenes and Paraffins by Silica Gel Adsorption," p. 20, 1946.
(3) Barnes, R. B., Gore, R. C., Stafford, R. W., and Williams, V. Z., ANAL. CHEM., **20**, 402 (1948).
(4) Breger, I. A., personal communication.
(5) Burton, V. L., and Breger, I. A., personal communication.
(6) Dinsmore, H. L., and Smith, D. C., ANAL. CHEM., **20**, 11 (1948).
(7) Field, J. E., Woodford, D. E., and Gehman, S. D., *J. Applied Phys.*, **17**, 386 (1946).

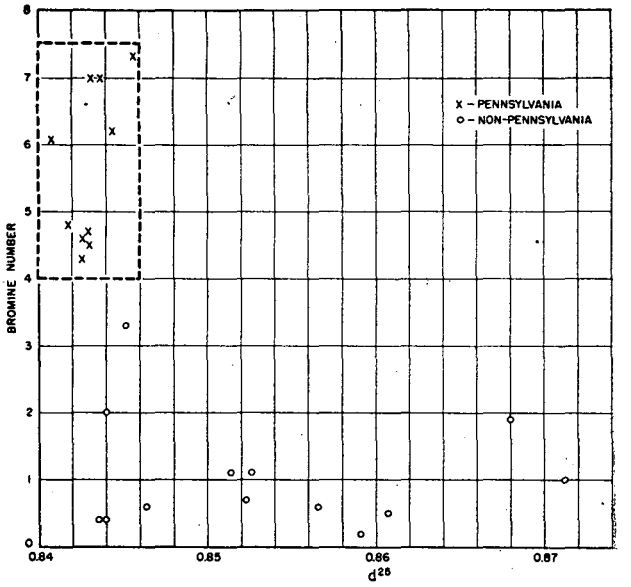


Figure 15. Bromine Numbers and Densities of Paraffin-Naphthene Silica Gel Fractions of Neutrals

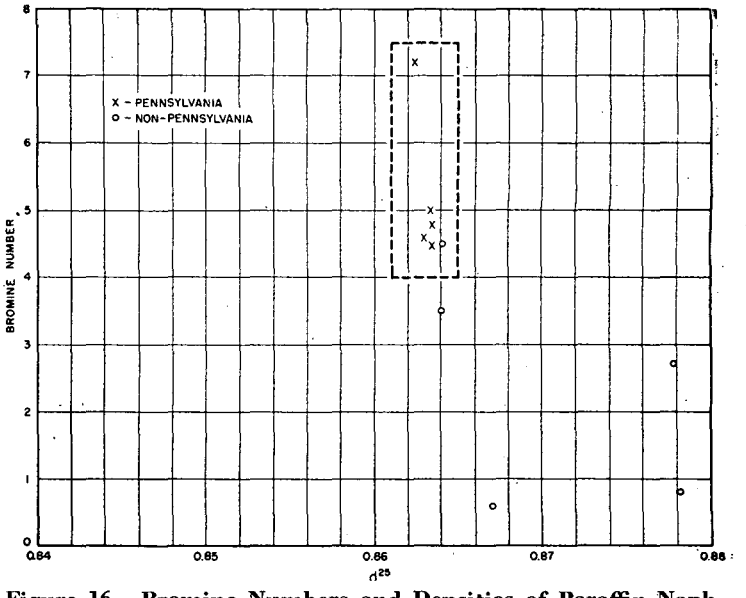


Figure 16. Bromine Numbers and Densities of Paraffin-Naphthene Silica Gel Fractions of Bright Stocks

(8) Gallaway, W. S., personal communication.
(9) Gore and Johnson, *Phys. Rev.*, 68, 283 (1945).
(10) Johnson, H. L., and Clark, R. A., ANAL. CHEM., 19, 867 (1947).
(11) Kilpatrick, J. E., and Pitzer, K. S., *J. Research Natl. Bur. Standards*, 38, 191 (1947); RP 1768.
(12) Komarewsky, V. I., and Kritchevsky, T. H., *J. Am. Chem. Soc.*, 65, 547 (1943).
(13) Lipkin, M. R., Joffecker, W. A., Martin, C. C., and Ledley, R. E., ANAL. CHEM., 20, 130 (1948).
(14) Mair, B. J., *J. Research Natl. Bur. Standards*, 34, 435 (1945).
(15) Mair, B. J., and Forziati, A. F., *Ibid.*, 32, 165 (1944).
(16) Petroleum Experiment Station, Bureau of Mines, Bartlesville, Okla., "Report on Possible Crude Oil Sources for Jet Propulsion Fuels," p. 10, October 1945.
(17) Rasmussen, R. S., Brattain, R. R., and Zucco, P. S., *J. Chem. Phys.*, 15, 135 (1947).
(18) Thompson, H. W., and Torkington, P., *Trans. Faraday Soc.*, 41, 246 (1945).

RECEIVED August 18, 1947.

Determination of Impurities in *n*-Heptane Concentrates

By Infrared Absorption

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In connection with experimental studies of the production of reference fuel grade *n*-heptane of 99% purity or better by fractional distillation of refinery stocks, an infrared procedure has been developed for the determination of individual impurities present in *n*-heptane concentrates of high purity (90 to 99%). Impurity components determined by the analysis are 2-methylhexane, 3-methylhexane, *trans*-1,3-dimethylcyclopentane, *trans*-1,2-dimethylcyclopentane, 2,2,4-trimethylpentane, *cis*-1,2-dimethylcyclopentane, and methylcyclohexane. Time requirements are approximately 2 hours for the complete determination. On the basis of analysis of synthetic samples, the method is indicated to have an average accuracy for individual impurities of ±0.2%, based on the total sample. Results for total impurities by the infrared and freezing point methods check on the average within ±0.3%. The method has been applied extensively in laboratory work for determination of the relative amounts of the individual impurities in *n*-heptane concentrates derived from different crude sources.

IN THE production of reference fuel grade iso-octane by direct fractional distillation of butylene alkylate (*2*), a product of 99.5% purity is separated from a material containing 18 to 25% iso-octane in admixture with about twenty other paraffinic compounds. The production of reference fuel grade *n*-heptane in a similar manner by distillation of available refinery virgin naphthas may be limited, however, by the presence in the naphtha of two compounds—namely, *cis*-1,2-dimethylcyclopentane and 2,2,4-trimethylpentane (iso-octane) which possess boiling points within 1.5° F. (0.8° C.) of that of *n*-heptane. In connection with studies of this potential process, an analytical procedure employing infrared absorption spectroscopy has been developed for determining the type and amount of impurities present in *n*-heptane concentrates from various crude sources. The technique employed for analysis is similar to that described in a previous paper (*1*) by one of the authors. The method, which has a time requirement of about 2 hours, is indicated to have an average accuracy for individual impurities of about ±0.2%, based on the total sample.

APPARATUS AND EXPERIMENTAL TECHNIQUES

The infrared instrument employed was a Perkin-Elmer Model 12-A equipped with a sodium chloride prism, adapted for the scanning of spectra with a synchronous wave-length drive, and equipped with a contact modulated direct current amplifier of the type developed by General Motors Corporation (*4*).

Stability of the instrument was found to be of primary importance. The instrument was housed in an air-conditioned room with temperature controlled to ±1° F.; under these conditions little or no drift was encountered. In order to reduce noise level as much as possible, and thereby improve accuracy of absorption measurements, the amplifier was operated at lower gain and the slits were opened wider than for conventional analytical work. Under these conditions, full scale deflection on the 0- to 10-millivolt Brown Electronik recorder used was obtained with a signal to the direct current amplifier of the order of 2 microvolts, instead of the usual 0.5 to 1 microvolt. This did not make an excessive increase in slit width necessary—for example, at 8.28 microns, a slit width of 0.200 mm. was used instead of the usual 0.130 mm.

The sodium chloride sample cell employed for this work was of the type manufactured by the Perkin-Elmer Corporation. The sample cell thickness was of the order of 0.4 mm. This particular thickness was chosen because it was great enough to bring out the absorption of the impurity components, but not thick enough to cause excessive absorption by the *n*-heptane. In order to obtain the high reproducibility required in the analysis, reference intensity measurements were made with a rock salt plate at each spectral position within a few seconds after completion of the measurement on the liquid-filled sample cell.

DEVELOPMENT OF METHOD

Study of the literature on the boiling points of hydrocarbons indicated that as many as eight impurities might be present in the *n*-heptane concentrates derived by distillation. These components are shown in Table I, together with their boiling points and wave lengths employed for analysis. Similar data are given for *n*-heptane. A detailed study of the spectra of these impurity components and the spectrum of the *n*-heptane showed that the "masking" absorption effects of *n*-heptane would prevent making qualitative analyses of the impurity components present in the *n*-heptane concentrates by direct inspection of their absorption spectra. However, the impurity components present in the concentrates could be determined by solution of simultaneous equations written for the absorption of the impurities at the spectral positions involved. Suitable wave lengths were found for the analysis of each of the impurities, with the exception of 3-ethylpentane, which was omitted from the analysis. The effect

Table I. Impurity Analyses of Synthetic Mixtures

Compound	Boiling Point, ° F.	Key Wave Length, Microns	Synthetic 1 Syn.	Synthetic 1 Found	Synthetic 1 Diff.	Synthetic 2 Syn.	Synthetic 2 Found	Synthetic 2 Diff.	Synthetic 3 Syn.	Synthetic 3 Found	Synthetic 3 Diff.
2,2,4-Trimethylpentane	210.6	8.28	0.21	0.26	0.05	0.77	0.73	0.04	1.29	1.30	0.01
trans-1,3-Dimethylcyclopentane	195.8	8.69	0.23	0.20	0.03	0.73	0.93	0.20	2.85	2.34	0.51
cis-1,2-Dimethylcyclopentane	210.6	9.62	0.21	0.11	0.10	0.74	0.53	0.19	2.85	3.08	0.23
trans-1,2-Dimethylcyclopentane	197.4	9.94[a]	0.32	0.35	0.03	0.77	0.58	0.19	2.85	3.23	0.38
2-Methylhexane	194.0	10.93	0.26	0.19	0.07	0.77	0.83	0.06	2.85	3.03	0.18
3-Methylhexane	197.5	11.33[b]	0.23	0.33	0.10	0.75	0.68	0.07	2.85	2.80	0.05
Methylcyclohexane	213.8	11.86	0.23	0.28	0.05	0.77	0.85	0.08	1.29	1.36	0.07
3-Ethylpentane	200.3	....	..	..	..	..	..	..	...	...	..
n-Heptane	209.1	....									
Total high boiling impurities	...	....	0.65	0.65	0.00	2.28	2.11	0.17	5.33	5.74	0.41
Total low boiling impurities	...	....	1.04	1.07	0.03	3.02	3.02	0.00	11.40	11.40	0.00
Total impurities	...	....	1.69	1.72	0.03	5.30	5.13	0.17	16.73	17.14	0.41

[a] Checked at 10.19 microns.
[b] Checked at 10.35 microns.

of this omission has not been indicated to be serious on the basis of checks of infrared and freezing point purities on several *n*-heptane concentrates.

Measurements on samples were made on a basis of direct comparison with absorption at the same spectral position for *n*-heptane of high purity. Because of the small quantity of the impurity components, base-line methods (*3*) were not considered applicable.

REFERENCE STANDARDS

The reference standard employed for this work was 99.67% pure *n*-heptane manufactured by Westvaco Chlorine Products Company. No attempt was made to identify the small amount of impurities present in the Westvaco product. In cases where the analysis was employed for determination of impurities in *n*-heptane concentrates of 99% purity or better, measurements were made directly against a primary reference standard of higher purity (National Bureau of Standards *n*-heptane with a purity of 99.90 ± 0.05 mole %) or else against a secondary standard that had been calibrated against the primary standard.

CALIBRATION AND CALCULATION PROCEDURES

The instrument employed for analysis was calibrated by measurement of the optical density differences at each spectral position between 5 volume % blends of each impurity compound in *n*-heptane and the *n*-heptane reference standard, using a rock salt plate for reference intensity measurements. These differential optical densities (the differences between the optical densities of the reference standard and blend of the impurity) were computed to a 100% basis—i.e., to absorption coefficients, for the impurities, assuming Beer's law to apply. Because the absorption coefficients for the impurities were measured against the reference standard *n*-heptane, on a differential basis, they were lower than the absolute absorption coefficients of the impurities at the given spectral positions. As such, these coefficients were called differential absorption coefficients, because they represented the difference in absorption between the impurity and *n*-heptane. This procedure of the use of differential optical densities was employed also for samples; differential optical densities were obtained between the samples and the reference standard. For the calculation of concentration values for the individual impurities, the differential optical densities obtained for the samples were substituted in a matrix composed of the differential absorption coefficients obtained by calibration for each of the impurities, and the resultant simultaneous equations were solved for the percentage of each of the individual components. The use of differential absorption coefficients for individual component analyses is described in more detail in the following paragraphs.

DIFFERENTIAL ABSORPTION COEFFICIENTS

The spectrometric analysis for several impurity components in *n*-heptane concentrates, which is based on direct comparison of the sample and a pure *n*-heptane reference, is similar to conventional spectroscopic multicomponent analysis employing a transparent solvent, except that *n*-heptane—i.e., the solvent—absorbs an appreciable quantity of radiation of all the wave lengths involved in the analysis, and its concentration is unknown. It has been found that if the absorption characteristics of the impurity compounds and of the samples are measured against a *n*-heptane reference, exact analysis for the impurity compounds can be made without knowledge of the *n*-heptane content of the samples, and corresponding correction for *n*-heptane absorption effects. This is shown in the following derivations.

The optical density of an absorbing solvent can be represented by the following equation:

$$d_{\lambda_i s} = \log \frac{I_{\lambda_i 0}}{I_{\lambda_i s}} = K_{\lambda_i s} \qquad (1)$$

where $I_{\lambda_i 0}$ = energy of wave length λ_i transmitted through a standard thickness sample cell filled with nonabsorbing solvent
$I_{\lambda_i s}$ = energy of wave length λ_i transmitted through the same standard thickness sample cell filled with absorbing solvent *s*
$d_{\lambda_i s}$ = absolute optical density of solvent in cell
$K_{\lambda_i s}$ = absorption coefficient of solvent, defined as optical density calculated for pure solvent in standard thickness cell, as the concentration expressed as volume fraction is unity in this case

If a sample is blended with absorbing solvent for absorption measurements, the absolute optical density, $d_{\lambda_i m}$, of the mixture of sample and solvent is as follows:

$$d_{\lambda_i m} = \log \frac{I_{\lambda_i 0}}{I_{\lambda_i}} = \log I_{\lambda_i 0} - \log I_{\lambda_i} \qquad (2)$$

where I_{λ_i} = energy of wave length λ_i transmitted by mixture of sample and solvent. From Equation 1,

$$K_{\lambda_i s} = \log I_{\lambda_i 0} - \log I_{\lambda_i s} \qquad (3)$$

$$\text{or } \log I_{\lambda_i 0} = K_{\lambda_i s} + \log I_{\lambda_i s}$$

Then, by substitution,

$$d_{\lambda_i m} = K_{\lambda_i s} + \log I_{\lambda_i s} - \log I_{\lambda_i} = K_{\lambda_i s} + \log \frac{I_{\lambda_i s}}{I_{\lambda_i}} \qquad (4)$$

But $d_{\lambda_i m} = X_1 K_{\lambda_i 1} + X_2 K_{\lambda_i 2} + X_3 K_{\lambda_i 3} + \ldots + \ldots + X_n K_{\lambda_i n} + X_s K_{\lambda_i s}$ where $X_1, X_2, X_3, \ldots, X_n, X_s$, represent the volume fraction of components 1, 2, 3, ..., *n*, and solvent *s* (volume changes on mixing are assumed to be negligible), and $K_{\lambda_i 1}, K_{\lambda_i 2}, K_{\lambda_i 3}, \ldots, K_{\lambda_i n}$ represent the absorption coefficient of compounds 1, 2, 3, ..., *n*—that is, the absolute optical density of these pure compounds in a standard thickness cell.

Substituting and collecting terms,

$$\log \frac{I_{\lambda_i s}}{I_{\lambda_i}} + K_{\lambda_i s}(1 - X_s) = X_1 K_{\lambda_i 1} + X_2 K_{\lambda_i 2} + X_3 K_{\lambda_i 3} + \ldots + X_n K_{\lambda_i n} \qquad (5)$$

As $1 - X_s = C$, where *C* is the volume fraction of the sample in the sample and solvent mixture, dividing every term of Equation 5 by *C* gives

$$\frac{1}{C}\left(\log\frac{I_{\lambda_i s}}{I_{\lambda_i}}\right) + K_{\lambda_i s} = \frac{1}{C}\,(X_1K_{\lambda_i 1} + X_2K_{\lambda_i 2} + X_3K_{i\lambda_3} + \ldots. + X_nK_{\lambda_i n}) \quad (6)$$

or

$$\frac{1}{C}\left(\log\frac{I_{\lambda_i s}}{I_{\lambda_i}}\right) + K_{\lambda_i s} = K_{\lambda_i}$$

where K_{λ_i} = the absolute absorption coefficient of the sample in the standard thickness cell on a solvent-free basis.

As $\log\frac{I_{\lambda_i s}}{I_{\lambda_i}}$ is the observed optical density obtained using the absorbing solvent for reference measurement, Equation 6 may be written as

$$\frac{d_{\lambda_i 0}}{C} + K_{\lambda_i s} = K_{\lambda_i} \quad (7)$$

$$\text{where } d_{\lambda_i 0} = \log\frac{I_{\lambda_i s}}{I_{\lambda_i}}$$

This equation is very useful, as $d_{\lambda_i 0}$ can be measured directly for a sample, whereas $d_{\lambda_i m}$ cannot usually be, because transparent solvents are not readily available for the entire wave-length region. The disadvantage of this equation lies in the fact that the term $K_{\lambda_i s}$ must be known for the entire wave-length region. In the following paragraphs a series of equations is developed that allows the use of the observed optical density, without having to know the absorption coefficient of the solvent used for reference measurement.

At any given wave length λ_i the absorption coefficient of the sample on a solvent-free basis may be expressed in terms of the absorption coefficients of its components. For example,

$$K_{\lambda_i} = X_1K_{\lambda_i 1} + X_2K_{\lambda_i 2} + X_3K_{\lambda_i 3} + \ldots. + X_nK_{\lambda_i n}$$

By writing similar equations for several different wave lengths until there is a number of equations equal to the number of components, the usual matrix encountered in spectrometric analysis is obtained.

$$K_{\lambda_i} = \sum_{j=1}^{n} X_jK_{\lambda_i j} \quad (\lambda_i = \lambda_1, \lambda_2, \lambda_3, \ldots, \lambda_n) \quad (8)$$

The handling of these equations and procurement of the necessary data can be simplified by introduction of a term ΔK, the differential absorption coefficient, defined by

$$\Delta K_{\lambda_i j} = K_{\lambda_i j} - K_{\lambda_i s} \quad (9)$$

Thus the absorption coefficients of the pure components may be expressed as

$$K_{\lambda_i j} = \Delta K_{\lambda_i j} + K_{\lambda_i s} \quad (10)$$

If Equation 7 is written in the same form as Equation 10, it is evident that

$$\frac{d_{\lambda_i 0}}{C} = \Delta K_{\lambda_i} = K_{\lambda_i} - K_{\lambda_i s}$$

Substituting these last equations into Equation 8 transforms it to

$$\Delta K_{\lambda_i} + K_{\lambda_i s} = \sum_{j=1}^{n} X_j(\Delta K_{\lambda_i j} + K_{\lambda_i s}) \quad (\lambda_i = \lambda_1, \lambda_2, \lambda_3, \ldots, \lambda_n)$$

Removing parentheses and collecting terms reduce this to

$$\Delta K_{\lambda_i} + K_{\lambda_i s} = \sum_{j=1}^{n} X_j\Delta K_{\lambda_i j} + \sum_{j=1}^{n} X_jK_{\lambda_i s} \quad (\lambda_i = \lambda_1, \lambda_2, \lambda_3, \ldots, \lambda_n)$$

As $\sum_{j=1}^{n} X_j = 1$, the term $K_{\lambda_i s}$ may be subtracted from both sides to give the following final matrix.

$$\Delta K_{\lambda_i} = \sum_{j=1}^{n} X_j\Delta K_{\lambda_i j} \quad (\lambda_i = \lambda_1, \lambda_2, \lambda_3, \ldots, \lambda_n) \quad (11)$$

The constant terms in these equations are very readily evaluated, inasmuch as for the pure components as well as for the mixture,

$$\Delta K_{\lambda_i} = \frac{1}{C}\log\frac{I_{\lambda_i s}}{I_{\lambda_i}}$$

This equation is valid even if the sample (or pure compound) is not blended with the absorbing solvent, if it is remembered for this case $C = 1$.

No correction need be made for the absorption of the solvent even if it has varying degrees of transparency at different wave lengths. This is true whether or not the solvent is one of the components in the sample mixture.

Table II illustrates the application of this method of calibration to iso-octane, using *n*-heptane as a reference standard. Wherever the iso-octane absorbs less than the *n*-heptane, the differential absorption coefficients are negative and must be used with their proper sign in the final matrix. A summary of the calibration data used in the analysis described in this paper is given in Table III. By using one of the components, *n*-heptane, as the reference standard, only seven wave lengths are needed for the exact mathematical analysis of this eight-component system.

Examination of these equations indicates several advantages and possible applications. Errors inherent in measurement of energy incident upon a sample by means of a rock salt plate due to varying optical density of the cell with respect to the plate and the deviation of the equivalent absorption of a plate from that of a transparent solvent are entirely eliminated. In addition, the optical density of the sample cell need not be known, and the cell changes that occur gradually with time do not affect the accuracy of the analysis. Even if the solvent used for the reference measurement is not one of the components in the analytical scheme, no correction need be made for its absorption. The matrices derived from these equations may be presolved by any of the usual methods.

EVALUATION OF METHOD

In order to check on the accuracy of the infrared procedure for the determination of individual impurities present in *n*-heptane concentrates from several crude sources, three synthetic samples were analyzed by the procedure described above. Results obtained on the analysis of these samples are shown in Table I.

As shown by these data, the accuracy for the individual impurities is within ±0.1 to ±0.2% except for those cases in which the concentration of the total impurities is well over 5%. In

Table II. Calculation of Δ Absorption Coefficients for Iso-octane, Using *n*-Heptane as I_0

λ_i, Microns	C, Volume Fraction of Iso-octane	$I_{\lambda_i s}$, Using *n*-Heptane	I_{λ_i}	$d_{\lambda_i}(iCs)$	$\Delta K_{\lambda_i}(iCs)$
8.28	0.0904	110.4	65.7	0.224	2.477
8.69	1.000	97.5	120.6	−0.093	−0.093
9.62	1.000	134.4	184.5	−0.137	−0.137
9.94	1.000	144.6	166.4	−0.061	−0.061
10.93	1.000	150.0	91.0	0.218	0.218
11.33	1.000	112.2	181.6	−0.210	−0.210
11.86	1.000	149.6	179.0	−0.078	−0.078

Table III. Differential Absorption Coefficients (Δ Values) for Eight-Component Mixture

(One component, *n*-heptane, used as I_0)

Compound Wave Length, Microns	Iso-octane	*trans*-1,3-Dimethyl-cyclo-pentane	*cis*-1,2-Dimethyl-cyclo-pentane	*trans*-1,2-Dimethyl-cyclo-pentane	2-Methyl-hexane	3-Methyl-hexane	Methyl-cyclo-hexane
8.28	2.477	0.088	0.112	−0.007	−0.072	−0.159	−0.082
8.69	−0.093	1.107	−0.076	0.258	0.398	0.687	−0.308
9.62	−0.137	0.057	0.913	0.004	0.195	0.219	0.063
9.94	−0.061	0.356	0.343	1.077	0.027	0.367	−0.074
10.93	0.218	−0.070	−0.168	−0.165	0.815	0.139	0.133
11.33	−0.210	−0.068	0.030	0.092	−0.123	0.366	0.116
11.86	−0.078	0.068	−0.064	−0.028	−0.113	−0.014	1.574

Table IV. Comparison between Infrared and Freezing Point Determinations for Total Impurities

	Sample Number			
	1	2	3	4
Purity by infrared, vol. % *n*-heptane	97.7	98.5	95.3	95.5
Purity by infrared, mole % *n*-heptane	97.4	98.1	94.8	95.0
Purity by freezing point, mole % *n*-heptane	97.62	97.84	94.82	94.75
Difference by infrared and freezing point, mole % *n*-heptane	0.2	0.3	0.0	0.2

addition, the results expressed as the sum of the total high boiling impurities, the sum of the total low boiling impurities, and the sum of the total impurities are also accurate and usually within ±0.3% on the total sample. A further check on the accuracy of the method was obtained for several samples by comparison of the infrared results for total impurities versus concentration of impurities as determined by the freezing point method. Results obtained on these samples are shown in Table IV. These data indicate agreement to within ±0.2% on the average between infrared and freezing point determinations.

The infrared method as described above has been used for study of the composition of impurities in *n*-heptane concentrates from eight crude sources. In a number of cases, calculated results showed a percentage of *cis*-1,2-dimethylcyclopentane relative to *n*-heptane greater than 1%. In no case was the concentration of 2,2,4-trimethylpentane relative to *n*-heptane greater than 0.3%. The calculated percentages of 2,2,4-trimethylpentane which were obtained were not considered significant, inasmuch as they were almost within the reproducibility of the determination.

Although this analysis requires a precision that is almost at the practical limit of the instrument, it may be applicable for fractional distillation column control for the plant production of high purity *n*-heptane in the same manner as a similar analysis was used for fractional distillation column control for the plant production of reference fuel grade iso-octane (*1*). In this case the analysis would be used to determine the relative amounts of high and low boiling impurities present in the *n*-heptane product. For this application, however, the results should always be examined for an unexpected or unusual distribution of the impurity components caused by the influx of another component not allowed for in the calculations. Periodic cross checks of analyses by the freezing point method will also assure that all the significant components have been included in the analytical scheme.

ACKNOWLEDGMENT

The work described in this publication was carried out in the laboratory of the Humble Oil and Refining Company and the authors wish to express their thanks for permission to publish details of the analytical method employed. The contributions of D. W. McDonald, who made arrangements for the segregation of *n*-heptane concentrates from various crude sources, and of G. A. Satterwhite, who performed nearly all calibration and calculation work involved in the development of the method, are hereby acknowledged.

LITERATURE CITED

(1) Anderson, J. A., Jr., ANAL. CHEM., 20, 801 (1948).
(2) Gerbes, Otto, Hall, H. J., and Becker, A. E., *Petroleum Processing*, 2, 734 (1947).
(3) Heigl, J. J., Bell, M. F., and White, J. U., ANAL. CHEM., 19, 293 (1947).
(4) Liston, M. D., Quinn, C. E., Sargeant, W. E., and Scott, C. G., *Rev. Sci. Instruments*, 17, 194 (1946).

RECEIVED December 29, 1947. Presented before the Southwest Regional Meeting, AMERICAN CHEMICAL SOCIETY, Houston, Tex., December 12 and 13, 1947.

Infrared Spectroscopic Determination of Ester Carbonyl

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Frequency and intensity of the carbonyl absorption have been measured for nineteen esters of low molecular weight and two polymers. Both the frequency and intensity of the ester carbonyl absorption vary considerably with the adjacent molecular structure, so that, for accurate analyses, it is necessary to know something about the molecular structure adjacent to the carbonyl group. Working curves have been prepared for the quantitative determination of polymethyl methacrylate and for ethyl adipate (selected as a representative ester).

IT IS nominally possible, by means of infrared absorption spectra, to identify and determine various types of organic functional groups such as hydroxyl, nitrile, amine, and carbonyl. Qualitative analysis is based on the presence or absence of absorption bands at frequencies characteristic of the groups to be identified (*2*). Quantitative analyses are based on measurements of intensities of the absorption bands. Usually quantitative analyses are made to determine a specific compound, and calibrations are based on a pure sample of the compound to be determined. However, it is frequently possible to determine (although less accurately) the amount of a specific functional group. This type of analysis can give useful information in many cases dealing with polymers or compounds whose exact structure is in doubt. For example, it should be possible to estimate the molecular weight of a compound when the number of functional groups per molecule is known or can be inferred, or to determine the composition of a tautomeric equilibrium mixture.

The determination of ester carbonyl is useful in the analysis of copolymers containing esters (such as the acrylates), in studying hydrolysis, lactone formation, and other problems involving esters. Before analyses can be made with reasonable confidence it is desirable to study the spectra of known compounds, to determine the effect of structure variation on the frequency and intensity of absorption, and to establish the quantitative relation between concentration and absorption intensity. Thompson and Torkington (*4*) have reported the absorption frequencies of a considerable number of esters, but their data were almost entirely on saturated aliphatic esters of monocarboxylic acids and did not give extinction coefficients.

Anderson and Seyfried (*1*) have recently made an excellent and

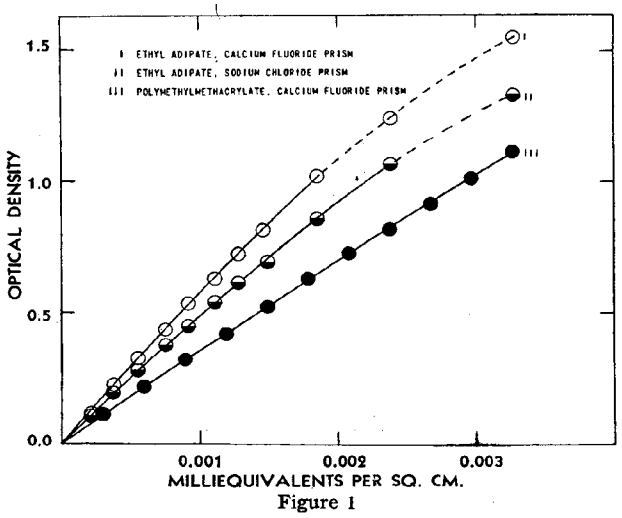

Figure 1

comprehensive study of the determination of specific functional groups, including ester carbonyl, in samples such as hydrocarbon synthesis naphtha. They did not give data on individual esters, but gave an average value for the wave length and intensity of absorption for the esters studied (presumably of the simple aliphatic type).

The authors have measured the frequency and intensity of the carbonyl absorption for nineteen esters of low molecular weight and two polymers. Working curves have been prepared for the quantitative determination of ethyl adipate (selected as a representative ester), and polymethyl methacrylate.

The model 12A Perkin-Elmer spectrometer used has been equipped with a blackened Thermistor bolometer, Western Electric amplifier Model KS-10281, and Speedomax Type A recorder. The recorder chart and Littrow mirror are driven through Selsyns to ensure accuracy of the wave-length calibration on the charts. Radiation is chopped at 15 cycles per second by means of a synchronous rotating sector at the globar. This recording system is stable, sensitive, and completely free from zero drift.

Except where otherwise specified, a calcium fluoride prism was used, with a slit width of 0.155 mm., equivalent to a half intensity band width of about 7 cm.$^{-1}$.

Each sample was measured twice, using two interferometrically calibrated (*3*) sodium chloride cells 0.01045 and 0.01856 cm. thick, respectively. The cells were vapor-tight, being made with amalgamated lead spacers and tapered lead plugs closing the hypodermic hub filling holes. Carbon tetrachloride was used as solvent for all samples except polymethyl methacrylate, which, being insoluble in carbon tetrachloride, was measured in chloroform solution. In every case the solution concentration was such as to give an optical density of about 0.45, after correction for solvent absorption. The optical density was measured with the spectrometer set at the frequency of maximum absorption by the carbonyl bond (for the particular sample being studied).

With the recorder running but the spectrometer drive stopped, the cell containing the sample solution was alternately placed in front of the slit, then removed, and the corresponding intensities were read from the chart. The zero, determined using a glass shutter, was subtracted from each reading, thus correcting for the small amount of stray short wave-length light present. The readings were repeated using the same cell filled with pure solvent. If the chart showed any change in the cell-out reading, the readings were corrected for this change. The molar extinction coefficient, ϵ, was calculated:

$$\epsilon = \frac{d}{cl}$$

where c is the solution concentration in gram-moles per liter
l is the length in cm. of absorbing path (cell depth)
d is the optical density

$$d = \log \frac{I_0}{I}$$

I is the intensity of radiation transmitted by the solution
I_0 is the intensity of radiation transmitted by the pure solvent

ϵ was determined for two different solutions of each sample, and the values (which in most cases agreed to within 2%) were averaged.

MATERIALS STUDIED

The two polymers were prepared in this laboratory. The polymethylmethacrylate was prepared by heating pure methylmethacrylate at 60° C. in the absence of air, and had been stored for 2 years before use, thus ensuring complete polymerization. It is believed to be pure. The pentamethylene pimelate was prepared by condensation of pentamethylene glycol and pimelic acid, and is probably considerably less pure.

The low molecular weight esters were all purified by fractional distillation through a packed column at reduced pressure. In each case a series of middle fractions of constant boiling point and refractive index were combined for use in this work. All unsaturated esters were distilled at reduced pressure in an atmosphere of carbon dioxide and stored in the dark at 3° C. until needed. Polymerizable monomers were inhibited by the addition of *tert*-butyl catechol (50 p.p.m.).

The infrared spectra showed all samples to be free from appreciable amounts of alcohol. Chemical analyses of many of the samples (Table I) showed negligible free acid (<0.25%) and approximately 100% ester by saponification (for those samples analyzed).

MOLECULAR STRUCTURE AND INFRARED ABSORPTION

The molar extinction coefficient and frequency of the carbonyl absorption for the esters studied are listed in Table I. For exact correlation, data from many more esters would be desirable. However, the data in Table I show the magnitude of variation to be expected, and permit some qualitative conclusions on the effects of structure. In most cases only the structure of the acid used in forming the ester is important, variation in the nature of the alcohol radical having little effect.

The simple aliphatic esters studied show little variation. All absorb near 1740 cm.$^{-1}$; ϵ varies from 537 to 610. Thompson and Torkington (*4*) studied a considerable number of formates, acetates, propionates, butyrates, and isobutyrates and found that the carbonyl absorption was very close to 1740 cm.$^{-1}$, except for

Table I. Effect of Structure on Carbonyl Absorption of Esters

	Frequency, Cm.$^{-1}$	Molar Extinction Coefficient	Boiling Point, ° C.	Refractive Index, n^{20}_D
Isoamyl acetate	1743	610	4115	1.4006
Propyl propionate[a]	1740	553	761.7	1.3934
Ethyl butyrate[a]	1738	600	4850	1.3925
Ethyl palmitate	1738	569	150–1521.5	1.4403
Ethyl phenyl acetate[a]	1740	537	701.6	1.4982
Ethyl α-bromobutyrate	1744	504	566	1.4475
Ethyl trichloroacetate	1770	720	6715	1.4502
Methyl methacrylate	1727	672	55147	1.4145
Methyl acrylate	1735	584	44195	1.4026
2-Ethylhexyl acrylate[a]	1728	634	8251	1.3456
Diallyl fumarate	1730	576[b]	.....	....
Diallyl maleate	1738	433[b]	.....	....
Dibutyl oxalate[a]	1746	459[b]	841.5	1.4238
Diethyl adipate[a]	1739	586[b]	891.6	1.4726
Dibutyl phthalate[a]	1732	523[b]	.....	1.4930
Butyl benzoate[a]	1723	767	70–710.8	1.4972
Benzyl benzoate[a]	1725	713	135–1361.6	1.5690
Ethyl cinnamate[a]	1717	697	981.7	1.5605
Methyl salicylate	1684	673	570.7	1.5365
Polymethyl methacrylate	1729	354[b]	.....	....
Polypentamethylene pimelate	1738	538[b]	.....	....

[a] Saponification equivalent indicated 100% purity within accuracy of analyses. These samples were also found to contain negligible amounts (<0.25%) of free acid. Chemical analyses were not made on other samples.
[b] For polycarboxylic esters equivalent weight, rather than molecular weight, was used in calculating extinction coefficients.

the formates, which had maxima near 1723 cm.$^{-1}$ Anderson and Seyfried (*1*) report an average wave length for ester carbonyl absorption of 5.71 microns (1751 cm.$^{-1}$), and an average absorption intensity equivalent to a value of about 342 for ϵ. Their use of a sodium chloride prism, and relatively wide slits, would be expected to give somewhat lower precision of wave-length measurement, and also lower absorption intensities. The present authors' absorption frequencies are believed to be accurate to ± 1 cm.$^{-1}$ In practice, because calibrations and analyses would be made on the same spectrometer, such systematic errors would be canceled out.

The effect of halogen on the α-carbon atom is to shift the carbonyl absorption to a higher frequency. With ethyl α-bromobutyrate ϵ is somewhat lower than usual, but with ethyl trichloroacetate, ϵ is higher than for the simple esters.

When an olefinic double bond is conjugated with the carbonyl bond, as in the acrylates and diallyl fumarate, there appears to be a shift to a lower frequency for the carbonyl absorption and a slight increase in the intensity of absorption. In the case of diallyl maleate there are considerable steric strain and electrostatic interaction between the two carbonyl groups, resulting in the abnormally low value of 433 for ϵ, and a frequency higher than for the other unsaturated esters. The effect of conjugation is not great, probably because the ester group is itself a resonance hybrid of considerable stability.

With the two benzoates, where the ester carbonyl is conjugated directly with an aromatic ring, and ethyl cinnamate where the carbonyl is conjugated through an olefinic double bond to the aromatic ring, the frequency shift and intensification of absorption are much more pronounced.

In the methyl salicylate molecule, where there is intramolecular hydrogen bonding between the hydroxyl hydrogen and the carbonyl oxygen, the carbonyl absorption is shifted to the extremely low frequency of 1684 cm.$^{-1}$ and ϵ has the value of 673, somewhat lower than for the benzoates, where hydrogen bond formation is not involved.

In dibutyl oxalate, with two immediately adjacent carbonyl groups, absorption is at a higher frequency and of lower intensity. In diethyl adipate, the two carbonyl groups are separated by four methylene carbon atoms, and both frequency and intensity of absorption are comparable with the simple aliphatic esters of monocarboxylic acids. Dibutyl phthalate, with two carbonyl groups at adjacent carbons of the benzene ring, has both frequency and intensity of absorption slightly lower than "normal."

Polypentamethylene pimelate, with the carbonyl groups reasonably far apart, appears to be comparable with the simple esters of low molecular weight. Polymethyl methacrylate has an extremely low value for $\epsilon(354)$ possibly because of steric effects. In this case the absorption band was unusually broad, and it is possible that the measurement of band area, rather than peak height, would give more consistent results.

QUANTITATIVE ANALYSES

It can be readily seen from the observed variation in ϵ that it is not possible to determine, with high accuracy, the ester content of a completely unknown sample. For the determination of a specific ester, for which a calibration curve is available, good accuracy (2% or better) is probably attainable. If the normal calibration curve is used for an unknown ester, errors as great as 50% may result. These results will be useful only where semiquantitative results are adequate. In other cases it will be possible to reduce considerably the margin of error by application of a correction based on structural similarity to some of the esters in Table I.

The value of ϵ measured experimentally is usually found to decrease somewhat at high optical densities, partly because a finite band of radiation frequencies is used rather than strictly monochromatic light. For accurate quantitative analyses, the observed values of optical density are plotted against the corresponding product (concentration) $\times$ (cell thickness), and analyses are made by reading directly from the curve. Such working curves have been prepared for the determination of ethyl adipate and polymethyl methacrylate using the calcium fluoride prism. For comparison a curve was also prepared for the normal ester, ethyl adipate, using the sodium chloride prism, with a slit width of 0.078 mm., equivalent to a half-intensity band width of about 18 cm.$^{-1}$

The lower slope of the curve with the sodium chloride prism is due to lower dispersion of the prism and consequent lower spectral purity of the radiation.

INTERFERENCES

The presence of aldehydes, ketones, or free carboxylic acids may prevent the determination of ester carbonyl, although in some cases the carbonyl absorption frequency is enough lower than the ester carbonyl frequency to reduce interference. Amides or soaps should not interfere.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of numerous members of the General Laboratories staff in supplying samples and making analyses, and particularly of F. M. Callahan, who made all the distillations and refractive index determinations, and J. Warnet, who made many of the infrared measurements.

LITERATURE CITED

(1) Anderson, J. A., Jr., and Seyfried, W. D., ANAL. CHEM., **20**, 998 (1948).
(2) Barnes, R. B., Gore, R. C., Stafford, R. W., and Williams, V. Z., *Ibid.*, **20**, 402 (1948).
(3) Smith, D. C., and Miller, E. C., *J. Optical Soc. Am.*, **34**, 139 (1944).
(4) Thompson, H. W., and Torkington, P., *J. Chem. Soc.*, **1945**, 640.

RECEIVED December 3, 1948.

Polarography of Cyclo-octatetraene

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Cyclo-octatetraene in the presence of tetramethylammonium ion produces a well defined polarographic wave. The half-wave potential is −1.51 volt *vs.* the saturated calomel electrode in a 50% alcohol solution. The reduction involves two electrons and no hydrogen ions. Because the diffusion current is linear with concentration over the range of 4.5×10^{-6} *M* to 2×10^{-3} *M*, a satisfactory polarographic method of analyzing cyclo-octatetraene is available. Some comments are offered on the significance of the polarographic data in the elucidation of the structure of cyclo-octatetraene.

THE unique physical and chemical properties of cyclo-octatetraene prompted the author to investigate its polarography. In the presence of tetramethylammonium ion a well defined wave with a half-wave potential of −1.5 volts *vs.* the saturated calomel electrode is obtained, which is independent of pH. The diffusion current is directly proportional to concentration over the entire concentration range studied. As a result, cyclo-octatetraene may be readily determined polarographically. Because of the low half-wave potential, other hydrocarbons should not interfere with the determination.

EXPERIMENTAL

Apparatus. The polarograph was constructed in this laboratory. For this polarograph the voltage increment is 3.75 mv. per second. The polarograms were recorded on a Leeds & Northrup Speedomax recorder which had a sensitivity of 2.5 mv.

The capillary was constructed of marine-barometer tubing obtained from the Corning Glass Works. At an applied voltage of −1.5 volts the drop time $t = 3.6$ seconds, the rate of flow of mercury $m = 1.42$ mg. per second, and $m^{2/3}t^{1/6} = 1.56$.

The electrolysis cells were placed in a thermostat at a temperature of 25.0° C. Because of the volatility of the cyclo-octatetraene, quantitative measurements required the use of sodium sulfite to remove dissolved oxygen. A stream of nitrogen was used only very briefly for stirring when quantitative measurements were made.

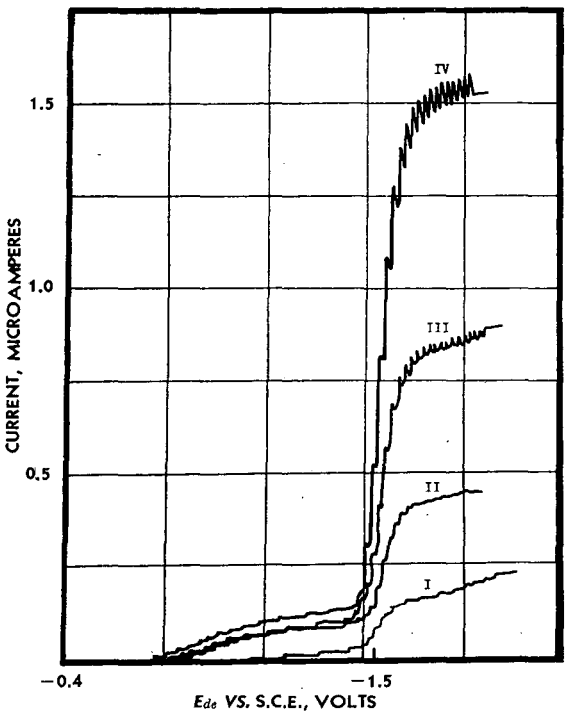

Figure 1. Polarograms of Cyclo-octatetraene in 50% Ethanol and 0.05 *M* Tetramethylammonium Hydroxide Containing Sodium Sulfite

I. 2.77×10^{-5} *M* III. 1.66×10^{-4} *M*
II. 7.4×10^{-5} *M* IV. 3.41×10^{-4} *M*

Cyclo-octatetraene. A sample of cyclo-octatetraene prepared in this laboratory was purified by fractional distillation; boiling point 141.5° C. (corrected), $n_D^{25} = 1.5342$. Prosser and Rossini (*3*) report $n_D^{25} = 1.5350$. Standard solutions were prepared by dissolving the material in absolute ethanol.

Media. Buffers were prepared according to Clark and Lubs. Tetramethylammonium bromide and iodide were Eastman C.P. chemicals. Tetramethylammonium hydroxide was prepared according to Perrachio and Meloche (*2*).

QUANTITATIVE DETERMINATIONS

For this purpose a cell containing 50 ml. of ethanol, 50 ml. of 0.1 *M* tetramethylammonium hydroxide, 5 ml. of saturated sodium sulfite, and 5 ml. of a known or unknown alcoholic solution of cyclo-octatetraene was prepared. Polarograms run on the resulting solution showed well defined waves with a half-wave potential of −1.51 volts *vs.* the saturated calomel electrode. In Figure 1 are illustrated typical polarograms. In Figure 2 are presented the results of plotting the diffusion current as a function of concentration. The linearity is excellent over the entire range of concentration from 4.5×10^{-6} *M* to 2.07×10^{-3} *M*, which were the practical limits of the polarograph. It is noteworthy that no maxima were observed in any of the polarograms. Except for a very slight change in half-wave potential, the sodium sulfite has no effect on the characteristics of the polarograms.

The number of electrons involved in the reduction appears to be two. Thus, for curves III and IV in Figure 1, the differences in values of E_{de} at $i = (3/4)i_d$ and $i = (1/4)i_d$ are both 0.034 mv., which, according to Tomes (*4*), would indicate a value for n of 2, providing that the reaction at the electrode is reversible. In Figure 3 is illustrated a plot of $\log i/(i_d - i)$ *vs.* E_{de}. The reciprocal of the slope of the resulting straight line is 0.0315, indicating that $n = 2$.

On the other hand, the limiting diffusion current for cyclo-octatetraene in tetramethylammonium hydroxide is about 50% greater than for an equal concentration of benzaldehyde. However, because the mechanism of reduction of benzaldehyde in such a medium is somewhat indefinite according to Kolthoff and Lingane (*1*), the lack of agreement may not be serious. In tetramethylammonium bromide solution containing 50% ethanol, a diffusion current of 0.80 microampere was recorded for a benzaldehyde concentration of 1.96×10^{-4} *M*, which compares favorably with a current of 0.77 microampere for cyclo-octatetraene according to Figure 2. This suggests that $n = 2$ for the reduction of cyclo-octatetraene. The lack of diffusion constant data in such a medium prevented the direct test of the Ilkovič equation.

Comparison of the wave heights of benzohydroquinone and cyclo-octatetraene indicates that they are about equal and hence $n = 2$ for the reduction of the cyclo-octatetraene in this medium, as two electrons are involved in the oxidation of the hydroquinone.

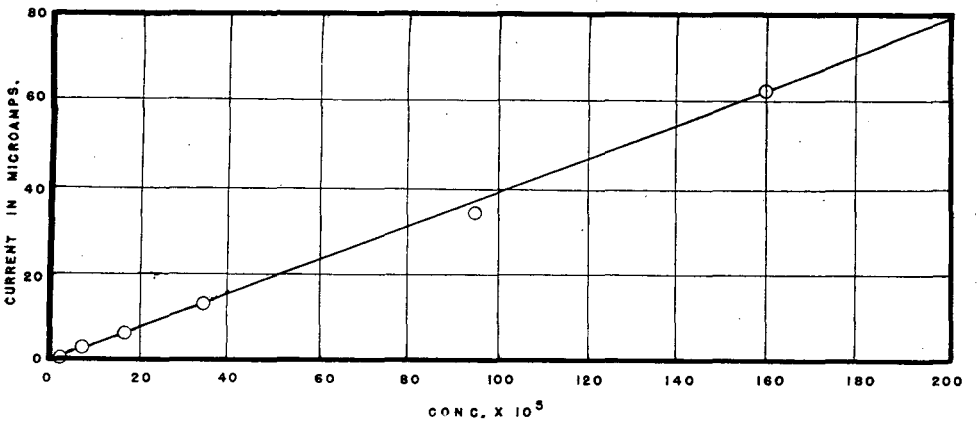

Figure 2. Limiting Current as a Function of Concentration in 0.047 *M* Tetramethylammonium Hydroxide plus 50% Ethanol

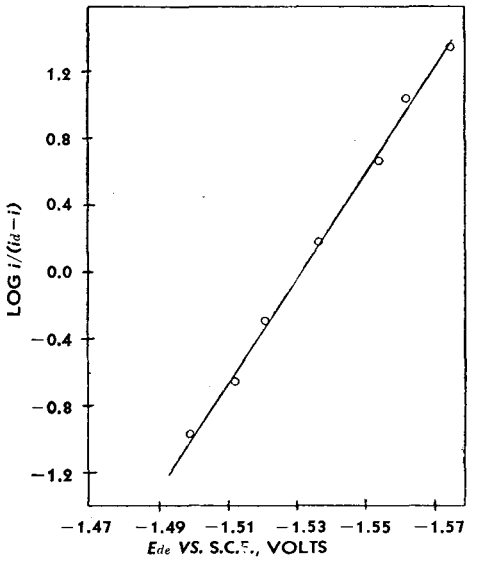

Figure 3. Plot of Log $i/(i_d - i)$ *vs.* E_{de} for Curve III of Figure 1

However, because of the interference of the medium with the hydroquinone wave exact data cannot be presented.

POLAROGRAMS IN MEDIA NOT CONTAINING TETRAMETHYLAMMONIUM IONS

In buffered and unbuffered solutions not containing tetramethylammonium ions, the polarograms are anything but well defined, and they are unsuitable for quantitative measurements. In Figure 4 are illustrated typical polarograms run in ammonium nitrate and sodium hydroxide solutions. Under these conditions, no definite wave is formed, although reduction does take place. In certain media such as a phosphate buffer at pH 10.2 or ammonia–ammonium chloride buffer, indistinct waves with something approaching a limiting current are obtained. The apparent half-wave potentials were −1.58 and −1.56 volts *vs.* the saturated calomel electrode for the phosphate and ammonia buffers, respectively. The addition of tetramethylammonium bromide to these cells results in the formation of well defined waves with $E^1/_2$ ≈ −1.50 volts *vs.* saturated calomel electrode, as illustrated in Figure 4. Similar results were obtained in other media such as ammonium chloride and phosphate buffers at pH 7.2. Addition of potassium bromide to the cell that contained ammonium chloride resulted in no change in the polarogram. These results indicated clearly that satisfactory waves were obtained only in the presence of tetramethylammonium ions. That the half-wave potentials were essentially independent of the anion was shown by the fact that in cells containing 5 ml. of ethanol, 1 ml. of 0.01 *M* cyclo-octatetraene, and, in turn, 5 ml. of 0.1 *M* tetramethylammonium hydroxide, bromide, and iodide, the half-wave potentials were, respectively, −1.50, −1.51, and −1.52 volts.

In Table I are illustrated the half-wave potentials of solutions containing tetramethylammonium bromide together with a number of buffers. As the results show, the half-wave potentials are completely independent of pH in the pH range of 7.25 to that of 0.1 *N* sodium hydroxide.

DISCUSSION

The fact that the half-wave potential is independent of pH indicates that no hydrogen is involved in the reduction. This suggests the formation of negatively charged ions as the primary electrode process. The fact that tetramethylammonium ions

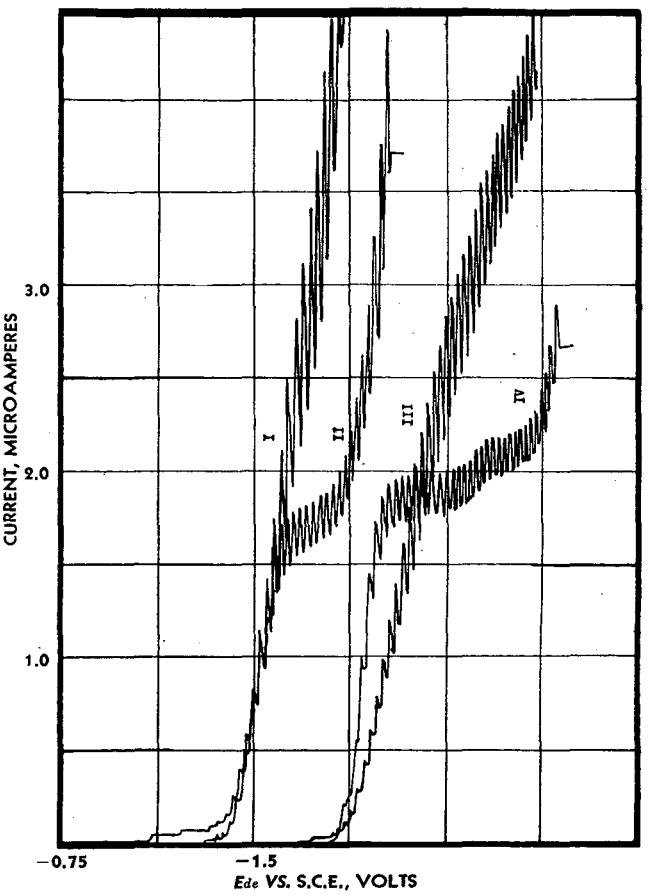

Figure 4. Polarograms of 0.001 *M* Cyclo-octatetraene in 50% Ethanol

I. 0.05 *M* NH_4NO_3
II. Same as I + 50% by volume 0.1 *M* $(CH_3)_4NBr$
III. 0.05 *M* NaOH
IV. Same as III + 50% by volume 0.1 *M* $(CH_3)_4NBr$

Table I. Half-Wave Potentials of Cyclo-octatetraene in Presence of Tetramethylammonium Ions

Temp. 25.0 ° C.

25 ml. of 0.1 *M* $(CH_3)_4NBr$
25 ml. of EtOH
25 ml. of buffer
1 ml. of 0.01 *M* cyclo-octatetraene

Buffer pH	$E^1/_2$, Volts
4.0	Hydrogen evolved
7.25	−1.491
9.37	−1.492
0.1 *N* NaOH	−1.495

are necessary for well defined waves would suggest that this ion was capable of stabilizing the anions formed in the reduction according to Equation 1:

$$[\text{Cyclo-octatetraene}] + 2e + 2(CH_3)_4N^+ \longrightarrow [(CH_3)_4N^+]_2\,[\text{Cyclo-octatetraene}]^{--} \quad (1)$$

In agreement with the reactions of the compound and its infrared and Raman spectra, the polarographic data show that the molecule is definitely not aromatic. This follows from the fact that reduction takes place at a much lower voltage than for any aromatic hydrocarbon such as benzene or naphthalene. Furthermore, no simple olefinic or acetylenic bond is known which can be reduced at such a low voltage. However, the low voltage necessary for reduction does parallel the yellow color of cyclo-octatetraene, for most colored compounds are reducible at the dropping mercury electrode.

The polarographic evidence suggests an unusually highly polarized or polarizable double bond or conjugated double bond system suggestive of Equation 2:

$$-HC{=}(CH{-}CH{=})_n{-}CH- \rightleftharpoons -\overset{+}{H}C{-}(CH{=}CH{-})_n\,\overset{-}{C}H-$$
$$+\,2e \longrightarrow -\overset{-}{H}C{-}(CH{=}CH{-})_n\,\overset{-}{C}H-$$
$$n = 0, 1, 2, \text{ or } 3 \quad (2)$$

Interpretation of this fact may require formulation of a structure to explain formation of the stable anion.

ACKNOWLEDGMENT

The author wishes to express his thanks to J. F. Shekleton of this laboratory for providing the cyclo-octatetraene, and to L. J. Lohr who fractionally distilled it. Thanks are also expressed to L. T. Hallett under whose direction this work was performed, and to R. H. Müller of New York University, a consultant of the company, who directed the construction of the polarograph.

LITERATURE CITED

(1) Kolthoff and Lingane, "Polarography," New York, Interscience Publishers, 1941.
(2) Perrachio and Meloche, *J. Am. Chem. Soc.*, 60, 1770 (1938)
(3) Prosser and Rossini, *Ibid.*, 69, 2068 (1947).
(4) Tomes, *Collection Czechoslav. Chem. Commun.*, 9, 12, 81, 150 (1934).

RECEIVED September 25, 1948.

Spectrographic Determination of Trace Elements in Silica

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A spectrographic method for the determination of trace elements in silica involves the use of silver nitrate as a buffer and internal standard. Quantitative working curves and qualitative limits of detection have been established for a number of elements. The method is also applicable to analysis of glass sands.

IN THE course of a spectrographic study of certain ceramic materials it was found desirable to investigate the applicability of spectrographic methods for the determination of trace elements in silica. Satisfactory quantitative working curves for 10 elements were established with the use of silver nitrate as a combination buffer and internal standard. Qualitative limits of detection were also determined for 45 elements. The method has also been applied to the analysis of iron in a series of glass sands.

A search of the literature indicates little information on the determination of trace elements in silica. Smith (*7*) mentions the use of a constant current arc for silica, and Schlegel (*5*) describes a solution method involving treatment of the sample with sulfuric and hydrofluoric acids with subsequent sparking on carbon electrodes. Several methods have been described for the determination of iron in glass sands (*1, 2, 6*).

The choice of silver nitrate as a combination buffer and internal standard was based upon experiments that gave satisfactory quantitative results. Although silver nitrate does not produce sensitivities comparable to those obtained by the use of buffers such as graphite, ammonium nitrate, or ammonium chloride, readily reproducible quantitative results are obtained with it which cannot be established with the other buffers. Moreover, silver nitrate as an internal standard offers a sufficient number of satisfactory spectral lines in the ultraviolet region. Photographic-quality silver nitrate was found to be of sufficient purity for this use.

The spectrographic method is preferable to existing wet or colorimetric methods of analysis because it offers a means of comprehensive analysis of silica in a short time with a sample as small as 100 mg. The total time of analysis including sample preparation, excitation, film processing, and interpretation of the photographic record is approximately 60 minutes. Where the film is not desired as a permanent record, processing conditions may be changed to decrease the time of analysis to 40 minutes. Where a number of samples are involved the time per sample is still less.

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Table I. Analysis of Glass Sands for Fe_2O_3 Content

Sample No.	Percentage of Fe_2O_3	
	Chemical	Spectrographic
21	0.050	0.051
29	0.076	0.077
30	0.095	0.089
31	0.045	0.045
32	0.035	0.034
33	0.131	0.136
34	0.033	0.034
41	0.068	0.066
46	0.063	0.058
47	0.134	0.122
81[a]	0.073	0.073

[a] Bureau of Standards glass sand.

APPARATUS

The 150-cm. original grating spectrograph, Multisource unit, and comparator-densitometer used were A.R.L.-Dietert. The spectrograph covered the range of 2300 to 4300 Å. with a uniform dispersion of 7 Å. per mm. in the first order. A revolving sector wheel was employed to give an exposure of varying transmittance levels for all exposures made. The Multisource was adjusted to direct current arc excitation and the exposures were made under the following conditions:

Charge *vs.* discharge	0°
Discharge point control	90°
Initiator switch	Strike
Initiator knob	High power
Voltage regulator	Off
Capacitance	60 microfarads
Resistance	20 ohms
Inductance	375 microhenries
Gap distance	2 mm.
Slit setting	3
Exposure time	10 seconds

PHOTOGRAPHY

Eastman spectrum analysis film No. 1 was used to photograph the ultraviolet spectrum. The film was developed for 3 minutes in PD-1 developer at 68° F. (*3*), fixed in sodium thiosulfate solution for 5 minutes, washed for 10 minutes in circulating water, and dried for 10 minutes in conditioned-air dryers. Standard spectrographic calibration techniques (*4*) were used to calibrate the film.

EXPERIMENTAL

Eimer and Amend C.P. silicic acid was used as the source of pure silica. The silicic acid was heated in a furnace at 900° C. for 1 hour and stored in a desiccator. Spectrographic analysis of the silica indicated the following elements present in trace amounts: iron, copper, titanium, and zirconium. Merck photographic grade silver nitrate was used as the source of the buffer–internal standard. Stock solutions of water-soluble salts were prepared for all contaminating elements.

The standards and samples were prepared by making a mixture of silica and silver nitrate in a weight ratio of 1 to 1 and grinding it thoroughly in a mullite mortar with the Fisher automatic grinder. To a weighed portion of this mixture the desired quantity of contaminating solution was added. After drying under infrared lamps the mixture was once more thoroughly ground. Uniformity of the sample was established by making a large number of determinations on a single sample; individual determinations did not vary from the mean outside of the range of error normally expected. Quartz dishes were used throughout the preparation and all precautions were taken to reduce extraneous contamination to a minimum.

In the direct current arc excitation of the silica–silver nitrate samples, 0.25-inch National Carbon Company spectroscopic carbon electrodes were used. They were preburned for 15 seconds in the arc before the addition of the sample in order to burn out residual contaminants. These electrodes proved satisfactory for both quantitative and qualitative analysis. Approximately 150 mg. of prepared sample were packed into the cup of the electrode.

Excessive background was produced in certain regions of the spectrum when silica–silver nitrate samples were burned in carbon electrodes. For the purpose of establishing quantitative working curves for some elements it was necessary to reduce this background by limiting the amount of exposed grating surface. By closing the grating gates to limit the surface to 50% of its total, working curves were obtained for elements whose lines would otherwise be obscured. This was accompanied by a reduction in sensitivity for the elements concerned (lead, chromium, nickel, and iron).

DISCUSSION OF RESULTS

With the procedure described, straight-line quantitative working curves were established for molybdenum, cobalt, iron, manganese, chromium, lead, indium, bismuth, nickel and tin, and similar curves could presumably be set up for other elements. These curves were within an approximate range of from 0.0002 to 0.02%, and the range can be extended upward by decreasing the ratio of silica to silver nitrate, as was done in the case of iron. Where a residual amount of a contaminant was present in the silica, the working curve was corrected so that a straight-line function could be obtained. This was necessary in the case of iron. In Figure 1 are shown several typical curves. The over-all relative mean deviation for the points obtained on the ten working curves was 4.2%.

In order to test further the applicability of the method, eleven glass sands were analyzed for their iron content. A comparison of the results obtained chemically and spectrographically will be found in Table I. The over-all relative mean deviation for these samples was approximately 11%. The glass sands and chemical analyses were kindly furnished by the Bureau of Mineral Research of Rutgers University.

Table II. Limits of Detection of Elements in Silica

Element	%	Element	%	Element	%
Al	0.01	Ge	0.01	Re	0.005
As	0.05	Hg	0.0025	Rh	0.0025
Au	0.005	In	0.005	Ru	0.01
Ba	0.5	Ir	0.0025	Sb	0.02
Be	0.005	K	0.2	Sn	0.0005
Bi	0.0001	Li	0.01	Sr	0.05
Ca	0.05	Mg	0.01	Ta	0.02
Cb	0.01	Mn	0.0005	Th	0.2
Cd	0.005	Mo	0.0001	Ti	0.005
Ce	0.2	Na	0.05	Tl	0.005
Co	0.0002	Ni	0.0001	V	0.0001
Cr	0.0001	Pb	0.0005	W	0.01
Cu	0.0005	Pd	0.0025	Y	0.01
Fe	0.0005	Pr	0.1	Zn	0.01
Ga	0.01	Pt	0.005	Zr	0.0005

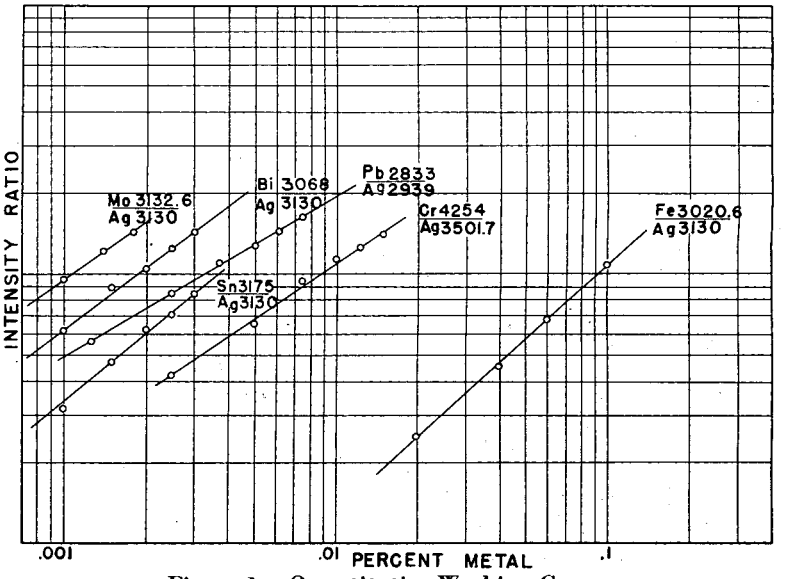

Figure 1. Quantitative Working Curves

Listed in Table II are the percentage limits of detection of the most common metallic elements. The sensitivities were determined under conditions similar to those under which the quantitative curves were obtained; the criterion for the limit of detection was the disappearance of the persistent lines as the concentrations were gradually decreased. In many cases the limits of detection could be increased by the use of such buffers as graphite or ammonium salts; however, this would be at some expense of uniformity of results. The limits could also be considerably improved for certain elements by using lines outside the working range of the spectrum analysis No. 1 film.

The method of analysis as described is simple, rapid, and reproducible. It is readily adaptable to routine analysis of silica samples, and with proper instruction all operations could be performed by nontechnical laboratory personnel.

LITERATURE CITED

(1) Ahrens, L. H., *J. S. African Chem. Inst.*, 27, 28 (1944).
(2) Gilard, P., Dubrul, L., and Jamar, F., *J. Soc. Glass Technol.*, 20, 326 (1936).
(3) Lester, H. M., "Photo-Lab-Index," Vol. I, p. 5, New York, Morgan and Lester, 1941.
(4) Pierce, W. C., and Nachtrieb, N. H., IND. ENG. CHEM., ANAL. ED., 13, 774 (1941).
(5) Schlegel, H., *Angew. Chem.*, 49, 411 (1936).
(6) Slavin, M., *Glass Ind.*, 22, 341 (1941).
(7) Smith, D. M., *Analyst*, 71, 368 (1946).

RECEIVED October 28, 1948.

Rapid Estimation of Ethylenes

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This paper describes a rapid procedure for the determination of styrene and certain homologs with an accuracy of ±0.5%. The procedure is based on the reaction of an excess of mercuric acetate in methanol solution with an ethylenic double bond. During the reaction one mole of acetic acid is liberated for each ethylenic bond. The acid liberated is, therefore, a measure of the unsaturation, and can be titrated directly with standard sodium hydroxide after the addition of an excess of sodium chloride. The applicability of the procedure to fifteen ethylenic compounds has been indicated.

A RAPID and simple procedure for the estimation of ethylenic compounds has been developed in this laboratory and used successfully for over 3 years. A reaction similar to the one described in this paper was recently reported by Marquardt and Luce (*2*) for the determination of styrene and styrene derivatives. Earlier Tausz used a similar procedure in the analysis of petroleum oils (*4*) and turpentine (*3*). Connor and Wright have also used such a reaction for the determination of geometric isomers (*1*).

In the procedure described by Marquardt and Luce, the styrene or styrene derivative is treated with an excess of aqueous mercuric acetate to form a hydroxy mercuric acetate addition product. The excess mercuric acetate in the reaction mixture is treated with sodium hydroxide to form mercuric oxide, which is reduced to metallic mercury by boiling with hydrogen peroxide. The mercury in the addition product is then titrated in an acid medium with standard ammonium thiocyanate as a measure of the unsaturation.

In the procedure described below styrene and its derivatives as well as fifteen other monomers used in the plastics or synthetic rubber industries reacted with an excess of mercuric acetate in methanol. Under such treatment a number of the ethylenic compounds added the elements of methoxy mercuric acetate to the double bond and liberated a mole of acetic acid for each mole of mercuric acetate reacting:

$$R{-}CH{=}CH{-}R' + (CH_3COO)_2Hg + CH_3OH \longrightarrow R{-}\underset{\displaystyle OCH_3}{\underset{|}{CH}}{-}\underset{\displaystyle HgOOCCH_3}{\underset{|}{CH}}{-}R' + CH_3COOH$$

The reaction in methanol is generally more rapid than the corresponding reaction in an aqueous medium. The addition of an excess of sodium chloride to the reaction mixture converts the excess mercuric acetate to mercuric chloride and permits one to make a direct titration with standard alkali of the acetic acid liberated in the reaction mixture. Because one mole of acetic acid is formed for each equivalent of ethylenic group reacting, the amount of sodium hydroxide consumed in the titration is a direct measure of the unsaturation.

Only part of the ethylenic compounds investigated reacted as outlined in the equation above. Tausz (*5*) loosely classified the reactions of mercuric acetate with ethylenes into four groups: (I) those that add the elements of methoxy mercuric acetate to the ethylenic group, (II) those that are oxidized by the reagent, (III) those that react by substitution (usually only at elevated temperatures), and (IV) those that form no mercuric addition compound and are not oxidized by the reagent. This paper describes a procedure for the estimation of those compounds in Group I where the reaction proceeds at a reasonable rate and is essentially complete under the experimental conditions chosen.

REAGENTS

Mercuric Acetate, Merck's C.P. reagent grade. Must be low in free acetic acid content.

Synthetic Methanol. The reagent should be substantially free of acids or aldehydes.

Sodium Chloride. Commercial grades of sodium chloride are satisfactory. The aqueous salt solution should be saturated with sodium chloride, then filtered and made neutral to phenolphthalein.

Sodium Hydroxide, 0.1 *N* solution. It was carbonate-free and standardized against Baker's C.P. reagent grade benzoic acid.

Vinyl Monomers. The unsaturated compounds were obtained from various sources. Where their purity was not indicated by the supplier, they were carefully redistilled and only a very narrow middle cut was used. In most cases the purified materials boiled over a 1° C. range, and never over more than a 3° C. range.

Sodium Nitrate, C.P. reagent grade, is used as a saturated solution in methanol.

ANALYSIS

Accurately weigh approximately 4 milliequivalents of the ethylenic compound in a weighing bottle and transfer the weighing

Table I. Analysis of Styrene and Styrene Derivatives

Compound	% Purity	Determined by	% Found, This Method
Dow styrene	99.8	Supplier	99.85, 99.75
Monsanto styrene	99.8	Melting point	99.80, 99.66
Styrene solution	2.02	Dilution of 99.8% styrene	2.06
Styrene solution	1.45	Dilution of 99.8% styrene	1.50
Divinyl- and ethylvinylbenzene	142.2	Method of Marquardt and Luce	142.3, 142.9[a]

[a] Calculated as ethylvinylbenzene.

Table II. Factors Affecting Reaction

Compound	Ethylene: $Hg(OAc)_2$	Temp., ° C.	Time, Min.	Catalyst	% Reaction
Styrene	1:1	25	10	O	95.4, 95.6
	1:1	45	10	O	97.0
	1:1.25	25	10	O	99.8
	1:1.50	25	10	O	99.9, 100.6
	1:1	25	120	O	99.1, 99.8
	1:1	25	240	O	100.1
	1:1	25	15	$NaNO_3$	99.7, 100.5
Methyl methacrylate	1:2	25	10	O	1.3
	1:2	25	420	O	6.8
	1:2	25	10	$NaNO_3$	4.0
Allyl alcohol	1:1	25	10	O	98.1
	1:1.5	25	15	O	98.7
2-Vinylpyridine	1:1	25	30	O	19.8
	1:2	25	30	O	36.1
	1:3	25	30	O	47.4
	1:2	25	30	$NaNO_3$	51.8
	1:2	25	960	$NaNO_3$	97.0
	1:3	25	1200	$NaNO_3$	97.3

bottle and contents to a 500-ml. Erlenmeyer flask containing 20 to 25 ml. of carbon tetrachloride. Empty the contents of the weighing bottle into the solvent. Add 4.00 grams of mercuric acetate and 30 ml. of methanol. If the ethylenic compound reacts slowly with the reagents, the use of 30 ml. of a saturated solution of sodium nitrate in methanol in place of the pure methanol will increase the rate of reaction. Stopper the flask, swirl the contents, and warm slightly, if necessary, to dissolve the mercuric acetate. Allow the reaction to proceed 10 to 15 minutes and then add 75 ml. of neutral saturated sodium chloride solution and 50 to 100 ml. of water. Add 20 drops of phenolphthalein solution and titrate to the first pink end point with standard 0.1 *N* sodium hydroxide. Shake the reaction mixture vigorously during the titration, so as to ensure complete removal of the acetic acid from the carbon tetrachloride layer.

A blank should be run immediately after mixing the reagents, omitting only the unsaturated compound. If the blank is allowed to stand too long, its titer has a tendency to increase slowly. Each milliequivalent of sodium hydroxide consumed in the titration, after subtraction of the blank, represents one milliequivalent of ethylenic group. The analyses reported in Tables I and III were obtained using the above procedure; the analyses reported in Table II were obtained by modifying the procedure as indicated.

FACTORS INFLUENCING REACTION

When the elements of methoxy mercuric acetate add to an ethylenic double bond, a number of factors influence the rate and extent of the reaction. The effect of some of these factors can be seen from an inspection of the data in Table II where variations in the regular procedure were employed. Increasing the temperature and adding sodium nitrate (*6*) accelerate the reaction. An excess of mercuric acetate not only increases the rate of reaction but also serves to force the reaction to completion, the excess necessary depending on the particular ethylenic compound. In practice 1 gram of mercuric acetate was used for approximately 1 milliequivalent of ethylenic compound. If this amount of mercuric acetate was not sufficient to give complete reaction in 15 minutes, the method was considered impractical so far as that particular compound is concerned. As indicated in Table II, longer reaction periods give more complete reaction, but may result in some inaccuracies due to an increase in the blank.

The structure of the compound is the ultimate factor in determining the extent, rate, and type of reaction occurring when an ethylenic compound is treated with mercuric acetate in methanol. Although this paper deals primarily with the analysis of styrene and styrene derivatives, Table III shows the results of one or two determinations on fifteen other ethylenic compounds. These exploratory results indicate that the procedure will give satisfactory results with allyl and crotyl alcohol, certain allyl ethers and esters, certain vinyl ethers, and vinylcarbazole. Unsatisfactory results were obtained with acrylate, methacrylate, itaconate, and maleate esters as well as with acrylonitrile and vinylpyridine. Vinyl acetate and vinyl benzoate gave results approximately twice that expected.

Of major importance in the use of this procedure is the quality of the mercuric acetate. This reagent should not only be low in free acid but the entire supply should be thoroughly blended, so that each portion used for an analysis has the same acid content. If this is not the case, checks will be poor. If the mercuric acetate contains appreciable amounts of acid, it is often advisable to place it in a vacuum desiccator for 2 or 3 hours and then blend carefully before use. The bottle containing this reagent should be tightly stoppered except when in use.

Some difficulty was experienced in detecting the end point. However, after a few titrations and the addition of somewhat more than the usual amount of phenolphthalein the end point gave little trouble.

The procedure described has been in use for over 3 years in this laboratory for the rapid routine estimation of styrene and divinyl and ethylvinylbenzene samples. It has been entirely satisfactory and in general gave results checking within a few tenths of 1%. Typical data on styrene and ethylvinyl and divinylbenzene appear in Table I.

Table III. Analysis of Unsaturated Compounds

Compound	% Purity	Purification	% Found, This Method
Methyl methacrylate	99[a]	Redistillation	1.3
Diallyl phthalate	...	Redistillation	94.5
Diethyl itaconate	...	Redistillation	Trace
Methyl acrylate	98[a]	....	43.5
Vinyl acetate	99[a]	....	201
Vinyl benzoate	...	3° C. boiling range	187
Diethyl maleate	...	1° C. boiling range	4.0
Acrylonitrile	...	1° C. boiling range	3.4
Allyl alcohol	...	1° C. boiling range	99.48, 98.71
Crotyl alcohol	...	1° C. boiling range	101.5
β-Chloroallyl alcohol	...	2° C. boiling range	15
N-Vinylcarbazole	...	1° C. melting point range	99.4, 99.7
2-Vinylpyridine	92.8[b]	....	39.8
Diallyl ether	...	1° C. boiling range	99.9
Vivinyl ether	96.5[a]	Anesthetic grade	96.8

[a] As given by commercial supplier.
[b] Determined by acid titration using $Fe(OH)_3$ indicator.

CONCLUSION

A known reaction has been applied to the estimation of styrene and styrene derivatives. The procedure is rapid and simple, requires no special equipment or reagents, and in general gives results within ±0.5% of theory or better. Substitution, which often occurs when halogens are added to ethylenes, is avoided. An indication of the range of applicability of the procedure to a number of vinyl monomers, commonly used in the plastics and synthetic rubber industries, is indicated.

LITERATURE CITED

(1) Connor, T., and Wright, G. F., *J. Am. Chem. Soc.*, **68**, 256–8 (1946).
(2) Marquardt, R. P., and Luce, E. N., ANAL. CHEM., **20**, 751–3 (1948).
(3) Tausz, J., *Chem. Ztg.*, **42**, 349–51 (1918).
(4) Tausz, J., *Petroleum*, **13**, 649–54 (1918).
(5) Tausz, J., *Z. angew. Chem.*, **32**, I, 233 (1919).
(6) Wright, G. F., *J. Am. Chem. Soc.*, **57**, 1994 (1935).

RECEIVED November 19, 1948.

Infrared Analysis of Low Temperature Polymers

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Because of the considerable interest in methods of polymer analysis by which the amount of each type of unsaturation can be determined, this paper gives a description of the method used in obtaining the data on 1,2 and trans-1,4 addition and an extension of the method that permits determination of cis-1,4 addition and combined styrene, and gives more accurate results for 1,2 and trans-1,4 addition.

MOST of the rubberlike polymers (both natural and synthetic) are diene addition polymers or copolymers. In diene polymerization, addition may take place in several ways. With butadiene, for example, cis-1,4, trans-1,4, and 1,2 addition are all possible (Figure 1). With substituted butadienes, such as isoprene, there is the additional possibility of 3,4 addition. (Resonance considerations, *5*, indicate that addition will normally be "head to tail.") Natural rubber is polyisoprene, that is all, or nearly all, in the cis-1,4 configuration. Balata is *trans*-1,4-polyisoprene. Synthetic polyisoprene, on the other hand, is a random mixture of at least three of the possible configurations and does not have the desirable physical properties of either rubber or balata. Ordinary GR-S contains a random mixture of styrene units and the three possible types of butadiene units. Recently Hart and Meyer have shown (*6*) that, when butadiene polymers are prepared at low temperatures, the addition of the butadiene units is preponderantly trans-1,4. Low temperature butadiene polymers have also been shown to have significantly improved (*15*, *19*) physical properties.

Accordingly, there has been considerable interest in methods of polymer analysis by which the amount of each type of unsaturation can be determined. The present paper describes the method used to obtain the data for 1,2 and trans-1,4 addition, as previously reported (*6*), and an extension of that method which permits the determination of cis-1,4 addition and combined styrene and gives more accurate results for 1,2 and trans-1,4 addition.

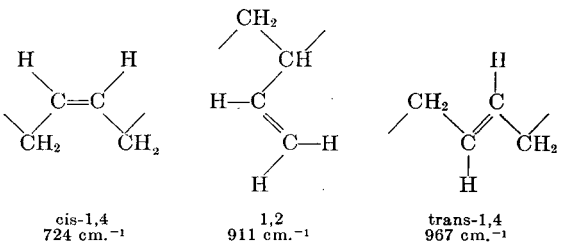


Figure 1. Polybutadiene Structures

Ozonization (*1*, *13*) and perbenzoic acid (*7*, *8*) methods have been applied to the chemical determination of 1,2 addition in butadiene polymers. Infrared spectroscopic methods of analysis have also been described by various workers.

Thompson and Torkington (*17*) published spectra showing the presence of a large amount of 1,2 addition in sodium-polymerized butadiene, but did not give quantitative methods. Field, Woodford, and Gehman (*4*) attempted to determine the relative amounts of 1,2 and 1,4 addition in polybutadiene by measuring the relative intensities of absorption at 996 and 967 cm.$^{-1}$, and comparing with results on known mixtures of 1-octene and 2-octene. However, they did not distinguish between cis and trans forms, and only the trans isomer has strong absorption at 967 cm.$^{-1}$ Anderson and Seyfried (*3*) described infrared methods for the determination of unsaturation in complex samples such as hydrocarbon synthesis naphthas. They recognized that absorption at 967 cm.$^{-1}$ is characteristic of trans internal double bonds, whereas the cis form absorbs near 700 cm.$^{-1}$ Extinction coefficients for a number of pure compounds were averaged for use in the analyses. Such methods are extremely useful for mixtures with more than one component having a given type of unsaturation.

As shown by Table I, the characteristic infrared extinction coefficients for olefins of low molecular weight vary somewhat for the individual compounds. It therefore seemed desirable to determine extinction coefficients for the specific polymers being analyzed. Such methods have now been developed, and found satisfactory for the analysis of polybutadiene, and butadiene-styrene copolymers (in which styrene is also determined). In the near future it is planned to extend these methods to substituted butadiene polymers such as polyisoprene.

Table I. Characteristic Absorption for National Bureau of Standards Olefins

Sample	N.B.S. No.	Absorption Maximum, Cm.$^{-1}$	$\epsilon = \frac{\text{Log } I_0/I \times \text{Molecular Weight}}{\text{(G./Liter)} \times \text{(Cm. Cell Depth)}}$
cis-2-Pentene	282-5S	698	45
4-Methyl-*cis*-2-pentene	537-5S[a]	719	126
Average for *cis*-R—CH=CH—R		ca. 700	85
trans-2-Pentene	283-5S	965	141
4-Methyl-*trans*-2-pentene	536-5S[a]	966	138
trans-2-Hexene	527-5S	966	153
trans-3-Hexene	529-5S	966	133
trans-4-Octene	548-5S	968	132
Average for *trans*-R—CH=CH—R		967	139
3-Methyl-1-pentene	531-5S	910	163
3,3-Dimethyl-1-butene	287-5S	910	145
4-Methyl-1-pentene	532-5S	910	143
4,4-Dimethyl-1-pentene	547-5S	912	153
1-Heptene	520-5S	909	153
1-Octene	521-5S	910	155
1-Nonene	551-5S	909	160
1-Decene	552-5S	910	159
1-Undecene	555-5S	909	168
Average for R—CH=CH$_2$		910	155

[a] From a study of the infrared absorption spectra in the 3-, 6-, and 10- to 15-micron regions, it is believed that N.B.S. samples 536 and 537 have the configurations listed in this table rather than the reverse configurations as the samples are at present labeled. Rossini (*14*) is conducting a further investigation to establish the true configuration.

Early attempts to use infrared spectroscopy in studying polymer unsaturation were seriously handicapped by the lack of suitable pure reference compounds. One of the first standards obtained was a commercial material, stated to be "95% pure 2-octene." Later work showed this sample to contain about 50% 1-octene, and of course nothing was known regarding the relative amounts of *cis*- and *trans*-2-octene. However, through the efforts of the National Bureau of Standards and A.P.I. Project 46, several pure olefin samples are now available and their number is being steadily increased.

The infrared spectra of the olefins listed in Table I show characteristic differences, depending upon the type of olefin, in three regions. The C=C stretching frequencies in the 6-micron region are weak and not sufficiently well characterized to be useful in analysis. The C—H stretching frequencies in the 3-micron region are considerably more intense and are characteristic when examined with high resolving power. However, the C—H bending absorption bands in the 10- to 15-micron region are very intense and apparently highly specific, if only hydrocarbons are being analyzed. Much less is known about the C—H bending frequencies in samples containing strongly polar atoms such as oxygen or halogen.

MATERIALS STUDIED

The hydrocarbons of low molecular weight studied in this work were all National Bureau of Standards standard samples and are listed in Table I. Polymers of butadiene and copolymers of butadiene-styrene from a variety of sources have been measured and the results are shown in Tables II to V. All samples were examined in carbon disulfide solution. The polymers were purified by solution in carbon disulfide, filtration through 300-mesh silk to remove gel, precipitation with methanol, and final re-solution in carbon disulfide. In actual analyses the removal of gel in this manner would cause erroneous results, if the gel were of different composition than the sol portion of the polymer, as might well be the case. However, for this experimental work results on the sol portion were satisfactory. In some cases the gel was so finely divided that it could not be removed by filtration and this gel was left suspended in the polymer solution for the analysis. Polymers may also be examined as thin films, molded in optically flat molds, or deposited from solution. In comparison with carbon disulfide solutions, the solid films have spectra with slightly different absorption frequencies. Preliminary data indicate that extinction coefficients may be considerably different.

Table II. Comparison of Results on Polybutadiene Samples

Sample	Polymerization Temp., ° C.	% cis (by Difference)		% 1,2		% trans	
		[a]	[b]	[a]	[b]	[a]	[b]
26-E-57-76	−19	0.8	7.6	19.6	16.4	79.6	76.0
26-E-41-56	−10	3.1	9.2	20.6	17.0	76.3	73.8
J-946-4	+5	7.7	16.0	20.8	16.0	71.5	68.0
26-E-77-96	+5	7.0	14.4	21.3	18.3	71.7	67.3
14-R-3	+50	14.8	21.5	23.2	20.0	62.0	58.5
14-R-6	65	19.6	27.3	23.8	19.1	56.6	53.6
14-R-5	97	23.8	33.8	24.8	18.0	51.4	48.2

Table III. Comparison of Results on Butadiene Styrene Copolymers

Sample	Polymerization Temp., ° C.	% Styrene[a]	% 1,2		% trans	
			[a]	[b]	[a]	[b]
GR-S	50	23.4	16.8	13.7	49.6	46.3
GR-S-10	50	21.8	15.7	12.1	45.4	42.1
J-888-3	5	23.3	16.1	12.5	58.3	54.7
J-889-3	−18	24.3	14.8	11.8	63.5	58.3
J-948-B-2	+5	7.6	17.9	15.2	67.1	63.6
J-947-2	+5	3.8	20.2	16.5	68.7	64.7

[a] Results reported by Hart and Meyer (*6*). % 1,2 and % trans based on octene standards; styrene estimated from refractive index.
[b] Results based on original data obtained for Hart and Meyer, recalculated according to present method, using polymer standards.

Table IV. Complete Analyses of Polybutadiene Samples

Sample	Source[a]	Polymerization Temp., ° C.	% cis	% 1,2	% trans	Total Found
P-7	P	−20	7.5	17.5	77.6	102.6
P-6	P	−10	7.4	17.6	74.3	99.3
7 HRZF-1	A	+50	18.5	19.9	59.2	97.6
XP-132-L 7	A	50	18.6	19.7	59.3	97.6
HM-7	G	70	23.5	19.9	57.3	100.7
IC	G	70	21.2	19.1	53.7	94.0
63PC-1D4	A	87	24.2	20.5	56.2	100.9
63PC-4D4	A	90	22.5	20.5	55.6	98.6
21444[b]	R	..	15.2	65.8	17.9	98.9
184C[b]	I	30	13.9	67.6	19.9	101.4

[a] Sources of samples measured.
A. Government Laboratory, University of Akron.
G. General Laboratories, U. S. Rubber Co., Passaic, N. J.
I. University of Illinois, C. S. Marvel.
P. Produced by Phillips Petroleum Co.
R. Rubber Reserve (imported from Russia).

[b] Sodium-catalyzed; all other samples emulsion-polymerized.

Table V. Complete Analyses of Butadiene-Styrene Copolymers

Sample	Source[a]	Polymerization Temp., ° C.	% Styrene	% cis	% 1,2	% trans	Total Found
K-2524	F	−38	26.6	12.0	11.0	54.0	103.6
J-1351-5-4	N	+5	26.1	9.7	12.0	50.9	98.7
J-1446-4	N	10	25.8	11.8	12.0	50.0	99.6
63PB7D3-4	A	109	24.7	22.6	14.3	39.8	101.4

[a] Sources of samples measured.
A. Government Laboratory, University of Akron.
F. R. L. Bebb, Firestone Tire and Rubber Co.
N. U. S. Rubber Co., Synthetic Rubber Division, Naugatuck, Conn.

INSTRUMENTAL TECHNIQUES

Infrared measurements were made using a Model 12A Perkin-Elmer spectrometer equipped with a blackened Thermistor bolometer, Western Electric amplifier Model KS-10281, and a Speedomax Type A recorder. The recorder chart and Littrow mirror are driven through Selsyns to ensure accuracy of the wave-length calibration on the charts. Radiation is chopped at 15 cycles per second by means of a synchronous rotating sector at the globar. This recording system is stable, sensitive and completely free from zero drift. Although prisms of lithium fluoride, calcium fluoride, and potassium bromide were used for some of the preliminary studies, all analyses reported here were made using a sodium chloride prism, with slit widths of 0.210 mm. at 967 cm.$^{-1}$, 0.215 mm. at 911 cm.$^{-1}$, and 0.360 mm. at 724 cm.$^{-1}$ corresponding, respectively, to equivalent half-intensity widths of 7, 6, and 4.5 cm.$^{-1}$ (Measurements were made on the 909 cm.$^{-1}$ band of 1-heptene for slit widths from 0.100 to 0.600 mm. Extrapolation to zero slit width indicated about 20% higher extinction coefficient than that found using the standard slit width of 0.215 mm.)

In order to reduce short wave-length scattered radiation a plane grating (*18*) of 3610 lines per inch is used in place of the flat mirror which directs the light from the globar to the condensing mirror. Correction was made for residual stray light (about 0.6%), and also for a slight nonlinearity in response of the bolometer-amplifier combination (maximum correction 0.5%). Under these conditions it was found that Beer's law is obeyed accurately at optical densities less than 0.8. Measurements were made by two different methods. In the scanning method a portion of the spectrum including the band to be measured was recorded and measurements were made at the peak relative to a similarly recorded spectrum for the same cell containing pure carbon disulfide. Measurements were made also with the recorder running and the spectrometer drive stopped at the frequency of maximum absorption for the particular band being measured. The cell containing the sample solution was alternately placed in front of the slit, then removed, and the corresponding intensities were read from the chart. These readings were then repeated using the same cell filled with the pure solvent. If the chart showed any change in the cell-out reading, the readings were corrected for this change.

The vapor-tight cells used, made with amalgamated lead

spacers, were filled by means of a glass syringe, and closed by clamping a sheet of tin foil–covered rubber against the rim of the hypodermic needle hub opening of the cell. Six different cells were used, varying in thickness from 0.0052 to 0.0905 cm., so that in most cases measurements could be made at optical densities between 0.4 and 0.7. Cell thicknesses were determined interferometrically (*16*) except for the 0.0905-cm. cell, which was measured microscopically.

For the National Bureau of Standards hydrocarbons, molar extinction coefficients were calculated:

$$\epsilon = \frac{\log I_0/I}{cl}$$

where c is the solution concentration in gram-moles per liter, l is the length in centimeters of absorbing path (cell depth), I is the intensity of radiation transmitted by the solution, and I_0 is the intensity of radiation transmitted by the pure solvent.

For the polymer samples, the specific extinction coefficient, E, is calculated like the molar extinction coefficient, except that the concentration is expressed in grams per liter.

EXPERIMENTAL

The data reported by Hart and Meyer (*6*) were obtained by a method similar to that since described by Anderson and Seyfried (*3*), using 1-octene as a standard for the determination of 1,2 addition, and *trans*-4-octene as a standard for the determination of trans-1,4 addition. Measurements were made by scanning, as described above, except that no grating was used, and the amplifier response was not corrected for lack of linearity. Stray light, as measured with a lithium fluoride shutter, was subtracted from all readings. An empirical calibration curve was used, thus avoiding dependence on Beer's law and automatically compensating for amplifier and stray light errors. It was realized that slight errors were probably inherent in the assumption that extinction coefficients for polybutadiene were identical with those found for 1-octene and *trans*-4-octene. For the determination of cis-1,4 addition, low molecular weight standards are much less satisfactory.

The method finally developed uses polybutadiene standards for the determination of unsaturation, and has been extended to cover the determination of styrene in copolymers, and cis-1,4 addition of butadiene, as well as giving more accurate values than the original method for 1,2 and *trans*-1,4 addition.

Polybutadiene exhibits infrared absorption bands at 724, 911, and 967 cm.$^{-1}$, the relative intensities of which vary, depending on the method of preparation of the polymer. The band at 967 cm.$^{-1}$ is attributed to *trans*-1,4-polybutadiene as suggested by Pitzer (*12*) and since confirmed by Anderson and Seyfried (*3*), and by study of the spectra of the olefins listed in Table I, and numerous A.P.I. spectra (*2*). The band at 911 cm.$^{-1}$ has been similarly attributed to 1,2-polybutadiene. For emulsion polybutadiene prepared at high temperatures, the sum of 1,2- plus *trans*-1,4 polymer (estimated from the 911 and 967 cm.$^{-1}$ bands) is considerably lower than for other polybutadiene samples; by difference, the amount of cis-1,4 polymer should be relatively high. This material shows an absorption band at 724 cm.$^{-1}$, which is very weak in the other samples, and is considered characteristic of *cis*-1,4-polybutadiene.

From measurement of the absorption at the three positions (724, 911, and 967 cm.$^{-1}$) it is possible to calculate the isomeric composition of a sample of polybutadiene, by solution of three simultaneous equations

$$E_{724} = \underline{CE^c_{724}} + VE^v_{724} + TE^t_{724} \quad (1)$$

$$E_{911} = CE^c_{911} + \underline{VE^v_{911}} + TE^t_{911} \quad (2)$$

$$E_{967} = CE^c_{967} + VE^v_{967} + \underline{TE^t_{967}} \quad (3)$$

where C, V, and T represent the respective weight fractions of cis-, vinyl (1,2), and trans polymer in the sample; E_{724}, E_{911}, and E_{967} represent specific extinction coefficients found for the sample at the three frequencies; and E^c_{724}, E^v_{911} . . . etc., represent specific extinction coefficients for the three pure isomers of polybutadiene at the same three frequencies.

The underscored coefficients $\underline{E^c_{724}}$, $\underline{E^v_{911}}$, and $\underline{E^t_{967}}$ are the important characteristic coefficients for *cis*-, vinyl-, and *trans*-polybutadiene and are determined as described below. The other "correction" coefficients are so small that considerable errors can be tolerated without seriously affecting the final result. All except E^v_{724} were estimated by using average values from low molecular weight olefins. The 1-alkenes all have a moderately strong band near 724 cm.$^{-1}$, whose exact position varies from different samples; so E^v_{724} cannot be satisfactorily estimated from low molecular weight compounds. With 1,2-polybutadiene, this interfering band is found at 680 cm.$^{-1}$ Measurements were made on a sample of sodium-polymerized polybutadiene, assuming that the 680 cm.$^{-1}$ band is symmetrical, so that $E^v_{636} = E^v_{724}$, and that only 1,2-polybutadiene absorbs at 636 cm.$^{-1}$ Measurements at 636 cm.$^{-1}$ were made using a potassium bromide prism and slits of the same equivalent half-intensity width as for the sodium chloride prism measurements at 724 cm.$^{-1}$

Preliminary values of the major extinction coefficients, $\underline{E^c_{724}}$, $\underline{E^v_{911}}$, and $\underline{E^t_{967}}$, were obtained by dividing the corresponding average molar extinction coefficients for low molecular weight olefins by the molecular weight of butadiene. Using these preliminary values, the three equations given were solved to determine preliminary values for per cent *cis*- and per cent *trans*-polybutadiene in a sample of sodium-polymerized polybutadiene and the major component, 1,2-polybutadiene, was estimated by difference. This figure (per cent 1,2) was substituted in Equation 2 in order to calculate a new value for $\underline{E^v_{911}}$. In a similar manner E^t_{967} was re-evaluated using a sample of low temperature emulsion polymer, whose major component, *trans*-1,4-polybutadiene, was determined by difference, and E^c_{724} was calculated from data on a sample of high temperature emulsion polymer, on which per cent cis was determined by difference. Values were averaged, respectively, for three samples relatively high in cis, two samples high in vinyl, and four samples high in trans, to obtain new values for E^c_{724}, E^v_{911}, and E^t_{967}. Using the new extinction coefficients, the above series of calculations was repeated until further successive repetitions did not result in further change in the E values. For each repetition, a new value of E^v_{724} was also used, based on the new figures for per cent 1,2 in the sodium polymers.

Copolymers of styrene and butadiene are treated as four-component systems, and measurements are made at 699 (for styrene determination), 724, 911, and 967 cm.$^{-1}$ In this case four simultaneous equations result:

$$E_{699} = \underline{SE^s_{699}} + CE^c_{699} + VE^v_{699} + TE^t_{699}$$

$$E_{724} = SE^s_{724} + \underline{CE^c_{724}} + VE^v_{724} + TE^t_{724}$$

$$E_{911} = SE^s_{911} + CE^c_{911} + \underline{VE^v_{911}} + TE^t_{911}$$

$$E_{967} = SE^s_{967} + CE^c_{967} + VE^v_{967} + \underline{TE^t_{967}}$$

where S represents the weight fraction of styrene in the polymer, and the other symbols have the same meanings as before.

E^s values were determined directly on carbon disulfide solutions of pure polystyrene, and the other E_{699} values were calculated from measurements on a series of polybutadiene samples which had been analyzed as described above.

An electrical computer (12-equation electrical computer, Model 30–103, obtained from Consolidated Engineering Corp., Pasadena, Calif.) has been used for this work, although solution of the equations is not difficult by successive approximations or

the use of determinants. For most purposes the correction terms can be determined with sufficient accuracy by using estimated values for the weight fractions S, C, V, and T, and the final values calculated directly. Table VI lists the extinction coefficients found.

DISCUSSION

The above method involves the tacit assumption that no other components are present and that the samples examined had the theoretical total unsaturation, one double bond per butadiene unit. Lee, Kolthoff, and Mairs (*9*) have reported that sodium- and emulsion-polymerized polybutadiene, respectively, have approximately 92 and 98% of the theoretical unsaturation. If these representative values apply to the samples used in the present work, then the results reported for 1,2 addition are too high by about 8% of the amounts reported; results for cis and trans would not be affected. If accurate total unsaturation values were determined for the samples used as standards, more accurate extinction coefficients could be calculated on that basis. However, in view of the uncertainties and difficulties inherent in the chemical determination of unsaturation, as well as lack of experience with such methods, it seemed preferable tentatively to assume that the purified polymer samples used had the theoretical unsaturation, and accept the possible errors resulting. The 1,2 polymer, which would be the only one affected if the results of Lee, Kolthoff, and Mairs are applicable, is a relatively minor and unvarying constituent except in the case of sodium polymers.

The method for styrene is believed to be more reliable than the refractive index method (*10*), for it has been shown (*6*) that the refractive index of polybutadiene varies with temperature of polymerization. The ultraviolet absorption method (*11*) for combined styrene is presumably applicable to low temperature polymers, but is more subject to interference by traces of antioxidant than is the infrared method.

Table VI. Polymer Extinction Coefficients[a]

	699 cm.$^{-1}$	724 cm.$^{-1}$	911 cm.$^{-1}$	967 cm.$^{-1}$
Polystyrene	2.703	0.038	0.064	0.050
cis-1,4-Polybutadiene units	0.385	0.551	0.037	0.058
1,2-Polybutadiene units	0.153	0.050	3.193	0.098
trans-1,4-Polybutadiene units	0.005	0.007	0.055	2.542

[a] Extinction coefficients were measured for carbon disulfide solutions and are defined as follows:

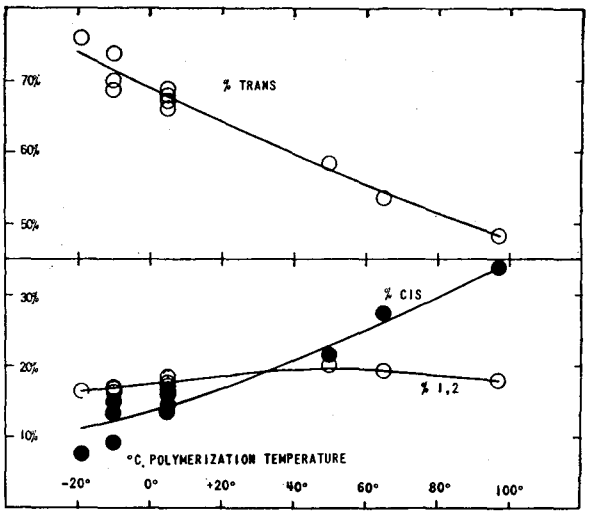
$$E = \frac{d}{xl}$$

d = optical density = $\log \frac{I_0}{I}$
I = intensity of radiation transmitted by solution
I_0 = intensity of radiation transmitted by pure solvent in same cell
x = solution concentration in grams per liter
l = cell thickness in cm.

RESULTS

Tables II and III compare some of the data obtained by Hart and Meyer (*6*) with results obtained by recalculating the original data according to the new method. Because no measurements were made on these samples at 699 or 724 cm.$^{-1}$, the equation involving E_{724} was replaced by the equation $S + C + V + T = 1.000$ (thus determining cis by difference), and the weight fraction of styrene, as estimated by refractive index measurement, was substituted for S, in order to estimate the correction terms for the copolymer samples. The new method gives somewhat higher values for cis, and lower values for 1,2 and trans, but confirms the general conclusion that lowering the temperature of polymerization lowers the cis–trans ratio.

Tables IV and V give the results of complete analyses by the new method, where cis, 1,2, and trans (and styrene, if present) were determined. In most cases the summation is reasonably close to 100%. These results further confirm the previous conclusion (*6*) regarding the effect of polymerization temperature.



Fignre 2. Effect of Temperature on Polybutadiene Structure

Infrared spectroscopy provides a valuable means of polymer analysis. By this method it is possible to distinguish between standard and "cold" GR-S in unknown samples. In studying other possible methods of polymerization it is now readily possible to determine the effect of various conditions on polymer composition, and thus guide research in the most fruitful channels.

ACKNOWLEDGMENT

The author wishes to thank the various members of the General Laboratories' staff who cooperated in supplying and purifying the polymer samples used, and in particular J. Warnet, who assisted in making the infrared measurements and W. A. Hermonat, who prepared many of the samples.

LITERATURE CITED

(1) Alekseeva, E. N., and Belitzkaya, R. M., *Zhur. Obshch. Khim.* (*U.S.S.R.*), **11**, Part 4, 358–62 (1941).
(2) American Petroleum Institute, Research Project 41, National Bureau of Standards, "Catalog of Infrared Spectral Data."
(3) Anderson, J. A., Jr., and Seyfried, W. D., ANAL. CHEM., **20**, 998 (1948).
(4) Field, J. E., Woodford, D. E., and Gehman, S. D., *J. Applied Phys.*, **17**, 386 (1946).
(5) Flory, P. J., *J. Polymer Sci.*, **2**, 36 (1947).
(6) Hart, E. J., and Meyer, A. W., *J. Am. Chem. Soc.*, **71**, 1980 (1949).
(7) Kolthoff, I. M., and Lee, T. S., *J. Polymer Sci.*, **2**, 206 (1947).
(8) Kolthoff, I. M., Lee, T. S., and Mairs, M. A., *Ibid.*, **2**, 220 (1947).
(9) Lee, T. S., Kolthoff, I. M., and Mairs, M. A., *Ibid.*, **3**, 66 (1948).
(10) Madorsky, I., and Wood, L. A., private communication to Office of Rubber Reserve, 1944.
(11) Meehan, E. J., *J. Polymer Sci.*, **1**, 175 (1946).
(12) Pitzer, K. S., private communication.
(13) Rabjohn, N., Bryan, C. E., Inskeep, G. E., Johnson, H. W., and Lawson, J. K., *J. Am. Chem. Soc.*, **69**, 314 (1947).
(14) Rossini, F. D., Natl. Bur. Standards, private communication.
(15) Schultze, W. A., Reynolds, W. B., Fryling, C. F., Sperberg, L. R., and Troyan, J. E., *India Rubber World*, **117**, 617 (1948).
(16) Smith, D. C., and Miller, E. C., *J. Optical Soc. Am.*, **34**, 130 (1944).
(17) Thompson, H. W., and Torkington, P., *Trans. Faraday Soc.*, **41**, 246 (1945).
(18) White, J. U., *J. Optical Soc. Am.*, **37**, 713 (1947).
(19) White, L. M., *Ind. Eng. Chem.*, **41**, 1554 (1949).

RECEIVED May 31, 1949. Presented before the Division of Rubber Chemistry, AMERICAN CHEMICAL SOCIETY, at Boston, Mass., May 23 to 25, 1949. Contribution 87 from the General Laboratories, United States Rubber Company.

Determination of Styrene in Hydrocarbon Copolymers

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A method has been developed which determines, with about 3% or better accuracy, the styrene content of polystyrene and of soluble or insoluble polymers of styrene and butadiene. It presents the advantages of greater speed and less hazard than the conventional carbon-hydrogen method. Butadiene does not interfere. Several substituted styrenes and other aromatics give different reactions. Thus they interfere with a styrene determination. If they are to be determined in their polymers, a separate standardization would be required.

SATISFACTORY methods of analysis of monomeric styrene are available in the literature (*8*, *16*). Soluble styrene–copolymeric hydrocarbon resins are also relatively easy to analyze by physical means (*3*, *19*, *27*). Insoluble ones, however, are almost impossible to analyze accurately (*6*, *7*, *29*, *30*, *33*); the carbon-hydrogen determination is the only method used to any great extent (*28*). This method is uncertain, because a small error in hydrogen content introduces errors more than ten times as great into the final styrene estimation. The carbon-hydrogen determination is also hazardous (*5*), as styrene-butadiene resins tend to explode in any oxygen atmosphere.

Other methods for the analysis of styrene, such as the proposed A.S.T.M. method (*2*), are long and involved. Thus, an attempt was made to develop a more satisfactory method for the determination of styrene in mixed hydrocarbon resins, especially polystyrene and butadiene-styrene copolymers and particularly insoluble polymers such as "popcorn" (*17*).

DISCUSSION OF PREVIOUS INVESTIGATIONS

Many methods have been developed for determining monomeric styrene and pure polystyrene, including halogen absorption, nitration, etc. Styrene may be identified in copolymers by oxidation to benzoic acid and benzaldehyde (*14*, *18*, *26*). This method is not quantitative, however, and materials other than styrene that oxidize to benzoic acid or benzaldehyde under these conditions interfere—for example, toluene and ethylbenzene.

Halogenated styrene copolymerized with a nonhalogenated compound can be determined from the halogen content of the polymer (*10*). Similarly, styrene copolymerized with a compound containing an additional element can be determined by difference (*1*, *13*, *15*, *23*, *36*). The direct chemical quantitative determination of styrene in copolymers with other pure hydrocarbons, however, has not been found in the literature; the carbon-hydrogen analysis (*4*, *16*) is the only available method, and of course, is applicable only when the styrene and other monomer differ in their carbon-hydrogen ratios and when no other constituents than carbon or hydrogen are present

Polymeric styrene may also be determined by certain physical methods requiring considerable equipment—fluorescent and absorption spectra (*24*, *31*), molecular spectra (*15*, *20*, *22*, *35*), electron diffraction (*32*), and x-ray diffraction (*9*). However with the widespread use of polystyrene in plastic materials, coatings, and many other applications, a simple method for the analysis of styrene has become a necessity. It must be adaptable to a wide variety of styrene-containing materials and, after proper standardization, must be fairly accurate. The method here described seems to meet most of these conditions.

A rapid procedure, lending itself readily to regular routine analysis, was developed from a combination of the colorimetric method of Rowe *et al.* (*25*) for monomeric styrene and the steam-distillation method of Frank *et al.* (*11*), in which the sample is depolymerized by superheated steam at about 300° C. The monomeric material is steam-distilled over and absorbed in a known volume of carbon tetrachloride.

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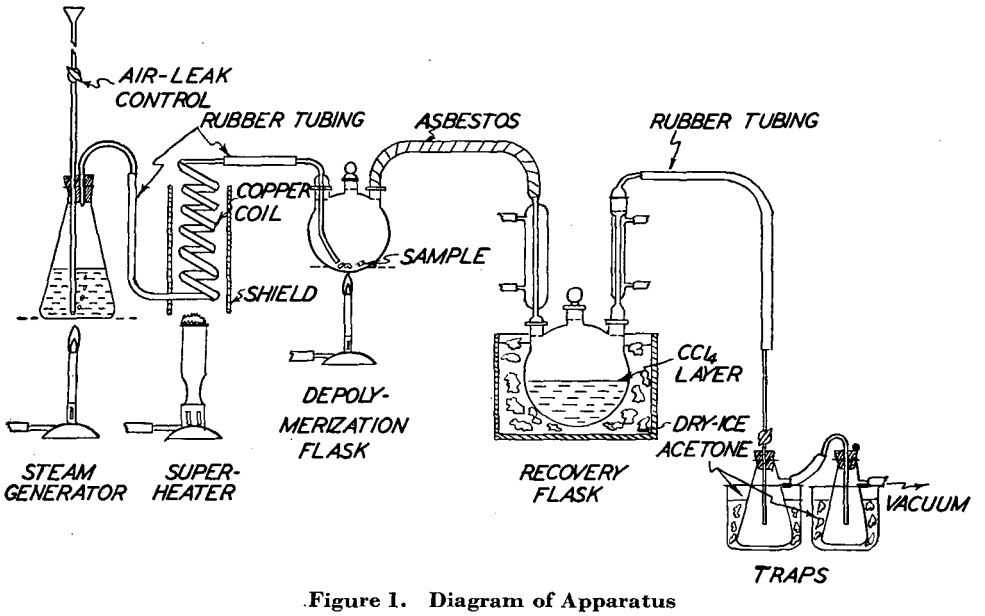

Figure 1. Diagram of Apparatus

ANALYTICAL PROCEDURE

Approximately 3 grams of sample are introduced into the depolymerization flask (Figure 1), 100 ml. of carbon tetrachloride are introduced into the recovery flask, and the entire system is sealed.

The recovery flask, as well as the trap ahead of the vacuum pump, is cooled with dry ice–acetone mixture. An additional trap prevents oil backing up from the pump or loss of material due to a rapid flow of vapors through the system. The system is evacuated to the vapor pressure of the cold carbon tetrachloride and the steam generator and superheater are turned on. The depolymerization flask should operate in the general neighborhood of 300° to 400° C. and 5 to 10 mm. of mercury absolute pressure. A slow stream of air bubbles to control the boiling rate is admitted to the steam generator by means of a capillary tube, hose, and pinchclamp.

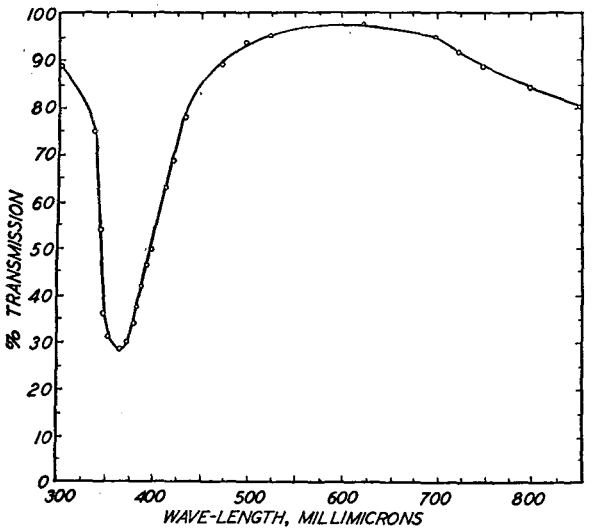

Figure 2. Spectral Transmittance of Nitrated Styrene

8.0 mg. per 50 ml. of solution. Coleman Universal spectrophotometer

A flame is applied slowly to the sample flask after the flow of steam has started. Water is turned on in the condensers and the sample is melted and evaporated slowly. After it has been distilled, the flame is played over the entire surface of the flask to distill splashes on the walls. Some 100 ml. of water will have been condensed at this point. The flames are then shut off and the entire system is allowed to cool. The vacuum is broken and the system dismantled for the warming up of the recovery flask and traps. The condenser tubes and flasks are rinsed with the carbon tetrachloride layer in the recovery flask.

After shaking, an aliquot of the carbon tetrachloride layer containing about 2 to 8 mg. of styrene is pipetted into a separatory funnel and diluted to 15 ml. with C.P. carbon tetrachloride. Two milliliters of nitrating mixture (equal volumes of concentrated sulfuric acid and freshly boiled and cooled nitric acid) are added, and the separatory funnel is stoppered and shaken vigorously for 10 seconds every 2 minutes over a period of 10 minutes. Fifteen milliliters of distilled water are added, the funnel is shaken, and the water layer is removed. Two additional 15-ml. water washes are employed and the combined washes are made up with distilled water to 50 ml. in a volumetric flask. This solution is filtered through ashless paper to remove any cloudiness or allowed to stand until the cloudiness settles out. The light transmittance is then read in a photoelectric colorimeter using a blue filter, or better yet, in a spectrophotometer at approximately 365 millimicrons wave length, which corresponds to a minimum in transmittance (Figure 2). The reading is compared to a calibration curve obtained on pure styrene and the corresponding weight of pure styrene is multiplied by an empirical calibration factor of 2.0 to give the amount of styrene in the polymer.

For extreme accuracy, it is essential that an exact technique be used and the same number and type of operations be performed on each sample.

STANDARDIZATION WITH STYRENE MONOMER

In the standardization, the method of Rowe *et al.* (*25*) is essentially followed.

Pure redistilled styrene monomer (99.5% purity or better, 1.1 ml. by volume or 1 gram by weight) is diluted to 100 ml. with C.P. carbon tetrachloride in a volumetric flask, and 25 ml. of this solution are then diluted to 250 ml. This latter solution represents 1.0 mg. of styrene per ml. Volumes from 1.0 to 15.0 ml. are made up to 15 ml. with carbon tetrachloride, treated with nitrating mixture, extracted, and filtered, and light transmittance is determined as previously described.

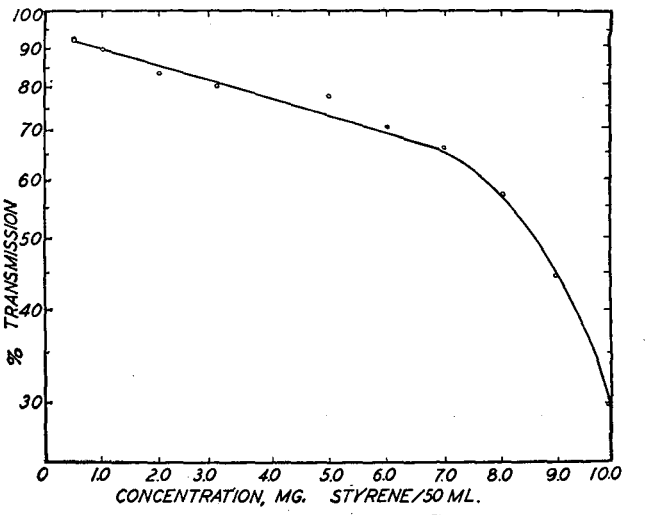

Figure 3. Transmittance Curve

Cenco photelometer with blue glass filter and 1 to 10 mg. of styrene per 50 ml.

A typical calibration curve with a photoelectric colorimeter is given in Figure 3 and with a spectrophotometer in Figure 4. The deviation from linearity at high concentrations is confirmed by Rowe (*25*).

STANDARDIZATION WITH POLYMERS

A number of commercial GR-S synthetic rubber and "popcorn" polymer samples (cross-linked insoluble polymer of styrene and butadiene) were analyzed by different methods to determine their styrene contents (Table I), and also by the superheated steam depolymerization method herein described. Attempts were made to secure samples of styrene copolymerized with drying oils and other polymerizable substances, but these were unavailable at the time. However, it was thought that this method could be adapted to such materials by working with the unsaponifiable fraction. One polymer sample containing *p*-chlorostyrene and one containing 3,5-dimethylstyrene were also run but calibration curves were not obtained. The nitration reaction gives different colors in these cases but it is believed that after suitable standardization, the method would apply equally well as with unsubstituted styrene.

DISCUSSION

As styrene has only one reactive double bond, it polymerizes linearly. Temperatures of about 100° C. or lower are employed. The reverse process, or depolymerization, requires some 75,000 calories per gram mole (*12*); thus temperatures of 300° to 400° C. are necessary. On heating, the polymer melts at first, then begins to bubble. In air at atmospheric pressure, charring occurs. Charring is decreased but still appreciable in air at reduced pressures. Replacing the air with steam makes charring negligible; the steam also minimizes subsequent recombination of the monomer molecules.

The color that develops on nitrating is probably due in part to organic compounds formed and in part to oxides of nitrogen. The similarity of the colors obtained with styrene monomer and with the products of disintegration of the polymers here investigated, indicates that monomeric styrene is a principal product and that other interfering compounds do not form. However, slightly high results may be obtained when other materials than butadiene

Table I. Determination of Styrene in Polymers

Description	Sample	Without Steam Depolymerization: Nominal	Micro C—H	Macro C—H	Average C—H	With Steam Depolymerization: Nitrosite	Photelometer	Spectrophotometer	Spectrophotometer *vs.* C—H
Popcorn (coml.)	9A	...	84.00	..	...	..	76.3	83.3	−0.7
	9B	...	85.07	...	...	..	77.5	83.7	−1.37
	9C	...	59.9	66.9	63.4	..	56.0	62.76	−0.64
	9D	...	77.48	..	...	..	78.5	77.9	+0.4
	10A	...	67.05	..	...	..	69.2	....	(+2.1)
	10B	...	76.50	...	...	..	....	77.2	+0.7
	13A	...	...	71.5	...	..	....	70.9	−0.6
	13B	...	...	49.4	...	..	....	50.3	+0.9
	1	...	78.7	79.2	78.95	..	78.7	79.1	+0.15
Popcorn (Bd) (coml.)	Phillips	0	0	..	...	0	Trace	0	0.0
	2A	...	91.52	..	...	..	90.2	....	(−1.32)
	2B	...	84.40	..	...	..	....	84.7	+0.3
	3A	...	73.60	..	...	..	75.1	74.0	+0.4
	BB	...	57.03	..	...	..	59.1	57.5	+0.47
	4	...	90.22	..	...	..	....	90.5	+0.28
	5	...	91.78	...	...	..	90.5	....	(−1.28)
	7	...	91.86	..	...	..	90.7	....	(−1.16)
Popcorn (lab.)	Syn. 85/15	85	...	79.5	...	...	80.2	82.6	+3.1
	Syn. 90/10	90	...	87.7	...	86.4	88.5	90.32	+3.6
GR-S synthetic rubber	BC-60428-A	23.2	...	23.5	...	...	....	24.0	+0.5
	BC-60428-B	23.3	...	23.8	...	22.8	....	23.9	+0.1
	BC-60426-DM	23.8	...	..	...	..	....	24.3	+0.5
	BC-60426-B	23.4	...	24.6	...	22.7	27.25	25.2	+0.6
	BC-60426-XBC	23.6	...	24.8	...	22.9	28.0	24.7	−0.1
	BC-60424-A	23.5	...	..	...	..	....	23.9	+0.4
	BC-60424-C	23.5	...	24.0	...	..	....	23.8	+0.3
Pure (lab.)	Polystyrene	100	99.5	99.7	99.6	98.7	97.2	98.8	−0.8
Mean, compared to C—H method							−0.94	+0.37	
Mean deviation, compared to C—H method							±2.7	±0.74	

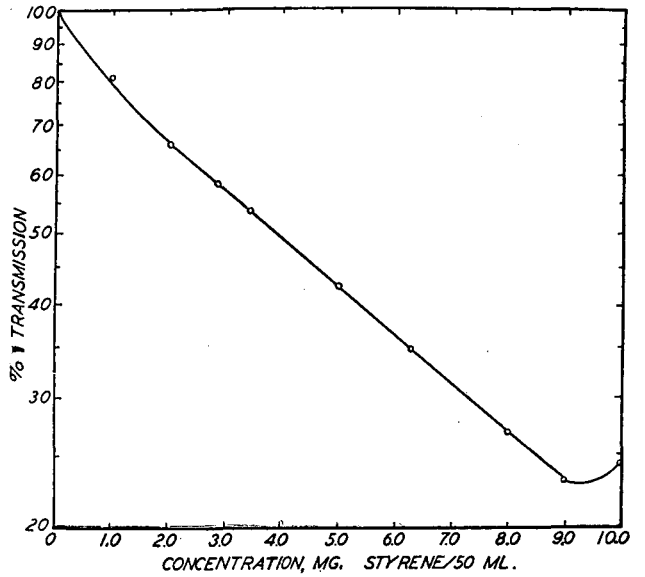

Figure 4. Styrene Standardization Curve

Coleman Universal spectrophotometer at maximum absorption of 365 mμ

and styrene are pyrolyzed, such as stearic acid in GR-S synthetic rubber. Leaching the polymer with sodium hydroxide and then water minimizes the effect in this case. Similarly, in oils work may be done with the unsaponifiable portion and the method adapted to styrenated oils.

The empirical correction factor of 2.0 was obtained by comparison of the carbon-hydrogen and colorimetric analyses of the samples in Table I. After applying this factor, the styrene content by spectrophotometer of the 23 samples averages 0.37% higher than the corresponding carbon-hydrogen figure, or the mean of the two carbon-hydrogen figures if both micro- and macromethods were employed. Thus a correction factor slightly below 2.0 would give better agreement for the particular equipment and conditions used. However, the mean deviation is ±0.74% and adequate accuracy is afforded by the round number of 2.0. For the 16 samples on which Photelometer determinations were also carried out the mean deviation is ±2.7% and the mean is 0.94% below the carbon-hydrogen figure. With this equipment, therefore, the reproducibility is not so good; a correction factor a little higher than 2.0 would give better agreement. More precise values of the correction factor are not reported because it should be determined by each laboratory for the exact equipment, operating conditions, and procedures used.

The need of a correction factor of about 2.0 is supported by Madorsky and Straus (*21*), who distilled polystyrene in high vacuum and analyzed the pyrolytic products. Forty per cent of their product was found to be styrene monomer and the balance dimer, trimer, etc., and some degradation products. Wall (*34*) at atmospheric pressure similarly found 33% of monomer in the products. The nitrosite method (*4*) also requires a correction factor. It was employed on several of the samples after depolymerization (Table I) but did not agree well with the other methods and was not utilized further.

The equipment required for this procedure can be set up from standard parts in approximately 0.5 hour. Exclusive of standardization an analysis can be completed within 1 hour, which is much less than other methods require. This general procedure may prove useful for the analysis of other types of polymers, after proper standardization, and may be adaptable to microprocedures.

ACKNOWLEDGMENT

Thanks for assistance are due to B. L. Harris, D. A. Edwards, and J. Grossman; and for samples to The Goodyear Tire and Rubber Company at Akron and at Houston, C. S. Marvel of the University of Illinois, Phillips' Petroleum Company at Borger, and Humble Oil Company at Baytown, Texas.

COMPREHENSIVE BIBLIOGRAPHY

(1) Alfrey, T., and Harrison, J. G., Jr., *J. Am. Chem. Soc.*, **68**, 299–301 (1946).
(2) Am. Soc. Testing Materials, Vol. III-B, p. 969, D 833-46T (1946).
(3) Barnes, R. B., Liddel, U., and Williams, V. Z., IND. ENG. CHEM., ANAL. ED., **15**, 681 (1943).
(4) Bond, G. R., Jr., *Ibid.*, **19**, 390–2 (1947).
(5) Bonilla, C. F., Edwards, D. E., Grossman, J., and Crippen, R. C., *Rubber Reserve Corp. Rept.*, **CD-850** (1946).
(6) Brajnikoff, B. J., *Plastics*, **6**, 276–84 (1942).
(7) *Ibid.*, **6**, 316–28 (1942).
(8) Coghill, R. D., and Sturtevant, J. M., "Identification of Organic Compounds," pp. 139, 140, 150, 168, New York, McGraw-Hill Book Co., 1936.
(9) Dzhelepov, B. S., *Phys. Z. Sowjetunion*, **11**, 157–67 (in German); *J. Exptl. Theoret. Phys. (U.S.S.R.)*, **7**, 61–7 (1937).
(10) Fieser, L., and Fieser, H., "Organic Chemistry," pp. 548, 550, 917, 918, New York, D. C. Heath Co., 1945.
(11) Frank, J. C., Amos, J. L., and Straubel, A. F. (to Dow Chemical Co.), U. S. Patent 2,359,212 (1944).
(12) Gilman, Henry, "Advanced Treatise in Organic Chemistry," pp. 743–50, New York, John Wiley & Sons, 1943.
(13) Glavis, F. J., Ryden, L. L., and Marvel, C. S., *J. Am. Chem. Soc.*, **59**, 707–11 (1937).
(14) Goff, J. H., *Paper Trade J.*, **122**, No. 5, 41–3 (1946).
(15) Horio, M., *Mem. Coll. Eng. Kyoto, Imp. Univ.*, **7**, 177–248 (1933).

(16) Kamm, Oliver, "Qualitative Organic Analysis," 2nd. ed., pp. 41, 141, 194, 195, 267, New York, John Wiley & Sons, 1937.
(17) Kharasch, M. S., Jensen, E. V., and Nudenberg, N., *Rubber Reserve Corp. Rept.* CR-870, CR-950 (1945).
(18) Kunke, W. F., *J. Assoc. Agr. Chemists*, 28, 723–5 (1945).
(19) Lebas, G., *Trans. Faraday Soc.*, 15, Pt. 3, 231–7 (1920).
(20) Ley, H., and Dirking, H., *Ber.*, 67B, 1331–8 (1934).
(21) Madorsky, S. L., and Straus, S., *Ind. Eng. Chem.*, 40, 848–52 (1948).
(22) Pestemer, M., and Wiligut, L., *Monatsh.*, 66, 119–28 (1935).
(23) Quilico, A., and Fleischner, E., *Atti acad. Lincei*, 7, 1050–6 (1928).
(24) Ramart-Lucas, Mme., and Amagat, P., *Bull. soc. chim.*, 51, 965–8 (1932).
(25) Rowe, V. K., Atchison, G. J., Luce, E. N., and Adams, E. M., *J. Ind. Hyg. Toxicol.*, 25, 348–53 (1943).
(26) Simonds, H. R., and Ellis, C., "Handbook of Plastics," pp. 770–1, New York, D. Van Nostrand Co., 1943.
(27) Smoker, E. H., and Burchfield, P. E., IND. ENG. CHEM., ANAL. ED., 15, 128–9 (1943).
(28) Snell, F. D., and Biffen, F. M., "Commercial Methods of Analysis," pp. 143–5, New York, McGraw-Hill Book Co., 1944.
(29) Staudinger, H., and Heuer, W., *Ber.*, 67B, 1159–64 (1934).
(30) Staudinger, H., Heuer, W., and Husemann, E., *Trans. Faraday Soc.*, 32, 323–35 (1936).
(31) Titeica, R., *Ann.* 11, 445–70 (1936).
(32) Trillat, J. J., *Kolloid-Z.*, 69, 378–89 (1934).
(33) Valyi, I., Janssen, A. G., and Mark, H., *J. Phys. Chem.*, 49, 461–71 (1945).
(34) Wall, L. A., paper presented before Division of Cellulose Chemistry, High Polymer Forum, at the 112th Meeting of AM. CHEM. SOC., New York, 1947.
(35) Williams, D., *J. Chem. Phys.*, 4, 460 (1936).
(36) Wulff, Carl, Dorrer, Eugen, and Hopff, Heinrich (to I. G. Farbenindustrie, Ger. Patent 627–037 (March 6, 1936).

RECEIVED August 21, 1948. Presented before the Division of Paint, Varnish, and Plastics Chemistry, Plastics Group, at the 114th Meeting of the AMERICAN CHEMICAL SOCIETY, Washington, D. C. Investigation carried out under the sponsorship of the Office of Rubber Reserve, Reconstruction Finance Corporation, in connection with the government synthetic rubber program. Presented in partial fulfillment for the degree of master of science in engineering in the Department of Chemical Engineering at the Johns Hopkins University, Baltimore, Md., June 1948.

Spectrophotometric Determination of Cobalt as Cobalt(II) Chloride in Ethanol

Determination of Water in Ethanol

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A photometric method has been developed for estimation of cobalt based upon the intense blue color of cobalt(II) chloride in ethanol solution. The solutions show minimum transmittancy at 655 mμ. The color is stable with respect to time, but the transmittancy is decreased considerably by rise in temperature; the temperature effect is completely reversible. The system shows some deviation from Beer's law. The optimum range for the spectrophotometric measurement with the instrument and procedure used is between 100 and 400 p.p.m. of cobalt, with an accuracy of 0.5%. The effect of diverse ions has been studied. The method has been tested by comparison with other methods (gravimetric and titrimetric) in the assay of a cobalt salt and in the analysis of a standard steel. Water modifies the color of the cobalt chloride–ethanol solutions; this effect is the basis of a method for estimating water in ethanol, for which the optimum range is about 1 to 5% water and the maximum accuracy is 0.3 to 0.5%, depending upon the concentration of cobalt chloride used.

SINCE 1737, when J. Hellot first observed color changes in cobalt(II) chloride, this substance has been the subject of extensive study on account of the varied colors exhibited by the solid and by solutions of varying concentration and temperature in many different solvent media. Dilute aqueous solutions, which are pink (spectrophotometrically, reddish purple), change to violet-blue on heating, corresponding in general to changes in color in passing from the hexahydrate to lower hydrates and anhydrous salt. Solutions of cobalt(II) chloride in sufficiently concentrated hydrochloric acid, and in certain salts such as the chlorides of calcium, magnesium, zinc, tin(II), and mercury(II), are blue in color; solutions in many organic solvents are deep blue in color at room temperature, and many of these change through violet to red at lower temperatures (*5*, pp. 632–6).

[All the following report is concerned with cobalt only in compounds in which it is bivalent, except in the analytical methods used for checking the proposed method; the bivalent state of the cobalt is not specifically designated hereafter.]

Winkler (*9*) observed that cobalt chloride is soluble in ethanol to give a blue solution; although a 1 to 2500 solution is distinctly blue, a 1 to 10,000 solution is colorless when cold, but blue when hot. (The ethanol used by Winkler was obviously not absolute, for a 1 to 10,000 solution of cobalt chloride in absolute ethanol is very distinctly blue even at room temperature; a 1 to 10,000 solution in 95% ethanol is visually almost colorless.) Winkler found that the addition of water to the blue alcoholic solution caused the color to change through violet to red; hence the blue solution could be used to detect the presence of water.

Many of the studies involving color changes of cobalt chloride in various solvents have been concerned with deducing the composition of the colored solute, and various theories have been proposed to account for the observed effects. It is not the purpose of the present investigation to study the mechanism of the color reactions, but rather to test the application of the cobalt chloride–ethanol color to determination of small amounts of water in ethanol, and to establish a colorimetric method for cobalt based on the intense blue color of the cobalt chloride–ethanol system.

REAGENTS

All chemicals were reagent grade; maximum limits of impurities likely to interfere are given below.

Cobalt chloride hexahydrate. Impurities: nickel, 0.15%; copper, 0.002%; iron, 0.001%.

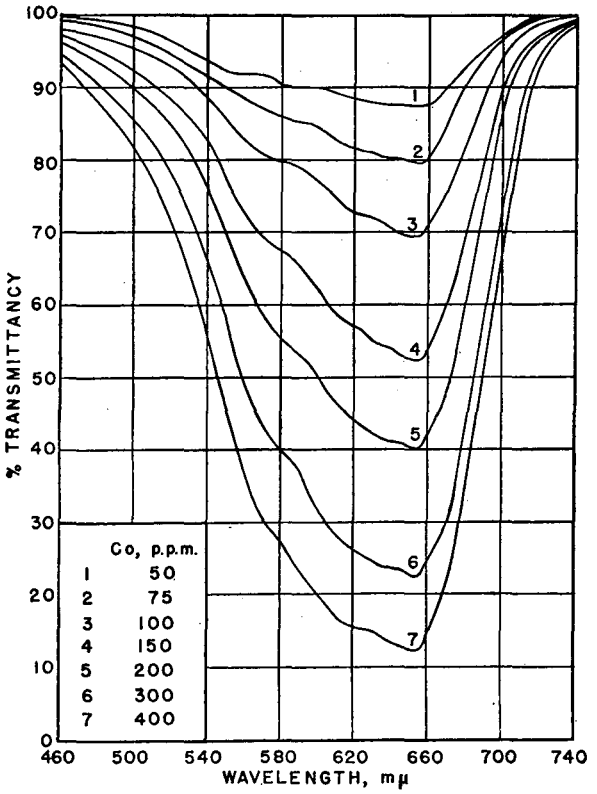

Figure 1. Transmittancy–Wave Length Curves for Cobalt Chloride–Ethanol Solutions

Cobalt nitrate hexahydrate. Impurities: nickel, 0.11%; copper, 0.005%; iron, 0.001%.

Copper(II) chloride dihydrate. Impurities: nickel, 0.01%; iron, 0.015%.

Iron(III) chloride hexahydrate. Impurity: copper, 0.003%.

Nickel(II) chloride hexahydrate, special, low in cobalt. Impurities: cobalt, 0.002%; copper, 0.001%; iron, 0.01%.

Ethanol, absolute.

National Bureau of Standards cobalt-molybdenum-tungsten steel No. 153. Cobalt, 8.45%; molybdenum, 8.38%; tungsten, 1.58%; chromium, 4.14%; vanadium, 2.04%; nickel, 0.107%; copper, 0.099%.

Potassium permanganate solution was standardized against National Bureau of Standards sodium oxalate. Sodium thiosulfate solution was standardized against pure potassium iodate.

APPARATUS

All transmittancy measurements for calibration and analysis were made with a Coleman Model 10-S spectrophotometer, using matched square cells of 1.30-cm. optical path and a 30-mμ slit. The wave-length calibration of the instrument had been factory-checked just prior to use in this investigation. Some comparison measurements were made with a Beckman model DU spectrophotometer, using matched square cells of 1.004-cm. optical path, and band widths of the order of 5 mμ.

Calibrated weights and volumetric ware were used throughout.

EXPERIMENTAL

General Procedure. A stock standard solution having a cobalt concentration of 1000 p.p.m. was prepared by dissolving 2.0186 grams of cobalt chloride hexahydrate in ethanol and diluting to exactly 500 ml. Substandards were prepared as needed by volumetric dilution of this stock solution. All volumetric measurements were made at 25° ± 0.05° C. All transmittancy measurements were made against a corresponding blank. In the preliminary measurements it was obvious that the transmittancy of the system was considerably influenced by temperature; all final measurements were therefore made on samples that had been brought to 25° C. in a thermostat, then measured without delay in the spectrophotometer. The increase in transmittancy (in the wave-length region of minimum transmittancy) with decrease in temperature is consistent with the report by Toporescu (*7*) that the blue ethanol solution changes to red upon cooling.

Effect of Cobalt Chloride Concentration. Data for transmittancy–wave-length curves were taken by measuring the transmittancy, at 10-mμ or 5-mμ intervals, over the range 460 to 740 mμ. The solutions ranged in cobalt concentration from 50 to 600 p.p.m. Typical transmittancy–wave-length curves are shown in Figure 1; all have minimum transmittancy at 655 mμ, with faint indication of bands at about 585 and 630 mμ, when measured with the Coleman instrument. Measured with the Beckman spectrophotometer, the minimum transmittancy appeared at 655 mμ, and band indications in the vicinity of 585 and 630 mμ were more pronounced. Brode (*2*) reported the absorption maximum at 660 mμ, with slight indications of bands at 580, 595, 610, and 630 mμ.

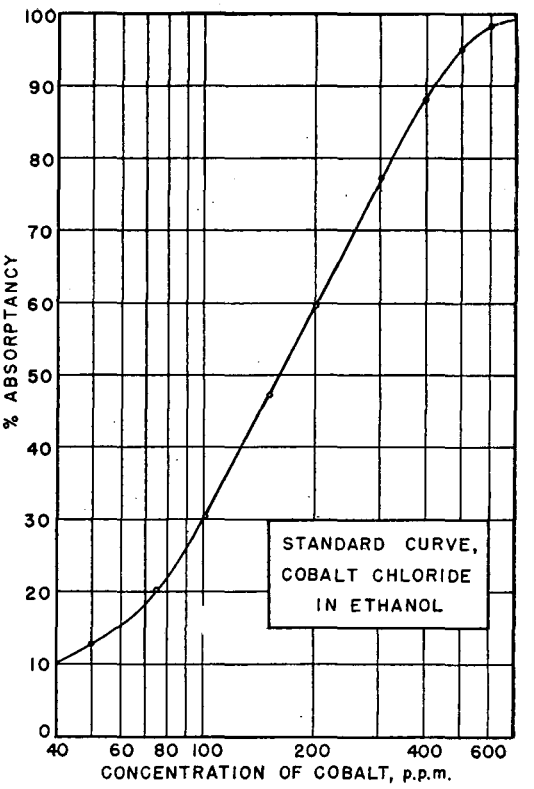

Figure 2

A plot of log per cent transmittancy against concentration showed poor conformity to Beer's law for cobalt concentrations below 75 and above 300 p.p.m. The nearly linear portion between 75 and 300 p.p.m., extrapolated to zero concentration, did not pass through the point of 100% transmittancy, but corresponded to about 130% transmittancy. Deviations from Beer's law are probably due to shifts in the equilibria involved in the cobalt chloride–ethanol system.

In Figure 2, the data are plotted in the form of per cent absorptancy—i.e., 100 − % transmittancy—against log concentration, according to the original method of Ringbom (*6*). The advantages of this plotting method have been discussed by Ayres (*1*). The optimum range is from about 100 to 400 p.p.m. In this range, the maximum accuracy is represented by 2.5% relative error per 1% absolute photometric error. For a precision (reproducibility) of measurement to 0.2%, the maximum accuracy is therefore 0.5% of the concentration.

Stability of Color. Solutions of various concentrations were measured again 2 weeks after preparation and first measure-

ments. Within the limits of precision of the measurement, the transmittancies were identical.

Effect of Temperature. Although the influence of temperature on transmittancy was not studied extensively, several measurements indicated that the transmittancy decreased approximately 1% per 1° C. rise in temperature in the range 15° to 30° C. The change was completely reversible, and was not associated with any detectable shift in the main absorption band at 655 mμ.

Effect of Diverse Ions. Transmittancy–wave-length curves were determined for ethanol solutions of chlorides of nickel (II), copper(II), and iron(III). The shape of these curves indicated that there might be considerable interference in the case of nickel and of copper, but little, if any, interference by moderate amounts of iron. The tolerance of the cobalt chloride–ethanol solution for the diverse ions was studied by measuring the transmittancy of solutions containing 200 p.p.m. of cobalt and successively smaller amounts of the diverse ion (added in the form of an ethanol solution of the metal chloride) until the transmittancy was such as to correspond to an error of 1% or less on the amount of cobalt present; this error is represented by a difference of about 0.4 in the measurement of per cent transmittancy. In all cases, measurements were made at several different wave lengths covering the region of the cobalt minimum transmittancy. The tolerances thus determined are summarized in Table I, and indicate that nickel up to 0.5%, copper up to 1%, and iron up to 5% of the amount of cobalt in the standard and/or unknown sample would not interfere with the estimation of cobalt.

Table I. Tolerance of Cobalt Chloride–Ethanol Solution for Diverse Ions

(All solutions, 200 p.p.m. cobalt)

Diverse Ion	Amount Tolerated, P.P.M.	Ratio of Diverse Ion to Cobalt	Effect on Transmittancy
Ni^{++}	1	1:200	Decrease
Cu^{++}	2	1:100	Decrease
Fe^{+++}	10	1:20	Increase

Assay of a Cobalt Salt. Cobalt nitrate hexahydrate was analyzed for cobalt by the colorimetric procedure proposed herein, and the results were compared with those obtained by two established methods.

Nitrite-Permanganate Method. The procedure given by Kolthoff and Sandell (*3*) was used. To an acetic acid solution of the sample, a large excess of potassium nitrite was added to precipitate potassium hexanitritocobaltiate. After standing for 24 hours, the precipitate was filtered off, washed with 2% ammonium nitrate solution, then dissolved in an excess of acidified standard solution of potassium permanganate at 50° C. The excess permanganate was determined iodometrically by adding excess potassium iodide and titrating the liberated iodine with standard sodium thiosulfate solution. In this process 1 mole of cobalt corresponds to 11 equivalents of permanganate,

Electrodeposition. Following the procedure given by Treadwell and Hall (*8*), the cobalt nitrate sample was fumed down with sulfuric acid to expel nitric acid, and the cobalt was deposited cathodically from strongly ammoniacal solution containing ammonium sulfate. Electrolysis was conducted at 2.8 to 3.2 volts and 0.5 ampere for 2 hours. The remaining solution (acidified with acetic acid) gave a negative test for cobalt with hydrogen sulfide.

Colorimetric Method. The cobalt salt to be analyzed must first be converted to the chloride, because the other common salts, such as the nitrate and sulfate, do not show the characteristic blue color when dissolved in ethanol. Weighed samples of cobalt nitrate hexahydrate were dissolved in concentrated hydrochloric acid and evaporated to dryness. This operation was repeated twice, then the residue was dissolved in a small amount of water and again evaporated just to dryness, after which it was taken up in ethanol and made up to exactly 100 ml. Volumetric dilution with ethanol was made to bring the cobalt content into the optimum range, the transmittancy at 655 mμ was measured, and the cobalt was calculated by reference to the calibration curve, Figure 2.

Table II. Assay of Cobalt Nitrate

Method	Cobalt Found, % 1	2	Average
Nitrite-permanganate	20.09	19.92	20.00
Electrodeposition	20.10	20.06	20.08
	20.04	20.13	
Colorimetric	20.4	20.2	20.3
Calculated for $Co(NO_3)_2.6H_2O$			20.26

The results of the determinations are summarized in Table II.

Analysis of Cobalt Steel. National Bureau of Standards steel No. 153, containing 8.45% cobalt, was analyzed by the methods outlined below.

1-Nitroso-2-Naphthol Method. This method, used by most of the National Bureau of Standards analysts, is described by Lundell, Hoffman, and Bright (*4*). It consisted of double precipitation of the oxidized iron with zinc oxide, followed by precipitation of the cobalt as the cobalt(III) salt of 1-nitroso-2-naphthol, which was ignited and weighed as Co_3O_4.

Nitrite-Permanganate Method. From the solution of the steel sample in acid, iron was removed by double precipitation with zinc oxide as above. The filtrate was evaporated to dryness to remove mineral acids, the residue taken up in acetic acid, and the cobalt precipitated with potassium nitrite. The precipitate was then handled as described under assay of cobalt nitrate.

Colorimetric Method. After removal of the iron with zinc oxide, the filtrate was evaporated just to dryness with hydrochloric acid and then with water, and the residue was taken up in ethanol and made to a volume of exactly 100 ml. By using an appropriate aliquot, the concentration of cobalt was adjusted to be in the optimum range for measurement of transmittancy at 655 mμ, and the cobalt was calculated by reference to the calibration curve, Figure 2.

Table III shows the results of the three methods.

Effect of Small Amounts of Water. Solutions were prepared containing a constant amount of cobalt chloride in ethanol containing varying amounts of water. Three such series were

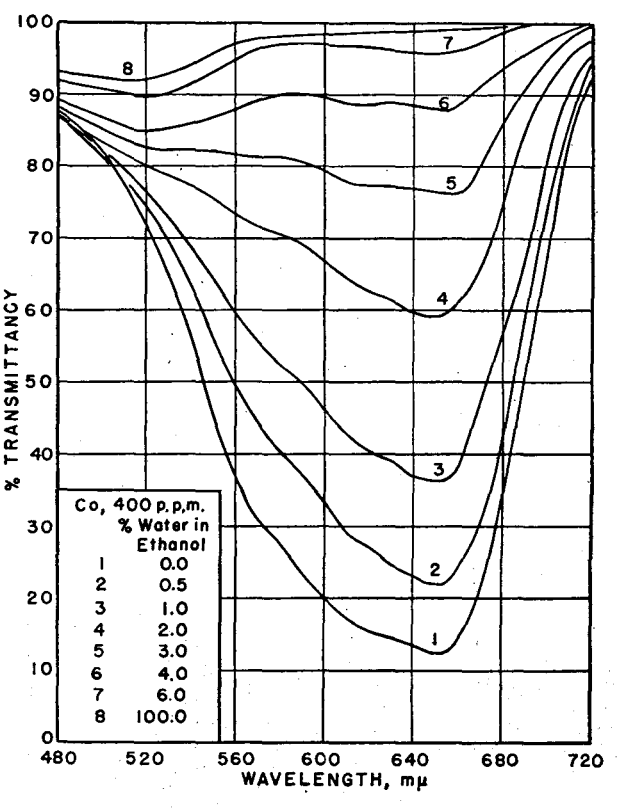

Figure 3. Effect of Water on Transmittancy of Cobalt Chloride–Ethanol Solution

made, using cobalt concentrations of 200, 400, and 1000 p.p.m., and water up to 5 or 6% by volume. For a given cobalt concentration, increasing amounts of water caused the color to change from deep blue through blue-violet to red-violet, and finally to pink. The smaller the amount of cobalt present, the smaller the amount of water required to cause the visible color changes. In transmittancy measurements, however, a given amount of water produced a greater change the higher the cobalt concentration.

Some transmittancy–wave-length curves for solutions containing 400 p.p.m. of cobalt in solutions containing varying amounts of water are shown in Figure 3. Curves for an aqueous solution and a pure ethanol solution of cobalt chloride of the same cobalt concentration are included for reference. The pink aqueous solution shows transmittancy minimum at about 515 mμ; the influence of this absorption region on solutions containing 3% or more water is apparent from the curves. Measured at 655 mμ, the solutions, especially those containing 400 and 1000 p.p.m. of cobalt, showed considerable deviations from Beer's law; the transmittancy changed with water concentration more rapidly than corresponds to the law. At this wave length, good agreement with Beer's law would hardly be expected in the presence of more than small amounts of water, as the intense blue color of the ethanol complex gives way to the pink of the aquo complex. However, the deviations from the law are in a direction that increases the accuracy attainable in the determination of water.

Table III. Analysis of Cobalt Steel

Method	Cobalt Found, % 1	2	3	Average
1-Nitroso-2-naphthol	8.39	8.39		8.39
Nitrite-permanganate	8.41	8.50	8.45	8.45
Colorimetric	8.40	8.38		8.39
Certified by National Bureau of Standards				8.45
Variation among N.B.S. analysts				8.40–8.54

In Figure 4 the data for the three series of solutions are plotted as per cent transmittancy against logarithm of the water concentration. Because the process is a "subtraction" method—that is, increasing amounts of water increase the transmittancy—application of Ringbom's method of plotting (*1*, *6*) is more convenient if the ordinates are transmittancies rather than absorptancies, so that low values of photometric data correspond to low percentages of constituent determined. Using 1000 p.p.m. of cobalt, the optimum range for the measurement is from 2 to 5% water, with a relative analysis error of 1.5% per 1% absolute photometric error, or 0.3% in terms of the precision of the measurement; this is about twice the accuracy attainable with systems that follow Beer's law (2.7% relative analysis error per 1% absolute photometric error). Using 400 p.p.m. of cobalt, the optimum range for the measurement is from 1 to 4% water, with a relative analysis error of 2.5% per 1% absolute photometric error, or 0.5% in terms of the precision of the measurement. Although the use of only 200 p.p.m. of cobalt covers a range of slightly lower water concentration, it does so only at the expense of considerable decrease of accuracy.

For the application of the method to ethanol solutions containing more than 5% water, the appropriate volume of a concentrated cobalt chloride–ethanol reagent solution (to give, in the final solution measured, a standard amount of cobalt—e.g., 400 or 1000 p.p.m.) is added to an aliquot of the sample suitable to get within the optimum range, and the mixture is made up to definite volume with absolute ethanol for the transmittancy measurement. For original ethanol samples containing less than 1% water, a known amount of water can be added to bring the final solution within the optimum range for measurement of transmittancy, the original water content being found by difference.

Although the results confirm Winkler's (*9*) statement that the color change could be used to detect water in ethanol, the application of the method of the quantitative estimation of water in ethanol suffers somewhat from the rather large influence of temperature on the transmittancy, amounting to about −2% transmittancy per 1° C. temperature rise in the vicinity of 20° to 25° C.; rigorous temperature control is therefore required. Heat from the lamp of the instrument causes some change of transmittancy during the time required to make a measurement; for accurate work, a thermostated cell compartment should be used.

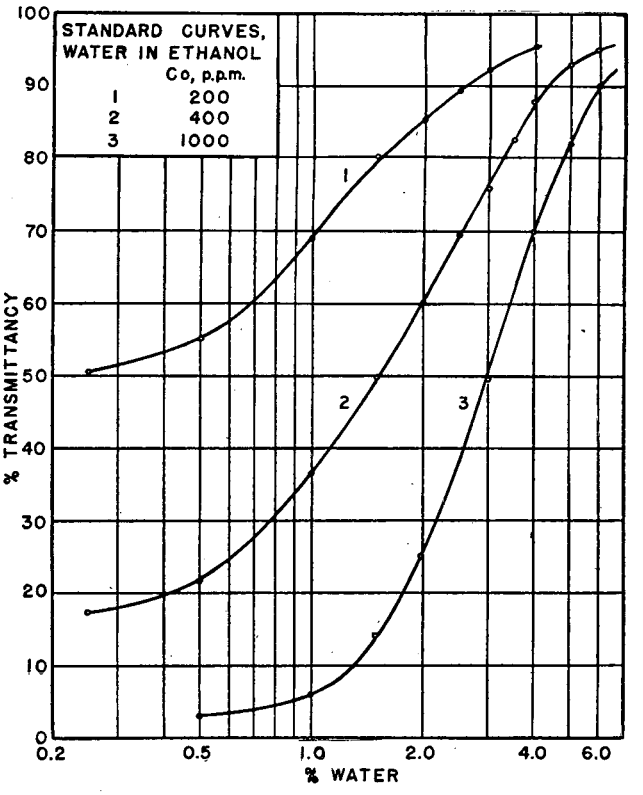

Figure 4

DISCUSSION

In view of the fact that small amounts of water modify the color of cobalt chloride–ethanol solution, the question arises as to the possible effect of the hydrate water when cobalt chloride hexahydrate is used as the standard for calibration of the method, whereas evaporation to dryness, during determinations, results in the formation of the monohydrate (*5*, p. 612).

To test this point, a standard solution having a cobalt concentration of 1000 p.p.m. was prepared by dissolving 0.4037 gram of cobalt chloride hexahydrate in ethanol to make exactly 100 ml. An identical weight of the hexahydrate was dissolved in a small amount of water and the solution was evaporated just to dryness; care was taken not to bake the residue. The solid was then dissolved in ethanol and made up to exactly 100 ml. Substandards, containing 200 p.p.m. of cobalt, of each solution were prepared and their transmittancies were measured at several wave lengths covering the 655 mμ transmittancy minimum. There was no significant difference in transmittancy between the two solutions. For a solution containing 200 p.p.m. of cobalt (near the middle of the optimum range for cobalt determination) the difference between hexahydrate and monohydrate would account for about 0.03% water in the ethanol solution, an amount which is not significant in relation to the precision of the measurements involved in determinations. Use of the hexahydrate for constructing the standard curve for cobalt determination is therefore recommended.

Another standard solution, prepared from cobalt chloride hexahydrate which had been heated for 2 hours in an oven at 110° C. was faintly turbid, and gave transmittancy readings about

0.8% (absolute) lower than the hexahydrate standard. A fourth sample, dissolved in water, evaporated to dryness, and baked for about 10 minutes, gave a very turbid suspension in ethanol, and was therefore unsuitable.

The formation of the sparingly soluble basic salt when the monohydrate is heated above 100° C. precludes this method of preparing standards, and indicates that care must be taken, in analyses, to evaporate just to dryness, avoiding baking of the residue.

For estimating water in ethanol by the use of cobalt chloride reagent, it would be immaterial whether hexahydrate or monohydrate is used, inasmuch as the calibration and determination are made by the use of the same standard solution.

If desired, the determination of cobalt can be made using ordinary commercial (about 95%) ethanol instead of absolute ethanol; in this case the calibration curve, for measurements at 655 mμ, is nearly parallel to the curve of Figure 2, but covers a cobalt concentration range about ten times as high. Spectrophotometrically, solutions of cobalt chloride in 95% ethanol have considerable components of both blue and red; solutions containing about 5000 p.p.m. (5.00 mg. per ml.) of cobalt are visually blue, and with decreasing cobalt concentration the solutions show gradations through bluish purple to reddish purple. Measured at 655 mμ (the absorption maximum of blue ethanol solutions), the solutions in 95% ethanol showed considerable deviations from Beer's law, but in such a way as to increase the analysis accuracy; at 515 mμ (the absorption maximum of pink dilute aqueous solutions; see Figure 3, curve 8) the measurements followed Beer's law, but a calibration curve based on these measurements is flat and if used for analysis would give larger relative error.

The specifications of range and accuracy given herein for the cobalt determination apply to the measurements made against a blank, using the Coleman Model 10-S spectrophotometer with 1.30-cm. absorption cells. As with other spectrophotometric methods, for a given wave length and cell thickness the range of the cobalt determination can be extended upward by measuring against a standard solution of concentration somewhat lower than that of the solution measured; the standard is so chosen that the transmittance ratio is near the optimum—theoretically 37%, although the analysis accuracy is almost as good at transmittancies from about 20 to 60%. When a Beckman spectrophotometer is used, the range can also be extended upward, and with some increase in accuracy, by the use of the 0.1 selector switch for measuring transmittancies below 11% (*1*).

The rather high concentration range of the method for cobalt is a good illustration of the fact that spectrophotometric methods of analysis are not necessarily limited to the determination of small amounts of constituent, but can apply to concentrations comparable to those used in gravimetric and titrimetric methods (*1*, *6*).

In comparison with gravimetric and titrimetric methods for cobalt, the proposed spectrophotometric method is somewhat more rapid, and gives results of comparable precision and accuracy. The 1-nitroso-2-naphthol method requires filtration, washing, and ignition to constant weight, all of which are time-consuming. The nitrite-permanganate method requires 12 to 24 hours' standing for precipitation of the hexanitritocobaltiate, followed by filtration, washing, dissolving, and back-titrating. The electrodeposition method requires fuming down with sulfuric acid, and an electrolysis time of 2 hours. In contrast, the proposed colorimetric method, although requiring evaporation of the solution just to dryness, is very rapid from that point on to the measurement of the desired constituent.

LITERATURE CITED

(1) Ayres, G. H., ANAL. CHEM., **21**, 652 (1949).
(2) Brode, W. R., *Z. physik. Chem.*, **A187**, 211 (1940).
(3) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," rev. ed., p. 603, New York, Macmillan Co., 1946.
(4) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A., "Chemical Analysis of Iron and Steel," pp. 339–42, New York, John Wiley & Sons, 1931.
(5) Mellor, J. W., "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. XIV, New York, Longmans, Green and Co., 1935.
(6) Ringbom, A., *Z. anal. Chem.*, **115**, 332 (1939).
(7) Toporescu, E., *Compt. rend.*, **192**, 280 (1931).
(8) Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," 9th English ed., Vol. II, p. 197, New York, John Wiley & Sons, 1942.
(9) Winkler, C., *J. prakt. Chem.*, [1] **91**, 209 (1864).

RECEIVED November 8, 1948. Condensed from a thesis submitted by Betty Vining Glanville to the faculty of the Graduate School of the University of Texas in partial fulfillment of the requirements of the degree of master of arts, August 1948.

Colorimetric Determination of Capsaicin in Oleoresin of Capsicum

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A method for the colorimetric determination of capsaicin in oleoresin of capsicum has been developed in which the readily available vanillin is employed for the standard solution in place of capsaicin.

THE most important constituent of red pepper is the pungent principle known as capsaicin, discovered by Thresh (*8*) in 1876. In 1898 Micko (*6*) showed that the substance had the properties of a weak phenol and contained one methoxyl group. He found also that with an alcoholic solution of platinic chloride an odor of vanilla was developed on standing. In 1919 the structure of capsaicin was established by Nelson (*7*), who showed it to be the vanillyl amide of isodecenoic acid.

Because the pungency of different varieties of peppers varies enormously, there has long been a demand for an accurate method for the determination of capsaicin content. The organoleptic method formerly official in the United States Pharmacopoeia and later in the National Formulary has now been discarded entirely. Tice (*9*) brought out a colorimetric method based on Fodor's (*2*) reaction in which capsaicin gives a blue color with vanadium oxytrichloride. A study of this method by Hayden and Jordan (*5*) showed that the results were unreliable. However, some of Tice's recommendations relative to the isolation of capsaicin, modified to meet the requirements of an analytical procedure, have been incorporated in the method described here.

Folin and Denis (*3*) devised a solution of phosphotungstic-phosphomolybdic acid which gives a blue color with phenols, and applied this reagent to the determination of vanillin (*4*) in vanilla extracts. This is now an official method (*1*) of the Association of Official Agricultural Chemists.

A colorimetric method of analysis of capsaicin using the pure drug as a standard would be unsatisfactory, as the preparation of pure capsaicin is a difficult, tedious, and very unpleasant task. This isolation of pure capsaicin has been circumvented by the discovery that vanillin, which like capsaicin contains a phenolic hydroxy group in the same relative position, serves just as well as capsaicin for the standard solution. The molecular weight of vanillin is 152, while that of capsaicin is 305. For practical purposes the latter may be considered double the former, so that 5 ml. of a solution containing 0.5 mg. of vanillin are equivalent to 1.0 mg. of capsaicin. Although this relationship is assumed, we have in hand a practical means for comparing the pungencies of different oleoresins of capsicum.

Before applying the colorimetric test, however, it is necessary to isolate the capsaicin present in the sample to be tested, in a sufficient degree of purity, in order to eliminate other substances of a phenolic nature which also give a blue color with the phosphotungstic-phosphomolybdic acid reagent. It is believed that the number of steps necessary to accomplish this purpose has been reduced to the minimum possible under the circumstances.

Duplicate results obtained by the application of this method are in excellent agreement, as evidenced by the following table:

Sample No.	% Capsaicin	
1	4.96	4.81
2	3.07	3.07
3	1.63	1.63
4	1.19	1.20
5	1.12	1.04
6	0.51	0.45
7	0.38	0.36

The substitution of vanillin for capsaicin in the present analytical procedure suggests that similar procedures may be applicable to some other colorimetric determinations in which the substances to be determined are not readily obtainable in a pure state and in which other substances of suitable composition are so obtainable and can be used for the preparation of the standard solutions.

ANALYTICAL PROCEDURE

Special Reagents. Ultrasene. This is purified, deodorized kerosene much more suitable for analytical work than kerosene itself. Kerosene can be used, if it is treated with sulfuric acid and redistilled.

Acetone, 60% by volume.

Phosphotungstic-Phosphomolybdic Acid. To 100 grams of pure sodium tungstate and 20 grams of phosphomolybdic acid (free from nitrates and ammonium salts) add 100 grams of sirupy phosphoric acid (containing 85% H_3PO_4) and 700 ml. of water; boil over a free flame for 1.5 to 2 hours; then cool, filter if necessary, and make up with water to a volume of 1 liter. An equivalent amount of pure molybdic acid may be substituted for the phosphomolybdic acid.

Standard Vanillin Solution. Dissolve 0.1 gram of vanillin in sufficient distilled water to make 1000 ml. This solution must be freshly prepared each day.

Procedure. Weigh 1.0 gram of oleoresin red pepper in a small beaker and transfer to a 125-ml. Squibb separatory funnel by solution in 20 ml. of ultrasene, using the ultrasene in portions. Dissolve 1.0 gram of sodium chloride in 80 ml. of 60% acetone (by volume) and wash out the beaker with 20 ml. of this solution, in portions, transferring the washings to the separatory funnel. Shake the funnel sufficiently to keep the liquids well mixed and continue this gentle shaking for about 5 minutes. On standing, the mixture separates within 2 or 3 minutes into two sharply defined layers but the lower layer is always cloudy. Draw the lower layer into a 125-ml. Squibb separatory funnel and continue the extraction of the solution of oleoresin in like manner, using the balance of the acetone solution in 20-ml. portions. To the combined extractions add 5 ml. of ultrasene and shake gently for a few minutes. Let stand 1 hour to separate. Draw off the still hazy lower layer into a 100-ml. volumetric flask containing 0.5 gram of Filter-Cel, cork the flask, and shake 0.5 hour in a machine. Make up to the mark with 60% acetone, mix thoroughly, a filter through a dry double filter. The filtrate should be p fectly clear.

Pipet 50 ml. of the clear filtrate into a 250-ml. beaker marked 20 ml. and evaporate on top of a steam bath (not directly over t steam) at a temperature not over 65° C., using a small thermo eter as a stirring rod, until the volume of liquid is reduced to 20 By this treatment the acetone is removed from the solution a the crude capsaicin separates as an oily sediment. Solutions capsaicin should be heated as little as possible and at as low a te perature as possible.

Cool the liquid to room temperature, add 10 ml. of 0.5 sodium hydroxide, and stir until the oily sediment has dissolv Pour the solution into a 250-ml. Squibb separatory funnel, a wash the beaker with two further 5-ml. portions of 0.5 *N* sodi hydroxide and finally with two 5-ml. portions of water, pouring t washings into the separatory funnel. Now add to the funnel grams of sodium bicarbonate and 150 ml. of petroleum eth shake moderately 15 minutes, and let stand until the layers sep rate sharply (overnight, if necessary). The amount of petroleu ether is sufficient for 1.0 gram of a normal oleoresin. In spec cases it may be necessary to use a larger quantity of solvent.

Draw off and reject the lower layer and carefully filter t upper layer into a clean 250-ml. Squibb separatory funnel, was ing the separatory funnel and the filter with small portions petroleum ether. It is essential that the yellow substance whi separates at this point be carefully excluded from the filtra Shake the petroleum ether solution with 10 ml. of 0.5 *N* sodi hydroxide, add 10 drops of 95% ethyl alcohol, and without furth shaking let stand until the layers separate sharply. Filter t lower layer into a 50-ml. volumetric flask and extract the pet leum ether further with three 10-ml. portions of water, passing t extractions successively through the filter into the flask. Fill the flask with water to the 50-ml. mark and mix thoroughly. Th solution should be nearly colorless. The concentration remai the same as the 50 ml. of clear filtrate originally taken for eva ration.

Pipet 5 ml. of the solution into a 50-ml. volumetric flask a into another 50-ml. volumetric flask pipet 5 ml. of standard van lin solution. To each flask add from a pipet 5 ml. of the ph photungstic-phosphomolybdic acid reagent, allowing it to fl down the neck of the flask in such a way as to wash do the solution that may be on the sides of the flask. Mix co tents of flasks by rotating and after 5 minutes dilute contents 50 ml. with saturated sodium carbonate solution. Mix th oughly by inverting the flasks several times and shaking and th place the flasks in a shaking machine until 30 minutes ha elapsed since the phosphotungstic-phosphomolybdic acid reage was first added to the solutions. This thorough shaking is nec sary in order to precipitate the sodium phosphate completely a prevent the filtrate from becoming hazy while the solution is bei read in the colorimeter. Filter the solutions through dry doul filters and compare the blue colors of the clear solutions witho delay in a colorimeter.

With samples poor in capsaicin, there may be a slight hue d ference between the standard solution and the test solution cause then the traces of color carried through from the oleore have a greater influence on the total color. This does not int fere in any way with the usefulness of the method. In this labo tory it is customary for two observers to read the color and th results uniformly agree within one or two tenths of the col imeter scale. It is essential that the blue solutions be perfec clear. Ordinarily the standard blue color is set at 20, but if t test solution is pale it may be necessary to set the standard at or even 5. After a reading is made, the positions of the cu should be reversed and another reading made. The average these two readings is used for the calculation. The zero points the colorimeter should be checked and corrected if necessary fore the instrument is used.

If it is a question of determining capsaicin in the spice, 5 to grams of the ground material are extracted with acetone ether in a Soxhlet extraction apparatus and the extract is tested above described.

ACKNOWLEDGMENT

Acknowledgments are due to V. H. Fischer, vice president Dodge & Olcott, Inc., who assigned this problem for study, Herman Wachs, director of research, who supervised the prepa

ion of this material for publication, and to Thaddeus Ptaszynski, Philip Catanzaro, and Thomas Medwick who performed a large part of the experimental work.

LITERATURE CITED

1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 6th ed., p. 366, 1945.
2) Fodor, *Z. Untersuch. Lebensm.*, **61**, 94 (1931).
(3) Folin and Denis, *J. Biol. Chem.*, **12**, 239 (1912).
(4) Folin and Denis, *J. Ind. Eng. Chem.*, **4**, 670 (1912).
(5) Hayden and Jordan, *J. Am. Pharm. Assoc.*, **30**, 107 (1941).
(6) Micko, *Z. Nahr. Genussm.*, **1**, 818 (1898); **2**, 411 (1899).
(7) Nelson, *J. Am. Chem. Soc.*, **41**, 1115 (1919).
(8) Thresh, *Pharm. J. Trans.* (3), **7**, 21, 259, 473 (1876–77); **8**, 187 (1877–78).
(9) Tice, *Am. J. Pharm.*, **105**, 320 (1933).

RECEIVED October 9, 1948.

Rapid Identification of Manganese Dioxide Ores

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Manganese dioxide ores obtained from different geographic locations and as a result of different methods of preparation differ in their depolarizing ability when used in the common Leclanché type of dry cell. Ores are commonly tested for their quality by constructing an actual cell and making suitable measurements on its current capacity and shelf life. Because such tests are time-consuming, a rapid method for testing ores would be of great interest. Through the use of the pulse polarizer, it was possible to differentiate in a few minutes between the poor and good ores in a set of samples. The ores were rated independently on the basis of test cells, and the comparisons between predicted and actual depolarizing ability were, in the majority of cases, found to be good.

IN A dry cell of the common Leclanché type, a depolarizer or oxidizing agent is used to provide the cathodic reaction. Manganese dioxide is ordinarily used for this purpose, but the depolarizing characteristics of different commercial batches vary considerably. Ordinary chemical and x-ray analytical methods have not been used extensively in the evaluation of manganese dioxide ores. The method presented here shows promise of providing a rapid and reliable means of identifying good and poor battery depolarizer ores.

The depolarizing characteristics of manganese dioxide ores are ordinarily evaluated on the basis of dry cell tests, carried out in special test cells, which are rather complicated and time-consuming to construct. Rapid evaluation of the characteristics of manganese dioxide ores, from the standpoint of not only immediate capacity but also shelf life, is highly desirable from the standpoint of battery makers and ore suppliers. Although these characteristics have not been studied, the work described here indicates that it may be possible to establish rapidly the depolarizing characteristics of an ore as measured by the initial capacity of the cell.

THE PULSE POLARIZER

The method described here involved use of the pulse polarizer, developed during 1947. The instrument employs a system of electronic circuits to polarize an electrode over a brief time interval. The polarization and depolarization at the surface of the electrode are recorded continuously on a high-speed strip chart (Brown Instruments Division, Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.; single record; full scale, 5 mv.) and show up as a curve which is distinctive for each set of conditions. The polarizing circuit consists of a condenser (125 mfd.) which is charged to 310 volts. Discharge of this condenser brings about the polarization at the electrode surface. The polarization potential is amplified by means of an electronic direct current voltage amplifier, and then recorded. The pulse polarizer has been successfully employed in the field of corrosion research (*2–4*).

EXPERIMENTAL TECHNIQUE

A technique has been developed which permits rapid study of the depolarization characteristics of manganese dioxide ores. A platinum cylinder 0.5 inch in diameter is filled with ore and tamped until tightly packed. In the top of the cylinder an electrolyte is added, in which an "inert" electrode is immersed. This electrode, which merely completes the electrical circuit, is a fine iron wire. A thin calomel half-cell is then lowered into contact with the ore surface. The experimental setup is shown in Figure 1.

Either the cathodic or anodic polarization may be studied with the apparatus, but the cathodic polarization is of interest in this particular case. By cathodic polarization is meant the change in the electrode potential of the manganese dioxide when electrons are forced into it from the external circuit.

The voltage applied is constant for each pulse, and the time during which the ore surface is polarized is between 0.05 and 0.1 second. Experiments showed that the curves obtained with platium alone are long but very narrow, radically unlike those obtained with the ores. On this basis, the results must be attributed almost entirely to the depolarizing characteristics of the ore.

The electrical discharge obtained from the pulse polarizer is standardized to the extent that curves obtained are, for the most part, reproducible in minute detail, and curves obtained with a

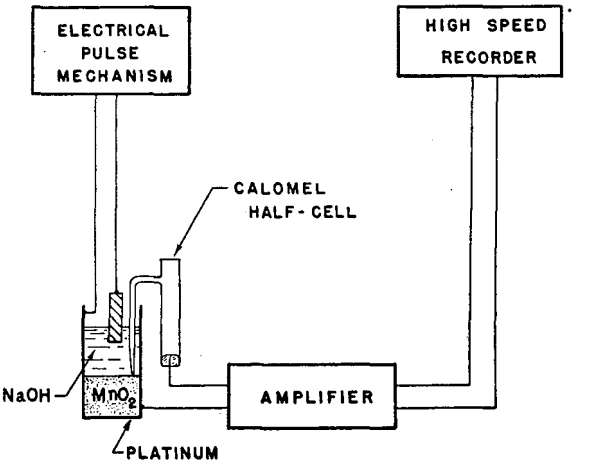

Figure 1. Experimental Setup

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Table I. Summary of Manganese Dioxide Data

Sample	Signal Corps Laboratory Rating	Reciprocal Pulse Polarizer Slope	Average Deviation, 2 Runs
A	Excellent	0.264	0
B	Very good	0.252	0.022
C	Very good	0.615	0.012
D	Good	0.873	0.050
E	Fair	0.788	0.008
F	Poor	0.787	0.002
G	Poor	0.808	0.012
H	Poor	Curved line	..

given sample on different days are entirely comparable. Thus the depolarization characteristics of the ores might furnish a method of identifying them.

When a neutral or acidic electrolyte was used, the curves obtained with different ores did not appear to differ markedly. However, a 2.5 *N* sodium hydroxide solution when used as the electrolyte gave curves that varied widely with different samples. Any particular sample gave a reproducible curve, however.

RESULTS AND CONCLUSIONS

The results with seven different ores are given in Figure 2. These curves were redrawn directly from the chart paper. The vertical axis, representing relative potential, has a full scale of about 0.25 volt. The horizontal axis represents relative time, about 2.5 minutes being equal to the full width. All the curves start approximately at a common point, which might be considered the "normal" potential of the ore. When a pulse is applied, the potential drops to a new value corresponding to the polarization under extremely high current density. Then the curves return toward the normal potential at various rates. In most cases, the test is complete within 2 or 3 minutes, and curves for even the very slowly depolarizing ores return within 5 minutes.

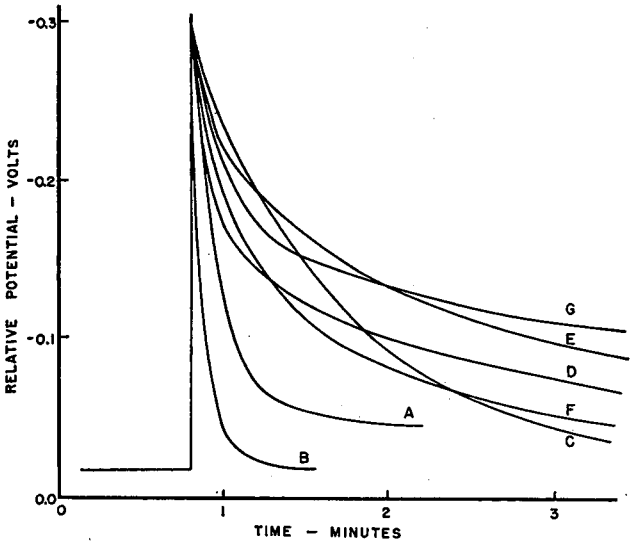

Figure 2. Depolarization Curves for Manganese Dioxide Ores

The ores in this figure (A to G), were also tested in actual dry cell construction. The ores that performed best in the dry cell test, as far as initial capacity goes, were the ones that produced the quickly depolarizing curves.

Up to this time, no further work has been done on the best choice of electrolyte. Inasmuch as manganese dioxide undoubtedly adsorbs hydroxyl ions, these may play a part in the depolarization. The depolarization of a cathode has been found by Hickling (*1*) to follow a logarithmic course, and hence one should expect a linear plot when potential is plotted against log of time. This linear plot assumes that the depolarization is a diffusion process; both diffusion from and into the bulk of the liquid could be involved. However, the plots discussed in this report indicate that the diffusion of polarization products away from the cathodic surface contributes most to the logarithmic decay of polarization.

The curves in Figure 2 were redrawn in terms of voltage or polarization potential as a function of "log of time." Representative graphs for an ore rated as excellent (sample A) and one rated as poor (sample H) are shown in Figure 3. The two curves for each ore were obtained a month apart, and indicate the reproducibility of the method.

From a study of the graphs, it appeared that a more readily detectable correlation might be obtained between "the slopes of the linear portions of the curves" and "the quality of the ore" than from the original curves drawn by the recorder (Figure 2). The relative slopes were therefore determined and are given in Table I.

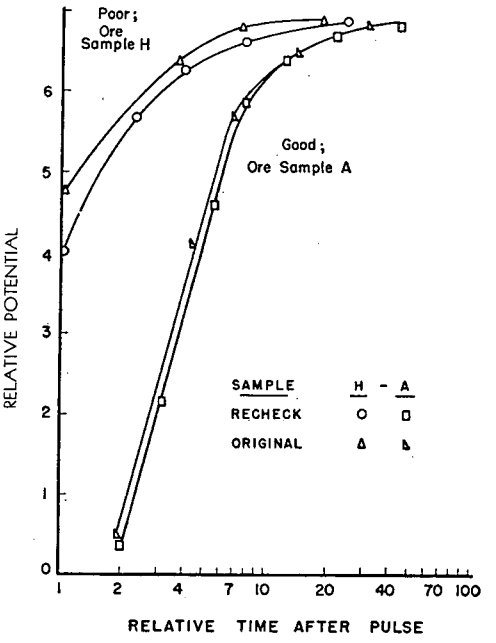

Figure 3. Representative Curves for Excellent (A) and Poor (H) Ores

In general, the ores fell into line. The only notable exception was ore D which was rated as "good" on the basis of actual cell test but on the basis of pulse polarization curves must be rated as poor, from the standpoint of a depolarizer, because of its relatively large slope. The theoretical justification for this criterion for classifying the ores as regards their value as depolarizers is, of course, that the slopes represent the rate of depolarization.

An essentially linear plot was given by all ores, for the greater part of the graph (especially the initial part), except that sample H was rated "very poor" on the basis of test cells. This ore gave a curved line as indicated in Figure 3. This is an important clue—namely, that it is the logarithmic diffusion away from the surface which is responsible for the character of the pulse polarizer curves. The poor ores apparently do not respond with the same depolarizing mechanism and hence are easily identified.

Figures 4 and 5 show curves obtained when eleven additional ores supplied by the Signal Corps were tested by means of the pulse polarizer. These ores were furnished as unknowns and the Signal Corps data were supplied after the pulse polarizer results were made known. The order of decreasing slope was: 4, 8, 9, 1, 7, 10, 6, 5, 3, 2, 11.

Table II summarizes the predictions made with the pulse polarizer data for these eleven ores. The predictions were first

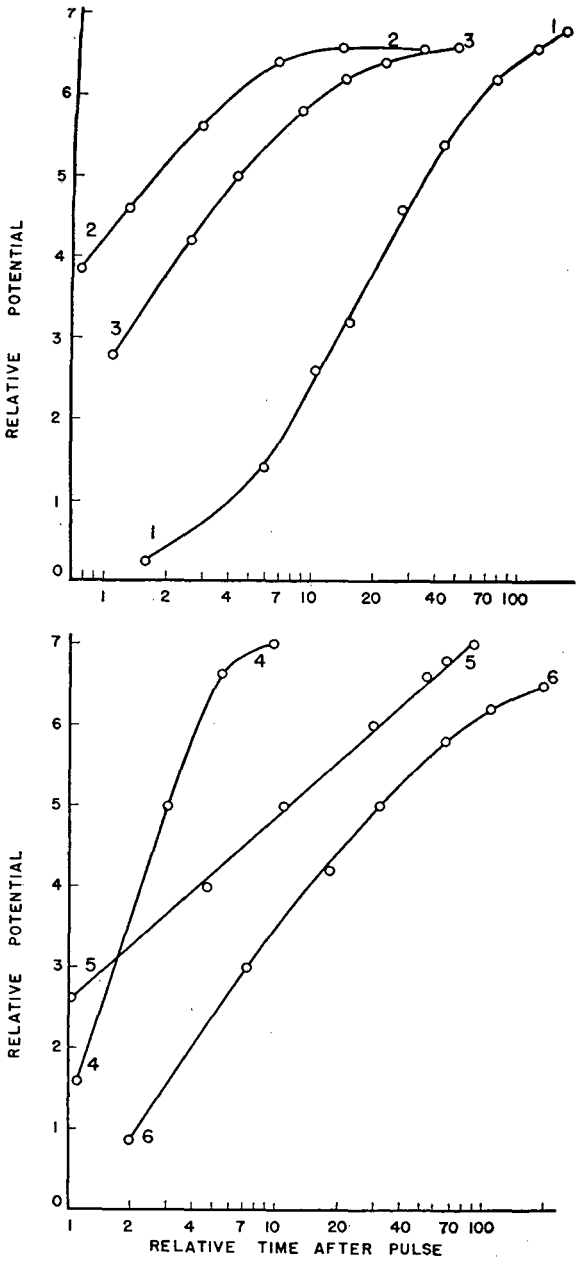

Figure 4. Pulse Polarizer Tests on Ores

made on the basis of the shape of the curve; however, five of the ores were not like those previously studied and consequently their curves did not lend themselves to comparison with known data. Based on the slope of the linear part of the curve, predictions made with the pulse polarizer were surprisingly accurate. It was immediately possible to differentiate the good and excellent ores from the poor ones.

It is not known whether pulse polarizer data are relevant as far as shelf life is concerned. It appears, however, that through the use of this method, the initial capacity of the ore can be reasonably well predicted.

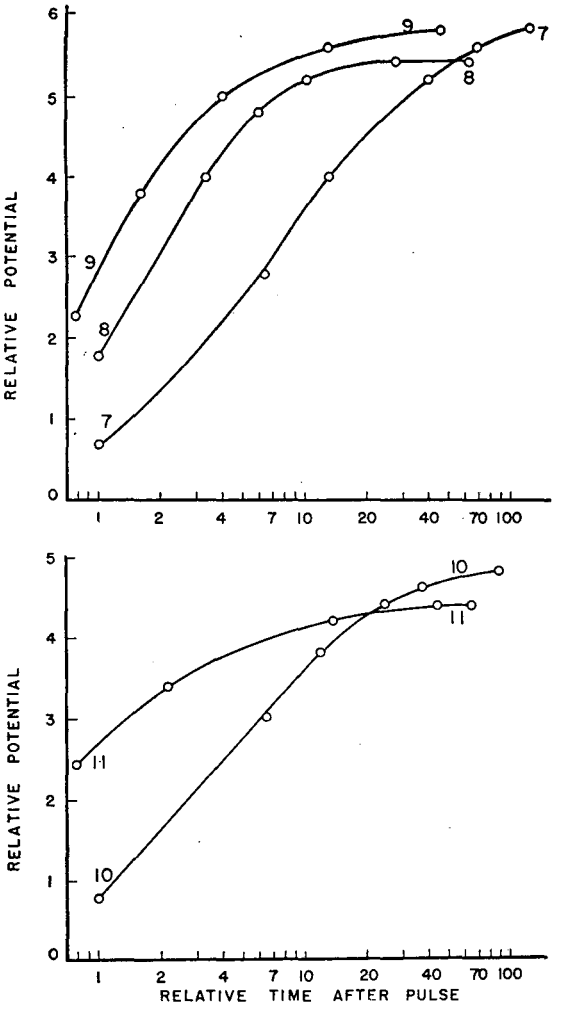

Figure 5. Pulse Polarizer Tests on Ores

Table II. Predictions on Eleven Ores

Rating	Predictions Based on: Shape of curve	Predictions Based on: Slope of linear part	Signal Corps Rating
Good-excellent	4	4	4
		8	8
		9	9
Good-fair	1	1	1
	6	7	5
	7	10	3
	10	6	7
Poor	2[a]	5	2
	3	3	6
	5	2	10
	8		
	9		
No depolarizing ability	11	11	11

[a] This group was not like any previously studied.

LITERATURE CITED

(1) Hickling, A., and Salt, F. W., *Trans. Faraday Soc.*, 37, 224 (1941).
(2) Marsh, G. A., *Corrosion and Material Protect.*, 5, No. 1, 15 (1948).
(3) Marsh, G. A., and McDonald, H. J., "Proceedings of the Pittsburgh International Conference on Surface Reactions," Pittsburgh, Pa., Corrosion Publishing Co., June 1948.
(4) Misch, R. D., Marsh, G. A., and McDonald, H. J., "Proceedings of the First University Conference on Corrosion and Metal Protection," Pittsburgh, Pa., Corrosion Publishing Co., in press.

RECEIVED November 2, 1948. Presented before the Division of Physical and Inorganic Chemistry, Symposium on Galvanic Cells and Batteries, at the 113th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill. The research work discussed in this paper was done under Contract No. W-36-039-sc-32065, with the U. S. Signal Corps, Squier Signal Laboratory, Fort Monmouth, N. J.

Quantitative Spectrochemical Determination of Lead and Zinc in Ores

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A spectrochemical method applicable to the determination of 0.05 to 6% of lead and 0.05 to 6% of zinc in antimony, tin, and copper ores is described. The specific examples discussed are the determination of lead and zinc in antimony sulfide and oxide ores. This method employs the high amperage direct current arc, external standards, lithium carbonate buffer, and fine wire-mesh screen light filters. Accurately weighed samples and carefully controlled arc conditions make possible the elimination of an internal standard. A statistical study of the results obtained by this method compared with the results obtained by chemical analysis indicates no significant difference.

UNITED States Customs Laboratories make numerous determinations of the lead (Public Law No. 725, June 19, 1948, suspends import duties on lead-bearing ores until June 30, 1949) and zinc content of a wide variety of ores such as antimony, copper, tin, and tungsten (*4*). Available chemical procedures are both tedious and time-consuming, and they involve difficult fusions and troublesome separations, particularly where the concentrations of lead and zinc are low. This paper deals with a spectrochemical procedure which has to a large extent replaced the chemical methods in this laboratory and which has at least comparable accuracy as well as great advantage in time economy.

PROCEDURE

Preparation of Standards and Samples. The preparation of standards consists of incorporating into a base of stibnite known amounts of lead and zinc as the oxides. The initial standard containing 6% each of lead and zinc is diluted successively to give a series of standards containing 3, 1.5, 0.75, 0.38, 0.19, and 0.09% each of lead and zinc.

A systematic search for a suitable spectroscopic buffer indicated that a mixture of 1 part of graphite to 2 parts of lithium carbonate (reagent grade) was suitable. By mixing 1 part of ore or standard with 2.5 parts of buffer it is possible to get a smooth-burning, nonwandering arc which gives very reproducible excitation. A highly satisfactory method of mixing the ore and flux involves the use of the Wiggle Bug shaker described by Helz and Scribner (*2*). The samples as received by this laboratory are generally commercially prepared samples requiring no further preparation. Crude samples are pulverized, quartered, and finally ground to −200-mesh.

Description of Equipment. The spectrograph is a 3-meter grating spectrograph manufactured by Baird Associates. This instrument has a 15,000-line-per-inch grating and gives a reciprocal dispersion of 5.6 Å. per mm. in the first order. Auxiliary optical equipment consists of a quartz condensing lens used to form an image of the electrodes on the grating mask, and light transmission filters made of fine wire-mesh screen. A combination of a 325- and 100-mesh screen placed in front of the slit gives lines of suitable density for densitometry with a minimum of background when used in combination with a 75-micron slit and S.A.1. plates. The excitation unit is an A.R.L. direct current arc source giving a maximum current of 15 amperes with an initial gap voltage of 250 volts. Line densities are measured with an A.R.L. nonrecording comparator-densitometer. The small charges necessary for analysis are weighed on a Roller-Smith torsion type microbalance and are transferred to cratered electrodes by means of a small ared pan. A water-cooled excitation stand, designed by Scribner nd Corliss (*3*), is used to hold the electrodes.

Electrodes. Regular grade graphite is used throughout. The bwer electrode (anode), made from 0.6-cm. (0.25-inch) graphite rds, has the following dimensions: depth of crater, 1 mm.; inside cater diameter, 5 mm.; diameter of center post, 2 mm. It was observed that the use of the center post to receive the initial impact of the arc served to minimize mechanical loss of the powdered charge. The counterelectrode (cathode), is a 0.3-cm. (0.125 inch) graphite rod tipped in a pencil sharpener.

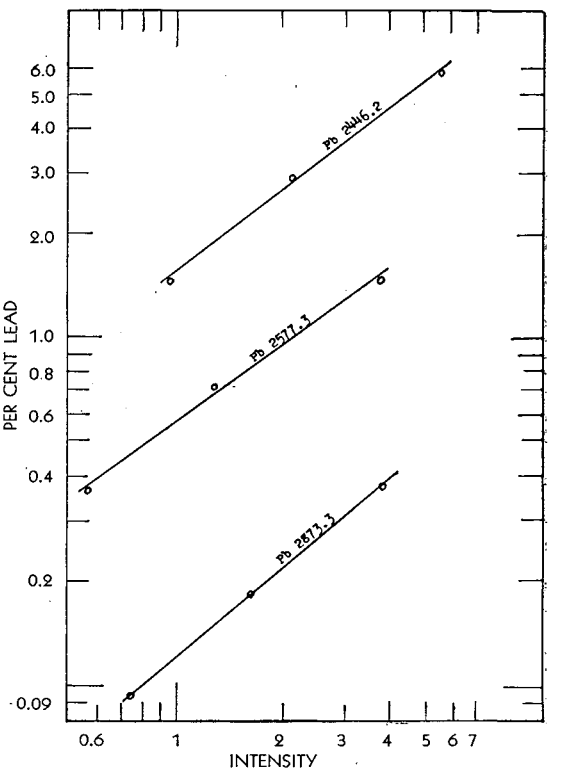

Figure 1. Working Curve for Determination of Lead

Excitation and Photography. Charges (20 ± 0.2 mg.) of previously prepared mixtures of the buffer and samples or standards are transferred to cratered electrodes, each sample mixture in triplicate and each standard mixture in duplicate. A drop of saturated alcoholic sugar solution is added to the packed electrodes and allowed to evaporate, thus cementing the charges firmly and minimizing mechanical loss. The charges are ignited in the direct current arc at 14 amperes for 20 seconds; a moving plate study indicated that the lead and zinc are completely vaporized in this period.

An auxiliary image aids in maintaining an arc gap of 2.5 mm., a distance which keeps the image of the glowing tips of the electrodes tangent to the grating mask and permits the sampling of energy from the entire arc. Spectra are recorded on Eastman Kodak S.A.1 emulsions and are developed for 2.5 minutes at 70° F. using Developer D-19 with continuous agitation.

Photometry. The analysis lines are indicated in Table I.

The analytical curves are prepared by plotting log per cent concentrations against log intensity. The intensities are obtained from plate calibration curves using the Dieke method of homogeneous lines (*1*). Typical analytical curves are indicated in Figures 1 and 2 for lead and zinc, respectively. In the procedure outlined here the upper limit of the zinc is 2%. To extend this range to 6% for zinc it is necessary to weigh a 5-mg.-charge instead of 20 mg. of both ores and standards.

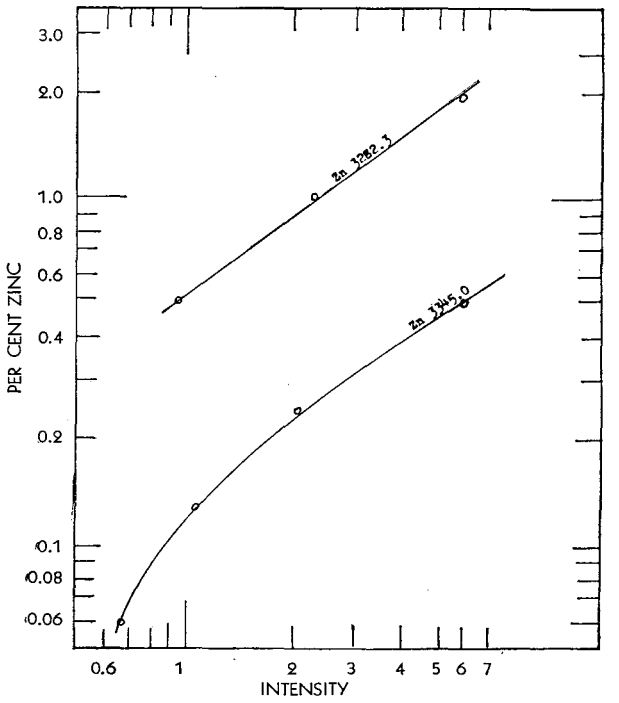

Figure 2. Working Curve for Determination of Zinc

Table I. Analysis Lines

Pb Concentration, %	Line	Zn Concentration, %	Line
6.0 -1.5	2446.2	2.0-0.5	3282.3
1.5 -0.38	2577.3	0.5-0.05	3345.0
0.38-0.05	2873.3		

RESULTS

Table II shows the results and statistical treatment of the spectrochemical lead determinations, in triplicate, of 17 antimony ores. Sample 7 is out of control and is not included in the calculations. The standard deviation of a single determination using $2 \times 16 = 32$ degrees of freedom is 0.061%. The standard error of the mean of three determinations is 0.035%. This indicates that replicate analyses by the spectrochemical method yield very close and acceptable results.

A comparison of the spectrochemical results with the corresponding lead results reported by commercial laboratories on certificates of analysis is given in Table III. As the assay certificates did not have values for zinc, a similar comparison of the zinc results was not possible.

The statistical treatment for precision and accuracy at the bottom of Table III follows the technique described by Youden (*5*). The results of sample 7 were not included in the calculations because one such exceptional case is given too great a weight in fitting a straight line by the method of least squares. The chemical results are the usual accepted values and are here considered "accurate" or without error.

Table II. Precision of Spectrochemical Method for Determination of Lead in Antimony Ores

Sample No.	Per Cent Lead Test 1	Test 2	Test 3	Average	Sum of Deviations Squared
1	0.20	0.22	0.23	0.217	0.00047
2	0.11	0.10	0.11	0.107	0.00007
3	0.54	0.62	0.75	0.637	0.02247
4	0.46	0.36	0.49	0.437	0.00927
5	0.14	0.19	0.17	0.167	0.00127
6	0.36	0.34	0.57	0.423	0.03247
7[a]	4.90[a]	4.35[a]	4.55[a]	4.600[a]	0.15500[a]
8	0.08	0.08	0.08	0.080	...
9	0.18	0.21	0.25	0.213	0.00247
10	0.11	0.13	0.15	0.130	0.00080
11	0.57	0.56	0.56	0.563	0.00009
12	0.20	0.25	0.22	0.223	0.00127
13	1.10	1.25	1.33	1.227	0.02727
14	1.17	1.03	1.08	1.093	0.01007
15	0.55	0.67	0.59	0.603	0.00747
16	0.72	0.66	0.65	0.677	0.00287
17	0.70	0.68	0.70	0.693	0.00027
				Total	0.11860

Standard deviation of single determination $= \sqrt{\frac{0.1186}{2 \times 16}} = 0.061\%$

Standard error of mean of three determinations $= \frac{0.061}{\sqrt{3}} = 0.035\%$

[a] Out of control, not included in calculations.

The straight line intercepts the y axis at about -0.002%. The slope is practically identical with the average ratio and shows no marked disagreement with the ratio of unity. Based upon the available data the line may be considered to pass through the origin, thus indicating that the spectrochemical method is free from systematic error.

Table III. Accuracy of Spectrochemical Method for Determination of Lead in Antimony Ores

Sample No	Per Cent Lead: Chemical results x	Spectrochemical results y	Ratio y/x
1	0.23	0.22	0.956522
2	0.13	0.11	0.846154
3	0.65	0.64	0.984615
4	0.46	0.44	0.956522
5	0.14	0.17	1.214286
6	0.43	0.42	0.976744
7[a]	4.70[a]	4.60[a]	0.978723[a]
8	0.07	0.08	1.142857
9	0.17	0.21	1.235294
10	0.17	0.13	0.764706
11	0.46	0.56	1.217391
12	0.24	0.22	0.916666
13	1.21	1.23	1.016529
14	1.06	1.09	1.028302
15	0.60	0.60	1.000000
16	0.66	0.68	1.030303
17	0.72	0.69	0.958333
Total	7.40	7.49	16.245224
Av.	0.4625	0.468125	1.0153265

Σx^2, 5.1420 Σy^2, 5.2979 Σxy, 5.2108

Intercept, $a = -0.001684$ Slope, $b = 1.015804$
$(n-2)s^2 = 0.0173643$
Standard deviation of single analysis (equivalent to standard error of means of three determinations), $s = 0.03522$
Standard deviation of intercept, $s_a = 0.01523$; $t_a = 0.11$, $P = 0.9$
Standard deviation of slope, $s_b = 0.02686$; $t_b = 0.18$, $P = 0.8$

[a] Not included in calculations.

LITERATURE CITED

(1) Dieke, O. H., "Study of Standard Methods for Spectrographic Analysis," War Metallurgy Committee Report, *Serial* **W-54** (1943).
(2) Helz, A. W., and Scribner, B. F., *J. Research Natl. Bur. Standards*, **38**, 439 (1947) **R.P. 1786.**
(3) Scribner, B. F., and Corliss, C. H., *J. Optical Soc. Am.*, **33**, 515 (1943); *J. Research Natl. Bur. Standards*, **30**, 41 (1943).
(4) Tariff Act 1930, Public Law No. 361, U. S. Government Printing Office, Washington, D. C., 1930.
(5) Youden, W. J., ANAL. CHEM., **19**, 946 (1947).

RECEIVED September 7, 1948.

Total Phenols in Gasolines and in Cresylic Acids

Spectrophotometric Determination

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A rapid spectrophotometric method for determination of total phenols in small samples of gasoline is described. The phenolic compounds are extracted from the sample with aqueous sodium hydroxide and the transmittance of the extract is measured at 290 mμ, near which phenolates have a strong absorption maximum. An approximate, empirical, specific extinction coefficient of 24 is used for the composite of phenols in gasoline when an actual value is not determinable. Thiophenols are included in the total and, in the absence of large quantities of alkyl mercaptans, their concentration can be roughly estimated from a transmittance measurement of the caustic extract at 265 mμ. The determination of phenols in gasoline is not affected by components such as nitrogen bases, carboxylic acids, hydroperoxides, and alkyl mercaptans. The method is applicable to the analysis of other phenol-containing materials, such as cresylic acids.

CRACKED gasolines generally contain small but significant and, nowadays, commercially important quantities of phenolic compounds. Such phenols, several years ago, were made the subject of an extended investigation (*1*). However, the method of analysis involving distillation, crystallization, and chemical separation gives far more data than are usually called for, is time-consuming, and cannot be applied to the determination of phenols in small samples of gasolines.

For the determination of phenols in small samples of gasoline and other materials, a colorimetric method (*2–4*), which takes advantage of the color produced by the quinoid form of the nitrosophenols, is fairly rapid and rather widely applicable. The absorption curves of the individual nitrosophenols in ammoniacal solution vary so widely, however, that for mixed phenols, such as are present in gasoline, large errors are possible.

In the present work it was noted that phenol, cresols, and xylenols, the main phenolic components of gasoline, show in alkaline solution a strong, broad maximum absorption near 290 mμ. Although the wave length of the absorption maximum for the various phenolates examined ranges from 288 to 296 mμ, the breadth of the band in all cases is such that none is far from its maximum absorption at 290 mμ. The specific extinction coefficients of the alkaline solution of the individual phenols tested all fall within the limits of 21 to 30 (see Table III).

Briefly, phenols are determined in gasoline by extraction of the sample with a solution of 10% aqueous sodium hydroxide. The alkaline solution is then diluted and its optical density at 290 mμ is measured. Unless typical purified petroleum phenols are available for calibration, it is recommended that an extinction coefficient of 24 liters per gram cm. be used for the phenols in gasoline.

The elapsed time required for the analysis is generally no more than 15 minutes, the volume of gasoline sample needed is usually only a few milliliters, and a minimum of sample preparation is required. In favorable cases, analyses may be made on gasolines containing as little as 0.002% phenols.

The method may be applied not only to phenols in gasolines but also to other phenolic samples such as cresylic acids.

APPARATUS

The absorption measurements were made on a Beckman spectrophotometer, model DU, using 1-cm. quartz cells. These cells, with reasonable care, are not appreciably attacked by the dilute sodium hydroxide solution. Indeed, in the author's laboratory a pair of such cells has been used for a year in several hundred analyses with practically no change in transmission characteristics. However, the alkaline solutions are not allowed to remain in the cells unnecessarily—for example, overnight.

PROCEDURE

When the range of concentration is not known, place a convenient volume—for example, 15 ml.—of sample in a small separatory funnel and shake vigorously for 1 minute with an equal volume of 10% sodium hydroxide. Allow the layers to separate and withdraw a portion of the alkaline solution. Dilute 1 ml. of this solution with water to exactly 25 ml. Compare the optical density of the diluted solution at 290 mμ with that of 0.4% aqueous sodium hydroxide at this wave length.

If the optical density of the diluted solution is too great for accurate work—i.e., about 0.9 on the Beckman unit—dilute the solution further with 0.4% aqueous sodium hydroxide. If the optical density is too low—i.e., about 0.3 on the Beckman unit—employ a larger ratio of gasoline to alkaline solution in the extraction step.

When the approximate range of concentration of the phenolic material is known, the data in Table I on ratios of sample to caustic solution and the suggested dilutions will be helpful, though the optical densities of the solutions to be tested cover a range somewhat wider than the 0.3 to 0.9 recommended above.

Calculation. Calculate the percentage of phenols in the sample from the expression:

$$\text{Phenolic content, } \% = \frac{D_{290} \times A \times B \times 100}{V \times \text{sp. gr.} \times 24}$$

where D_{290} is optical density observed at 290 mμ; A is milliliters of sodium hydroxide used in extraction of sample; B is liters to which 1 ml. of alkaline extract was diluted; V is milliliters of sample extracted; and 24 is the approximate, empirical, specific extinction coefficient (liters per gram cm.) of the sodium hydroxide solution of the mixed phenol aggregate generally found in gasoline.

PHENOLS IN CRESYLIC ACIDS AND SIMILAR PRODUCTS

Weigh out a quantity of sample according to the estimated concentration range given in Table II, dissolve in iso-octane, and dilute to 25 ml. Extract with 25 ml. of 10% sodium hydroxide, dilute, and measure the absorption at 290 mμ as directed under the procedure for phenols in gasoline.

Table I. Suggested Sample Size, Phenols in Gasolines

Approximate Concentration Range % From	To	Volume of Gasoline *Ml.*	Volume of 10% NaOH *Ml.*	Volume to Which 1 Ml. of Caustic Extract Should Be Diluted *Ml.*
0.001	0.005	100	5	25
0.005	0.02	25	5	25
0.02	0.1	15	15	25
0.1	0.5	15	15	125
0.5	2.5	5	25	125

Table II. Suggested Sample Size for Petroleum Phenols, Cresylic Acids, etc.

Approx. Concentration Range % From	To	Wt. of Sample *Gram*	Vol. of Hydrocarbon as Solvent *Ml.*	Vol. of 10% NaOH *Ml.*	Vol. to Which 1 Ml. of Caustic Extract Should Be Diluted *Ml.*
2.5	10	0.2	25	25	25
10	50	0.05	25	25	25
50	100	0.1	25	25	125

Calculation. If the volume of solvent used for the sample is equal to the volume of 10% sodium hydroxide employed in extraction:

$$\% \text{ phenols} = \frac{D_{290} \times B \times V \times 100}{W \times 24}$$

where D_{290} is optical density at 290 mμ; B is liters to which 1 ml. of sodium hydroxide extract was diluted; W is grams of sample; and V is milliliters of hydrocarbon used as solvent for the sample.

DISCUSSION

Representative Specific Extinction Coefficient. A known single phenol can be readily and accurately determined by the method described above after determination of the specific extinction coefficient of a sample of the pure phenol in 0.4% sodium hydroxide solution. On the other hand, because phenols either in, or isolated from, gasoline consist of a number of compounds, the extinction coefficient used must represent the absorption of a composite of these compounds and the value must either be determined in some way or chosen arbitrarily.

One method of selecting a coefficient is to consider the spectra of a number of phenols likely to be present in gasoline and, from the data obtained, to select a reasonable value. This is not too difficult because the range in the values of the extinction coefficients of the compounds studied is not very great (Table III).

The samples of cresols and xylenols available for this study were not of high purity but each was completely soluble in sodium hydroxide. Impurities, therefore, are probably largely isomeric phenols which, when present in small amounts, would make little difference in the band position or absorption coefficient at 290 mμ. Water and carboxylic acids, if present, would cause the values observed to be somewhat lower than actual, for neither has absorption near 290 mμ.

Although the list of phenols examined is incomplete and no consideration was given to the relative abundance of the individual phenols in gasoline, it is felt that this method of calibration would justify a choice of extinction coefficient of near 25. This is based upon the premise that phenol, cresols, and xylenols make up by far the major portion of the phenols in typical gasolines. The presence of some high molecular weight phenols in gasoline would favor adoption of a value for the coefficient slightly lower than 25.

A second method of calibration is to isolate and study typical petroleum phenols such as are described in Table IV. Such crude samples, when completely soluble in aqueous sodium hydroxide, may contain up to about 15% water and significant quantities of carboxylic acids, lower-boiling alkyl mercaptans (thiols), thiophenols, and tarlike material. The center cuts of the two petroleum phenols described in Table IV are water free, and are low in sulfur compounds and carboxylic acids. They would appear to be representative of the average phenols found in gasoline. These data, together with information gained from the study of a number of other petroleum phenol samples, indicate that an average, specific extinction coefficient of between 23 and 25 should be satisfactory.

After consideration of the two methods of calibration, the author has arbitrarily chosen the value 24 as a representative, but empirical, specific extinction coefficient for the phenols present in gasolines for use when a direct determination is not feasible.

POSSIBLE INTERFERENCES

Mercaptans. Alkaline solutions of alkyl mercaptans have negligible absorption at 290 mμ and hence do not in general interfere with the determination of phenols by this method. On the other hand, thiophenols in alkaline solution show absorption at 290 mμ of the same order of magnitude as do the phenolates. At 265 mμ, where the phenolates have extinction coefficients approxi-

Table III. Ultraviolet Absorption of Phenols in 0.4% Aqueous Sodium Hydroxide Solution

	Specific Extinction Coefficient, E, L./G. Cm. At 265 mμ	At 290 mμ	At max.[a] absorption	$\frac{E_{265\ m\mu}}{E_{290\ m\mu}}$	λ Max.[a], mμ
Phenol	8.8	27.5	28.0	0.32	288
o-Cresol	9.1	30.0	30.0	0.30	289
m-Cresol	6.9	24.5	24.5	0.28	289
p-Cresol	6.0	22.1	24.6	0.27	295
2,5-Dimethylphenol	8.2	28.2	28.4	0.29	291
2,4-Dimethylphenol	7.3	23.0	25.8	0.32	296
3,4-Dimethylphenol	6.9	22.5	24.0	0.31	294
3,5-Dimethylphenol	7.5	21.2	21.2	0.35	290
Salicylic acid	3.3	22.7	25.8	..	298
Thiophenol	140	22.2	141	..	263
p-Thiocresol	128	22.3	128	..	265
tert-Butyl mercaptan	14	0.1	57	..	243

[a] For longer wave-length band. Phenolates have stronger band near 240 mμ not utilized in present study.

Table IV. Ultraviolet Absorption Characteristics of Fractions from Typical Petroleum Phenol Mixture

Cut No.	Distillation Cut Point °C.	Volume of Cut %	Total Sulfur % w	Thiophenols Est. by Ultraviolet % w	Specific Extinction Coefficient of Solution in 0.4% Aqueous NaOH At 290 mμ L./g. cm.	At 265 mμ L./g. cm.
			Sample A[a]			
1[b]	180	11.6	9.2[c]	..	9.2	22.5
2	196	9.2	6.0	18	26.1	30.2
3	205	13.7	2.2	8	25.8	17.3
4	208	15.7		4	25.5	13.2
5	215	16.7	0.48	1	24.0	8.8
6	220	13.4		1	22.8	8.3
7	225	13.6	1.22	4	21.2	11.2
8	240	1.6	6.9	19	18.0	28.7
Residue		4.4	3.4			
Undistilled sample		100	2.3	6	21.6	13.8
			Sample B			
1[d]	187	5.2	..	6	24.1	14.4
2	193	20.3	..	3	26.2	11.2
3[e]	207	20.4	..	2	25.2	9.8
4	210	20.5	..	1	24.2	8.8
5	214	7.2	..	1	22.0	8.4
6	217	20.1	4.7	15	20.7	25.2
Residue		6.3[f]	..			
Undistilled, air oxidized		100	2.5		21.6	8.15

[a] Examination by infrared absorption revealed negligible quantities of carboxylic acids.
[b] 80% water. Examination was made of oil layer having unknown water content.
[c] A considerable portion of this amount is alkyl mercaptan sulfur.
[d] Cut contained only small amount of water. Examination was made of oil layer saturated with water.
[e] Examination by infrared absorption showed cut contained less than 0.3% carboxylic acid.
[f] Only partially soluble in 10% NaOH.

mately 30% as great as at 290 mμ, the spectra of the alkaline thiophenolate solutions show a very strong maximum. At 265 mμ the specific extinction coefficients for the thiophenols of lower molecular weight are greater than 100.

These facts make possible the detection of any significant quantity of thiophenols in petroleum phenols and provide for a means of roughly approximating their amount.

In the absence of large amounts of alkyl mercaptans, thiophenols are probably present if the absorption for the alkaline solution at 265 mμ is significantly greater than 30% of the coefficient found at 290 mμ. To estimate the amount, 30% of the specific extinction coefficient of the alkaline solution measured at 290 mμ is subtracted from the specific extinction coefficient found at 265 mμ. The difference divided by 125 (an assumed average specific extinction coefficient for the aryl mercaptans in gasoline) and multiplied by 100 gives a very rough measure of the percentage of thiophenols. This percentage is usually small compared to the percentage of phenols. In case, however, the sample should contain a relatively large amount of thiophenols, a more accurate true phenol determination can be made if, prior to alkaline extraction, the thiophenols are removed by "copper sweetening." This may be accomplished by shaking about 15 ml. of the gasoline sample for 5 minutes, with an equal volume of an aqueous solution containing 10% cupric sulfate pentahydrate and 20% sodium chloride.

The percentages of thiophenols in the various cuts of two petroleum phenols, as determined by the above method, are listed in Table IV. In sample A, where the analysis for sulfur was more complete, there is a fairly constant ratio of 0.3 between the sulfur content and the estimated amount of thiophenols; this observed ratio is near that calculated for thiocresols.

The alkyl mercaptans of lower molecular weight are readily soluble in aqueous sodium hydroxide and have in the alkaline solution a strong absorption maximum at 243 mμ. At the wave length used for determining thiophenols, 265 mμ, the specific extinction coefficient for the alkyl sulfur compounds has dropped considerably, but is still significant in comparison to that of thiophenols. Hence, when rather large quantities of alkyl mercaptans are present, they will interfere somewhat with the estimation of thiophenols. Thus far, this interference has been observed only in the small initial fraction obtained in the distillation of petroleum phenols and in relatively sour gasolines of low phenol content. A comparison of the absorption spectra of alkaline solutions of a phenol, a thiophenol, and an alkyl mercaptan is given in Figure 1.

The behavior of petroleum phenols on standing at room temperature in contact with air is interesting. Petroleum phenol (sample B, Table IV), contained, as received, 2.5% of sulfur. In several fractions, the ratio of the 265 and 290 mμ extinction coefficients was considerably greater than 0.3, which indicates the presence of appreciable thiophenols.

After the sample had stood for about 4 weeks in a loosely stoppered bottle, it was re-examined. The solution in 10% sodium hydroxide was turbid instead of clear, the specific extinction coefficient at 290 mμ was low (only 21.6), and the coefficient at 265 mμ was even lower than the value of any single fraction previously measured on the fresh, distilled sample. Distillation of the aged sample showed that the greater part of the sulfur remained in the residue. The absorption at 290 mμ for the alkaline solution of the distillate was increased to the values expected for phenols. These observations are explainable on the basis that the thiophenols during storage undergo oxidation to disulfides. The disulfides thus formed are insoluble in aqueous sodium hydroxide; consequently, the solution (suspension) does not absorb strongly at 265 mμ. Hence, if one wishes to obtain a useful estimation of thiophenolic content in the petroleum phenol or gasoline, it is necessary either to use fresh samples or to protect them from oxidation.

Carboxylic Acids. These compounds show no interference even in the case of benzoic acid. An alkaline solution of salicylic acid absorbs strongly at 290 mμ, but this is caused by the phenolic rather than the carboxylic group. Naphthenic acids do not interfere with the phenol determination and have little influence on the thiophenol analysis. This statement is based on the following experiment:

Cut 3 of petroleum phenol B (Table IV) was analyzed for phenols and thiophenols by dissolving the sample in iso-octane extracting with sodium hydroxide, and completing the analysis as usual. The procedure was repeated using a known weight of the same phenol sample to which was added an approximately equal weight of commercial, low-boiling naphthenic acid. The resulting phenol analysis checked within experimental limits of the spectrophotometric measurements—i.e., 1%. The apparent thiophenol content, though low in the sample, was only slightly affected, in spite of the fact that the naphthenic acid concentration in the synthetic sample was much higher than would usually be expected for petroleum phenols.

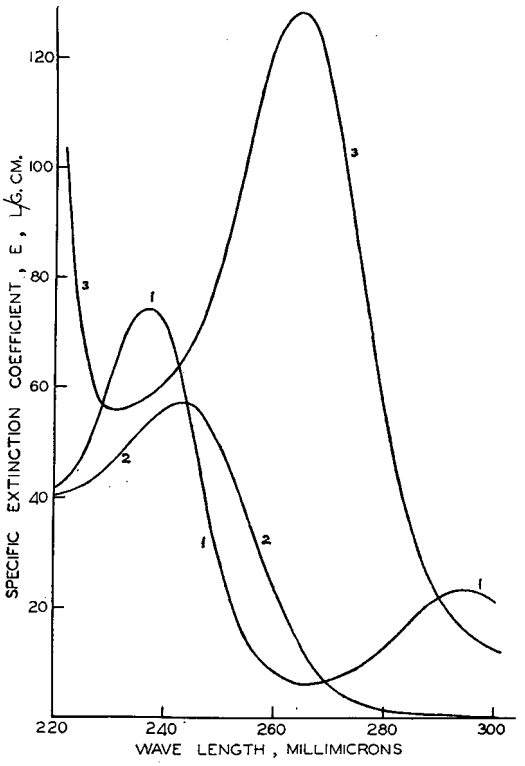

Figure 1. Ultraviolet Absorption Spectra of Aqueous Sodium Hydroxide Solutions
1. 3,4-Dimethylphenol. 2. *tert*-Butyl mercaptan. 3. *p*-Thiocresol

Nitrogen Bases. An experiment similar to the one described with naphthenic acids was performed except that quinoline was added. Neither the phenol nor the thiophenol determination was significantly affected in spite of the fact that the quinoline was present in the sample in a much higher concentration than would normally be expected in petroleum phenols.

Hydroperoxides. Several peroxides were tested and found to interfere only very slightly with the analysis for phenols. For example, analysis of a gasoline which contained equal quantities of phenols and *tert*-butyl hydroperoxide showed the apparent concentration of phenols to be higher than the true value by about 1% of the true phenolic concentration. Deviations of the same magnitude were observed for a sample containing cumene hydroperoxide and for one containing Uniperox 60.

Oxidation Products and Tarry Materials. Of the several hundred gasolines examined to date, two gave obviously incorrect answers. One of these was a sample of thermally cracked gasoline which had been in storage for a number of months at elevated temperatures in the presence of air. The alkaline extract had a

Table V. Summary of Experiments Designed to Remove Interference Caused by Colored Oxidation Products in California Thermally Cracked Gasoline

Age of Sample, Days	Appearance of Sample	Modification of Analysis of Caustic Solution for Phenols	Apparent % Phenols
14[a]	Reddish	None	0.45
21	Dark red	None	0.53
50	Brownish	None	0.54
80	Brownish	None	0.57
60	Brownish	Sample extracted with 2% H_2SO_4 then copper sweetened	0.46
80	Brownish	Sample extracted with 2% H_2SO_4	0.49
80	Brownish	Extracted caustic extract once with iso-octane	0.44
80	Brownish	Extracted caustic extract once with toluene	0.36
80	Brownish	Extracted caustic extract thrice with toluene	0.18
80	Brownish	Extracted caustic extract once with ether	0.19
80	Brownish	Sample extracted with caustic; acidified caustic extract; extracted acid layer with iso-octane; extracted iso-octane solution with caustic and analyzed	0.31[b]
80	Brownish	Check analysis	0.33[b]

[a] Estimated. Protected from air by nitrogen; after this period, sample was stored in refrigerator without nitrogen protection.
[b] No more than 0.02% of phenolic material was recoverable by further extraction of acidified solution.

distinctly yellowish color and the absorption curve for the caustic solution showed the maximum near 290 mμ to be masked by general absorption.

A second sample which gave difficulty was a California thermally cracked naphtha which, though protected by nitrogen up to the time of analysis, had turned a reddish color before the analysis was attempted. Some of this color, during extraction, was taken up by the alkaline layer and caused apparent phenol percentages to be too high and the curve to be of an unusual type. With further aging in air, both the depth of color and the apparent phenol concentration increased. This sample of oxidized, dark-colored gasoline was subjected to various treatments in attempts to eliminate the interference (Table V). Of the methods tried, only the following was successful.

The dark-colored sample was first extracted with aqueous sodium hydroxide. The alkaline solution was made acid with hydrochloric acid and the resulting solution was extracted with iso-octane. This extract was then shaken with sodium hydroxide and analyzed for phenols in the usual manner. In this way, the resulting absorption curve was fully characteristic of phenolates and the analysis approximated that reported by use of a macro-method. (Examination of the aqueous solution after iso-octane extraction showed that all but minor amounts of the phenols appeared in the final alkaline extract.) This modification appears at first to be somewhat cumbersome, but in reality is not too time-consuming for the occasional off-color, partially oxidized sample which gives a visibly colored alkaline extract.

The cause of this interference is not clear, but it is believed that certain light-absorbing products, at least some of which are formed during oxidation, are solubilized by the phenolates or by salts of organic acids. The modification suggested for analysis of such gasolines so dilutes the interfering material that during the second caustic extraction negligible quantities of interfering products are taken into the water layer.

For the pure phenols tested, as well as for most of the cresylic acid fractions, the observed extinction coefficient was found to be the same whether the sample was dissolved directly in sodium hydroxide and diluted or was first dissolved in a hydrocarbon and then extracted with sodium hydroxide. In the case of some very dark-colored cresylic acid fractions which contain considerable tarlike material, however, direct solution of the sample in strong alkali results in appreciable interference. It is believed that the tarry products are solubilized by the phenolates to an extent sufficient to cause significant nonphenolate absorption at 290 mμ. For such samples, the difficulty can readily be overcome by dissolving the sample first in a hydrocarbon, such as iso-octane, and extracting the resulting solution with aqueous sodium hydroxide. Under these conditions, the interfering tars remain almost entirely in the hydrocarbon. Inasmuch as a fair proportion of cresylic acid samples are dark colored, this technique is made a part of the regular procedure for phenols in cresylic acids.

COMPLETENESS OF EXTRACTION PROCESS

Certain phenols of higher molecular weight, such as those having large groups ortho to the hydroxyl, may be extracted with difficulty or hardly at all from hydrocarbon solutions by the aqueous sodium hydroxide reagent. Because the phenols in gasoline are predominantly in the lower molecular weight range, the writer doubts that incomplete extraction would introduce appreciable error into the analysis for phenols in gasoline.

That the ordinary phenols found in gasoline are extracted completely by the alkaline solution was shown by the following:

1. As with pure phenols, it makes no difference in the value of the extinction coefficient at 290 mμ whether the pure phenol is dissolved directly in the sodium hydroxide or dissolved in iso-octane and then extracted.
2. A solution of 0.0295 gram of 3,4-dimethylphenol in 25 ml. of iso-octane was extracted with an equal volume of 10% sodium hydroxide. The hydrocarbon layer was carefully removed to a clean separatory funnel and re-extracted with 5 ml. of fresh sodium hydroxide solution. No phenol was detectable in the extract.
3. Experiment 2 was repeated by dissolving 0.369 gram of sample B, cut 3 (Table IV), in 25 ml. of iso-octane. The second extraction with 10% sodium hydroxide removed only 0.2% as much phenolic material as the first extraction.

Table VI. Apparent Phenol Results on Fractions from Typical Petroleum Phenol Mixtures

Cut No.	Distillation Cut Point, °C.	Volume of Cut %	Specific Extinction Coefficient At 290 mμ	Specific Extinction Coefficient At 265 mμ	Apparent Phenol Content % w
			L./g. cm.		
		Sample C, 0.86% Sulfur			
1	180	20.4[a]	19.0	14.4	79
2	200	17.7	26.7	9.4	111
3	210	10.9	25.2	9.6	105
4	220	14.4	22.6	8.3	94
5	240	15.2	20.7	8.4	86
		Sample D, 1.3% Sulfur			
1	180	12.8[b]	16.7[c]	31	70
2	200	14.0	26.9	12.1	112
3	210	14.4	21.4	8.9	89
4	220	21.5	23.6	9.2	98
5	240	20.0	19.8	8.0	82

[a] 70% of this fraction is water. Examination was made of oil layer saturated with water.
[b] 60% of this fraction is water. Examination was made of oil layer saturated with water.
[c] No maximum observed; this is due to relatively large thiophenol concentration.

PRECISION AND ACCURACY

Analysis of a given sample, whether a gasoline or a petroleum phenol, is highly repeatable; the deviation is seldom greater than 1% of the phenol content in the range of 1.0 or higher.

The greatest uncertainty in the method is believed to arise in general from the arbitrary value of 24 selected for the specific extinction coefficient for the aggregate of phenols found in gasoline. Testing of the method by analysis of synthetic mixtures is meaningless, but some idea of the reliability of the method can be gained by examining the data for the lower-boiling fractions of petroleum phenols such as are shown in Table VI.

It is important to allow the concentration of sodium hydroxide in the solution, whose absorption is being measured, to drop no lower than 0.4%, because at lower concentrations there is danger of partial hydrolysis of phenolates. This phenomenon would cause a shift in the absorption maximum with a consequent lowering of the intensity of absorption at 290 mμ.

At a concentration of 0.4 N sodium hydroxide, the optical densities at 290 mμ of several concentrations of 3,4-dimethylphenol were measured. A plot of concentration against optical density yielded a straight line from which the individual points deviated no more than 1% over the optical density range 0.2 to 1.2.

APPLICATION OF METHOD

The spectrophotometric method for phenols has been applied in the author's laboratory thus far to the determination of phenols in several hundred samples of thermally and catalytically cracked gasolines and to a few straight-run gasolines. Approximately one hundred petroleum phenols or fractions thereof have been studied. The method should apply to other petroleum products outside the gasoline range, provided it is realized that the specific extinction coefficient changes with molecular weight of the phenols and that an appropriate coefficient must be used in the calculation. For calibration purposes, actual isolation and careful purification of a sample of the phenols from a distillate in the boiling range to be studied are highly desirable.

ACKNOWLEDGMENT

The author wishes to express his appreciation for the suggestions and cooperation given by Warren W. Johnstone and Charles Wankat. Thanks also are due to W. S. Gallaway for the infrared examination of the petroleum phenols and to Charles Berg for a portion of the ultraviolet absorption study.

LITERATURE CITED

(1) Field, E., Dempster, F.H., and Tilson, G. E., *Ind. Eng. Chem.*, 32, 489 (1940).
(2) Lykken, Louis, Treseder, R. S., and Zahn, Victor, IND. ENG. CHEM., ANAL. ED., 18, 103 (1946).
(3) Stoughton, R. W., *J. Biol. Chem.*, 115, 293 (1936).
(4) Wetlaufer, L. A., Van Natta, F. J., and Quattlebaum, H. B., IND. ENG. CHEM., ANAL. ED., 11, 438 (1939).

RECEIVED May 20, 1948.

Determination of Carboxy Group in Aromatic Acids

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The carboxy group attached to an aromatic nucleus can be split off in the form of carbon dioxide by heating the acid with quinoline in the presence of a catalyst. In the apparatus described, the carboxy group can be estimated on samples of from 0.002 to 0.02 mole of acid. A comparative study of inorganic catalysts showed that basic cupric carbonate was the most efficient in most cases. Where this proved inefficient, silver carbonate acted as a decarboxylation catalyst.

IN MANY cases the carboxy group attached to an aromatic ring can be split off by merely heating; however, in the majority of cases, this procedure alone will not work. More than half a century ago, several chemists (*1*, *6*) observed that the cleavage of the carboxy group in hydroxy acids can be speeded up by the presence of certain tertiary amines. Later, Willstätter and Pummerer found that copper exerts a catalytic effect in eliminating the two carboxy groups from chelidonic acid (*9*). Shepard, Winslow, and Johnson were the first to combine these two observations and thus found an efficient method for the decarboxylation of aromatic carboxylic acids (*7*). Though this reaction has been increasingly utilized in synthesis since 1930, the author has been unable to find a comparative study on the relative merits of the various catalysts proposed for this reaction, nor a simple apparatus for the quantitative study.

The carbon dioxide developed in this reaction can be determined by recording the increase in pressure, or the increase in volume, or by weighing. In this work, the last two methods were employed. The apparatus shown in Figures 1 and 2 was constructed for this purpose. By this means, the carboxy group can be determined quantitatively, and at the same time there is an opportunity to study the speed of reaction.

The carboxy group can usually be determined accurately and precisely by titration. The method described in this paper is of special interest in cases where the carboxy group is of such weak acidity that it cannot be titrated, or when the acid contains other acidic groups besides the carboxy group that make titration valueless.

APPARATUS

The reaction flasks, A (Figure 1), of 30-ml. capacity, has a side neck which is closed by a glass stopper, a. The deflagrator, or spoon, c, has a cup on one end to hold the catalyst, and a flat handle on the other end. In experiments where nitrogen gas is introduced, tube b is used. This has a cup on one end and a small hole right behind it. The function of the condenser, B, is to hold back the quinoline which is condensed mostly in the lower air-cooled part. The eudiometer, E, is connected with B by means of a short rubber tube which ensures more flexibility than a taper joint at that point. The graduation on E is from 0 to 140 ml., with 1-ml. subdivisions, and is shielded from the hot flask, A, by means of an asbestos board, F. Slightly above the zero mark of the eudiometer is a sintered-glass plate, D, of coarse porosity, which serves to hold back any mercury that might surge back into the condenser, and at the same time permits passage of the gas. C is a three-way stopcock. The whole apparatus is clamped to a special stand having two vertical rods 13 cm. apart; one rod is used only for the leveling bulb support. Not shown in Figure 1 is a thermometer hugging E. A dips into a beaker containing Fisher bath wax, heated by a microburner.

If more than 140 ml. of carbon dioxide is developed, it can be measured in this manner: After the gas has filled the eudiometer to the 140-ml. mark, stopcock C is turned to position 3 and the mercury is raised to the zero mark, thereby ejecting the gas through the side tube without losing any carbon dioxide developed during this operation. The stopcock is then turned back to position 2.

If it is desired to weigh the carbon dioxide, the top of B is connected with the assembly shown in Figure 2. The gas first passes through a tube containing 10 ml. of concentrated sulfuric acid, which retains not only water but also traces of quinoline. Then it is passed through a weighing tube filled with 6 to 7 grams of Ascarite, sufficient for eight to ten determinations of 0.005 mole of carbon dioxide each. This tube weighs 27 to 30 grams when filled and has an indentation on one end to keep it from rolling off the balance pan.

Table I. Decarboxylation of 0.005 Mole of Acid in 5 Ml. of Quinoline (Method A)

Catalyst		Time	Temperature	Carbon Dioxide Volumetric	Carbon Dioxide Gravimetric	Decarboxylated Acid
	Mg.	*Min.*	° C.	*% of theory*		*% of theory*
o-Benzoylbenzoic Acid						
Cupric carbonate	50	6	240	100.1	98.5	88
Cupric carbonate	10	20	240	99.3	97.9	90
Cupric acetate	50	9	240	97.0	98.4	82
Copper metal, powder	50	34	240	99.7	97.9	89
Copper chromite	50	15	240	101.3	97.4	84
Silver carbonate	50	6	240	99.2	98.7	85
Silver carbonate	5	37	240	98.0	99.2	94
3,5-Dinitrobenzoic Acid						
Cupric carbonate	50	18	240	108.4	100.3	77
Cupric acetate	50	34	240	104.3	99.3	86
Copper metal, powder	50	11	240	107.6	100.2	72
Copper chromite	50	46	240	103.3	99.1	84
Silver carbonate	50	13	240	100.3	98.7	95
4′,4″-Dihydroxytriphenylmethane-2-carboxylic Acid						
Cupric carbonate	50	28	240	91.8	89.6	74
Cupric carbonate	20	77	240	93.9	87.6	79
Cupric acetate	50	62	240	91.4	89.4	70
Copper metal, powder	50	933	240	90.3	82.4	63
Copper chromite	50	244	240	89.6	85.6	80
Coumarilic Acid						
Cupric carbonate	50	51	176	99.2	96.7	80
Cupric acetate	50	72	176	97.1	96.9	70
Copper metal, powder	50	201	176	96.9	97.1	73
Copper chromite	50	102	176	97.6	97.4	83
Silver carbonate	50	23	176	99.7	97.2	82
2,4-Dichlorobenzoic Acid						
Silver carbonate	50	94	176	98.0	97.9	86

REAGENTS

The quinoline was synthetic material, redistilled before use. All melting points are corrected.

As catalysts, copper, silver, and nickel salts from the J. T. Baker Chemical Company and the Mallinckrodt Chemical Works were used. The copper chromite catalyst 25 KAF was prepared according to Connor, Folkers, and Adkins (*2*).

The *o*-benzoylbenzoic acid (Eastman Kodak No. 2242), as crystallized from benzene, melted at 128.8–129.2° C. and assayed 100.1%. The 3,5-dinitrobenzoic acid (Eastman Kodak No. 635), after one recrystallization from ethanol, melted at 204.0–207.3°, and titrated 100.3%. The 4′,4″-dihydroxytriphenylmethane-2-carboxylic acid (Eastman Kodak phenolphthalein, No. 1657) was recrystallized from acetic acid and then from dilute ethanol; it melted at 237.0–237.5° and assayed 98.0%. The coumarilic acid, prepared according to the method of Perkin (*4*), was purified by sublimation at 130° and 20 microns pressure (*5*), melted at 192.1–194.3° and assayed 99.1%. The 2,4-dichlorobenzoic acid (Eastman Kodak No. 5568), after one sublimation at 110° in vacuo (*5*) and subsequent crystallization from benzene, melted at 161.5–162.0° and titrated 99.9%. The acids used in the experiments summarized in Table II were of equally high quality, purified preferably by sublimation in vacuo.

PROCEDURE

Method A. Many aromatic acids are relatively stable and do not split off the carboxy group even when heated in boiling quinoline, but may be decarboxylated by adding a catalyst to such a solution. The following procedure was used with these compounds:

The acid (0.005 to 0.02 mole) is weighed into flask *A* (Figure 1) and 5 to 20 ml. of quinoline are added. The catalyst is weighed and placed in the cup of deflagrator *c*. *A*, closed by *a*, is attached to the apparatus, and a preheated oil bath is placed around it. In order to maintain the temperature of the solution in the flask at 176° C., the temperature of the oil bath should be at 180° to 183°; a bath temperature of 250° to 254° will maintain a temperature of 240° in the flask. *C* is placed in position 1 and the mercury is brought to the zero mark. When temperature equilibrium is reached after about 3 minutes, *a* is replaced by *c*, *C* is turned to position 2, and the catalyst is dropped into the solution by turning *c* through 180°. In most cases, the catalyst will fall easily, but when cupric acetate is used, a sticky mass is formed on contact with the quinoline vapors. However, within a few minutes, the vapors will wash it down.

The five acids in Table I were decarboxylated in this manner.

Method B. Some aromatic carboxylic acids, when heated in quinoline to 250°, will slowly give off carbon dioxide. In such cases, the acid-quinoline solution is heated to a lower temperature—for example, 120°—at which no reaction takes place. After temperature equilibrium has been reached, the catalyst is added. Because at this relatively low temperature, even in the presence of the catalyst, gas develops but slowly, the temperature is raised to a point where a reaction takes place at a reasonable rate—for example, 230°. After the evolution of gas has ceased, the temperature of the oil bath is lowered to the initial temperature and the gas volume is read after constancy of volume has been attained.

Method B is also used when the decarboxylated acid is a liquid boiling below 250°, such as benzene or chlorobenzene obtained in the decarboxylation of benzoic acid and chlorobenzoic acid, respectively.

In Table II, the temperature at which the system was closed and at which the gas volume was read at the end, is called the initial temperature. The temperature at which most of the reaction takes place is marked as the reaction temperature.

Acids whose behavior on decarboxylation is unknown are first heated in quinoline, to a low temperature—for example, 120°. Then, if no reaction takes place, to 200°, and finally to 250°. If no gas evolution is observed at 250°, the catalyst, such as 50 mg. of basic cupric carbonate, is added.

When either a carbonate or an acetate is used as catalyst, a correction is made. The carbon dioxide content of silver carbonate is equal to the theoretical, and that of basic cupric carbonate corresponds to the formula $CuCO_3.Cu(OH)_2$. The correction for cupric acetate, $Cu(C_2H_3O_2)_2.H_2O$, was determined by dropping 0.2 gram into 20 ml. of quinoline at 240°: 11 ml. of gas were evolved.

Quinoline absorbs carbon dioxide at lower temperatures; thus 5 ml. were found to absorb 1.1 ml. of carbon dioxide at 100°.

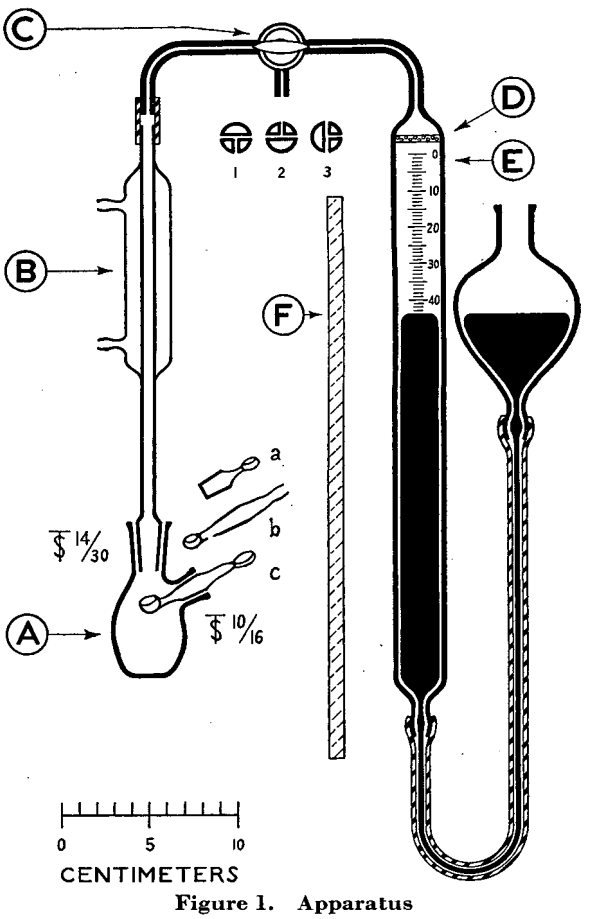

Figure 1. Apparatus

Table II. Determination of Aromatic Carboxyl

Acid	Melting point	Catalyst		Reaction Conditions: Temperature of Oil Bath, ° C. Method	Initial	Reaction	Reaction time, minutes	Per Cent —COOH: Theory	Determined Volumetrically	Determined Gravimetrically
	° C. corr.	Mg.	Formula							
Benzoic	121.2–121.6	50	$CuCO_3$	B	120	250	230	36.9	36.2	34.8
m-Chlorobenzoic	152.2–154.1	50	$CuCO_3$	B	130	250	197	28.7	27.1	27.0
2,4-Dichlorobenzoic	161.5–162.0	50	$AgCO_3$	A	...	180	93	23.6	23.1	23.0
Salicylic	158.8–159.0	50	$CuCO_3$	B	120	220	134	32.6	29.4	29.8
m-Hydroxybenzoic	202.2–202.4	50	$CuCO_3$	A	...	250	310	32.6	31.9	29.7
p-Hydroxybenzoic	214.8–215.4	50	$CuCO_3$	B	120	220	80	32.6	31.3	31.8
Anthranilic	144.7–145.7	50	$CuCO_3$	B	180	250	73	32.8	31.9	31.9
m-Aminobenzoic	177.5–178.0	50	$CuCO_3$	A	...	250	217	32.8	32.0	29.8
p-Aminobenzoic	186.2–186.6	50	$CuCO_3$	B	200	250	170	32.8	32.0	31.0
3,5-Dinitrobenzoic	204.0–207.3	50	$CuCO_3$	A	...	250	18	21.2	23.0	21.3
o-Benzoylbenzoic	128.8–129.2	50	$CuCO_3$	A	...	250	6	19.9	19.9	19.6
Coumarilic	192.1–194.3	50	$AgCO_3$	A	...	250	23	27.8	27.7	27.0
3-Hydroxy-2-naphthoic	221.2–222.3	50	$CuCO_3$	A	...	250	215	23.9	23.7	22.6
4,4″-Dihydroxytriphenylmethane-2-carboxylic	237.0–237.5	50	$CuCO_3$	A	...	250	28	14.0	13.0	12.6
Isophthalic	343.0–345.0	50	$CuCO_3$	B	130	250	270	54.2	55.0	50.6

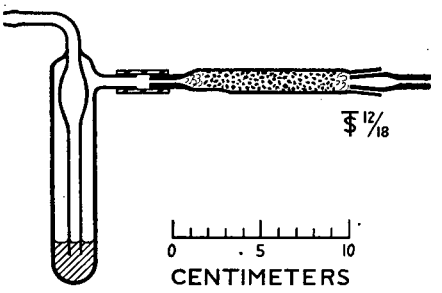

Figure 2

Some small errors, such as varying atmospheric pressure at the beginning and end of the determination, or different vapor pressure of the decarboxylated acid as compared with the acid, are inherent in this method. For these reasons, the method is not precise. In order to check its accuracy, another series of experiments was undertaken, in which the carbon dioxide formed was weighed. The catalyst was introduced by means of tube *b*, which also was connected to a source of nitrogen. In place of the eudiometer, the assembly shown in Figure 2 was connected with the top of the condenser.

In one series of experiments, the efficiency of the various catalysts, as well as the yield of decarboxylated acid, was studied (Table I). The decarboxylated acids were isolated from the dark quinoline solution as follows:

The mixture was washed with a total of about 25 ml. of ether into a small separatory funnel. In the case of *m*-dinitrobenzene, benzene was used as a solvent. The ethereal solution was washed subsequently with 15 and 5 ml. of 3 *N* hydrochloric acid, 5 ml. of 2 *N* sodium carbonate, and finally a little water. In most cases it is necessary to filter the content of the funnel through a fritted-glass filter, so that the two layers separate more sharply. After drying, the ether solution was evaporated. The crude benzophenone was treated with 1 ml. of petroleum ether, seeded, and cooled to 5°; after the mother liquor had been siphoned off, the crystals were freed from a little oily material by pressing them between filter paper. The purified benzophenone solidified at 45.1°. The crude *m*-dinitrobenzene was sublimed at 70° and 10 to 20 microns' pressure; the sublimate showed a solidification point of 85.9°. The crude 4,4′-dihydroxytriphenylmethane, after one crystallization from 220 ml. of 20% ethanol, melted at 162.9–164.0°. The crude coumarone was distilled and the fraction boiling at 168° to 180° was weighed. The crude *m*-dichlorobenzene was distilled and the fraction boiling at 162° to 172° was taken.

The percentage of carboxy group in various aromatic acids was determined in this apparatus. The results are tabulated in Table II. The percentage of —COOH in the sample is calculated according to the formula: % —COOH = 0.2009 v/s, where v = ml. of carbon dioxide at 0° and 760 mm., and s = grams of the acid.

DISCUSSION

The data on the efficiency of the six catalysts in the decarboxylation of five acids are shown in Table I. Basic cupric carbonate was found to be the best all-around catalyst. It did not work with 2,4-dichlorobenzoic acid, nor did other copper catalysts, but in this case silver carbonate acted catalytically. On the other hand, silver carbonate did not catalyze the decarboxylation of the other four acids. Nickel carbonate had no effect with any of the five acids mentioned in Table I.

The yield figures represent the average of three determinations. The accuracy, as well as the precision of the method, in determining the carboxy group is only fair. Nevertheless, this method is of value in organic research.

That quinoline, in addition to an inorganic catalyst, is necessary for smooth decarboxylation has been known and was confirmed again. When any of the five acids in Table I was heated with the inorganic catalyst, in the absence of quinoline, the decarboxylation proceeded much more slowly. This same observation was made recently on substituted cinnamic acids (*8*). When coal tar quinoline was used, the results were somewhat erratic; therefore, synthetic quinoline was preferred.

In experiments not specifically reported here, hydroxyethylmorpholine was substituted for quinoline, but this secondary amine had no catalytic effect at its boiling point of 226°.

Terephthalic acid resisted decarboxylation. Phthalic acid gave only low values, probably because part of it goes over to the anhydride at higher temperatures.

The yield of decarboxylated acid is good, as shown in the last column of Table I.

The data on the yield of carbon dioxide, measured volumetrically, are generally slightly higher than when the gas is weighed, probably because the vapor pressure of the decarboxylated acid is usually higher than that of the corresponding acid.

As Fieser (*3*) points out, the mechanism of the decarboxylation still awaits solution. Metallic copper and silver, respectively, are formed during the decarboxylation.

ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of A. Horner in carrying out the experimental work.

LITERATURE CITED

(1) Cazeneuve, M. P., *Bull. soc. chim. Paris*, 7, 550 (1892); 15, 72 (1896).
(2) Connor, R., Folkers, K., and Adkins, H., *J. Am. Chem. Soc.*, 54, 1139 (1932).
(3) Fieser, F. L., "Experiments in Organic Chemistry," p. 198, Boston, D. C. Heath & Co., 1941.
(4) Fuson, R. C., Kneisley, J. W., and Kaiser, E. W., *Organic Syntheses*, 24, 33 (1944).
(5) Hubacher, M. H., IND. ENG. CHEM., ANAL. ED., 15, 448 (1943).
(6) Lauth, C. M., *Bull. soc. chim. Paris*, 9, 971 (1893).
(7) Shepard, A. F., Winslow, N. R., and Johnson, J. R., *J. Am. Chem. Soc.*, 52, 2083 (1930).
(8) Walling, C., and Wolfstirn, K. B., *Ibid.*, 69, 852 (1947).
(9) Willstätter, R., and Pummerer, R., *Ber.*, 37, 3745 (1904).

RECEIVED November 16, 1948.

5,6-Dimethyl-1,10-phenanthroline

Spectrophotometric Constants as Ferrous Complex and Use as Redox Indicator for Determination of Iron by Oxidation with Dichromate

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The spectrophotometric constants and conformance to Beer's law of 5,6-dimethyl-1,10-phenanthroline ferrous sulfate have been demonstrated. Its formal oxidation potential in 1 *F* sulfuric acid was found to be 0.97 volt by a potentiometric evaluation. This dimethylferroin was shown to be a desirable indicator for the titration of ferrous ion by dichromate in sulfuric acid and hydrochloric acid solutions.

THE phenanthrolinium ferrous ion (ferroin), as an oxidation-reduction indicator, was first described by Walden, Hammett, and Chapman (*7*), and its use has been extensively adopted particularly in cerate oxidimetry (*4*). A series of substituted 1,10-phenanthrolinium ferrous ions for use as redox indicators has been described by Smith and Richter (*5*).

Ferroin as a redox indicator is oxidized from an intense red form to the faint blue of the phenanthrolinium ferric ion, the oxidation potential of the system being 1.06 volts in formal acid solutions (*2*). At 2 formal acidity, and successively 3, 4, 5, and 6 formal acidity, the oxidation potentials are, respectively, 1.03, 1.00, 0.96, 0.925, and 0.89 volt (*5*).

The 5-methyl-1,10-phenanthrolinium ferrous ion (methylferroin) has the oxidation potential 1.02 volts in formal acid solutions. At 2 formal acidity, and successively 3, 4, 5, and 6 formal acidity, its oxidation potentials are, respectively, 1.00, 0.96, 0.93, 0.86, and 0.81 volt (*5*).

Both ferroin and methylferroin are stable redox indicators in 1 to 8 *F* mineral acid solutions, provided they are not heated (*3*). Neither indicator is completely satisfactory for rapid and convenient use in the oxidimetric determination of iron by dichromate at less than 5 or 6 *F* sulfuric acid, which is inconveniently high. Such determinations are therefore carried out at lower acidities using the diphenylamine series of indicators as less desirable substitutes.

The present work has for its objective the determination of the spectrophotometric constants of dimethylferroin (5,6-dimethyl-1,10-phenanthroline ferrous ion), the evaluation of its formal oxidation potential, and the demonstration of its practical use in the titration of ferrous iron by dichromate.

PREPARATION OF REAGENTS

5,6-Dimethyl-1,10-phenanthroline. This compound was prepared by Case, and data concerning the synthesis of this substituted phenanthroline together with other substituted polymethyl 1,10-phenanthrolines have been published (*1*). The reagent being described and the dimethylferroin indicator made from it are now commercially available (G. Frederick Smith Chemical Company, Columbus, Ohio).

Sulfatoceric Acid. A sample of pure ceric hydroxide, $Ce(OH)_4$, made from pure ammonium nitratocerate, $(NH_4)_2Ce(NO_3)_6$, by precipitation using a twofold excess of ammonium hydroxide served as starting material. A sample was dissolved in sufficient hot dilute (*1–4*) sulfuric acid to dissolve the hydroxide and provide for dilution to a 0.1 *N* sulfatocerate solution which was 1 *F* in free sulfuric acid. (Pure ceric hydroxide is commercially available.)

Potassium Dichromate and Ferrous Sulfate. Pure potassium dichromate and ferrous sulfate heptahydrate were dissolved in 1 *F* sulfuric acid in sufficient amounts to make approximately 0.1 *N* solutions.

Formal Sulfuric Acid. One molecular weight of sulfuric acid (56 ml. of sulfuric acid, specific gravity 1.84) was diluted to 1000 ml.

Dimethylferroin. A 0.01 *M* solution prepared by reaction of 0.03 mole of the dye base with 0.01 *M* of ferrous sulfate heptahydrate.

Apparatus and Operational Technique. The potentiometric apparatus consisted of a student potentiometer and the usual accessories, including a sensitive lamp and scale galvanometer. The electrode pair consisted of a saturated calomel electrode and internal salt bridge with contact to the test solution through an unsealed asbestos fiber microleak. A bright platinum wire completed the electrical circuit. Solutions were stirred with a magnetic stirring device. Ample time intervals were allowed for the attainment of equilibria before electrode potentials were recorded.

The spectrophotometric studies were made using a G.E. recording spectrophotometer, using cells of 1-cm. thickness.

POTENTIOMETRIC TITRATION OF FERROUS IRON BY DICHROMATE

The results of the potentiometric titration (in a 1 *F* sulfuric acid solution throughout) of potassium dichromate with ferrous sulfate and ferrous sulfate by potassium dichromate are shown in Figure 1. The two titrations are seen to be markedly different.

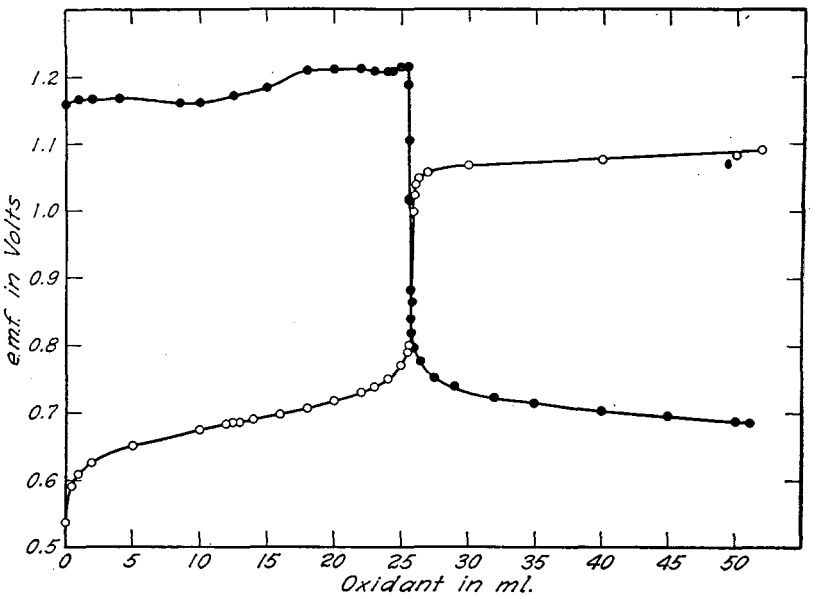

Figure 1. Potentiometric Titration of Ferrous Iron

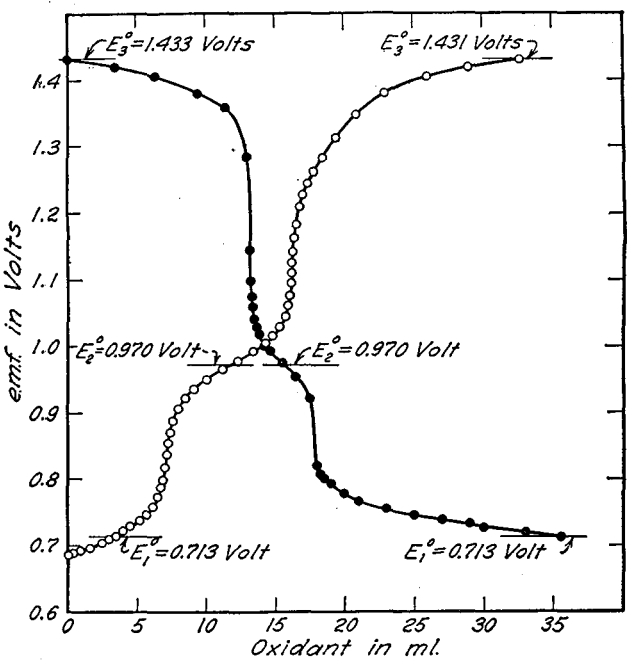

Figure 2. Oxidation Potential of Dimethylferroin-Dimethylferriin Indicator System

Table I. Calculation of Extinction Coefficient of Solutions of Dimethylferroin

Fe	Transmittancy	Dimethylferroin Concn.	Extinction Coefficient
P.p.m.	%	*Mole/liter*	
0.99	58.4	1.767×10^{-5}	13220
2.01	36.4	3.596×10^{-5}	12205
2.98	21.9	5.334×10^{-5}	12365
3.90	12.5	6.978×10^{-5}	12942
4.92	8.3	8.812×10^{-5}	12264
5.93	4.8	10.57×10^{-5}	12476
6.77	3.1	12.12×10^{-5}	12365
		Av.	12562

The abrupt fall in potential of the ferrous sulfate titration of dichromate gives an equivalence point break in potential from 1.22 to 0.85 volt. For this titration ferroin may be employed as indicator. The reverse titration of ferrous sulfate by dichromate produces an equivalence point break from 0.85 to 1.00 volt. Obviously, the ferroin-ferriin transition is not applicable as an indicator in this case. An indicator with a transition potential of some value between 0.85 and 1.00 volt is required. Barium diphenylamine sulfonate has been employed. The transition point in this case is 0.84 volt. *N*-Phenylanthranilic acid (*6*) is not suitable because its oxidation potential is 1.08 volts. By the use of dimethylferroin a favorable indicator potential is available.

DETERMINATION OF OXIDATION POTENTIAL OF DIMETHYLFERROIN-DIMETHYLFERRIIN REDOX INDICATOR SYSTEM

The oxidation potential of this system in 1 *F* sulfuric acid solution was determined by the titration of a mixture of ferrous sulfate and dimethylferroin. The sulfatocerate ion was employed as oxidant and the sulfuric acid solution was held at 1 *F* throughout. By attaining correct oxidation potentials for the Fe(III)–Fe(II) system and for the Ce(IV)–Ce(III) system the determined oxidation potential for the indicator system was considered to be reliable. This determination was made in both the forward and reverse titrational scheme and the same result was obtained for the unknown oxidation potential. The data are shown graphically in Figure 2. From these titrations the oxidation potential of the dimethylferroin-dimethylferriin indicator system in 1 *F* acid is shown to be 0.97 volt. (The ability to carry out these titrations successfully in both directions indicates a high degree of stability for both the oxidized and reduced forms of the indicator.)

SPECTROPHOTOMETRIC CONSTANTS OF DIMETHYLFERROIN COMPLEX ION

Solutions of the 5,6-dimethyl-1,10-phenanthrolinium ion containing approximately 1 to 7 p.p.m. of iron were prepared and finger printed spectrophotometrically with the results shown in Figure 3.

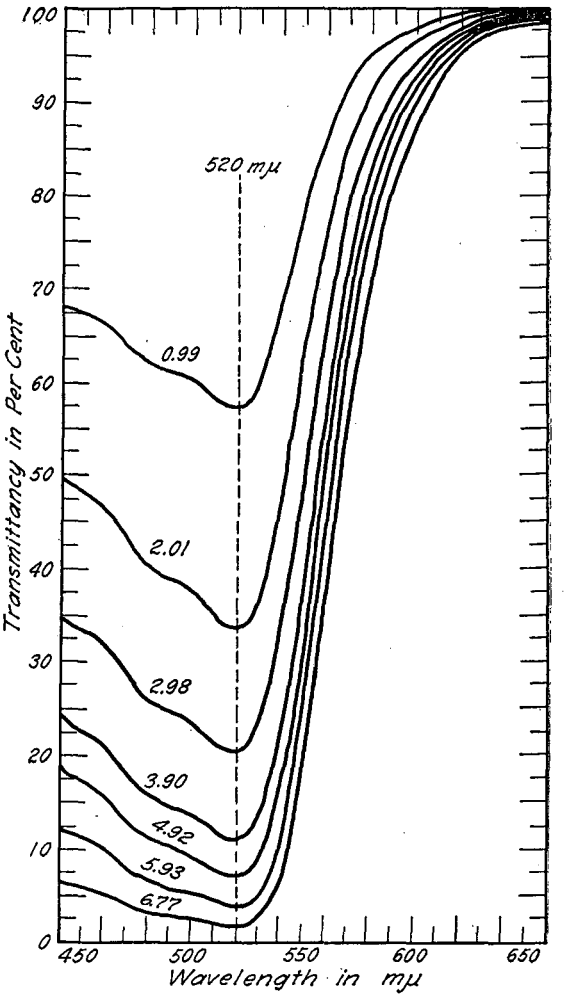

Figure 3. Spectrophotometric Constants of Dimethylferroin Complex Ion

From an examination of the data shown graphically in Figure 3 the wave length of maximum absorption is found to be 520 mμ. This value is constant over the entire range 1 to 7 p.p.m. Conformity to Beer's law was verified by plotting log transmittancy against parts per million of iron, which gives a linear plot. When the plot of data shown in Figure 3 was duplicated using the same solutions after a period of 180 days, duplicate results were obtained within 1.5%; the change was attributed to change in concentration of the solutions, even though they were stored in glass-stoppered containers.

The molecular extinction coefficient calculated from data given in Table I was found to be 12,560. This value is the highest by a small amount of any phenanthroline derivative so far studied.

Titration of Ferrous Iron by Dichromate with Dimethylferroin as Indicator. This procedure may be applied in either hydrochloric or sulfuric acid solutions which are 1 to 2 *F* in

Table II. Standardization of Approximately 0.05 *N* Ferrous Sulfate

(Forward titration employing dimethylferroin indicator. Reverse titration using ferroin as indicator)

Acid Formality	No. of Titrations	Fe^{++} Normality, Forward Titration	Fe^{++} Normality, Reverse Titration
1 *F* H_2SO_4	6	0.04781	0.04785
	3	0.04733	0.04734
	6	0.04698	0.04702
2 *F* H_2SO_4	6	0.04749	0.04753
	6	0.04626	0.04630
			Diphenylamine Sulfonate Forward Titration
1 *F* HCl	6	0.04958	0.04961
			Potentiometric
2 *F* HCl	6	0.04972	0.04969

acid concentration. Dimethylferroin gives, as color change, a transition from orange to green if hydrochloric acid is present and no phosphoric acid is employed to complex ferric ions. The color change in the presence of sulfuric acid is from red to yellowish green.

The indicator may be reversed indefinitely at the equivalence point by alternate dropwise excess of ferrous and dichromate solution additions. Thirty such reversals over a period of 30 minutes did not affect the sharpness of the color change. A 1-ml. excess of 0.1 *N* dichromate did not affect the sharpness of the indicator color change in a titrated solution during 1 hour's time. The use of 0.05 ml. of 0.025 *M* dimethylferroin in a volume of 150 ml. of solution gives a sharp indicator color change. Comparison titrations of ferrous iron by dichromate using the new indicator with the reverse titration of dichromate by ferrous iron using ferroin as indicator are recorded in Table II. These latter tests were made in hydrochloric acid solution.

LITERATURE CITED

(1) Case, F. H., Abstracts of 112th Meeting, Am. Chem. Soc., p. 35L, 1947.
(2) Hume and Kolthoff, *J. Am. Chem. Soc.*, 65, 1895 (1943).
(3) Kolthoff, Lee, and Leussing, Anal. Chem., 20, 985 (1948).
(4) Smith, G. F., "Cerate Oxidimetry," Columbus, Ohio, G. Frederick Smith Chemical Co., 1942.
(5) Smith, G. F., and Richter, F. P., "Phenanthroline and Substituted Phenanthroline Indicators," Columbus, Ohio, G. Frederick Smith Chemical Co., 1944.
(6) Syrokomsky and Stiepen, *J. Am. Chem. Soc.*, 58, 928 (1936).
(7) Walden, Hammett, and Chapman, *Ibid.*, 53, 3908 (1931).

Received July 22, 1948.

Determination of Starch and Cellulose with Anthrone

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A procedure is presented for colorimetric analysis of starch and cellulose at a wave length of 625 mμ, using a 0.1% solution of anthrone in concentrated sulfuric acid. The method is accurate for ranges of 10 to 200 micrograms and sensitive to 2 micrograms of these substances. Because of the instability of the reagent, a known standard must be used with each set of analyses to determine the correct Beer's law constant. Color intensity studies of the effect of heat upon the reaction between anthrone reagent and starch and cellulose are presented. Spectral transmittance curves of carbohydrate-anthrone colors prepared under different conditions are also included.

DREYWOOD (*1*) initially demonstrated the use of anthrone in a specific qualitative test for carbohydrates and suggested its possible quantitative use. Morse (*3*) used anthrone for determining low concentrations of sucrose, and Morris (*2*), in a report that appeared while this article was in preparation, investigated its applications to carbohydrates and some conditions of the reaction. He also studied the relationship of color intensity with various carbohydrates.

The authors have applied this reagent to analysis of starch and cellulose (cotton lint) in air samples collected in plants manufacturing cotton textiles. The discussion that follows includes only analyses of these two substances and pertinent facts concerning the reagent and reaction conditions.

Anthrone can be made as described by other investigators (*1–3*) or obtained commercially (Paragon Testing Laboratories, Orange, N. J., Panrone Chemical Company, Farmington, Conn., and National Biochemical Co., 3106 West Lake St., Chicago 12, Ill.). For the present work, a commercial product obtained in 1946 from the Paragon Testing Laboratories was used without purification.

The test is made by rapidly adding a solution of anthrone (0.05 to 0.20%) in concentrated sulfuric acid to an aqueous solution or suspension of the carbohydrate and mixing immediately. Under controlled conditions the amount of green color produced is proportional to the carbohydrate content. The heat produced by mixing acid and water appears to be a necessary part of the reaction. In analyses for cellulose, sulfuric acid (60% by volume) is used to digest the material prior to analysis and therefore is present (in aliquots of 0.5 ml.) with water and the anthrone reagent. (For dissolving cellulose, 60% sulfuric acid was found to give optimum results regarding rapidity of solution. Cotton is the only form of cellulose referred to in this work.)

Morse (*3*) used unfiltered and Morris (*2*) filtered light, for photoelectric determination of carbohydrates. Neither investigator presented any spectral transmittance data for the anthrone-carbohydrate color to permit proper selection of wave length for maximum sensitivity. Morris (*2*) arrived at an adequate choice (620 mμ) from measurements with three light filters.

SELECTION OF WAVE LENGTH FOR COLOR MEASUREMENT

The purpose of this study was the proper selection of wave length for colorimetric analysis of starch and cellulose with anthrone. Spectral transmittance curves (Figure 1) of the colors

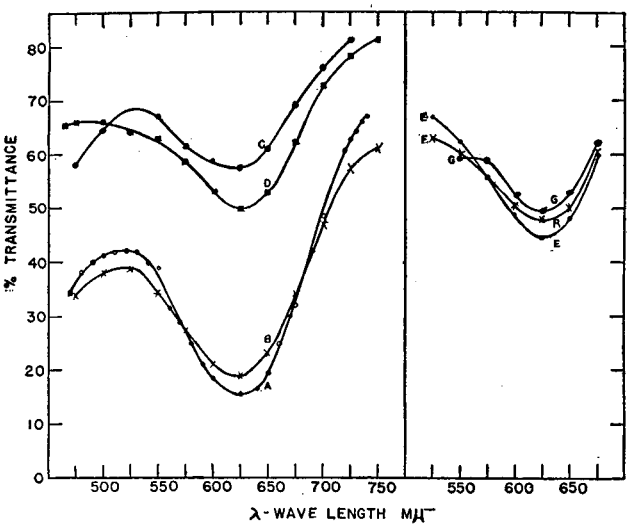

Figure 1. Spectral Transmittance Curves for Anthrone-Cellulose and Anthrone-Starch Colors

A. 90 micrograms of cellulose
B. 90 micrograms of cellulose (reagent 1 day old)
C. 25 micrograms of cellulose
D. 50 micrograms of cellulose (different conditions, see text)
E. 50 micrograms of starch
F. 50 micrograms of starch (reagent 1 day old)
G. Sample *E* after standing several hours

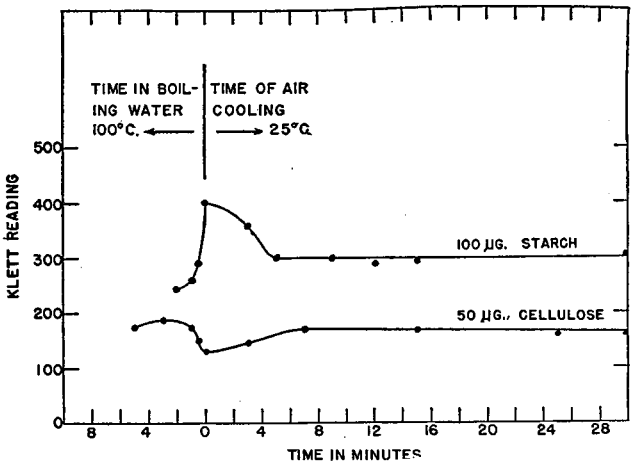

Figure 2. Time of Heating or Cooling before Immersion in Cold Water Bath

Klett-Summerson colorimeter readings, λ = 625 mμ
Klett reading = (constant) log $1/T$ = (constant) (optical density)

resulting from the reaction between anthrone and starch or cellulose were determined by a Coleman Model 11 Universal spectrophotometer. These colors were prepared under different conditions to determine their effect on the spectral absorption bands, particularly the wave length of maximum absorption. The conditions involved were: variations in age of the anthrone reagent, water content, anthrone concentration, age of the color, and because two different carbohydrates were used, variations in the carbohydrate. Reference blanks used for transmittance measurements consisted of the same reagents used for the carbohydrate analysis.

In Figure 1, curves *A* and *C* are for 90 and 25 micrograms of cellulose, respectively, prepared from 0.5 ml. of 60% sulfuric acid, 1.5 ml. of water, and 4.0 ml. of a 0.2% solution of anthrone in concentrated sulfuric acid. A wave length of minimum transmittance of 625 mμ is indicated. Curve *B* represents the same cellulose content and reactants as curve *A* (90 micrograms of cellulose) except that the anthrone solution used was 1 day old. The spectral transmittance curve changes with age of the anthrone solution but the wave length of maximum absorption remains at 625 mμ. The color represented by curve *D* is based on reaction between 50 micrograms of cellulose, 0.5 ml. of sulfuric acid, 2.0 ml. of water, and 4.0 ml. of 0.1% solution of anthrone in concentrated sulfuric acid. This differed from curves *A* and *C*, which contained less water (0.5 ml. less) and twice as much anthrone and different amounts of carbohydrate. Although the spectral transmittance curve shape is altered by these factors (different water and anthrone content), the maximum absorption band remains at 625 mμ.

Curves *E* and *F* are spectral transmittance curves for colors prepared from 50 micrograms of starch, 2.0 ml. of water, and 4.0 ml. of 0.1% anthrone in concentrated sulfuric acid. Curve *F* involved an anthrone solution 1 day old. The similarity of these curves with *A* and *B* can be readily noted. The wave length of minimum transmittance is also 625 mμ, although a different carbohydrate is used. Curve *G* is based on the same solution used for curve *E* after standing for several hours. Fading on standing changes the intensity of color but not the wave length of maximum absorption (625 mμ).

The use of a filter of this wave length for colorimetric analysis of starch and cellulose with anthrone is therefore recommended and it is probable that this wave length is suitable for reactions of other carbohydrates with anthrone.

EFFECTS OF HEAT UPON REACTION

Previous workers (*1–3*) have found that the color intensity of the anthrone reaction with carbohydrates is widely influenced by heat. Experiments were undertaken to determine the optimum reaction conditions with regard to heat. Consistent results with minimum error rather than maximum sensitivity were desired.

Standard solutions containing 100 micrograms of starch or 50 micrograms of cellulose were placed in Klett-Summerson colorimeter tubes and the anthrone reagent was added by means of a pipet or buret. The components were mixed immediately after addition and subjected to air cooling or immersion in a boiling water bath for definite time intervals. Following this interval, the tubes were immersed in a cold water bath for 15 minutes or more. The color was then read on a Klett-Summerson colorimeter using a 625 mμ (±1.5 mμ) filter (evaporated metal film filter obtained from Baird Associates, Cambridge, Mass.). For starch, 2.0 ml. of distilled water and 4.0 ml. of 0.1% solution of anthrone in concentrated sulfuric acid were used; however, because 60% sulfuric acid was used in the analytical procedure for dissolving cellulose, this procedure required 0.5 ml. of 60% sulfuric acid, 2.0 ml. of water, and 4.0 ml. of 0.1% anthrone reagent. Reference blanks consisted of mixtures of similar amounts of acids, water, and anthrone reagent which were allowed to air-cool completely. Because the Klett-Summerson colorimeter contains a logarithmic (optical density) scale, dial readings are directly proportional to color intensity or concentration of carbohydrate if the solution obeys Beer's law.

The results obtained (Figure 2) show consistent readings after 10 minutes of air cooling, although the maximum sensitivity is not obtained. The quantity of heat produced by the reaction of starch with the reagent appeared to be detrimental to the color developed. Thus maximum color development occurred upon immediate cooling in cold water; and in the hot water immersion tests rapid deterioration of color took place. The cellulose reaction with anthrone apparently did not create sufficient heat to develop maximum color, for increased color results on air cooling and still additional color increase takes place with immersion in boiling water. Deterioration of color begins after immersion for 3 minutes in boiling water. Because of the consistent results obtained in both cases after air cooling for 10 minutes, this method of treatment was adopted for the analysis of these two carbohydrates.

Different amounts of water used in the test also result in changes in amount of heat accompanying the reaction and, therefore, affect color intensity. In the present work 2.0 ml. of water were found to be satisfactory. Tests for cellulose using 2.5 ml. of water resulted in turbidity; this indicates that the minimum concentration of sulfuric acid in the final solution should be 65% for the amounts of anthrone specified.

Table I. Stability of Starch-Anthrone Color

Time of Standing	Average Klett-Summerson Readings for 50 γ of Starch, λ = 625 mμ
Min.	
5	120 ± 2
10	120 ± 2
25	120 ± 2
45	120 ± 2
Hours	
1	120 ± 2
3	120 ± 2
4	118 ± 2
24	113 ± 1

Average deviation based on 3 samples

METHOD OF ADDING REAGENTS

Variations in the method of adding anthrone reagent may cause variations in maximum solution temperature and affect the color intensity. Morse (*3*) added all the anthrone reagent to form two layers and then mixed the solutions. The authors' experience with this method for cellulose and starch showed less consistent results than those obtained with rapid addition and mixing. Apparently it is difficult to form two layers with water and concentrated sulfuric acid without evolution of appreciable amounts of heat which create convection mixing currents. Undoubtedly partial evolution of heat caused by such mixing is a significant variable and causes inconsistencies. Any method of addition that yields consistent results is satisfactory.

COLOR STABILITY

Although tests have been made on the rate of color formation of anthrone with a carbohydrate (*3*), information on the deterioration of anthrone-carbohydrate color has not been presented.

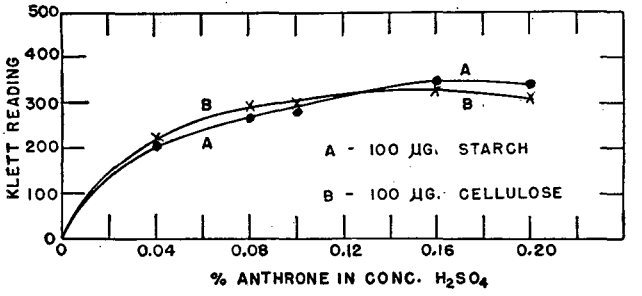

Figure 3. Percentages of Anthrone in Concentrated Sulfuric Acid with Cellulose and Starch Standards
Klett-Summerson colorimeter readings, λ = 625 mμ

Tests made with anthrone and starch indicate complete stability (Table I) from 5 minutes to 3 hours, after which slight fading occurs.

ANTHRONE CONCENTRATION AND COLOR FORMATION

Observations made with solutions of increasing anthrone concentration revealed increased sensitivity. Data from tests made on starch and cellulose with different anthrone solutions are presented in Figure 3. Reference zero standards consist of the same reactants in each case minus the carbohydrate. Maximum sensitivity occurs with 0.16% anthrone solution. Greater concentration of anthrone reduces sensitivity. As there is only a slight increase in sensitivity using 0.16% instead of 0.10% anthrone, a 0.10% solution was selected. Slight turbidity has been observed in some cases when anthrone solutions of higher concentration (0.2%) were used.

STABILITY OF REAGENT

A disturbing feature of this method is reagent instability, which has been investigated previously (*2*, *3*). This instability is characterized by a darkening of the reagent over a period of time. Further investigation was made with various anthrone concentrations in concentrated sulfuric acid using the Klett-Summerson colorimeter to determine reagent color changes at 625 mμ, over a 5-day period (Figure 4). The results clearly show the greater stability of the more concentrated solutions.

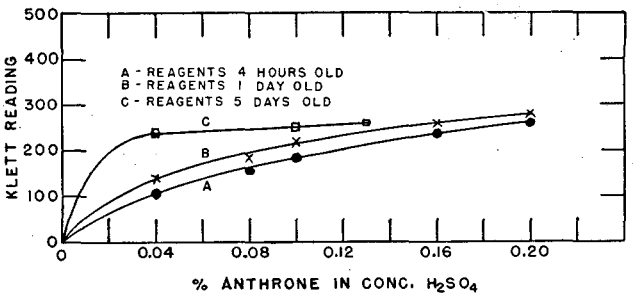

Figure 4. Percentages of Anthrone in Concentrated Sulfuric Acid over 5 Days
Concentrated H_2SO_4 as reference blank
Klett-Summerson colorimeter readings, λ = 625 mμ

STANDARDIZATION AND APPLICATION OF BEER'S LAW

Because anthrone reagent exhibits instability, and in the authors' experience inconsistencies in sensitivity, the use of a single standard curve is not practicable. Accurate results can be obtained by the use of one or more known standards for each group of analyses. Obviously this method depends upon the application of Beer's law for all such anthrone solutions. In investigations with solutions up to 9 days old (maximum age of any solution tested) Beer's law was followed, as shown in Figure 5. This observation is in agreement with Morris's (*2*) that even after 1 month straight-line calibrations were obtained at 620 mμ. Freshly prepared solutions of anthrone (less than 2 hours old) should not be used, as they cause inconsistent results. Reagents at least 4 hours old are recommended, with one or more known standards for each group of analyses. Such solutions will give a sensitivity of approximately 2 micrograms of starch or cellulose with the following procedures.

ANALYSIS OF CELLULOSE AND STARCH

Starch. The solid sample is boiled in distilled water, cooled, and made up to a measured volume with distilled water so that a 2.0-ml. aliquot contains 10 to 200 micrograms of starch. If the solution is turbid, part of it is filtered through a dry filter paper. A 2.0-ml. aliquot in a colorimeter tube is treated with 4.0 ml. of 0.1% anthrone in concentrated sulfuric acid (4 hours to 9 days old) rapidly added from a pipet or buret. The solution is mixed immediately and allowed to air-cool. After approximately 10 to 15 minutes, the tube is cooled completely in a cold water bath. A

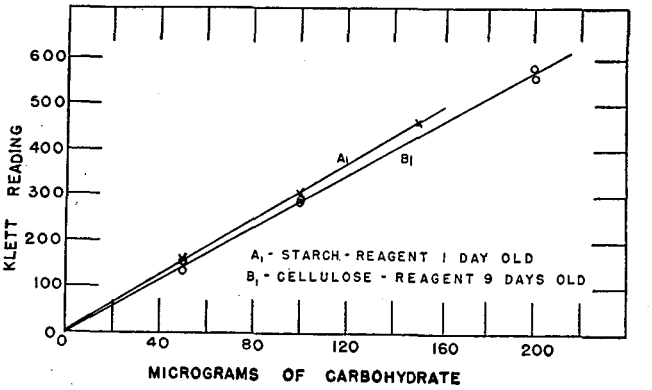

Figure 5. Standard Curves for Starch and Cellulose, Indicating Application of Beer's Law
Klett-Summerson colorimeter readings, λ = 625 mμ

reference blank containing 2.0 ml. of distilled water is treated similarly. At the same time one or more 2.0-ml. starch standards (100 micrograms suitable) receive the same treatment. The colorimeter is adjusted to zero with the reference blank and the samples and standards are then read. When a logarithmic scale colorimeter is used, concentrations are proportional to scale readings. The proportionality constant is determined by the standards used. Colorimeters with a transmittance scale require a semilogarithmic calibration curve.

Cellulose. The solid sample is digested (cold) in 60% sulfuric acid for 15 to 30 minutes. The solution is made up to a measured volume with 60% sulfuric acid, so that a 0.5-ml. aliquot contains 10 to 200 micrograms of cellulose. If the solution contains a residue, it is filtered through a dry asbestos mat previously washed with 60% sulfuric acid and then with water. A 0.5-ml. aliquot is then added to 2.0 ml. of water, and allowed to cool. Then 4.0 ml. of a 0.1% anthrone solution are added and the starch procedure above is employed. A reference blank is prepared from 0.5 ml. of 60% sulfuric acid, 2.0 ml. of water and 4.0 ml. of 0.1% anthrone reagent. Standards are prepared from 0.5-ml. aliquots of known amounts of cellulose in 60% sulfuric acid.

Mixtures of Cellulose and Starch. Mixtures of starch and cellulose (cotton) in the presence of each other are analyzed as follows:

The sample after boiling in water is filtered through a fine porosity sintered-glass filter or asbestos Gooch pad. The filtrate is analyzed for starch and the residue retained by the filter is digested in 60% sulfuric acid for 15 to 30 minutes. This solution is then filtered again if necessary and the filtrate is analyzed for cellulose as outlined above.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Maria Frenkel.

The work was done on a grant provided by the Nashua Manufacturing Company and the Saco-Lowell Shops.

LITERATURE CITED

(1) Dreywood, Roman, IND. ENG. CHEM., ANAL. ED., 18, 499 (1946).
(2) Morris, D. L., *Science*, 107, 254–5 (1948).
(3) Morse, E. E., IND. ENG. CHEM., ANAL. ED., 19, 1012–13 (1947).

RECEIVED May 3, 1948.

Dichromate Reflux Method for Determination of Oxygen Consumed

Effectiveness in Oxidation of Organic Compounds

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Although the proposed method for the determination of oxygen consumed has definite limitations, nevertheless it will be of value in estimating the strength of industrial wastes and sewage. Hydrocarbons and straight-chain acids are oxidized very slightly. The end products obtained in the oxidation of amino acids vary with the type of acid used. Branched-chain acids and alcohols as well as phenolic compounds are readily attacked. Sugars are quantitatively broken down to carbon dioxide and water. When 50% by volume of sulfuric acid is used in the reflux mixture, chlorides are quantitatively oxidized. The oxygen consumed values of industrial wastes containing high chloride concentrations can, therefore, be corrected for their chloride content.

SINCE the inception of a biochemical oxygen demand test in 1870 by Frankland (*9*) and in 1884 by Dupré (*7*) for the determination of the strength of waste products of human or industrial origin, numerous attempts have been made to devise a chemical method that would give the same results in a much shorter time. Inasmuch as the metabolic activities of the flora and fauna in different samples of waste do not necessarily follow a constant rate, a chemical method would not necessarily correlate with the biochemical determination of oxygen demand.

However, it is frequently desirable to know in the minimum time the approximate oxygen-absorbing power of a waste. A chemical method for determining oxygen consumed is most satisfactory for this purpose, although it will give a value that is not comparable to B.O.D. on toxic wastes and will give higher results on stabilized biological treatment plant effluents, because it is impossible for a chemical method to differentiate between organic matter in biologically stable and unstable forms. Because a chemical method for determining oxygen consumed seems desirable as an additional criterion of pollution control, it is also necessary to study such procedures more thoroughly in order to understand, apply, and interpret the data obtained with them.

Of the various oxidizing agents available, in general, only four have been used to any extent for determining the oxygen-consuming power of sewage and industrial wastes—namely, potassium permanganate, potassium dichromate, ceric sulfate, and iodic acid.

Potassium permanganate is still used in the recommended procedure (*2*). Stamm (*22*) carried out the oxidation with permanganate in an alkaline solution and prevented the reaction of $MnO_4^{--} \rightarrow MnO_2$ by the addition of a barium salt which allows a better end point to be obtained. Benson and Hicks (*4*), in determining the pollution in sea water, found that application of the Zimmerman-Reinhardt procedure in titrating the excess permanganate gave more reproducible results. Haupt (*10*) tried to correlate the permanganate oxygen consumed with the B.O.D. on wastes from paper pulp factories. He found, however, that the chemical method gave much higher results, due to the fact that cellulose is not readily attacked by either dissolved oxygen or bacteria.

As with most chemical methods, variation in conditions affect the result obtained. Matubara (*16*) found that increased values could be obtained by increasing the boiling time, increasing the concentration of potassium permanganate used, saponifying the fats or oils, and neutralizing water-soluble fatty acids. Lovett (*15*) states that totally different values can be obtained depending on whether 0.125 *N* or 0.0125 *N* potassium permanganate is used. Kashkin and Karasik (*13*) added an initial excess of potassium permanganate calculated to be equivalent to 0.3 to 0.5 mg. of oxygen, and determined the final excess by titration with oxalic acid at boiling temperature. Shutkovskaya (*21*) compared the discoloration of the potassium permanganate by the sample

Table I. Oxidation of Organic Compounds by the Dichromate Reflux Method

Compound	Formula	Oxygen Consumed: 33% H_2SO_4 by Volume Replicates	33% H_2SO_4 Average	50% H_2SO_4 by Volume Replicates	50% H_2SO_4 Average	Theoretical	% Deviation from Theoretical: 33% H_2SO_4	50% H_2SO_4
Glucose	$C_6H_{12}O_6$	1036 1035 1023 1032	1033	...	...	1066	3.1	...
Lactose	$C_{12}H_{22}O_{11}$	1021 1017	1019	1027 1049	1038	1066	4.4	2.6
Acetic acid	CH_3CO_2H	15.0 20.0	17.5	52.8 54.8	53.8	1066	98.3	94.9
Lactic acid	$CH_3CHOHCO_2H$	443 438	441	549 491	520	1066	57.7	48.8
Citric acid	CH_2CO_2H $\vert$ $CHOHCO_2H$ $\vert$ CH_2CO_2H	698 651	675	760 744	752	787	14.1	3.2
Tartaric acid	$CHOHCO_2H$ $\vert$ $CHOHCO_2H$	536 525	531	528 526	527	533	0.37	1.1
Malic acid	CH_2CO_2H $\vert$ $CHOHCO_2H$	657 662	660	680 685	683	716	7.8	4.6
Furoic acid	$CH=C-CO_2H$ $\vert \quad \diagdown O$ $CH=CH \diagup$	701 691	697	1107 1101	1104	1285	45.9	14.1
Benzoic acid	$C_6H_5CO_2H$	251 241	246	1963 1941	1952	1967	87.5	0.76
Salicylic acid	$C_6H_4(OH)(CO_2H)$ (*o*)	1534 1531	1533	1602 1575	1589	1622	5.4	2.0
m-Hydroxybenzoic acid	$C_6H_4(OH)(CO_2H)$ (*m*)	1537 1527	1532	1620 1587	1602	1622	5.5	1.2
Glycine	$CH_2(NH_2)CO_2H$	202 197	200	632 627	630	640	68.2	1.5
Alanine	$CH_3CH(NH_2)CO_2H$	163 164	164	408 400	404[a]	1258[a] 359	86.9	67.9 12.5
Tyrosine	$OHC_6H_4CH_2CH(NH_2)CO_2H$ (*p*)	1435	1435	1659 1624	1642	1678	14.5	2.1
Valine	CH_3 $\prime$ $CH_3 \diagup CHCH(NH_2)CO_2H$	252 261	257[b]	1332 1314	1323	1641	83.4	19.4
α-Amino isobutyric acid	CH_3 $CH_3 \diagup C(NH_2)CO_2H$	183 167	175	1240 1153	1197	1398	87.4	14.3
Glutamic acid	$CO_2HCH_2CH_2CH(NH_2)CO_2H$	213 215 206	211	827 820 855	834	980[c] 218	78.4 3.2	14.9
Isopropyl alcohol	CH_3 $CH_3 \diagup CHOH$	842 834	838	1611 1599	1605	1600	47.6	0.31
Ethyl alcohol	C_2H_5OH	667 676	672	747 747	747	2087 695[d]	66.7 3.3	63.7 7.5
Catechol	$C_6H_4(OH)(OH)$ (*o*)	1737 1738	1738	2024 1872	1948	1891	8.1	3.0
5-Me-2-isopropyl phenol (thymol)	$(CH_3)(C_3H_7)C_6H_3OH$	1667	1667	2225 2217	2221	2346	24.6	5.3
p-Cumyl phenol	CH_3 $\vert$ $OHC_6H_4C-C_6H_5$ $\vert$ CH_3	1111 1115	1113	2648 2568	2608	2596	57.1	0.46
o-Cresol	$C_6H_4(OH)(CH_3)$ (*o*)	2174 2158	2164	2267 2277	2272	2518	14.0	9.7
m-Cresol	$C_6H_4(OH)(CH_3)$ (*m*)	2139 2155	2147	2334 2324	2329	2518	14.7	7.4
2-4,6-Trinitrophenol	$(NO_2)_3C_6H_2OH$	526 449	488	923 923	923	976	50.0	5.1
2-Naphthol	$C_{10}H_7OH$	2360 2380	2370	2526 2518	2522	2556	7.2	1.3
Benzene	C_6H_6	10.0 7.5	8.8	263 280	272	3072	99.7	91.1
Pyridine	C_5H_5N	0.0	...	0.0	...	2531	100	100
Toluene	$C_6H_5CH_3$	205 215	210	752 742	747	3130	93.3	76.1
Cellulose	$(C_6H_{10}O_5)_x$	1150	1150	...	...	1185	2.9	...

[a] $CH_3CH(NH_2)CO_2H + 3\frac{1}{2}O_2 \longrightarrow 3CO_2 + 3H_2O + NH_3$. Theory is 1258 but reaction obviously does not follow this course.
$CH_3CH(NH_2)CO_2H + O_2 \longrightarrow CH_3CO_2H + NH_3 + CO_2$. Theory is 359 p.p.m. However, as shown with CH_3CO_2H, there is an oxygen consumed of 54 p.p.m.; hence observed value would be expected to be high.

[b] $CH_3 \diagup CHCH(NH_2)CO_2H + O_2 \longrightarrow CO_2 + NH_3 + CH_3 \diagup CHCO_2H$. Theory is 273. This reaction evidently takes place.
Odor of isobutyric acid noticeable.

on heating and allowing to stand for 5 minutes to standard colored-glass plates calibrated in p.p.m. of oxygen.

The amount of research carried out with the permanganate method shows that it is not entirely satisfactory for the determination of oxygen consumed values. For this reason, workers in this field have turned their attention to the use of other oxidizing agents.

Klein (*14*) made a comparison of the permanganate, dichromate, and ceric sulfate methods of determining the strength of sewage. He found that ceric sulfate gave values two thirds that of the dichromate but two to three times that of the permanganate. Bezel (*5*) also found that ceric sulfate was superior to permanganate because of the greater stability of the reagent and of the titer.

Adeney and Dawson (*1*) were among the first to use dichromate in the presence of sulfuric acid to determine the organic matter in water. They heated the mixture of 100° to 110° C. for 2 hours and titrated the excess dichromate with ferrous sulfate using an outside indicator. Popova (*19*) also used dichromate to determine the "oxidizability" of sewage and found it gave results of about 86% of the B.O.D. Ostrovskaya (*18*) and Rhame (*20*) made use of the iodometric procedure for determining the excess of dichromate present. This method, however, required rather careful manipulation and gave rather wide variations in the calculated B.O.D. and the standard B.O.D. test. Ingols (*11*) modified Rhame's procedure by refluxing the sample and the oxidizing mixture for 60 minutes at about 145° C. He determined excess dichromate iodometrically.

In 1938 Dzyadzio (*8*) used potassium iodate in a 65 to 80% sulfuric acid solution as the oxidizing agent, heated the mixture at 200° C., and determined excess iodate iodometrically. He claimed that the error obtained in the oxidation of 14 organic compounds did not exceed 2 to 3% and that the method is superior to the dichromate method. Johnson, Tsuchiya, and Halvorson (*12*) also used the iodic acid method. In their work, they refluxed the mixture if the sample was high in volatile acids.

Another approach to the problem of determining the oxygen consumed by sewage and industrial wastes was tried by Mohlman and Edwards (*17*), who used chromic acid as the oxidizing agent and absorbed the liberated carbon dioxide in 0.1 *N* barium hydroxide. The excess barium hydroxide was titrated and the oxygen consumed calculated from the amount of barium hydroxide used. This method, as well as that of Burtle and Buswell (*6*), requires very careful manipulation and rather complicated apparatus. In the latter case, the precipitated barium carbonate is filtered and weighed.

In samples of sewage and industrial wastes there are present a wide variety of organic compounds. In order to evaluate the usefulness of any chemical method for determining the oxygen consumed, it is helpful to known just how efficient that method is in the oxidation of various organic compounds. The dichromate method proposed was, therefore, studied with about 30 organic compounds of various classes.

APPARATUS AND PROCEDURE

The reflux apparatus used consisted of a 300-ml. round-bottomed flask with a 24/40 taper-joint neck connected with a Friedrich's reflux condenser. All samples were run in duplicate and a blank containing 50 ml. of distilled water was run simultaneously.

One gram of the organic compound under study was weighed out, dissolved in distilled water, and diluted to 1 liter. In the case of phenolic compounds, it was necessary to add alkali to effect the solution of the compound. With compounds such as benzene, it was necessary to homogenize the mixture of water and the organic compound in a colloid mill and dilute the emulsion to 1 liter. The size of sample taken for the oxidation was based on the theoretical amount of oxygen necessary for complete combustion to carbon dioxide and water. This amount of sample was diluted with distilled water to 50 ml. and placed in the round-bottomed flask. To the sample 25 ml. of 0.25 *N* potassium dichromate were added, followed by 75 ml. of 95% sulfuric acid. A few granules of pumice were added to prevent bumping and the flask was connected to the reflux condenser. The mixture was refluxed for 2 hours, cooled, transferred to a 500-ml. Erlenmeyer flask, and diluted to about 300 ml. The excess potassium dichromate was titrated with 0.25 *N* ferrous diammonium sulfate using *o*-phenanthroline ferrous complex as an indicator.

The end point is sharp, changing from a gray-green to red. When the concentration of sulfuric acid was 50% by volume or less, no difficulty was encountered in determining the end point. However, if a higher acid concentration, or stronger dichromate was used, it was necessary to dilute the refluxed mixture three to four times with distilled water in order to reach the correct end point. The standard ferrous diammonium sulfate was standardized each day. The blank determination rarely exceeded 0.2 ml. of 0.25 *N* potassium dichromate. The temperature of refluxing, using the 50% by volume of sulfuric acid was 145° to 150° C.

EFFECT OF CHLORIDES

In 1932 Bach (*3*) found that the oxygen consumed value of raw sewage was increased from 258 to 291 p.p.m. when the sodium chloride content varied from 20 to 2000 p.p.m. In Figure 1 the effect of chlorides on oxygen consumed values obtained with 0.25 *N* potassium dichromate is shown. When 50% by volume of sulfuric acid is used, quantitative oxidation of chlorides is obtained over the range from 250 to 20,000 p.p.m. However, when 33% by volume of sulfuric acid is employed the results obtained are somewhat erratic, the amount of oxidation depending on the amount of 0.25 *N* potassium dichromate used. With 50.0 ml. of the dichromate, the results are not so erratic as with 25.0 ml. The chloride correction in the latter instance is subject to a larger error.

OXIDATION OF ORGANIC COMPOUNDS

In Table I, the results obtained with 32 organic compounds are given. These compounds represent several different types, such as sugars, aliphatic and aromatic acids, amino acids, alcohols, phenolic compounds, and hydrocarbons. No attempt was made to repurify any of these compounds and for this reason the oxidation values obtained may be slightly low.

With the two sugars, glucose and lactose, the oxidation to carbon dioxide and water is about 97% complete. Cellulose (represented by filter paper) is 100% oxidized under the experimental conditions used. As expected, the straight-chain acids are hardly attacked. When (as with lactic acid) an OH group is introduced into the straight chain, slightly better oxidation is obtained. Approximately 51% of this acid is broken down to carbon dioxide and water, using the 50% acid concentration. No difficulty was encountered in oxidizing the branched-chain or aromatic acids, as shown in Table I. Heterocyclic acids, such as furoic acid, were not so easily attacked under the conditions set up. Furoic acid was oxidized to only 85% of completion. Of the amino acids studied only glycine and tyrosine were quantitatively oxidized to carbon dioxide, water, and ammonia. If we assume, as shown in the following equation, that a mole of acetic acid is formed in the oxidation of alanine:

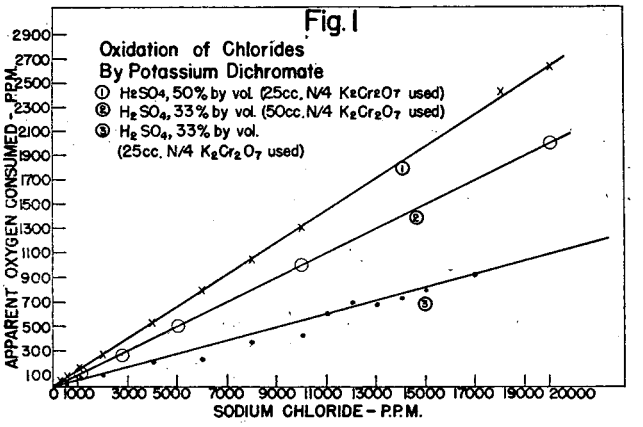
$$CH_3CH(NH_2)CO_2H + O_2 \longrightarrow CH_3CO_2H + NH_3 + CO_2$$

about 88% breakdown is obtained and, as pointed out above, the acetic acid formed would not undergo further oxidation. Valine, representing a branched-

[c] $CO_2HCH_2CH_2CH(NH_2)CO_2H + 4\frac{1}{2}O_2 \longrightarrow 5CO_2 + NH_3 + 3H_2O$. Theory is 980.
$CO_2HCH_2CH_2CH(NH_2)CO_2H + O_2 \longrightarrow CH_3CH_2CO_2H + 2CO_2 + NH_3$. Theory is 218.
[d] $C_2H_5OH + O_2 \longrightarrow CH_3CO_2H + H_2O$. Theory is 694. However, 50% H_2SO_4 gave oxygen consumed of 54 p.p.m. for CH_3CO_2H which will account for higher value obtained.

Table II. Comparison of Dichromate and Iodic Acid as Oxidizing Agents

Compound	% Deviation from Theoretical	
	Dichromate	Iodic acid
Glucose	3.1	13.0
Lactose	2.6	14.0
Acetic acid	94.9	77.1
Tartaric acid	1.1	5.8
Benzoic acid	0.76	3.3
Glycine	1.5	0.0
Tyrosine	2.1	8.1
Glutamic acid	14.9	0.13
Pyridine	100	80.9
Benzene	91.1	74.8
Toluene	76.1	88.7



chain amino acid, is apparently broken down with the 33% sulfuric acid in accordance with the following equation:

$$\begin{matrix}CH_3\\ \diagdown\\ \quad CHCH\ (NH_2)CO_2H\\ \diagup\\ CH_3\end{matrix} + O_2 \longrightarrow NH_3 + CO_2 + \begin{matrix}CH_3\\ \diagdown\\ \quad CHCO_2H\\ \diagup\\ CH_3\end{matrix}$$

as evidenced by the odor of isobutyric acid in the reaction flask. With the 50% acid concentration, complete oxidation is not obtained, about 81% of the valine being broken down to carbon dioxide, water, and ammonia. Glutamic acid is broken down about 86% of the theoretical with 50% by volume of sulfuric acid. With the 33% acid concentration, it is possible that the following reaction takes place:

$$CO_2HCH_2CH_2CH(NH_2)CO_2H + O_2 \longrightarrow \begin{matrix}CH_2CO_2H\\ |\\ CH_2CO_2H\end{matrix} + CO_2 + NH_3$$

The theoretical amount of oxygen required for this reaction is 218 p.p.m. and the experimental amount obtained was 211 p.p.m. Of the two alcohols studied isopropyl alcohol is quantitatively oxidized, whereas the straight-chain ethanol is oxidized to acetic acid which is not further attacked. No difficulty was encountered in oxidizing the phenolic compounds studied, the amount of oxidation ranging from 90% for *o*-cresol to 99.5% for *p*-cumylphenol. Benzene was about 10% oxidized and pyridine was not attacked at all under the experimental conditions employed. The one substituted aromatic hydrocarbon used was toluene. It would normally be expected that this compound would be easily oxidized to benzoic acid, which was completely oxidized as shown in Table I. However, on the basis of complete oxidation only 24% of the theoretical value was obtained. Neither increasing the acid concentration to 66% by volume nor increasing the potassium dichromate strength to 0.5 *N* had any effect on the oxidation of toluene. Various catalysts were also employed, such as selenium, copper, iron, nickel, and platinum, but still only about 25% of the theoretical value was obtained.

In Table II a comparison of dichromate and iodic acid as oxidizing agents is shown. The oxidation with iodic acid was carried out by refluxing the compound with potassium iodate in a mixture of phosphoric and sulfuric acids. Iodic acid is not superior to dichromate in the oxidation of many of these compounds and the analytical work involved is more complicated.

RECOMMENDED PROCEDURE

Dilute an appropriate amount of sample to 50 ml. with distilled water in a 300-ml. round-bottomed flask with a taper-joint neck, add 25.0 ml. of 0.2500 *N* potassium dichromate and 75 ml. of concentrated sulfuric acid, and reflux for 2 hours. For the best quantitative results, use 50% by volume sulfuric acid. Cool, transfer the mixture to an Erlenmeyer flask, and titrate the excess potassium dichromate with approximately 0.2500 *N* ferrous ammonium sulfate, using *o*-phenanthroline ferrous complex as an indicator. Reflux a blank at the same time, using the same amount of reagents and substituting 50 ml. of distilled water for the sample. The ferrous ammonium sulfate must be standardized daily.

Calculation.

$$O.C.,\ \text{p.p.m.} = \frac{(a - b) \times \text{normality (standard Fe)} \times 8000}{\text{Volume of sample}}$$

where

$O.C.$ = oxygen consumed
a = ml. of $Fe(NH_4)_2(SO_4)_2$ used for blank
b = ml. of $Fe(NH_4)_2(SO_4)_2$ used for sample

CONCLUSION

As with most wet combustion methods employed, the method proposed has its limitations. However, the results obtained are reproducible and the method should be useful in determining the approximate strength of sewage and industrial wastes. Hydrocarbons as well as straight-chain acids and alcohols, are scarcely attacked. In contrast to this, the aerobic bacteria are able to utilize and oxidize the latter two types of compounds as a food source. Branched-chain aliphatic acids and alcohols are, as a rule, readily oxidized by the proposed chemical method, and no difficulty is encountered in the oxidation of sugars. Phenolic compounds are also oxidized quantitatively. Chlorides are shown to be quantitatively oxidized at the higher acid concentration. Correction for the chloride content of industrial wastes may be made. With the lower acid concentration, however, the amount of oxidation is dependent upon the volume of 0.25 *N* potassium dichromate used.

LITERATURE CITED

(1) Adeney, W. E., and Dawson, B. B., *Sci. Proc. Roy. Dublin Soc.*, **18**, 199–202 (1926).
(2) Am. Pub. Health Assoc. and Am. Water Works Assoc., "Standard Methods for the Examination of Water and Sewage," 9th ed., p. 122, New York, 1946.
(3) Bach, H., *Z. anal. Chem.*, **89**, 439–41 (1932).
(4) Benson, H. K., and Hicks, J. F. G., Jr., IND. ENG. CHEM., ANAL. ED., **3**, 30–1 (1931).
(5) Bezel, L. I., *J. Applied Chem.* (*U.S.S.R.*), **18**, 361–6 (1945).
(6) Burtle, Jerome, and Buswell, A. M., *Sewage Works J.*, 224–38 (1937).
(7) Dupré, "Stream Sanitation," E. B. Phelps, p. 66, New York, John Wiley & Sons, 1944.
(8) Dzyadzio, A. M., *Vodosnabzhenie i Sanit. Tekh.*, No. 8–9, 117–25 (1938).
(9) Frankland, Sir Edward, "Stream Sanitation," by E. B. Phelps, p. 65, New York, John Wiley & Sons, 1944.
(10) Haupt, H., *Vom Wasser*, **10**, 60–77 (1935).
(11) Ingols, Robert, and Murray, P. E., *Water & Sewage Works*, **95**, 113–17 (1948).
(12) Johnson, D. W., Tsuchiya, H. M., and Halvorson, H. O., Abstracts of 109th Meeting, AM. CHEM. SOC., p. 2 S, Atlantic City, 1946.
(13) Kashkin, M. L., and Karasik, R. M., *Med. exptl.* (*Ukraine*), No. 4, 9–13 (1940).
(14) Klein, L. J., *Proc. Inst. Sewage Purif.* (England), **1941**, 174–91.
(15) Lovett, M. J., *Ibid.*, **1940**, 194–5
(16) Matubara, Tamenaka, *Mitt. Med. Akad. Kioto*, **28**, 563–78 (1940).

(17) Mohlman, F. W., and Edwards, G. P., IND. ENG. CHEM., ANAL. ED., 3, 119–23 (1931).
(18) Ostrovskaya, R. E., *Lab. Prakt. (U.S.S.R.)*, Sammelband, 84–6 (1939).
(19) Popova, E. S., *Trav. comm. recherches épurat. eaux (Moscow)*, No. 12, 143 (1930).
(20) Rhame, G. A., *Water & Sewage Works*, 94, 192–94 (1947).
(21) Shutkovskaya, L. A., *Hig. i Sanit. (U.S.S.R.)*, 10, No. 10/11,54–7 (1945).
(22) Stamm, Hellmuth, *Angew. Chem.*, 47, 791–5 (1934).

RECEIVED June 30, 1948. Presented before the Division of Water, Sewage, and Sanitation Chemistry at the 113th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Extraction of Carotene from Green Leaves

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Hot light petroleum (85° C.) extracted nearly all the carotene from dried grass meal but only very little from fresh green leaves. Higher temperature (130° C.) increased the extraction from leaves but destroyed some carotene. A mixture of cold light petroleum, acetone, and quinol easily extracted the carotene from leaves. Extraction of carotene by hot petroleum is hindered by large particle size and to a less extent by water. In support of these conclusions it has been shown that rehydration of grass meal reduced the extraction rate; extraction of whole dried leaves by hot petroleum was incomplete; and the finer the disintegration of the dried leaves the better the extraction. By grinding fresh leaves to a fine pulp, almost complete extraction with hot petroleum was achieved. But carotene was lost by oxidation, and although this could be countered by grinding with solid quinol, such a technique offers no advantage over that using cold petroleum-acetone.

IN A collaborative investigation, initiated by the Crop Driers Association in England, a simple method was evolved for the rapid estimation of total carotene in dried grass meal (*12*). (The term "dried grass" is used for convenience and covers other dried fodder crops including alfalfa or lucerne. Alfalfa meal was in fact used for many of the experiments.) The extraction part of this technique had been worked out by J. R. Edisbury. In this method the meal is heated with light petroleum for 1 hour on a water bath at about 90° C. Light petroleum, boiling range 80° to 100°, is used in a Kjeldahl flask, whose long neck acts as a condenser. The temperature attained by the extractant is about 85°. One extraction dissolves over 97% of the carotene. The solution is cooled and decanted directly through a column of bone meal (*9*) which adsorbs unwanted pigments. Experience has shown that the new method must be carried out at about the temperature stated. In this it is different from the method of Kernohan (*7*).

It is known that 40° to 60° light petroleum is inefficient for extracting carotene from fresh leaves. It was therefore not surprising to find that carotene could not be extracted from fresh grass by 80° to 100° petroleum. This paper is concerned with the cause of the different behavior of fresh grass and of dried grass meal towards hot petroleum.

REAGENTS

Light petroleum, boiling range 40° to 60°.
Light petroleum, nominal boiling range 80° to 100°.
High boiling petroleum means petroleum boiling above 130°.
Petroleum-acetone is made by mixing 40° to 60° light petroleum with an equal volume of acetone and adding 1 gram of quinol to each liter of the mixture.
Petroleum-ethanol is the foaming mixture of Moore and Ely (100 ml. of 95% ethanol and 75 ml. petroleum ether, *11*). The ethanol was purified by distillation from zinc and potassium hydroxide.

METHODS

Method with Light Petroleum. The method with 80° to 100° petroleum was used as described above, except that the pigments were purified on a mixture of alumina and sodium sulfate as described below. This adsorbent is faster and more specific than bone meal, and the elution of carotene is under visual control.

Method with Petroleum-Acetone. The method with cold petroleum-acetone-quinol mixture (*2*), modified in minor detail, was used for the determination of control values as well as for the determination of carotene that remained after one extraction of fresh or dried leaves with 80° to 100° petroleum. The method can be used for most types of fresh and dried vegetable tissue. It is particularly convenient for grass and other plants with small leaves.

A gram of the material is weighed into a very thick 50-ml. squat beaker. About 1 gram of quartz powder (not sand) and 10 ml. of petroleum-acetone are added at once and the tissue is ground with a flat-bottomed glass pestle in the beaker. The beaker is much more convenient than a conventional mortar. Dry samples are damped with water before extraction. The supernatant solution is decanted (without filtering in most cases, but through a filter when dried grass meal is being extracted) into a separating funnel containing water. The residue is ground again and about six or seven extractions (taking 5 or 6 minutes altogether) are sufficient to remove all pigment. The acetone, etc., are removed in a simple automatic washing arrangement in which drops of water fall through the petroleum-acetone solution in the separating funnel to an overflow made from a bent glass tube. The pigments remain in the light petroleum. The solution is purified by adsorbing all the pigments on a chromatographic column of alumina–sodium sulfate and eluting the carotene with 2% acetone in petroleum.

Because aluminum hydroxide is hygroscopic and alterations in its water content change its power of adsorption, each portion must be heated before use (*2*). Alumina was therefore stabilized by mixing it with an equal weight of anhydrous sodium sulfate and heating for 12 hours at 150°. The mixture may be stored in bottles and is ready for use at all times.

Proving the Control Method. In work of this type the choice of a control method is very important. The method of Moore and Ely (*11*) using the Waring Blendor appears to be one of the most popular of published methods for extracting carotene from plant materials. On the other hand the beaker method (*2*) using petroleum-acetone-quinol is much neater and requires fewer vessels. In order to study their relative merits the two methods were compared.

One operator practiced the blender method for several days, following the directions given by Peterson (*13*) until a routine for interlocking replicates was well developed. Minor modifications were essential for quantitative transfer of the extract from blender to separator. All apparatus being ready, a specimen of fresh grass was obtained. One operator weighed and extracted sextuplicates of about 3 grams each by the blender method. Simultaneously another operator weighed and extracted sextuplicates of 600 to 800 mg. each by the beaker method. The latter completed his weighing and extractions and had the

Table I. Comparison of Methods for Extracting Fresh Grass

	Blender Method (*13*)	Beaker Method (*2*)
Extraction solvent	Petroleum-ethanol and petroleum	Petroleum-acetone-quinol
Replicates	6	6
Time for weighing and extracting, min.	115	35
Time for washing with water, min.	40	45[a]
Chromatography and colorimetry, min.	60	50
Total time for 6 estimations	3 hr. 35 min.	2 hr. 10 min.
Operational time	3 hr. 35 min.	1 hr. 40 min.
Carotene, mg. per kg.	112	116
Recovered from solid residues[b]	2	0
Total	114	116
Standard deviation	4.1	6.1
Coefficient of variation	3.7	5.3

[a] Total washing time, continuous drip method, 45 min.; operator's time less than 15 min.
[b] Time taken on these recoveries not included in recorded times.

six extracts ready for washing in 35 minutes. In spite of performing necessary manipulations (according to the practiced plan) while the blender was running, the other operator took 115 minutes to prepare his six extracts. Washing the blender extracts six times by shaking with water took 40 minutes as against 45 minutes for the beaker extracts washed on the automatic overflow apparatus, but the latter left the operator free to do other work during most of the time. The chromatography took longer for the blender extracts than for the beaker extracts because the volumes were greater.

The means of the results were about the same by each method. The standard deviations were also similar. The comparison is summarized in Table I. The dry solid residues from the six blender extractions were extracted with petroleum-acetone in beakers. A small quantity of carotene was recovered from the combined extracts. Because the residues from the beakers contained quartz they could not be extracted in the blender. They were therefore digested with ethanolic potassium hydroxide and extracted with light petroleum. No carotene was found in the combined extracts.

The conclusion is reached from this and other experiments that the beaker method has a similar accuracy to, but is very much faster than, the blender method. The blender is able to deal with larger samples and is invaluable for soft bulky material such as fruit. The beaker method exploits the advantages of small samples. Dried grass is more difficult than fresh leaves to extract in a beaker. According to Zscheile and Whitmore (*15*) this difference applies also to the blender. The blender obviates grinding. However, grinding in a small beaker with a sensibly shaped pestle and with quartz is much easier than with the conventional mortar and pestle and is well worth doing to save the laborious extractions of the hypophase which are necessary in the blender-petroleum-ethanol method. Confirmation of the speed of beaker extractions is afforded by the fact that the author and one assistant have on many occasions completed 120 assays of total carotenoids (this obviates pigment fractionation) in individual carrots by the cylinder method (*3*) in one 7.5-hour day. Because the samples are small, sets of 10 extractions performed in parallel by one operator took only 20 minutes. For these reasons the beaker technique has been used for the control method in the present investigation. The accuracy of the method has already been demonstrated (*2*).

EXPERIMENTAL RESULTS

Hot Light Petroleum as a Solvent for Extracting Carotene from Dried Grass Meal. It had been recognized that one extraction of grass meal with 80° to 100° petroleum left 2 to 3% of the carotene in the solid residues (*4*). Some of this residual carotene could be recovered in a second extraction but it was considered that for routine analyses the smallness of the amount did not justify the work involved.

In the present investigation the residual carotene, after one extraction of several different meals by 80° to 100° petroleum, has been determined, in some cases by a second extraction with 80° to 100° petroleum and in others by cold petroleum-acetone. The results are shown in Table II. On the assumption that all the carotene was recovered from the residues the first treatment yielded an average of 100 − 2.6 = 97.4% of the total.

The result of an extensive comparison of the 80° to 100° petroleum method with a hot petroleum-acetone method has been recorded (*12*).

In order to obtain another measure of the accuracy of the method 16 replicates of about 300 to 700 mg. of a sample of dried grass meal were extracted. The average particle size of this meal was about 70-mesh. The 16 complete analyses took 3.75 hours. The results ranged from 215 to 221 mg. of carotene per kg. with an average of 218.2. The standard deviation was 1.47, which makes the coefficient of variation only 0.67. Some of the residues were damped with water and extracted by the Moore and Ely method. These yielded a further 6.9 mg. of carotene per kg. of meal. Other residues which were damped with water and extracted with petroleum-acetone, yielded a further 6.5 mg. per kg. Six replicates of about 300 mg. of the same meal were damped with water and extracted with petroleum-acetone in beakers. The average value found by this method was 226.0 mg. of carotene per kg. with a standard deviation of 1.27. If the residual carotene is added to that extracted by 80° to 100° petroleum the total is 218.2 + 6.7 = 224.9 mg., which compares well with 226.0 mg. found by the beaker method.

These experiments show that satisfactory extraction and highly satisfactory replication may be obtained within a reasonable operational time. Could this very simple method be easily adapted for use with fresh leaves it would indeed be useful.

Light Petroleum as Solvent for Extracting Carotene from Green Leaves. Although 80° to 100° petroleum effectively extracts carotene from grass meal, it is ineffective for fresh leaves.

Carrot leaves were cut into pieces about 1 cm. long and heated for 1 hour in 80° to 100° petroleum on a near-boiling water bath. The residue was dark, shrunken, and brittle as though desiccated. This treatment yielded only 5% of the carotene found by the control method with cold petroleum-acetone. Much carotene remained in the residue and was extracted by petroleum-acetone, although the low total carotene in the combined extracts suggested that some destruction had occurred during the treatment with 80° to 100° petroleum. Similar results were obtained with fresh grass and with clover. Whole clover leaves were also immersed in 40° to 60° light petroleum for 3 days at 18° but only a small amount of carotene was extracted, although this procedure has been found satisfactory for dried grass meal by Kernohan (*7*) and by Hoffman, Lum, and Pitman (*6*).

Effect of Higher Temperatures. Edisbury (*4*) found that a temperature of 80° to 85° was necessary for extracting carotene from grass meal into light petroleum within a reasonable time.

Table II. Carotene in Residues of Samples of Dried Grass Meal after One Extraction with 80° to 100° Petroleum

First Extraction	Carotene in Residue		Solvent for Determining Residual Carotene[a]
Mg./kg.	*Mg./kg.*	*% of total*	
255	2.2	0.8	paq
220	1.8	0.8	hbp
231	2.5	1.1	paq
180	2.5	1.4	hbp
157	2.9	1.8	hbp
220	4.0	1.8	ek
172	3.6	2	paq
290	6.6	2.2	hbp
143	3.6	2.4	paq
217	6.2	2.9	paq
220	6.8	3.1	paq
218	6.9	3.2	pep
218	6.9	3.2	pep
157	5.4	3.4	paq
290	11.3	3.8	paq
143	5.7	3.8	hbp
190	7.5	4	paq
161	8.1	4.9	paq
	Mean	2.6	

[a] hbp = 80°–100° petroleum at 85°; paq = cold petroleum-acetone-quinol; pep = cold petroleum-ethanol and petroleum in Waring Blendor; ek = ethanolic KOH.

This has been confirmed here. Hence an even higher temperature might be effective in extracting fresh grass.

Accordingly, grass was heated with high boiling petroleum, on a glycerol bath, at a temperature which was brought steadily up to 130° where it was maintained for an hour. Water distilled off first, leaving the grass in a dry state. Although much carotene was found in the petroleum, the extraction was incomplete; a further 10% was yielded by grinding the grass residue with cold petroleum-acetone. Moreover, the total carotene yielded by combining the two extracts (hot and cold) was lower than that yielded by the control (a paired sample of grass extracted with petroleum-acetone), which suggested that some destruction had occurred during the heating. From a sample of dried grass meal less carotene was yielded by heating it at 130° than by heating a similar sample in the standard manner at 85° to 90°. This lower yield at 130° confirmed the loss observed with the fresh grass at 130°. Evidently the destruction in dried grass meal, and part of the destruction in fresh grass, at 130° was associated with the high temperature rather than with this specimen of petroleum, because the same yield of carotene was obtained from dried grass meal heated on a near-boiling water bath whether the solvent used was the standard 80° to 100° petroleum or the high boiling petroleum. (The destruction of carotene in fresh grass during extraction by 80° to 100° petroleum is probably enzymic and doubtless occurs during the warming up.)

Further investigation of extraction at high temperature appears to be unprofitable.

Effect of Rehydration. Dried grass meal differs from fresh grass in at least two important respects: The water has been removed and the leaves have been smashed to small pieces. Experiments were therefore done to find out whether replacing the water interfered with extraction.

To 1 gram of grass meal in a Kjeldahl flask 6 ml. of water were added. The water was allowed to soak into the meal for an hour. On being heated with 80° to 100° petroleum the sample yielded 233 mg. of carotene per kg. A dry sample of the same meal yielded 255 mg. Thus rehydration had hindered extraction by only 9%. In further tests rehydrated samples were almost completely extracted in those cases in which the water had been allowed to evaporate—e.g., by using a short-necked flask—while from similar samples in which the water was retained—e.g., by using a slightly less volatile petroleum or by fitting a reflux condenser—about half the carotene was extracted.

Under standard conditions most of the water is distilled off from fresh leaves during the extraction process. Hence the difference in extraction rates of fresh grass and dried grass meal cannot be accounted for by the water content.

Importance of Comminution. As water in itself does not play a major part in preventing the extraction of carotene, it seemed that the break-up of the leaf tissue might be essential for extraction. The following two groups of experiments were done to test this hypothesis.

In the first group dried grass which had not been ground to meal was extracted with 80° to 100° petroleum. In 1 hour only 50% of the carotene had been extracted although the other 50% was extractable from the residue by cold petroleum-acetone. From the unground leaves of dried alfalfa 85% of the carotene was extracted by 80° to 100° petroleum in 1 hour, and from the stalks of the same dried alfalfa 70% was extracted. The greenest parts of dried cabbage were selected and half the sample was ground to powder. Much more carotene was extracted by 80° to 100° petroleum from the powdered than from the unground portion.

In the second group of experiments a commercial sample of dried grass meal was divided into three grades by sieves of 40- and 60-mesh. The medium fraction was discarded. The coarse fraction, which comprised only a small part of the whole, and a portion of the fine fraction were extracted first with 80° to 100° petroleum, then with cold petroleum-acetone, and the carotene in each extract was determined separately.

Table III shows that more of the carotene was extracted by 80° to 100° petroleum from the fine (passing 60-mesh) than from the coarse (retained by 40-mesh) particles. Powdered dried carrot was similarly divided into two grades. One extraction with 80° to 100° petroleum yielded 94% of the carotene from the fine but only 70% from the coarse powder. A sample of the extremely finely powdered dried dust which accumulates in the filter sock from the hammer mill was compared with ordinary grass meal. Each was heated for 45 minutes with 40° to 60° light petroleum on a water bath at 47°. The results in Table IV show that carotene was more effectively extracted from the fine than from the ordinary meal at this temperature.

Table III. Extraction Rates from Fine and Coarse Grass Meals and Carrot Powders at 85°

	Carotene Extracted, Mg./Kg.			First, as % of Total
	First extraction	Second extraction	Total	
Dried grass meal, fine	220	3.6	223.6	98.4
Dried grass meal, coarse	153	11.3	164.3	93
Dried carrot, fine powder	86.3	5.4	91.7	94
Dried carrot, coarse powder	84	36	120	70

Table IV. Extraction Rates from Extra Finely Powdered and Ordinary Grass Meal at 47°

	Carotene Extracted, Mg./Kg.			First, as % of Total
	First extraction	Second extraction	Total	
Extra fine meal	168	7.5	175.5	96
Ordinary meal	193	24	217	89

It is clear from these findings that the carotene can be satisfactorily extracted by 80° to 100° petroleum from dried plant material only if the latter is finely divided, and the finer the state of division the lower the temperature necessary for a given extraction rate.

To see whether this applies also to fresh leaves, grass and other leaves were ground with quartz in small beakers. The pulps were extracted once with 80° to 100° petroleum, and the pulp residues were extracted with cold petroleum-acetone. The carotene in the first (hot) extract of the leaf pulp expressed as a percentage of the total (hot and cold) was much higher than when whole leaves were so extracted. The percentage varied in different experiments from 45 to 95 and seemed to be related to the degree of dehydration which occurred during heating. In cases in which the 80° to 100° petroleum was encouraged to boil away (by using short-necked flasks) the water also evaporated and the degree of extraction of carotene into the petroleum from the dry pulp was high.

The results of this comparison between pulped and whole leaves prove that an essential condition for extraction of leaves by 80° to 100° petroleum is prior disintegration.

DISCUSSION

Extraction from Dried Grass or Alfalfa Meal. The incomplete extraction of carotene from grass meal by one treatment with 80° to 100° petroleum and the variation from sample to sample (both factors are small) may perhaps have their explanation in the finding that the degree of extraction depends upon fineness of division. Grass meals from different sources vary in particle size. The size of particles varies not only with mechanical details of the mill and screen used in their manufacture but with the moisture content of the material being milled and with the proportion of leaf to stalk.

Resistance to Extraction from Fresh Leaves Due to Mechanical Obstruction. The water in pulped leaves and in rehydrated dried grass hinders extraction of the carotene by hot petroleum more or less according to the amount that remains unevaporated during the heating. But even in cases in which the pulp remained wet, the hindrance due to water (approximately 50%) cannot wholly account for the low extraction from fresh whole leaves (approximately 5%). Moreover at 130°, although the water was completely evaporated from the whole leaves, some carotene (10%) still resisted extraction.

The evidence makes it clear that very fine comminution of leaves (whether fresh or dried) is a necessary condition for the extraction of carotene by the 80° to 100° petroleum technique. But not all the carotene is extracted from fresh leaf pulp by 80° to 100° petroleum even after very thorough grinding. This may be partly because the sticky pulp is not easily penetrated by the

immiscible petroleum: the pulp is not dispersed and suspended in the petroleum in the way in which dry grass meal is seen to be. Or it may be that a protein-carotene linkage similar to that in the carrot, described by Kreula (*8*), is not easily ruptured by hot petroleum.

Thus the low extraction of carotene from whole leaves by 80° to 100° petroleum is probably mainly due to types of mechanical hindrance which can be overcome by dehydration and disintegration, but not by either alone. This conclusion is in keeping with the fact (which the author has confirmed) that carotene cannot be satisfactorily extracted from fresh green leaves within a few hours by grinding under cold light petroleum or by soaking, without grinding, under acetone (which dehydrates the leaves), but can be easily extracted in a few minutes by a combination of grinding and the use of acetone.

Enzymic Destruction before Extraction. The problem of adapting the 80° to 100° petroleum technique to fresh leaves is not solved merely by grinding prior to extraction, for carotene disappears from pulped leaf tissue sufficiently rapidly to interfere with accurate recovery. Under some conditions 25% may be lost from leaf pulp in 6 minutes (*2*), which is about the shortest practicable time for thorough grinding and transference to the extraction vessel. The destruction is associated with lipoxidase activity (*1*, *10*, *14*, and others). In the experiments in which leaves were ground and then extracted with 80° to 100° petroleum the total yield of carotene, including that recovered from the residue, was less than that found by the control method. The loss could be considerably reduced by excluding oxygen with carbon dioxide during grinding, and almost eliminated by adding about 0.25 gram of quinol to 1 gram of leaves before grinding. Two difficulties, however, remain. The quinol makes the grinding physically difficult, and quantitative transference of the grist to the extraction flask is tedious. No doubt these difficulties and the incomplete extraction could be overcome by the development of appropriate techniques—for instance, by first disintegrating leaves in cold 80° to 100° petroleum and quinol in a Folley-Watson (*5*) homogenizer and then heating in the same glass vessel. But then the main elegance of the 80° to 100° petroleum technique—its direct simplicity—will be lost and no improvement is had over the method using cold petroleum-acetone-quinol for the extraction.

ACKNOWLEDGMENT

The author thanks L. J. Harris and T. Moore for their interest in this work.

LITERATURE CITED

(1) Booher, L. E., Hewston, E. M., and Marsh, R. L., *Food Research*, 6, 493 (1941).
(2) Booth, V. H., *J. Soc. Chem. Ind.*, 64, 162T (1945).
(3) *Ibid.*, 64, 194T (1945).
(4) Edisbury, J. R., private communication, 1946.
(5) Folley, S. J., and Watson, S. C., *Biochem. J.*, 42, 204 (1948).
(6) Hoffman, E. J., Lum, F. G., and Pitman, A. L., *J. Agr. Research*, 71, 361 (1945).
(7) Kernohan, G., *Science*, 90, 623 (1939).
(8) Kreula, M., *Suomen Kemistilehti*, 18B, 69 (1945).
(9) Mann, T. B., *Analyst*, 69, 34 (1944).
(10) Mitchell, H. L., and Hauge, S. M., *J. Biol. Chem.*, 163, 7 (1946).
(11) Moore, L. A., and Ely, R., IND. ENG. CHEM., ANAL. ED., 13, 600 (1941).
(12) Nelson, W. A. G., *Analyst*, 72, 200 (1947).
(13) Peterson, W. J., *Biol. Symposia*, 12, 43 (1947).
(14) Sumner, J. B., and Dounce, A. L., *Enzymologia*, 7, 130 (1939).
(15) Zscheile, F. P., and Whitmore, R. A., ANAL. CHEM., 19, 170 (1947).

RECEIVED September 22, 1948.

Stability of Vitamin A Acetate under Laboratory Conditions

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The stability of vitamin A acetate was studied under conditions comparable to those usually involved in biological and chemicophysical studies. The results show that vitamin A acetate, in the crystalline state or when dissolved in oils of low peroxide value, may be stored without appreciable deterioration for considerable periods of time. Vitamin A acetate may be dissolved in any one of several organic solvents and the solution stored with more than the required stability for its use as a vitamin A standard in spectrophotometric and other chemicophysical studies. Batches of crystalline vitamin A acetate having essentially the same absorption properties may be prepared in the same or in different laboratories.

IN A previous report, certain properties of crystalline vitamin A acetate were discussed in relation to its use as a standard in vitamin A assays (*4*). Included were data obtained through a preliminary investigation of the storage stability of vitamin A acetate under certain experimental conditions, inasmuch as stability under these conditions is one of the primary requisites of an acceptable vitamin A standard. This investigation, enlarged to include a study of the stability of vitamin A acetate in a number of organic solvents, has been completed and the results are presented here.

STABILITY OF VITAMIN A ACETATE

In Crystalline State and in Oil Solutions. The procedure employed in preparing the crystalline vitamin A acetate used in these studies has been described (*4*), as has the preparation of the oil solutions of vitamin A acetate in a commercially refined and deodorized cottonseed oil (Wesson oil), corn oil (Mazola oil), and peanut oil for the stability studies. (The peroxide values of cottonseed oil, corn oil, and peanut oil were, respectively, 2.0, 1.5, and 11.7. In the previous publication it was reported that the peanut oil was purchased through a laboratory supply company; therefore it may not have been a fresh product or even representative of the peanut oils generally used as vitamin A carriers.) However, the following brief description of the essential procedures seems worthy of repetition.

The oil solutions were made to contain approximately 10,000 U.S.P. units of vitamin A per gram of solution. Portions of these oil solutions (250 mg.) and of the vitamin A acetate in the

crystalline state (4 to 7 mg.) were sealed in clear Pyrex ampoules in vacuo and in an atmosphere of nitrogen, and stored in the absence of light at 5° C. and at room temperature (25° to 30° C.). Samples from freshly sealed ampoules and samples from ampoules removed from storage at intervals were examined spectrophotometrically by means of a Beckman quartz spectrophotometer in order to determine the effect of the time and condition of storage on the vitamin A content.

A summary of the results of these studies is presented in Table I.

Dissolved in Certain Organic Solvents. To study the stability of vitamin A acetate in solution in some of the solvents commonly used in spectrophotometric and other chemicophysical investigations pertaining to this vitamin, the following procedure was adopted.

Duplicate portions of the crystalline vitamin (4 to 7 mg.), which previously had been stored in evacuated glass ampoules at 5° C., were weighed on a microbalance and dissolved in the following solvents: absolute ethanol (100% U.S.P. from U. S. Industrial Chemical Co.), isopropyl alcohol (Baker's C.P.), cyclohexane (Eastman No. 702), benzene (Baker's C.P.), and a mixture of 10% cyclohexane and 90% absolute ethanol. From each of these solutions more dilute solutions having optical densities ranging between 0.5 and 0.8 were prepared in duplicate for spectrophotometric examination. In all instances the vitamin A solutions were prepared and maintained at room temperature in amber glass volumetric flasks (*2*). For each of the above-mentioned solutions, one of the duplicate portions was examined spectrophotometrically within 60 minutes after removing the crystalline vitamin from the glass ampoule. At varying intervals up to and including 24 hours following the preparation of the solutions, the $E^{1\%}_{1\,cm.}$-maxima of each type of solution was determined at specific wave lengths, as was also the optical density of the solution. These measurements were also made by means of the Beckman quartz spectrophotometer while using the hydrogen discharge tube as the source of illumination.

When no significant differences could be detected in the extinction ratios or the $E^{1\%}_{1\,cm.}$-maxima of any of the solutions within the first 24-hour period, the spectrophotometric absorption examination was continued at specific intervals during the next 130 hours, the solutions remaining at room temperature (approximately 25° C.) in the interim. In the meantime, solutions of vitamin A acetate in absolute ethanol, isopropyl alcohol, and cyclohexane, having optical densities ranging between 0.6 and 0.7, were prepared by diluting portions (120 to 135 mg.) of a Wesson oil solution of the vitamin with the appropriate solvent. The $E^{1\%}_{1\,cm.}$-maxima of these latter solutions were determined immediately and then at specific intervals during the next 165 hours. These solutions were likewise stored at room temperature. The stability of vitamin A acetate and of Wesson oil solution of vitamin A acetate in the above organic solvents is shown in Figures 1 and 2.

In addition, optical densities at specific points over the spectral range of 220 to 400 mμ were determined for the freshly prepared

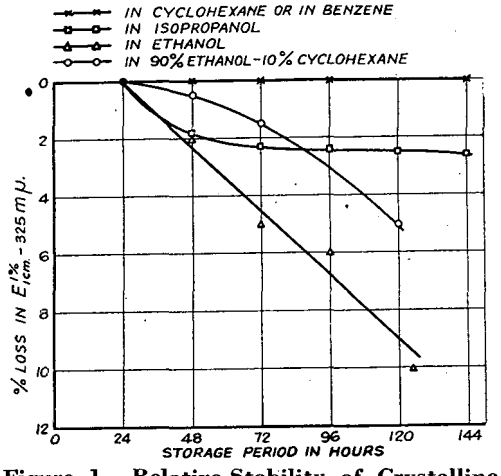

Figure 1. Relative Stability of Crystalline Vitamin A Acetate at Room Temperature Dissolved in Different Solvents

Table I. Stability of Vitamin A Acetate and Its Oil Solutions

[As indicated by change in extinction coefficient $\left(E^{1\%}_{1\,cm.}\text{-325 m}\mu\right)$]

Time in Storage	Retention of Vitamin A Stored at Room Temperature: Under nitrogen	Retention of Vitamin A Stored at Room Temperature: In vacuum	Retention of Vitamin A Stored at 5° C.: Under nitrogen	Retention of Vitamin A Stored at 5° C.: In vacuum
Days	%	%	%	%
		In crystalline state		
0	100	100	100	100
30	...	101	100	99
60	83, 97[a]	100	101	...
120	...	100	96, 98[a]	100
151	83	99	95	99
187	...	100	...	100
244	89	99	93	99
307	80, 97[a]	99	91, 99[a]	99
		Dissolved in Wesson oil		
0	100	100	100	100
31	98	100	100	101
61	90	99	98	...
117	89	98	98	98
152	84	97	97	98
184	...	97	96	99
217	88	95	94	100
301	87	94	93	98
		Dissolved in corn oil		
0	100	100	100	100
30	100	99	98	98
60	97	98	...	...
101	83	96	98	...
120	88	97	97	97
156	83	96	99	98
189	83	97	99	97
257	82	96	100	99
290	80	...	98	97
		Dissolved in peanut oil		
0	100	100	100	100
30	85	94	...	...
73	69	94	97	98
90	62	93	96	99
124	59	90	93	97
189	59	91	87	98
249	51	...	79	...

[a] Ampoules filled with nitrogen by means of manifold system; other ampoules charged with nitrogen by vacuum desiccator.

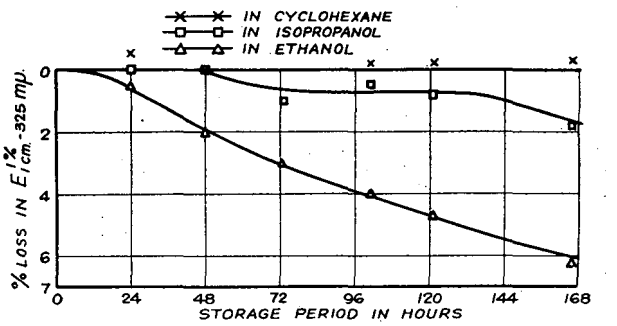

Figure 2. Relative Stability of Vitamin A Content of Wesson Oil Solution of Vitamin A Acetate Dissolved in Different Solvents

solutions of crystalline vitamin A acetate in the above-mentioned solvents. Similar ultraviolet light absorption measurements were made on absolute ethanol solutions of a sample of the crystalline vitamin A acetate supplied for a U.S.P. collaborative study (*12*, *14*). The concentrations of the solutions used in these latter studies were adjusted so that the optical densities fell within the limits of 0.5 to 1.9, as suggested by Vandenbelt *et al.* (*13*). The optical density data expressed as extinction ratios (*9*) are presented in Table II. For comparison, previously reported data for crystalline vitamin A acetate when dissolved in absolute ethanol and in cyclohexane (*7*, *8*, *12*) are also presented in Table II.

MANIPULATION OF BECKMAN QUARTZ SPECTROPHOTOMETER

In carrying out the above measurements, the sensitivity knob of the Beckman spectrophotometer was set at three turns from

the extreme clockwise position. The slit width was then varied to adjust the galvanometer needle to 100% transmission when the solvent occupied the absorption cell. In following this procedure of instrumentation, the slit width, with rare exception, never varied more than 0.04 mm. from a setting of 0.40 mm. when transmission measurements were made at 325 mμ. This practice was believed justifiable, inasmuch as varying the slit width in steps from 0.2 to 0.55 mm. and compensating by sensitivity adjustments produced no appreciable differences in the optical densities of a series of solutions of varying concentrations of a distilled vitamin A ester concentrate (obtained from Distillation Products, Inc.) in isopropyl alcohol when the measurements were made at 325 mμ.

The performance of the Beckman spectrophotometer was checked routinely by means of an isopropyl alcohol solution 2-phenylazo-*p*-cresol dye (obtained from Shohan Laboratories) as suggested by Taylor (*11*) and Kreider (*5*). For this purpose, a 15 mg. % solution of the dye in isopropyl alcohol was kept in the refrigerator. The solution under this condition of storage remained practically stable for 3 months.

DISCUSSION

Stability of Vitamin A Acetate during Storage in Crystalline State and Dissolved in Oils. On examining ampoules of crystalline vitamin A acetate at various intervals during storage under the different conditions, it was found that those samples of vitamin A stored in vacuum under refrigeration showed the least change in extinction coefficient. In fact, vitamin A acetate in the crystalline state and in oil solutions, when packed in vacuum, showed no serious decrease in extinction coefficient when subjected to the two conditions of storage (see Table I). From these data it is evident that storage in vacuum resulted in a more favorable retention of the vitamin than storage under nitrogen. This instability of the vitamin (*4*) could have been due to oxidation, in that traces of oxygen could have been admitted into the ampoules while filling with nitrogen. This possibility was suggested by the observation that the crystalline vitamin A acetate from those ampoules which had been prepared by attaching the vitamin-containing ampoule to a manifold, evacuating, filling with nitrogen, and sealing (*4*) showed less deterioration than did crystalline vitamin A acetate from similar ampoules which had been filled with nitrogen by means of a vacuum desiccator. In the latter method of filling, the open ampoules were removed from the desiccator before being sealed.

To investigate the possibility of oxygen contamination as the cause of vitamin A deterioration, a new solution of vitamin A acetate was prepared as previously described (*4*) by dissolving the required weight of crystals in Wesson oil from the same batch and diluting to volume. The oil solution was injected in 300-mg. portions through an extended capillary into small glass ampoules. The oil-filled ampoules were then charged with carbon dioxide in a vacuum desiccator, removed, and immediately sealed. In contrast to the previously mentioned nitrogen-flushed ampoules, only a very small gas space remained in these latter ampoules after sealing. These ampoules were stored in the absence of light at 5° C. and at room temperature (25° to 30° C.) as were the nitrogen-charged ampoules. Subsequent spectrophotometric examination of the contents of the ampoules revealed that much higher stability of vitamin A had been attained. After 138 days of storage at 5° C. and at room temperature, the average $E^{1\%}_{1\text{ cm.}}$–325 mμ values obtained on the contents of triplicate ampoules indicated that the oil retained 100 and 98%, respectively, of its original vitamin content.

The above observations indicate that vitamin A acetate in the crystalline state and when dissolved in a refined cottonseed oil is sufficiently stable to warrant its use as a vitamin A standard.

Stability of Vitamin A Acetate Dissolved in Organic Solvents. The data presented in Figures 1 and 2 show that vitamin A acetate, in the crystalline state or when dissolved in Wesson oil, remained stable for 24 hours under all the conditions investigated except when the oil solution of the vitamin was dissolved in absolute ethanol. Even the extinction coefficient of this solution was sufficiently stable to permit the use of the solution as a spectrophotometric standard over the time interval usually required. The extended stability of crystalline vitamin A acetate and of Wesson oil solutions of the vitamin, when dissolved in cyclohexane or in isopropyl alcohol, is a further advantage of using vitamin A acetate as the standard in routine spectrophotometric analysis for this vitamin. Perhaps the instability of vitamin A acetate dissolved in absolute ethanol may be explained in part by cyclization, an effect observed by Gray and Cawley (*3*).

Vitamin A acetate, dissolved in anhydrous C.P. chloroform, remained stable for 48 hours when the solution was stored in the dark at 5° C. This observation is of particular interest in connection with the antimony trichloride method of estimating vitamin A.

Effect of Type of Solvent on Ultraviolet Light Absorption Characteristics of Vitamin A Acetate. It is evident from the data presented in Table II that the extinction ratios obtained on ethanol solutions of the vitamin A acetate prepared in this laboratory and of that supplied for the U.S.P. collaborative assay (*12*, *14*) showed excellent agreement. Likewise, the extinction ratios obtained for both ethanol and cyclohexane solutions of the vitamin A acetate prepared in this laboratory agreed reasonably well with values reported by other investigators (*7*, *8*). Inasmuch as Morton and Stubbs (*7*) reported that no vitamin A_2 could be detected in the vitamin A acetate used in their studies and the extinction ratios for the two products were remarkably similar, it was assumed that the vitamin A acetate prepared in this laboratory was relatively free of vitamin A_2.

The data presented in Table II also show good agreement in the position of the absorption maximum for the three different lots of vitamin A acetate when dissolved in the different solvents. The shift in the point of maximum absorption of vitamin A acetate toward the shorter wave lengths when polar solvents are used had been reported by Morgareidge (*6*). However, Rawlings and

Table II. Comparison of Ultraviolet Light Absorption Characteristics of Independently Produced Samples of Crystalline Vitamin A Acetate in Different Organic Solvents

(Determined by Beckman quartz spectrophotometer)

Solvent	Position of Maxima	$E^{1\%}_{1\text{ cm.}}$ at Maxima	Extinction Ratios for Designated Wave Lengths, mμ											
			220	240	250	260	280	300	310	325	328	332	350	370
Ethanol[a]	325–7	1545	0.09	0.11	0.11	0.11	0.23	0.58	0.82	1.00	0.99	0.96	0.52	0.12
Ethanol[b]	325–8	1550	0.10	0.11	0.13	0.12	0.24	0.58	0.82	1.00	0.99	0.95	0.51	0.13
Ethanol[c]	325	1545	0.09	0.10	0.12	0.12	0.23	0.58	0.82	1.00	...	...	0.52	0.13
Ethanol[d]	326.5	1525	0.11	0.11	0.12	0.12	0.25	0.60	0.83	1.00	0.99	...	0.56	0.13
Isopropyl alcohol[a]	325–7	1521	0.09	0.10	0.11	0.10	0.22	0.58	0.82	1.00	0.99	0.96	0.52	0.12
Isopropyl alcohol[b]	325–8	1533	0.09	0.10	0.11	0.11	0.23	0.58	0.82	1.00	0.98	0.94	0.52	0.13
Cyclohexane[a]	327–9	1500	0.09	0.10	0.11	0.11	0.22	0.56	0.80	0.99	1.00	0.98	0.54	0.12
Cyclohexane[b]	328	1477	0.10	0.12	0.13	0.13	0.24	0.58	0.85	0.99	1.00	0.97	0.54	0.13
Cyclohexane[e]	327–9	1482	...	...	0.14	0.14	0.24	0.57	0.80	0.99	1.00	...	0.57	0.15
10% cyclohexane and 90% ethanol[a]	325–7	1519	0.08	0.11	0.11	0.10	0.22	0.58	0.82	1.00	0.98	...	0.52	0.12
Benzene[a]	331–3	1393	...	...	...	...	0.20	0.47	0.69	0.93	0.95	1.00	0.70	0.25
Benzene[b]	328–32	1402	...	...	...	...	0.21	0.46	0.71	0.94	0.97	1.00	0.68	0.23

[a] Vitamin A acetate prepared in authors' laboratory from concentrate of natural esters.
[b] Synthetic crystalline vitamin A acetate obtained through courtesy of Hoffmann-LaRoche, Inc., Nutley, N. J.
[c] Calculated from data obtained in connection with U.S.P. Collaborative assay (*12*).
[d] Taken from data of Morton and Stubbs (*8*). $E^{1\%}_{1\text{cm.}}$ value for vitamin A acetate was calculated on a molecular weight basis from value of 1700 given by authors for free vitamin.
[e] Calculated from data of Morton and Stubbs (*7*).

Wait (*10*) did not report any difference in the point of maximum absorption for natural vitamin A esters when dissolved in cyclohexane from that observed when this form of the vitamin was dissolved in isopropyl alcohol or in ethanol. Awapara *et al.* (*1*) have reported that vitamin A alcohol, when dissolved in benzene, exhibits a maximum absorption at 322 mμ. Rawlings and Wait (*10*) noted differences in the magnitude of the $E^{1\%}_{1\text{ cm.}}$-maxima of solutions of the vitamin A in ethanol and in isopropyl alcohol similar to those shown in Table II. They also noted that the $E^{1\%}_{1\text{ cm.}}$-maxima of a vitamin A ester concentrate decreased in the following order of the solvents used: ethanol, isopropyl alcohol, and cyclohexane.

LITERATURE CITED

(1) Awapara, J., Mattson, F. H., Mehl, J. W., and Deuel, H. J., *Science*, **104**, 602 (1946).
(2) Embree, N. D., IND. ENG. CHEM., ANAL. ED., **13**, 144 (1941).
(3) Gray, E. LeB., and Cawley, J. D., *J. Nutrition*, **23**, 301 (1942).
(4) Guerrant, N. B., Chilcote, M. E., Ellenberger, H. A., and Dutcher, R. A., ANAL. CHEM., **20**, 465 (1948).
(5) Kreider, H. R., IND. ENG. CHEM., ANAL. ED., **17**, 694 (1945).
(6) Morgareidge, K., *Ibid.*, **14**, 700 (1942).
(7) Morton, R. A., and Stubbs, A. L., *Analyst*, **71**, 348 (1946).
(8) Morton, R. A., and Stubbs, A. L., *Biochem. J.*, **42**, 195 (1948).
(9) Oser, B. L., Melnick, D., and Pader, M., IND. ENG. CHEM., ANAL. ED., **15**, 717 (1943).
(10) Rawlings, H. W., and Wait, G. H., *Oil & Soap*, **23**, 83 (1946).
(11) Taylor, R. J., *Nature*, **149**, 474 (1942).
(12) U. S. Pharmacopoeia Vitamin Advisory Board, *Letter* **172**, p. 534 (1947).
(13) Vandenbelt, J. M., Forsyth, J., and Garrett, A., IND. ENG. CHEM., ANAL. ED., **17**, 235 (1945).
(14) Wilkie, J. B., U. S. P. Vitamin Advisory Board, *Letter* **164**, p. 508 (1946).

RECEIVED September 16, 1948. Authorized for publication August 20, 1948, as Paper 1463 in the Journal Series, Pennsylvania Agricultural Experiment Station. Financed in part by a grant from The Nutrition Foundation, Inc.

Amperometric Determination of Primary and Tertiary Mercaptans in Their Mixtures

By Iodometric Combined with Argentometric Titrations

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All mercaptans upon amperometric titration in ammoniacal medium with silver nitrate react in a molar ratio of 1 to 1. Upon titration with iodine a primary mercaptan reacts with 0.5 mole of iodine, whereas a tertiary mercaptan reacts with 1 mole of iodine. The titrations can be carried out amperometrically, using a rotating platinum electrode as indicator electrode and a saturated calomel electrode as reference electrode. When a mixture of a primary and tertiary mercaptan is titrated iodometrically, the amount of iodine used is much less than that calculated because part of the tertiary mercaptan R'SH reacts with the formation of a mixed disulfide: (prim.) RSH + (tert.) R'SH + I_2 → RSSR' + 2HI. Transformation of the mercaptans into slightly dissociated mercaptides, such as lead mercaptide, prevents this reaction from taking place. When mixtures of primary and tertiary mercaptans are titrated iodometrically in the presence of an excess of lead nitrate or perchlorate, the primary and tertiary mercaptans react, giving the disulfide and the sulfenyl compound, respectively. The composition of a mixture of a primary and tertiary mercaptan is found by an argentometric amperometric titration combined with an iodometric amperometric titration in the presence of lead salt. Best results are obtained when mercaptan solutions are dilute and cold. Concentrated mercaptan solutions favor partial disulfide formation by the tertiary mercaptan. The method is of limited accuracy because it is indirect and some tertiary mercaptans react incompletely with iodine.

THE application of the rotating platinum electrode as indicator electrode in the amperometric titration of mercaptans (thiols) with silver nitrate has been reported (*2*). When a primary, secondary, or tertiary mercaptan is titrated amperometrically in ammoniacal solution with silver nitrate, 1 mole of silver is used for each mole of mercaptan present. Hence, in a mixture of primary and tertiary mercaptans no distinction between the two can be made by amperometric titration with silver nitrate. The reactions can be represented by the following equations:

$$\text{(prim.) RSH} + \text{Ag(NH}_3)_2^+ \longrightarrow \text{RS Ag} + \text{NH}_4^+ + \text{NH}_3 \quad (1)$$

$$\text{(tert.) R'SH} + \text{Ag(NH}_3)_2^+ \longrightarrow \text{R'SAg} + \text{NH}_4^+ + \text{NH}_3 \quad (2)$$

where RSH represents a mercaptan and (prim.) and (tert.) indicate that the —SH group is attached to a primary and tertiary carbon atom, respectively.

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Primary mercaptans are readily oxidized by iodine to the disulfide.

$$\text{(prim.) 2RSH} + \text{I}_2 \longrightarrow \text{RSSR} + \text{2HI} \quad (3)$$

An iodometric method of analysis, based on this reaction, has been worked out by Kimball, Kramer, and Reid (*1*). In the analysis an excess of iodine is added to the mercaptan and then back-titrated with sodium thiosulfate. More recently Rheinboldt (*4*) has shown that tertiary mercaptans react with iodine to form sulfenyl iodides.

$$\text{(tert.) R'SH} + \text{I}_2 \longrightarrow \text{R'SI} + \text{HI} \quad (4)$$

A method for the iodometric determination of tertiary mercaptans analogous to the method of Kimball, Kramer, and Reid (*1*) for primary mercaptans has been described by Tyler and Brown (*5*). According to Equation 3, when a primary mercaptan is titrated with iodine the equivalence point corresponds to a consumption of 0.5 mole of iodine per mole of mercaptan. On the other hand, according to Equation 4 a tertiary mercaptan consumes 1 mole of iodine per mole of mercaptan.

In the present investigation it has been found that mercaptans can be titrated directly amperometrically with standard iodine solutions, using a rotating platinum electrode as indicator electrode and a saturated calomel electrode as reference electrode. Experimentally it was found that when a primary mercaptan is titrated amperometrically with iodine the end point corresponds to a consumption of 0.5 mole of iodine, per mole of mercaptan and with dilute solutions of tertiary mercaptans 1 mole of iodine is used at the end point. However, when a mixture of primary and tertiary mercaptans is titrated, much less than the calculated amount of iodine is consumed. Undoubtedly, this is due to the formation of a mixed disulfide by the following reaction:

$$(\text{prim.})\ \text{RSH} + (\text{tert.})\ \text{R'SH} + \text{I}_2 \longrightarrow \text{RSSR'} + 2\text{HI} \qquad (5)$$

Tertiary mercaptan which participates in a reaction of this nature reacts with only one instead of two atoms of iodine.

Transformation of the free mercaptan groups into insoluble or slightly dissociated mercaptides impedes this undesirable reaction. Lead ion can be used for this purpose, because it forms a slightly dissociated or complex lead mercaptide. When mixtures of primary and tertiary mercaptans are titrated with iodine under the proper conditions in the presence of lead nitrate or perchlorate, the mercaptans in the mixture react according to Equations 3 and 4.

In the iodometric amperometric titration the current remains zero or nearly zero until the end point is reached, then increases rapidly. Plotting the galvanometer or microammeter deflection against volume of reagent added yields two straight lines whose point of intersection near or on the abscissa gives the location of the end point.

APPARATUS AND MATERIALS

The apparatus used for the iodometric amperometric titration is the same as for the argentometric amperometric titration (*2*). The reference cell, *F*, of Figure 1 in that article is changed to a saturated, calomel electrode for the iodometric titration in place of the mercury–mercuric iodide reference electrode used in the argentometric titrations.

Approximately 0.002 to 0.005 *N* alcoholic iodine solution was made by dissolving solid iodine in alcohol. This iodine solution is standardized by titrating amperometrically against 10 to 20 ml. of standard 0.01 *N* arsenic trioxide solution added to 100 ml. of water containing approximately 1 gram of sodium bicarbonate. Because dilute alcoholic iodine solutions are not stable over long periods of time, they need to be standardized every day or two.

Standard 0.005 *N* silver nitrate solution was made by weighing the correct amount of pure dry silver nitrate and dissolving in a known volume of water.

Lead nitrate (1 *M*) and perchloric acid (1.0 *M*) were made up as aqueous solutions.

PROCEDURE

Procedure A. Take an aliquot portion of a solution containing 1 to 40 mg. of mercaptan and titrate with standard silver nitrate solution amperometrically as described in (*2*).

Procedure B. Dilute a second aliquot portion of the solution containing not more than about 5 mg. of mercaptan to about 100 ml. with 95% ethanol. To the solution add 1 ml. of 1 *M* lead nitrate or perchlorate solution and enough 1 *M* perchloric acid to make the solution 0.01 *M* in perchloric acid. Titrate amperometrically in the cold with standard 0.005 *N* iodine, using a rotating platinum electrode short-circuited with the saturated calomel electrode through the microammeter.

The end point in the titration of Procedure A corresponds to a consumption of 1 mole of silver for every mole of mercaptan; no distinction is made between primary and tertiary mercaptans.

The end point in the titration of Procedure B corresponds to an iodine consumption of 0.5 mole for every mole of primary mercaptan and of 1 mole for every mole of tertiary mercaptan. If the total amount of mercaptan sulfur present from the silver nitrate titration (Procedure A) is known, the amounts of primary and tertiary mercaptans present are readily calculated as follows:

Milliequivalents of silver nitrate (Procedure A)

$$A = V_{\text{AgNO}_3} \times N_{\text{AgNO}_3} \times \frac{V \text{ total sample}}{V \text{ aliquot}} \qquad (6)$$

Milliequivalents of iodine used (Procedure B).

$$B = V_{\text{iodine}} \times N_{\text{iodine}} \times \frac{V \text{ total sample}}{V \text{ aliquot}} \qquad (7)$$

$$\text{Per cent of mercaptan present as tertiary mercaptan} = \frac{B - A}{A} \times 100 = C \qquad (8)$$

$$\text{Per cent of mercaptan present as primary mercaptan} = 100 - C \qquad (9)$$

EXPERIMENTAL

The argentometric part of the procedure has been demonstrated to be reliable (*2*). The iodometric titration portion of the procedure has been tested with pure *n*-dodecyl mercaptan (dodecanethiol) and two tertiary mercaptans, dimethyl-*n*-nonylcarbinthiol and di-*n*-butyl-*n*-propylcarbinthiol. Results in Table I illustrate the accuracy that can be expected of the method in the titration of primary and tertiary mercaptans alone and in mixtures of various concentrations. The figures under the heading "mercaptan present" are based upon the results of amperometric titration of the mercaptan with silver nitrate in ammoniacal medium.

Table I. Titration of Mercaptans and Their Mixtures Amperometrically in Presence of Lead Ion with Iodine

Mercaptan Present, Mg.			Ml. of 0.00495 *N* Iodine	
n-Dodecyl[a]	Dimethyl-*n*-nonyl-carbinthiol[a]	Di-*n*-butyl-*n*-propyl-carbinthiol[a]	Used	Calculated[b]
8.22	...	..	8.25, 8.24	8.22
...	1.06	..	2.03	2.12
...	1.77	..	3.40	3.54
...	3.55	..	6.87, 6.81	7.10
...	5.32	..	10.13	10.64
...	8.88	..	16.82	17.76
...	17.75	..	33.1	35.50
...	35.50	..	63.2	71.0
1.84	2.96	..	7.27	7.76
3.68	2.96	..	9.18	9.60
3.68	14.82	..	29.97	33.32
18.42	7.41	..	29.43	33.24
...	...	6.40	12.71	12.80
3.68	...	2.56	8.84	8.80
3.68	...	6.40	16.30	16.48
9.21	...	2.56	14.31	14.33
9.21	...	6.40	21, 51, 21.47	22.01

[a] Samples prepared and supplied by C. S. Marvel, University of Illinois.
[b] Calculated on basis that 1 mole of primary mercaptan requires 0.5 mole of iodine, while 1 mole of tertiary mercaptan requires 1 mole of iodine.

It is apparent from Table I that, at least with the mercaptans tested, most reliable results are obtained when the total concentration of the mercaptan is small. At higher concentrations the amount of iodine consumed by tertiary mercaptans alone or in mixtures is less than expected. This is no doubt due to disulfide formation by the tertiary mercaptan. The data also indicate that the sample of dimethyl-*n*-nonylcarbinthiol used may not be completely a tertiary mercaptan, for even at very low concentrations theoretical quantities of iodine are not consumed or it may react abnormally. On the other hand, the di-*n*-butyl-*n*-propylcarbinthiol sample is probably relatively pure and has very little of other types of mercaptan as contaminants.

Attempts to titrate secondary mercaptans by the amperometric iodometric procedure have shown that they react with neither 0.5 nor 1 mole of iodine per mole of mercaptan but with a quantity of iodine between these two values. The exact amount of iodine consumed depends upon the conditions of reaction. At high dilutions the number of moles of iodine consumed approaches 1. Titration of concentrated solutions requires amounts of iodine approaching only 0.5 mole per mole of mercaptan. Apparently at high concentrations the formation of disulfide is favored, while at low concentrations formation of the sulfenyl iodide is favored. Disulfide formation is also favored if the solutions are titrated hot.

The end point determined amperometrically with secondary mercaptans is somewhat indefinite. At or even before the end

point some of the RSI formed apparently decomposes slowly with the formation of disulfide and free iodine.

$$2RSI \longrightarrow RSSR + I_2 \quad (10)$$

This formation of free iodine gives a constantly increasing current at the microelectrode. For this reason the end point must be determined very rapidly if the amperometric method is used. Such a procedure is not practicable and the results are not accurate.

Some evidence that all tertiary mercaptans do not react stoichiometrically to form the sulfenyl iodide has been obtained by Laitinen (*3*). A qualitative measure of the tendency to disulfide formation by a tertiary mercaptan can sometimes be obtained at the completion of the iodometric titration. If Reaction 10 is taking place to any appreciable extent, the microammeter will register the constantly increasing current due to the formation of free iodine. If the rate of increase is small—i.e., less than a few tenths of a microampere per minute—the formation of disulfide by tertiary mercaptan is probably slight. By the above test it has been noted that the dimethyl-*n*-nonylcarbinthiol has a much higher tendency to form disulfide than does the di-*n*-butyl-*n*-propylcarbinthiol.

Some commercial mercaptans have been analyzed by the method described in this paper (Table II).

Table II. Analysis of Commercial Mercaptans for Their Primary and Tertiary Mercaptan Content

Mercaptan Titrated	Mercaptan (Calcd. as $C_{12}H_{25}SH$), %	Primary Mercaptan, %	Tertiary Mercaptan, %
Main Fraction 3B, Sharples Chemical Co.	99.0	5.6	93.4
Sulfole, Phillips Petroleum Co.	95.8	5.3	90.5
D.D.M. No. 337, Naugatuck Chemical Co.	91.1	88.6	2.5

Table III. Diffusion Currents at Rotating Platinum Electrode with Varying Excesses of Iodine in Some Solutions

	Solutions to Which Iodine Is Added		
	100 ml. 95% ethanol 0.01 *M* $HClO_4$	100 ml. 95% ethanol 0.01 *M* $HClO_4$ 0.001 *M* KI	100 ml. 95% ethanol 0.01 *M* $HClO_4$ 0.001 *M* KI 0.01 *M* $Pb(NO_3)_2$
0.005 *N* I_2 Solution in Excess, Ml.	Microamperes		
0.07	1.7	0.3	1.9
0.14	3.2	0.5	3.7
0.21	4.7	0.7	5.3
0.30	6.3	1.0	7.6

INTERFERENCES

The substances that interfere in the argentometric titration have been discussed (*2*). Substances oxidizable by iodine under the conditions of the titration will interfere in the iodometric procedure. In addition, substances that form insoluble precipitates with lead should be absent or enough excess lead nitrate should be added to precipitate all of the interfering substance. Although in many instances the iodometric procedure could be applied to pure mercaptans without the addition of lead, this is undesirable because the iodide formed by the reaction reduces the accuracy of the determination of the equivalence point. When iodide is present the current at the rotating electrode for any given excess of iodine is greatly reduced (see Table III). The addition of excess lead as recommended in the procedure precipitates the iodide as lead iodide, so that iodide desensitization is eliminated. In all cases the end point obtained is the same whether or not iodide is present, although less precisely determined when iodide is present.

The same electrode should not be used for the iodometric titration as is used for the argentometric titration unless the silver plated onto the platinum has first been removed with nitric acid.

LITERATURE CITED

(1) Kimball, J. W., Kramer, R. L., and Reid E. E., *J. Am. Chem. Soc.*, 43, 1199 (1921).
(2) Kolthoff, I. M., and Harris, W. E., IND. ENG. CHEM., ANAL. ED., 18, 161 (1946).
(3) Laitinen, H. A., private communication.
(4) Rheinboldt, H., *Ber.*, 72, 657 (1939).
(5) Tyler, W. P., and Brown, W. E., B. F. Goodrich Co., private communication.

RECEIVED August 11, 1948. Investigation carried out under sponsorship of the Office of Rubber Reserve, Reconstruction Finance Corporation, in connection with the government synthetic rubber program.

Determination of Inorganic Phosphate

Modification of Isobutyl Alcohol Procedure

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THE procedure employing extraction of phosphomolybdic acid by isobutyl alcohol described by Berenblum and Chain (*2*) and recently evaluated by Pons and Guthrie (*4*) affords a good method for determination of inorganic phosphate in colored solutions. In studies of phosphatases and of phosphorus fractions in alfalfa, the use of this method seemed desirable because colored solutions were encountered. Investigation of the isobutyl alcohol extraction procedure has led to improvements that simplify the color development, shorten the extraction procedure, and eliminate the interference from proteins. In the improved procedure the sample is in contact with an acid solution for only a short time (60 to 90 seconds). This should enhance the value of the method for the determination of inorganic phosphate in the presence of easily hydrolyzable phosphate compounds. Ferric ions do not interfere in the isobutyl alcohol extraction procedure.

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REAGENTS

Isobutyl Alcohol–Benzene Solution. Mix equal volumes of isobutyl alcohol and thiophene-free benzene. (Some technical grades of benzene impart a cloudiness to the solutions after color development.)

Molybdate Reagent. Dissolve 50 grams of ammonium molybdate in 400 ml. of 10 *N* sulfuric acid and dilute to 1 liter with water.

Silicotungstate Reagent. Dissolve 5.7 grams of sodium silicate nonahydrate and 79.4 grams of sodium tungstate dihydrate in about 500 ml. of water. Add 15 ml. of concentrated sulfuric acid, boil for 5 hours, cool, and dilute to 1 liter with water.

Stannous Chloride Stock Solution. Dissolve 10 grams of stannous chloride dihydrate in 25 ml. of concentrated hydrochloric acid and store in a small glass-stoppered brown bottle.

The method of Berenblum and Chain for the determination of inorganic phosphate has been modified. The number of extractions and washings has been decreased from three to one and the shaking time reduced to 15 seconds. Color development is effected by direct addition of stannous chloride solution to an aliquot of the extract which has been diluted with ethyl alcohol–sulfuric acid solution. The modified procedure may be applied directly to solutions containing proteins by use of a silicotungstate reagent.

Stannous Chloride Dilute Solution. Dilute 1 ml. of the stannous chloride stock solution to 200 ml. with approximately 1 *N* sulfuric acid. Prepare fresh daily.

Sulfuric Acid in Ethyl Alcohol. Dissolve 20 ml. of concentrated sulfuric acid in 980 ml. of 99.5% ethyl alcohol. (Instability of the molybdenum blue color has been attributable at times to some contaminant in 95% ethyl alcohol.)

Phosphate Standard. Dissolve 0.4950 gram of reagent grade potassium dihydrogen phosphate (dried at 110° C.) in water and dilute to 1 liter. Dilute 50 ml. of this solution to 200 ml. to obtain a solution containing 28.2 micrograms of phosphorus per ml.

ANALYTICAL PROCEDURE

Pipet an aliquot of the solution for inorganic phosphate analysis into a 25 × 200 mm. test tube. (The aliquot should contain 20 to 80 micrograms of phosphorus if a light absorption cell 2 cm. thick is to be used for the final colorimetric reading.) Add water to bring the volume to 15 ml. Add 25 ml. of the isobutyl alcohol–benzene solution. (Use a rubber bulb or other mechanical source of suction for pipetting this solution to avoid inhaling the toxic fumes of benzene.) Add 5 ml. of the silicotungstate reagent followed by 5 ml. of the molybdate reagent. Stopper the tube with a rubber stopper and immediately shake the mixture 15 seconds. Allow the phases to separate. Pipet a 10-ml. sample of the isobutyl alcohol–benzene layer into a 50-ml. volumetric flask and wash the sample from the pipet with alcoholic sulfuric acid solution. (Reproducible drainage of the isobutyl alcohol–benzene solution is difficult to obtain.) Dilute the sample to about 45 ml. with the alcoholic sulfuric acid, add 1 ml. of dilute stannous chloride solution, dilute to volume with alcoholic sulfuric acid, and mix. Carry a blank through the same procedure. Measure the per cent transmittance of the sample in a photometer adjusted to read 100% transmittance for the blank. A filter system transmitting between 625 and 725 millimicrons should be used in the photometer. The authors have isolated a satisfactory wave length band by the combination of a Corning 243 filter with 2.5 cm. (1 inch) thickness of 2% copper sulfate solution. Prepare a standard curve by the same procedure, substituting aliquots of the dilute phosphate standard for the sample aliquots.

Table I. Recovery of Inorganic Phosphate by Isobutyl Alcohol–Benzene Mixtures as a Function of Extraction Time

Extraction Time *Seconds*	P Added γ	P Recovered γ
5	56.4	56.2
10	56.4	56.2
15	56.4	56.3

Table II. Effects of Sulfuric, Hydrochloric, and Acetic Acids on Stability of Molybdenum Blue Color Developed by Homogeneous Reaction with Stannous Chloride

Acid	Vol., Ml. per 50 Ml.	Per Cent Transmittance 15 min.	30 min.	45 min.
Concd. H_2SO_4	0.1	32.2	32.2	32.2
	0.2	32.2	32.0	32.0
	0.5	32.7	32.0	32.1
Concd. HCl	0.2	36.4	38.1	39.5
	0.5	35.8	35.0	36.5
	2.0	37.0	35.0	35.6
Glacial acetic	1.0	36.1	38.2	39.4
	5.0	34.8	36.0	36.6
	25.0	33.5	33.9	34.2

INVESTIGATION OF PROCEDURE

Consideration of such factors as extraction time, number of extractions, method of color development, and interference from proteins led to the modifications embodied in the analytical procedure.

A check on the rate at which isobutyl alcohol would extract high concentrations of phosphomolybdic acid from aqueous solutions revealed that 500 micrograms of phosphorus could be extracted quantitatively in 10 seconds' shaking time. Normal phosphorus concentrations were recovered quantitatively by as little as 5 seconds' shaking (Table I).

In previous methods (*2*, *4*) an acid wash of the isobutyl alcohol layer and color development by shaking with stannous chloride solution are required. Because the stannous chloride solution may be added to the organic solution of phosphomolybdic acid to give a homogeneous, stable, colored solution in the presence of alcoholic sulfuric acid solution (Table II), it was possible to eliminate all except the initial extraction by aliquoting from the organic layer.

The color stability was exceptionally good, as per cent transmittance readings after 24 hours were identical with the initial value. Some lots of 95% ethyl alcohol caused the molybdenum blue color to be unstable; however, the use of 99.5% ethyl alcohol has eliminated difficulties of this nature.

The mutual solubilities of isobutyl alcohol and water are different. To overcome the appreciable change in volume of the phases during mixing, an investigation was made of the extraction of phosphomolybdic acid by mixtures of isobutyl and other alcohols with benzene (Table III). Dilution of alcohol extractants for phosphomolybdic acid with benzene up to 50% by volume does not actually reduce the amount of phosphorus extracted. Apparently the decrease in phosphorus recovery at low benzene concentrations results from the higher solubility of water in the organic phase, which causes an error from dilution. At high benzene concentrations the extraction is actually inhibited. The data (Table III) indicate that *n*-butyl alcohol or possibly the amyl alcohols could be substituted for isobutyl alcohol as an extractant.

No information has been found in the literature as to equilibrium compositions in the ternary system water–isobutyl alcohol–benzene. Washburn and Strandskov (*5*) indicate that in the system water–*n*-butyl alcohol–benzene a 1 to 1 mixture of *n*-butyl alcohol and benzene in equilibrium with water gives a two-phase system having nearly equal mutual solubilities. A mixture of isobutyl alcohol and benzene in the proportion of 1 to 1 by volume has been adopted for use in the proposed procedure for phosphorus determination.

Because changes in the composition of the aqueous phase might cause variations in the mutual solubilities, the volume of the organic layer in equilibrium with aqueous solutions of varying pH and salt content has been measured and found to be constant (Table IV). The equilibrium volume of the isobutyl alcohol–benzene phase will vary inversely with the water content of the initial isobutyl alcohol–benzene solution.

Protein interference in determination of inorganic phosphorus appears to be of two types: interference due to turbidity resulting

Table III. Extraction of Phosphomolybdic Acid from Aqueous Solutions by Organic Alcohols and Esters Alone and in Mixtures with Benzene

Organic Solvent	Vol. Proportion, Solvent: Benzene	T	P Added	P Found[a]	Deviation
		%	γ	γ	%
Isobutyl alcohol	10:1	35.8	56.4	50.8	−10.0
	3:1	33.0	56.4	55.0	−2.5
	1:1	32.0	56.4	56.4	0.0
	1:2	34.2	56.4	53.2	−5.7
	1:4	40.5	56.4	44.8	−13.0
	1:9	91.0	56.4	4.6	−92.0
n-Butyl alcohol	1:0	46.0	56.4	38.6	−32.0
	1:1	32.4	56.4	55.9	−0.9
	1:4	36.0	56.4	50.7	−10.0
	1:9	66.7	56.4	20.2	−64.0
Isoamyl alcohol	1:0	58.3	28.2	26.9	−4.6
	1:1	33.5	56.4	54.1	−4.1
Benzyl alcohol	1:0	58.1	28.2	27.0	−4.3
	1:4	86.3	56.4	7.3	−87.0
Amyl acetate	1:0	59.6	28.2	25.7	−9.0
	1:4	100.0	56.4	0.0	−100.0
Ethyl acetate	1:0	33.0	56.4	55.0	−2.5
	1:4	100.0	56.4	0.0	−100.0

[a] Aliquots taken from organic layer after extraction. % transmittance referred to standard curve prepared using 1:1 isobutyl alcohol–benzene extraction.

from coagulation of protein, and loss of phosphorus as phosphomolybdic acid precipitating with the protein during coagulation.

These sources of error from proteins have been eliminated by the modifications incorporated in this method. When a sample is taken from the isobutyl alcohol–benzene layer rather than the entire layer withdrawn for analysis, the coagulated protein that collects at the interface is not a source of turbidity in the solution after color development. To eliminate the interference from protein precipitation of phosphomolybdic acid it was necessary to find a satisfactory protein precipitant that could be added to the solution prior to the formation of the phosphomolybdic acid. Silicotungstic acid, recommended by Mitchell, Shaw, and Frary (*3*) as a good precipitant for gelatin, was satisfactory. Good recoveries of quantities of inorganic phosphate added to protein-containing solutions were obtained by the addition of the reagent prior to the addition of the molybdate reagent (Table V). The silicotungstic acid did not interfere in the colorimetric determination, for it contributed no color to a phosphate blank solution.

The effect of variations in the use of the silicotungstate reagent is worth noting. The boiling treatment of the silicotungstate produces a reagent which has no tendency to form a complex with the phosphate of the solution. Optimum reproducibility is obtained when additions of silicotungstate and molybdate reagents and the extraction with isobutyl alcohol–benzene are performed in successive operations with minimum intervening time intervals. Good recovery of the phosphate may be obtained in the presence of proteins even when the order of addition of silicotungstate and molybdate reagents is reversed, provided the extraction time is increased to approximately 2 minutes. Silicotungstate appears to replace phosphomolybdate in a precipitated protein phosphomolybdate complex.

Table IV. Volume of Isobutyl Alcohol–Benzene Phase in Equilibrium with Aqueous Phases

Aqueous Phase	Volume of Isobutyl Alcohol–Benzene, Initial	Volume of Isobutyl Alcohol–Benzene, After equilibrium
Ml.	*Ml.*	*Ml.*
25 H_2O	25.00	24.00
25 0.4 *N* H_2SO_4	25.00	23.90
25 1.0 *N* H_2SO_4	25.05	24.05
20 H_2O 5 Molybdate reagent	25.05	24.00
10 H_2O 5 Molybdate reagent 10 Silicotungstate reagent	25.00	23.95

If the molybdenum blue color shows evidence of instability, the ethyl alcohol stock should be considered as a likely source of the difficulty and other supplies of alcohol should be tested.

COMPARISON OF METHODS

Analyses for inorganic phosphate obtained by the modified isobutyl alcohol method have been compared with analyses on the same samples by the Berenblum and Chain procedure (*2*), and the A.O.A.C. sulfite reduction methods (*1*) (Table VI).

The A.O.A.C. method gave consistently higher values for inorganic phosphate in the tissue extracts. The color of the extracts introduced errors that could not be eliminated. During color development a turbidity often formed which was probably related to the protein content of the sample. This turbidity could be largely removed by centrifuging.

Differences between results by the Berenblum and Chain method and the modified method were small and erratic. Apparently the effect of proteins in the extracts was not a factor in this study.

The modified method showed the least deviation between different extracts.

PRECISION OF METHOD

Sixteen analyses of five extracts of one alfalfa sample gave an average of 0.127% phosphorus with a standard deviation of 0.0016%.

Table V. Recovery of Inorganic Phosphate in Presence of Proteins by Use of Silicotungstic Acid Reagent

P added	Phosphate Found in Presence of: 10 mg. gelatin	50 mg. gelatin	10 mg. blood albumin	10 mg. hemoglobin
γ	γ	γ	γ	γ
0.0	1.0	0.5	7.8	7.4
56.4	57.0, 55.0	54.4, 53.3	51.0, 54.4	55.7, 55.7
84.6	83.8, 82.5	83.3, 79.8	78.8, 79.4	82.8, 82.8

Table VI. Inorganic Phosphorus in Alfalfa

Sample[a]	A.O.A.C.[b,c]	Berenblum-Chain	Modified
	Mg./g.	*Mg./g.*[c]	*Mg./g.*[c]
1 A	1.50	1.47	1.41
1 B	1.50	1.37	1.42
2 A	1.12	1.10	1.07
2 B	1.15	1.07	1.09
3 A	1.34	1.25	1.27
3 B_1	1.38	1.27	1.28
3 B_2	1.38	1.25	1.27

[a] All A samples were obtained by boiling ground alfalfa after phospholipide extraction for 5 minutes with 0.1 *N* sodium acetate buffered to pH 4.8, filtering, washing, and diluting to volume; B by 60-minute room temperature extraction with 12.5% trichloroacetic acid, filtering, washing, and diluting to volume.

[b] Solutions that showed turbidity were centrifuged before reading in photometer.

[c] All values averages of at least triplicate colorimetric determinations on each extract.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mary Dell Springer for her assistance with some of the analyses performed during the course of this investigation.

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 6th ed., p. 137, 1945.
(2) Berenblum, I., and Chain, E., *Biochem. J.*, 32, 295 (1938).
(3) Mitchell, D., Shaw, E. H., Jr., and Frary, G. G., *Proc. S. Dakota Acad. Sci.*, 24, 108 (1944).
(4) Pons, W. A., Jr., and Guthrie, J. D., IND. ENG. CHEM., ANAL. ED., 18, 184 (1946).
(5) Washburn, E. R., and Strandskov, C. V., *J. Phys. Chem.*, 48, 241 (1944).

RECEIVED July 7, 1948. Presented before the Division of Agricultural and Food Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J. Journal paper 298 of the Purdue University Agricultural Experiment Station. Herman Frasch Foundation for Agricultural Research paper 232.

Quantitative Determination of Thiophenol, Diphenyl Disulfide, and Phenyl Thiolacetate

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Quantitative methods have been developed for the determination of thiophenol, phenyl thiolacetate, diphenyl disulfide, and benzyl phenyl sulfide. Thiophenol is titrated with alcoholic iodine in the presence of pyridine; diphenyl disulfide is reduced to thiophenol with zinc and acetic acid; and phenyl thiolacetate is hydrolyzed to thiophenol with alcoholic alkali. Benzyl phenyl sulfide is determined by oxidation to the sulfoxide in aqueous *tert*-butyl alcohol solution.

FOR some studies on the cleavage of benzyl phenyl sulfide by aluminum bromide and other acidic reagents (*5*), it became necessary to develop rapid methods of analysis for thiophenol, (benzenethiol), diphenyl disulfide, and phenyl thiolacetate (acetyl phenyl sulfide). The present paper describes volumetric methods of moderate accuracy for these compounds, and certain combinations of them, and gives some data on the determination of benzyl phenyl sulfide.

The titration of mercaptans (thiols) in alcohol (*7*) according to the equation

$$2C_6H_5SH + I_2 \longrightarrow C_6H_5SSC_6H_5 + 2HI \qquad (1)$$

has been used in the present work. Other methods (*6, 8, 11*) were found unsuitable because the solutions contained some benzyl bromide and some hydrobromic acid. The reaction represented by Equation 1 was found to be much more rapid and suitable for analytical use in the presence of a small amount of pyridine. The appearance of the iodine color was a suitable end point.

Diphenyl disulfide was determined quantitatively by reduction of the disulfide to thiophenol by zinc and acetic acid, followed by titration with iodine as above.

$$C_6H_5SSC_6H_5 \xrightarrow[HOAc]{Zn} 2C_6H_5SH \qquad (2)$$

Phenyl thiolacetate was determined by hydrolysis of the ester, followed by iodometric titration of the thiophenol.

$$C_6H_5SCOCH_3 + KOH \longrightarrow C_6H_5SK \xrightarrow{H^+} C_6H_5SH \qquad (3)$$

The hydrolysis of the thiol ester was complete in 20 minutes at room temperature, when 30% alcoholic potassium hydroxide was used.

Satisfactory determinations of one component in several combinations of the preceding compounds are reported below. In elaborating these methods, two difficulties arose. In the hydrolysis of the thiol ester, in the presence of large amounts of disulfide (which may have arisen from the oxidation of mercaptan), some cleavage of the disulfide to mercaptan occurred if the solution was allowed to react for more than 0.5 hour with the alcoholic potassium hydroxide. This could be avoided by acidifying the hydrolysis mixture after 20 minutes; the thiol ester was completely hydrolyzed after this interval. The second difficulty was in the reduction of the disulfide to mercaptan with zinc dust and acetic acid. In the presence of benzyl phenyl sulfide, there was 2 to 6% reductive cleavage of the latter under these conditions.

The quantitative determination of benzyl phenyl sulfide was also investigated; of the methods proposed for sulfides (*1, 4, 12*), the oxidation by bromine water (*12, 13, 15*) was found suitable. The reaction was followed by determining the uptake of bromine. Equation 4 was verified by isolation of benzyl phenyl sulfoxide in 92% yield; apparently no nuclear substitution occurred. The quantitative determination of the sulfide was carried out in a medium consisting of some organic solvent, bromine water, and enough *tert*-butyl alcohol to make the solution homogeneous.

$$C_6H_5SCH_2C_6H_5 + Br_2 + H_2O \longrightarrow \underset{\displaystyle O}{C_6H_5SCH_2C_6H_5} + 2HBr \qquad (4)$$

The determination of benzyl phenyl sulfide in the presence of diphenyl disulfide was examined; the latter reacts with bromine as follows (*16*):

$$C_6H_5SSC_6H_5 + 5Br_2 + 6\,H_2O \longrightarrow 2C_6H_5SO_3H + 10HBr \qquad (5)$$

Four bromometric determinations of total sulfur in mixtures containing 0.861 millimole of diphenyl disulfide and 1.250 to 1.426 millimoles of benzyl phenyl sulfide gave an average result of 96.9%. The mean deviation from the average was ±1.2%. If all the error in the determination is assigned to the sulfide, and the disulfide is assumed to react quantitatively according to Equation 5, the amount of the sulfide found is 7 to 23% too low. The amount of disulfide present is known from the results of the preceding determinations.

The use of pyridine hydrobromide perbromide (*2, 3*) instead of bromine water as the oxidant was investigated. The oxidation of the disulfide was much slower, requiring 17 hours at room temperature; the sulfide was oxidized rapidly to the sulfoxide, but the bromine consumption proceeded beyond 1 mole (sulfoxide formation) to 3 moles within 17 hours, if a large excess of oxidizing agent was present.

REAGENTS

The thiophenol used was obtained from Eastman Kodak Company, and used without preliminary purification. Diphenyl disulfide was prepared by the iodine oxidation of thiophenol. The directions found in the literature were followed for the preparation of benzyl phenyl sulfide (*14*) and phenyl thiolacetate (*9*). The solvents were commercial grade, freed from sulfur.

APPARATUS

Standard solutions (of the range 0.05 to 0.1 molar) were prepared in organic solvents (alcohol, ether, benzene, or chlorobenzene) and aliquots of these solutions were measured into glass-stoppered flasks using the glass pump system shown in Figures 1 to 3. The automatic refilling device for the glass pump (a copy of one used by F. T. Martin, Department of Chemistry, University of Maine, Orono, Maine) was used to obtain rapidly reproducible aliquots.

The plunger of the syringe was inserted through the spring coils and Bakelite ring into the syringe after the syringe had been clamped into a slot *A* by means of the Bakelite ring. Screw *B* was adjusted so that the syringe plunger (forced back against tip *C* by the spring) withdrew from the stock solution (at the base of the pump) the desired volume of solution for the aliquots. On dis-

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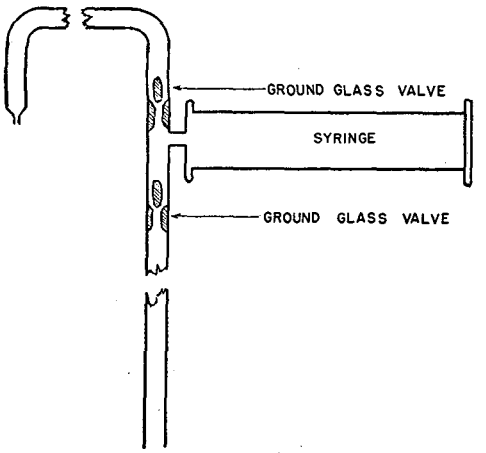

Figure 1. Glass Pump

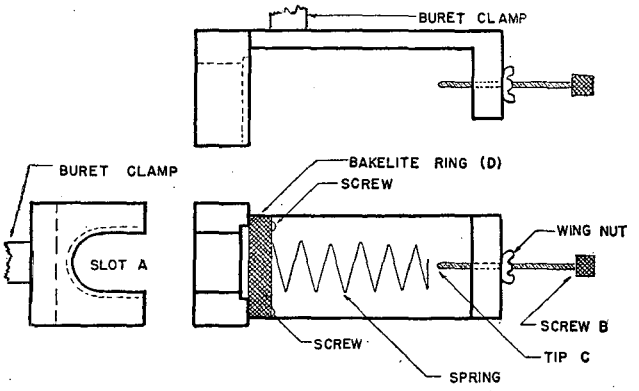

Figure 2. Automatic Refilling Device for Glass Pump

charging the syringe, the desired aliquot was delivered to the flask at the outlet of the pump. Release of the plunger allowed the automatic refilling of the syringe with the exact volume of solution already used. The complete setup is shown in Figure 3.

Determination of Thiophenol. Aliquots of a standard solution in ether, benzene, *tert*-butyl alcohol, or chlorobenzene were titrated with 0.02 *N* alcoholic iodine, after the addition of a few drops of pyridine; the end point was taken as the first appearance of the brown iodine color. Eleven determinations on quantities of 0.3900 to 1.818 millimoles of thiophenol gave an average result of 99.6%. The mean deviation of the individual values from the average was ±0.5%.

Determination of Phenyl Thiolacetate. About 10 ml. (an excess) of 30% alcoholic potassium hydroxide were added to the aliquots to be titrated, and the resulting solutions, made homogeneous by addition of methanol or *tert*-butyl alcohol, were allowed to stand at room temperature for 20 minutes. They were then acidified with glacial acetic acid, a few drops of pyridine were added, and the solution was titrated as above. All the solvents mentioned above proved satisfactory. Seven determinations on 0.285 to 0.712 millimole of phenyl thiolacetate gave an average result of 99.6%. The mean deviation of the individual values from the average was ±1.4%.

Determination of Diphenyl Disulfide. Glacial acetic acid (3 to 5 ml.) and a large excess of zinc dust were added to the aliquots, which were left at room temperature for 16 hours. The supernatant liquid was decanted, the zinc dust was washed twice with ether, and the combined organic layers were titrated for thiophenol. The solvents used were the same as those already mentioned. Ten determinations on 0.1195 to 0.1945 millimole of diphenyl disulfide gave an average result of 99.4%. The mean deviation of the individual values from the average was ±1.4%.

Bromometric Determination of Benzyl Phenyl Sulfide. An excess of bromine water (about 0.1 *N*) was added to the aliquots to be titrated, with enough *tert*-butyl alcohol to form a homogeneous solution. The solutions were allowed to stand at room temperature for 5 to 10 minutes, and then the excess of bromine was titrated iodometrically. This method was satisfactory for the titration of benzyl phenyl sulfide in ether or benzene, if a large amount of *tert*-butyl alcohol was present. With chlorobenzene the results were not so satisfactory, and these results are not reported. Nine determinations on 1.2222 to 1.0949 millimoles of benzyl phenyl sulfide gave an average result of 102.8%. The mean deviation of the individual values from the average was ±2.2%.

To show that the sulfoxide was the end product under these conditions, 2.00 grams of benzyl phenyl sulfide were dissolved in 30.0 ml. of *tert*-butyl alcohol, 3 to 5 ml. of water were added, and a solution of bromine in *tert*-butyl alcohol was added until it was no longer decolorized. The mixture was poured into water; the resulting precipitate, after recrystallization from petroleum ether (boiling point 90° to 100°), gave 2.00 grams of crystals, melting point 124 to 125°. The melting point of benzyl phenyl sulfoxide is reported at 125.5° (*10*).

Determinations on Mixtures. Seven determinations on 0.0780 to 0.1890 millimole of thiophenol in the presence of 0.0438 to 0.239 millimole of diphenyl disulfide, 0.0570 to 0.1424 of phenyl thiolacetate, and 0.0444 to 0.0968 of benzyl phenyl sulfide, gave an average result of 100.7%, for the thiophenol present. The mean deviation of the individual results from the average was ±1.7%.

Eight determinations of phenyl thiolacetate on 0.1424 to 0.2937 millimole of phenyl thiolacetate in the presence of 0.000 to 0.1600 millimole of diphenyl disulfide and 0.000 to 0.444 millimole of benzyl phenyl sulfide, gave an average result of 100.7%. The mean deviation of the individual results from the average was ±1.1%.

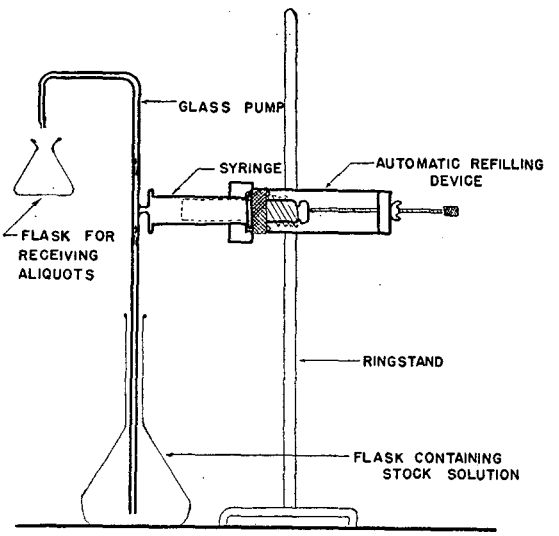

Figure 3. Setup of Apparatus

Four determinations of disulfide on 0.1600 to 0.3068 millimole of diphenyl disulfide in the presence of 0.2047 to 0.444 millimole of benzyl phenyl sulfide gave an average result of 100.1%. The mean deviation of the individual results from the average was ±3.2%.

LITERATURE CITED

(1) Bell and Agruss, IND. ENG. CHEM., ANAL. ED., **13**, 297 (1941).
(2) Djerassi and Scholz, *J. Am. Chem. Soc.*, **70**, 417 (1948).
(3) Englert and McElvain, *Ibid.*, **51**, 863 (1929).
(4) Faragher, Morrell, and Monroe, *Ind. Eng. Chem.*, **19**, 1281 (1927).
(5) Harnish and Tarbell, *J. Am. Chem. Soc.*, **70**, 4123 (1948).
(6) Kimball, Cramer, and Reid, *Ibid.*, **43**, 1199 (1921).
(7) Klason and Carlson, *Ber.*, **39**, 738 (1906).
(8) Kolthoff and Harris, IND. ENG. CHEM., ANAL. ED., **18**, 161 (1946).
(9) Michler, *Ann.*, **176**, 178 (1875).
(10) Pummerer, *Ber.*, **43**, 1406 (1910).
(11) Sampey and Reid, *J. Am. Chem. Soc.*, **54**, 3404 (1932).
(12) Sampey, Slagle, and Reid, *Ibid.*, **54**, 3401 (1932).
(13) Sease *et al.*, ANAL. CHEM., **20**, 431 (1948).
(14) Shriner, Struck, and Jorison, *J. Am. Chem. Soc.*, **52**, 2060 (1930).
(15) Siggia and Edsberg, *Ibid.*, **20**, 938 (1948).
(16) Young, *Ibid.*, **59**, 811 (1937).

RECEIVED June 28, 1948. References (*13*) and (*14*) appeared after the authors' work had been completed.

Titration of Thiocyanate

Iodine Monochloride End Point in Titration with Iodate, Permanganate, and Ceric Solutions

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Direct titration with iodate of soluble thiocyanates in hydrochloric acid solutions to the iodine monochloride end point does not yield quantitative results; less than the stoichiometric volume of iodate is required because of partial decomposition of the thiocyanate before oxidation. Quantitative determinations can be made by adding the thiocyanate to a solution containing iodine monochloride and then titrating with iodate. The titration is not quantitative under similar conditions with permanganate or ceric sulfate; negative errors result.

THE determination of copper by precipitation of cuprous thiocyanate, followed by titration of the precipitate with iodate to the iodine monochloride end point, was originally suggested by Jamieson, Levy, and Wells (*3*) and has since been extensively used (*8*). Similar procedures for the determination of zinc (*1*) and mercury (*2*) based upon titrations of zinc mercuric thiocyanate precipitates have been proposed. Jamieson, Levy, and Wells (*3*) show data on the titration of two samples of ammonium thiocyanate which indicate that the method could be applied to the general titration of thiocyanate solutions.

The use of iodate solutions for the determination of thiocyanate was studied by Lang in the course of investigations on the iodine cyanide method (*6*, *7*). He found that a quantitative determination could be made by the rapid addition of excess iodate to an acid solution, followed by back-titration with thiosulfate; also by direct titration with iodate by the iodine cyanide procedure, provided that the iodate was added rapidly or that iodine cyanide or monochloride was added prior to the titration. The erratic results obtained by slow addition of the iodate or without prior addition of iodine cyanide or chloride were attributed to atmospheric oxygen. Lang stated that the iodine monochloride method of Andrews is applicable to the titration of thiocyanate solutions but gave no experimental verification.

Because the iodine monochloride method for the determination of thiocyanate in acid solutions would be of considerable value, preliminary titrations were made in order to determine the accuracy of the method. The values obtained were so erratic that an experimental study was undertaken in order to investigate the causes of the erratic results, to ascertain whether conditions could be found under which quantitative titrations could be made with iodate solutions, and to determine if by the use of the iodine monochloride end point the method could be extended to titrations with permanganate and with ceric sulfate solutions (*10*).

EXPERIMENTAL

Reagents. Standard thiocyanate solutions were made from potassium thiocyanate prepared by the procedure of Kolthoff and Lingane (*5*). Check titrations on the thiocyanate solution used for the final test analyses, made by the Volhard method with a standard silver nitrate solution, gave a formality of 0.015295 (a normality with respect to the oxidation of thiocyanate to sulfate and hydrocyanic acid of 0.0917_7) as compared with a formality of 0.01528_8 calculated from the original weight of the thiocyanate and the volume to which the solution was diluted; the latter value is believed to be the more reliable. (Volume formal concentrations, formula weights per liter of solution, are used except in the case of standard titrating solutions in order to avoid uncertainty regarding type of reaction or oxidation changes.) Permanganate solutions were standardized against Bureau of Standards sodium oxalate or arsenious oxide; the iodate and ceric sulfate solutions were standardized against the latter.

Titration with Iodate. In Table I are shown the procedure used and the results obtained from a series of preliminary experiments made in order to ascertain the effect of various conditions on titrations made with iodate solutions.

General Procedure. Five milliliters of carbon tetrachloride were taken in an iodine flask, followed by that volume of 12 *F* hydrochloric acid which when mixed with the 25 ml. of potassium thiocyanate next added would give the initial hydrochloric acid concentration stated. The mixture was allowed to stand the time stated, then titrated. In some cases more hydrochloric acid was added during the titration. Calculated volume of iodate was 43.50 ml. The titrations required from 7 to 15 minutes.

The following conclusions can be drawn from Table I. The sign of the error is consistently minus—that is, not enough iodate is being required. The error tends to increase with increase in initial concentration of the acid. With concentrations above 2 *F* it tends to increase with increased length of time the thiocyanate stands in the hydrochloric acid. With 2 *F* acid initially it amounts to about 0.25%, and appears to be independent of time of standing. At least in 2 *F* acid, it is not caused by oxygen. This last conclusion is not in accord with the conclusions of Lang (*6*, *7*).

Because soluble thiocyanates are known to undergo polymerization and decomposition in concentrated acid solutions (*9*), experiments were made to ascertain whether this source of error could be eliminated by providing iodine monochloride in the acid to which the thiocyanate was added. In Table II the data obtained from such experiments indicate that by previous addition to the acid of approximately four tenths the equivalent amount of iodine monochloride required for the oxidation of the thiocyanate the error is considerably decreased, provided that the initial acid is not too concentrated and that the mixture is not allowed to stand. That slowly reacting products of the

Table I. Effect of Conditions on Titration of Thiocyanate with Iodate (Iodine Monochloride End Point)

Expt. No.	HCl Formality: Initial	HCl Formality: At end point	Time of Standing, Min.	% Error	Remarks
1	6.5	3.6	1	−0.9	
2	6.5	3.6	2	−1.3	
3	6.5	3.6	26	−2.6	
4	6.5	3.6	30	−2.6	
5	4.3	2.0	1	−0.5	Care required in 2 *F* HCl
6	4.3	2.0	2	−0.5	to avoid overtitrating
7	4.3	2.0	18	−0.6	because of slow oxida-
8	4.3	2.0	20	−0.6	tion of iodine
9	2.0	2.0	1	−0.16	
10	2.0	2.0	1	−0.25	
11	2.0	2.0	10	−0.25	
12	2.0	2.0	19	−0.25	
13	0.5	2.0	7	−0.25	O_2 excluded by CO_2
14	0.5	2.0	15	−0.25	O_2 excluded by CO_2

Table II. Effect of Prior Addition of Iodine Monochloride on Titration of Thiocyanate with Iodate

Expt. No.	HCl Formality Initial	HCl Formality At end point	Time of Standing, Min.	Ratio Equivalents ICl/SCN	% Error
1	2	2	2	0.35	−0.13
2	2	2	19	0.35	−0.20
3	2	2	1	0.77	−0.02
4	2	2	2	0.77	−0.04
5	2	2	2	1.5	−0.07
6	2	2	15	1.5	−0.07
7	2	2	10	0.77	−0.18
8	2	2	2	0.45	−0.11
9	2	2	2	0.37	−0.16
10	6	2	1	0.37	−0.09
11	6	2	10	0.37	−0.09
12	6	2	20	0.37	−0.25
13	4	2	1	0.37	−0.10
14	4	2	10	0.37	−0.16
15	4	2	20	0.37	−0.30

acid decomposition of the thiocyanate, rather than atmospheric oxygen, are largely responsible for the error was indicated by the dependence of the error on the time of standing, especially in the more concentrated acid, and by the fact that titrated solutions showed a return of end point on standing; in several cases solutions that had stood for several days and were then retitrated gave substantially correct titrations.

Side reactions involving the formation of products such as the thionic acids may be a significant source of error, especially at the lower acid concentrations.

Procedure. The procedure is the same as that given for Table I, except that iodine monochloride was added to the hydrochloric acid before addition of the thiocyanate. Ratios of oxidation equivalents of iodine monochloride first added to reduction equivalents of thiocyanate are shown in Table II.

As a result of these experiments the procedure outlined below was adopted and a series of test titrations was made, not only with iodate, but with permanganate and ceric solutions.

Procedure Adopted. The indicated volume of thiocyanate solution was pipetted into an iodine flask, 15 ml. of tested carbon tetrachloride were added, and the mixture was cooled in an ice bath (to less than 10° C.). The indicated volume of 0.5 *F* iodine monochloride in 3 *F* hydrochloric acid, similarly cooled, was added, and the stoppered flask was shaken until most of the resulting iodine was dissolved in the carbon tetrachloride. Then there was added that volume of cold 12 *F* hydrochloric acid required to give desired acid concentration at the end point (3.5 *F* for the iodate and permanganate and 4.5 for the ceric titrations). A small portion of the acid was added first around the neck of the flask and the stopper was partly withdrawn so that possible loss of iodine was minimized. After addition of the acid the flask was again stoppered and shaken, then immediately titrated.

The data obtained are shown in Table III. The data for the iodate titrations show that a high order of precision and accuracy is possible by the procedure described.

An incompleted study of the determination of copper by precipitation of cuprous thiocyanate and direct titration of the precipitate—that is, without preliminary addition of iodine monochloride—indicated that although the tendency was towards negative errors, these errors were smaller than those found in the direct titration of a soluble thiocyanate. Thus in three determinations in which 78.75 mg. of copper were taken there were found 78.64 and 78.55 mg. when the hydrochloric acid concentration was 5 *F* initially and 3 *F* at the end point; and 78.49 with the acid 7 and 5 *F*, respectively. It seems probable that decomposition of the thiocyanate by the acid is minimized because of the low solubility of the precipitate.

Titration with Permanganate. The direct titration of thiocyanate in an acid solution with permanganate has been the subject of numerous investigations, which have shown that too little permanganate is used, apparently because of an induced reaction between oxygen and permanganate. Kolthoff (*4*) reviews these investigations, confirms the induced oxygen error, and states that in his study (unpublished) of the volumetric determination of thiocyanate he, under no conditions, obtained very good results with permanganate. In the hope that the preliminary oxidation of the thiocyanate with iodine monochloride might eliminate the "oxygen error" and permit the titration with permanganate to an iodine monochloride end point, a series of preliminary experiments was made. The procedure was essentially that used with the experiments shown in Table II. The initial hydrochloric acid concentration was varied from 2 to 6.5 *F;* the final from 2 to 3.5. The time of standing ranged from 1 to 15 minutes; the ratio of the equivalents of iodine monochloride added to thiocyanate present was 0.37.

Contrary to the iodate titration, in no case was the error less than about −0.2; with the higher acid concentrations and with the longer periods of standing it amounted to as much as −0.5%. Furthermore, the test analyses made with permanganate titrations (shown in Table III) have an average error of −0.3%. When these latter titrated solutions had stood for 2 hours an iodine color developed in the carbon tetrachloride; upon again titrating to an end point the average volume of total permanganate used was 43.41, with a maximum deviation of 0.02 ml.; this average volume gives a formality of 0.01534, which is approximately 0.3% high.

In order to check the factors that might cause the error with permanganate, titrations of thiocyanate with iodate were made in which an amount of manganous chloride equivalent to that formed during the permanganate titration was added. The

Table III. Titrations of 0.01528$_8$ Formal Thiocyanate Solution with Iodate, Permanganate, and Ceric Sulfate

(Iodine monochloride end point)

Expt. No.	Thiocyanate Taken, Ml.	Standard Oxidizing Solution, Ml.	Iodine Monochloride (0.5 *F* in 3 *F* HCl) Added, Ml.
	I. With Iodate (0.10201 *N*)		
1a	50.03	45.00	5.0
1b		45.00	5.0
1c		45.02	5.0
		Av. 45.01	
	Calculated formality of thiocyanate solution 0.01529$_0$		
2a	50.03	44.98	5.0
2b		44.98	5.0
2c		45.00	5.0
2d		45.02	5.0
		Av. 45.00	
	Calculated formality of thiocyanate solution 0.01529$_2$		
3a[a]	50.03	45.02	5.0
3b		45.01	5.0
3c		45.00	5.0
3d		45.02	5.0
		Av. 45.01	
	Calculated formality of thiocyanate solution 0.01529$_0$		
4	24.95	Av. 22.45 (7 titrations, max. deviation 0.06 ml.)	2.5
	Calculated formality of thiocyanate solution 0.01529$_6$		
	II. With Permanganate (0.1061$_2$ *N*)		
5a	50.03	43.11	5.0
5b		43.10	5.0
5c		43.12	5.0
		Av. 43.11	
	Calculated formality of thiocyanate solution 0.01523$_8$		
	III. With Ceric Sulfate (0.0924$_5$ *N*)		
6a	50.03	49.50	5.0
6b		49.35	5.0
6c		49.21	5.0
		Av. 49.35	
	Calculated formality of thiocyanate solution 0.0151$_6$		

[a] In the third set of experiments 1 millimole of manganese chloride was added to the solution to be treated.

Table IV. Titration of Iodide with Permanganate (Iodine Monochloride End Point) in Presence of Cyanide and Sulfate

Expt. No.	Permanganate (0.1061_2 *N*) Used, Ml.	Potassium Iodide, Gram Taken	Found
1	44.05	0.3881	0.3880
2	44.03	0.3879	0.3880
3	43.85	0.3862	0.3863
4	44.07	0.3883	0.3882
5	44.18	0.3891	0.3892

results of these titrations are shown in Table III, (Experiments 3, a, b, c, and d) and indicate that the manganese is without noticeable effect. Weighed samples of potassium iodide were titrated with permanganate after addition of amounts of cyanide and sulfate equivalent to those formed in the titration of thiocyanate. The results, tabulated in Table IV, show no evident interference. The procedure was that described above.

These data show that under the above conditions iodide can be accurately titrated by permanganate to the iodine monochloride end point, and that the presence of cyanide and sulfate is without effect. The reason for the error in the titration of thiocyanate with permanganate by the above procedure is therefore still uncertain.

Titration with Ceric Sulfate. Preliminary experiments indicated, and the data shown in Table III (Experiments 6, a, b, and c) confirm that the titration with ceric sulfate is not stoichiometric; negative errors are again observed. Titration of iodide solutions with ceric solutions with and without addition of cyanide and sulfate again indicated that, as with iodate and permanganate, these constituents were without effect.

The titrations with ceric sulfate were more critical on the final hydrochloric acid concentration than were these with iodate or permanganate, and in all cases required longer for attainment of apparent stability. Negative errors of around 10% were obtained when the final acid concentration was 3 *F*.

ACKNOWLEDGMENT

The authors are indebted to David Pressman for a series of preliminary experiments carried out in 1934–35, to Miyoshi Ikawa for the experiments on the determinations of copper, and to Dale Meier for helpful comments on the manuscript.

LITERATURE CITED

(1) Jamieson, *J. Am. Chem. Soc.*, 40, 1036 (1918).
(2) Jamieson, *J. Ind. Eng. Chem.*, 11, 296 (1919).
(3) Jamieson, Levy, and Wells, *J. Am. Chem. Soc.*, 30, 760 (1908).
(4) Kolthoff, I. M., and Furman, N. H., "Volumetric Analysis," Vol. II, p. 300, New York, John Wiley & Sons, 1929.
(5) Kolthoff, I. M., and Lingane, J. J., *J. Am. Chem. Soc.*, 57, 2126 (1935).
(6) Lang, Rudolph, *Z. anorg. allgem. Chem.*, 142, 289 (1925).
(7) Oesper, "Newer Methods of Volumetric Chemical Analysis," p. 85, New York, D. Van Nostrand Co., 1938.
(8) "Scott's Standard Methods of Chemical Analysis," N. H. Furman, ed., 5th ed., Vol. 1, p. 375, New York, D. Van Nostrand Co., 1939.
(9) Stokes and Cain, *Bull. U. S. Bur. Standards*, 3, 157 (1907).
(10) Swift, *J. Am. Chem. Soc.*, 52, 894 (1930).

RECEIVED June 18, 1948. Contribution 1301 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology.

Determination of High Percentages of Copper

With a Beckman Spectrophotometer

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A spectrophotometric method for the determination of high percentages of copper with an accuracy and precision of 1 to 3 parts per thousand utilizes the color of the cupric ion contained in 10% perchloric acid solution. The stated precision is obtained by working with copper solutions possessing extinctions greater than 2.0. Such high densities are not read in the normal way, which would involve a large percentage error, but a differential method is used. The optical density scale is set for zero with a solution containing 1.5000 grams of copper per 100 ml. rather than with distilled water. This is accomplished by working at a much larger slit width than is normally employed. Higher concentrations of copper are then read against this zero. The commonly occurring colored metal ions, cobalt, iron, chromium, and nickel, in concentrations up to 4% each, do not interfere with the determination. This method has been applied to a lead brass, a phosphor bronze, and a synthetic sample.

THE colorimetric or spectrophotometric determination of major constituents has been delayed because of the limit of accuracy imposed by conventional methods. If one proceeds in the normal way, it can be shown that the precision of measurement of the intensity of a given color does not increase indefinitely with increasing concentration of the color. Rather, for colors that obey Beer's law, the maximum precision is obtained when the extinction of the given colored solution is 0.434, corresponding to a transmittancy of 36.8%. Actually, the error remains nearly constant in the range of 20 to 60% transmittancy (*5*), and above or below this region it increases rapidly.

The error in the determination will depend upon the accuracy with which the extinction measurement can be made. Assuming an error of 0.1 division in the scale reading (based on 100 scale divisions) at an optical density of 0.434, the concentration error is 0.27%. In practice, however, this is difficult to achieve. Sandell says (*5*), "The error in setting the microammeter needle at 100 and making the transmittancy or absorption reading should not exceed 0.2 scale division, which represents an error of 0.6% in concentration at 50% transmittancy, if it is assumed that the standard curve is not in error." At the optimum point, this scale error corresponds to a concentration error of 0.54%, which is too high for the determination of major constituents. Moreover, this treatment assumes that no error is present in the standard curve; in actual practice, a concentration error of 1% is not unusual.

Methods for decreasing this error have been described. Ringbom (*4*) has shown that much better results can be obtained if an unknown concentration of a given color contained in one cuvette is matched by adding a standard solution to a second cuvette containing the color-forming reagents only. This is done with the galvanometer at full sensitivity. Under such conditions, which amount to a colorimetric titration, an error of as little as 0.15% at a transmittancy of 50% is indicated (*6*).

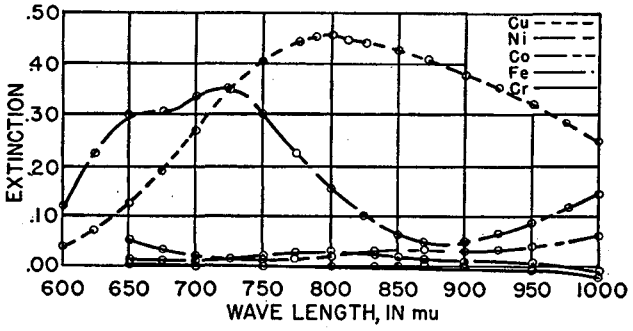

Figure 1. Extinction-Wave Length Curves for Metals in 10% Perchloric Acid Solution

Copper concentration, 0.25 gram per 100 ml. Other metals, 1.0 gram per 100 ml.

Kortüm (*2*) describes a method for comparing a color with a similar standard and obtaining an error of 0.2%. Rabinovitch and Wood (*3*) have been able to distinguish differences in concentration of 0.002% using an arc source and special apparatus.

The above methods indicate that the problem can be solved but they are too inconvenient or tedious for general use. The method described below employs a model DU Beckman spectrophotometer and is almost as simple to use as conventional methods.

For doing this work, copper was employed. The blue color of the cupric ion itself contained in a 10% perchloric acid solution was selected because of its expected stability, and because its spectral characteristics are such that interference from other colored metal ions is low.

PRINCIPLE OF METHOD

Assume that a concentration of 0.2 gram of copper per 100 ml. can be determined by the normal method with an accuracy of 1%. If the color obeys Beer's law at higher concentrations, a difference in concentration of 0.2 gram of copper per 100 ml. will always give the same difference in extinction.

Yet if an attempt is made to take ten times the concentration of copper in the hope of getting ten times as much accuracy, the density reading will be off the scale; and even if such a high density could be read, the percentage error in making such a reading would be greater than the error in reading the lower concentration.

This is all based on the assumption that the optical density scale is set for zero (or the transmittancy scale for 100%) using distilled water.

Suppose, however, instead of using distilled water for this purpose, a solution containing 1.8 grams of copper per 100 ml. were used. A solution containing 2.0 grams of copper per 100 ml. read against this standard should then give the same scale reading that a concentration of 0.2 gram of copper per 100 ml. gives against distilled water.

If it is possible to determine the 0.2-gram difference at the higher concentration to an accuracy of 1%, the total concentration is automatically determined to an accuracy of 0.1%. Such a process could be carried farther. If 19.8 grams of copper per 100 ml. were taken for the zero setting, and all the above conditions still applied, an accuracy of 0.01% could be obtained.

The above treatment assumes that there is no loss in accuracy in measuring differences in concentrations at higher concentrations, provided Beer's law is obeyed. No comparative experimental data are given to prove this in this paper. However, it is clear that if the error in determining such differences does not increase by a factor of 10 or more as the concentration is increased ten times, the method will prove advantageous.

A limitation to the method is that some provision must be made for getting enough light through the high concentration of colored material to make the necessary zero setting. On the Beckman instrument this is accomplished by working at a much wider slit width than is normally employed. This naturally increases the band width of light which emerges.

Actually, a compromise was reached so as to provide reasonable monochromaticity and yet a reasonably high concentration of copper. A concentration of 1.5000 grams of copper per 100 ml. was finally selected for the zero point, and a slit width of 0.34 mm. was employed. This corresponds to a band width of 25.5 millimicrons. Attempts to use the slit wide open (2.0 mm.) and 3.0 grams of copper per 100 ml. yielded a curved rather than a straight line.

SELECTION OF WAVE LENGTH

All the work was done using 1.000-cm. Corex cells at a wave length of 870 millimicrons. The selection of the wave length is based upon the absorption data shown in Figure 1 for the commonly occurring colored metal ions plotted against a distilled water zero with the instrument used in normal fashion. The slit widths varied from about 0.02 to 0.06 mm. The concentration of copper shown is 0.25 gram per 100 ml., the other metals 1.0 gram per 100 ml.

MATERIALS

The sources of these metals were 99.85% C. P. iron wire, C. P. nickel, C. P. cobalt chloride, C. P. chromic acid, and 99.99 + % oxygen-free, high conductivity copper.

The materials were dissolved in nitric acid, treated with 10 ml. of 60% perchloric acid, taken to fumes, and fumed for a few minutes. Such treatment leaves iron and chromium in oxidized states. After cooling, 50 ml. of water were added, and the solutions were boiled to remove chlorine, cooled, and diluted to 100.0 ml. in volumetric flasks.

Nickel is the only metal besides copper that shows appreciable absorption in the region shown on the graph. Because its absorption is nearly at a minimum at 870 millimicrons while the copper absorption is still high, this wave length was selected for the subsequent work.

PREPARATION OF STANDARD CURVE

To prepare the standard curve, 1.5000 grams of oxygen-free, high conductivity copper and varying greater quantities were weighed and placed in 250-ml. beakers. (In all this work the 1.5000-gram standards varied in weight from 1.4995 to 1.5005 grams. Corrections were applied for the slight deviations.) After solution in 20 ml. of 1 to 1 nitric acid, 10 ml. of 60% perchloric acid were added and the samples were taken to heavy perchloric acid fumes. After fuming, the samples were cooled, diluted with 50 ml. of water, and boiled for 2 minutes to remove chlorine. The samples were cooled to room temperature, transferred to 100-ml. Exax volumetric flasks, and diluted to the mark.

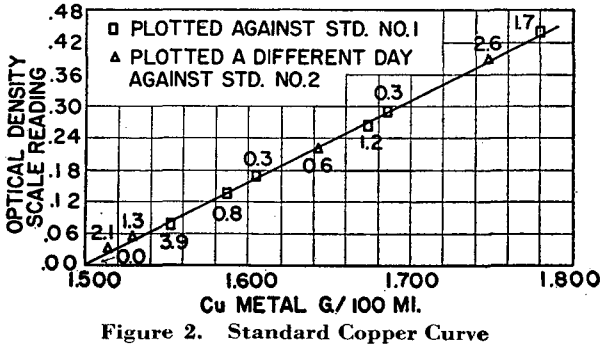

Figure 2. Standard Copper Curve

870 mμ, slit at 0.34 mm. 1.000-cm. Corex cells

The 1.5000-gram standard was placed in two adjacent cells, the slit opened to 0.34 mm., and the wave length set at 870 millimicrons. The dark current adjustment was made and then the galvanometer zeroed using the sensitivity knob alone. Under these conditions the knob was used at from 2 to 4 turns from the clockwise end, thus giving satisfactory sensitivity.

After this the companion cell was slid into position and if any difference in reading was observed (generally 0.002 to 0.005 on the density scale), a corresponding correction was made on the solutions measured in that cell. The standard solutions were then read in the second cell, always against the 1.5000-gram

Table I. Effect of Colored Ions

(Solutions contained 0.08 gram each of iron, nickel, chromium, and cobalt, plus indicated amount of copper per 100 ml.)

Cu Taken, Grams	Cu Found, Grams	Error, Parts per Thousand
1.6495	1.6518	+1.4
1.6478	1.6443	−2.1
1.6466	1.6503	+2.2
1.6443	1.6405	−2.3
Av. 1.6471	1.6467	±2.0

Error = −0.2 part per thousand
Computed error = +2.4 parts per thousand
Maximum error = −2.6 parts per thousand

standard as a zero point and adjusting for zero with the sensitivity control only. Figure 2 (squares) shows the results obtained. The following day a new 1.5000-gram standard was prepared and the triangular points were obtained against this standard.

The deviation of the points from the line in parts per thousand of concentration are indicated on the graph. The maximum deviation is +3.9 parts per thousand and the average deviation ±1.5 parts per thousand, including the zero point in the average.

Assuming that the increase in accuracy over the normal method is directly proportional to the increase in copper concentration over that required to give an extinction of 0.434 against a distilled water zero, this method should yield about six times the normal accuracy over the range shown on the graph.

EFFECT OF OTHER COLORED IONS

To test the effect of other colored ions, four samples of copper were analyzed, to which 0.08 gram each of iron, nickel, cobalt, and chromium were simultaneously added. On the weight of copper taken these amounts represent about 4% each of the metals. These samples were treated exactly like the standards and read against a freshly prepared 1.5000-gram copper standard using Figure 2. The values are given in Table I.

Computing the interference from the other ions on the assumption that the absorption at the wider slit is about the same as under the conditions in Figure 1, and assuming that the colors obey Beer's law and are additive, the result should be 2.4 parts per thousand high. The maximum error under these conditions would be −2.6 parts per thousand.

From the above, it may be concluded that nickel, chromium, iron, and cobalt in amounts up to 4% each do not appreciably interfere. Inasmuch as these concentrations are well above the normal amounts contained in many copper-base alloys, the method should be excellent for such materials.

Any tin present in such materials will be precipitated as metastannic acid, and this must be filtered off. If appreciable amounts of tin are present, the precipitate will retain copper, but this situation arises in the standard electrolytic method as well.

RESULTS ON COPPER-BASE ALLOYS

Table II gives the results obtained on two copper-base alloys.

Accurately weighed 2.7-gram samples were employed for sample B. Because a trace of tin was present, causing a faint turbidity, the samples were diluted to the mark and then filtered into the absorption cells before reading.

In the case of sample A, accurately weighed 2-gram samples were dissolved in 20 ml. of 1 to 1 nitric acid. After decomposition, 50 ml. of hot water were added, the solutions were filtered, and the residue was washed twice with hot water. The filter papers were placed in the original beakers and treated with 20 ml. of nitric acid and 10 ml. of perchloric acid. After heating to fumes to destroy the paper, the solutions were cooled somewhat, and a little water was added, followed by 20 ml. of 48% hydrobromic acid (*1*). The solutions were heated to fumes again to remove tin. This was repeated three times. The small residue of antimony and tin remaining was then filtered off and washed thoroughly and the filtrate was combined with the original filtrate. This was boiled to fumes and diluted to 100 ml. in the volumetric flask as in the other methods. The solution was very faintly turbid (probably owing to the incomplete removal of tin, antimony, or silica) and therefore was filtered into the cell before reading.

It is felt that the errors indicated here are higher than those which need occur under the best conditions. Because the original 1.5000-gram standards used in plotting the points were discarded, the author had to prepare fresh ones for subsequent analyses. Any error in the standard, therefore, was added to the sample error. In routine analysis it would be much better to preserve this standard and eliminate that source of error. In addition, the volumetric flasks were not calibrated. Some fluctuations due to this source are likely. (Upon the suggestion of one of the reviewers, some time after this work was done, all thirteen 100-ml. volumetric flasks possessed by the laboratory were calibrated. Barring the possibility of breakage, these would include all the flasks used. The maximum difference between any two flasks was 1.0 part per thousand and the average deviation from the mean ±0.3 part per thousand. It is probable that the error introduced was between these two limits.)

Table II. Determination of Copper in Copper-Base Alloys

Sample A. Bureau of Standards Phosphor Bronze 63b[a]		Sample B. Lead Brass[b]	
Cu present, %	Cu found, %	Cu found, electrolytic, %	Cu found, colorimetric, %
77.96	78.30	61.26[c]	61.50
	77.88		61.30
	78.08		61.38
	77.85		61.50
	Av. 78.03 ± 2.1 parts per thousand		61.42 ± 1.3 parts per thousand
Error = +0.9 part per thousand			Error = +2.6 parts per thousand (taking electrolytic value as correct)

[a] Provisional analysis, 77.96% Cu, 9.35% Pb, 9.78% Sn, 0.71% Zn, 0.54% Sb, 0.47% Fe, 0.44% P, 0.33% Ni, 0.17% S, 0.12% Si, 0.05% Al, 0.04% Ag, and 0.015% As.
[b] Approximate composition. 3.4% Pb, 61 to 62% Cu, 0.13% Fe, trace of Sn, remainder Zn.
[c] Average of 3 determinations.

This paper is intended only to illustrate a method of approach in colorimetric work. The method described should be applicable to other metals and in cases where the colors are stable enough to the more sensitive color reactions. If very stable colors can be found, it should be possible to obtain very high precision on small samples by applying the method. A more intense source of light than is at present contained on the Beckman would be very helpful because it would permit the use of even higher concentrations of color, at the same time maintaining narrow slit widths.

ACKNOWLEDGMENT

Many thanks are expressed to Richard Weberling for performing the electrolytic analysis of copper (sample B), to Frank Bassani and his drafting department for preparation of the graphs, and to M. J. Rafale and Mrs. A. H. Childers for their assistance in checking over the manuscript.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "Methods of Chemical Analysis of Metals," p. 186, 1946.
(2) Kortüm, G., *Angew. Chem.*, 50, 193 (1937).
(3) Rabinovitch, E., and Wood, W., *Trans. Faraday Soc.*, 32, 547 (1936).
(4) Ringbom, A., *Z. anal. Chem.*, 115, 332 (1938/39).
(5) Sandell, E. B., "Colorimetric Determination of Traces of Metals," p. 53, New York, Interscience Publishers, 1944.
(6) *Ibid.*, p. 54.

RECEIVED September 17, 1948. Presented before the Division of Analytical and Micro Chemistry at the 114th Meeting of the AMERICAN CHEMICAL SOCIETY, St. Louis, Mo.

Iodometric Determination of Copper

Effect of Thiocyanate on End Point and Use of Sulfate–Hydrogen Sulfate Buffers

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The use of thiocyanate in the iodometric determination of copper has been studied. Evidence has been obtained that at the conventional end point, without thiocyanate, the cuprous iodide precipitate contains adsorbed iodine and small amounts of starch-iodine compound and cupric copper. The effect of the point of addition, of the amount of thiocyanate used, and of "protective" agents, such as shellac and certain commercial wetting agents, has been investigated. The permissible pH range for the iodometric determination of copper in solutions buffered by sulfate and hydrogen sulfate has been found to be from about 0.5 to 3.0. In this range stable end points were obtained and no significant "oxygen error" was observed. A series of test analyses of pure copper made with pH values from 2.0 to 2.5, when thiocyanate was added near the end point, gave results with an accuracy within that of the volumetric measurements involved. The use of this method for the standardization of thiosulfate solutions is indicated. When thiocyanate was not used, the same series of analyses gave values having an average deviation from their mean of only 0.05%, but with a mean value 0.34% low. Analyses of two typical copper alloys gave values agreeing within 2 parts per 1000 with those reported by Bureau of Standards.

THE iodometric determination of copper has been studied, with special attention to the effect of thiocyanate on the end point and the use of sulfate–hydrogen sulfate buffers.

EFFECT OF THIOCYANATE UPON END POINT

The suggestion of Foote and Vance (*7*) that a soluble thiocyanate be added near the end of the titration with thiosulfate has been widely recommended. Foote and Vance stated that when thiocyanate is used the additional thiosulfate required is about 0.15 ml. of a 0.1 *N* solution, that the error in the absence of thiocyanate is "due partly to absorption of iodine by cuprous iodide," and that "cuprous thiocyanate is more insoluble than cuprous iodide, thus tending to make the reaction more complete." No experimental evidence was given to indicate the relative importance of these two effects; nor is there a complete statement of the conditions under which the difference of 0.15 ml. was observed. The experiments reported below were made as a part of an investigation of these effects and their causes.

The solutions were buffered to pH values of from 2.0 to 2.5 by means of sulfate and hydrogen sulfate. The sulfate–hydrogen sulfate system was used in order to minimize the complex-ion formation observed by Crowell (*3*) in other systems and because, as is shown below, the sulfate buffer system can be used to maintain pH values at which neither hydrolysis of the cupric ion nor "oxygen error" causes significant errors.

EXPERIMENTAL

Chemicals and Reagents. The sodium thiosulfate solutions, usually slightly greater than 0.1 formal, were prepared and stored according to the instructions given by Swift (*19*). These solutions were standardized before and after each series of experiments against National Bureau of Standards potassium dichromate; the procedure of Bray and Miller (*1*) was used. Three determinations were made, and the maximum deviation was less than 1 part per 1000; volume burets were used exclusively. Some of the solutions were used over a period of 30 to 60 days, but in no case was a change in normality of as much as 1 part per 1000 observed.

The copper sulfate solutions, approximately 0.1 *F*, were prepared by dissolving cupric sulfate pentahydrate in water or by treating Bureau of Standards copper, sample 45 A having a melting point of 1083° C., with just enough dilute nitric acid to dissolve the copper and then fuming the solution with sulfuric acid; the sulfuric acid was then neutralized with standard ammonium hydroxide, and more ammonium sulfate was added when necessary. These solutions were made approximately 1.8 *F* in ammonium sulfate. This concentration was selected because in 50 ml. of the solution the sulfate present would be equivalent to that formed by neutralizing 5 ml. of 18 *F* sulfuric acid with ammonium hydroxide; this volume of sulfuric acid is frequently recommended for the removal of the nitric acid used in dissolving copper ores or alloys. The pH values of these cupric sulfate–ammonium sulfate solutions were approximately 3.6 at 20° C. The pH of the solution to be titrated was adjusted by the addition of sulfuric acid or ammonium hydroxide. The pH measurements were made with a Beckman glass electrode pH meter.

Various brands of reagent grade potassium thiocyanate were used. Because in some cases this material showed evidence of decomposition and of impurities that required a significant correction because of reaction with iodine, recrystallized material was used in the later experiments. Several preparations of ammonium thiocyanate gave evidence of extensive decomposition and abnormal reactions with iodine solutions; therefore the use of this compound is not recommended.

The starch solutions were 0.5%, prepared as needed from a "soluble starch" which had been tested for sensitivity.

All other chemicals used were of reagent grade; they were tested for reaction with iodine and iodide and appropriate corrections were made when necessary.

General Titration Procedure. A portion of the copper sulfate solution, usually 50 ml., was pipetted into a 200-ml. conical flask and sulfuric acid or ammonium hydroxide was added to give the desired initial pH. Three grams of potassium iodide, iodate- and iodine-free, were dissolved in 5 ml. of water and added to the swirling solution. The mixture was then titrated with thiosulfate until the triiodide color became indistinct. Five milliliters of starch solution were added and the titration was continued until the starch-iodine color just disappeared. This end point was recorded and is designated as the iodide end point. Four grams of potassium thiocyanate were then added and the titration was continued until the color was again bleached. This end point is designated as the thiocyanate end point.

The modifications of this procedure used for the analysis of pure copper and of certain alloys are described below.

DISCUSSION AND DATA

Reactions of Iodine and Cupric Copper with Thiocyanate. The addition of solid potassium thiocyanate at or near the iodide end point imparts a pale blue-purple color to the mixture. This color is not caused by the characteristic starch-iodine complex; it will form in the absence of starch when the thiocyanate is added at the equivalence point. If the thiocyanate is added at the iodide end point, in the presence of starch, approximately 0.15 ml. of 0.1 *N* thiosulfate will be required to decolorize the mixture. If the thiocyanate is added at the equivalence point, without starch, the color will form and will last from about 30

Table I. Effect of Addition of Potassium Thiocyanate at Various Points during Titration

(Iodide and thiocyanate end points, determined by general procedure were at 45.08 and 45.18 ml.)

Expt. No.	Volume of Thiosulfate, Ml. At which thiocyanate was added	At thiocyanate end point
1	31.00	44.91
2	35.00	45.12
3	40.00	45.15
4	45.00	45.18
5	45.05	45.18
6	45.18	45.21
7	45.20[a]	45.20

[a] When thiocyanate was added a definite color appeared and lasted about 1 minute.

Table II. Effect of Amount of Potassium Thiocyanate Added

(Iodide and thiocyanate end points, determined by general procedure with 4 grams of thiocyanate were at 44.95 and 45.08 ml., respectively. Each thiosulfate volume shown below represents average of three determinations in which maximum deviation from mean value was 0.02 ml.)

Expt. No.	Potassium Thiocyanate Added	Thiosulfate Added: At iodide end point	Thiosulfate Added: At thiocyanate end point	Difference
	Grams	*Ml.*	*Ml.*	*Ml.*
1	8	44.95	45.08	0.13
2	4	44.95	45.08	0.13
3	2	44.95	45.08	0.13
4	1	44.95	45.06	0.11
5	0.5	44.95	45.01	0.06

to 40 seconds; if added at the iodide end point, without starch, the color will be less intense and will last about 10 to 15 seconds. If starch is added at this point, after the color has faded, the usual starch-iodine color will appear.

It has not been possible to determine the nature of the colored substance. However, because it could not be caused to appear in the absence of a copper salt and similar colors are known to be caused by the reaction between cupric copper and thiocyanate (*12, 13, 18*), the possibility is suggested that the cuprous iodide precipitate contains a significant amount of cupric copper. Walker and Dover (*20*) have presented evidence of compounds such as copper tetraiodide in cuprous iodide precipitates produced in the presence of iodine. Efforts to determine the ratio of cupric to cuprous copper by the method used by Walker and Dover were not successful.

It is known that the thiocyanate cannot be added early in the titration because of oxidation of thiocyanate, and Foote and Vance (*7*) recommended that the addition be made near the end of the titration. The experiments recorded in Table I were made in order to determine the effect of the addition of the thiocyanate at various points during the titration.

The data of Table I show that under the conditions of this procedure the thiocyanate can be added as much as 5 ml. before the iodide end point without serious error. Because of the color mentioned above there is a tendency to overrun the equivalence point if the thiocyanate is added after the iodide end point.

Amount of Thiocyanate Needed. Foote and Vance (*6*) recommended that 4 grams of potassium thiocyanate be used but gave no experimental basis for this amount. Experiments were made in which the amounts of potassium thiocyanate were varied from 0.5 to 8 grams (Table II). There is a definite trend toward low values for the thiocyanate end point with less than 2 grams of thiocyanate; above this amount no difference was apparent.

Effect of pH on End Points. A series of experiments was made in order to determine the effect of the pH of the solution on the end points (Table III). Even at pH 4 the titration volumes are roughly 1% less than those at pH 2. There is also evidence that the use of thiocyanate is particularly advantageous where there is hydrolysis of the cupric ion or where complex ion formation with ammonia or with other complex-forming agents, such as the organic anions mentioned by Crowell (*3*), is a significant factor. These data also fail to show evidence of "oxygen error" even at pH values of 1.

The difference between the iodide and thiocyanate end points appears to be less at lower pH values, leading to the conclusion that in the absence of thiocyanate the titration should be made in more acid solutions than have been generally used.

Causes of Difference between Iodide and Thiocyanate End Points. Repeated experiments have shown that even when the titration is carried out under conditions such that hydrolysis and complex formation of the cupric copper are not significant factors there remains a definite difference between the iodide and thiocyanate end points. Possible causes of this difference are: (1) iodine adsorbed on the cuprous iodide precipitate, (2) cupric copper in the precipitate, and (3) retention of the starch-iodine compound by the precipitate.

The evidence most frequently used in support of the view that iodine is adsorbed on the cuprous iodide is the buff color of the precipitate at the iodide end point. There seems some confusion as to the color of cuprous iodide. Most handbooks state that it is white; Groth (*8*) states that in the absence of iodine it is white; Latimer and Hildebrand (*14*) state that it is gray-brown.

Table III. Effect of pH on Difference in End Points

Expt. No.	pH (before Titration)	End Points: Iodide	End Points: Thiocyanate	Difference
1	4.7	No real end point		
2	4.0	43.70	44.00	0.30
3	3.5	44.38	44.48	0.10[a]
4	3.0	44.40	44.50	0.10
5	2.0	44.40	44.50	0.10
6	1.0	44.47	44.53	0.06

[a] Copper sulfate solution used for these experiments was about 0.07 *F*, and difference between iodide and thiocyanate end points is correspondingly smaller than that found with 0.1 *F* solutions.

In an effort to prepare a pure precipitate of cuprous iodide repeated experiments were made in which specimens of cuprous iodide, known to contain iodine, were dissolved in a concentrated potassium iodide solution and the iodine was reduced with thiosulfate, starch being used as an outside indicator. Then one drop of 0.1 *N* thiosulfate was added in excess and the cuprous iodide was reprecipitated by addition of water. The precipitates were not white but gray-brown in color. The precipitates were separated from the solution, and washed with water, ethyl alcohol, and finally with diethyl ether. When such a precipitate was again dissolved in a fresh potassium iodide solution, no iodine test was obtained with starch. These experiments show that the precipitate may be buff colored and yet not contain either iodine or cupric copper.

Table IV. Titration of Copper Solutions and Analysis of Resulting Precipitate and Solution

The volume of thiosulfate indicated in the second column was added, giving the calculated excess over the iodide end point shown in column 3. The cuprous iodide precipitate was centrifuged, separated, and dissolved in concentrated potassium iodide, and the resulting iodine was titrated; the volume of thiosulfate required is shown in column 4. The centrifugate was titrated with a triiodide solution; the volume required is shown in column 5 and the equivalent volume of thiosulfate in column 6. The net volume of thiosulfate required is shown in column 7. The previously determined iodide and thiocyanate end points were at 44.55 and 44.68 ml. of $Na_2S_2O_3$.

Expt. No.	$Na_2S_2O_3$ in Original Solution: Added initially	$Na_2S_2O_3$ in Original Solution: Excess present	$Na_2S_2O_3$ Required for CuI Precipitate	Centrifugate: KI_3 added	Centrifugate: Equivalent volume $Na_2S_2O_3$	Net Volume $Na_2S_2O_3$
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Ml.*
1	44.55	0.0	0.15	0.00	0.00	44.70
2	44.77	0.22	0.13	0.20	0.09	44.81
3	44.83	0.28	0.12	0.39	0.17	44.78
4	44.95	0.40	0.08	0.79	0.34	44.69
5	45.05	0.50	0.03	0.90	0.38	44.70
6	45.10	0.55	0.05	1.10	0.47	44.68
7	45.20	0.65	0.08	1.45	0.62	44.66
8	45.30	0.75	0.05	1.53	0.65	44.70
9	45.50	0.95	0.05	2.00	0.85	44.70
10	45.05[a]	0.50	0.00	0.85	0.36	44.69
11	45.30[a]	0.75	0.00	1.50	0.64	44.66

[a] Starch not added until after separation of precipitate.

Table V. Titration of Excess Thiosulfate in Presence and Absence of Cuprous Iodide Precipitate

Values are from the average of two determinations in which the deviation of each was of the order of 1 part per 1000. The triiodide was standardized and its normality adjusted to be just equal to that of the thiosulfate solution. Starch was not added until the triiodide titration. The iodide and thiocyanate end points for these solutions were at 49.75 and 49.85 ml., respectively.

	Thiosulfate Added		Triiodide Solution		
Expt.	Total volume	Excess	CuI present	Without CuI	Difference
	Ml.	*Ml.*	*Ml.*	*Ml.*	*Ml.*
1A	49.85	0.00	0.13		
B	49.85	0.00		0.05	0.08
2A	49.95	0.10	0.23		
B	49.95	0.10		0.10	0.13
3A	50.10	0.25	0.35		
B	50.10	0.25		0.25	0.10
4A	50.35	0.50	0.63		
B	50.35	0.50		0.50	
5A	50.85	1.00	1.17		
B	50.85	1.00		1.07	0.10

In Table IV are recorded the data from a series of experiments in which the cuprous iodide precipitates were separated from the titrated solutions, in some cases at the iodide end point and in others when an excess of thiosulfate had been added.

The mixture was centrifuged until the solution was clear, then the centrifugate was decanted, the precipitate was washed with about 5 ml. of sulfate buffer solution, and this washing was added to the centrifugate. The precipitate was dissolved in potassium iodide solution, and the liberated iodine was titrated with 0.1 *N* thiosulfate; the centrifugate was titrated with standard triiodide solution. In Experiments 1 through 9 the starch was added at 0.5 to 1 ml. before the iodide end point; in Experiments 10 and 11, the starch was not added until after separation of the precipitate.

The results indicate that the precipitate contains iodine in some form, or cupric copper, or both at the iodide end point; and that if starch is present when the cuprous iodide is separated from the filtrate, some iodine or cupric copper remains with the precipitate even when an excess of thiosulfate is present. It is also seen from Experiments 10 and 11 that in the absence of starch and in the presence of an excess of about 0.5 ml. of thiosulfate, the precipitate does not contain oxidizing material.

This last observation led to experiments in which it was observed that if a small excess of thiosulfate were added, the cuprous iodide removed from the mixture, starch then added, and the excess thiosulfate back-titrated with a standard triiodide solution, the net volume of thiosulfate used corresponded closely to that at the thiocyanate end point; if the cuprous iodide were not removed the net volume of thiosulfate corresponded to that at the iodide end point. In a series of experiments made for the purpose of studying this effect the iodide and thiocyanate end points were accurately determined by the general procedure, then portions of the same copper and thiosulfate solutions were used for all other experiments. The data obtained, which are tabulated in Table V, show that below 1 ml. of excess thiosulfate the final end point is in good agreement with the thiocyanate end point when the cuprous iodide is removed. With 1 ml. of 0.1 *N* thiosulfate there was evidence of decomposition of the thiosulfate and with volumes above 1 ml. the results were erratic. The time required for the back-titration varied from about 2 to 5 minutes. The difference between the volume of triiodide required for the titration in the presence of the precipitate and that required in its absence is in general agreement with the difference between the iodide and thiocyanate end points; this indicates that adsorption of iodine by the precipitate is a reversible process and is probably the major factor causing this difference.

It had been reported by Caldwell (*2*) that a small amount of shellac in an alcoholic solution would give a sharper iodide end point and prevent the coprecipitation of the "starch-iodine complex" along with the cuprous iodide. Caldwell added 0.5 to 1 ml. of a 4% solution of white shellac in alcohol "after most of the iodine was consumed." In experiments made to test the effect of this procedure a commercial brand known as Siller pure shellac, an alcoholic solution, was precipitated with water, dried, then redissolved in ethyl alcohol to make a 4% solution. Titrations were made without shellac, with the shellac added at the iodide end point, 2 ml. before reaching the iodide end point, and before adding the potassium iodide. The results are recorded in Table VI.

It is the opinion of the experimenter (EWH) that under the conditions of this procedure the iodide end point is not as sharp with the shellac as without it. Floating material, perhaps shellac, interfered with sharpness of the end point. Some of the solutions were centrifuged in an attempt to clear them. The addition of thiocyanate to the mixture gave the usual color at the iodide end point, and, as the results show, the end points agree with or without the shellac.

In an attempt to find an effective substitute for shellac unsuccessful experiments were made with the following commercial wetting agents: Tween 20, morpholine, tetraethylene, pentaamine, and polyvinyl alcohol.

Table VI. Effect of Alcoholic Shellac on Titration End Points[a]

	Thiosulfate Required, Ml.			
End Points	Without shellac	Shellac added before KI	Shellac added at iodide end point	Shellac added 2 ml. before iodide end point
Iodide	43.83	43.85	43.80	43.80
Thiocyanate	44.03	44.03	44.05	44.05
Difference	0.20	0.18	0.25	0.25

[a] Volumes are result of three determinations in each case.

USE OF SULFATE-HYDROGEN SULFATE BUFFERS

The pH range within which the iodometric determination of copper can be made, and buffer systems for maintaining this range, have been the subject of numerous investigations (*3*, *4*, *6*, *7*, *16*). There seems to be general agreement that in the absence of interfering elements, such as arsenic, the minimum pH is determined by an increasing oxygen error, but no agreement as to this minimum pH, as procedures are to be found in which it varies from 3.7, established by an acetate buffer, to whatever value is obtained by the use of "not more than 2 ml. of concentrated mineral acid in a volume of 50 ml." There is uncertainty as to both the value of the maximum limit and the factors establishing it. The statement is made in a text (*17*) that the reaction between cupric and iodide ions is "catalyzed by hydrogen ions and the assumption that equilibrium conditions obtained during the titration is justified only if the pH is below 4.5." Both Park (*16*) and Crowell (*3*) have shown that the maximum limit is influenced by the buffer system used when that system contains constituents that may cause a precipitate or form un-ionized compounds, and Crowell has found evidence of the latter with the anions of the organic acids commonly used as buffer agents for this titration.

Because sulfuric acid is commonly employed to displace the nitric acid used in dissolving copper ores or alloys, and there is no evidence of cupric complexes in sulfate solution, it seemed worth while (1) to establish the minimum and maximum pH values permissible in such solutions and (2) to determine the effectiveness with which the pH could be maintained within these values by the use of a sulfate–hydrogen sulfate system.

The results of experimental studies of the pH limits in sulfate solutions, the magnitude of the resulting errors on exceeding these limits, the effect of the presence of certain anions, and test analyses with certain alloys are presented below.

EXPERIMENTAL

Titration Procedure. The general titration procedure described above was used in the experiments described below. Iodide and thiocyanate end points have the meaning stated there.

Table VII. Effect of pH in Sulfate Buffered Solutions

[Titrations made with solutions buffered by means of ammonium sulfate (90 millimoles) and sulfuric acid]

Expt. No.	$3F$ H_2SO_4 Added, Ml.	pH Values			Thiosulfate Added, Ml.			Maximum Deviation, Ml.
		Initial	At iodide end point	At thiocyanate end point	Iodide end point	Thiocyanate end point	Difference	
1A	0.00	3.60	...	6.15	44.59	44.97	0.38	0.05
2A	0.30	2.45	...	2.56	45.03	45.17	0.14	0.01
3A	0.50	2.13	...	2.30	45.05	45.18	0.13	0.00
4A	1.0	1.80	...	2.10	45.06	45.20	0.14	0.02
5A	2.5	1.45	...	1.60	45.09	45.20	0.11	0.00
6A	6.0	1.00	...	1.15	45.09	45.20	0.11	0.02
7A	15.0	0.49	...	0.69	45.10	45.20	0.10	0.00
1B	0.00	3.62	6.05	6.05	43.60	43.95	0.35	...
2B	0.05	3.15	3.41	3.37	43.80	44.05	0.25	0.00
3B	0.10	2.91	3.11	3.08	43.83	44.05	0.22	0.02
4B	6.0	1.00	1.27	1.20	43.90	44.03	0.13	0.00
5B	15.0	0.50	0.77	0.70	43.95	44.05	0.10	0.05
6B	4.15 (18 F)	0.0	...	0.37	43.90	44.03	0.13	...
7B	5.0 (18 F)	...	...	...	43.95	44.10	0.15	...
8B	25.0 (18 F)	...	...	...	44.10	Decomp.	...	...
9B	30.0 (18 F)	...	...	...	44.60	Decomp.	...	...

Permissible pH Range. The data collected in Table VII, selected from over 90 titrations, show the effect of making the titration at various pH values. Series A and B represent experiments with two different sets of solutions. Duplicate, in many cases triplicate or more, titrations were made in all cases except for Experiments 6B to 9B, inclusive. The maximum deviations in milliliters of each group of such titrations are shown in the last column of the table. Although the concentrations of the copper sulfate solutions used in these experiments were not established exactly it is believed, as the results of the experiments cited below, that the volumes obtained at the thiocyanate end point when the initial pH values lie between 3.15 and 0.5 are correct within 1 part per 1000. The pronounced drop in hydrogen ion concentration in the absence of added acid in Experiments 1A and 1B is probably due to two causes: The thiosulfate solution was stabilized by the addition of 0.1 gram of sodium carbonate per liter as recommended by Kilpatrick and Kilpatrick (*10*), and the removal of the acidity contributed by the hydrolysis of the cupric ion. This latter effect was confirmed by experiments in which it was found that a pure cupric sulfate solution, approximately 0.1 F, had a pH of 4.06; however, after addition of potassium iodide and titration with a thiosulfate solution containing no added carbonate the pH at the iodide end point was 5.35.

The experiments indicate that for practical use the maximum initial pH limit with the sulfate system is not greater than approximately 3.0, for above that value so little acid is present that inadequate buffering action is obtained. The results with the experiments at low pH values are surprising in that they indicate that so long as a significant amount of sulfate is present no serious deviations are observed. For example, in experiment 6B, where 4.15 ml. of 18 F sulfuric acid were added, the calculated ratio of hydrogen sulfate to sulfate is 10 to 1, yet the volume of thiosulfate is the same as at the higher pH values; with 5 ml. of 18 F sulfuric acid, experiment 7B, which should represent a solution of hydrogen sulfate with no excess sulfate, the thiosulfate used for the thiocyanate end point indicates an upward trend. With larger excess of 18 F sulfuric acid decomposition of thiocyanate occurred. The abnormally high hydrogen ion activity of these solutions is attributed to the high ionic strength of the solutions and is in agreement with the trend observed with sulfate–hydrogen sulfate buffers by Jeffreys and Swift (*9*).

Oxygen Error. In the general procedure no effort was made to use air- or oxygen-free solutions, yet in the time required for a normal titration, 2 to 5 minutes, no appreciable oxygen error is apparent until after as much as 5 ml. of 18 F sulfuric acid have been added. On stoppering the flasks containing the titrated solutions and allowing them to stand, a marked difference was observed in the time required for a return of the starch-iodine color. With no added acid and an initial pH of 3.6, no return of color was observed even after 24 hours; with initial pH values of 1 to 2 the thiocyanate end point was stable for only 5 to 10 minutes. In all cases where the pH value was below 3.6, the time required for the return of the starch-iodine color varied directly with the pH value. The difference between the iodide end point and the thiocyanate end point decreases as the pH is decreased to about 1.5 to 1.0; therefore if one is not to use thiocyanate more accurate values are obtained nearer the lower pH limit. In experiments with procedures in which the initial pH varied from 2.2 to 2.3 the solutions were saturated with carbon dioxide, and the containers swept out with carbon dioxide and then titrated, the titration values were the same within the experimental limits as those with air-saturated solutions.

Confirmatory Analyses of Pure Copper. The data in Table VIII were obtained by the analysis of weighed samples of Bureau of Standards copper, sample 45A. Although this is a melting point standard, it was assumed to be pure copper because the melting point (1083° C.) agrees with that given in the International Critical Tables for pure copper, and because six electrolytic determinations gave 100.06% copper with an average deviation from the mean of 0.02%. The samples were dissolved in a minimum amount of 6 F nitric acid, 5.5 ml. of 18 F sulfuric acid were added, the mixture was fumed, diluted to 30 ml., and boiled, and 15 F ammonium hydroxide was added until a barely perceptible blue color was obtained. Then 1 ml. of 3 F sulfuric acid was added, and the solution was diluted to 50 ml. The pH of such solutions was approximately 2.2. The solution was titrated as outlined in the general procedure. The results obtained indicate that under the conditions of this procedure the volume of thiosulfate found at the thiocyanate end point can be used without a correction factor and confirm the conclusion of Foote (*5*) that pure copper can be used as a satisfactory primary standard for thiosulfate solutions. The iodide end point gave results which, while having an average deviation from their mean of only 0.05%, averaged 0.34% low. If the iodide end point is to be used for the standardization of thiosulfate solutions by this procedure, a correction factor should be used. These conclusions are in agreement with those reached by Crowell (*3*).

EFFECT OF CERTAIN CONSTITUENTS

Chloride Ion. There has been some uncertainty as to the effect of chloride ion on this titration. Moser (*15*) claimed it to be undesirable, whereas Kolthoff (*11*) found that a small concentration of hydrochloric acid was not troublesome; he attributed the effect of high concentration to the formation of cupric chloride complexes. The experiments in Table IX indicate that if as much as 5 grams of sodium chloride is present under the conditions of these procedures, the only significant effect is a slight tendency toward larger differences between the iodide and thiocyanate end points.

Effect of Certain Metallic Elements. The effect of certain metallic elements (iron, arsenic, and antimony) is considered, because such elements may be found in small amounts in copper alloys and when in their higher oxidation states may oxidize iodide to iodine. In order to ascertain their effect in sulfate-hydrosulfate buffered solutions the experiments recorded in Table X were made. The initial pH value was approximately 2.4, obtained by the use of 90 millimoles of ammonium sulfate

Table VIII. Analyses of Pure Copper Using Sulfate Buffers

Expt.	Thiosulfate Used, Ml. Iodide end point	Thiosulfate Used, Ml. Thiocyanate end point	Copper, Gram Taken	Copper, Gram Found Iodide end point	Copper, Gram Found Thiocyanate end point	Deviation, % Iodide end point values	Deviation, % Thiocyanate end point values
1	47.40	47.51	0.3115	0.3108	0.3117	−0.23	0.00
2	47.55	47.70	0.3128	0.3118	0.3127	−0.32	−0.03
3	47.30	47.45	0.3114	0.3101	0.3111	−0.42	−0.10
4	...	47.60	0.3120	...	0.3121	...	0.03
5	...	47.30	0.3111	...	0.3110	...	−0.03
6	45.37	45.52	0.2902	0.2891	0.2900	−0.38	−0.07
7	45.38	45.52	0.2901	0.2892	0.2900	−0.31	−0.03
8	45.45	45.60	0.2906	0.2896	0.2906	−0.34	−0.00
9	45.55	45.70	0.2913	0.2902	0.2913	−0.38	0.00
					Av.	−0.34	−0.02

Normality of thiosulfate, Expts. 1 to 5, 0.10314; Expts. 6 to 9, 0.10024.

Table IX. Effect of Chloride Ion

(End-point volumes are average of three titrations; maximum deviation was 0.03 ml.)

Expt.	NaCl Added *Grams*	Thiosulfate Used, Iodide end point *Ml.*	Thiosulfate Used, Thiocyanate end point *Ml.*	Difference *Ml.*
I	0	44.54	44.69	0.15
II	1.0	44.53	44.69	0.16
III	2.0	44.51	44.70	0.19
IV	5.0	44.52	44.70	0.18

and 1 millimole of sulfuric acid. Approximately 5 millimoles of copper were taken. In the absence of phosphoric acid there is reduction of ferric iron which causes a positive error. In fact, there is some evidence that the reduction of the iron is accelerated by the presence of the copper and this effect is being investigated further. The addition of 1 ml. of 18 *F* phosphoric acid (instead of the sulfuric acid) minimized the reduction of small amounts of iron, but the phosphate complex was not sufficiently stable to prevent partial reduction with larger amounts; the use of an acid fluoride as both a buffering and complex-forming agent as proposed by Crowell (*3*) is recommended with these quantities. Antimony and arsenic caused no significant effect in the amounts used; these amounts are larger than those usually present in copper alloys.

Effect of Nitrates. In the early phases of this study it was observed that the presence of small amounts of nitrates did not cause an error in the titrations, and that even 2 ml. of freshly boiled 16 *F* nitric acid could be added to the solution just before the titration without noticeable effect. Verification of this observation seemed worth while, as nitric acid is widely used for dissolving copper ores and alloys and it would be advantageous to eliminate the sulfuric acid fuming.

Experiments were made by treating weighed samples of pure copper, Bureau of Standards sample 45 A, with just enough nitric acid to cause complete solution and then boiling until the volume was about 2 ml. This solution was diluted to about 30 ml., 10 grams of ammonium sulfate were added, and the solution was boiled, cooled to room temperature, and diluted to 50 ml. The solution was then titrated by the general procedure. The results shown in Experiments 1 to 3 in Table XI indicate that the presence of nitric acid does not interfere with the determination of pure copper.

Experiments 4 through 12, in Table XI, present data obtained with alloys that contain about 10% tin, 10% lead, 0.5% iron, and traces of arsenic and antimony. A heavy white precipitate formed when the alloy sample was heated with the nitric acid; therefore 5 ml. of 12 *F* hydrochloric acid were added in Experiments 7, 8, and 9, and the volume was reduced to about 3 ml. In Experiments 10, 11, and 12 two 5-ml. portions of the hydrochloric acid were added. The results indicate that the presence of the hydrochloric acid had little effect on the value obtained by the thiocyanate end point.

Confirmatory Analyses of Alloys. Finally, confirmatory analyses were made (by the method used for the confirmatory analyses of pure copper) of Bureau of Standards samples 63 and 37 D. The average values given for sample 63 are 78.05% copper, 9.74% lead, 9.91% tin, 0.27% iron, 0.55% antimony, and 0.19% arsenic; for sample 37 D 70.78% copper, 0.96% tin, 0.94% lead, and 0.075% iron in addition to zinc and nickel. Four analyses of sample 63 gave values for the copper ranging from 77.99 to 77.93%, the average being 77.96%. It is possible that compensating errors may occur when tin and iron are both present. The average value resulting from the iodide end points was 3.3 parts per 1000 lower. Five analyses of sample 37 D gave values for the copper ranging from 70.73 to 70.80%, the average being 70.77%. Values from the iodide end point averaged 2.7 parts per 1000 lower.

Table X. Effect of Certain Metallic Elements

Expt. No.	Element	Element Added, Mg.	Thiosulfate, Iodide end point	Thiosulfate, Thiocyanate end point	Thiocyanate End Point, After 5 min.	Thiocyanate End Point, After 10 min.
Ia		0.0	43.66	43.80	43.80	43.81
b	Fe^{III}	0.6	43.75	43.89	43.89	43.89
c	Fe^{III}	6	44.59	44.76	44.76	44.76
d	Fe^{III}	60	53.40	53.55	53.60	53.60
IIa		0.0	48.85	49.00	49.05	49.05
b	Fe^{III} also H_3PO_4	0.6	48.85	49.00	49.07	49.07
c	H_3PO_4	6	48.90	49.10	49.17	49.17
d	(18 millimoles)	60	58.40	59.25	59.50	59.50
IIIa		0.00	44.00	44.12		
b	Sb^V	0.6	43.98	44.12		
c	Sb^V	6	43.98	44.11		
d	Sb^V	60	44.00	44.10		
IVa	As^V	0.6	43.98	44.11		
b	As^V	6	43.98	44.12		
c		60	44.00	44.12		

Table XI. Effect of Nitric and Hydrochloric Acid on Determination of Copper

(Samples 1 through 6 treated with nitric acid only; 7 through 9 treated with nitric and 5 ml. of concentrated hydrochloric acid; last three treated with two 5-ml. portions of concentrated hydrochloric acid)

Sample	Thiosulfate, Ml. Iodide end point	Thiosulfate, Ml. Thiocyanate end point	Difference	pH	Copper, Gram Taken	Copper, Gram Found (by thiocyanate end point)	% Error
Pure copper							
1	44.18	44.40	0.22	1.72	0.2804	0.2803	−0.05
2	44.40	44.61	0.21	1.50	0.2818	0.2816	−0.05
3	43.94	44.16	0.22	1.65	0.2789	0.2788	−0.03
Copper alloy							
4	49.10	49.36	0.25	1.51	0.3127	0.3117	−0.32
5	49.20	49.40	0.20	1.72	0.3123	0.3119	−0.13
6	49.06	49.31	0.25	1.83	0.3120	0.3112	−0.26
7	49.15	49.40	0.15	1.80	0.3126	0.3119	−0.23
8	49.05	49.33	0.28	1.87	0.3127	0.3118	−0.30
9	49.20	49.35	0.15	1.85	0.3128	0.3116	−0.39
10	49.15	49.34	0.19	1.50	0.3120	0.3115	−0.16
11	49.10	49.28	0.18	1.52	0.3119	0.3111	−0.26
12	49.10	49.32	0.22	1.55	0.3121	0.3114	−0.26

LITERATURE CITED

(1) Bray and Miller, *J. Am. Chem. Soc.*, 46, 2204 (1924).
(2) Caldwell, *Ibid.*, 57, 96 (1935).
(3) Crowell, IND. ENG. CHEM., ANAL. ED., 11, 159 (1939).
(4) Crowell *et al.*, *Ibid.*, 8, 9 (1936).
(5) Foote, *J. Am. Chem. Soc.*, 60, 1349 (1938).
(6) Foote and Vance, IND. ENG. CHEM., ANAL. ED., 8, 119 (1936).
(7) Foote and Vance, *J. Am. Chem. Soc.*, 57, 845 (1935).
(8) Groth, "Chemische Krystallographie," Vol. I, p. 199, Leipzig, W. Engelmann, 1918.
(9) Jeffreys and Swift, *J. Am. Chem. Soc.*, 54, 3220 (1932).
(10) Kilpatrick and Kilpatrick, *Ibid.*, 45, 2132 (1923).
(11) Kolthoff, "Volumetric Analysis," Vol. II, p. 429, New York, John Wiley & Son, 1929.
(12) Krüger, Bussem, and Tschirch, *Ber.*, 69B, 1601 (1936).
(13) Krüger and Tschirch, *Z. anal. Chem.*, 97, 161 (1934).
(14) Latimer and Hildebrand, "Reference Book of Inorganic Chemistry," p. 526, New York, Macmillan Co., 1940.
(15) Moser, *Z. anal. Chem.*, 45, 597 (1907).
(16) Park, IND. ENG. CHEM., ANAL. ED., 3, 77 (1931).
(17) Rieman, Neuss, and Neiman, "Quantitative Analysis," 2nd ed., p. 226, New York, McGraw-Hill Book Co., 1942.
(18) Speter, Max, *Z. Medizin. Chemie*, 3, 14; *Chem. Zentr.*, 1925, I, 2069.
(19) Swift, "System of Chemical Analysis," p. 80, New York, Prentice-Hall, 1940.
(20) Walker and Dover, *J. Chem. Soc. (London)*, 87, 1584 (1905).

RECEIVED November 22, 1948. Contribution 1262 from Gates and Crellin Laboratories of Chemistry, California Institute of Technology.

Indirect Colorimetric Determination of Gaseous Fluorine

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A method suitable for semicontinuous automatic determination of fluorine in mixtures with nitrogen and/or air is described. A stoichiometric exchange of fluorine for bromine is secured by passing the gas mixture over heated sodium bromide, and the bromine in the effluent stream is determined colorimetrically. The most satisfactory operating conditions are indicated.

IN VIEW of the increasing industrial utilization of gaseous fluorine, a method for the continuous determination of this component in mixtures with air and/or nitrogen may be of value in monitoring stack gases, and in other operations. Such a method should, if possible, avoid the use of reagents in solution because of the disastrous effects which may ensue if such solutions are sucked back into the fluorine conduit by a sudden pressure drop.

In connection with the work of this laboratory an instrumental method that is automatic and semicontinuous, and involves the use of only solid reagent(s) has been developed. The concentration range of primary interest was 0 to 20% fluorine in nitrogen or air, and particular importance was attached to the 0 to 2% range. The accuracy desired was ±0.5% fluorine or better.

The principle on which this instrument is based, suggested by J. L. Culbertson, is the stoichiometric exchange of gaseous fluorine for gaseous bromine, by reaction of the fluorine with a solid bromide, followed by a colorimetric determination of the displaced bromine in a flowing system.

A plot of the extinction coefficient of gaseous bromine against wavelength shows a wide peak in the region 4000 to 4300 Å. This peak falls off rapidly toward the ultraviolet and rather more slowly in the blue-green region. It is plain that a colorimetric determination of optimum sensitivity should be made with light of wave length corresponding to the absorption maximum; and, fortunately, absorption measurements in this region may be conducted satisfactorily with a photoelectric colorimeter. For visual work it would probably be more advantageous to operate in the wave-length region around 5100 Å., where the extinction coefficient is still large enough to give promise of adequate accuracy, while the sensitivity of the eye becomes sufficiently great to allow reasonably precise observation. However, despite its greater cost, a photoelectric colorimeter is, in the present case, far more satisfactory than any direct visual observation.

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EXPERIMENTAL METHODS AND RESULTS

A Fisher photoelectric colorimeter, provided with filters transmitting bands in the 4250 and 5250 Å. regions, was used throughout this work. The scale covered the range from 0 to 90% extinction, and the cell holder accommodated cylindrical cells of 25-mm. outside diameter.

A preliminary experiment was made to provide an empirical calibration curve, and to establish whether a modified Beer-Lambert law could be used to correlate the observed extinctions with the partial pressure of bromine vapor in the absorption cell. A light path of approximately 25 mm. was considered favorable for the range 0 to 10% bromine (fluorine) in a gas at atmospheric pressure if 4250 Å. radiation is used; and the same cells will serve in the 10 to 20% range if 5250 Å. radiation is then employed.

A number of glass cells 25 mm. in outside diameter were made from one length of Pyrex tubing. Although these cells were probably not accurately matched, they proved adequate for the present purposes. The cells were attached through heavy-walled constrictions to a glass manifold, to which was also affixed a glass thimble containing about 2 ml. of C.P. bromine. The bromine was frozen with liquid nitrogen, after which the line was thoroughly evacuated and flamed. The connection to the pump was then fused shut, and one of the cell capsules was pulled off as a blank. The liquid nitrogen was then removed and the bromine reservoir was surrounded by a Dewar flask containing ether cooled to −54° C. by the cautious addition of finely powdered dry ice. This bath was allowed to warm to −53.3° C. over a period of about 20 minutes, and one of the cells was then pulled off. All temperatures were read with a pentane thermometer, and it is believed that accurate temperature control was secured. At −53.3° C. bromine has a vapor pressure of 0.76 mm. of mercury (I.C.T. data)—that is, a pressure corresponding to 0.1% bromine (fluorine) in a gas at atmospheric pressure.

The temperature of the bath was raised to approximately −47.5° C. by the addition of uncooled ether, and was then allowed to rise very slowly to −46.7° C., at which point another cell was sealed off. At this temperature bromine has a vapor pressure of 1.52 mm. of mercury—i.e., a pressure corresponding to 0.2% bromine (fluorine) in a gas at atmospheric pressure. By repeating these operations at various predetermined temperatures a series of sealed cells containing bromine at pressures

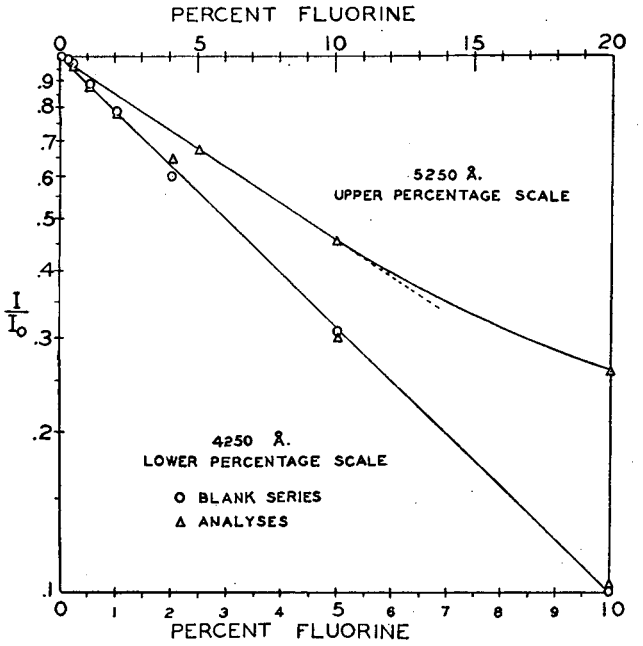

Figure 1. Determination of Fluorine

corresponding to 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0% of atmospheric pressure was secured. The various cells were examined with the colorimeter, using the 4250 Å. filter, and the data obtained are shown (in circles) in Figure 1.

It is apparent that a plot of log I/I_0 versus "per cent" bromine is satisfactorily linear for bromine pressures varying from 0 to 10% of atmospheric pressure. Rough readings were also made with the 5250 Å. filter and, as expected, a plot of much reduced slope was obtained. Because of the reduced sensitivity in this wave-length region, the departure of the individual points from the line was somewhat greater. It appears then that the Beer-Lambert law may be safely applied to this system, replacing the concentration term by the partial pressure (or per cent) of bromine. In the present case the cells contained nothing but bromine vapor, but the absence of other gases—e.g., nitrogen, oxygen, or possible traces of silicon tetrafluoride, hydrogen fluoride, etc.—cannot affect the colorimetric values, for these gases all have low extinctions in the wave-length ranges used in these measurements.

The exchange of fluorine for bromine was now examined, to establish that the displacement reaction is both rapid and stoichiometric at all fluorine concentrations within the range of interest.

To this end a series of determinate fluorine-nitrogen mixtures was prepared. A partially exhausted tank of fluorine was available. The fluorine pressure in the tank was accurately determined with a large Bourdon gage attached to it. This mixing tank was then connected, through a long copper coil immersed in a dry ice bath, with a high pressure cylinder containing water-pump nitrogen. The nitrogen was admitted to the mixing tank until the pressure therein, as read on the attached Bourdon gage, was just five times its original value. The valve on the mixing tank was then closed, the tank was detached, and its contents were mixed thoroughly by running hot water down one side of the tank and cold water down the other. A homogeneous 20% mixture of fluorine in nitrogen was thus obtained. After completion of the analytical trial with this sample, the mixture was further diluted by bleeding down the pressure in the mixing tank to a suitable value, and then refilling with dried nitrogen to the desired pressure—i.e., dilution. The mixture was stirred convectively after each dilution. In this way mixtures containing 20, 10, 5, 2, 1, 0.5, and 0.2% fluorine in nitrogen were successively prepared and separately analyzed.

As a check on the adequacy of this dilution system a known volume of the 1% mixture was bubbled through a series of three gas-scrubbing bottles containing an aqueous solution of potassium iodide acidified with acetic acid. The volume used was metered with a rotameter attached to the line after the last scrubber. The iodine content of the combined solution from the three bottles was determined by titration, the fluorine concentration was calculated from the iodine found, and the gas volume used was 1.1%. The agreement of this value with the anticipated figure of 1.0% is satisfactory, particularly because the measurement of the gas volume used, made with a new but uncalibrated rotameter, may well be somewhat in error. Furthermore, the potassium iodide method is itself subject to small errors. It was concluded that the mixtures that had been prepared were sufficiently determinate for the purpose in hand.

Each mixture was analyzed with a train arranged so that a stream of the sample gas delivered under positive pressure from the mixing tank passed successively through (1) an "exchanger" consisting of a tube packed with coarse crystals of Baker's C.P. anhydrous sodium bromide and heated to 150° C. by a small sleeve furnace; (2) a cylindrical glass cell 25 mm. in outside diameter, which could be sealed off for later (colorimetric) examination; (3) a trap immersed in liquid oxygen, intended to remove the bulk of the bromine from the gas stream; and (4) a rotameter which delivered the effluent gas directly to the atmosphere. The sodium bromide used in the exchanger was freed from superficial moisture by heating at 150° C. for several hours in high vacuum. The charge was not re-exposed to the atmosphere until after completion of the trials.

The determinate fluorine mixtures were examined with this train, and in Figure 1 the results of the colorimetric determinations of the evolved bromine are plotted (in triangles) against the known fluorine concentrations. A good degree of correlation obtains—that is, within experimental error, the points fall closely on the calibration line drawn through the readings obtained in the blank trials with pure bromine. Thus the fluorine concentrations deduced from the colorimeter readings and the calibration curve are in good agreement with those predicted on the basis of the determinate dilutions in the mixing tank. The 20% point falls somewhat off the extrapolated 5250 Å. calibration curve, but it was not determined whether this discrepancy was due to a breakdown of the Beer-Lambert law at higher concentrations of bromine, or to some experimental aberration.

DISCUSSION

Completeness of Reaction. A test for traces of fluorine that might have escaped reaction in the exchanger was made by uncoupling the analytical train after the liquid oxygen trap and examining the effluent gas for fluorine. Starch-iodide paper gave a weak test, indicating a trace of oxidant, but this was probably due to the low but appreciable vapor pressure of bromine at liquid oxygen temperatures. A more specific test for fluorine, with zirconium-alizarin paper (courtesy of R. Kunin) was entirely negative. It appears then that the displacement reaction proceeds substantially to completion.

Temperature of Exchanger. This is a factor of prime importance. When a stream of test gas containing more than 5% fluorine is passed into the exchanger the inlet end of the sodium bromide charge is strongly heated. However, this heating does not appear to be sufficient to ensure completion of the reaction. When the whole exchanger was heated gradually in a sleeve furnace it was found that the maximum (stoichiometric) concentration of bromine was not developed until the temperature reached 100° to 125° C. Therefore, to leave a margin of safety, the tube was regularly heated to 150° C. during the analyses. Temperatures greatly in excess of this value are undesirable not only because some sintering of the charge may occur, but also because of the increased danger of a side reaction between the strongly heated sodium bromide and any oxygen that might be present in the gas stream.

Reaction of Oxygen. At 150° C. oxygen should not react appreciably with sodium bromide, but, to check this point in the present system, about 6 liters of tank oxygen were passed through the exchanger while the latter was heated to 175° C. Only a minute amount of bromine was found in the liquid oxygen trap,

and this material corresponded to much less than 0.1% "fluorine" in the gas stream. Therefore, under the prescribed conditions, the presence of oxygen in the gas mixture causes no difficulty.

Effect of Hydrogen Fluoride. This component may have a deleterious effect on the validity of the results if it is allowed to pass through the exchanger, because etching of the colorimeter cell may then occur. Therefore a small amount of sodium fluoride is packed into the exit end of the exchanger tube, to absorb any hydrogen fluoride that may be present during the earlier stages of operation. After the exchanger has been operated for some time, more sodium fluoride is formed internally, and additional quantities of hydrogen fluoride can be completely absorbed.

Operating Lifetime. Although the rest of the analytical train is capable of indefinite operations, the chemical capacity of the packing in the exchanger is limited. When crystalline sodium bromide was used the operating lifetime of the exchanger was found to be much shorter than that corresponding to complete exhaustion of the charge. It appears that the surface coating of sodium fluoride prevents adequate contact of the gas stream with the underlying bromide. That a surface effect is involved was demonstrated by the complete recovery of activity observed when a sample of sodium bromide inactivated by prolonged exposure to dilute fluorine was finely ground, dried, and reintroduced into the exchanger. The operating lifetime with crystalline sodium bromide varied from 6 to 10 hours, according to the flow rates and fluorine concentrations that prevailed. The useful lifetime of the charge may be prolonged by any method that increases its active surface. Thus the sodium bromide may be ground to a fine powder before it is packed into the exchanger; or, even better, a highly porous charge of enormous active surface may be prepared by the cautious vacuum dehydration of the salt, sodium bromide dihydrate.

It is conceivable that this inactivation on fluorine exposure might be less marked in some other bromide that could be used as a packing for the exchanger. However, as acceptable results were obtained with sodium bromide, no other salt was investigated.

Rate Factors. When a sample was passed into the analytical train, the equilibrium concentration of bromine was established rapidly, and no perceptible variation in the (colorimetrically) measured concentrations could be detected in trials continued up to 3 hours.

With an active charge in the exchanger there was also no measurable variation in the bromine concentration in the cell when the flow rate in the train was varied between 20 and 100 ml. per minute. (The volume of the exchanger was about 75 ml.) Thus under optimum conditions the results are independent of the flow rate over a rather wide range of rates. With a charge that is on the verge of exhaustion, the maximum concentration of bromine may be obtained at very low flow rates (about 5 ml. per minute), but at higher rates spurious (low) results are obtained. Periodic replacement of the exchanger avoids this difficulty.

Operating Range. The proposed method yields results accurate to at least 0.5% fluorine in the 0 to 10% range of fluorine concentrations. The Beer-Lambert law applies throughout this range. For the 10 to 20% concentration region an empirical calibration curve is required. A further complication is encountered at fluorine concentrations in excess of 10% in that the light absorption in the 25-mm. cell is then so strong that the extinction of 4250 Å. radiation is more than 90% complete and no readings can be secured with the standard photoelectric colorimeter. If the entire 0 to 20% range of fluorine concentrations must be covered, one of two measures may be adopted:

The 10 to 20% range may be handled satisfactorily with the 25-mm. cell if the readings in this region are made while using a 5250 Å. filter, as the extinction coefficient of bromine vapor at 5250 Å. is notably smaller than that at 4250 Å. This was the expedient adopted in the present investigation.

The entire 0 to 20% range may be satisfactorily covered using a colorimeter set for 4250 Å. radiation if a duplex cell of the type shown in Figure 2 is employed. At the higher concentrations adequate transmission may be secured through the zone of smaller diameter; the section of larger diameter may be employed to obtain measurements of optimum sensitivity in dealing with mixtures containing relatively lower fluorine concentrations.

Instrumental Design. With the data collected in this investigation a design for an instrument for routine fluorine analysis was evolved. This design, shown in Figure 2, differs from that used in the developmental work only in so far as is necessary to make the analytical operations automatic and foolproof. An apparatus based on this design has been constructed and successfully operated in another laboratory.

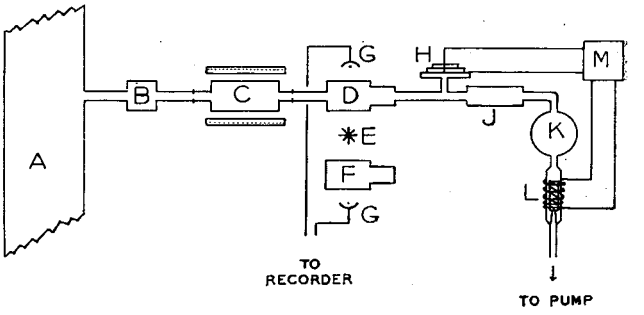

Figure 2. Automatic Fluorine Analyzer

The automatic shut-off Kerotest valve, *B*, is essential to prevent back-diffusion from the analytical system into the conduit, *A*, in case of a catastrophe to the pump, etc. The rate of flow of the gas mixture may be controlled from this valve; and, to secure a maximum operating lifetime for the exchanger, *C*, the flow rate should be the minimum required to replace the gas in cell *D* completely within the time interval chosen as appropriate for successive readings of the concentration. *C* is enclosed within a 150° C. sleeve furnace, and is so attached to the line that it may be easily replaced with a duplicate exchanger whenever the charge is exhausted. An absorbent packed in tube *J* serves to remove the bromine from the gas stream before the latter passes into the pump.

The sealed comparison cell, *F*, is similar to cell *D*, and stands equally distant from the light source, *E*. The outputs from the two photocells, *G*, used in the colorimeter system may be fed through a standard electronic circuit which makes it possible to record on a Micromax the ratio of the two outputs—i.e., I/I_0—whence, with the aid of a calibration curve, the fluorine concentration is readily deduced.

Accurate control of the pressure in the analytical system, and, in particular in the colorimeter cell, is essential. Such control is readily attained by using a Booth-Cromer (diaphragm-contact) gage, *H*, to activate a pressure stabilizer, *L*. The pressure in the external chamber of the gage is preset at the desired value (necessarily somewhat below the minimum pressure expected to prevail in the conduit). Then, as soon as the pressure in the analytical train rises slightly above this value, electrical contact in the gage is established and the Thyratron relay, *M*, is activated. The output from the relay is fed into an electromagnet which lifts an iron-cored plunger in *L* away from the outlet to the vacuum pump. Discharge of gas through this outlet continues until the pressure in the system is slightly below that prevailing in the outer chamber of the Booth-Cromer gage, whereupon the contact in the gage is broken, the Thyratron circuit is deactivated, and the plunger drops into place on the pump outlet. The pressure fluctuations may be kept within narrow bounds by attaching a ballast flask, *K*, to the line and by suitably throttling the lead to the pump.

A pressure regulator of this type has been built and operated successfully.

RECEIVED August 23, 1948

Routine Determination of Nickel in Cobalt-Base Alloys

Ferricyanide Oxidation of Cobalt

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In ammoniacal solution, cobaltous salts are oxidized to the trivalent stage by ferricyanide and nickel is subsequently precipitated by dimethylglyoxime in acetate buffered solution. One part of nickel may be detected in the presence of 200 parts of cobalt. The usual components of high temperature alloys—tungsten molybdenum, chromium, iron, manganese, columbium, and titanium—do not interfere and no special separations are needed.

THE principle of the ferricyanide method (*2, 8*) for the determination of cobalt may be applied to the gravimetric determination of nickel in the presence of very large amounts of cobalt. In ammoniacal citrate solution, one atom of trivalent cobalt forms a Wernerlike complex with two molecules of dimethylglyoxime (*1*); and this complex forms no precipitate with ferric iron. Thus, the proposed procedure does not require the preliminary removal of iron. Chromium (300 mg.), columbium (20 mg.), and tungsten or molybdenum (60 mg.) do not interfere. Tantalum, whose reactions are similar to those of columbium, should not interfere. Materials insoluble in aqua regia (silica, columbium carbide, etc.) are removed, after perchloric acid dehydration, by filtration.

The precautions observed in the determination of nickel in steel, such as regulation of acidity, volume of alcohol, amount of reagent dimethylglyoxime, time of standing, and temperature of filtration are followed.

The minimum amount of dimethylglyoxime reagent required is 10 ml. of a 1% solution for each 10 mg. of nickel expected to be present, plus 10 ml. for each 6 mg. of cobalt expected, plus an excess of 10% of reagent to obtain complete precipitation. However, it is simpler to add 1 gram of solid dimethylglyoxime for each 200 mg. of cobalt expected, and later reprecipitate the nickel under more closely controlled conditions. An acetate solution with a pH of 8 is preferred. The time of standing after precipitation is 1 hour for nickel in amounts of 1 to 4%, 2 hours for nickel from 0.5 to 1%, and 4 or more hours for less than 0.5%. The volume of alcohol should be less than 25% (*4*) and the nickel precipitate should be filtered at room temperature. One important precaution must be taken. In hypochlorite solution (*7*), nickel is quickly oxidized to a higher valence, in which form the glyoxime is soluble; hence an acid mixture of hydrochloric acid and nitric acids, or a perchloric acid solution, must be diluted and boiled until free of chlorine.

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RESULTS

In Table I are listed results obtained by this method as compared to those obtained by other acceptable methods.

Sample 1 is Bureau of Standards No. 153 in which the cobalt content is more than 50 times that of the nickel and the iron content is more than 500 times that of nickel. Molybdenum, vanadium, and tungsten are also present. One-, 2-, and 5-gram samples were analyzed by the proposed method; the 5-gram samples yielded the more accurate results. Samples 2 to 7 are Westinghouse alloys with approximate analyses as listed. Samples 2 and 3 were checked by the cyanide procedure of Feigl and Kapulitzas (*3, 6*). Samples 4, 5, 6, and 7 show check results with the method of Kirtchik (*5*). Sample 6 is the same as Sample 5 with columbium added in the form of ferrocolumbium and titanium added as ferrotitanium. Spectrographic analysis showed only traces of columbium and titanium in the final nickel precipitate.

Table II shows check results by different analysts of some high-cobalt alloys.

SOLUTIONS

Cobalt Buffer Solution. Dissolve 500 grams of citric acid in 675 ml. of ammonium hydroxide and 1000 ml. of water.

Alkaline Dimethylglyoxime Solution. Dissolve 5 grams of potassium hydroxide in 100 ml. of water, add 10 grams of dimethylglyoxime, and stir until dissolved. Dilute to 250 ml. with water.

Alcoholic Dimethylglyoxime Solution. Dissolve 10 grams of dimethylglyoxime in 1 liter of alcohol. Filter before using.

Tartaric Acid Solution, 50%. Dissolve 500 grams of tartaric acid in 1 liter of water. Filter before using.

Potassium Ferricyanide Solution, 10%. Dissolve 10 grams of potassium ferricyanide in 100 ml. of water. One milliliter is approximately equivalent to 0.02 gram of cobalt or 0.02 gram of manganese. Discard after 30 days.

Table I. Nickel Determination in Cobalt Alloys

Sample No.	Sample Size	Co	Cr	W	Mo	V	Cb	Ti	Ni Proposed Method	Ni Other Methods
	Grams	%	%	%	%	%	%	%	%	%
1 (B.S. 153)	1	8.43	4.14	1.58	8.36	2.03	...	...	0.10	0.107[a] 0.11[b]
	2	...	...	...	...	...	...	...	0.10	...
	5	(420 mg.)	...	(70 mg.)	(410 mg.)	...	...	...	0.11	...
2	1	60	20	6	...	...	...	...	0.54	0.52[c]
3	1	60	20	6	...	...	...	...	0.59	0.59[c]
4	1	60	26	6	0.2	...	...	...	1.98	2.00[b]
5	1	60	26	6	0.2	...	...	...	2.01	2.03[b]
6[d]	1	60	26	6	0.2	...	2	2	2.02	2.02[b]
7	1	60	26	6	0.1	...	...	...	1.97	1.96[b]

[a] Value listed on Bureau of Standards certificate.
[b] Cyanide-peroxide procedure of Kirtchik (*5*).
[c] Cyanide method of Feigl and Kapulitzas (*6*).
[d] Same as sample 5 with columbium and titanium added.

PROCEDURE

The sample should not contain more than 50 mg. of nickel. Weigh a 1.000-gram sample and transfer to a 400-ml. beaker. Digest the alloy with 30 ml. of hydrochloric acid until most of the sample has been dissolved. Add 10 ml. of nitric acid and 15 ml. of perchloric acid (70%); then evaporate to fumes of perchloric acid. Boil 3 to 5 minutes longer, cool, add 100 ml. of water, and boil for 5 minutes to remove free chlorine. Pour this solution into

Table II. Reproducibility of Nickel Results

Sample No.	Sample Size	Nickel	
		Analyst A	Analyst B
	Grams	%	%
1	5	0.11	0.11
2	1	1.85	1.86
3	1	2.47	2.48
4	1	2.62	2.60
5	1	2.73	2.72
6	1	1.97	1.98
7	1	0.12	0.13

a 600-ml. beaker containing 100 ml. of cobalt buffer solution and 60 ml. of ammonium hydroxide. Wash the 400-ml. beaker with dilute ammonium hydroxide to remove any tungstic oxide, and stir until a clear solution is obtained. Add sufficient 10% potassium ferricyanide solution to oxidize the cobalt and manganese plus a 10% excess (6 ml. for each 0.1 gram of cobalt or manganese present), and mix well. The red color of the cobaltic ion appears. Add 50 ml. of alcohol and 100 ml. of alkaline dimethylglyoxime solution, and stir well. Allow to stand for 10 minutes. Carefully adjust the solution with glacial acetic acid to pH 8, using a pH meter. Allow to stand for the prescribed time and if necessary, readjust the acidity with acetic acid or ammonium hydroxide. A white precipitate of excess dimethylglyoxime along with the nickel glyoxime forms. Filter through a 12.5-cm. No. 40 Whatman paper, and wash 6 times with warm (50° C.) water. Discard the filtrate.

Return the paper to the 600-ml. beaker and add 25 ml. of nitric acid and then 10 ml. of perchloric acid (70%). Break the paper with a stirring rod, heat gently until the paper decomposes, and then evaporate to fumes of perchloric acid. Boil 3 to 5 minutes longer, cool, add 100 ml. of water, and boil for 5 minutes to volatilize free chlorine. Filter through an 11-cm. No. 40 Whatman paper into a 400-ml. beaker and wash 6 times with hot water. Discard the paper (silica). Add 10 ml. of a 50% tartaric acid solution to the filtrate. Neutralize to litmus paper with ammonium hydroxide and add 1 ml. in excess.

Dilute the solution to 250 ml. with water, heat to 60° C., and add a 1% alcoholic dimethylglyoxime solution (10 ml. for each 10 mg. of nickel expected). Stir and allow to warm for 0.5 hour (longer for low nickels). Cool, filter through a weighed Gooch crucible, and wash 15 times with warm (50° C.) water. Dry the crucible at 110° C. for at least 2 hours and weigh. The increase in weight is nickel dimethylglyoxime.

$$\% \text{ Ni} = \frac{\text{weight of nickel glyoxime} \times 20.32}{\text{weight of sample}}$$

ACKNOWLEDGMENT

The authors wish to express their thanks to Walter Motz who performed some of the check analyses and to George Wiener for the spectrographic work.

LITERATURE CITED

(1) Ablov, A., *Bull. soc. chim.*, **7**, 151 (1940).
(2) Dickens, P., and Maassen, G., *Mitt. Kaiser-Wilhelm Inst., Eisenforschung, Düsseldorf*, **17**, 191 (1935).
(3) Diehl, H., "Applications of the Dioximes to Analytical Chemistry," Chap. II, Part E, Columbus, Ohio, G. Frederick Smith Chemical Co., 1940.
(4) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 315, New York, John Wiley & Sons, 1929.
(5) Kirtchik, H., ANAL. CHEM., **19**, 95 (1947).
(6) Lundell, G. E. F., Hoffman, J. I., and Bright, H. A., "Chemical Analysis of Iron and Steel," p. 279, New York, John Wiley & Sons, 1931.
(7) McAlpine, R. K., *J. Chem. Education*, **23**, 301 (1946).
(8) Steele, G. J., and Phelan, J. J., *Gen. Elec. Rev.*, **42**, 218 (1939).

RECEIVED May 5, 1947.

Determination of Potash in Fertilizers

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A modification of the official A.O.A.C. method for the determination of potash in fertilizers introduces a very rapid wet combustion procedure which operates simultaneously with the precipitation of potassium chloroplatinate. As a result the method offers a saving in time and a simplification of apparatus.

A RAPID and accurate method for determining potash is badly needed by laboratories controlling fertilizer manufacture. Thornton (*10*) emphasizes the importance of prompt analysis of fertilizer samples by control laboratories. This paper describes a modification of the official A.O.A.C. method, wherein the elimination of ammonium salts is effected by a new wet combustion technique. Use of this proposed method makes it possible to run a potash analysis in less than 2 hours and thus improve laboratory service and efficiency.

The wet combustion method for the destruction of ammonium salts and organic matter, suggested by DeRoode in 1895 (*2*) and studied by Keitt and Shiver (*7*), was modified in an effort to make it applicable to a variety of fertilizer mixtures (*3*, *8*). However, collaborative trials (*4*, *5*) of the improved procedure gave disappointing results and interest has decreased considerably.

Joy (*6*) reviews some disadvantages associated with the official (*1*) A.O.A.C. method: possible losses of potassium due to spattering or volatilization during ignition and the formation of water-insoluble residues. Shuey (*9*) points out advantages of avoiding the ignition step.

Some advantages of wet combustion, as practiced in the proposed method, include:

A new and extremely rapid method of destroying interfering substances, combined with precipitation of the weighing form, results in a substantial saving in time and simplification of procedure. Former methods making use of wet combustion of ammonia offered little or no saving in time.

There is little danger of loss of potassium from spattering or decrepitation, whereas in the official method these losses frequently occur.

It is unnecessary to wash the precipitate with the regular ammonium chloride solution (saturated with respect to potassium chloroplatinate). In the official method this wash is frequently necessary to remove visible impurities.

The precipitate is completely soluble in water, whereas in the official method it is frequently necessary to make allowance for insoluble residues. At no stage in the new method is it necessary to evaporate solutions to dryness (as in former wet combustion methods) or ignite residues (as in the official method). Thus the danger of forming insoluble or difficultly soluble impurities is reduced and phosphates cause no interference.

The precipitate does not adhere to the containing vessel and can be transferred to the crucible without the aid of a rubber policeman. In the official method this transfer is occasionally troublesome.

The precipitate obtained is more uniform in particle size and appearance than in the official method.

The precipitate formed is purer than that secured in the official A.O.A.C. method before the ammonium chloride wash.

Table I. Comparative K_2O Analyses on Magruder Check Fertilizer Samples

(Distributed by F. S. Royster Guano Co.)

Check Sample Formula	Date	Average of Approx. 70 Labs. %	New Method %	Official A.O.A.C. Method %
3-12-6	Nov. 1947	6.25	6.20 6.18	5.90
3-9-6 Tob.	Feb. 1948	6.22	6.17 6.26	6.25
6-8-6	March 1948	6.42	6.44 6.39	6.55
0-9-27	April 1948	27.95	27.91 28.07	28.40
Cocoa tankage	May 1948	0.64	0.66	0.64
3-9-6	June 1948	6.61	6.61 6.65	6.60
3-12-6	Aug. 1948	6.44	6.46 6.51	6.27
6-8-6	Sept. 1948	6.22	6.28 6.29	6.04
0-8-30	Oct. 1948	30.45	30.27 30.46	29.34
Work done by R. St. Arnaud in Montreal Laboratory of Canada Packers Limited				
3-9-9	March 1947	9.07	9.19	9.46
3-12-6	April 1947	6.54	6.70	6.71
0-8-30	May 1947	31.16	31.00	31.63
10-6-4	July 1947	4.09	4.23	4.22
2-12-12 (Borax 80 lb.)	Sept. 1947	13.04	12.87	12.73
3-9-9	Oct. 1947	9.66	9.67	9.70
3-12-6	Nov. 1947	6.25	6.20	6.27
4-10-6	Dec. 1947	6.25	6.26	6.24

REAGENTS

Platinum Solution. Use a solution containing the equivalent of 0.5 gram of platinum (1.05 grams of chloroplatinic acid) in every 10 ml.

Diglycol Stearate Solution. Dissolve 20 grams of technical diglycol stearate in 1 liter of equal parts of benzene and ethyl alcohol.

Preparation of Solution. Place 2.5 grams or the factor weight, 2.425 grams, of sample in a 250-ml. volumetric flask, and add 125 ml. of water and 50 ml. of saturated ammonium oxalate solution. Add 1 ml. of diglycol stearate solution when necessary to prevent foaming. Boil 30 minutes, add a slight excess of ammonium hydroxide; after cooling, dilute to 250 ml., mix, and pass through dry filter.

DETERMINATION

Place a 50-ml. aliquot of solution (or a 25-ml. aliquot and 25 ml. of water, if the sample contains over 20% K_2O) in a 500-ml. Kjeldahl flask. Add 10 ml. of nitric acid and a silica granule (about 1 cm. long) previously weighed with a prepared Gooch or medium fritted crucible (Pyrex M porosity). Boil 2 minutes and add 10 ml. of hydrochloric acid. Boil down to approximately 25 ml. and add 5 ml. of hydrochloric acid and excess platinum solution. Boil down to 10 to 15 ml., rotating the flask occasionally, and then add 5 ml. of hydrochloric acid. Reduce heat and boil down to 3 to 5 ml. (depending on amount of precipitate), rotating the flask frequently near the end of the evaporation.

Remove the flask from heat and swirl to dissolve any soluble residue on walls. After cooling, immediately add 25 ml. of 95% alcohol so that it washes down neck of flask. Chill under tap, swirl, and allow to stand for at least 5 minutes. Decant into the tared crucible and transfer precipitate and granule with the aid of a stream of 95% alcohol. Dry crucible to constant weight (5 to 7 minutes usually are sufficient) in an aluminum plate (having depressions to fit crucibles loosely) maintained at 130° C. Weigh and subtract weight of crucible plus the silica granule. $K_2PtCl_6 \times 0.19376 = K_2O$.

DISCUSSION

Use of 250-ml. Pyrex volumetric flasks in place of Kjeldahl flasks is recommended when more than about six determinations are carried out simultaneously. These flasks (containing the silica granules) are placed on a hot plate. The heat is reduced considerably near the end of the evaporation period to avoid spattering and the flasks are successively removed from the hot plate when the volume of the contained liquid reaches 3 to 5 ml. (depending on the amount of precipitate). In general, transfer of the precipitate from the small flasks to the crucible requires somewhat less skill than when a Kjeldahl flask is used. A disadvantage is that the evaporation must be slower, particularly near the end, to avoid loss due to bumping.

In the preparation of solution 15 minutes' boiling is sufficient for most fertilizer mixtures. (An exception was the cocoa tankage mentioned in Table I. Using a 15-minute boiling period, a low value of 0.49% potash was obtained.)

If the platinum solution is added too early, when considerable ammonia is present, ammonium chloroplatinate may precipitate out. This passes completely into solution before the end of the combustion period and no harm is done. Normally no precipitate is observed until the volume of liquid is reduced to less than 10 ml.

Transfer of precipitate from the Kjeldahl flask to the crucible seems clumsy at first. However, after a little practice it can be accomplished in 2 minutes with only about 30 ml. of alcohol from the wash bottle. No attempt is made to remove the last 0.5 mg. or less from the flask.

In place of the aluminum plate it is permissible to dry the crucible and precipitate for 30 minutes in a 100° oven as in the official A.O.A.C. method.

The addition of oxidizing agents such as chromium trioxide, ammonium persulfate, and sodium permanganate to the aqua regia caused a more complete removal of the oxalic acid and other organic matter without interfering with the accuracy of the method. It is possible that some fertilizer combination will be encountered which will require a more powerful oxidation than that produced by the aqua regia alone. However, no practical fertilizer mixture has been discovered which cannot be analyzed accurately for water-soluble potash by the procedure given above. Apparently the residual impurities from the aqua regia digestion cause no significant error in the determination.

Similarly, no fertilizer mixture has been encountered which requires, for its accurate analysis, purification of the weighing form by washing with the usual ammonium chloride solution. In the official A.O.A.C. method the need for this ammonium chloride wash probably is associated with the ignition step.

Table II. K_2O Analyses on Laboratory Mixtures of Known Potash Content

Sample	K_2O Calculated, %	K_2O New Method, %	Composition of Sample
A	12.05	12.05 12.07	Mixture of 7 Magruder check samples
B	7.57	7.54	0.300 g. C.P. dried KCl 2.200 g. superphosphate
C	7.57	7.54 7.54	0.300 g. C.P. dried KCl 2.200 g. C.P. diammonium phosphate
D	7.57	7.56	0.300 g. C.P. dried KCl 2.200 g. C.P. urea
E	7.57	7.56	0.300 g. C.P. dried KCl 2.200 g. pure gelatin
F	29.14	29.07	C.P. dried KCl mixed with March 1948 check sample (see Table I)

Tables I and II illustrate the precision and scope of the method. In Table I results are given of the analysis of 17 fertilizer check samples. Table II shows that excessive quantities of ammonia, phosphate, and soluble nitrogenous organic matter are destroyed or tolerated.

The method has been in continuous use since April 1948 in the routine analysis of several hundred fertilizer samples. Company chemists are of the opinion that it is more accurate and more reliable than the official A.O.A.C. method.

To test the effect of a simultaneous high level of sulfate, phosphate, and ammonium ion, the regular wet combustion was carried out on a 50-ml. aliquot containing 0.100 gram of C.P. dried potassium sulfate and 0.5 gram of C.P. diammonium phosphate. The indicated percentage of potash or K_2O was 54.07, and the calculated value was 54.05%.

To test the effect of a very high percentage of magnesium, to a weighed portion of the Magruder check sample for June 1948 (see

Table I) an equal weight of C.P. magnesium sulfate heptahydrate was added and mixed. A potassium determination on a 2.5-gram sample using the proposed method gave a result of 3.28% and the calculated value was 3.30% K_2O.

In another experiment 2.5 grams of C.P. urea and 2.5 grams of C.P. diammonium phosphate were mixed with 0.300 gram of C.P. dried potassium chloride in a 250-ml. volumetric flask. The regular quantities of ammonia and ammonium oxalate were added and a 50-ml. aliquot was digested with aqua regia exactly as in the normal procedure. The weight of potassium chloride recovered was 0.300 gram, showing that larger quantities of urea and ammonia are decomposed than could possibly be encountered in practice.

A final experiment showed the effect of excessive amounts of protein matter. The regular procedure was carried out on a 2.4-gram sample consisting of 0.300 gram of C.P. dried potassium chloride mixed with 0.700 gram of peanut meal, 0.700 gram of soybean meal, and 0.700 gram of tankage. One 50-ml. aliquot was treated by the new method and another was analyzed by the official A.O.A.C. method. The new method indicated 8.93% K_2O and the official method indicated 9.10% K_2O. It is probable that the new method will tolerate any quantity of protein likely to be met with in mixed fertilizers.

REASONS FOR DIFFICULTIES WITH WET COMBUSTION

In an effort to learn why the proposed method gives more satisfactory results than past methods depending upon wet combustion, some comparative tests were made.

The wet combustion procedure in the Fraps method (*3*) was selected as a good example of past technique and was applied to a 1-gram sample of C.P. ammonium sulfate in a Pyrex evaporating dish. In order to avoid spattering, the evaporation was carried out very slowly. In spite of this precaution, a residue of ammonium salt was observed on the walls of the dish after each evaporation with aqua regia and a heavy precipitate of ammonium chloroplatinate was obtained. Therefore, the experiment was repeated using distilled water to wash down the residue after each evaporation in the hope that this would ensure the complete oxidation of ammonia. However, on the final evaporation with platinum solution, a 0.3435-gram precipitate of ammonium chloroplatinate was obtained. (When the experiment was repeated on 0.5 gram of ammonium sulfate a precipitate of only 9 mg. resulted.)

For comparison a 50-ml. aliquot containing 1 gram of C.P. ammonium sulfate was placed in a Kjeldahl flask and analyzed by the proposed method. As there was no precipitate of ammonium chloroplatinate it would appear that the wet combustion employed in the proposed method is more effective than that in the Fraps method, in so far as the elimination of large amounts of ammonia is concerned.

The erratic results obtained with past techniques may have been due to spattering, which could lead to low results through loss of potassium and high results through removal of ammonium salts from the field of action of the aqua regia. In the proposed method the danger of loss of potassium by spattering is greatly reduced. Furthermore, the condensation in the neck of the flask causes a reflux action which continually returns spattered ammonium salts to the acid mixture. Another favorable condition is the violent agitation, particularly around the silica granule, caused by rapid boiling. This increases the speed of oxidation.

ACKNOWLEDGMENT

The author is grateful to A. L. VanLuven and R. St. Arnaud of Canada Packers' Toronto and Montreal laboratories, respectively, for their kind assistance in testing the method under routine conditions.

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," 6th ed., p. 31, 1945.
(2) DeRoode, *J. Am. Chem. Soc.*, **17**, 85 (1895).
(3) Fraps, G. S., *J. Assoc. Offic. Agr. Chemists*, **9**, 192 (1926).
(4) Haigh, *Ibid.*, **14**, 213 (1931).
(5) *Ibid.*, **15**, 277 (1932).
(6) Joy, A. B., IND. ENG. CHEM., ANAL. ED., **16**, 383 (1944).
(7) Keitt, T. E., and Shiver, H. E., *J. Ind. Eng. Chem.*, **10**, 219 (1918).
(8) *Ibid.*, **11**, 1049 (1919).
(9) Shuey, P. M., IND. ENG. CHEM., ANAL. ED., **15**, 633 (1943).
(10) Thornton, S. F., *Am. Fertilizer*, **107**, No. 12, 7 (1947).

RECEIVED August 20, 1948.

Isoquinoline as a Reagent in Inorganic Analysis

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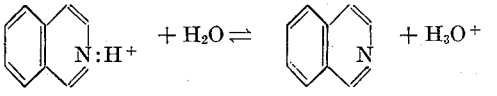
Isoquinoline, a tar base recently made commercially available, is desirable as an analytical reagent because it is easily purified, can effect useful separations, and has a large combining weight. Some general aspects of the nature of the reaction between isoquinoline, the thiocyanate ion, and certain divalent cations are considered. The reagent is applied to the determination of copper in alloys and in copper ores with an accuracy ranging from 3 to 4 parts per thousand in brasses to 10 to 13 in the ores. The reagent also provides a means for the separation of copper and zinc and thereby permits gravimetric determination of zinc in brass with an average accuracy of ±0.2 mg.

MANY heterocyclic nitrogen compounds have proved useful as reagents in inorganic analysis (*12*). Pyridine, for example, in neutral solution and in the presence of a thiocyanate yields with Cu(II) a green precipitate having the composition $CuPy_2(CNS)_2$ (*9*). By means of this reaction copper can be detected at a concentration of 1 to 300,000, while the reaction of pyridine and thiocyanate with Zn(II) is sensitive to 1 part of zinc in 200,000 (*10*). Because isoquinoline (2-benzazine), recently made commercially available, is easily purified and has a large equivalent weight, both desirable in a reagent, an investigation of the analytical properties of this interesting compound was undertaken. Although addition compounds of isoquinoline and metallic salts are known—e.g., $Hg(C_2H_3O_2)_2.2C_9H_7N$ (*11*), $HgCl_2.2C_9H_7N$ (*11*), $AuBr_4.C_9H_7N$ (*6*), $Cu(N_3)_2.2C_9H_7N$ (*1*), $H_2PbCl_6.2C_9H_7N$ (*7*), $H_2SnCl_6.2C_9H_7N$ (*3*), $2Na_4Fe(CN)_6.2C_9H_7N.H_2O$ (*2*), and $CaCl_2.2C_9H_7N$ (*8*)—the analytical possibilities of these compounds are virtually unexplored.

When a solution containing isoquinoline and a thiocyanate is added to solutions of certain divalent cations, precipitates corresponding to the formula $Me(C_9H_7N)_2(CNS)_2$ are formed. The following equations best describe the reactions involved:



$$2\ C_9H_7N + Me^{++} + 2\ CNS^- \longrightarrow Me(C_9H_7N)_2(CNS)_2$$

As isoquinoline takes part in the formation of the precipitate, there is a shift in the hydrolysis equilibrium with a resulting increase in H_3O^+ ions. With both Cu(II) and Zn(II) a pH lowering was observed during the course of the precipitation. This was shown by the results of potentiometric titrations of cupric thiocyanate solutions with isoquinoline solutions using a glass electrode pH meter. Before titration, the pH of the separate solutions was adjusted to a value of 5.0. It was found that in each case the pH markedly decreased upon the addition of titrant until the equivalence point was reached. Visual observations could detect no further precipitate being formed after the minimum pH value (about 2.8) had been obtained. Addition of excess isoquinolinium chloride caused a rise in the pH value, as the pH at the equivalence point was well below that of the titrant.

This is further illustrated by results obtained from conductometric titrations with the isoquinoline reagent of both copper and zinc thiocyanates. In each case, the titration curve obtained was one in which the conductance increased sharply to the equivalence point and then became almost constant. Inasmuch as in most precipitation titrations the conductance usually decreases, the increase is a strong indication of the liberation of hydronium ions during the reaction.

PHYSICAL PROPERTIES

Isoquinoline is a white crystalline material melting at 26.6° C. when carefully purified (*5*). Commercial isoquinoline (Koppers Company 2°–isoquinoline) is a brown liquid (melting point 24° C.) of about 90 mole % or better purity. Two simple methods of obtaining isoquinoline of high purity from this material are fractional crystallization (*5*) and formation of the calcium chloride addition salt (*8*). By employing one step of either process, a product of sufficient (approximately 95 mole %) purity for analytical work is obtained. Unlike most members of the pyridine or quinoline family, isoquinoline is a fairly pleasant smelling substance. It is sparingly soluble in water and was therefore used as the hydrochloride.

REAGENTS

A 0.2 *M* isoquinolinium hydrochloride solution was prepared by dissolving 26.0 grams (0.2 mole) of purified isoquinoline in 50 ml. of 3 *M* hydrochloric acid and diluting the mixture to 1 liter with distilled water. This solution keeps indefinitely.

A standard copper solution was prepared from reagent-grade copper sulfate pentahydrate. The copper concentration of the solution (in the neighborhood of 2.5 mg. of copper per ml.) was determined by electrodeposition.

A 0.5 *M* ammonium thiocyanate solution was prepared from reagent-grade salt.

The standard zinc solutions were prepared by dissolving Coleman and Bell's reagent grade zinc oxide, which had been heated an hour over a full Bunsen flame, in dilute hydrochloric acid and then diluting to 1 liter.

The solutions used in studying the possible interference of various ions on the reaction were prepared from reagent-grade salts and generally contained 1 mg. of the desired ion per ml.

Thymol blue indicator was prepared by making a 0.4% alcoholic solution of the dye.

All pH measurements were made using a Beckman Model H line-operated pH meter.

SENSITIVITY

Copper solutions were made up in 10-ml. portions with concentrations ranging from 1 part in 1000 to 1 part in 50,000,000. To each portion were added 4 drops each of 0.2 *M* isoquinolinium hydrochloride and 0.5 *M* ammonium thiocyanate solutions. Precipitates began to form immediately. With concentrations above 1 part in 256,000, coarse green precipitates were formed. As the copper concentration decreased to 1 part in 2,000,000, the precipitate formed was pale green and very fine. When the concentration fell to 1 part in 20,000,000, the lowest with which a reaction was observed, the superfine precipitate lost its original green color and became white.

In another series of tests, 4 drops each of Cu(II) (2.5 mg. per ml.) and 0.5 *M* ammonium thiocyanate solutions were added to 10-ml. portions of isoquinoline solution. With isoquinoline concentrations as low as 1 part in 13,000 the characteristic green precipitate appeared. Similarly, thiocyanate concentrations of 1 part in 30,000 produced a green precipitate upon the addition of copper and isoquinoline solutions.

Zinc solutions were prepared in a similar manner with concentrations ranging from 1 part in 1000 to 1 part in 20,000,000. To each of these solutions were added 8 drops each of 0.5 *M* ammonium thiocyanate and 0.2 *M* isoquinolinium hydrochloride solutions. The pH was then adjusted to a value between 6.5 and 7.0, using 0.2 *M* ammonium hydroxide solution. White crystalline precipitates began to form immediately with zinc concentrations as low as 1 part in 1,000,000. The lowest zinc concentration with which a reaction was observed was 1 part in 10,000,000.

The increase in the sensitivity of this isoquinoline reaction with both Cu(II) and Zn(II) over that found for pyridine serves as an illustration of the principle that an increase of molecular weight of an organic reagent will increase the sensitivity of its reactions.

PROPERTIES OF COPPER AND ZINC PRECIPITATES

Thermal Stability. A quantity of dry green copper precipitate was introduced into a capillary melting tube and heated slowly. No visible change occurred below 190° C., at which temperature the compound decomposed. Similar treatment of the white zinc precipitate revealed that this substance could be heated to about 200° C. without visible change. The zinc precipitate melted sharply at 205° C. and the solidified melt took on a light tan color. The conclusion that 105° to 110° C. was a safe temperature at which to dry both precipitates was confirmed by the ease with which these compounds could be heated to constant weight in this temperature range.

Solubility. The solubility of the copper addition compound in various organic solvents was determined in a qualitative manner. The precipitate was found to be insoluble or only slightly soluble in chloroform, carbon tetrachloride, ethyl ether, toluene, methyl and ethyl alcohols, and acetone. The last three solvents were somewhat more effective. The zinc addition compound was insoluble in carbon tetrachloride and ethyl ether, slightly soluble in toluene and bromobenzene, and very soluble in acetone, absolute methyl and ethyl alcohols, and chloroform. All the resulting solutions were colorless.

Composition. The composition of the copper addition salt was determined in the following manner:

A given quantity of the precipitate heated at 105° to 110° C. to constant weight was dissolved in a mixture of concentrated sulfuric acid and nitric acid, and the solution was made strongly alkaline with sodium hydroxide. The isoquinoline thus liberated was then removed by evaporation (heating accomplished by infrared lamp from above solution to prevent bumping usually encountered with caustic solutions). The solution was carefully acidified with dilute sulfuric acid and heated until sulfur trioxide fumes were evolved. The solutions were then diluted to 150 ml. and electrodeposition was carried out in the usual manner. The theoretical percentage of copper in $Cu(C_9H_7N)_2.(CNS)_2$ is 14.513%. The corresponding value obtained by an average of six determinations on separately prepared precipitates is 14.51 ± 0.005% copper.

The composition of the zinc complex was shown to correspond to a 2 to 1 mole ratio of isoquinoline to zinc by the results of the conductometric titration. The gravimetric factor of zinc in $Zn(C_9H_7N)_2(CNS)_2$, 0.1486_4, was utilized in the calculations for all the zinc determinations with good results.

INTERFERENCES

To various cation solutions an excess of isoquinolinium hydrochloride and ammonium thiocyanate solutions was added, and the resulting mixture was shaken for a few minutes and then set aside. White precipitates were obtained with the following cations: Ag, Hg(II), Cd, Sb(III), Bi(III), Ni(II), Co(II), and Zn. None of the other common cations, such as those in the alkali and alkaline earth groups and Al(III), Cr(III), Sn(II), Sn(IV), Pb(II), As(III), and Mn(II), yielded any precipitate, although Fe(III) gave a characteristic red color due to the formation of the thiocyanate complex.

Table I. Influence of pH on Precipitate Formation[a]

Cation	Minimum pH at Which Ppt. Forms	Maximum pH at Which Ppt. Is Stable	pH Ppt. Stability
Cu(II)	2.0	10.2	2.5–9.5
Zn(II)	4.0	9.0	4.5–8.5
Cd(II)	4.5	9.0	5 –8.5
Co(II)	3.9	ca. (11–12)	4.5–11
Ni(II)	4.2	ca. (11–12)	4.7–11

[a] No ammonia present in solutions. pH changed by means of HCl and NaOH.

Table II. Determination of Copper

Detn.	Copper Taken G.	pH at Precipitation	Weight of Precipitate G.	Copper Found G.	Error Mg.
1	0.0502	2.38	0.3452	0.0501	−0.1
2	0.0376	2.53	0.2590	0.0376	0.0
3	0.0697	3.10	0.4798	0.0696	−0.1
4	0.0740	3.25	0.5106	0.0741	+0.1
5	0.1039	3.25	0.7170	0.1041	+0.2
6[a]	0.0498	2.42	0.3435	0.0498	0.0

[a] 0.0081 gram of zinc added.

In order to determine the extent of the interference of zinc, cadmium, cobalt, and nickel, with the copper-isoquinoline reaction, the dependence of precipitate formation on pH was studied. The results, which are summarized in Table I, indicate that copper may be separated from the other metals listed.

Cations such as nickel, copper, and cadmium form precipitates at closely the same pH ranges as does the zinc and hence must be absent to ensure successful determination of zinc.

The various anions to be tested were mixed with an excess of copper-isoquinoline solution, and the resulting solutions were shaken and observed. Many of the anions were found to give interfering precipitates. Noteworthy among these are bromide (light blue precipitate), sulfate (brown precipitate), nitrite (green precipitate), arsenate (pale blue precipitate), and chromate (yellow precipitate). Chloride in high concentration yielded a light blue precipitate. Fortunately, nitrate and sulfate do not form any precipitate with the copper-isoquinoline mixture. Thus most interfering anions may be effectively removed by treatment with concentrated sulfuric acid and evaporation to sulfur trioxide fumes. [If arsenic is present, this may be preceded by evaporation from concentrated hydrochloric acid solution, while chromate may be reduced to Cr(III).]

OPTIMAL PRECIPITATING CONDITIONS FOR COPPER

A series of runs was made to determine the effect of pH on the completeness of precipitation.

The solution was made up to about 100 ml. and an excess of ammonium thiocyanate (based on the requirement of 2.5 mg. of thiocyanate for 1 mg. of copper) was added. An excess of isoquinoline solution (4.1 mg. of isoquinolinium hydrochloride required for 1 mg. of copper) was then added with constant stirring. The pH was adjusted by means of dropwise addition of 0.2 *M* sodium hydroxide solution. The resulting precipitate and solution were heated to 70° C. to aid the digestion. The mixture was allowed to stand for 1 hour and filtered through a sintered-glass crucible (porosity M). The precipitate was washed with water and then dried in an oven maintained between 105° and 110° C. until constant weight was obtained. Table II lists the results obtained in this manner. The filtrates were tested for Cu(II) by both ammonium sulfide and potassium ferrocyanide but no Cu(II) was detected. Other runs performed at pH values of 2.0 or below yielded only a fraction of the desired precipitate.

Inasmuch as it was found that zinc would not yield a precipitate unless pH were raised to 4.0, a run (No. 6 in Table II) was made in the presence of enough zinc to approximate conditions encountered in brasses. As the results indicate, a good separation of copper and zinc can be effected.

RECOMMENDED PROCEDURE FOR COPPER

The following procedure has proved successful in such diversified copper-containing materials as brass, German silver, and copper ore (sulfide type).

The alloy samples were dissolved by use of dilute nitric acid. In the case of brass, 10 ml. of concentrated phosphoric acid may be added to prevent tin from precipitating or the usual procedure for brass may be adhered to if the determination of tin is desired. A volume of 20 ml. of phosphoric acid was used in all the bearing-metal runs because of the large quantities of tin present. The ore samples were brought into solution in the usual manner with nitric and hydrochloric acids (*4*).

The solution containing about 50 mg. of copper is adjusted to about 150 ml. and the pH of the solution is adjusted to 3.0 by dropwise addition of 0.2 *M* sodium hydroxide. The pH determination can be made either with a pH meter or by using 5 drops of thymol blue solution, a color change from pink to yellow indicating a properly adjusted acidity. Eight milliliters of 0.5 *M* ammonium thiocyanate and 16 ml. of 0.2 *M* isoquinolinium hydrochloride are then added to the solution with constant stirring.

The solution is heated to 70° C. with stirring to aid in the digestion of the precipitate, allowed to stand for an hour, and then filtered through a sintered-glass (medium porosity) or a Gooch crucible and the precipitate is washed with several portions of water. If the precipitation was carried out properly, addition of a few drops of sodium hydroxide solution to the filtrate will not cause the formation of any further green precipitate. The filtrate will either remain clear or upon further addition of alkali will exhibit a white precipitate due to zinc or nickel. The precipitate is brought to constant weight at a temperature of 105° to 110° C. Some typical results are presented in Table III.

Table III. Determination of Copper in Various Substances

Brasses	Detn. No.	Sample Weight G.	Weight of Precipitate G.	Weight of Cu Taken G.	Weight of Cu Found G.	Error Mg.
A	1	0.1502	0.7755	0.1130	0.1126	−0.4
	2	0.1123	0.5818	0.0845	0.0844	−0.1
	3	0.0559	0.2901	0.0421	0.0421	0.0
B	4	0.1018	0.4809	0.0701	0.0697	−0.4
C	5	0.1081	0.5800	0.0843	0.0842	−0.1
D	6	0.1211	0.6920	0.1009	0.1004	−0.5
E	7	0.1221	0.6258	0.0909	0.0908	−0.1
N.B.S. 37	8	0.1201	0.5849	0.0844	0.0849	+0.5
	9	0.1201	0.5840	0.0844	0.0847	+0.3
German silver (61.02% Cu, 22.99% Zn; 15.15% Ni; 0.94% Pb)						
A	1	0.1214	0.5200	0.0741	0.0755	+1.4
	2	0.1254	0.5296	0.0765	0.0769	+0.4
	3	0.1250	0.5216	0.0763	0.0757	−0.6
Bearing metal (N.B.S. 54, 3.76% Cu; 88.24% Sn; 7.33% Sb)						
	1	1.4872	0.3854	0.0558	0.0559	+0.1
	2	1.0079	0.2597	0.0378	0.0377	−0.1
	3	0.9972	0.2554	0.0374	0.0371	−0.3
	4	1.0241	0.2665	0.0384	0.0387	+0.3
	5	1.3326	0.3469	0.0500	0.0503	+0.3
Copper ore						
A	1	0.7338	0.4068	0.0606	0.0590	−1.6
	2	0.6260	0.3696	0.0517	0.0536	+1.9
	3	0.7610	0.4330	0.0629	0.0629	0.0
	4	0.7022	0.4015	0.0580	0.0583	+0.3
	5	0.9845	0.5628	0.0813	0.0817	+0.4
B	6	0.8379	0.2960	0.0431	0.0430	−0.1
	7	1.4868	0.5139	0.0764	0.0746	−1.8
	8	1.3046	0.4644	0.0671	0 0674	+0.4

OPTIMAL PRECIPITATING CONDITIONS FOR ZINC

A series of runs was made to study the effect of pH on the completeness of precipitation.

The standard zinc solutions were made up to about 150 ml. and a 10 to 15% excess of ammonium thiocyanate (based on the requirement of 2.3 mg. of ammonium thiocyanate for 1 mg. of zinc) was added. A 10 to 15% excess of isoquinolinium hydrochloride solution (4.0 mg. of isoquinoline required for 1 mg. of zinc) was then added with constant stirring. Precipitation at a pH of 6.5 was found to serve satisfactorily; the precipitates formed and coagulated more rapidly than at somewhat lower pH values. The mixtures were heated to 70° C. and set aside for about an hour. The precipitates were then filtered through sintered-glass crucibles (porosity M), washed with cold water, and dried to constant weight at 105° to 110° C. The results of three runs are listed in Table IV.

A second series of runs was made with known amounts of copper and zinc in which the copper-zinc ratio varied between 0.1 and 0.5. The copper was removed by precipitating it as $Cu(C_9H_7N_2)(CNS)_2$ at a pH of 3.0, using the procedure developed above but substituting aqueous ammonia for sodium hydroxide to adjust the pH. To the filtrate from the copper determination, additional amounts of isoquinoline and thiocyanate solutions were added.

Table IV. Determination of Zinc

Detn.	Zinc Taken G.	Weight of Precipitate G.	Zinc Found G.	Error Mg.
1	0.0257	0.1738	0.0258	+0.1
2	0.0310	0.2092	0.0311	+0.1
3	0.0388	0.2580	0.0384	−0.4
4	0.0401	0.2700	0.0401	0.0
5	0.0426	0.2841	0.0422	−0.4
6	0.0465	0.3155	0.0469	+0.4
7	0.0814	0.5485	0.0815	+0.1
8	0.0775	0.5216	0.0775	0.0

The pH of the solution was then increased to 6.5 with 0.2 *M* ammonium hydroxide, and the mixture was heated to 70° C. and set aside for an hour. The precipitate was then filtered, washed with cold water, and brought to constant weight at 105° to 110° C. The results are shown in Table V.

Table V. Determination of Zinc in Presence of Copper

Detn.	Zinc Taken G.	Copper Taken G.	Weight of Precipitate G.	Zinc Found G.	Error Mg.
1	0.0387	0.0152	0.2508	0.0388	+0.1
2	0.0426	0.0200	0.2829	0.0421	−0.5
3	0.0678	0.0099	0.4578	0.0680	+0.2
4	0.0678	0.0101	0.4575	0.0679	+0.1
5	0.0717	0.0215	0.4830	0.0718	+0.1
6	0.0717	0.0176	0.4802	0.0714	−0.3
7	0.0698	0.0155	0.4710	0.0700	+0.2
8	0.0756	0.0203	0.5086	0.0756	0.0

RECOMMENDED PROCEDURE

The following procedure has proved successful in the determination of zinc in brass.

After the brass sample has been put into solution and tin removed as metastannic acid, the copper may be removed as the isoquinoline-thiocyanate complex. To the filtrate from the copper precipitation an excess of ammonium thiocyanate and isoquinoline is added. The pH of the solution is increased to a value between 6.5 and 7.0 with 0.2 *M* ammonium hydroxide. The mixture is heated to 70° C., allowed to stand an hour, and then filtered through a sintered-glass (porosity M) or Gooch crucible. The zinc complex is washed with cold water and heated to constant weight at 105° to 110° C. The weight of the precipitate multiplied by 0.14864 gives the weight of zinc. Results of the determination of zinc in various brass samples are given in Table VI.

Table VI. Determination of Zinc in Brass

Brass	Detn. No.	Sample Weight G.	Weight of Precipitate G.	Weight of Zinc Taken G.	Weight of Zinc Found G.	Error Mg.
A	1	0.3578	0.3701	0.0548	0.0550	+0.2
	2	0.3858	0.4007	0.0591	0.0596	+0.5
	3	0.3997	0.4135	0.0612	0.0615	+0.3
	4	0.4901	0.5009	0.0750	0.0745	−0.5
B	5	0.3463	0.4863	0.0719	0.0723	+0.4
C	6	1.2191	0.4048	0.0603	0.0602	−0.1
	7	1.4114	0.4714	0.0699	0.0701	+0.2
D	8	1.8277	0.2474	0.0367	0.0368	+0.1
	9	2.1208	0.2866	0.0426	0.0426	0.0
E	10	0.3603	0.0896	0.0134	0.0133	−0.1
	11	0.2918	0.0748	0.0109	0.0111	+0.2
F	12	0.2208	0.0075	0.0009	0.0011	+0.2
	13	0.3101	0.0097	0.0012	0.0014	+0.2
G	14	0.2670	0.2737	0.0409	0.0407	−0.2
H	15	0.3665	0.0596	0.0087	0.0088	+0.1

CONCLUSIONS

The method described is sensitive, the procedure is simple, and the precipitates obtained have comparatively small gravimetric factors. The isoquinoline reagent may be used for the determination of both copper and zinc in brass. Although the reagent is now not so specific as desired, the use of complexing ions such as citrate and tartrate is being investigated to determine the possibility of more extensive separations.

LITERATURE CITED

(1) Cirulis, A., and Straumanis, M., *J. prakt. Chem.*, **162**, 307 (1943).
(2) Cumming, W. M., *J. Chem. Soc.*, **123**, 2457 (1923).
(3) Druce, J. G. F., *Chem. News*, **125**, 265 (1922).
(4) Fales, H. A., and Kenny, F., "Inorganic Quantitative Analysis," p. 464, New York, D. Appleton-Century, 1939.
(5) Freiser, H., and Glowacki, W. L., *J. Am. Chem. Soc.*, **71**, 514 (1949).
(6) Gutbier, A., and Huber, J., *Z. anorg. Chem.*, **85**, 353 (1913).
(7) Gutbier, A., and Wismiller, M., *Ibid.*, **90**, 491 (1914).
(8) Reimers, U. S. Patent 2,391,270, (Dec. 18, 1945).
(9) Spacu, G., *Bul. Soc. Stiinte Cluj.*, **1**, 284 (1922).
(10) *Ibid.*, **1**, 348 (1922).
(11) Ukai, T., *J. Pharm. Soc. Japan*, **51**, 542 (1931).
(12) Welcher, F. J., "Organic Analytical Reagents," Vol. III, New York, D. Van Nostrand Co., 1947.

RECEIVED August 11, 1948. Presented in part before the Division of Analytical and Micro Chemistry at the 114th Meeting of the AMERICAN CHEMICAL SOCIETY, St. Louis, Mo. Abstracted from a thesis to be presented by Adolph E. Spakowski in partial fulfillment of the requirements for the degree of master of science in chemistry at the University of Pittsburgh.

Determination of Benzaldehyde in Benzyl Alcohol

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BENZALDEHYDE is almost a universal contaminant in benzyl alcohol. Chemical methods such as those recommended by Donnally (*1*), Parkinson and Wagner (*4*), and Iddles and Jackson (*2*) are not satisfactory for determining traces of benzaldehyde in benzyl alcohol. They involve procedures that are somewhat time-consuming, which may thus lead to changes in the benzaldehyde content due to the possibility that benzaldehyde or benzyl alcohol may undergo chemical changes.

The spectrophotometric method outlined here involves few manipulations and is rapid. It minimizes the possibility that some benzyl alcohol might be oxidized during the analytical procedure. It is, of course, applicable only to solutions containing no interfering substances.

REAGENTS

Benzaldehyde, purified from Eastman Kodak Company No. 30 chlorine-free benzaldehyde, was washed with dilute sodium hydroxide solution, dried, and distilled at reduced pressure in a nitrogen atmosphere. The purity was tested by measuring the infrared transmission in the range 2 to 16 microns with a Baird recording spectrophotometer. The absorption bands due to benzyl alcohol and benzoic acid were not present in the freshly purified material.

Methanol-water diluent was prepared by adding sufficient distilled water to 500 ml. of spectrophotometric-quality methanol to produce 1 liter of solution.

Several small portions of benzaldehyde-free benzyl alcohol were prepared from commercial benzyl alcohol by treatment with active Raney nickel and hydrogen in a Parr low pressure hydrogenation apparatus. The sample of benzyl alcohol having the lowest absorbancy at 283 mμ was assumed to contain the least amount of benzaldehyde and was used to establish the intercept of the calibration curve.

APPARATUS

The apparatus consisted of a Beckman spectrophotometer, Model DU, with accessories for measuring ultraviolet energy, and fused silica absorption cells, 1-cm. light path.

The amount of benzaldehyde present in benzyl alcohol can be determined by making ultraviolet absorption measurements at 283 millimicrons, at which wave length a significant difference exists between the absorbancies of benzyl alcohol and benzaldehyde. The uncertainty in the amount of benzaldehyde present in the range 0 to 0.1% is probably less than 0.006 absolute % of benzaldehyde. The deviation of eight values determined by four operators on two spectrophotometers is about 1% of benzaldehyde.

EXPERIMENTAL

A 10-ml. sample of benzyl alcohol was dissolved in water and diluted to 1 liter. Then a 5-ml. aliquot was diluted to 100 ml. to give a solution with a satisfactory absorbancy (optical density, *3*) which was read against water as a blank. The absorption curve of this solution is curve *D* in Figure 1. Curves *A*, *B*, and *C* were obtained by determining the absorbancies of solutions to which known amounts of benzaldehyde had been added.

If the benzaldehyde concentration is in the range above about 0.1%, measurements at 255 mμ permit making a determination.

Table I. Absorbancy of Benzyl Alcohol Containing Known Added Amounts of Benzaldehyde

Benzaldehyde Added, % by Weight	Absorbancy at 283 mμ[a]	Average
0.00	0.192	
	0.194	
		0.193
0.022	0.335	
	0.335	
		0.335
0.031	0.412	
	0.413	
		0.412
0.043	0.489	
	0.487	
		0.488
0.063	0.631	
	0.632	
		0.632
0.097	0.865	
	0.865	
		0.865

[a] These values obtained using slit width of 0.53 mm., band width of 1.9 millimicrons.

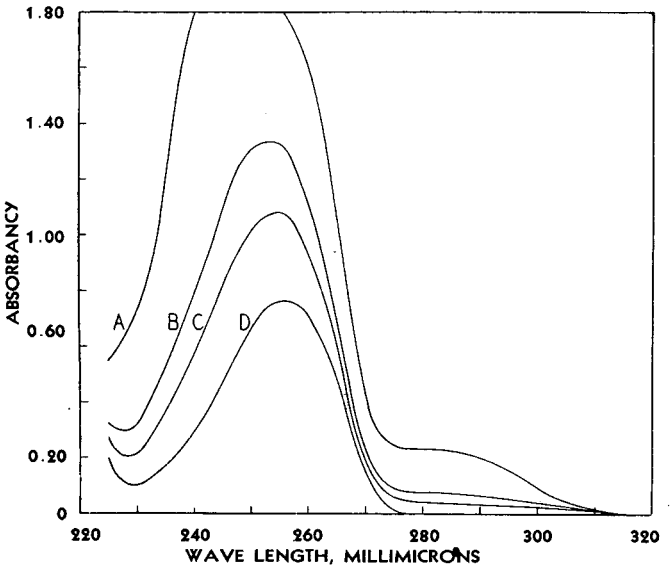

Figure 1. Absorption of Benzyl Alcohol–Benzaldehyde Solutions

A. 3% benzaldehyde
B. 1% benzaldehyde
C. 0.5% benzaldehyde
D. Benzaldehyde-free benzyl alcohol

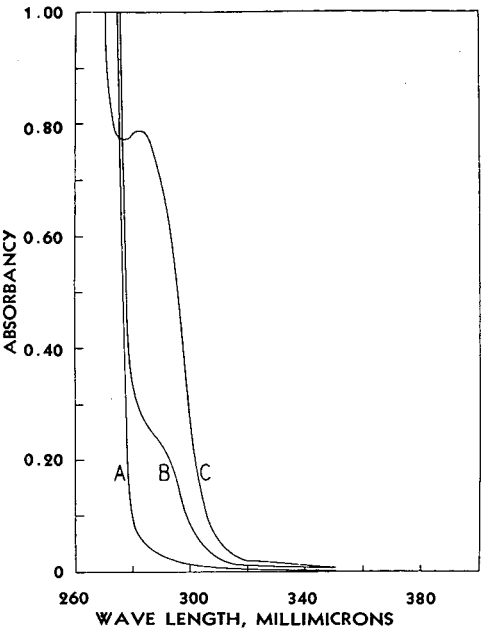

Figure 2. Absorption in Methanol–Water Diluent

A. Benzaldehyde-free benzyl alcohol
B. Benzyl alcohol with 0.029% benzaldehyde
C. Benzaldehyde with no benzyl alcohol
Concentration about 0.05 gram per liter

For analyses of benzaldehyde concentrations lower than 0.1% there are two limitations to the above procedure: the high absorbancy of the benzyl alcohol at 255 mμ, and the limited solubility of benzyl alcohol in water, which makes it impossible to get enough material into solution to give a satisfactory absorbancy. These objections can be overcome by determining the absorbancy at 283 mμ, where the absorbancy of benzyl alcohol is low, and by employing a solution of methanol in water which allows the use of a larger sample size. In Figure 2, the absorption curves are given for solutions of benzaldehyde, benzyl alcohol, and benzyl alcohol containing 0.029% benzaldehyde, all dissolved in the methanol-water diluent and measured against this diluent as a blank. A series of benzyl alcohol solutions was prepared, to which known amounts of purified benzaldehyde were added. The samples were diluted 5 ml. to 100 ml. with methanol-water diluent and their absorbancies at 283 mμ were determined (Table I).

Experiments show that the diluted solutions will give constant absorbancy values for periods up to 0.5 hour. The methyl alcohol diluent does not need to be deaerated. Trials made with nitrogen-swept diluent gave results identical with those using the reagent that had stood in a glass-stoppered bottle one-half full for 2 or 3 days.

Because the benzyl alcohol used to prepare the standard samples in Table I contained some benzaldehyde, these data were used to calculate the slope of the calibration curve using the technique described by Youden (*5*). The calculated slope was 7.0

with a standard deviation of 0.06. A calibration curve was drawn to pass through the intercept obtained by analysis of the best sample of purified benzyl alcohol. The data for these analyses are shown in Table II.

PROCEDURE

Pipet 5 ml. of the benzyl alcohol sample into a 100-ml. volumetric flask, dilute to the mark with methanol-water diluent, and invert several times to dissolve the sample.

Transfer a portion to a 1-cm. fused silica absorption cell and determine the absorbancy at 283 mμ. The blank cell should contain the methanol-water diluent.

ACCURACY AND PRECISION

It is possible that there was a slight residue of benzaldehyde in the benzyl alcohol having the lowest absorbancy at 283 mμ and so any statement regarding the accuracy is subject to criticism on this point.

If pure benzyl alcohol has no absorption at 283 mμ, it is evident that the best sample prepared contains material which, if calculated as benzaldehyde, would amount to 0.006% benzaldehyde. However, the actual error, if any, is probably less than 0.006% of benzaldehyde.

To secure an indication of the precision of the determination, four analysts using two spectrophotometers analyzed a sample of benzyl alcohol (Table III). One dilution was made by each analyst and in no case was a duplicate or check dilution prepared by any analyst.

Variance analysis indicates that there is no significant systematic variability between the spectrophotometers or among the test operators. The best estimate of the variability of the method is the standard deviation of the eight values and this is 0.00022% benzaldehyde. The average error is about 1%.

Table II. Absorbancy of Purified Benzyl Alcohol

Absorbancy at 283 mμ
0.042
0.043
0.041
0.042
0.044
0.044
Av. 0.043

Table III. Benzaldehyde Found by Four Analysts Using Two Beckman Spectrophotometers

Analyst	Spectrophotometer 1380, %	Spectrophotometer 2323, %	Average, %
A	0.0126	0.0127	0.01265
B	0.0122	0.0126	0.01240
C	0.0125	0.0126	0.01255
D	0.0127	0.0130	0.01285
Av.	0.01250	0.01272	0.01261

ACKNOWLEDGMENT

The benzaldehyde-free benzyl alcohol was prepared by D. B. Glass and Stephen Michel, Color Control Department, Eastman Kodak Company.

LITERATURE CITED

(1) Donnally, L. H., Ind. Eng. Chem., Anal. Ed., 5, 91 (1933).
(2) Iddles, H. A., and Jackson, C. E., *Ibid.*, 6, 454 (1934).
(3) Natl. Bur. Standards, *Letter Cir.* LC 857 (May 19, 1947).
(4) Parkinson, A. E., and Wagner, E. C., Ind. Eng. Chem., Anal. Ed., 6, 433 (1934).
(5) Youden, W. J., Anal. Chem., 19, 946 (1947).

Received October 4, 1948.

Direct Colorimetric Method for Carbohydrates

MALTOSE

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The molybdenum blue reaction as applied to glucose has been extended to maltose in such a way that mixtures of maltose and glucose may be analyzed. Maltose reacts in conformity with Beer's law, but gives values which are about one tenth as great as values for glucose under identical conditions. Aliquots of mixtures containing not more than 5 mg. of the two sugars combined were analyzed to yield *B*, the absorption value at 650 for the glucose-maltose mixture. Complete hydrolysis of the maltose without any destruction of glucose was effected in an autoclave in 1 hour by using small concentrations of hydrochloric acid. After this hydrolysis the mixture contained only glucose, which was analyzed to yield *A*, the absorption value for all the glucose now present. By calculation from the most favorable points obtained from the standard curves, two equations were obtained: $B = (0.084G + 0.0245) + (0.0097M - 0.0009)$ and $A = 0.084(G + M) + 0.0245$, where G = glucose and M = maltose. Solving for M, $A - B = 0.0743M + 0.0009$. Substitution of the values for M in the second equation yields the value for G. Mixtures so analyzed yield recoveries of 98 to 100% when 2 to 5 mg. are taken for analysis.

THE molybdenum blue reaction as used for small quantities of glucose by Benham and Despaul (*1*) has been adapted for the determination of maltose and mixtures of maltose and glucose. The method as previously outlined was used throughout this work, with strict attention to details of time and temperature.

In order to analyze an unknown mixture of glucose and maltose in terms of each of these sugars, it was decided to evaluate the blue color before hydrolysis and after complete hydrolysis. The value before hydrolysis (B) is due to glucose alone only if the maltose color is entirely eliminated. The value after hydrolysis (A) is due to the sum of the original glucose and the glucose obtained by total hydrolysis of the maltose present.

This approach hinges upon three conditions: (1) the complete elimination of any color due to maltose in determination B, (2) the complete hydrolysis of maltose for determination A, and (3) absence of any destructive effect upon glucose with the conditions of hydrolysis chosen.

Table I. Effect of Acid Concentration on Hydrolysis of Maltose

(2.5 mg. of maltose in an autoclave for 1 hour at 120° C.)

Acid Used		Recovery	
Ml.		*Mg.*	%
5	Concd. HCl	2.0	80
2	Concd. HCl	2.2	88
1	Concd. HCl	2.3	92
5	10% HCl	2.4	96
4	10% HCl	2.45	98
3	10% HCl	2.45	98

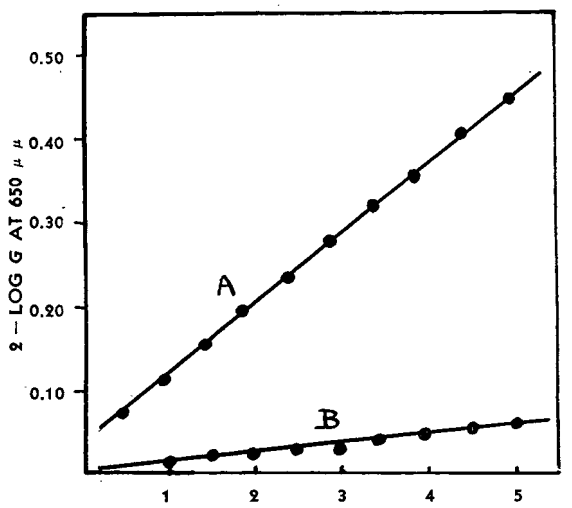

Figure 1. Standard Curves for Glucose and Maltose

A. Mg. of glucose
B. Mg. of maltose

Benham and Despaul (*1*) found that under the standard conditions adopted for glucose, the presence of sucrose, except in high concentrations, could be discounted, inasmuch as the blue color developed with sucrose was substantially zero. Under the same conditions, maltose gives a slight blue color, as shown in Figure 1, which represents standard curves for glucose and for maltose plotted on the same graph.

An attempt was made to eliminate the color due to maltose by shortening the heating time to 10 minutes. Under these conditions 1 mg. of maltose does not have time to react, but 5 mg., the upper limit for the procedure, still yield a very slight blue color. Moreover, the shorter heating time decreases the blue color due to glucose to a point where the sensitivity of the method is considerably impaired. Accordingly, no attempt was made to eliminate the maltose color, but a procedure involving two steps was adopted for an unknown mixture of the two sugars.

With dilute acid hydrolysis for 1 hour at a temperature of 120° C. in an autoclave, the recovery of maltose as glucose after hydrolysis is 98 to 100%.

The acid concentration in the final hydrolysis mixture was 3 ml. of 10% hydrochloric acid in a total of 50 ml. This is approximately 0.07 *N* with respect to hydrochloric acid and has a pH of 1.7. More concentrated acid causes some destruction, as shown in Table I.

Analysis of known mixtures of maltose and glucose, each containing the equivalent of 5 mg. of glucose when hydrolyzed, yields satisfactory agreement, as shown in Table II.

Standard curves were obtained for glucose alone after identical acid treatment followed by neutralization. In each case, these curves matched precisely the standard curves obtained for glucose directly. It is evident that glucose itself is unaffected when submitted to the acid treatment finally adopted for maltose hydrolysis.

Accordingly, the conditions for the determination of unknown mixtures of glucose and maltose were established as follows:

A sample containing not more than 500 mg. of glucose and maltose is accurately weighed out and made up to 100 ml.; 50 ml. of this are taken and made up to 100 ml. with water, and 2 ml. of this solution, which contains not more than 5 mg. of the mixed sugars, are used for direct analysis without hydrolysis. To it are added 5 ml., of 0.02 *M* potassium dihydrogen phosphate and 10 ml. of 7.5% ammonium molybdate. The solution is adjusted to the mark in 25-ml. volumetric flasks and mixed and the stoppers are removed. The flasks are introduced into a preheated autoclave and covered with a piece of sheet metal to prevent any condensation, then heated for exactly 30 minutes with open steam at 100° C. to develop the blue color. They are removed after the heating period and plunged immediately into an ice bath to arrest the reaction; when cooled to room temperature, the volumes are adjusted to the mark if necessary. The color is read in a Coleman Model II spectrophotometer at a wave length of 650 mμ. This reading gives value *B*. To the other 50 ml. of original solution are added 3 ml. 10% hydrochloric acid, and hydrolysis is carried out in an autoclave at 68-kg. (15 pounds) pressure for 1 hour. The flask is cooled, and the solution is neutralized with 0.1 *N* sodium hydroxide to the methyl red end point and made up to 100 ml. The presence of methyl red in the neutralized solution has no effect on the subsequent colorimetry. Two milliliters of this solution are taken and treated for development of the blue color in exactly the same way as the unhydrolyzed sample. This gives value *A*.

In order to evaluate with some precision the maltose and glucose content of unknown mixtures from the two colorimeter readings *B* and *A*, the method of mean squares is employed to analyze the most probable curve for both sugars.

Table II. Analysis of Known Mixtures of Maltose and Glucose

Maltose *Mg.*	Glucose *Mg.*	Transmittancy after Hydrolysis %	2 − log *G*	Recovery *Mg.*
5	0	36.7	0.435	4.95
4	1	36.8	0.433	4.95
3	2	36.2	0.440	5.0
1	4	36.1	0.441	5.0
0	5	36.2	0.440	5.0

Equations 1 and 2 are solved from the actual observed values for x and y listed in Table III.

$$\Sigma ny - m\Sigma x - nb = 0 \quad (1)$$

$$\Sigma xy - m\Sigma x^2 - b\Sigma x = 0 \quad (2)$$

By substitution in Equations 1 and 2, Equations 3 and 4 are obtained for maltose and Equations 5 and 6 for glucose.

$$0.254 - 27m - 9b = 0 \quad (3)$$

$$0.908 - 96m - 27b = 0 \quad (4)$$

Table III. Standard Curves

x	y	x^2	xy
	Maltose Standard Curve		
1.0	0.010	1.0	0.010
1.5	0.014	2.25	0.021
2.0	0.018	4.0	0.036
2.5	0.023	6.25	0.058
3.0	0.027	9.0	0.081
3.5	0.032	12.25	0.112
4.0	0.038	16.0	0.152
4.5	0.044	20.25	0.198
5.0	0.048	25.0	0.240
$\Sigma x = 27.0$	$\Sigma y = 0.254$	$\Sigma x^2 = 96.0$	$\Sigma xy = 0.908$
	Glucose Standard Curve		
1.0	0.106	1.0	0.106
1.5	0.147	2.25	0.221
2.0	0.199	4.0	0.398
2.5	0.230	6.25	0.575
3.0	0.279	9.0	0.837
3.5	0.325	12.25	1.138
4.0	0.359	16.0	1.436
4.5	0.403	20.25	1.814
5.0	0.440	25.0	2.20
$\Sigma x = 27.0$	$\Sigma y = 2.488$	$\Sigma x^2 = 96.0$	$\Sigma xy = 8.725$

$$2.488 - 27m - 9b = 0 \quad (5)$$

$$8.725 - 96m - 27b = 0 \quad (6)$$

Solutions of these pairs of equations yield:

For maltose $m = 0.0097$, $b = -0.00088$

For glucose $m = 0.0840$, $b = 0.0245$

Hence the values of 2 − log transmittance before hydrolysis (B) and after hydrolysis (A) are represented by:

$$B = (0.0840\,G + 0.0245) + (0.0097M - 0.00088) \quad (7)$$

$$A = 0.0840\,(G + M) + 0.0245 \quad (8)$$

Whence

$$A - B = 0.0743\,M + 0.00088 \quad (9)$$

The value of M so obtained is substituted in Equation 8 to evaluate G.

A sample calculation is given in which not more than 5 mg. of a glucose-maltose mixture were taken. The 2 − log G values were $A = 0.435$ and $B = 0.146$.

Solving for M by Equation 9, $M = 3.9$ mg. and for G by Equation 8, $G = 0.98$ mg.

Actually this mixture contained 4 mg. of maltose and 1 mg. of glucose. The experimental results were satisfactory, as the ratio of maltose to glucose was exactly as taken.

This type of determination and calculation may be applied to commercial products to analyze for maltose and glucose. One typical product (Dextrimaltose) was labeled as containing "55% maltose as total reducing sugars." Analysis by the Munson and Walker method gave a value of 57.5% as total reducing sugars. By using the method outlined in this paper, and resolving the equations, the results obtained were: 47.7% maltose and 10% glucose.

Such a procedure is rapid and extremely useful in cases where the ratio of maltose to glucose must be controlled, as in the preparation of partially hydrolyzed starch products. In these instances, it is necessary to remove oligosaccharides and dextrins of higher molecular weight in a quantitative manner prior to the determination.

LITERATURE CITED

(1) Benham, G. H., and Despaul, J. E., ANAL. CHEM., 20, 933–5 (1948).

RECEIVED October 10, 1948.

Determination of Lactose in Milk Products

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The ferricyanide method may be used for the determination of lactose and sucrose in dairy products. It is simple, convenient, and time-saving, and requires no special equipment. The method can be used for the analysis of dairy products containing lactose and lactose in the presence of sucrose, but cannot be used when the products contain other reducing sugars.

THE unsatisfactory nature of existing methods for the determination of maltose in flour led Blish and Sandstedt (*2*) to seek a method that possessed reliability, simplicity, convenience, and minimum requirement for special equipment. Accordingly they adapted the ferricyanide method of Hagedorn and Jensen (*3*) to the estimation of maltose in flour. This adaptation was modified by Sandstedt (*4*), who also applied it to the determination of sucrose in flour (*5*).

In view of the agreement in the maltose values secured by the ferricyanide method as compared to the official method of the association of official agricultural chemists (*1*), the procedure has been extended to the determination of lactose in milk and milk products. Therefore, comparisons were made by both the ferricyanide and the official method on the lactose determination in milk and milk products.

REAGENTS

Acid Buffer Solution. Dissolve 3 ml. of glacial acetic acid, 4.1 grams of anhydrous sodium acetate, and 4.5 ml. of sulfuric acid (specific gravity 1.84) in water and dilute to 1 liter with water.

Sodium Tungstate, 12%. Dissolve 12.0 grams of sodium tungstate dihydrate in water and dilute to 100 ml. with water.

Acetic Acid–Salt Solution. Dissolve 70 grams of potassium chloride and 400 grams of zinc sulfate heptahydrate in water, add slowly 200 ml. of glacial acetic acid, and dilute to 1 liter with water.

Soluble Starch–Potassium Iodide Solution. Suspend 2 grams of soluble starch in a small quantity of cold water and pour slowly into boiling water with constant stirring. Cool thoroughly (or the resulting mixture will be dark colored), add 50 grams of potassium iodide, dilute to 100 ml., and add one drop of saturated sodium hydroxide solution.

Thiosulfate Solution, 0.1 N. Dissolve 24.82 grams of sodium thiosulfate pentahydrate and 3.8 grams of borax and make up to 1 liter. Standardize against pure copper. Dissolve 45 to 55 mg. of pure copper in 2 ml. of concentrated nitric acid, and heat carefully until brown fumes are driven off. Dilute to 10 ml. with water. Add concentrated ammonium hydroxide a drop at a time until the last drop produces a deep blue solution. Add 5 ml. of concentrated acetic acid and 1 ml. of potassium iodide–starch solution and titrate with the thiosulfate solution until the starch-iodide color fades out. From the milligrams of copper and the milliliters of thiosulfate used the normality can readily be calculated.

Alkaline Ferricyanide Solution, 0.1 N. Dissolve 33 grams of pure dry potassium ferricyanide and 44 grams of anhydrous sodium carbonate and dilute to 1 liter. To standardize, add to 10 ml. of this solution 25 ml. of acetic acid–salt solution, and 1 ml. of soluble starch–potassium iodide solution, and titrate with 0.1 N thiosulfate. Exactly 10 ml. should be required to discharge the blue color.

STANDARD LACTOSE CURVE

A water solution of high purity α-lactose was prepared so that 1 ml. contained 50 mg. of the anhydrous sugar. Increasing amounts of this solution were added to 50-ml. volumetric flasks which contained 43 ml. of acid buffer and 2 ml. of sodium tungstate solution. Sufficient water was added, when needed, to give a final volume of 50 ml. The mixture was vigorously agitated and 5-ml. aliquots were immediately added to 10-ml. quantities of the alkaline 0.1 N ferricyanide contained in 50-ml. Pyrex test tubes (18 to 20-mm. diameter). The tubes were immersed in boiling

water, so that the liquid in the tubes was 1.25 cm. (0.5 inch) below the surface of the water.

After 20 minutes the tubes were removed from the hot water bath and cooled in running tap water, and the contents were poured into 125-ml. Erlenmeyer flasks. The tubes were rinsed twice with 12.5-ml. portions of the acetic acid reagent and the rinsings were added to the same flasks into which the previous digests were transferred. Then 1 ml. of 50% potassium iodide–starch mixture was added to the contents of each flask. The samples were titrated against 0.1 *N* thiosulfate solution. Five individual replications were made for each of the five quantities of lactose used.

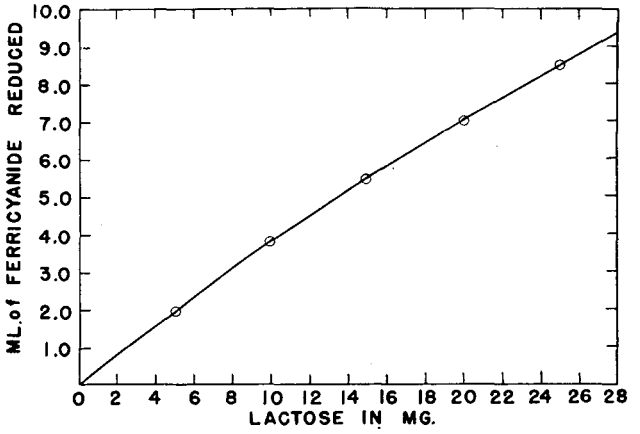

Figure 1. Standard Lactose Curve

The results of the titrations, given in Table I, show the close agreement between the replicate determinations.

Table I. Milliliters of Ferricyanide Reduced

Lactose, Mg.	Replicates 1	2	3	4	5	Av.
5	1.97	1.96	1.96	1.96	1.96	1.96
10	3.78	3.79	3.79	3.82	3.79	3.80
15	5.43	5.41	5.43	5.46	5.44	5.43
20	7.02	6.97	7.02	7.02	6.99	7.00
25	8.48	8.50	8.46	8.44	8.44	8.46

Figure 1 shows a standard ferricyanide-lactose curve that was drawn through the points obtained from the data in Table I. All the following results for the determination of lactose, except those for sweetened condensed milk, were obtained from this curve.

DETERMINATION OF LACTOSE IN MILK

Add a 5-ml. aliquot of the milk sample representing a given weight of the material to 43 ml. of the acid buffer in a 125-ml. Erlenmeyer flask. (Take 5-ml. aliquots of the following milk samples that have been diluted to 20 ml. with water: 10 grams of whole or skimmed milk, buttermilk, cream, or ice cream mix; 5 grams of unsweetened condensed milk; 4 grams of sweetened condensed milk; and 1.5 grams of either dried whole milk or nonfat dry milk solids. Direct weights of the dried milks may be preferable to taking aliquots of prepared suspensions of these materials.) Add 2 ml. of sodium tungstate solution to the mixture, agitate, and immediately filter through a No. 4 Whatman filter paper. Discard the first 10 or 12 drops of the filtrate. Transfer 5 ml. of the filtrate to a 50-ml. Pyrex tube (18- to 20-mm. diameter), and introduce 10 ml. of 0.1 *N* alkaline ferricyanide solution. Immerse the tube and contents in a boiling water bath, so that the liquid in the tube is 0.5 inch below the surface of the boiling water.

After 20 minutes remove the tube from the bath and cool under running tap water. Pour the contents of the tube into a 125-ml. Erlenmeyer flask, rinse the tube twice with 12.5 ml. of the acetic acid reagent, and add the rinsings to the flask. Add 1 ml. of the 50% potassium iodide–starch mixture to the contents of the flask and mix thoroughly. Titrate the mixture against 0.1 *N* thiosulfate solution. Subtract the number of milliliters of thiosulfate used from the milliliters of thiosulfate required for the blank determination.

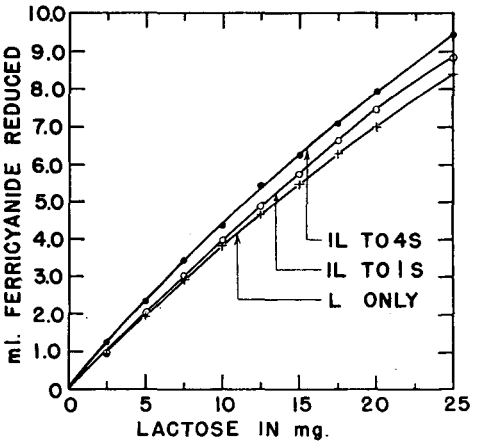

Figure 2. Ferricyanide Reduced

1L. 1 part of lactose
1S. 1 part of sucrose
4S. 4 parts of sucrose

Conduct a blank determination in the absence of milk or sugar by mixing together 43 ml. of acid buffer, 2 ml. of sodium tungstate solution, and 5 ml. of water. Transfer 5 ml. of the mixture to 10 ml. of the alkaline ferricyanide in a test tube, heat this preparation in a boiling water bath, and titrate in the same manner as the regular sample. The lactose in the 5-ml. aliquot is obtained from the standard curve (Figure 1). The lactose value for milk is then readily calculated.

Increasing amounts of lactose were added to constant weights of whole milk and the lactose was determined by both the ferricyanide and official methods.

A comparison of the results obtained by the two methods, given in Table II, shows them to be in excellent agreement; the nonprecipitable milk constituents do not interfere with the ferricyanide oxidation.

Table III shows the results of the determination of lactose in whole milk, several milk products, and ice cream mix by the two methods. The residual copper was titrated with thiosulfate in the presence of potassium iodide and starch instead of directly weighing the copper oxide. The results give a direct comparison between the two procedures. Variations between the official and the ferricyanide methods are no greater than those between duplicate determinations for any particular sample. The agreement between these two procedures is very close.

Table II. Added Lactose Recovered from Whole Milk

Lactose Added	Ferricyanide Total	Ferricyanide Added	Copper Reduction Total	Copper Reduction Added
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0	62	...	67	...
50	110	48	113	46
75	137	75	142	75
100	162	100	167	100
125	185	123	192	125
150	215	153	217	150
175	240	178	243	176
200	...	...	265	198

LACTOSE IN PRESENCE OF SUCROSE

More ferricyanide is reduced when lactose is oxidized in the presence of sucrose than when sucrose is absent. The greater the ratio of sucrose to a given weight of lactose the larger will be the amount of ferricyanide reduced. Therefore, a different set of values must be determined for constructing standard curves when this sugar is oxidized in the presence of sucrose. Table IV, column I, shows the number of milliliters of 0.1 *N* ferricyanide

Table III. Lactose Content of Milk Products

Product	Copper Reduction 1	Copper Reduction 2	Ferricyanide 1	Ferricyanide 2
	%	%	%	%
Whole milk	4.80	4.88	4.84	4.80
Condensed milk	9.76	9.76	9.84	9.68
Dry whole milk	36.2	36.5	36.0	36.2
Nonfat dry milk solids	48.2	48.0	48.0	48.0
Sweetened condensed milk	13.7	13.6	13.7	13.7
Buttermilk	7.86	7.84	7.84	7.92
Ice cream mix	9.60	9.48	9.44	9.48

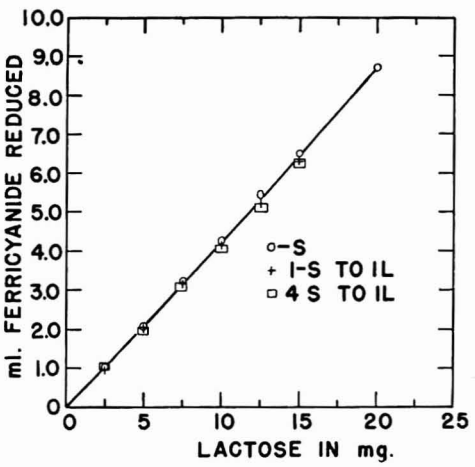

Figure 3. Ferricyanide Reduced

o-S. Sucrose
+ 1-S to 1L. 1 part of sucrose to 1 part of lactose
□ 4S to 1L. 4 parts of sucrose to 1 part of lactose

reduced by increasing quantities of lactose alone, column II for 1 part of lactose to 1 part of sucrose, and column III for 1 part of lactose to 4 parts of sucrose. These data show that if lactose is to be determined in the presence of relatively large quantities of sucrose, standard curves must be made for the lactose in the presence of sucrose in about the same ratios that they are present in the sample being analyzed. Figure 2 shows the curves constructed from the data given in Table IV. The curve for lactose alone was drawn from the data shown in column I, the curve for lactose oxidation in a mixture of 1 part of lactose to 1 part of sucrose from column II, and the curve for 1 part of lactose to 4 parts of sucrose from column III. The approximate quantity of lactose in a given sample may be obtained from the lactose curve constructed from data of column I and the amount of sucrose from the curves shown in Figure 3. This observation gives the ratio of lactose to sucrose in the sample and the true amount of lactose is then obtained from the curve that was constructed for lactose in the presence of this quantity of sucrose. For milk products not supplemented with sucrose the curve for lactose only is used.

SUCROSE IN MILK PRODUCTS

The ferricyanide method as applied to the determination of sucrose in flours by Sandstedt (*5*) may be revised for application to the analysis of milk products that contain added sucrose.

Determination. Dissolve a 2-gram sample of sweetened condensed milk and make to a 100-ml. volume with water (other dilutions may be necessary). After agitating this mixture, add 5 ml. of this solution to a 125-ml. Erlenmeyer flask which contains 43 ml. of the acid buffer and 2 ml. of 12% sodium tungstate solution. Shake the contents of the flask and filter through a No. 4 Whatman filter paper. Discard the first 10 or 12 drops of the filtrate. Determine the milliliters of ferricyanide required to oxidize the lactose in a 5-ml. aliquot of the filtrate.

Transfer a second 5-ml. aliquot of the filtrate to (18- to 20-mm. diameter) 8-inch test tube, place in a boiling water bath for 15 minutes, remove from the bath, cool, and add 10 ml. of the alkaline ferricyanide. Place the tube and contents in the boiling water bath for 20 minutes and proceed according to directions given for the determination of lactose. The milliliters of ferricyanide equivalent to the sucrose in the sample are obtained by subtracting the milliliters of ferricyanide required for the lactose oxidation from the total milliliters required for the inverted sucrose and lactose combined. The amount of sucrose in the 5-ml. aliquot may be obtained from a calibration curve (Figure 3) constructed as given in the following procedure.

STANDARD SUCROSE CURVES

Sucrose and Sucrose Plus Lactose. When lactose was oxidized in the presence of sucrose more ferricyanide was reduced than when it was oxidized alone. Table V shows the milliliters of ferricyanide reduced by increasing amounts of sucrose (I), by 1 part of sucrose in the presence of 1 part of lactose (II), and by 4 parts of sucrose to 1 part of lactose (III). These data show that slightly less ferricyanide is reduced by these levels of sucrose in the presence of lactose than by the same amounts of sucrose alone. Nearly the same quantities of ferricyanide were reduced by the sucrose present in the two different lactose mixtures. Hence the results obtained for either of the two mixtures of sucrose and lactose may be used to construct a calibration curve which can be used for determining the sucrose in mixtures having about these compositions of the two sugars without introducing a very large error. The points plotted in Figure 3 represent the milliliters of ferricyanide reduced by increasing amounts of sucrose in the two mixtures as compared to the curve drawn through the points obtained from the oxidation of the inverted sucrose alone. These results indicate that a calibration curve should be made for sucrose in the presence of lactose of the concentration typical of the product analyzed. The ratio of 4 parts by weight of sucrose to 1 part by weight of lactose was chosen for one set of the experiments because it approximates the ratio of this sugar to the lactose found in sweetened condensed milk.

Table IV. Milliliters of Ferricyanide Reduced

Lactose, Mg.	Ratio of Lactose to Sucrose I 1 to 0	II 1 to 1	III 1 to 4
2.5	0.96	0.97	1.25
5.0	1.96	2.00	2.33
7.5	2.85	2.96	3.43
10.0	3.80	3.90	4.38
12.5	4.66	4.82	5.26
15.0	5.43	5.72	6.10
17.5	6.33	6.66	7.05
20.0	7.00	7.46	7.97
25.0	8.46	8.88	9.45

Table V. Milliliters of Ferricyanide Reduced

Sucrose, Mg.	Ratio of Sucrose to Lactose I 1 to 0	II 1 to 1	III 4 to 1
2.5	1.03	1.07	1.11
5.0	2.07	2.12	2.06
7.5	3.22	3.17	3.11
10.0	4.22	4.21	4.05
12.5	5.44	5.30	5.21
15.0	6.33	6.36	6.31
20.0	8.76	...	...

ACKNOWLEDGMENT

This work was financed in part by a grant from the Roberts Dairy Company, Lincoln, Neb.

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, "Official and Tentative Methods of Analysis," p. 309, 1945.
(2) Blish, M. J., and Sandstedt, R. M., *Cereal Chem.*, **10**, 189–202 (1933).
(3) Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, **135**, 45–58 (1923).
(4) Sandstedt, R. M., *Cereal Chem.*, **14**, 603–4 (1937).
(5) *Ibid.*, **14**, 767–8 (1937).

RECEIVED July 19, 1947. Published with the approval of the director as Paper 426, Journal Series, Nebraska Agricultural Experiment Station.

Evaluation of Paint Films

The Interchemical Adherometer

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A method is presented for resolving the stripping force measurements of organic coatings on metal surfaces into their basic factors of plasticity and adhesion. The application of this method should make the adherometer a useful research tool in the evaluation and formulation of organic finishes.

GREEN and Lamattina (*1*) recently described a new instrument for measuring the adherence of organic coatings to metal surfaces. They stated that "adherence" or "stripping force" as measured by this instrument is not synonomous with "adhesion"; other factors involved include tear resistance, plastic resistance, and mechanical entrapment of the paint.

The instrument, now called the Interchemical adherometer, measures the force required for a sharpened ivory knife to remove or strip a 4-mm. width of film from the test panel. Smooth-surfaced test panels must be used, and it is not the purpose of this instrument to determine the force with which a coating is held mechanically in pits or pores of a roughened surface.

The stripping force, F, as measured on the instrument can be expressed in terms of its three component forces by the equation

$$F = T + P + A$$

These individual forces may be identified as:

T = tear resistance of film on either side of knife as it cuts its path through coating
P = plastic resistance of film to pushing action of cutting face of knife. This might also be considered a measure of the toughness of the film.
A = force of adhesion at metal-paint interface

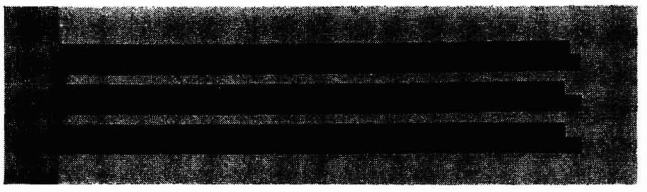

Figure 1. Film Stripping

The previous paper described the method of determining the value of T by shifting the cutting knife exactly 4 mm. laterally, and measuring the force used in stripping a second band of film of the same width, which in this case has only one edge subject to tearing. However, because it is difficult to move the knife by precisely this amount, a more accurate procedure has now been adopted. The knife is shifted somewhat less than 4 mm. for the second cut. By stopping this second cut a little short of the first one, as shown in Figure 1, the width of both cuts can be accurately measured, using a low-power microscope with hairline eyepiece. A sliding stage is provided, whose motion is controlled by a micrometer reading to 0.01 mm. By taking readings as the hairline falls consecutively on the first edge, the stepped-in edge, and the last edge of the cut, the accurate width of both strips is obtained. This has the added advantage of saving the rather time-consuming job of grinding the ivory knives to exactly 4-mm. width.

It is evident that component force T is independent of strip width whereas P and A are directly proportional thereto. Therefore, as a result of the two readings, it is possible to set up two

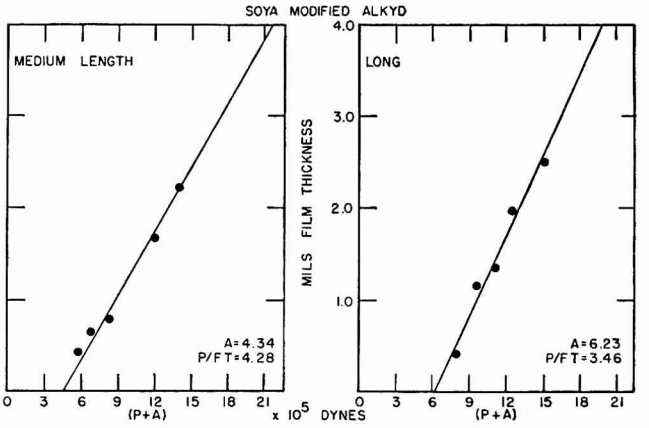

Figure 2

simultaneous equations and eliminate T (W_1 and W_2 being the widths of the first and second strips, respectively).

$$F_1 = T + (P + A)$$

$$F_2 = 1/2T + \frac{W_2}{W_1}(P + A)$$

$$2F_2 = T + \frac{2W_2}{W_1}(P + A)$$

$$2F_2 - F_1 = \left(\frac{2W_2}{W_1} - 1\right)(P + A)$$

$$P + A = \frac{2F_2 - F_1}{\frac{2W_2 - W_1}{W_1}} = \frac{W_1(2F_2 - F_1)}{2W_2 - W_1}$$

The figure for $P + A$ here obtained will be that for strip width W_1, from which it readily follows that the value for a unit width of 1 mm. will be $(P + A)/W_1$, or $(2F_2 - F_1)/(2W_2 - W_1)$.

All methods of measuring the stripping force of paint films which have been used up to this time, including the time-honored thumbnail test, must necessarily result in a value which is the sum of these two basic factors. This, however, does not provide the fullest information for a true evaluation of the film. A highly plasticized film might have good adhesion with a low toughness value. In a different formulation the reverse might be true, yet the sum of the two, as represented by the measured stripping force, could be the same in the two cases. Either condition might be entirely desirable for the specific application for which it was designed, but separate values for each component factor would be of greatest importance to the formulator.

With the present instrument, it is possible to do this on the following basis:

Adhesion factor A is purely an interfacial attraction between the upper surface of the metal and the under surface of the paint film. It should be independent of the film thickness of the applied paint.

Plastic resistance factor P is an intrinsic property of the film regardless of the metal on which it is applied. The value obtained, however, should be directly proportional to the thickness of the film being stripped.

The validity of these assumptions was proved over a wide range of typical organic coatings by preparing a set of four or five panels of each formula with a successively increasing number of applied coats in order to obtain a varying set of film thicknesses. The metal used throughout was 24-gage cold-rolled steel. The stripping forces for these films were now measured and the value of $P + A$ was calculated as previously described. If these values for $P + A$ are now plotted against the corresponding film thicknesses, they should show a straight-line relationship, due only to the varying values of the P factor. The A factor, remaining constant throughout the series, should merely cause a lateral shift in the curve to the right of the origin. Its exact value will then be represented by the distance from the origin to the intercept of the curve with the horizontal axis (Figure 2).

These results are in close agreement with the previously outlined theories. In order to eliminate any personal factor, the location of the curve which best fits the recorded points is calculated from the data by the method of least squares. After the value of the adhesion factor, A, is determined the value of P easily follows by subtraction of A from each value of $(P + A)$. The final step is to reduce the varying values of P to its specific value for a unit film thickness of 1 mil—in other words, calculate the average value for P/FT (FT representing film thickness). This term, which is actually represented by the slope of the curve, can be considered a measure of the film's toughness or resistance to deformation. The values shown in Figure 3 are for strips of 4-mm. width.

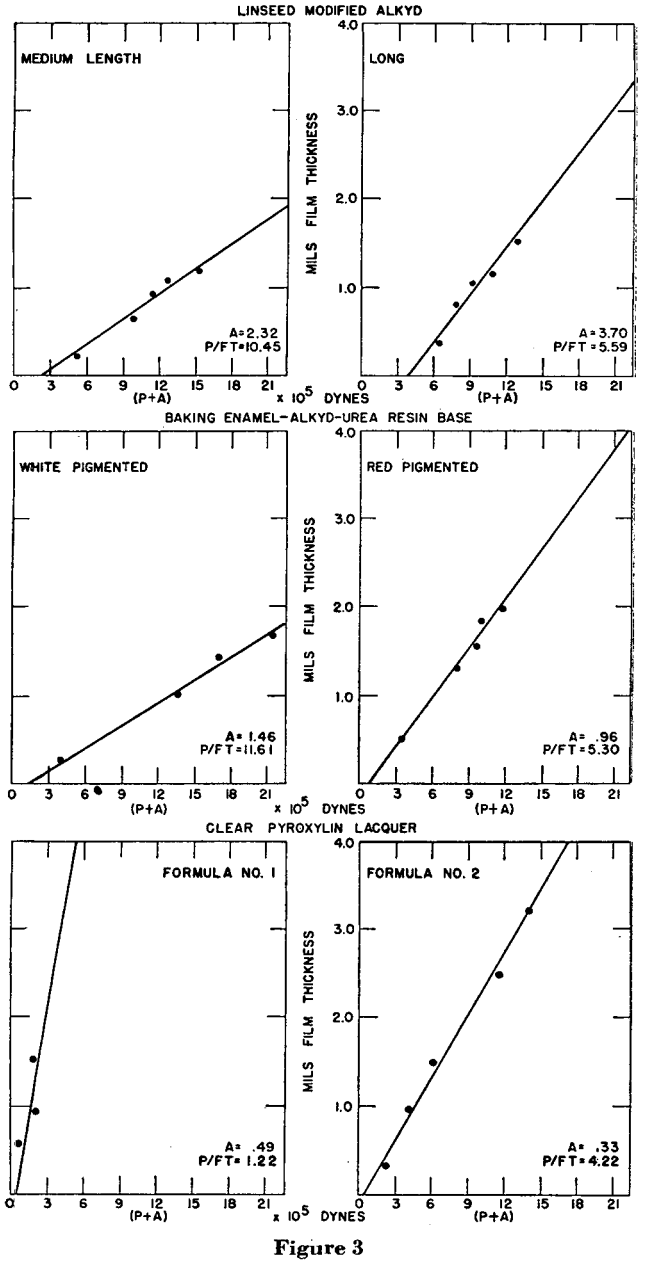

Figure 3

Table I. Stripping Force

Metal	A, Dynes	P/FT, Dynes
Cold-rolled steel	4.54×10^5	5.73×10^5
Hot-rolled steel	1.13×10^5	5.62×10^5
Stainless steel	5.56×10^5	6.29×10^5
24ST aluminum alloy	3.06×10^5	5.65×10^5
Electrolytic galvanized iron	0.17×10^5	3.14×10^5

A further confirmation of the theory is attained by extending the investigation to films on other metals besides the cold-rolled steel used in the previous work.

The case of a single paint formulation applied to a variety of metals may be considered. For each metal, a set of panels is again prepared by varying film thickness, so that in each case the separate values of A and P can be calculated as above. It is to be expected that the adhesion factor will now show a varying set of values as we pass from one metal surface to another. On the other hand, the plasticity or toughness factor, being a specific property of the film composition, should not be affected by the nature of the surface on which the paint is applied; hence it should maintain a constant value.

An air-drying, gray pigmented, alkyd paint was selected, and sets of test panels were prepared on cold-rolled steel, hot-rolled steel, stainless steel, 24S-T aluminum alloy, and galvanized iron. Stripping force measurements were carried out, and from the data the values of A and P/FT were calculated. The results are shown in Table I.

The plasticity value of the film on the first four metals maintains a very uniform figure, averaging 5.80×10^5 dynes, while the force of adhesion covers a range from 1.13 to 5.56×10^5 dynes, as we pass from one metal to another. The results are, therefore, entirely in agreement with the deduced theory.

The set of galvanized panels, however, presents a value for P/FT which is out of line with the other readings. Here we are dealing with an extremely low adhesion factor. The result was that, in the stripping operation, the cohesion of the film was so much greater than the adhesion, that portions of the film were loosened and flaked off for appreciable distances beyond the sides of the cutting knife, producing jagged edges and a strip of uneven width. A true value for P/FT is thus not obtainable in this particular case. The authors believe, however, that this does not invalidate the theory, as evidenced by the results with other metals which show a reasonable degree of adhesion for the paint film.

ACKNOWLEDGMENT

The work described in this paper is part of a general investigation of the adhesion of organic coatings on metal surfaces, conducted by Interchemical Corporation Research Laboratories under contract with the U. S. Navy Department, Washington, D. C., for the Bureau of Ships. The authors gratefully acknowledge permission granted by the U. S. Navy Department to publish this work. The opinions herein expressed are those of the authors, and are not to be construed as reflecting the views of the Navy Department.

LITERATURE CITED

(1) Green and Lamattina, ANAL. CHEM., 20, 523 (1948).

RECEIVED November 4, 1948. Presented before the Division of Paint, Varnish, and Plastics Chemistry at the 114th Meeting of the AMERICAN CHEMICAL SOCIETY, Washington, D. C.

Analysis of Hydrocarbon Mixtures

Application of Barrett Distillation

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A simple analytical procedure for the determination of small amounts of impurities in aromatic hydrocarbon mixtures is presented. The method was developed for the analysis of ethylbenzene containing small amounts of benzene and diethylbenzene and is based on the influence of impurities on the true boiling point of the major component where the impurities boil at temperatures considerably removed from that of the main constituent.

MOST of the styrene produced is made by the catalytic dehydrogenation of ethylbenzene (*5*), which may be produced by the catalytic alkylation of benzene with ethylene. The alkylate, containing benzene, ethylbenzene, diethylbenzene, and higher polyethylbenzenes, is fractionally distilled to recover ethylbenzene of high purity. In this distillation it is vitally important that the diethylbenzene content of the product be kept at the lowest possible value. In the dehydrogenation step, ethylbenzene is partially converted to styrene and any diethylbenzene present is partially converted to divinylbenzene. The presence of divinylbenzene in the dehydrogenated mixture can be harmful if the styrene is recovered by fractional distillation, because divinylbenzene will copolymerize with styrene to form a cross-linked insoluble polymer. The presence of as little as 0.01% divinylbenzene will create this form of polymer (*6*). In practice it has been found that if the diethylbenzene content of the ethylbenzene is kept below 0.03% there are no serious consequences in plant operation.

Various methods have been developed for the determination of small amounts of diethylbenzene in ethylbenzene. Quantities as low as 0.045% by weight have been determined by mass spectrometric methods with indications that amounts as small as 0.003% by weight could be detected (*3*, *9*). There is evidence that the sensitivity in spectrographic methods is low, in the range of 0.5 weight % (*10*). As little as 0.011% by weight can be determined by a method which combines ebulliometric measurements with fractional distillation (*7*). The German ethylbenzene industry employed a Barrett distillation method by which amounts less than 0.05% by weight (*4*, *8*) were determined. Information available indicates that this German method is similar to the method described in this paper.

This paper presents a simple analytical procedure which was developed for the analysis of ethylbenzene containing small amounts of benzene and diethylbenzene. The method can be handled by laboratory technicians and is based on the influence of minor components on the boiling range of the mixture where the minor components boil at temperatures considerably removed from the boiling point of the main constituent. The method appears to be capable of general application for the determination of small amounts of impurities in aromatic hydrocarbon mixtures.

APPARATUS

The apparatus consists of a 200-ml. Barrett distilling flask, a 600-mm., West, improved-type, Pyrex condenser supported by a ring stand, a 100-ml. graduate, an asbestos board 15 × 15 cm. (6 × 6 inches) with a 3.75-cm. (1.5-inch) hole supported on a ring stand, a Tirrill type burner, and an M.C.A. R-3 thermometer with a 70° to 160° C. range graduated in 0.2° divisions. It is assembled by the A.S.T.M. method (*2*) for distillation of industrial aromatic hydrocarbons.

[1] Deceased.

Table I. Effect of Benzene on Distillation Range of Ethylbenzene

Weight % Benzene Added	Temperature Difference between 5 and 50% Distilled, ° C.
0.0	0.5
1.0	2.0
2.0	3.4
3.0	4.7
5.0	8.0

METHOD

A 100-ml. sample of ethylbenzene is measured in the 100-ml. graduate and carefully transferred to the 200-ml. Barrett distilling flask, allowing 15 seconds for draining the graduate. The flask is placed in position on the asbestos board and connected to the condenser so that the side arm of the flask enters into the condenser tube at least 5 cm. (2 inches). The graduate is placed at the end of the condenser to receive the distillate. The thermometer is supported in the neck of the flask by means of a cork stopper in such a position that the top of the mercury bulb of the thermometer is just opposite the lower edge of the side arm and central in the neck of the flask.

Table II. Effect of Diethylbenzene on Distillation Range of Ethylbenzene

Weight % Diethylbenzene Added	Temperature Difference between 50% and Dry Point, ° C.
0.00	0.1
0.01	0.1
0.02	0.2
0.04	0.4
0.05	0.5
0.10	1.0
0.20	1.7
0.50	3.3
1.00	7.7

The flask is heated gently by the burner at a distance of 5 cm. (2 inches) from the bottom of the asbestos board. Heating is continued gently until ebullition has started and vapors reach the bottom of the thermometer bulb, when the flame is withdrawn. This process is repeated twice to permit complete expansion of the mercury.

The flame is then replaced and regulated to give a distillation rate of 5 to 7 ml. per minute, until the dry point is reached. Three temperature readings are recorded during the distillation: 5% distilled, 50% distilled, and dry point. The temperature is followed closely as the dry point is approached and the dry point is recorded as the temperature at which the liquid just disappears from the bottom of the flask.

The temperature difference between 5% distilled and 50% distilled is recorded and the per cent benzene is read from a graph

constructed from values given in Table I. The temperature difference between 50% distilled and dry point is recorded and the per cent diethylbenzene is read from a graph constructed from data given in Table II. This distillation will indicate the amount of benzene to ±0.10% and diethylbenzene to ±0.01% in the ethylbenzene, providing reasonable skill is exercised, particularly in the determination of the dry point.

DEVELOPMENT OF METHOD

Various concentrations of benzene and diethylbenzene in ethylbenzene were prepared using Baker's C.P. thiophene-free benzene, diethylbenzene prepared in this laboratory by fractional distillation, and Monsanto ethylbenzene purified by fractional distillation by M. R. Fenske of Pennsylvania State College. These mixtures were used to determine the effect of benzene and diethylbenzene on the distillation range of ethylbenzene. Data are presented in Tables I and II.

Because it is difficult to obtain a reproducible value for the initial boiling point, the 5 to 50% value is taken for the benzene determination.

Table III. Effect of Isopropylbenzene on Determination of Diethylbenzene in Ethylbenzene

Weight % Isopropylbenzene Added	Temperature Difference between 50% and Dry Point, ° C.
0.0	0.2
0.1	0.2
0.2	0.4
0.3	0.6
0.4	0.5
0.5	0.7

Ethylbenzene used contained 0.1% diethylbenzene.

The method is limited by the quantity of benzene and diethylbenzene in ethylbenzene. If benzene exceeds 3%, the 50% temperature reading may be suppressed, leading to a higher and incorrect value for diethylbenzene. The diethylbenzene content cannot be determined accurately above a concentration of 0.2%. Above this value the temperature is rising too rapidly at the dry point to give reproducible values.

Thermometer corrections, emergent stem corrections, and barometric pressure corrections may be ignored, as all results are based on temperature differences.

A comparison between this Barrett distillation and the shielded A.S.T.M. distillation (*1*) showed that more reproducible dry points could be obtained using the unshielded flask distillation. It was necessary to look directly down onto the flame when observing the dry point using the A.S.T.M. shielded distillation equipment, and thus more difficult to observe the true dry point.

The presence of small quantities of other impurities may interfere with the determination. This has been shown by the addition of isopropylbenzene to the sample. A sample of ethylbenzene containing 0.01% diethylbenzene was used in determining the effect of variable quantities of isopropylbenzene on the distillation. Values are presented in Table III.

To a sample of pure ethylbenzene were added 0.3% isopropylbenzene and 0.05% diethylbenzene. The 50% to dry point range was 1.0° C. For 0.3% isopropylbenzene alone the 50% to dry point range is 0.5° C. For 0.05% diethylbenzene the 50% to dry point range is 0.5° C. When both isopropylbenzene and diethylbenzene are present together in ethylbenzene they have a cumulative effect on the 50% to dry point range, each exerting its own individual effect just as though the other were not present.

The higher boiling impurity of a ternary mixture consisting of a major component and two impurities can be determined with greater accuracy than the lower boiling impurity by this distillation method. This is evident in the temperature spread shown for benzene and diethylbenzene in ethylbenzene. Further evidence of the lower limit characteristic is shown by data on the presence of toluene in ethylbenzene presented in Table IV.

Table IV. Effect of Toluene on Distillation Range of Ethylbenzene

Weight % Toluene Added to Ethylbenzene	Temperature Difference between 5 and 50% Distilled, ° C.
0.0	0.2
0.8	0.3
1.6	0.6
2.4	0.8
3.2	0.9

CONCLUSION

Utilizing the method described, benzene may be determined in ethylbenzene with an accuracy of ±0.10%, provided the concentration does not exceed 3.0%. Diethylbenzene may be determined in ethylbenzene with an accuracy of ±0.01%, provided the concentration is not greater than 0.20%. This method is applicable to the analysis of similar ternary hydrocarbon mixtures. Traces of a compound boiling above the main component can be determined with greater accuracy than one boiling below the main product. The presence of a fourth constituent similar in character to one of the impurities being determined interferes with the determination.

The 50% point was steadily depressed with increasing concentrations of toluene. The upper limit method, however, is applicable to a limited extent for the presence of ethylbenzene in toluene and toluene in benzene, as shown by the data in Table V.

Table V. Effect of Ethylbenzene in Toluene and of Toluene in Benzene

Weight % Ethylbenzene Added to Toluene	Temperature Difference between 50% and Dry Point, ° C.
0.0	0.05
0.1	0.20
0.2	0.50
0.3	0.65
0.4	0.80
0.5	0.95
Weight % Toluene Added to Benzene	
0.0	0.2
0.5	2.1
1.0	3.9
2.0	6.0

LITERATURE CITED

(1) Am. Soc. Testing Materials, *Method* D 850–47.
(2) *Ibid.*, D 86–46.
(3) Johnsen, S. E. J., ANAL. CHEM., **19**, 305 (1947).
(4) Livingston, J. W., "German Report on Ethylbenzene, Styrene, and Polystyrene, at Ludwigshafen, Germany," Technical Industrial Intelligence Committee *Rept.* **RM-332**, June 1945.
(5) Mitchell, J. E., Jr., *Trans. Am. Inst. Chem. Engrs.*, **42**, 293 (1946).
(6) Staudinger, H., and Heuer, W., *Ber.*, **67**, 1164 (1934).
(7) Swietoslawski, W., "Ebulliometric Measurements," pp. 135–40, New York, Reinhold Publishing Corp., 1945.
(8) Technical Industrial Intelligence Committee (Rubber Subcommittee) "Styrene Production at Huls, Germany," *Rept.* **RM-303**, Sept. 27, 1945.
(9) Washburn, H. W., Wiley, H. F., and Rock, S. M., IND. ENG. CHEM., ANAL. ED., **15**, 541 (1943).
(10) Woernley, D., and Munch, R. H., "Analysis of Ethylbenzene for Diethylbenzene by Spectrographic Methods," report submitted to Rubber Reserve Corp. by Monsanto Chemical Co.'s Central Research Department, July 20, 1943.

RECEIVED December 27, 1948. Presented at the Fourth Southwest Regional Meeting of the AMERICAN CHEMICAL SOCIETY, Shreveport, La., December 10 and 11, 1948.

DETERMINATION OF HYDROGEN

Universal Gasometric Micromethod

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A new universal gasometric micromethod is presented for the determination of hydrogen in organic, inorganic, and metal-organic compounds and low melting metals. The basis of the method is the evolution of the hydrogen in the sample within a sealed iron capsule and the complete diffusion of the liberated hydrogen through the walls of the capsule into a simple vacuum system. The procedure is rapid; a complete determination can be made within an hour. The average error (0.2%) is comparable with that of the standard combustion procedure (0.3%). There are no interferences from other elements.

THE many difficulties of the combustion procedure for the determination of hydrogen (carbon and hydrogen determination) are well known. Many attempts (*4*) have been made to simplify it and to adapt it to a universal method independent of composition of the sample. This has resulted in numerous modifications (*4*) to eliminate interferences from elements such as nitrogen by use of lead peroxide with all its attendant difficulties, the halogens and sulfur by use of the "universal" filling, etc. The high vacuum fusion procedures (*1*, *3*, *5–7*) used in metallurgical analyses involve extensive and intricate equipment, and are not adaptable to organic and inorganic compounds or low melting metals. The method described in the present paper obviates these difficulties, and is generally applicable for the determination of total hydrogen in organic, inorganic, and metal-organic compounds and the low melting metals.

The basis of the present method is the evolution of the hydrogen in the sample within a sealed iron capsule and complete diffusion of the liberated hydrogen through the walls of the capsule into a simple vacuum system. The hydrogen is then determined by measuring the reduction in pressure which occurs in a static system when the hydrogen is converted into water over hot copper oxide. Because hydrogen is the only gas that diffuses through the walls of the capsule at the operating temperature (700° C.), interferences by other elements are eliminated. To ensure the complete reduction of the sample and the products of the pyrolysis such as water, an excess of hydrogen-free magnesium turnings or sodium is sealed into the capsule. This procedure has proved satisfactory for all samples, organic and inorganic, to which it has been applied thus far. In the case of sodium hydroxide, which was selected as a representative inorganic compound whose hydrogen content could not be determined by available methods because of its stability toward thermal decomposition, oxidation, or reduction, an excess of distilled sodium was used as the reducing agent. The following reaction takes place quantitatively under the conditions of the determination:

$$2Na + 2NaOH \xrightarrow[450^\circ C.]{} 2Na_2O + H_2.$$

APPARATUS

Vacuum System. The simple vacuum system employed is diagramed in Figure 1. A photograph of the equipment is given in Figure 2.

The furnace tube, *C*, can be of Pyrex and is cooled by a blast of compressed air. The iron capsule, *B*, is supported on a molybdenum wire rack, *A*. The thermocouple gage, *D*, is used for the qualitative indication of the pressure in the system and as a leak detector. The cold trap, *E*, is cooled with liquid nitrogen, which has been found more convenient to use than dry ice mixtures. The double McLeod gage, *F*, provides for two ranges of pressure measurements, 2 and 10 mm. For some applications where

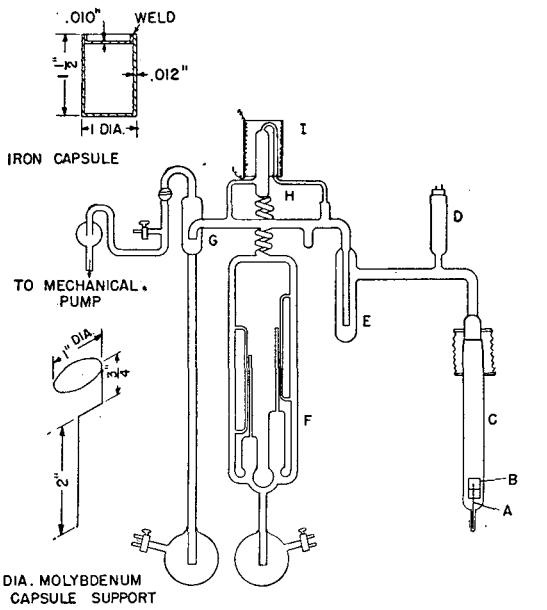

Figure 1. Vacuum Apparatus

larger amounts of hydrogen are to be measured, a suitable manometer may be used. The McLeod gage is connected to the system by a spiral to reduce the risk of breakage. The mercury cut-off, *G*, is preferred to a stopcock for a static system. The copper oxide tube, *H*, is made of quartz in order to withstand the rapid heating and cooling which it undergoes. Because the adjacent graded seals fatigue after a short time, the copper oxide tube is supported by a glass helix, as shown. With such a device, the copper oxide tube has been used continuously for over a year. The vacuum is produced by a mechanical pump only, because a vacuum of greater than 1 to 2 microns is unnecessary. The original apparatus included a Toepler pump, but this was eliminated, as the thermal cycling is sufficient to convert the hydrogen in the system to water in 35 minutes. The Toepler pump decreased this time by only 5 minutes.

Iron Capsules. The iron capsules (Figures 1 and 3), are the shells of 6C5 metal radio tubes, with the flange machined off. The covers are a press-fit cap stamped from 10-mil sheet iron. It is estimated that the capsules have withstood pressures as high as 4 atmospheres. In many hundreds of analyses, only one rupture has been encountered. In this instance, too large a sample of very low hydrogen content was used and the capsule heated too rapidly, so that an estimated internal pressure of 10 atmospheres was reached. The capsule ruptured on the side and not at the weld, and the apparatus was not broken.

Welding. The closure is made with a simple shielded argon arc welding technique, utilizing a tungsten electrode. Direct current is preferable, although 110-volt alternating current can be used. For rapid heat conduction, the capsules are held in a copper block

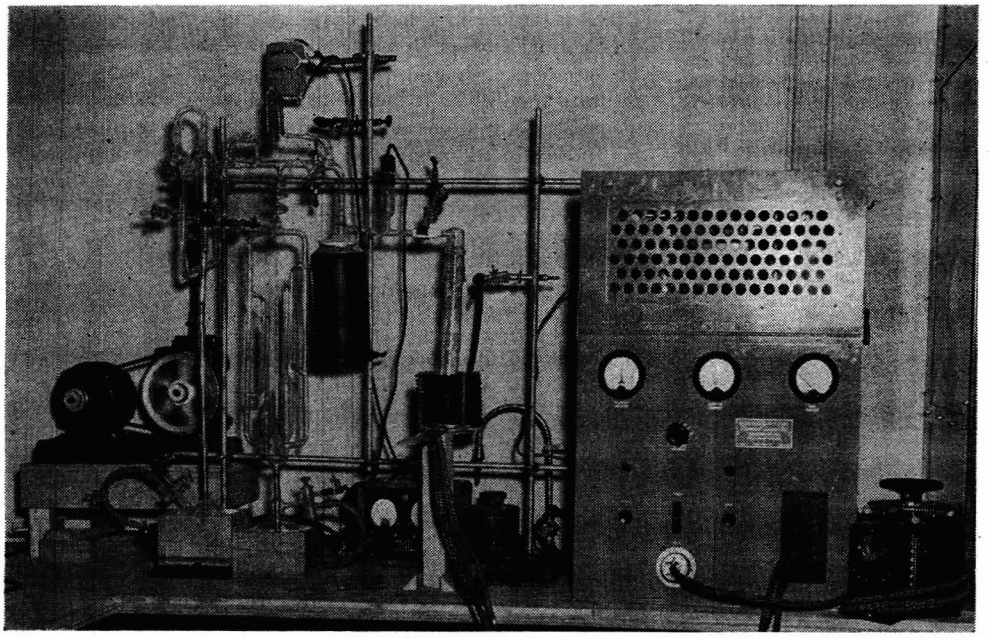

Figure 2. Apparatus for Microdetermination of Hydrogen

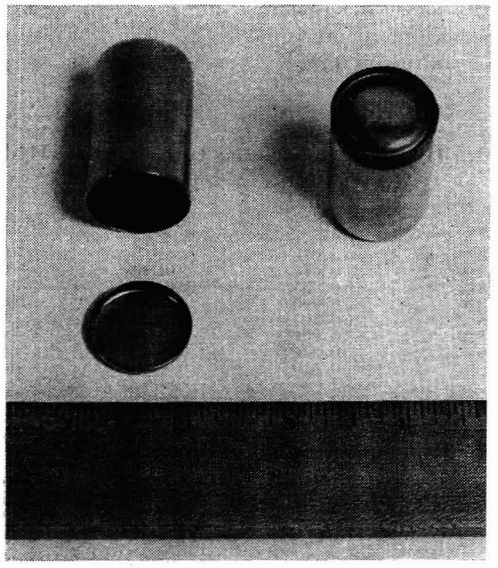

Figure 3. Iron Capsules

(Figure 4) which is rotated by a 1 r.p.m. Telechron motor. An easily constructed converter unit is diagramed in Figure 5. The selenium rectifiers are G.E. Type 18 HI with 8 plates. The best welds were obtained with 5 to 6 amperes across the arc. The complete sample preparation from weighing to introduction into the vacuum system requires less than 5 minutes.

Induction Heating. The capsules are heated inductively to the operating temperature (700° C.). However, the actual temperature is not critical and a variation in the range from 650° to 750° C. has no effect upon the reaction.

PROCEDURE

Capsule Preparation. The capsules and lids are degreased by washing with acetone and thoroughly dried in the oven. They are then degassed in the apparatus by pumping out the system to 2 to 3 microns, and heating the capsules to 700° C. with continuous pumping until the pressure again drops to 2 to 3 microns. The capsules are allowed to cool in the vacuum and tank helium is bled into the vacuum system until atmospheric pressure is attained. The capsules are then stored in a desiccator until used, care being taken not to touch them with the fingers. In actual practice, the capsules are degassed two at a time and enough are prepared (8 to 10) for the day's analyses.

Sampling. Standard Sodium Hydroxide Samples. Standard samples were made up by weighing out sodium hydroxide of known composition into the iron capsules, adding a small bulb containing hydrogen-free, triple-distilled sodium (ca. 1 gram), which had its drawn tip broken off just previous to the addition and then welding the capsule shut. The procedure proved to be simple and reproducible. The standard sodium hydroxide was prepared by allowing C.P. flakes to come to equilibrium with the atmosphere of a large desiccator containing silica gel. The titer, carbonate content, and moisture content (by difference) were determined on large samples. From these data it was calculated that there was 2.48% of hydrogen in the sodium hydroxide. The theoretical value for pure sodium hydroxide is 2.52%. There has been no significant change in the hydrogen value of this material for more than 6 months.

Raw Sodium Samples. Sodium is used as an example of a low melting metal with a high vapor pressure at moderate temperatures. Samples of raw brick sodium were taken from the interior of a brick inside a dry box with a dry helium atmosphere. The samples were cut out of the center of the brick, placed in the iron capsules, and weighed on a torsion balance, and the cap was pressed into place. The capsules were placed in a desiccator inside the dry box and removed from the desiccator only for welding.

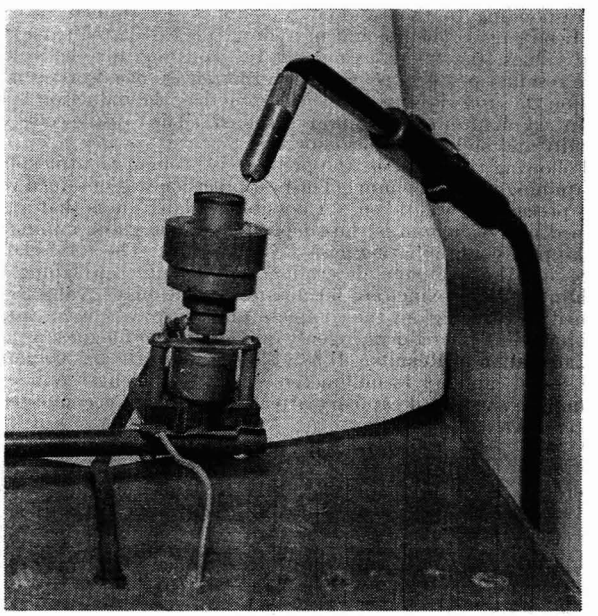

Figure 4. Shielded Argon Arc Welding Apparatus

Other Samples. The various compounds were weighed by difference into the iron capsules. An excess of hydrogen-free magnesium turnings was then added and the capsule was welded. In all cases the capsules were stored in a desiccator until used.

Evolution of Hydrogen. The sealed capsule is introduced into the vacuum system and with the copper oxide heated to 400° C. the system is pumped down to 1 to 2 microns' pressure. This precaution of degassing the copper oxide is essential to obtain the best

precision. When the desired pressure is obtained, the system is closed with the mercury cut-off and the copper oxide tube is cooled to room temperature. The cooling is accelerated with an air blast. During this interval, the thermocouple gage is checked to ascertain the tightness of the system. The capsule is then heated somewhat slowly to 700° C., over a period of 5 minutes, in order for the hydrogen to diffuse through the walls of the capsule as it is generated, so that an excessive pressure is not built up inside the capsule. The pressure is read at intervals on the McLeod gage.

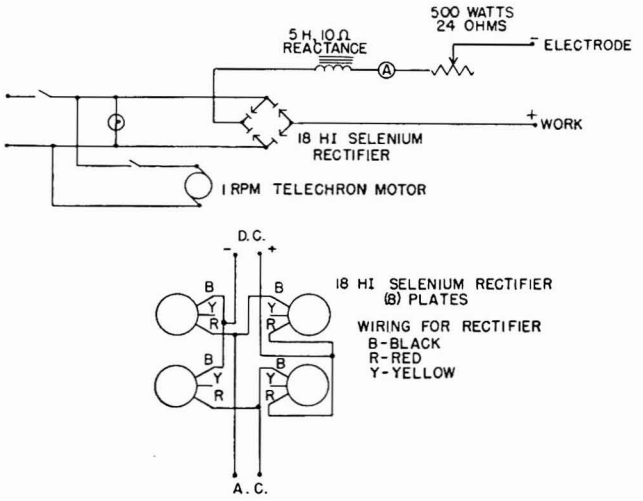

Figure 5. Wiring Diagram of Converter for Shielded Argon Arc Welder

When a constant reading (h_t) is attained (usually in 15 to 20 minutes), the furnace which has been kept at a temperature of 350° C. for rapid heating is lowered over the copper oxide tube. The hydrogen is oxidized to water and frozen out in the cold trap. The complete conversion requires about 35 minutes. The pressure is read at intervals until a minimum value is reached. After some experience, the thermocouple gage will serve to indicate complete conversion but not the actual pressure. The reading (h_r) corresponding to the residual pressure is determined with the McLeod gage. The amount of hydrogen is calculated from these two values, the blank value and the constant for the system. The residual pressure is caused by the release of adsorbed nitrogen on the iron capsule. In actual practice, the induction heater is turned off after equilibrium is attained. The capsule cools very rapidly and no back-diffusion occurs.

Following the analysis, the pressure is brought to atmospheric by running in tank helium. The best precision was obtained when this precaution was taken. A possible explanation is that an adsorbed film of helium is thus deposited on the glass, minimizing pick-up of contaminating gases from the air. The total elapsed time for the complete determination, including weighing and welding, is approximately 60 minutes. The blank value is obtained by carrying out the complete procedure without a sample but including the reducing agent—i.e., sodium or magnesium.

Calculation of Results. If h_t is the length of the gas column in the sealed capillary in millimeters and h_r is the length of the gas column in the sealed capillary after the hydrogen is converted to water, then

$$(h_t^2 - h_r^2)k_1 = P_{H_2} \quad (1)$$

where P_{H_2} is the pressure of hydrogen in millimeters of mercury and k_1 is the McLeod gage constant. Accordingly,

$$\text{Mg. of } H_2 = (h_t^2 - h_r^2)k_1k_2 - b \quad (2)$$

where

$$k_2 = \frac{V}{T} \times \frac{273}{760} \times \frac{2.016}{22.415} \quad (3)$$

b = blank, mg. of H_2
V = volume of system
T = temperature, ° A., of the system at time of measurement

In actual practice the equation

$$\text{Mg. of } H_2 = (h_t^2 - h_r^2)K - b \quad (4)$$

is used where K is the experimentally determined constant for the entire system. K is determined by carrying known amounts of standard sucrose through the procedure, using magnesium turnings as the reducing agent.

The variations in T have a negligible effect on the value of K over normal ambient room temperatures and can be neglected. The use of the experimentally determined value gives the most accurate results, for it includes such unknown variables as the temperature variations in the apparatus, deviations from a perfect gas, precise determination of the McLeod gage constant, and adsorption of hydrogen in the apparatus. The experimentally determined value for K for the authors' apparatus is 7.97×10^{-6} when h_t and h_r are measured in millimeters.

EXPERIMENTAL RESULTS

Table I gives the recoveries of hydrogen from sodium hydroxide with triple-distilled sodium as the reducing agent. The amount of sodium present in each determination was approximately 1 gram. To minimize the pick-up of water, the samples were weighed rapidly on a damped analytical balance. Accordingly, the sample weights are accurate only to the nearest 0.1 mg. but because of the very favorable factor (2.48%), the hydrogen values are given to the nearest 5 micrograms.

Table II gives some hydrogen values obtained from samples of raw brick sodium taken from the interior of the brick as described above.

The data in Table III indicate the wide applicability of the method to organic and inorganic compounds. The average sample used was from 3 to 5 mg. In terms of hydrogen, the average deviation for the analyses is only 0.009 mg. The accepted error for the determination of hydrogen by the carbon and hydrogen microprocedure is 0.3% (*4*). The average error for the present method is 0.2%.

Table I. Hydrogen Recoveries from Sodium Hydroxide

$$2Na + 2NaOH \longrightarrow 2Na_2O + H_2$$

Sample Weight	H_2 Present	H_2 Found	ΔH_2
Mg.	*Mg.*	*Mg.*	*Mg.*
2.9	0.070	0.074	+0.004
3.0	0.075	0.069	−0.006
3.4	0.085	0.094	+0.009
3.6	0.090	0.086	−0.004
8.2	0.205	0.210	+0.005
8.2	0.205	0.184	−0.021
9.3	0.230	0.222	−0.008
20.4	0.510	0.519	+0.009
			Av. ±0.008

Table II. Hydrogen Content of Raw Sodium from Interior of Brick

Sample Weight	H_2	H_2	Deviation from Mean
Grams	*Mg.*	*Wt. %*	%
2.536	0.142	0.0056	−0.0004
2.570	0.172	0.0067	+0.0007
3.253	0.169	0.0052	−0.0008
3.654	0.305	0.0084	+0.0024
3.884	0.238	0.0061	+0.0001
4.182	0.171	0.0041	−0.0019
		Av. 0.0060	±0.0010

Table III. Hydrogen Recoveries from Various Compounds

Compound	H_2, Theory	H_2, Experimental	No. of Determinations	Mean Deviation
	%	%		%
Sucrose	6.48[a]	6.49	20	0.01
Potassium acid phthalate	2.47[a]	2.46	8	0.14
Acetanilide	6.70[a]	6.68	5	0.24
Cystine	5.03[a]	4.89	8	0.48
Dithio-oxamide	3.36	3.37	5	0.16
Dithiobiuret	3.73	3.75	4	0.22
Sodium hydroxide	2.48[b]	2.48	10	0.09
Barium chloride dihydrate	1.66[c]	1.65	8	0.09
Water	11.13[d]	11.10	3	0.60
Silicone	5.29[e]	5.57	3	0.28
				Av. 0.23

[a] Bureau of Standards reagents.
[b] Determined by volumetric analysis as described above.
[c] 0.2% hydrogen in compound not due to water of crystallization.
[d] Samples used were 1 mg. or less because of capacity of McLeod gages.
[e] Combustion values.

Because only hydrogen will diffuse with any appreciable rate (*2*) through the walls of the iron capsule at this temperature, no other element will interfere with the determination and no special precautions need be taken for different samples. Originally it was thought that carbon monoxide might diffuse through at the temperatures used. Carbon monoxide resembles hydrogen in that it would be oxidized to carbon dioxide and frozen out in the cold trap. To test this, 0.5 ml. of iron carbonyl [$Fe(CO)_5$], which decomposes at 100° C. to iron and carbon monoxide, was sealed in a capsule and heated to 700° C. in the apparatus. No indication of any increase in pressure was noted, indicating no diffusion of carbon monoxide through the walls of the iron capsule during the heating interval. Furthermore, the excess magnesium will reduce carbon monoxide to magnesium oxide and carbon in the course of an actual analysis.

The nature of the blank value is still undetermined. However, it is very constant and reproducible and is equivalent to 0.015 mg. of hydrogen. All attempts to ascribe the blank value to the moisture content of the air in the capsule, or adsorbed moisture on the capsule, were without success. Nevertheless, for any group of capsules, the blank is very constant, and need be redetermined only once a week during normal operations. During very humid days the blank increased somewhat, probably owing to adsorbed water on the exterior of the capsules. Perhaps degassing the capsules at a higher temperature and lower pressure than that used for the actual determination will lower the blank value.

The residual gas is nitrogen, which is adsorbed on the capsules after degassing when exposed to air. The identification was made with a visual spectroscope using the glow discharge which is formed as the gas is liberated. The actual amount of nitrogen is very small and is corrected for by the method of calculation.

It should be possible to apply the procedure to the determination of hydrogen in higher melting metals without any changes except for a higher combustion temperature. Experiments along this line are contemplated for the future.

ACKNOWLEDGMENT

The authors are indebted to Saul Dushman for informative discussions at the inception of this project, to J. Rynasiewicz for devising the "standard" sodium hydroxide, and to W. Moak for some of the analyses included in this paper.

LITERATURE CITED

(1) Bobalek, E. G., and Shrader, S. A., IND. ENG. CHEM., ANAL. ED., **17**, 544 (1945).
(2) Dushman, Saul, "Scientific Foundations of Vacuum Technique," New York, John Wiley & Sons, 1949.
(3) Naughton, J., *Trans. Am. Inst. Mech. Engrs.*, **162**, 385 (1945).
(4) Niederl, J. B., and Niederl, V., "Micromethods of Quantitative Organic Analysis," New York, John Wiley & Sons, 1942.
(5) Norton, F. J., and Marshall, A. L., Am. Inst. Mining Met. Engrs., *Tech. Pub.* **1643** (1944).
(6) Prescott, C. H., and Morrison, J., IND. ENG. CHEM., ANAL. ED., **11**, 230 (1939).
(7) Reeve, L., *Trans. Am. Inst. Mech. Engrs.*, **113**, 82 (1934).

RECEIVED November 16, 1948. The Knolls Atomic Power Laboratory is operated by the General Electric Research Laboratory for the Atomic Energy Commission. The work reported here was carried out under Contract W-31-109 Eng-52

Microdetermination of Iodine in Materials with High Organic Content

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A simple and accurate method for the determination of iodine in organic materials containing less than 0.01% iodine uses the chromic acid digestion technique of Leipert followed by reduction with phosphorous acid. After distillation the iodine is oxidized to iodate by means of chlorine in carbon tetrachloride solution. The iodine is then released by the addition of potassium iodide solution and measured spectrophotometrically.

TO DETERMINE iodine in compounds containing less than 0.01% iodine, the material is digested in a mixture of chromic and sulfuric acids as described by Leipert (*4*). The digest is then reduced by the addition of phosphorous acid as described by Fashena and Trevarrow (*3*, *7*). The liberated iodine is distilled and trapped in a special apparatus, which was developed with the cooperation of the Microchemical Specialties Company, Berkeley, Calif. After oxidation with chlorine and liberation by potassium iodide, the free iodine is determined in the Beckman quartz spectrophotometer at a wave length of 353 mμ if only a tungsten lamp is available. If the ultraviolet attachment can be employed, it is preferable to use a wave length of 290 mμ. Custer and Natelson (*2*) have shown that at this wave length a 33% increase in sensitivity is achieved. The precision of the method at 353 mμ is such that 0.5 microgram of iodine, uncorrected for reagent blank, may be determined with an accuracy of ±0.05 microgram. At this range the spectrophotometer would give a maximum error of 10%. It is obviously desirable to maintain the blank at a low value for most precise work (Table I).

REAGENTS

Sulfuric Acid. C.P. grade sulfuric acid contains such minute quantities of iodine that ordinarily there is no need for further purification. However, it can be made iodine-free by adding 5 drops of 70% phosphorous acid and boiling for 30 minutes. A 70% solution of sulfuric acid is prepared from the cold concentrated sulfuric acid.

Chromic Acid. If chromic acid does not contain more than 4.0 micrograms of iodine per 100 grams of solid matter, there is no need for purification. This amount of impurity which will be recovered in the blank is not sufficient to interfere with the accuracy of the results. If purification is desired the method described by Matthews, Curtis, and Brode (*5*) is satisfactory. A final solution of chromic acid is prepared so as to contain 70.0 grams of chromic acid per 100 ml. of water.

Alkaline sulfite solution, prepared by adding 0.25 gram of sodium sulfite to 250 ml. of 0.5 *N* sodium hydroxide.

2.0 *N* Hydrochloric acid, prepared from concentrated C.P. hydrochloric acid which has a very low iodine content.

Phosphorous acid, 70%, prepared from crystalline phosphorous acid in distilled water.

Solution of chlorine in carbon tetrachloride, prepared according to the method described previously (*6*), or by bubbling

Table I. Determination of Iodine in Organic Materials

Material	Quantity Used, Gram	I Recovered, γ	Av.	I in Sample, γ	I Recovered, γ	% Recovery
Blank I	0	0.22				
Blank II	0	0.22	0.22	0	0	0
Purified casein	0.3	0.22				
	0.3	0.22	0.22	0	0	0
Purified casein plus 0.1 γ I (iodoalbumin solution)	0.3	0.32				
	0.3	0.33	0.325	0.105	0.098	93
Purified casein plus 0.3 γ I (iodoalbumin solution)	0.3	0.54				
	0.3	0.52	0.53	0.31	0.30	97
Serum A (pooled from 9 normal men)	3.0	0.40				
	3.0	0.38	0.39	0.17	0.17[a]	100
Serum A plus 0.2 γ I (thyroxine solution)	3.0	0.58				
	3.0	0.58	0.58	0.36	0.37	102
Serum A plus 0 γ I (thyroxin solution)	3.0	0.87				
	3.0	0.89	0.88	0.66	0.63	95

[a] Average of 10 determinations. Equivalent to 5.6 γ of iodine per 100 ml. of serum. Normal values for human serum are 5 to 7 γ of iodine per 100 ml. of serum.

Table II. Rate of Recovery of Iodine in Distillation Apparatus

Iodine Added to Digestion Mixture, γ	Distillation Period: 25 Minutes		10 Minutes		7.5 Minutes		5 Minutes	
	Iodine Recovered							
	γ	%	γ	%	γ	%	γ	%
0 (blank)	0.22	100	0.22	100	0.20	91	0.20	91
0.25	0.48	102	0.47	100	0.44	93	0.36	75
1.0	1.20	98	1.24	102	1.15	94	0.92	75

chlorine gas through carbon tetrachloride until the solution assumes a bright green color. The solution is then filtered and stored in a glass-stoppered bottle; it should be resaturated with chlorine once each week.

Potassium iodide solution, 0.1%, prepared fresh each day just before use. It should be discarded after 1 hour.

Distilled Water. Small quantities of redistilled water are needed in the last stage of the determination. Distilled water is redistilled from a potassium carbonate solution through a glass condenser. This water is stored in a glass-stoppered wash bottle.

METHOD

Digestion of Sample. The material containing less than 0.3 gram of organic material is transferred quantitatively to a 500-ml. Kjeldahl flask followed by 25 ml. of 70% sulfuric acid and 3 ml. of 70% chromic acid. As the reagents are not free from iodine, it is essential that the solutions be measured only by means of volumetric pipets. The flask is slowly heated. When the foaming subsides heating is continued until the mixture begins to fume, by which time the contents of the flask should appear green. After the flask is cool a piece of antibump material and about 50 to 75 ml. of distilled water are added to the mixture, which is then boiled until it again begins to fume. During the digestion, volatile acids are produced which must be removed before distillation. These acids, if present, would pass over in the distillation process, and would neutralize the alkaline solution used for trapping the iodine.

Distillation. Figure 1 is a diagram of the distillation apparatus, which employs the distillation principle used by Chaney (*1*).

Tube *H* connected to the aspirator is closed, funnel *B* is unclamped, and jacket *C* is filled with distilled water to the level of opening *D*. The digest is added through funnel *A*. The flask is washed twice, each time with about 15 ml. of distilled water, and the washings are added to the digest followed by 5.0 ml. of phosphorous acid.

Funnel *A* and trap opening *E* are closed by means of pinch clamps, and 3.0 ml. of alkaline sulfite solution are added to the trap through tube *F*. The water in jacket *C* is heated and funnel *B* is closed. The steam carrying the iodine is allowed to bubble through the mixture for 10 minutes. At the end of this time the flame is removed and *B* and *A* are opened. As soon as the boiling stops, the liquid in trap *K* is allowed to run into a 50-ml. Erlenmeyer flask through *E*. The trap is washed twice, each time with 5 to 10 ml. of distilled water. The final volume in the flask should be around 25 ml. In order to wash the apparatus funnel *B* is closed and tube *H* which is connected to an aspirator is opened. The liquid in jacket *L* is sucked out. Water is added through *A* and the liquid is again emptied through *H*. The apparatus is then ready for the next sample.

As shown in Table II, about 75% of the iodine is recovered after 5 minutes and almost 100% recovery is obtained after 10 minutes' distillation in this apparatus.

Blank. If phosphorous acid were added to chromic acid in the distillation apparatus, there would be a violent reaction in the apparatus. In order to avoid this the following procedure should be used.

In a Kjeldahl flask 3.0 ml. of chromic acid, 30 ml. of distilled water, and 25 ml. of sulfuric acid are placed. To this are added 3.0 ml. of phosphorous acid. This quantity of phosphorous acid is not sufficient to reduce all of the chromic acid, but it will reduce enough of the acid to prevent a violent reaction. When the reaction is complete, the mixture is cooled and transferred to the distillation apparatus, 2.0 ml. of phosphorous acid are added, and the iodine is distilled as in the case of the sample.

Oxidation. A piece of antibump material and 1.0 ml. of 2.0 *N* hydrochloric acid are added to the Erlenmeyer flask. The flask is shaken well, 0.5 ml. of the chlorine solution is added, and the sample is boiled until less than 3.0 ml. of liquid remains in the bottom of the flask. It is essential that the last traces of chlorine be removed from the solution and this can be accomplished only by reducing the volume of the liquid to the above-mentioned limit. However, in no case should the contents of the flask be allowed to dry.

Determination. The liquid in the flask is then transferred to a test tube (100 mm. in diameter) which has previously been marked to the 4-ml. level with a diamond pencil. The level is brought to the 4-ml. line with redistilled water; 1.0 ml. of 0.1% potassium iodide solution is blown into the tube, the contents of the tube are transferred to the spectrophotometer cells, and the liberated iodine is determined in the spectrophotometer at 353 mμ. Carefully matched cuvets should be employed and the transmission density should be checked against distilled water just prior to each reading of an unknown solution.

The Beckman spectrophotometer is used in all determinations, and the authors' experience has been limited to this model. In addition to the directions given by the manufacturers for the operation of the spectrophotometer, the following precautions should be observed for greater accuracy in these determinations:

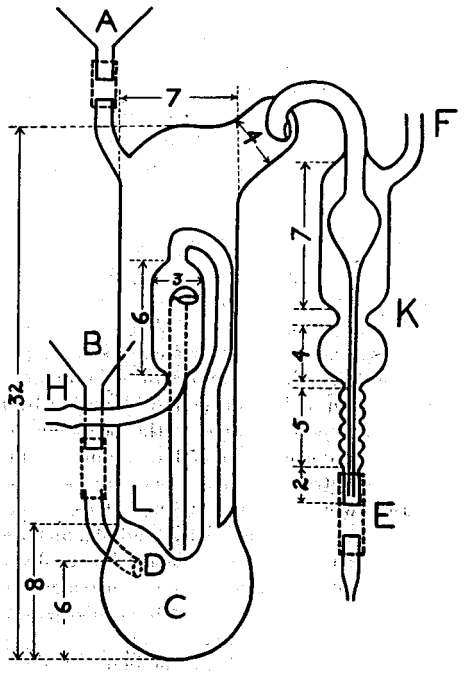

Figure 1. Distillation Apparatus

1. The cells should be checked against the control cell. Any discrepancy in the reading should be noted and corrections made in the final reading.

2. It is of the utmost importance that just before each reading the 0 mark on the transmission density knob be checked against the distilled water control. For this purpose the switch pointer remains on 1.0, the transmission density is set at 0, and the shutter is opened, allowing the light to pass through the cell containing distilled water. The adjustment is made with the sensitivity control and the dark current as required in the operation of the instrument. The reading of the unknown is made immediately after this adjustment. The spectrophotometer should be calibrated against standard iodate solutions in 0.1 *N* hydrochloric acid.

Next, 1.0 ml. of the standard is pipetted into the 4-ml. calibrated test tubes, the volume is brought to 4.0 ml. by the addition of redistilled water, 1.0 ml. of 0.1% potassium iodide solution is added to each tube, and the reading is made according to the above-mentioned directions. The slightly higher concentration of hydrochloric acid does not influence the results in any way.

LITERATURE CITED

(1) Chaney, A. L., IND. ENG. CHEM., ANAL. ED., **12**, 180 (1940).
(2) Custer, J. J., and Natelson, S., ANAL. CHEM., **21**, 1003 (1949).
(3) Fashena, G. J., and Trevarrow, V., *J. Biol. Chem.*, **114**, 351 (1936).
(4) Leipert, T., *Biochem. Z.*, **261**, 437 (1933).
(5) Matthews, N. L., Curtis, G. M., and Brode, W. R., IND. ENG. CHEM., ANAL. ED., **10**, 612 (1938).
(6) Shahrokh, B. K., *J. Biol. Chem.*, **154**, 517 (1944).
(7) Trevarrow, V., and Fashena, G. J., *Ibid.*, **110**, 29 (1935).

RECEIVED July 7, 1948.

Spectrophotometric Determination of Microquantities of Iodine

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Procedures are described for the spectrophotometric microdetermination of iodine. Light absorption spectra are reported for iodine in water, potassium iodide solutions, benzene, toluene, alcohol, and chloroform. The high absorption peaks in the ultraviolet region for elemental iodine in toluene, benzene, and potassium iodide solutions permit these solvents to be used for the determination of microquantities of iodine with the spectrophotometer. Sensitivity is increased sixfold by converting the iodine to iodate with alkaline permanganate. The iodate reacts with iodide in the presence of acid to liberate the iodine to be determined. Iodine is transferred by extraction procedures to the benzene, toluene, or potassium iodide solutions. As little as 0.2 microgram of iodine is easily determined by these methods.

THE need for a method, suitable for routine use for the determination of iodine in quantities on the order of 0.2 microgram as found in 1 ml. of human serum, has become of increasing importance. This determination is not performed in most biological laboratories because of the elaborate procedures or large quantities of blood serum required for a single determination. The authors' purpose was to devise a procedure which could be applied with reasonable precision and accuracy to the routine determination of microquantities of iodine.

Thiosulfate titration of iodine is limited to the determination of a concentration of 7.5 micrograms of iodine per ml. (*25*). The use of organic solvents such as benzene, petroleum ether, chloroform, and carbon tetrachloride as indicators in the titration of iodine has been proposed (*7*, *40*, *45*). These procedures increase the sensitivity of the titration so that 6.0 micrograms per ml. of iodine may be detected (*25*). A sensitivity of approximately 2 micrograms of iodine per ml. in the presence of excess of iodide ion is claimed (*26*).

Arsenious oxide, trivalent antimony (*26*), sulfurous acid (*8*), hydrogen sulfide (*24*), stannous ion, and thiocyanate (*21*) have been recommended for the titration of iodine. However, none of these appears to have a greater sensitivity for the determination of minute quantities of iodine than thiosulfate. Organic compounds such as formaldehyde (*42*), chloral hydrate (*41*), aldoses (*3*), acetone (*14*, *36*, *49*), and hydroquinone (*51*) have also been suggested for this purpose. These methods have not suggested a procedure wherein the sensitivity would be great enough to determine the quantities of iodine found in 1 ml. of blood serum.

Titration methods, using adsorption indicators, based upon the precipitation of insoluble iodides have also been proposed (*10*, *11*, *22*, *23*, *28*, *29*, *37*, *38*, *47*, *52*). The sensitivity of these methods is less than that for the thiosulfate titration. Electrometric titration of the reaction between iodine and thiosulfate (*30*) was not found practicable for routine determinations of minute quantities of iodine.

The methods wherein iodine is used as a catalyst for the reaction between ceric sulfate and nitrite (*18*) or arsenite (*9*, *48*, *50*) are capable of determining amounts of iodine in the required range. However, these catalytic methods are delicate, and require accurate timing, careful temperature control, and special apparatus. In this laboratory, these methods were found to be time-consuming and not uniformly successful.

In view of the chromophoric character of elemental iodine itself, it was felt that a colorimetric procedure might be developed with the use of the spectrophotometer. Various colorimetric methods for the determination of inorganic iodine have been proposed (*1*, *12*, *13*, *34*, *38*, *43*, *46*). These methods use the visible portion of the spectrum in reading iodine concentration. In the visible range the extinction coefficient for iodine is not high enough to be useful for minute quantities of iodine in water or other solvents (*46*). Higher peaks have been reported for elemental iodine in potassium iodide solutions in the ultraviolet (*4*, *46*).

Because the state of iodine varies with the nature of the solvent, the authors decided to investigate the absorption spectra of iodine in several solvents to see whether a higher extinction coefficient could be found. This study was extended over the entire range of the Beckman spectrophotometer for the purpose

of finding a suitable peak. The solvents investigated included water, potassium iodide solutions, ethanol, chloroform, benzene, and toluene. The extinction coefficients are plotted in Figures 1 and 2 against the wave length. For extinction coefficients above 600 the graph in Figure 1 has been condensed to permit the demonstration of high peaks of iodine in potassium iodide solution. Figure 2 is drawn to a different scale to show the peaks for the poorly absorbing solvents.

It is apparent from an inspection of Figures 1 and 2 that potassium iodide solution would be the most suitable solvent. In order to avoid the need for a hydrogen lamp and to adapt the procedure for use with other spectrophotometers, the peak at 352 mμ was chosen.

The effect of change in concentration of potassium iodide on the extinction coefficient was studied. Table I lists the extinction coefficients of three different concentrations of potassium iodide.. In each case the concentration employed was 2.13 micrograms of iodine per ml.

It is evident from Table I that 5% potassium iodide solution is perfectly suitable for this determination, for slight changes in potassium iodide concentration would not affect the extinction coefficient beyond the requirements of the method.

Prior to the actual isolation of iodine in the potassium iodide solution it is necessary to destroy any organic material associated with the iodine. Two methods for the destruction of organic matter have been employed: alkaline fusion (*15, 19, 31, 32, 39, 44*) and wet oxidation (*5, 6, 16, 17, 35*).

Alkaline fusion in Pyrex test tubes is suitable, provided the fusion temperature is not allowed to rise above

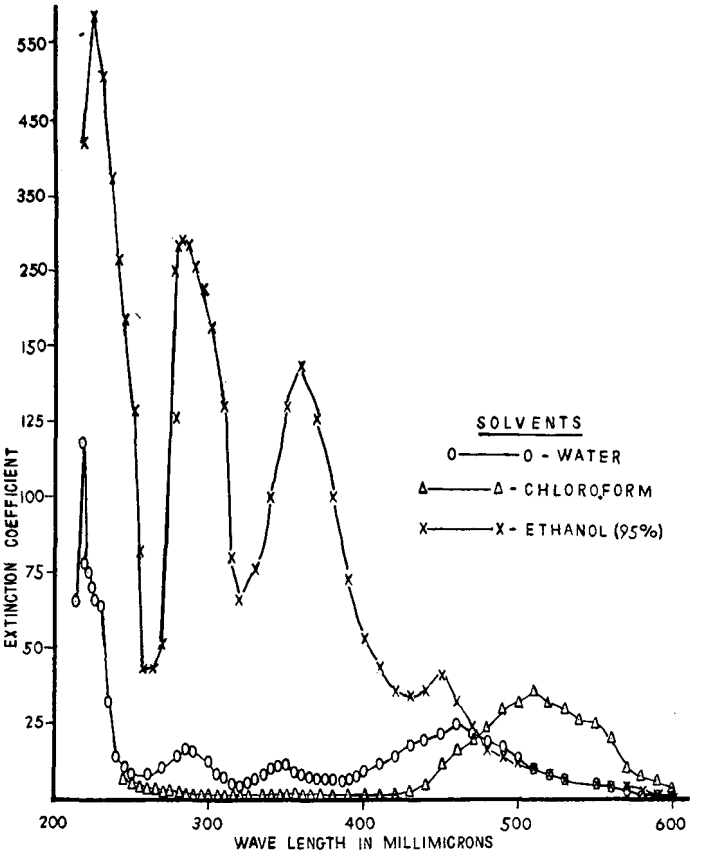

Figure 2. Absorption Spectra of Iodine Dissolved in Water, Chloroform, and 95% Ethanol

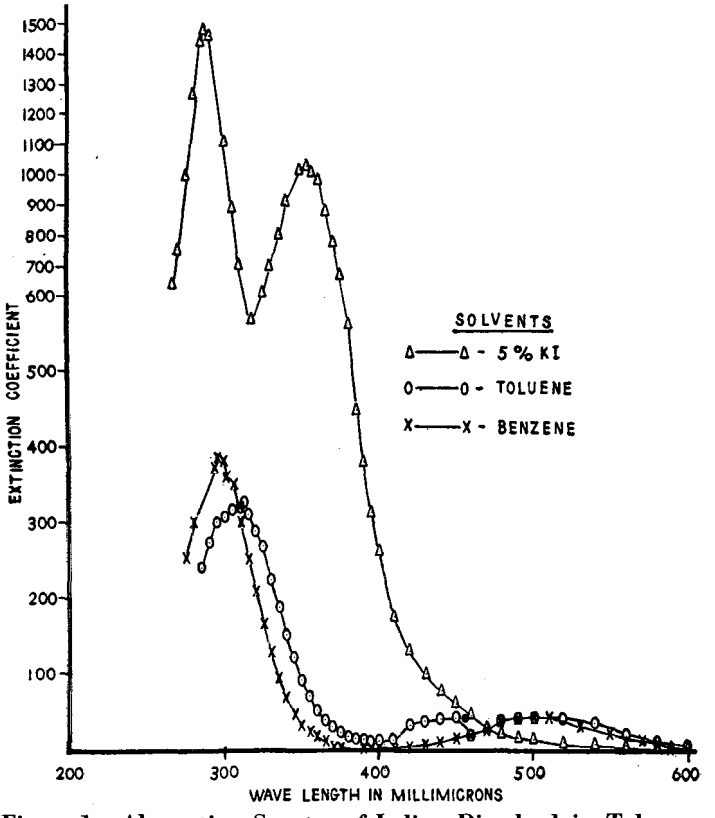

Figure 1. Absorption Spectra of Iodine Dissolved in Toluene, Benzene, and 5% Aqueous Potassium Iodide

450° to 475° C. Thus, a large number of test tubes may be placed in the oven simultaneously. Etched tubes must not be used.

The final problem of transferring the iodine to a potassium iodide solution remained. The most commonly used method for iodine transfer, distillation (*20, 27, 33, 53*), was discarded because in one apparatus only one distillation may be carried out at one time, the procedure is tedious, and the final volume in which the iodine is contained is too large for the present purpose.

The authors finally resorted to extraction procedures, using those solvents wherein the partition coefficient favored complete extraction. The iodine was therefore converted to iodate by alkaline permanganate and then liberated by the action of iodate ion on iodide ion in acid solution, extracted with chloroform, and re-extracted from the chloroform with 5% potassium iodide solution. The potassium iodide solution can then be read in the Beckman spectrophotometer at 352 or 289 mμ with the 1-ml. quartz cuvettes or in a Coleman spectrophotometer using the 3-ml. cuvettes with the 5-cm. light path at 352 mμ.

As shown in Figure 3, the light absorption of iodine in potassium iodide solution at 352 mμ follows Beer's law. This figure also shows that after iodine has been extracted with chloroform and then re-extracted with potassium iodide solution, the curve obtained is also a straight line. This indicates, as would be expected, that the partition coefficient has an effect which is constant for different concentrations of iodine. The

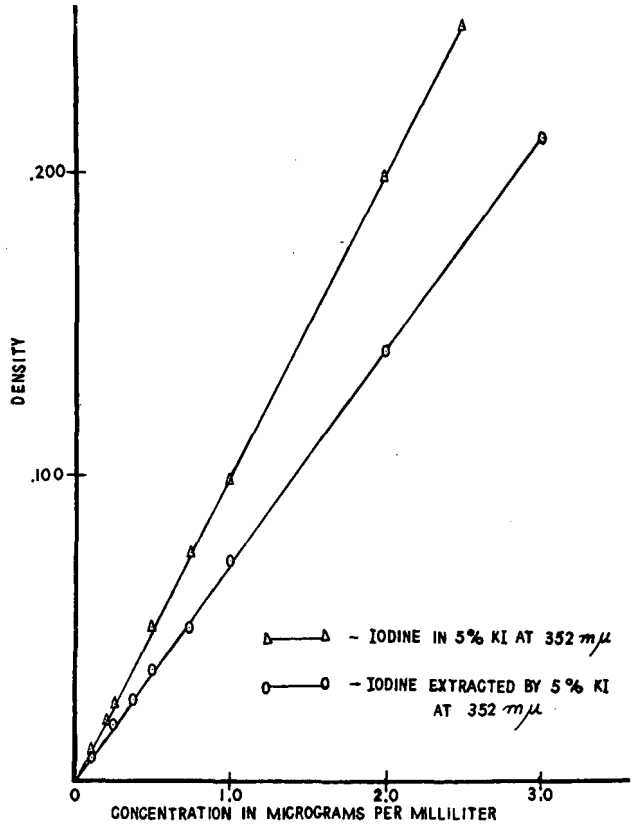

Figure 3. Density of Potassium Iodide Solutions

Before and after extraction procedure

Table I. Change in Extinction Coefficient for Iodine in Potassium Iodide Solution with Change in Potassium Iodide Concentration

(I − 2.13 γ per ml.)

	1% KI		5% KI		10% KI	
λ mμ	Density	Extinction coefficient	Density	Extinction coefficient	Density	Extinction coefficient
352	0.213	1000	0.217	1018	0.226	1060

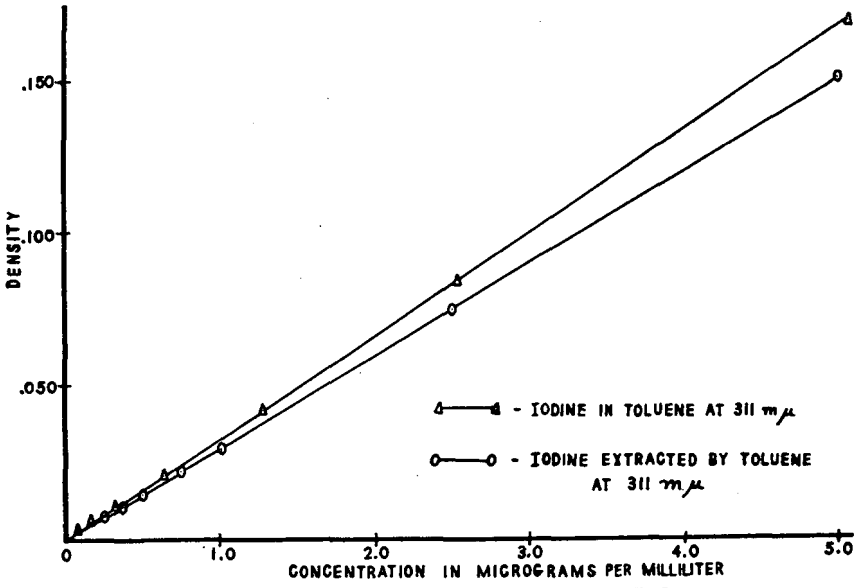

Figure 4. Density of Toluene Solutions of Iodine

Before and after extraction procedure

difference in the slope of the two lines is an over-all measure of the partition coefficients between the solvents.

By this method 0.1 microgram of iodine per ml. can be detected and 0.8 microgram per ml. can be determined. To obtain the amount of iodine in the original sample these values should be divided by the factor of 6, inasmuch as the iodine in the original sample is oxidized to iodate which in turn liberates six equivalents of iodine (*2*, *7*) to be read on the spectrophotometer. Thus approximately 0.02 microgram of iodine in the original sample may be detected and approximately 0.2 microgram determined.

In order to simplify this procedure, the use of toluene solutions of iodine was investigated at the absorption maximum, for this would eliminate an extra extraction and it would not be necessary to prepare potassium iodide solutions daily. Toluene also has the advantage of a lower density than water. Thus, an aliquot may be removed from the upper layer without fear of contaminating the pipet by the aqueous layer.

Iodine is more soluble in benzene and toluene than in chloroform. Moreover, chloroform does not show a peak worthy of use for iodine determination for the entire range of the Beckman spectrophotometer. Iodine in aromatic hydrocarbon solvents shows relatively high peaks in the near ultraviolet (see Figure 1). This may indicate compound formation.

Figure 4 shows two curves. The curve obtained by plotting iodine concentration in toluene against density at 311 mμ follows a straight line as far as it was investigated (to 10 micrograms per ml.). The second curve, the standard curve for analysis, is obtained by liberating iodine by the action of known amounts of potassium iodate on excess acidified potassium iodide solution followed by extraction with toluene. This curve is also a straight line, indicating that the partition coefficient between the aqueous and toluene phases is constant for different amounts of iodine.

From Figure 4 it is apparent that 1.0 microgram of iodine can be determined. This method is therefore adequate for the determination of 0.2 microgram of iodine, if it is first converted to iodate by permanganate.

Benzene was similarly studied and found to yield a straight line when concentration was plotted against density at 300 mμ with the hydrogen lamp and 305 mμ with the tungsten lamp. Toluene was chosen as the preferred solvent because of its higher boiling point.

To test this procedure, determinations were carried out on solutions of potassium iodide. The iodide ion was oxidized to iodate with alkaline permanganate. Excess permanganate was destroyed by hydrogen peroxide, and the manganese dioxide formed was subsequently removed by centrifuging. An aliquot was taken from the supernatant liquid. Potassium iodide and acid were added to this aliquot to liberate the iodine. The iodine was extracted with a measured volume of toluene. The toluene extract was then read directly at 311 mμ against a toluene extract of distilled water which had been carried through the oxidation steps as for the unknown.

Table II shows the amount of iodine recovered from known concentrations of potassium iodide on 28 analyses run consecutively. The results are indicative of the accuracy that may be obtained under routine conditions.

It is apparent from this series of determinations and numerous others, that when 0.2 microgram of iodine is being

Table II. Iodine Recovered from Potassium Iodide Solutions

Sample No.	Iodine Added, γ	Iodine Recovered, γ	% Recovered	Sample No.	Iodine Added, γ	Iodine Recovered, γ	% Recovered
1	0.79	0.79	100.0	15	0.20	0.19	95.0
2	0.79	0.78	98.7	16	0.20	0.19	95.0
3	0.79	0.78	98.7	17	0.15	0.15	100.0
4	0.79	0.77	97.5	18	0.15	0.15	100.0
5	0.40	0.40	100.0	19	0.15	0.15	100.0
6	0.40	0.40	100.0	20	0.15	0.16	106.7
7	0.40	0.38	95.0	21	0.10	0.091	91.0
8	0.40	0.37	92.5	22	0.10	0.091	91.0
9	0.29	0.29	100.0	23	0.10	0.091	91.0
10	0.29	0.29	100.0	24	0.10	0.11	110.0
11	0.29	0.29	100.0	25	0.080	0.080	100.0
12	0.29	0.27	93.1	26	0.080	0.080	100.0
13	0.20	0.20	100.0	27	0.080	0.066	82.5
14	0.20	0.20	100.0	28	0.080	0.091	113.8

Mean % recovery = 98.3.
Average deviation from mean % recovery = 4.2.

determined, accuracy within 5% may be obtained regularly with the toluene extraction procedure. The average deviation from the mean per cent recovered includes determinations on less than 0.1 microgram, which is apparently beyond the range of this method. However, these results were included to indicate the fact that such amounts of iodine can be detected.

Because of the varying results that are obtained in determining protein-bound iodine in serum, depending upon the method employed in precipitating and washing the serum proteins, these results are not included in this study. The protein-bound hormone iodine in serum is being investigated and will be reported in a separate study.

REAGENTS

Potassium Iodide Stock Standard. Anhydrous potassium iodide (analytical reagent, 1.308 grams) is made up to 1 liter. 1 ml. = 1 mg. of iodine. One milliliter of this solution diluted to 100 ml. is equivalent to 10 micrograms per ml.

Potassium Permanganate, 1%. One gram of the analytical reagent is made up to 100 ml. with distilled water.

Sodium Hydroxide, 1%. One gram of the analytical reagent is made up to 100 ml. with distilled water.

Hydrogen Peroxide, 6%. Hydrogen peroxide (30%, reagent grade) is diluted to five times its volume with distilled water.

Sulfuric Acid, 5%, is prepared by diluting 5 grams of analytical grade concentrated sulfuric acid to 100 ml. with distilled water.

Toluene, analytical reagent.

Potassium Iodide, 1%. One gram of potassium iodide (analytical reagent) is made up to 100 ml. with distilled water. This solution is best made up fresh daily.

Potassium Iodide, 5%. Five grams of the analytical reagent are made up to 100 ml. This solution is best made up fresh daily.

PROCEDURE

The potassium-iodide solutions to be determined (0.2 ml. each) are pipetted into 5-ml. centrifuge tubes with a mark at 2 ml., and 0.1 ml. of the 1% sodium hydroxide solution is added, followed by 0.1 ml. of 1% potassium permanganate.

If protein is present, it is dissolved in 1% sodium hydroxide in Pyrex test tubes, brought to dryness in a 100° C. oven, and ashed at 450° to 475° C. The residue is redissolved in water and a 0.2-ml. aliquot is treated as for the potassium iodide solution.

The contents of the tubes are mixed, and the rack with the tubes is placed in a boiling water bath for 30 minutes. The rack is removed from the boiling water bath, allowed to cool to room temperature, and placed in a refrigerator until the tubes have reached refrigerator temperature. Cold 6% hydrogen peroxide is added from a dropper to each tube, which is kept immersed in a beaker of cracked ice and salt during the decolorization of the permanganate. If these precautions are not followed, excessive amounts of peroxide will be needed, for the precipitated manganese dioxide catalyzes the decomposition of hydrogen peroxide at room temperatures. The tubes are now placed in a 37° C. oven for 1 hour, or allowed to stand at room temperature overnight to decompose excess peroxide. The volume is made up to the 2-ml. mark, and the tube is shaken and then centrifuged at 2500 r.p.m. for 15 minutes. A 1.5-ml. aliquot is taken, preferably by filtering the supernatant liquid into another test tube before taking the aliquot, so as to ensure complete separation from the manganese dioxide.

Method A. The 1.5-ml. aliquot is transferred to a 12-ml. centrifuge tube with ground-glass stopper. Silicone grease may be used to prevent leakage when shaken. Then 0.1 ml. of 1% potassium iodide solution is added, followed by 0.2 ml. of 5% sulphuric acid; 1.8 ml. of toluene are added, and the tubes are shaken in a mechanical shaker for 10 minutes. The tubes are now centrifuged at 2000 r.p.m. for 5 minutes. The toluene layer is transferred to 1-ml. quartz cuvettes and then read on the Beckman spectrophotometer at 311 mμ.

Method B. The 1.5-ml. aliquot is transferred to a centrifuge tube with ground-glass stopper, and 0.1 ml. of 1% potassium iodide solution is added, followed by 0.2 ml. of 5% sulfuric acid. Then 1.8 ml. of chloroform are added, and the tube is shaken for 10 minutes in a mechanical shaker. The tube is centrifuged at 1500 r.p.m. for 3 minutes. The upper layer is aspirated off, 1.5 ml. of the lower layer are transferred to a second centrifuge tube with ground-glass stopper, 1.5 ml. of 5% potassium iodide solution are added, and the tube is shaken for 10 minutes. The tube is centrifuged as before, and the upper layer, potassium iodide solution, is transferred to 1-ml. cuvettes and then read on the Beckman spectrophotometer at 352 mμ. For the Coleman spectrophotometer, 3.5 ml. of 5% potassium iodide solution are used for the extraction. The reading may then be made with the 3-ml. cuvettes with a 5-cm. light path at 352 mμ.

LITERATURE CITED

(1) Aldridge, W. N., *Analyst*, **70**, 474 (1945).
(2) Andrews, L. W., *J. Am. Chem. Soc.*, **25**, 756 (1903).
(3) Bland and Lloyd, *J. Soc. Chem. Ind.*, **32**, 948 (1914).
(4) Brode, W. R., *J. Am. Chem. Soc.*, **48**, 1877 (1926).
(5) Christensen, B. G., *Ugeskrift Laeger*, **105**, 866–9 (1943).
(6) Cook, *J. Chem. Soc.*, 47, 471 (1885).
(7) Dietz, H., and Margoshes, B. M., *Chem. Ztg.*, **28**, 1191 (1904).
(8) Dupasquier, A., *Ann. chim. phys.*, **73**, 310 (1840).
(9) Englis, D. T., and Knoepfelmacher, A. A., IND. ENG. CHEM., ANAL. ED., **17**, 393 (1945).
(10) Fajans, K., and Hassel, O., *Z. Elektrochem.*, **29**, 495 (1923).
(11) Fajans, K., and Wolff, H., *Z. anorg. allgem. Chem.*, **137**, 221, 233 (1924).
(12) Fellenberg, T. von, *Biochem. Z.*, **152**, 116 (1924).
(13) Flox, J., Pitesky, I., and Alving, A. S., *J. Biol. Chem.*, **142**, 147–57 (1942).
(14) Geelmuyden, *Z. anal. Chem.*, **35**, 503 (1896).
(15) Grauer, R. C., and Saier, E., *Endocrinology*, **24**, 553 (1939).
(16) Groak, B., *Biochem. Z.*, **175**, 455 (1926).
(17) *Ibid.*, **270**, 291 (1934).
(18) Hahn, F. L., and Adler, M., *Proc. 8th Am. Sci. Cong.*, **7**, *Phys. and Chem. Sci.*, 169–75 (1942).
(19) Hilty, W. W., and Wilson, D. T., IND. ENG. CHEM., ANAL. ED., **11**, 637 (1939).
(20) Jannasch, *Ber.*, **39**, 196 (1906); *J. prakt. Chem.*, **78**, 28 (1908).
(21) Kohler, B., *Chem. Listy*, **14**, 137–40, 195–9 (1920).
(22) Kolthoff, I. M., *Pharm. Weekblad.*, **54**, (1917); **58**, 917 (1921).
(23) *Ibid.*, **57**, 836 (1920).
(24) Kolthoff, I. M., *Z. Anal. Chem.*, **60**, 451 (1921).
(25) Kolthoff, I. M., Menzel, H., and Furman, N. H., "Volumetric Analysis," Vol. II, pp. 348–52, New York, John Wiley & Sons, 1929.
(26) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," pp. 618–21, New York, Macmillan Co., 1943.
(27) Lelièvre, J., and Menager, Y., *Compt. rend.*, **178**, 1315–16 (1924).
(28) Liebig, *Liebig's Ann. Chem.*, **185**, 289, 307 (1853).
(29) Lottermoser, A., Seifert, W., and Forstmann, W., *Kolloid Z. Zsigmondy Festschr.*, **36**, 230 (1925).
(30) McClendon, J. F., "Iodine and the Incidence of Goiter," Chap. III, University of Minnesota Press, 1939.
(31) McClendon, J. F., *J. Biol. Chem.*, **102**, 91 (1933).
(32) McClendon, J. F., and Remington, R. E., *J. Am. Chem. Soc.*, **51**, 394 (1929).
(33) McHargue, J. S., Young, D. W., Roy, W. R., IND. ENG. CHEM., ANAL. ED., **4**, 214 (1932).
(34) Martindale, W. H., "Extra Pharmacopoeia," Vol. II, p. 86, London, H. K. Lewis & Co., 1929.
(35) Matthews, N. L., Curtis, G. M., and Brode, W. R., IND. ENG. CHEM., ANAL. ED., **10**, 612 (1938).
(36) Messinger, *Ber.*, **21**, 3366 (1888).
(37) Mohr, Fr., "Lehrbuch der chemische-analytische Titrierenmethoden," Braunschweig, S. Vieweg & Sohn, 5th ed., Vol. 2, p. 81, 1859.
(38) Muller, and Diffenthaler, *Z. anorg. allgem. Chem.*, **67**, 418 (1910).

(39) Perkin, H. J., Brown, B. R., and Lang, J., *Can. Med. Assoc. J.*, **31**, 365 (1934).
(40) Rabourdin, *Compt. rend.*, **31**, 784 (1850).
(41) Rapp., E., *Arch. Pharmag.*, **241**, 328 (1903).
(42) Romijn, *Z. anal. Chem.*, **36**, 18 (1897); **39**, 60 (1900).
(43) Saifer, A., and Hughes, J., *J. Biol. Chem.*, **118**, 241 (1937).
(44) Salter, W. T., "Endocrine Function of Iodine," pp. 270–91, Cambridge, Mass., Harvard University Press, 1940.
(45) Schezow, *Z. anal. Chem.*, 44, 86 (1905).
(46) Sendroy, Julius, Jr., and Alving, A. S., *J. Biol. Chem.*, **142**, 159–70 (1942).
(47) Sherill, M. S., *Z. physik. Chem.*, 47, 104 (1904).
(48) Strickler, H. S., and Strickler, E. W., *Endocrinology*, 37, 220–2 (1945).
(49) Sy, A. P., *J. Am. Chem. Soc.*, **29**, 786 (1907).
(50) Taurog, A., and Chaikoff, I. L., *J. Biol. Chem.*, 163, 313–22 (1946).
(51) Valeur, *Bull. soc. chim.*, **23**, 58 (1900).
(52) Votecek, E., *Chem. Ztg.*, **42**, 257, 271, 317 (1918).
(53) Winterstein and Herzfeld, *Z. physiol. Chem.*, **63**, 49 (1909).

RECEIVED June 9, 1948. Part of a dissertation presented by John J. Custer to the Graduate Faculty of Brooklyn College in partial fulfillment of the requirements for the M.S. degree.

Microdetermination of Riboflavin by Synthetic Ion Exchange Resin

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A new method of chemical determination of riboflavin by using synthetic cation exchange resin (KH-9) has been studied, and a rapid and accurate microdetermination of riboflavin has been achieved with successful removal of nonriboflavin fluorescence.

NUMEROUS physicochemical methods have been presented for the determination of riboflavin, all of which may be classified as (1) direct measurement of color of riboflavin solution (*7*, *8*) or (2) measurement of fluorescence of riboflavin solution (*3*, *10*). The latter method is more sensitive than the former.

In applying the fluorescence method for the quantitative determination of riboflavin in biological materials, it is necessary to remove as completely as possible fluorescing substances, which interfere with the accurate determination of the yellow-green fluorescence due to riboflavin.

For this purpose, the following method has been generally employed. Riboflavin is adsorbed on adsorbents of the fuller's earth type, then eluted from such adsorbents with pyridine-acetic acid or pyridine-alcohol solution, and the eluate is treated with potassium permanganate and hydrogen peroxide prior to measuring the fluorescence. But very few have adopted a "dynamic" method, which is more effective than a static method for adsorption and elution.

In 1941 Conner (*2*) proposed adsorbing and eluting riboflavin by a dynamic method, using Supersorb, one of the fuller's earth group. In his combined determination of riboflavin and thiamine, he adsorbed riboflavin on Supersorb and thiamine on zeolite, and eluted riboflavin by pyridine–acetic acid solution and thiamine by potassium chloride. This method is comparatively complicated because the operation must employ a vacuum system with special apparatus.

For this reason, the authors studied a new adsorbent of riboflavin by which the operation can be carried out under normal pressure with a simple apparatus, and found that the synthetic cation exchange resin KH-9 is most suitable for this purpose.

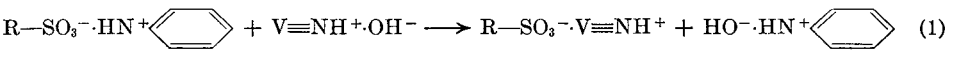
PRINCIPLE OF THE METHOD

Adsorption and Elution. The structure of riboflavin, in reference to the basic nitrogen, may be represented as: V≡N. Accordingly, in aqueous solution it undergoes dissociation as follows:

$$V{\equiv}N + H_2O \longrightarrow V{\equiv}NH^+{\cdot}OH^-$$

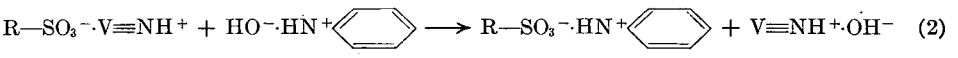
Therefore riboflavin can be adsorbed on a cation exchange resin by a cationic exchange reaction and eluted with pyridine as follows:

Adsorption



(1)

Elution



(2)

The cation exchange resin can be used repeatedly, as shown in Equations 1 and 2.

Removal of Nonriboflavin Fluorescence. Most of the interfering substances possessing fluorescence may be removed by the following procedures.

The nonriboflavin fluorescent substances in the extract, which have a stronger adsorption affinity than riboflavin, should be adsorbed on pyridine-treated zeolite, and those with an adsorption affinity equal to or less than riboflavin should be adsorbed on a synthetic resin. Then nonriboflavin fluorescent substances with a weaker affinity than riboflavin should be removed by rinsing the resin with hot water. The remaining nonriboflavin fluorescent substances with an equal adsorption affinity to riboflavin should be eluted with pyridine–acetic acid solution, and interfering substances possessing fluorescence should be reduced by oxidation with potassium permanganate solution and then by the use of a suitable yellow filter, the maximal transmission of which should be 560 mμ.

PRACTICAL PROCEDURES

Reagents. Synthetic Cation Exchange Resin KH-9. The Japanese Vitamin Pharmacal Co., Osaka, or the Oda Laboratory of Kyoto University.

Synthetic Zeolite. Takeda Chemical Co.

Pyridine–Acetic Acid Solution (pH 7.0), 20 or 30 volume %.

Glacial Acetic Acid.

Potassium Permanganate Solution. A 4% solution, freshly prepared each week.

Hydrogen Peroxide Solution. A 3% solution is prepared by diluting a 30% solution of hydrogen peroxide with distilled water.

Standard Riboflavin Solution. A 5 mg. % riboflavin solution is prepared with distilled water (adding 1 drop of glacial acetic acid to 100 ml. of water). It is diluted 1 to 50 with distilled water at the time of using.

Takadiastase. A 2% takadiastase solution is prepared and used after filtration through both zeolite and resin column.

Procedure. Two exchange tubes made of brown glass 0.7 to 0.8 cm. in diameter, are prepared. Figure 1 represents two assemblies.

The upper column is filled with 1.5 grams of activated zeolite of 60- to 100-mesh, and the lower column with the same amount of purified KH-9 of the same mesh. The former is treated with 50 ml. of 10% pyridine–acetic acid solution and then about 200 ml. of distilled water. The latter is treated with sufficient hot water and about 50 volume % pyridine solution until the resin gives no fluorescent substances in the eluate of 20 or 30 volume % pyridine–acetic acid solution, and is then rinsed with 200 ml. of distilled water. The eluate is preserved as a blank solution.

A finely pulverized sample, containing from 2 to 5 micrograms of riboflavin, is weighed, and about 40 ml. of distilled water are added. The pH of this mixture is adjusted to 4.5 with 1 *N* hydrochloric acid or sodium hydroxide. After addition of about 2 ml. of takadiastase solution and a few drops of toluene, the mixture is allowed to stand overnight in an incubator at 38° C. Then the mixture is heated on a boiling water bath for 15 minutes, being continuously stirred, after addition of 5 ml. of 1 *N* sulfuric acid. The extract is cooled to room temperature, distilled water is added to make the total volume of liquid 50 ml., and it is centrifuged at high speed until a clear supernatant liquid is obtained. The extract, which contains proteins, must be treated to precipitate with just enough 10% metaphosphoric acid.

A certain amount of clear supernatant solution, containing from 1 to 3 micrograms of riboflavin, is allowed to pass through the two columns at the rate of 1 to 2 ml. per minute, after pH has been adjusted to 4 to 5 with sodium hydroxide. Both columns are washed down thoroughly 6 to 7 times with 5-ml. volumes of distilled water successively at the same rate. Thiamine and the substances with stronger adsorption affinity than riboflavin are adsorbed on the pyridine-treated zeolite, but those with an adsorption affinity equal to or less than riboflavin pass through it and are adsorbed on the lower column of KH-9.

The resin column is washed with hot water (0.2 ml. of glacial acetic acid added to 100 ml. of distilled water), until no fluorescence is found in the solution passed through. Then riboflavin and the other substances with an adsorption affinity equal to riboflavin are eluted with 25 ml. of 20 or 30 volume % pyridine–acetic acid solution at a rate of 1 ml. per minute. The eluate is made up to exactly 25 ml. in a graduated cylinder. After mixing, 2 to 4 ml. of the eluate are pipetted into a test tube and acidified with 0.1 ml. of glacial acetic acid. Then 0.15 ml. of potassium permanganate solution is added, mixed, and allowed to stand 8 minutes. At the end of 8 minutes, a solution of 3% hydrogen peroxide is added until the color of the potassium permanganate disappears (the chief test solution). The blank solution is also oxidized by the same procedures (the blank test solution).

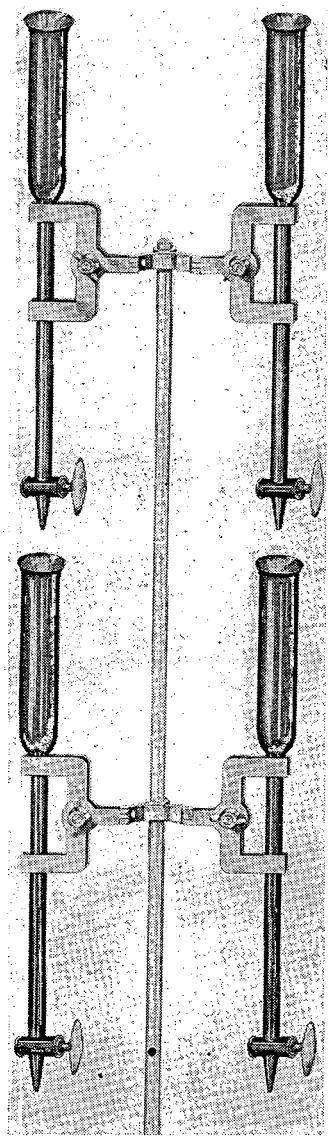


Figure 1. Exchange Tube Assemblies

The yellow-green fluorescence of the chief test solution is measured in comparison with the fluorescence of the riboflavin solution obtained by the titration of the blank test solution with standard riboflavin solution, by using a suitable yellow filter.

Table I. Riboflavin Content of Biological Materials

Material	Riboflavin Content, γ	Material	Riboflavin Content, γ
Rice, brown	68	Apricot	17
Rice bran	504	Peach	22
Soybean	116	Fresh cow's milk	279
Kidney bean (fresh)	69	Fresh goat's milk	351
Perilla	193	Liver of hens	1500
Leaf of beet	140	Heart muscle of hens	1280
Leaves of Japanese radish	250	Coffee, sample 1	927
Turnip greens	344	Coffee, sample 2	850
Japanese radish	30	Cocoa, sample 1	317
Pumpkin	40	Cocoa, sample 2	363
Potato	27	Green tea, Bancha UJI	635
Watermelon	24	Green tea, Sencha UJI	1050
Tomato	39	Green tea, Tencha UJI	1140
		Dried brewer's yeast	3750

ANALYTICAL RESULTS

The riboflavin content of various biological materials, analyzed by this method, is presented in Table I.

DISCUSSION

KH-9 is a synthetic resin with *p*- and *o*-phenolsulfonic acids as its base (*9*), which has a large ion exchange capacity and is very stable physicochemically. The break-through capacity is above 500 micrograms per gram of the resin. It can be used more than 200 times repeatedly in these determinations.

The adsorption of riboflavin on KH-9 under the conditions described above is always complete. But as an organic solvent interferes with adsorption, an extraction employing alcohol or acetone must be avoided.

Conner (*2*) recognized that the zeolite has a weak affinity for riboflavin. The authors have found that the pyridine-treated zeolite has less affinity for riboflavin than the potassium zeolite, and that the riboflavin adsorbed on pyridine-treated zeolite is eluted easily by a little distilled water without any leakage of thiamine. The authors' experiments show that 4 to 10 micrograms of riboflavin in 10 ml. of distilled water adsorbed on 1.5 grams of pyridine-treated zeolite are completely eluted by about 30 ml. of distilled water.

As the eluates obtained by this method always give yellow-green fluorescences due to riboflavin, more accurate results may be obtained by using a fluorometer, as generally employed in the United States for the determination.

The recovery of the pure riboflavin solution added to the extract was always more than 90% (Table II).

Table II. Percentage Recovery of Added Riboflavin

Sample	Riboflavin Content of Sample γ/g.	Riboflavin Added γ	Recovery of Riboflavin γ	Recovery %
Rice bran	5.04	1.0	0.97	97
Leaves of Japanese radish	2.50	1.0	0.95	95
Tomato	0.39	2.0	1.84	92
Urine	0.22	2.0	1.96	98

As thiamine is usually analyzed by using pyridine-treated zeolite (*4, 5*), a combined determination of thiamine and riboflavin can easily be achieved by this method.

Herr (*6*) and Brown (*1*) found that Amberlite IR-100 adsorbs riboflavin but they have not yet applied this resin to the determination. The authors have found that KH-9 is suitable not

only for the determination of riboflavin but also for the determination of pyridoxine and nicotinic acid by the dynamic method.

ACKNOWLEDGMENT

The authors wish to thank U. Miura, R. Oda, and M. Nishio of Kyoto University for valuable advice and assistance in this investigation.

LITERATURE CITED

(1) Brown, E. B., Bina, A. F., and Thomas, T. M., *J. Biol. Chem.*, **158**, 455 (1945).
(2) Conner, R. T., and Straub, G. J., IND. ENG. CHEM., ANAL. ED., **13**, 385 (1941).
(3) Euler, H. von, and Adler, E., *Z. physiol. Chem.*, **223**, 105 (1934).
(4) Fujiwara, M., and Kitamura, S., *Japan. J. Hyg.*, **2**, 12 (1948).
(5) Hennessy, D. J., IND. ENG. CHEM., ANAL. ED., **13**, 216 (1941).
(6) Herr, D. S., *Ind. Eng. Chem.*, **37**, 631 (1945).
(7) Kharit, A. Yu., and Khaustov, N. W., *Biochem. J.*, **29**, 34 (1935).
(8) Koshara, W., *Z. physiol. Chem.*, **232**, 101 (1935).
(9) Oda, R., Shimizu, H., and Nakayama, Y., *Chemistry of High Polymer*, to be published.
(10) Supplee, G. C., Ansbacher, S., Flanigan, G. E., and Hanford, Z. M., *J. Dairy Sci.*, **19**, 215 (1936).

RECEIVED September 22, 1948.

NOTES ON ANALYTICAL PROCEDURES

Volumetric Determination of Small Amounts of Iron

Chromous Chloride as Reducing Agent

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A PREVIOUS communication showed that chromous chloride gave excellent results when employed with liquid zinc amalgam as a reducing agent for ferric ions (*1*), and that its favorable application as a reducing agent depended upon the fact that the excess chromous ion is oxidized by atmospheric oxygen with no appreciable oxidation of the iron under the conditions of the experiment. Phenosafranine, a low potential redox indicator, was used to follow the reactions.

Chromous chloride alone is a satisfactory reducing agent for small amounts of iron. The method can be applied to solutions containing 0.1 mg. to 10 mg. of iron satisfactorily; with larger amounts of iron, the green color of the chromic ions interferes. A single determination can be carried out in 2 to 3 minutes without the use of an inert atmosphere.

The reagents were used at the concentrations shown in the following procedure and were prepared by methods already described (*1*). The average error for twelve determinations using between 1 and 10 mg. of iron was 0.25%. The average error was 0.9% in sixteen determinations using less than 1 mg. of iron.

Table I. Determination of Iron

Fe Taken, Mg.	Fe Found, Mg.	Error, %
9.047	9.022	−0.3
	9.013	−0.4
	9.026	−0.2
	9.018	−0.3
	Mean 9.020	
3.619	3.632	+0.3
	3.626	+0.2
	3.615	−0.1
	3.613	−0.2
	Mean 3.621	
1.810	1.805	−0.3
	1.820	+0.6
	1.810	0
	1.808	−0.1
	Mean 1.811	
0.724	0.730	+0.8
	0.724	0
	0.717	−1.0
	0.713	−1.5
	Mean 0.721	
0.362	0.358	−1.1
	0.357	−1.4
	0.357	−1.4
	0.363	+0.3
	Mean 0.359	
0.302	0.301	−0.3
	0.295	−2.3
	0.301	−0.3
	0.294	−2.6
	Mean 0.298	
0.120	0.119	−0.8
	0.120	0
	0.119	−0.8
	0.120	0
	Mean 0.120	

Five to 25 ml. of ferric iron solution containing 0.1 to 9 mg. of iron are acidified with either sulfuric or hydrochloric acid, and 2 or 3 drops of 0.01% phenosafranine indicator are added. Chromous chloride solution is then added dropwise until the pink color of the indicator disappears and the solution becomes a light clear green. The solution is swirled until the pink color reappears (this color is not the same as the original pink because of the added chromic ions). A few drops of 50% phosphoric acid and 0.05 to 0.1 ml. of a 0.16% diphenylamine sulfonate solution are added and the ferrous iron is titrated with a solution of potassium dichromate.

A 0.007 *N* solution of potassium dichromate is used for amounts of iron greater than 1 mg. A 0.003 *N* solution is used for less than 1 mg. The end point is a purple color which is easily detected over the light pink or violet color of the solution. A correction of 0.05 ml. of 0.01 *N* potassium dichromate is subtracted for each 0.1 ml. of indicator used in the titration. In the titration of less than 1 mg. a solution of the oxidized form of the diphenylamine sulfonate was used and gave a blank of 0.01 ml. of 0.01 *N* potassium dichromate for each 0.1 ml. of indicator.

LITERATURE CITED

(1) Cooke, Hazel, and McNabb, ANAL. CHEM., **21**, 643 (1949).

RECEIVED November 19, 1948. Presented before the Meeting-in-Miniature, Philadelphia Section, AMERICAN CHEMICAL SOCIETY, January 20, 1949.

Comparison of Tellurium and Selenium as Catalysts for Kjeldahl Digestion

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ALTHOUGH considerable work has been done on selenium and its compounds as catalysts for the Kjeldahl method, little has been published on the use of tellurium and its compounds. It might be reasoned that because selenium and tellurium are analogs, the catalytic effect of tellurium would be much the same as that of selenium.

Illarionov and Soloveva (*4*), in discussing selenium and tellurium, state that the catalytic effect of the elements is similar and proportional to the amount used. In a survey of the effect of various amounts of selenium on the Kjeldahl digestion, Bradstreet (*1*) found that when samples of approximately the same size were used, very little difference in time of clearing of the digest was found with an increase of selenium. Furthermore, a definite loss of nitrogen occurred with amounts of selenium in excess of 0.25 gram. If this is true of selenium, the possibility exists that tellurium may act in the same manner.

Gresin (*3*) states that tellurium is a good catalyst, and that the speed of decomposition of the sample depends on the amount of catalyst used.

In the present investigation, using varying amounts of tellurium, little difference in digestion times was noted, but in comparison with selenium, digestion was appreciably slower. In the majority of the determinations, tellurium catalysts gave results which were erratic, could not be correlated with an increase of catalyst, and were, in most cases, lower than the calculated percentages.

Operating conditions were standardized as far as possible to minimize errors. Electric heaters were used for digestion and distillation. The reduction of the nitro compounds was accomplished by the use of salicylic acid.

Table I. Relative Merits of Tellurium Catalysts

	Tellurium Used, Gram				
	0.10	0.25	0.50	0.75	1.00
	Per Cent Nitrogen Found				
	Acetanilide (10.36% N_2)				
Te	10.00	10.23	10.16	10.14	10.33
Te + $CuSO_4.5H_2O$[a]	10.22	10.16	10.27	10.28	10.30
Te + $FeSO_4.7H_2O$[b]	10.10	10.14	10.22	10.30	10.22
	Anthranilic Acid (10.21% N_2)				
Te	9.86	10.12	10.09	10.19	10.22
Te + $CuSO_4.5H_2O$[a]	9.94	10.21	10.00	10.19	10.18
Te + $FeSO_4.7H_2O$[b]	9.82	9.98	10.10	10.16	10.23
	p-Nitroaniline (20.30% N_2)				
Te	19.67	20.01	19.65	19.77	19.78
Te + $CuSO_4.5H_2O$[a]	19.92	19.97	19.31	19.1	19.18
Te + $FeSO_4.7H_2O$[b]	19.83	19.29	19.61	19.64	19.82
	m-Dinitrobenzene (16.68% N_2)				
Te	15.73	15.94	15.85	15.94	15.70
Te + $CuSO_4.5H_2O$[a]	15.93	16.06	16.20	15.95	15.65
Te + $FeSO_4.7H_2O$[b]	15.82	15.90	15.40	15.88	16.00

[a] 0.25 gram $CuSO_4.5H_2O$.
[b] 0.25 gram $FeSO_4.7H_2O$.

PROCEDURE

Samples were weighed into 300-ml. Kjeldahl flasks and 35 ml. of concentrated sulfuric acid containing 1 gram of salicylic acid were added. The flasks were allowed to stand for 30 minutes in the cold. Five grams of anhydrous sodium thiosulfate were added, and after the reaction had subsided the heaters were turned on low heat until the mixture blackened. The heat was then shut off and the flasks were cooled. Ten grams of potassium sulfate containing varying amounts of catalyst were added. Vigorous heat was applied until the digestion cleared, at which point the heat was reduced and the contents of the flasks were boiled gently for 1 hour. After the flasks had cooled, 125 ml. of distilled water were added.

To the diluted and cooled digest, 165 ml. of 35% sodium hydroxide were carefully added, so that two distinct layers were formed. A small piece of low melting paraffin and several pieces of mossy zinc were added and the flasks were connected to the distillation rack. Davisson (*2*) distilling bulbs were used. The flasks were swirled gently to mix the two layers and heat was applied. Distillation was continued for 1 hour after boiling started, and the distillate was collected in 500-ml. Erlenmeyer flasks containing 50 ml. of distilled water, 25 ml. of 0.1 *N* hydrochloric acid, and 4 drops of a 0.1% solution of methyl red. At the end of the distillation, the flasks were disconnected, and the condensers and delivery tubes were carefully washed out with distilled water. The distillate was titrated with carbonate-free 0.1 *N* sodium hydroxide. Blank determinations were run and suitable corrections applied.

The relative merits of tellurium, tellurium and copper sulfate, and tellurium and ferrous sulfate were compared; the amount of tellurium varied between 0.1 and 1.0 gram. Four typical organic compounds, the nitrogen of which was easily reducible to ammonia, were used. The results, shown in Table I, seem to indicate the unsuitability of tellurium as catalyst in elemental form or in combination with copper sulfate or ferrous sulfate.

The possibility of its use as a catalyst in the form of sodium salts led to further investigation and comparison with similar compounds of selenium. Ten different catalysts and catalyst combinations were used on six organic compounds containing nitrogen in various forms. Except in the cases of ferrous sulfate and copper sulfate in combination with selenium and tellurium, 0.25 gram of catalyst was used. The mixed catalysts contained 0.25 gram of each component. The time of clearing of the digestion mixture averaged 30 minutes, and all samples were given 1 hour afterboil.

The results of this comparison are shown in Table II. In

Table II. Per Cent Nitrogen

Compound	N_2 Calcd., %	Catalyst: Tellurium	Sodium tellurite	Sodium tellurate	Copper sulfate and tellurium	Ferrous sulfate and tellurium	Selenium	Sodium selenite	Sodium selenate	Copper sulfate and selenium	Ferrous sulfate and selenium
m-Dinitrobenzene	16.68	15.94	15.51	15.80	16.06	15.90	16.66	16.08	16.20	16.64	16.64
p-Nitrobenzoic acid	8.39	8.46	7.92	8.12	8.14	8.08	8.38	8.37	8.38	8.41	8.43
p-Nitroaniline	20.30	20.01	18.93	19.21	19.97	19.29	19.96	19.54	20.06	20.27	20.26
1-Aminobenzothiazole	18.67	14.06	13.93	18.80	18.56	...	18.53	18.47	18.51	18.49	18.46
Acetanilide	10.36	10.23	9.80	9.79	10.16	10.14	10.34	10.28	10.27	10.26	10.31
Anthranilic acid	10.21	10.12	10.15	10.17	10.21	9.98	10.25	10.23	10.22	10.26	10.25

nearly all cases, tellurium alone, tellurium compounds, and combinations of tellurium with ferrous sulfate or copper sulfate gave low results. Of the selenium catalysts, selenium alone, or with ferrous sulfate or copper sulfate, was satisfactory. Sodium selenite and sodium selenate gave slightly lower results in some cases. It may be concluded that tellurium is not generally suitable as a catalyst for the Kjeldahl digestion.

LITERATURE CITED

(1) Bradstreet, R. B., IND. ENG. CHEM., ANAL. ED., 12, 657 (1940).
(2) Davisson, B. S., *J. Ind. Eng. Chem.*, 11, 465 (1919).
(3) Gresin, Yu. D., *Farm. Zhur.*, 1937, No. 2, 104–9.
(4) Illarionov, V. V., and Soloveva, N. A., *Z. anal. Chem.*, 100, 328–43 (1935).

RECEIVED July 12, 1948.

Simplified Gas Microanalyzer

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ALTHOUGH the Saunders-Taylor method (*1, 2*) for the microanalysis of gas is rapid and accurate, it has certain disadvantages. The custom-built multiway stopcock around which the apparatus is designed is relatively expensive and inconvenient to obtain. Likewise, the design of the combustion chamber requires an undue amount of care to prevent overheating the grease on the ground joint through which the chamber is connected to the apparatus.

The desirable features of the method can be retained in a simplified form which is easily fabricated from standard stopcocks and ground joints.

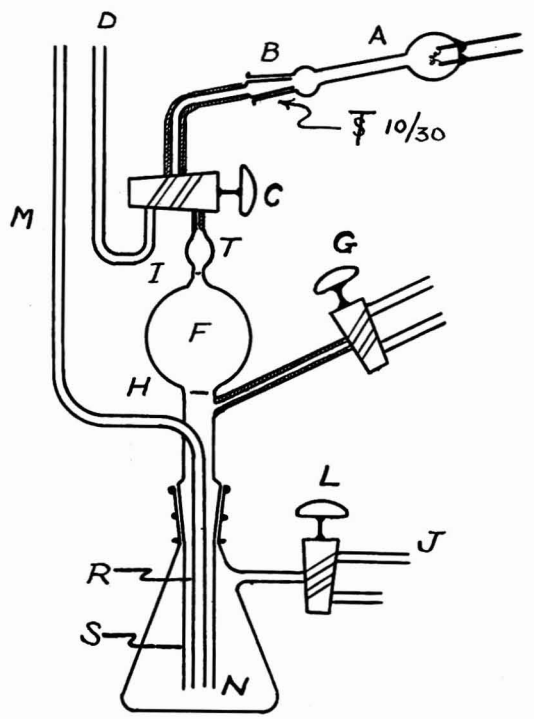

Figure 1. Diagram of Microanalyzer

The 1-mm. capillary two-way stopcock, *C*, replaced the multiway stopcock in the original Saunders-Taylor design. The stopcock plug and reaction tubes, *A*, were evacuated through *D*, which was connected to high vacuum. Gas to be analyzed was passed into *A* by rotating the plug 180°.

Tubes holding reagents for various determinations had 10/30 ground joints and were interchangeable at *B*. These tubes were similar to those used by Saunders and Taylor. The reaction tube shown was used for combustions and was a modification of the original design. The two leads were 1-mm. tungsten and the filament was made from three turns of platinum wire spot-welded to the tungsten electrodes. With this design the filament could be operated as long as required without cooling the ground joint, which was lubricated with Apiezon N.

In a typical analysis the reaction tube was evacuated through *D* and volume *F* was evacuated through one side of stopcock *G*. The other arm of *G* was connected to a reservoir of gas to be analyzed. During the evacuation the mercury level in *N* was kept below the mark at *H* by applying vacuum through stopcock *L* at *J*. When the pressure in *F* was 10^{-5} mm. or lower, a sample of gas was added through *G*. The mercury level was raised to *I* by cautiously opening *L* to the atmosphere. The difference in height of the mercury menisci at *I* and in manometer *M* was a measure of the gas in volume *T*. Stopcock *C* was then rotated and the gas was forced into the appropriate reaction tube by raising the mercury level. After a period of time determined by experiment, the mercury was lowered to *H* and *C* was closed. The gas was then compressed again to *I* and the manometer was read. The difference between the two manometer readings after correction for residual gas in the reaction tube was a measure of the volume change in the reaction tube used. The residual gas correction was calculated from the volume ratio of *A* and *F;* for the apparatus shown this value was 0.00441 or 0.41%.

A representative analysis of a four-component determinate mixture is given in Table I. The gases in these samples were purified by the techniques outlined by Saunders and Taylor (*2*).

The modified analyzer is easier to build and more readily kept in operation than previous designs. In general, the analytical difficulties encountered by Saunders and Taylor are still present.

Table I. Analysis of a Standard Sample

Component	Taken, Mm.	Found, Mm.
H_2	17.1	17.3 17.1
CO	32.4	32.8 32.0
CH_4	68.2	68.5 68.1
C_2H_6	14.7	14.9 14.8

However, the small empirical correction used by them in calculating carbon dioxide contractions can be neglected if not more than 10% excess oxygen was added to the hydrocarbon fraction prior to combustion. This correction, the need for which they attributed to stabilization of ozone on the walls of the water-cooled combustion tube, apparently may be neglected, if, as in the present design, the combustion tube is air-cooled and can, as a result, attain a higher wall temperature during combustion.

LITERATURE CITED

(1) Garrison, W. M., and Burton, M., *J. Chem. Phys.*, 10, 730–9 (1942).
(2) Saunders, K. W., and Taylor, H. A., *Ibid.*, 9, 616–25 (1941).

RECEIVED July 23, 1948.

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Laboratory Induction Stirrer for Closed Systems

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NUMEROUS internal stirrers have been devised for the agitation of liquids in closed systems. Most of the devices are of the magnetic type and are so designed that the mechanism required to establish a rotating or an alternating vertical field outside the reaction flask is cumbersome and impractical when the reaction flask must be immersed in a cooling or a heating bath. Other drawbacks are low power developed, complex construction details, and lack of versatility. An improved design of a simple induction stirrer which circumvents many of the above difficulties (*1*) is described in this paper.

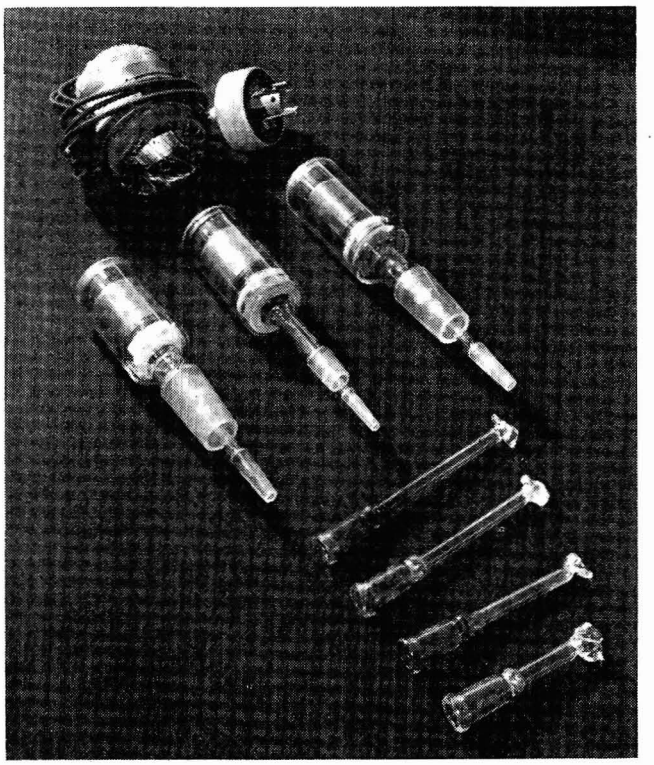

Figure 1. Stirrers

The induction motor consists of a three-phase selsyn generator stator, a glass-enclosed armature, and a bearing system (see Figure 1). Various sizes and types of stirrers may be conveniently attached to the armature. The unit is particularly useful with small scale reaction vessels. The maximum speed of the stirrer is over 3000 r.p.m. and sufficient power to stir viscous solutions is developed. The speed can be controlled with a variable transformer.

Two models of the stirrer have been developed which differ only in their bearing system. Figure 2 shows a sectional view of a unit which employs a ball bearing, and a corrosion-resistant model in which a Teflon (polymerized tetrafluoroethylene, made by E. I. du Pont de Nemours & Co., Wilmington, Del.) bearing is used. The motor is designed to have good vacuum characteristics and to be easily cleaned. The rotor is solid, and outgassing from windings is thus avoided. Holes in all solid bearings permit rapid evacuation of otherwise enclosed spaces and gas volumes are kept small. Both models have been used routinely in a C^{14} synthetic laboratory and show no memory effects.

Armature. The core of the armature (Figure 3) is turned from soft iron. The copper shell, which is made from copper tubing of 0.035-inch wall, and 1.25-inch outside diameter, and the iron core are soft-soldered together and made vacuum-tight at the edges. After the machining of the armature is finished, the entire armature, except for the area where the bearing fits on, is painted with a corrosion-resistant paint (plastic paint 4A, Interchemical Corp., 1073 Howard St., San Francisco, Calif.) and baked at 250° F. for 12 hours. The same armature is used with both models.

Cap. The cap is machined from hard copper and should fit the glass shell loosely (Figure 4). The cap, with screws in place, is also painted and baked as is the armature. The Teflon bearings (made from $^1/_{16}$-inch sheet) in the cap should fit loosely on the upper armature shaft, for Teflon expands when it is warmed.

The cap is sealed to the glass shell with a low melting sealing compound (de Khotinsky cement, sealing wax, etc.). In this process both the cap and the glass are warmed; the edges are covered with a thick layer of melted wax, and the parts are brought together. Enough wax should be used to form a definite fillet on the inside surface of the stirrer; excess wax on the outside may be cut off.

Bearings (Lower). The ball bearing (open type, 1.25-inch outside diameter, $^5/_{16}$ inch inside diameter, ball bearing 6210, SKF Industries, Inc., 440 East 34th St., New York, N. Y.) is centered in the glass shell by two or three small rubber bands or a metal ring and supported on an oil-resistant synthetic rubber washer; it is lubricated with a nonvolatile, nonreactive oil. (Some types of diffusion pump oil are very good for this purpose—for example, D.C. 703 silicone fluid, Dow-Corning Corp., Midland, Mich.)

The plastic bearing is machined from 0.25-inch Teflon sheet to give a slide fit inside the glass shell and a loose bearing fit on the armature. Rotation of this bearing is prevented by a V cut in the Teflon and an indentation on the lower end of the glass shell. Four 3-mm. holes (0.125 inch) bored slantwise through the body of the plastic permit evacuation of the upper chamber of the stirrer. A ring of $^1/_{16}$-inch Teflon provides a horizontal Teflon to Teflon bearing that has less friction resistance than a plastic to iron bearing; even so wear is appreciable and such bearings have a limited life.

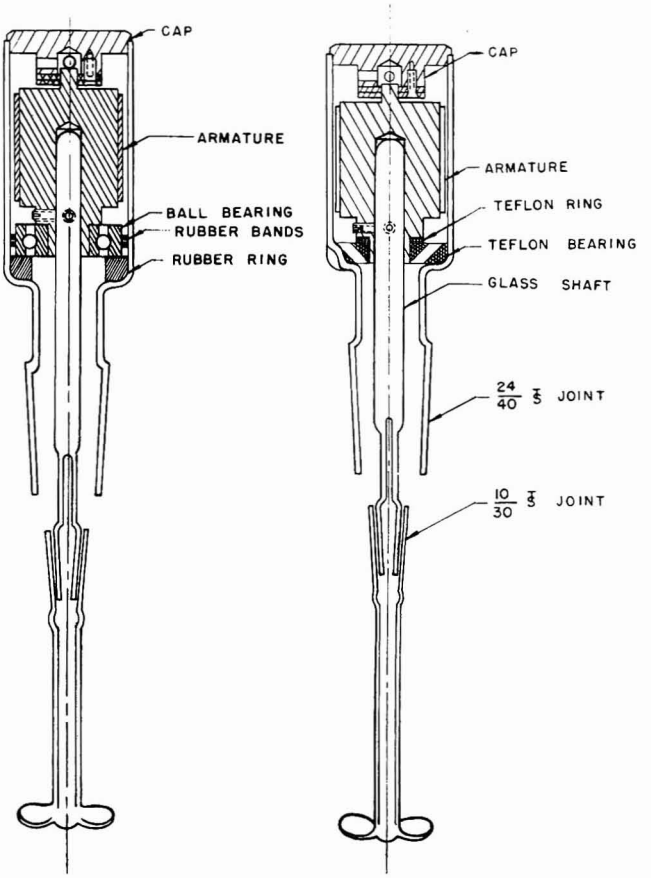

Figure 2. Induction Stirrers

Left. **Ball-bearing stirrer**
Right. **Corrosion-resistant model with lower bearing of Teflon**

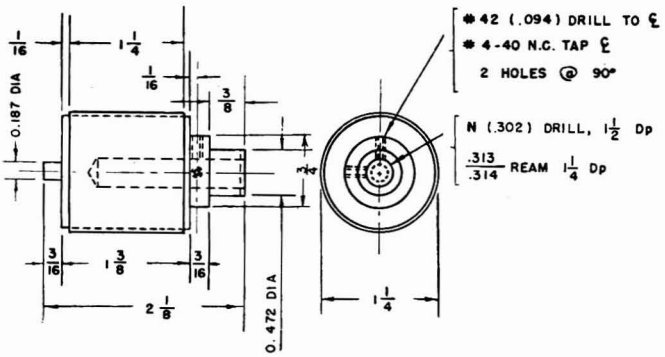

Figure 3. Armature

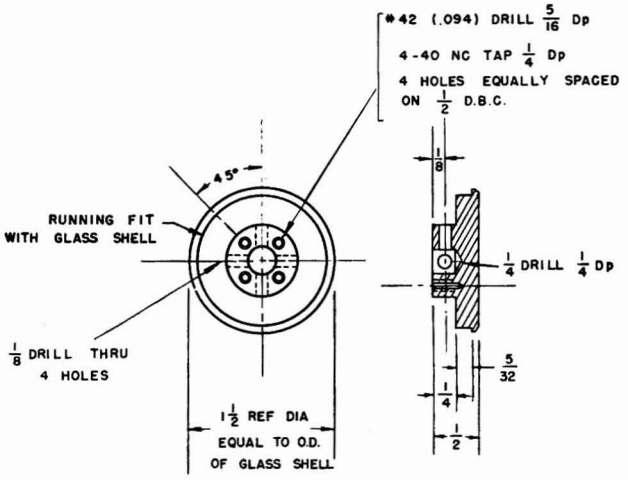

Figure 4. Cap

Stator. The stator is the field coil of a selsyn generator (Model 2J55V1, 110 volts, 60 cycles, General Electric Co., Schenectady, N. Y., 1.5-inch inside diameter). When the unit is operated above 30 volts a water jacket or air blast should be provided to prevent overheating of the windings of the stator and loosening of the wax seal of the armature housing. This stator is supported in the usual manner or on a collar (a cork ring) which rests on the reaction equipment. No alignment problem is encountered when the stirrer is assembled in the latter fashion.

The power supply for the stator is conveniently made from a variable transformer and a 50–100μF oil or paper condenser (Figure 5). (Pyranol Capacitor, Catalog No. 67X18X, Model 9CE1A318, 330 volts, 60 cycles, 50μF). This arrangement is a typical capacitor induction type motor circuit and permits operation of the motor on a 110-volt, 60-cycle single-phase line.

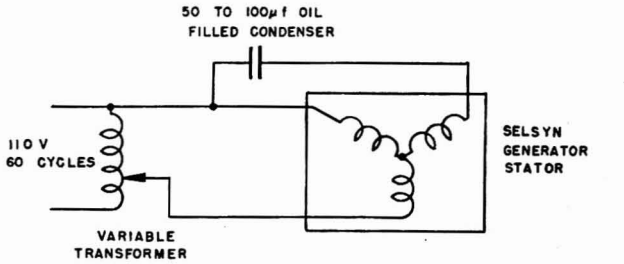

Figure 5. Wiring Diagram

Stirrers. Many types of stirrer assembly may be used with this motor. The stirrers are connected to the drive shaft of the motor by means of a ground joint and the joint is held together with a low melting solid (such as de Khotinsky cement or sealing wax). This method of connection allows for attaching different stirrers without opening the armature housing.

LITERATURE CITED

(1) Dauben, W. G., Reid, J. C., and Yankwich, P. E., ANAL. CHEM., 19, 828 (1947).

RECEIVED September 3, 1948. Based on work performed under contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, Calif.

Modified Method of Charging the Poth Carbon Dioxide Generator

P. L. PICKARD, *The University of Oklahoma, Norman, Okla.*

IN 1930, Poth (*2*) described a generator "which enables one to store a quantity of carbon dioxide with complete assurance that it will represent a gas of consistent high purity" for use in microanalysis of nitrogen by the Dumas method. The generator has since been modified (*3*). This author's first experience with the modified form of the Poth generator was with a commercial model which had been discarded because the difficulty of charging according to the directions given had led to the conclusion that it was incorrectly built. After several attempts the generator was successfully charged and served for the duration of a problem which involved an extraordinarily large number of nitrogen analyses (*1*).

Because new generators from the same source (Scientific Glass Apparatus Co.) have caused similar difficulty, a method of charging has been developed which is considered more convenient and rapid than that described by Poth.

With the generator (Figure 1) lying horizontally so that opening *E* is at the top, a solution of 1 to 2 sulfuric acid in water is sucked into *B* by connecting *D* to an aspirator. The acid solution is not cooled after mixing, as the heat generated aids in exclusion of air from the apparatus. The generator is then raised to a vertical position, the aspirator is connected to *C*, and 1200 ml. of a hot saturated solution of potassium bicarbonate are sucked into *A*. A few milliliters of water are pulled into *A* to rinse the bicarbonate from *D*.

Sufficient water is added to *D* to cover the tip of the bubbler. Mercury is added to *C* until the level is about half-way to the upper end of *F*. *C* is connected to the leg of a Y-type three-way stopcock. One arm of the stopcock is open to the atmosphere and the other connected through a T joint to a vacuum pump which is adequately protected by a drying column. The other leg of the T joint is connected to *D*. With *D* and *Y* closed the vacuum pump is started and *Y* opened into *C* very cautiously to prevent too violent bumping of the mercury. After the generator has been evacuated several minutes, *D* is opened slightly. By adjusting *D*, the level of sulfuric acid in *E* may be maintained nearly constant. Should the level of acid in *E* rise, *D* should be shut off momentarily.

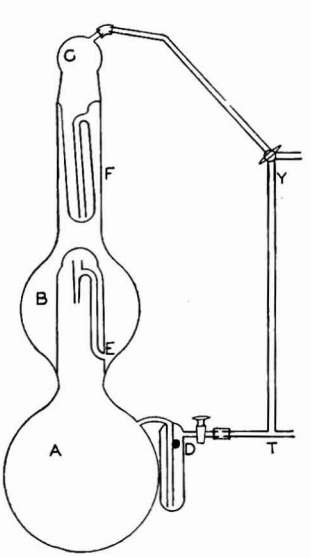

Figure 1

After 15 minutes' evacuation, *D* and then *Y* are closed. *D* is opened slowly, with the pump still operating, and acid is pulled up into *E*. As soon as a drop of acid falls, *D* is closed and the carbon dioxide evolved bubbles back through *E* and *F* to equalize the

pressure. D is again opened and the process is repeated several times.

With D closed, Y is opened slowly to the atmosphere. If the pressure in the generator is insufficient, mercury will be forced up into F, and more acid must be pulled into A as before. If the pressure exceeds atmospheric by an amount greater than the height of mercury in C, carbon dioxide will bubble through the mercury until equilibrium is attained. C and D are disconnected and more mercury is added to bring the level about to the top of F. The small amount of sulfuric acid solution on the mercury may be removed by a cotton swab.

Carbon dioxide is evolved more rapidly than it escapes through D. Consequently, while the generator is in use excess gas escapes through the mercury seal each time acid drops into the bicarbonate. Rapid delivery of gas causes large amounts of acid to be pulled into the bicarbonate and usually results in expulsion of mercury from the seal and contamination of the generator with air. To prevent this it is advisable to connect a section of capillary (a broken thermometer serves this purpose admirably) to D, so that gas may not be delivered more rapidly than is ordinarily required for the Dumas microprocedure.

LITERATURE CITED

(1) Pickard, P. L., and Lochte, H. L., *J. Am. Chem. Soc.*, **69**, 14 (1947).
(2) Poth, E. J., IND. ENG. CHEM., ANAL. ED., **2**, 250 (1930).
(3) *Ibid.*, **3**, 202 (1931); **11**, 518 (1939).

RECEIVED September 20, 1948.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

21. *o*-Aminobenzoic Acid (Anthranilic Acid)

o-Aminobenzoic acid exists in three different crystalline modifications differing only slightly in stability. Modification III is always obtained from fusion; modifications II and III are usually obtained from solvents unless the solution is cooled very slowly, in which case some modification I may be obtained. Good crystals are obtained from almost any solvent—e.g., acetic acid, ethyl acetate, aniline, nitrobenzene, water. The last three solvents are useful in obtaining crystals on a microscope slide

The commercial material usually contains modification III in largest amount, and very little modification I. Modification I is, however, the stable form at room temperature.

o-AMINOBENZOIC ACID I

CRYSTAL MORPHOLOGY (determined by W. C. McCrone).

Crystal System. Orthorhombic.

Form and Habit. Plates or tablets from water lying on 100; rods elongated parallel to c. Shows the forms: macropinacoid, {100}; brachypinacoid, {010}; bipyramid, {221}.

Axial Ratio. $a:b:c$ = 0.838:1:0.724. 0.6066:1:0.8715 (*1*).

Interfacial Angles (Polar). 101 Λ $\bar{1}01$ = 81.6°; 021 Λ $0\bar{2}1$ = 110.8°.

X-RAY DIFFRACTION DATA (determined by W. C. McCrone, I. Corvin, and J. Whitney).

Space Group. C_{2v}^{9} (*2*).

Cell Dimensions. a = 10.79 Å.; b = 12.87 Å.; c = 9.32 Å. a = 9.4 Å.; b = 10.8 Å.; c = 12.8 Å. (*2*).

Formula Weights per Cell. 8.

Formula Weight. 137.13.

Density. 1.408; 1.412 (*1*).

Principal Lines

d	I/I_1	d	I/I_1
8.02	0.45	3.62	Very weak
7.58	Very weak	3.46	Very weak
6.03	0.63	3.34	1.00
5.81	0.76	3.17	0.47
5.29	Very weak	3.04	0.11
4.71	Very weak	3.00	Very weak
4.03	0.80	2.83	Very weak
3.79	0.49	2.76	Very weak

OPTICAL PROPERTIES (determined by W. C. McCrone).

Refractive Indexes (5893 Å.; 25° C.). α = 1.500. β = 1.74. γ = 1.77.

Optic Axial Angles (25° C.). $2V$ = 39° (D); $2E$ = 70° (red); 68° (blue).

Dispersion. $r > v$.

Optic Axial Plane. 001.

Sign of Double Refraction. (−).

Acute Bisectrix. b.

Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.67. R (calcd.) = 36.8. R (obsd.) = 36.3.

Pleochroism. Colorless for light vibrations parallel to α; red for light vibrations parallel to β and γ.

o-AMINOBENZOIC ACID II

CRYSTAL MORPHOLOGY (determined by W. C. McCrone).

Crystal System. Orthorhombic.

Form and Habit. Rods and needles elongated parallel to c; shows brachypinacoid, {100}; macropinacoid, {010} and bipyramid, {111}.

Axial Ratio. $a:b:c$ = 0.727:1:0.447. [These ratios agree well with Groth (*1*) if a and b are reversed and our a doubled.]

Interfacial Angles (Polar). 011 Λ $0\bar{1}1$ = 48.2°; 101 Λ $\bar{1}01$ = 63.2°.

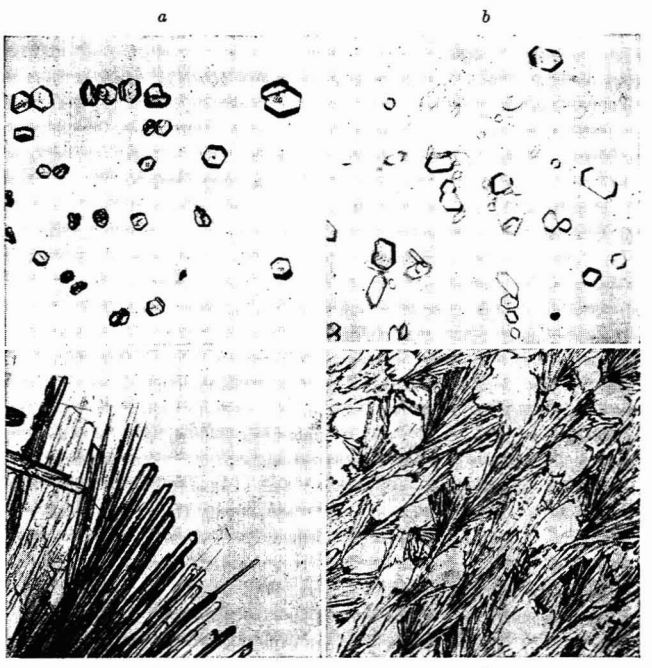

Figure 1. *o*-Aminobenzoic Acid

a. **Modification II from water on microscope slide**
b. **Modification I from aniline on microscope slide**
c. **Modification III from thymol on microscope slide**
d. **Modification III from melt on microscope slide**

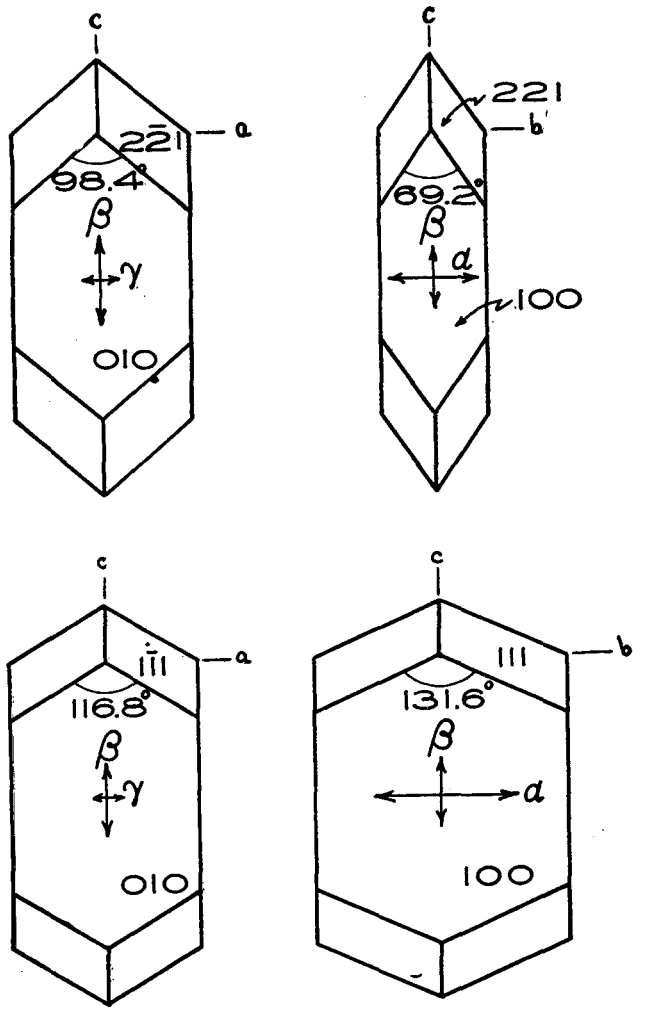

Figure 2. σ-Aminobenzoic Acid
Orthographic projections for modifications I (*upper*) and II (*lower*)

X-RAY DIFFRACTION DATA (determined by W. C. McCrone, J. Whitney, and I. Corvin).
Cell Dimensions. a = 11.66 Å.; b = 16.04 Å.; c = 7.18.
Formula Weights per Cell. 8.
Formula Weight. 137.13.
Density. 1.367 (*1*).

Principal Lines

d	I/I_1	d	I/I_1
8.21	0.11	3.38	0.12
6.42	0.24	3.21	0.36
6.16	0.30	3.01	Very weak
5.38	0.25	2.92	0.53
4.74	0.52	2.64	0.18
4.37	0.06	2.53	Very weak
4.11	Very weak	2.48	0.11
3.96	Very weak	2.44	Very weak
3.76	0.18	2.40	Very weak
3.66	1.00	2.32	Very weak
3.56	Very weak	2.27	0.11

OPTICAL PROPERTIES (determined by W. C. McCrone).
Refractive Indexes (5893 Å.; 25° C.). α = 1.560. β = 1.73. γ = 1.76.
Optic Axial Angles (25° C.). $2V$ = 46° (D). $2E$ = 84 (D); 81° (blue); 85° (red); 78½° (yellow); 73° (blue) (*2*).
Dispersion. $r > v$.
Optic Axial Plane. 001.
Sign of Double Refraction. (−).
Acute Bisectrix. b.
Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.68. R (calcd.) = 36.8. R (obsd.) = 37.9.

σ-AMINOBENZOIC ACID III

CRYSTAL MORPHOLOGY (determined by W. C. McCrone).
Crystal System. Monoclinic.
Form and Habit. From fusion elongated rods parallel to direction of growth (assumed c).
Interfacial Angles (Polar). 64°, 123°.
OPTICAL PROPERTIES (determined by W. C. McCrone).
Refractive Indexes (5893 Å.; 25° C.). α = 1.562. β = 1.707. γ = 1.78 (calcd.). (Note. α and β as listed are the Cargille liquids which when saturated with σ-aminobenzoic acid cause the crystals to disappear in those positions.)
Optic Axial Angles (25° C.). $2V$ = 68° (blue); 73° (yellow); 75° (red).
Dispersion. Very strong crossed dispersion, $r > v$.
Optic Axial Plane. ⊥ 010.
Sign of Double Refraction. (−).
Acute Bisectrix. b.
Extinction. β = 33° from length.
FUSION DATA (determined by W. C. McCrone).
σ-Aminobenzoic acid melts at 145° C. with sublimation. The melt solidifies spontaneously on cooling. The crystals are always those of modification III; they are characterized by large gas bubbles (Figure 1) and strong dispersion. The latter is manifest on those views showing an acute bisectrix interference figure; $2V$ = 68° (blue); 73° (yellow); 75° (red); (−).

LITERATURE CITED

(1) Groth, "Chemische Kristallographie," Vol. 4, p. 508, Leipzig Engelmann, 1910.
(2) Kitaigorodski, *Izvest. Akad. Nauk. U.S.S.R. Atdel. Khim. Nauk.*, 1948, 278–89.

CORRECTION. In the paper entitled "Microscope Hot Stage" by F. W. Matthews [ANAL. CHEM., 20, 1112 (1948)] Table I, page 1115, "anthraquinone" should read "anthracene."

F. W. MATTHEWS

Correspondence

Evaluation of Catalysts for Catalytic Cracking

SIR: In the article by Rescorla, Ottenweller, and Freeman [ANAL. CHEM., 20, 196 (1948)] we note some data given for natural catalyst which may be in error.

1. In Table I the natural catalyst density aerated is given as 49.1 pounds per cubic foot, and free settled density as 46.5. The values would be about right were they interchanged.
2. In Table II the sodium oxide content of natural catalyst is given as 0.32%. We believe this value is erroneously high. In the same table the iron content is given as a 0.32% Fe_2O_3. This value is apparently erroneously low. It appears that a typographical error is involved, as the iron and sodium contents in the case of both synthetic and natural are given as equivalent.
3. In Table III the sodium oxide content of natural catalyst appears erroneously high, and we do not agree that the cracking activity data given are typical of the average natural catalyst from fluid cracking units. In this case the data may be correct because of certain characteristics of the oil feed stock to the unit in question.
4. At the bottom of page 200, the volatile matter content of new natural catalyst is given as 25% when subjected to heat treatment at 850° F. Actually it would be expected to test between 17 and 20% on such heat treatment.

R. B. SECOR

Filtrol Corporation
Los Angeles, Calif.

SIR: Acknowledging our negligence in answering the comments of Dr. Secor, we discuss them in the order listed in his letter of April 29, 1948, to you.

1. This is correct and the column should read:

	Catalyst Densities, Natural, Pounds per Cubic Foot
Aerated	46.5
Freely settled	49.1
Compacted	55.7
Pressure, 5 pounds per square inch	54.6

2. Rather than go back to the data at the time the paper was written, we have averaged some recent chemical analyses of natural catalyst. These results are:

	New Natural Catalyst, %
Sodium, Na_2O	0.17
Iron, Fe_2O_3	1.23

It is probable that the iron content should have read 1.32%.

3. The choice of the word "typical" may not have been accurate, as this natural catalyst was used in a cracking unit charging deasphalted gas oil possessing considerable catalyst-contaminating characteristics. Thus the properties of the regenerated synthetic and natural catalysts are not comparable for a given operation. The sodium oxide content of recent samples has averaged 0.37% Na_2O.

4. Actually the new natural catalyst, as received, does test between 17 and 20%, or even lower, with 850° F. heat treatment. However, the sample after handling and elutriation with air (not completely dry) may contain more water.

J. H. OTTENWELLER

Cities Service Refining Corporation
Lake Charles, La.

BOOK REVIEWS

Kvalitativni Chemicka Analysa (Qualitative Chemical Analysis). *Jindrich H. Krepelka.* xii + 341 pp. Czechoslovak Chemical Society, Prague, Czechoslovakia, 1947. Price, 275 Czechoslovak crones (bound).

This book is one of the most recent and complete texts on qualitative analytical procecures published in the Czech language.

Krepelka has been professor of inorganic chemistry at the Charles University in Prague since 1931 and since 1938, dean of the faculty of natural sciences. His postgraduate work included studies under the direction of the prominent American scientist, T. W. Richards, at Harvard University during 1919 and 1920. In recognition of his contributions to science, he was awarded the distinguished honor of membership in the Czech Academy of Science and Arts. Krepelka is not only well known for his outstanding publications and teaching, but has also gained widespread recognition for his proficiency and valuable work in legal chemistry, a field in which he has worked untiringly since 1923.

This book consists of five major parts. In the first 7 pages the author discusses blowpipe analytical procedures and describes methods of preliminary tests for cations and anions, followed by 10 pages devoted to a discussion of the solubility characteristics of inorganic compounds. In 179 pages he discusses the group separation of cations and anions, followed by a systematic procedure for the qualitive analysis of inorganic compounds. In the last two sections the author discusses identification methods for organic substances of toxic nature and the last 29 pages present tables of physical constants and a list of the reagents employed. The material is clearly presented and illustrated by several figures and photographs.

This book was written primarily for university students, but will be helpful also to the practicing analytical chemist.

A. LASSLO

Table of Reagents for Inorganic Analysis. *C. J. van Nieuwenburg* and *P. E. Wenger.* xi + 201 pages. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y. Price, $10.

The third report of the International Commission on New Analytical Reactions and Reagents of the International Union of Chemistry is the successor to the first report, published in 1938 by C. J. van Nieuwenburg, W. Böttger, F. Feigl, A. S. Komarovsky, and N. Strafford. It is presented in the identical tabular form of the first report, in French, English, and German, and employs the same system of abbreviations. It covers the period 1937 to 1947 and lists some 1373 reactions, including additional references on previously described tests that are numerically designated to correspond with those given in the first report. There are new chapters on europium, ytterbium, hydroxylammonium, hydrazinium, silicofluoride, carbon oxychloride (phosgene), chlorite, sulfur dioxide, hydrosulfate, dithionate, water, and hydrogen dioxide.

The treatment in this volume is comprehensive, like that of the first report, rather than critical and selective, as was the second report, issued in 1945 [ANAL. CHEM., 19, 697 (1947)]. The volume is issued in the name of the commission, of which van Nieuwenburg and Wenger are, respectively, president and secretary. The compilation was prepared by Clement Duval, also a member of the commission.

Division of Analytical and Micro Chemistry List of Speakers

FOR the second successive year the Division of Analytical and Micro Chemistry through its Committee on Speakers has prepared a list of speakers on analytical topics and is planning to send copies of the list to secretaries of local sections in the belief that it will be of assistance to them in planning meetings of maximum interest to their members.

Absorption Spectroscopy
S. E. Q. ASHLEY, General Electric Co., Pittsfield, Mass.
A. O. BECKMAN, Laboratories of A. O. Beckman, Pasadena 2, Calif.
R. R. BRATTAIN, Shell Development Co., Emeryville 8, Calif
M. G. MELLON, Purdue University, Lafayette, Ind.

Analysis and Composition of Ancient Materials
E. R. CALEY, Ohio State University, Columbus 10, Ohio
M. FARNSWORTH, Metal and Thermit Corp., Rahway, N. J.

Chromatography
R. KUNIN, Rohm & Haas Co., Philadelphia, Pa.
H. H. STRAIN, Carnegie Institute of Washington, Stanford, Calif.
W. H. STEIN, Rockefeller Institute, New York, N. Y.
L. ZECHMEISTER, California Institute of Technology, Pasadena, Calif.

Determination of Organic Functional Groups
E. D. PETERS, Shell Development Co., Emeryville 8, Calif.
D. M. SMITH, E. I. du Pont de Nemours & Co., Wilmington, Del.

Distillation
M. R. FENSKE, Pennsylvania State College, State College, Pa.
E. S. PERRY, Distillation Products, Inc., Rochester, N. Y.
W. J. PODBIELNIAK, Podbielniak, Inc., Chicago 11, Ill.
A. ROSE, Pennsylvania State College, State College, Pa.

Electrometric, Related Methods
N. H. FURMAN, Princeton University, Princeton, N. J.
F. W. JENSEN, Texas A. and M. College, College Station, Tex.
I. M. KOLTHOFF, University of Minnesota, Minneapolis, Minn.
W. M. MACNEVIN, Ohio State University, Columbus, Ohio
L. B. ROGERS, Massachusetts Institute of Technology, Cambridge, Mass.
E. H. SWIFT, California Institute of Technology, Pasadena 4, Calif.

Electron Microscopy
L. L. MARTON, National Bureau of Standards, Washington, D. C.

Emission Spectroscopy
W. F. MEGGERS, National Bureau of Standards, Washington, D. C.
N. NACHTRIEB, Institute for the Study of Metals, University of Chicago, Chicago, Ill.
B. F. SCRIBNER, National Bureau of Standards, Washington, D. C.
L. W. STROCK, Saratoga Laboratories, Inc., Saratoga Springs, N. Y.

Extraction
C. J. RODDEN, U. S. Atomic Energy Commission, New Brunswick Laboratory, P. O. Box 150, New Brunswick, N. J.
N. H. FURMAN, Princeton University, Princeton, N. J.
T. MOELLER, University of Illinois, Urbana, Ill.

Fluorescence Methods
C. E. WHITE, University of Maryland, College Park, Md.
W. F. NEUMAN, University of Rochester, Rochester, N. Y.

Fundamentals of Analytical Chemistry
P. J. ELVING, Pennsylvania State College, State College, Pa.
I. M. KOLTHOFF, University of Minnesota, Minneapolis, Minn.

Gas Analysis
MARTIN SHEPHERD, National Bureau of Standards, Washington, D. C.

Gravimetric and Volumetric Analysis
E. R. CALEY, Ohio State University, Columbus, Ohio
I. M. KOLTHOFF, University of Minnesota, Minneapolis, Minn.
H. H. WILLARD, University of Michigan, Ann Arbor, Mich.

Infrared Spectroscopy
R. BOWLING BARNES, American Optical Co., Southbridge, Mass.
R. R. BRATTAIN, Shell Development Co., Emeryville 8, Calif.
N. WRIGHT, Dow Chemical Co., Midland, Mich.

Instrumental Analysis
T. R. P. GIBB, Metal Hydrides, Inc., Boston, Mass.
LOUIS LYKKEN, Shell Development Co., Emeryville 8, Calif.
A. WEISSBERGER, Eastman Kodak Co., Rochester, N. Y.
H. H. WILLARD, University of Michigan, Ann Arbor, Mich.

Instrumentation
V. W. MELOCHE, University of Michigan, Ann Arbor, Mich.
R. H. MÜLLER, Washington Square College, New York University, New York, N. Y.
D. J. POMPEO, Shell Development Co., Emeryville 8, Calif.

Ion Exchange Methods of Analysis
G. E. BOYD, Oak Ridge National Laboratory, Oak Ridge, Tenn.
E. R. TOMPKINS, Microchemical Specialties Co., Berkeley, Calif.

Low Pressure Techniques
S. E. Q. ASHLEY, General Electric Co., Pittsfield, Mass.
L. A. WOOTEN, Bell Telephone Laboratories, Summit, N. J.

Mass Spectroscopy
A. O. C. NIER, University of Minnesota, Minneapolis, Minn.
F. J. NORTON, General Electric Co., Schenectady, N. Y.
H. W. WASHBURN, Consolidated Engineering Corp., Pasadena 4, Calif.

Metallurgical Analysis, Nonferrous
D. R. EVANS, Western Electric Co., Kearny, N. J.

Microchemistry
A. A. BENEDETTI-PICHLER, Queens College, Flushing, L. I., N. Y.

Nucleonics
G. E. BOYD, Oak Ridge National Laboratory, Oak Ridge, Tenn.
D. HUME, Massachusetts Institute of Technology, Cambridge, Mass.

Organic Analytical Reagents
J. F. FLAGG, General Electric Co., Schenectady, N. Y.
G. F. SMITH, University of Illinois, Urbana, Ill.
F. WELCHER, Indiana University, Bloomington, Ind.

Polarography
H. A. LAITINEN, University of Illinois, Urbana, Ill.
E. F. ORLEMANN, University of California, Berkeley, Calif.

Raman, Ultraviolet Spectroscopy
D. R. LONG, Lane-Wells Co., Pasadena, Calif.
E. ROSENBAUM, Sun Oil Co., Norwood, Pa.

Standardization of Analytical Methods, Statistical Quality Control
F. D. TUEMMLER, Shell Development Co., Emeryville, Calif.
GRANT WERNIMONT, Eastman Kodak Co., Rochester, N. Y.

Ultrasonics
G. H. ROUNDY, Ultrasonic Corp., Cambridge, Mass.

X-Ray Methods
L. K. FREVEL, Dow Chemical Co., Midland, Mich.
D. HARKER, General Electric Co., Schenectady, N. Y.
H. A. LIEBHAFSKY, General Electric Co., Schenectady, N. Y.
W. PARRISH, North American Phillips Corp., New York, N. Y.

Analytical Round-Table Discussion at Atlantic City

"Local Analytical Groups Activities" is the subject of a round-table discussion scheduled for the 116th Meeting of the AMERICAN CHEMICAL SOCIETY at Atlantic City in September. This will be an informal meeting for the primary purpose of enabling representatives of the growing number of groups of analytical chemists being organized within the framework of AMERICAN CHEMICAL SOCIETY local sections to meet and discuss matters of mutual interest with each other and with the officers of the Division of Analytical and Micro Chemistry.

Brief talks will be made by H. F. Beeghly, chairman of the Local Groups Cooperation Committee; Wayne H. Hilty, chairman of the Society's Professional Relations Committee; and S. E. Q. Ashley of the division's Speakers Committee. A major portion of the time will be devoted to an informal question and answer period, with participation of representatives of local analytical groups, officers of the division, and members of the staff of ANALYTICAL CHEMISTRY.

The round-table discussion is scheduled tentatively for Monday afternoon, September 19. Representatives of organized local analytical groups of the AMERICAN CHEMICAL SOCIETY, and other analytical chemists interested in such groups, are invited to participate.

The Analyst's Calendar

Fourth Instrument Conference and Exhibit. Municipal Auditorium, St. Louis, Mo., September 12 to 16
American Society for Testing Materials. Fairmont Hotel, San Francisco, Calif., October 10 to 14
Optical Society of America. Hotel Statler, Buffalo, N. Y., October 27 to 29
American Council of Commercial Laboratories. Miami, Fla., December 5 to 7
Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

AIDS FOR THE ANALYST . . . . .

A Device to Prevent Bumping in Micro-Kjeldahl Digestions. John C. Henniker, Stanford Research Institute, Stanford, Calif.

DURING the determination of nitrogen in asphalts, bumping was often so severe as to break the sturdy Pyrex flasks ordinarily used. Neither glass beads nor Carborundum chips were effective; they often came to rest after a period of quiet boiling and allowed the acid to be superheated. The difficulty was overcome by attaching a vibrator to the metal stand on which the digestions are done.

The first vibrator used, a commercial alternating current hummer made for agitating small photographic developing tanks, was effective but was unnecessarily powerful and noisy. A very satisfactory vibrator was then improvised from the solenoid of a magnetic switch. The main U-shaped core was bolted to the metal digestion frame. The T-shaped plunger was free to move, but was prevented from touching the core by sheet rubber spacers. The thickness of the spacers was so adjusted that enough vibration was transmitted to the flasks to stop bumping, but not enough to jar the flasks from their sockets. The vibrator is used in conjunction with glass balls in the flasks. It is no more noisy than many stirring motors.

Blacet-Leighton Method of Gas Microanalysis. Robert Gomer, University of Rochester, Rochester, N. Y.

DURING photochemical studies the Blacet-Leighton apparatus [Smith, R. N., and Leighton, P. A., IND. ENG. CHEM., ANAL. ED., **14**, 758 (1942)] was used extensively, and several very minor but useful modifications were gradually evolved.

The accuracy of leveling the microburet and mercury reservoir can be greatly increased if a reference mark on the inner telescoping brass support tube is lined up with a similar mark situated on a slot cut in the outer tube, or directly with the top of the latter. This requires only that the mercury level in the large reservoir be kept constant, a very simple matter. This procedure avoids the necessity of lining up visually two mercury levels several centimeters apart, and thus eliminates parallax effects.

The useful life of potassium hydroxide beads may be prolonged almost indefinitely by periodically wiping them with a piece of filter paper moistened with distilled water. This removes the coatings of potassium carbonate, potassium sulfate, etc., which normally terminate the absorbing power of these beads, and also ensures the moist surface necessary for speedy absorption.

Phosphorus beads may be prepared very simply and safely.

A small glass thimble (such as is used for containing Blacet-Leighton samples) is filled with water. A small piece of yellow phosphorus, not necessarily clean, is introduced, the thimble is held under the warm water faucet, and a moderately intense stream of water is played on it. The phosphorus soon melts, and impurities are washed away. A straight platinum wire, attached in the usual manner to a reagent holder, is introduced into the molten phosphorus, and the system is held in a stream of cold water until the bead has solidified. It is then quickly removed from the water and submerged in the mercury reservoir. Excess moisture is easily removed by raising the bead a few times into a half-empty gas thimble.

This method avoids the danger of violent spontaneous combustion, inherent in the "dry" method, and minimizes the obstructive phosphoric acid coating on the bead.

Difficulty with cementing silver oxide beads onto platinum wires was avoided by making the beads cylindrical in shape, about 3 mm. in diameter, and 5 mm. in height. Ordinary Duco cement was then used, instead of the troublesome sodium silicate. Only 2 to 3 mm. of bead need be introduced into a gas sample, and contamination of or by the cement is avoided.

Analyses by means of the combustion coil are stated to be unsatisfactory for hydrocarbons higher than methane. This is indeed the case in determining hydrogen content, which necessitates a knowledge of the oxygen used. Carbon content, however, may be determined very accurately for higher hydrocarbons (and presumably other volatile compounds) by determining the carbon dioxide formed by combustion in the usual manner; water is first removed with a phosphorus pentoxide bead and then carbon dioxide is absorbed with a potassium hydroxide bead.

WORK supported by Contract N6onr-241, Task I, with the Office of Naval Research, United States Navy.

Preparation of Barium Carbonate for Assay of Radioactive Carbon 14. R. B. Regier, Phillips Petroleum Company, Bartlesville, Okla.

IN making assays for C^{14}, the carbon is frequently converted to barium carbonate, because this form is conveniently handled and may be deposited in a uniform and reproducible manner if certain precautions are observed. The procedure described by Dauben, Reid, and Yankwich [ANAL. CHEM., **19**, 828 (1947)] is not completely satisfactory. Careful evaporation of the alcohol from the barium carbonate in alcohol slurry sometimes produces very smooth and adherent layers, whereas at other times the barium carbonate is grossly marked with cracks, with the result that the deposit is flaky and easily lost from the aluminum disk.

In an effort to establish conditions that would consistently yield good barium carbonate layers, an investigation has been made of the relation between crystal size and the nature of the resulting layer when barium carbonate is deposited from an alcohol slurry by evaporation of the alcohol. It was found that the individual crystal size is the principal factor in determining the nature of the deposited layer.

A series of barium carbonate precipitates was prepared under varying conditions of temperature and alkalinity, using partially carbonated aqueous sodium hydroxide, ammonium chloride, and barium chloride as reagents, and examined under an optical microscope. The individual barium carbonate needles varied in length from 1 or 2 microns to approximately 150 microns. Sufficient quantities of the preparation comprising the smallest crystals, and of the preparation comprising the largest crystals, were mounted on aluminum disks to give a thick layer—i.e., about 25 mg. per sq. cm. The small-crystal barium carbonate exhibited most of the properties that are considered undesirable. It was difficult to manipulate into a layer of uniform thickness, and the resulting deposit was badly cracked and adhered loosely to the disk. In contrast, the large-crystal barium carbonate deposited as a layer with a uniformly smooth surface and was not loosened even when the disk was inverted and tapped lightly. It was concluded that barium carbonate behaves best when precipitated under conditions that cause slow crystal growth. These include using dilute reagents, elevated temperature, and lowered alkalinity (obtained by using ammonium chloride).

The procedure outlined by Dauben *et al.* has been modified to give consistently satisfactory results in preparing barium carbonate samples. An approximately 20% excess of ammonium chloride is used, instead of a quantity equivalent to the sodium hydroxide in the absorber. The resulting solution is then diluted to about 100 ml. and heated to near boiling before precipitating with barium chloride. After cooling, the precipitate is filtered off and treated in the usual way.