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WALTER J. MURPHY, Editor

The Analyst and IUPAC

THE trite saying "science is international and knows no geographial boundaries," has been employed so frequently we hesitate to use it again in comment on the International Union of Pure and Applied Chemistry.

How better can the universality of science, including chemistry, be desribed than by these words? The analyst has a numer of very special reasons for being vitally interested i the welfare of the union. Much of what we do requires a high degree of exactness, perhaps more than most other branches of chemistry or physics. This is jus another way of saying that precise measurement is the hief objective of original analytical research. Indeed, inall areas it is important that we as analysts continue todevelop international understanding and agreement. t is important to further advances in pure science as wel as in international trade.

In traveling abou the county and talking with chemists and chemical engineers we find an abysmal ignorance on the partof many members of the profession concerning the work and objectives of the union and its frequent confeences, despite the fact that the largest union conferene ever held took place in the United States in 1951 following the Diamond Jubilee Meeting of the AMERICAN CHEMICAL SOCIETY.

We strongly suspect the chief reason for this condition is the union's lck of funds, which makes it extremely difficult to publicize properly its activities here and abroad. To remedy this situation, IUPAC is endeavoring to raise, though voluntary contributions, the sum of $30,000 a yea tomake possible the employment of a full-time paid secetariat in Paris. American chemists are being asked to raise $5000 of this sum—a modest figure when we consider the total membership of the chemical professionin this country.

The National Researc Council is, of course, the official U. S. contact witIUPAC, not the AMERICAN CHEMICAL SOCIETY. NR suggests the desirability of small contributions ($5.00r more) from a large number of chemists and chemica engineers. Contributions may be sent to IUPAC Fnd, U. S. National Committee of IUPAC, Divisionof Chemistry and Chemical Technology, National Rsearch Council, Washington 25, D. C. Contributions to date, so we are informed, have been most encouraging, but the goal of $5000 for this year can be reachedonly if more chemists are willing to back their belief i theinternationalism of science with a modest sum of noney.

American analysts shouldbe aware of the fact that the Analytical Section of IUPAC is one of the most active, perhaps *the* most active group in the union. I. M. Kolthoff of the University of Minnesota and, as everyone knows, intensely interested for years in building up closer liaison between the analysts here and abroad, is president of the Section on Analytical Chemistry. Through his personal efforts, along with the work of other internationally minded analysts in various parts of the world, the program of the section has been revitalized and promises to bring to fruition many projects which have lain fallow for too many years.

The 18th conference of the union will be held in Zurich, July 20 to 28, 1955, together with the 14th International Congress of Pure and Applied Chemistry, which will feature organic chemistry. Our readers will recall that at the congress in New York in 1951, it was decided to discontinue congresses covering the broad spectrum of pure and applied chemistry and to devote each congress (to be held every two years instead of every four) to some one or two fields of specialization.

The reason advanced for this decision was the difficulty of finding adequate housing in many European countries desirous of acting as hosts to IUPAC and congresses. We were not in accord with that decision then. We still believe that by proper advance planning, housing difficulties could be solved.

However, this is neither the time nor the place to advance the pros and cons of the two meeting approaches. Kolthoff is also chairman-elect of the ACS Division of Analytical Chemistry and he is, therefore, in a peculiarly advantageous position to explore the possibility of staging, within the next couple of years, a large congress of analytical chemistry in the United States.

Gazing into the crystal ball for a moment—we suggest that analysts seriously investigate the possibility of such cooperative action that would assure the success of an international meeting in the U. S. in 1956, 1957, or 1958. For one year it should be possible to make the annual Louisiana State University Analytical Symposium, the annual Summer Analytical Symposium of the Division of Analytical Chemistry, and the annual Pittsburgh Analytical Symposium, sponsored by the Pittsburgh Section of the ACS, all part of a huge international meeting.

How better could analysts focus further attention on the present stature of the field in this country than by staging such an impressive event? Much planning, much hard work would be needed, but the many and varied tangible and intangible benefits to be derived should constitute powerful stimuli.

Infrared Absorption of the Aldehydic C—H Group

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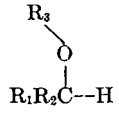
Various aldehydes, substituted benzaldehydes in particular, were measured in the 3000- to 2600-cm.$^{-1}$ region. The aldehydic C—H group usually absorbs at about 2720 cm.$^{-1}$, but in the case of some orthosubstituted benzaldehydes this band rises considerably. This effect is tentatively attributed to a new kind of hydrogen bonding involving the polar aldehydic hydrogen atom. Other characteristic bands in this region were also observed and their origin is discussed. These bands are of much value in elucidating the structure of substituted benzaldehydes.

ACCORDING to Linnett's calculations (*23*), the force constants of the aldehydic C—H bonds in acetaldehyde and formaldehyde are appreciably lower than those of the C—H bonds in both saturated and unsaturated hydrocarbons. This fact is attributed to the strong polarity of the carbon atom in the aldehydic group, which is assumed to polarize the C—H binding electrons, thereby increasing the ionic character of this bond and decreasing its strength (*32*). The chemical behavior of aldehydes as compared with ketones also suggests that this bond is somewhat weak. It can therefore be expected that all the infrared absorption bands due to the C—H stretching of the O=C—H groups will be at a lower frequency, relative to the parallel C—H stretching bands of the corresponding olefinic groups, C=C—H. Indeed, it was found that while the C—H band

of the C=CHR groups (R = alkyl) appears at about 3020 cm.$^{-1}$ (*10*), it appears at 2710 cm.$^{-1}$ in tetrahydrofurfural (*18*). This is in accord with the fact that while the symmetrical C—H stretching band of the $C{=}CH_2$ group is found at 2979 cm.$^{-1}$ in propylene (*10*), it occurs at 2780 cm.$^{-1}$ in formaldehyde (*26*). Similarly, this band appears in glyoxal (in the emission spectrum) at 2757 cm.$^{-1}$ (*13*). The C—H frequency of the $R_1R_2R_3C$—H group is shown at 2890 cm.$^{-1}$ (*10*) and that of a



group at 2980 cm.$^{-1}$ (*18*).

Acetaldehyde also shows a strong fundamental absorption band at about 2710 cm.$^{-1}$ (*25*) [Thompson and Harris (*30*) report a triplet at 2688, 2704, 2732 cm.$^{-1}$]. This band appears in the Raman spectrum at 2732 cm.$^{-1}$ (*21*). Although Morris (*25*) attributed this band arbitrarily to the symmetrical stretching of the methyl group and the strong band at 2788 cm.$^{-1}$ [Thompson and Harris (*30*) give 2758, 2778, 2800 cm.$^{-1}$] to the aldehydic C—H group, Thompson and Harris' assignment (*30*) of the 2710-cm.$^{-1}$ band to a C—H stretching other than the symmetrical one, due to the methyl group, is more likely. This C—H frequency seems to belong to the stretching of the formyl C—H bond, as can be judged by comparison with the band at about 2710 cm.$^{-1}$ of tetrahydrofurfural and many other aldehydes and the much higher frequency of even the symmetrical stretching of the methyl group, especially in oxygenated hydrocarbons (*28*). Thompson and Harris' other assignment of the band at about 2800 cm.$^{-1}$ to the first overtone of the formyl C—H bending vibration appearing at 1405 cm.$^{-1}$ (Morris 1414 cm.$^{-1}$) also seems to be correct. [This assignment of the 1405-cm.$^{-1}$ band is in accordance with the similar conclusion of Kohlrausch and Koeppl (*21*) regarding the 1390 cm.$^{-1}$ Raman band (which appears in the Raman spectra of all the aldehydes) and is therefore adopted here rather than that of Morris] The polarity of this bond is probably responsible for the intensity of this band.

The assignment of the 2710-cm.$^{-1}$ band rather than the 2800-cm.$^{-1}$ band [which appears in the case of other aldehydes at about 2820 cm.$^{-1}$—e.g., Pozefsky and Coggeshall (*28*)] as the fundamental (O=) C—H stretching frequency, is supported by the fact that its intensity, in the case of aliphatic aldehydes, is usually higher than that of the already strong 2800-cm.$^{-1}$ band [see the curves of Pozefsky and Coggeshall (*28*) and Thompson and Harris (*30*)].

The mean value of 2795 cm.$^{-1}$ for the (O=) C—H stretching frequencies in glyoxal (*13*) as well as the value of 2800 cm.$^{-1}$ for this stretching frequency in perfluoroacetaldehyde (*17*) also seems to show that the normal value of the (O=) C—H frequency is much less than 2800 cm.$^{-1}$, as an appreciable increase in this frequency can be expected in these aldehydes. This increase would seem to be the result of the strong negative inductive effect of the other carbonyl group or the fluorine atoms, which suppresses the polarization of the carbonyl group responsible for the lower (O=)C—H stretching frequency in aldehydes.

Pozefsky and Coggeshall (*28*) observed these bands in a carbon tetrachloride solution of acetaldehyde at 2724 and 2830 cm.$^{-1}$ They also found similar bands in the following aldehydes:

Propanal	2718	2772	2816	2897
Butanal	2718	...	2818	2882
Isobutyric aldehyde	2712	2783	2810	2876
Isovaleric aldehyde	2715	...	2820	2878
Heptanal	2716	...	2820, 2863	2873

These authors concluded therefore that the characteristic aldehyde bands at about 2720 and 2820 cm.$^{-1}$ are due to a resonance between the formyl C—H stretching level and that of the first overtone of the symmetrical CH_3 bending vibration which appears at 1380 cm.$^{-1}$, assuming both levels to be responsible for original frequencies at about 2780 cm.$^{-1}$

They admit, however, that benzaldehyde, although devoid of a methyl group, still shows these bands at the usual places. Tetrahydrofurfural (*18*), 1-naphthaldehyde, 2-naphthaldehyde, and salicylic aldehyde (*16*) all show this doublet clearly, although none of them contains a methyl group. It seems, therefore, that these bands must be assigned as above—namely, the 2720-cm.$^{-1}$ band to the aldehydic C—H stretching and the 2820-cm.$^{-1}$ band to the overtone of the aldehydic C—H bending vibration. The bands observed in some cases at about 2780 cm.$^{-1}$ seem to be related to the 2778-cm.$^{-1}$ band found by Thompson and Harris in acetaldehyde and ascribed by them to a combination of the symmetrical CH_3 bending frequency at about 1355 cm.$^{-1}$ and the formyl C—H bending frequency at about 1405 cm.$^{-1}$ (Morris, 1414 cm.$^{-1}$).

It is thus seen that apart from special cases—e.g., formaldehyde (two aldehydic hydrogen atoms) and glyoxal (a doubling of the formyl group)—the aldehydes so far investigated in the 2800 to 2600-cm.$^{-1}$ region appear to show their formyl C—H stretching frequency in the narrow range of 2700 to 2730 cm.$^{-1}$ In the light of the assumed polar character of the formyl C—H bond (*32*) however, drastic changes can still be expected in this stretching frequency in the case of aldehydes with substituted groups which are known to posses a strong electronic effect. It is significant that according to Linnett's calculations (*23*), the value of the force constant of this bond is, in units of 10^5 dynes per cm., 4.43 in formaldehyde and 4.37 in acetaldehyde but 4.48 in chloral and 4.67 in benzaldehyde [this value seems to

have been calculated from the frequency of the Raman band attributed by Linnett to the aldehydic C—H stretching. This stretching is, however, at about the normal frequency (2720 cm.$^{-1}$) in the infrared spectrum of benzaldehyde (*16*, *28*)]. It seemed desirable therefore to study the aldehydic C—H frequency in various substituted benzaldehydes. Such an investigation was undertaken and the results obtained are summarized in Table I, with data on some other aldehydes.

EXPERIMENTAL

Most of the measurements were carried out with a Perkin-Elmer infrared spectrophotometer, Model 12C, equipped with a rock salt prism. Some were made with a Beckman spectrophotometer, Model IR-2. The materials were mainly of commercial origin. Most of them were of the Eastman Kodak White Label grade. When this grade was not available, lower grades were used, but only seldom was a special purification undertaken.

2-Methylbenzaldehyde (*o*-toluic aldehyde) was synthesized according to Hass and Bender (*15*).

4-Cyanobenzaldehyde and 4-carbamidobenzaldehyde were synthesized as described by Bergmann and Pinchas (*5*). The synthesis of 2-ethoxy-1-naphthaldehyde is described in detail in the literature (*9*).

DISCUSSION

Aliphatic Aldehydes. All the aldehydes investigated (Table I) show the 2710-cm.$^{-1}$ band in the narrow range of 2695 to 2720 cm.$^{-1}$, whatever is the length of the aliphatic chain and whether it is branched in a position α or β relative to the formyl group or not. All these aldehydes show the second characteristic band in the region of 2810 to 2830 cm.$^{-1}$, although very often only as a shoulder on the CH_2 and CH_3 bands at about 2900 cm.$^{-1}$

The band appearing sometimes at about 2580 cm.$^{-1}$ seems to be related to that found in acetaldehyde at about 2550 cm.$^{-1}$ (*30*) and is probably due to some combination or overtone vibration. This may be the first overtone of the frequency of about 1300 cm.$^{-1}$ observed by Kohlrausch and Koeppl (*21*) in the Raman spectra of many aliphatic aldehydes as well as by Thompson and Harris (*30*) in the infrared spectrum of acetaldehyde (at 1295 cm.$^{-1}$) and by Barnes *et al.* (*3*) in the infrared spectra of formaldehyde and methacrolein. This band was found in isovaleric aldehyde at about 1305 cm.$^{-1}$ (ϵ = 37) and in butanal at about 1290 cm.$^{-1}$ (ϵ = 11). The region of 1260 to 1310 cm.$^{-1}$ was also assigned by Colthup (*7*) to the absorption of aromatic aldehydes and, indeed, various benzaldehydes show a strong band in this region (*3*, p. 80). If it is further assumed that this band at about 1300 cm.$^{-1}$ in aldehydes is due to the wagging of the aldehydic hydrogen atom (while that at about 1400 cm.$^{-1}$ is due to its rocking), then this band is analogous to that found at 1297 cm.$^{-1}$ for propylene, which was assigned by Wilson and Wells (*33*) to the =CH wagging in this molecule. The various benzaldehydes (including benzaldehyde itself) measured in this region by Barnes *et al.* (*3*) all show two strong bands in this region, at about 1300 and 1400 cm.$^{-1}$

In the two instances in which an additional band appears at about 2650 cm.$^{-1}$ (Nos. 3 and 4), an isopropyl group is present in the absorbing molecule. Attention is also drawn to the rise in the intensity of the CH_2 and CH_3 bands as the molecular weight of the aldehydes increases.

Benzaldehydes. A survey of the results for the benzaldehydes shows immediately that these may be divided into two groups: (1) the aldehydes that absorb at about 2730 cm.$^{-1}$ and (2) those that absorb at about 2760 cm.$^{-1}$. The first group includes a variety of aldehydes, different in their electronic structure one from the other—e.g., *p*-dimethylaminobenzaldehyde (No. 20) and terephthaldehyde (No. 19)—and still the frequency of about 2730 cm.$^{-1}$ remains almost the same for all the members of this group, being only slightly higher than the usual aliphatic frequency of 2710 cm.$^{-1}$ The second group includes only ortho-

Table I. Infrared Absorption of Aldehydes in 3000- to 2600-Cm.$^{-1}$ Region

(Cell thickness 2 mm. and solvent 1 ml. of carbon tetrachloride unless otherwise stated. Corresponding molar absorbancy index, in liter mole^{-1} cm.$^{-1}$, in parentheses after each frequency)

No.	Material	Weight, G.	Band Frequencies, Cm.$^{-1}$				
			Aliphatic Aldehydes				
1	Propionaldehyde	0.008	...	2710 (16.5)	2810 (16)	2900 (13)	2970 (13)
2	Butyraldehyde	0.008	...	2710 (20)	2820 (19)	2890 (24)	2960 (32)
3	Isobutyric aldehyde	0.016	(2580) 2650 (12.5)	2710 (13)	2810[a] (16)	2890[a] (34)	2960[b]
4	Isovaleric aldehyde	0.010	(2585[a]) 2645[a] (17)	2705 (18)	2825[a] (24)	2890[a] (43)	2970 (56)
5	2-Ethylbutanal	0.008	(2580[a]) 2625[a] (17)	2695 (24)	2810[a] (28)	2890[a] (55)	2980 (86)
6	Heptanal	0.009	...	2710 (16)	2820[a] (22)	2885 (54)	2970 (72)
7	2-Ethylhexanal	0.010	...	2710 (26)	2830 (32)	2900 (51)	2980 (73)
			Substituted Benzaldehydes				
8	Benzaldehyde	0.013	2670 (19)	2730 (26)	2820 (32)	...	...
9	2-Chloro-	0.014	2635 (6)	...	2760 (10)	2890 (21)	...
10	2-Methyl-	0.024[c, d]	...	2725	...	...	...
11	2-Nitro-	0.010	2650 (5)	...	2760 (7)	2890 (17)	
12	2-Methoxy-	0.010	...	...	2760 (8)	2860 (27)	2950 (20)
13	2,6-Dichloro-	0.028	...	...	2760 (6)	2870 (12)	
14	2-Hydroxy-3-methoxy-	0.012[e]	...	2740 (13)	...	2865 (38)	2990 (25)
15	3-Nitro-	0.010	2640 (2)	2725 (15)	2830 (22)	...	...
16	4-Nitro-	0.010[e]	...	2725 (12)	...	2865 (24)	...
17	4-Hydroxy-	0.005[e]	...	2730 (40)	2820 (49)	...	...
18	4-Cyano-	0.001	...	2720 (39)	2830 (52)	...	...
19	4-Formyl-	0.022[c, d, e]	...	2735	...	...	...
20	4-Dimethylamino-	0.010[e]	... 2650 (8)	2740 (26)	2830 (40)	...	2940 (36)
21	4-Carbamido-	0.010[e]	... ...	2730 (11)	2855 (21)	...	...
22	4-Methoxy-	0.030	(2590) 2635 (4)	2730 (18)	2820 (18)	...	2950 (16)
			Various Aldehydes				
23	Chloral	0.02	2650 (v.w.) 2680 (v.w.)	...	2850 (7)	2930 (1)	...
24	Tiglaldehyde[c, d]	0.018	...	2710 (14)	2790 (18)	...	...
25	Cinnamaldehyde	0.029	...	2730 (16)	2820 (17)	...	...
26	Alpha-*n*-amyl-cinnamaldehyde	0.013	2655[a] (25)	2710 (26)	2830[a] (36)	2885 (61)	2970 (88)
27	2-Ethoxy-1-naphthaldehyde	~0.01	...	2745[a] (w.)	2790 (15)	2890 (40)	2960 (24)

[a] Shoulder.
[b] Very broad and very strong.
[c] In 0.5-mm. cell.
[d] Not measured at ends of region.
[e] In 1 ml. of chloroform.

substituted benzaldehydes—but for *o*-toluic aldehyde and perhaps also 2-hydroxy-3-methoxybenzaldehyde, all the orthoaldehydes reported here. 2-Ethoxy-1-naphthaldehyde (No. 27) should also be included in this group.

The constancy of the aldehydic frequency in the first group seems to mean that in spite of the variations in the extent of the polar character of the carbonyl group, brought about by the conjugated aromatic ring and its various substituents [which very much affect the stretching frequency of the carbonyl group (*4*)], the strength of the adjacent C—H bond does not

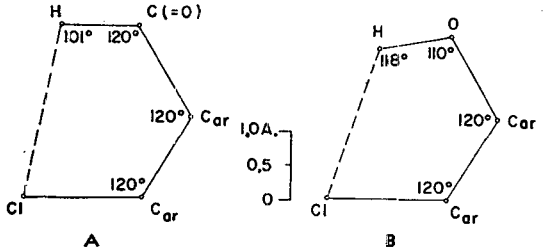


Figure 1. Structural Models

A. Functional part of *o*-chlorobenzaldehyde
B. Functional part of *o*-chlorophenol

change appreciably. This can partly be explained by the assumption that the net positive charge on the carbon atom does not rise in parallel to the rise (with conjugation) in the polar character of the carbonyl group, as most of it is smeared out over the aromatic ring. There is therefore little possibility that this charge will further weaken this bond. This constancy in frequency can also be explained in part by the opposite effect of the partial double bond character of the C_{ar}—CHO linkage, brought about by the resonance between the ring and the carbonyl group. This character becomes more pronounced the stronger the resonance, and because a double bond tends to strengthen the other bonds of the participating atoms (see *32* for an analogous interplay of such effects), a stronger opposite effect—to that of the weakening of the C—H linkage with higher resonance and greater charge on the carbon atom—is formed with a greater resonance. The effect of the latter is thus damped and even overpowered and the strength of the C—H bond in benzaldehydes rises somewhat as compared with aliphatic aldehydes.

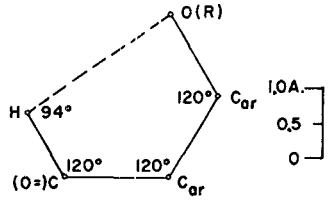


Figure 2. Structural Model of Functional Part of an *o*-Alkoxybenzaldehyde

The higher values of the C—H frequency for the orthoaldehydes of the second group cannot be ascribed to electronic effects of the substituents, as there is no difference in frequency between so different aldehydes in this respect as *o*-anisaldehyde (No. 12, 2760 cm.$^{-1}$) and *o*-nitrobenzaldehyde (No. 11, 2760 cm.$^{-1}$). Neither can they be ascribed to a steric interaction between the ortho substituent and the formyl group, because such an interaction would be expected to be stronger in the case of *o*-toluic aldehyde (No. 10) than in the case of *o*-chlorobenzaldehyde (No. 9)—the radius of the methyl group being greater than that of the chlorine atom—and still *o*-toluic aldehyde behaves normally (absorbs at 2725 cm.$^{-1}$) while *o*-chlorobenzaldehyde absorbs only at 2760 cm.$^{-1}$ A molecular model also shows that there is no steric interaction between the chlorine atom and the formyl group in *o*-chlorobenzaldehyde; even in the case of an *o*-methyl group, Kadesh and Weller (*20*) have found no appreciable interaction with the aldehydic group.

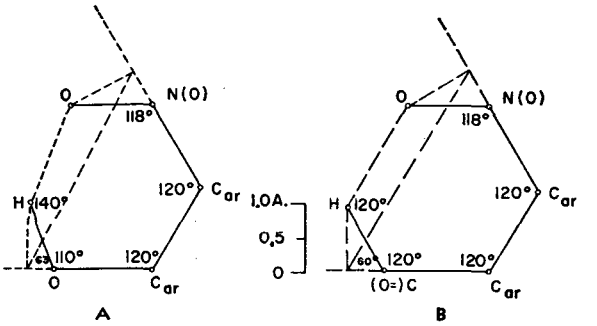


Figure 3. Structural Models

A. Functional part of *o*-nitrophenol
B. Functional part of *o*-nitrobenzaldehyde

A possible explanation of this phenomenon seems to be that in all these orthoaldehydes which show an unusual high C—H frequency there exists a new and unusual kind of hydrogen bonding between the aldehydic hydrogen atom, which according to Walsh (*32*) possesses a partial positive charge (in contradistinction to an ordinary hydrogen atom bound to a carbon atom), and a nucleophilic acceptor in the ortho position, or near it. Such an acceptor can be a chlorine atom which, for example, forms hydrogen bonds in *o*-chlorophenol (*31*), etc., an etheric oxygen atom (*11*), or an oxygen atom of a nitro group (*2*, *24*).

It is true that the line joining the center of the aldehydic hydrogen atom to that of the chlorine atom in *o*-chlorobenzaldehyde or to that of the etheric oxygen atom in the *o*-alkoxybenzaldehydes, appears from a drawing to scale (Figures 1 and 2) to form a somewhat obtuse angle (101° and 94°) with the direction of the C—H bond. It could, therefore, be argued that such a hydrogen bond, which is mainly electrostatic in its nature (*29*), would have a component of attraction opposite to that of the valency bond and hence would decrease rather than increase the energy of the C—H stretching. But one has to take into account that a hydrogen bond is brought about by an attraction between the involved hydrogen atom and the lone pair of electrons belonging to the acceptor (*29*) which usually is not symmetrically distributed

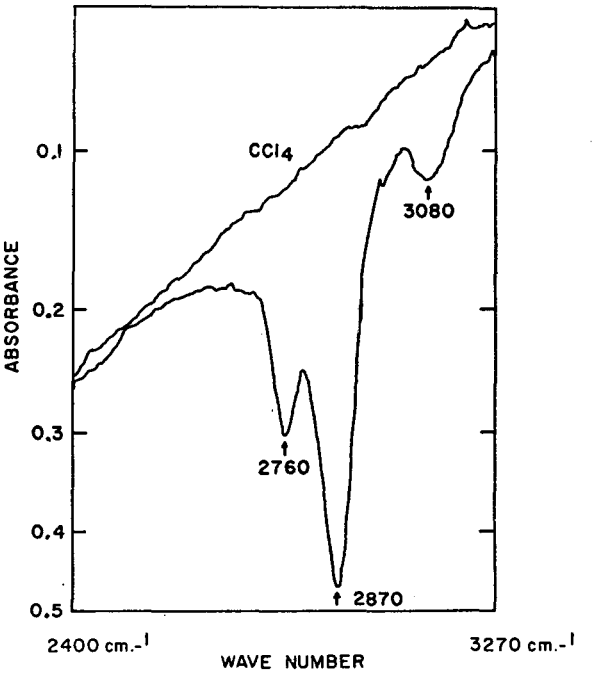


Figure 4. Absorption of 2,6-Dichlorobenzaldehyde in C—H Region

Cell thickness. 2 mm.
Concentration. 0.028 gram plus 1 ml. of CCl_4

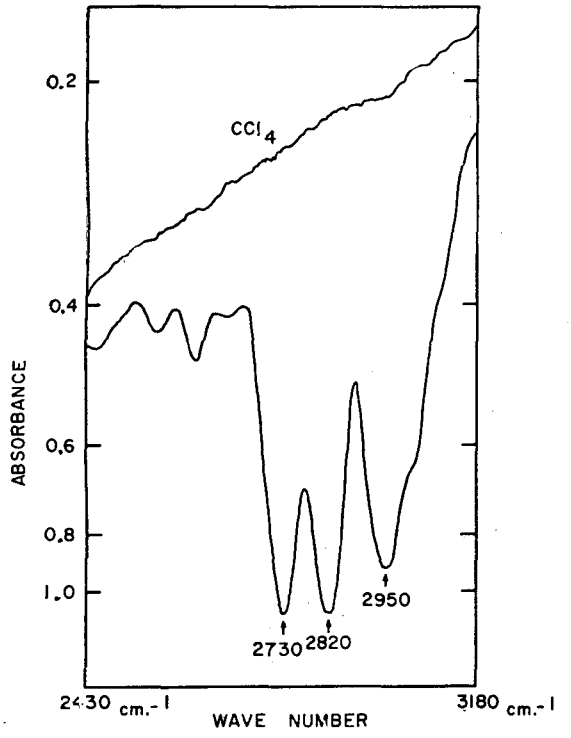


Figure 5. Absorption of 4-Methoxybenzaldehyde in C—H Region

Cell thickness. 2 mm.
Concentration. 0.030 gram plus 1 ml. of CCl_4

(*12*). Because of the interaction of these electrons with the aromatic ring in these aldehydes, it can be assumed that these electrons are localized in its neighborhood. Their attraction can, therefore, no longer be regarded as concentrated in the centers of their respective atoms, but would seem to be directed at an acute angle to the C—H stretching direction. This leads to a higher energy content for the C—H stretching, since this stretching becomes at the same time also a stretching of the hydrogen bond; the higher C—H frequency in these aldehydes is thus explicable.

Figure 1,*B* shows that the O—H. . .Cl angle in the case of *o*-chlorophenol is equal to about 118°. Therefore, even if a dissymmetry effect brings about a decrease of about 20° in this angle, this angle remains obtuse. It is thus in agreement with expectation that this phenol is known to show a decrease of its O—H stretching frequency because of an internal hydrogen bond (*31*).

Things are more complicated in *o*-nitrobenzaldehyde and *o*-nitrophenol. Although Pauling assumes (*27*) a coplanar configuration for the latter, a drawing shows (Figure 3, *A*) that the distance between the center of the near $—NO_2$ oxygen atom and that of the hydroxylic oxygen atom is only about 2.4 A. with this assumption. Remembering that even in the very strongly hydrogen bonded dihydrate of oxalic acid, the O—O distance is 2.51 A., it seems that this assumption is somewhat doubtful. A molecular model shows that *o*-nitrobenzaldehyde also cannot be coplanar. In these compounds a moderate rotation of the $—NO_2$ group about the C—N axis seems therefore more likely. As will be seen from Figure 3,*A*, the O—H. . .O angle is equal to about 140° in a coplanar *o*-nitrophenol molecule and to about 63° if both the O—H and the N—O linkages are rotated 90° away one from the other. The corresponding C—H . . .O angle is, however, only 120° in the coplanar *o*-nitrobenzaldehyde molecule and 60° in the perpendicular rotational isomer (Figure 3,*B*). It is thus clear that even a moderate rotation about the C—N axis and the C—C axis, together with the dissymmetry effect of the lone pair (which is probably strong in the $—NO_2$ group because of the partial double bond character of the N—O linkages there), will suffice to make the angle between the hydrogen bond and the C—H bond in *o*-nitrobenzaldehyde acute, while a far greater rotation (against the hydrogen bond) must be applied to *o*-nitrophenol before this result is obtained in that case.

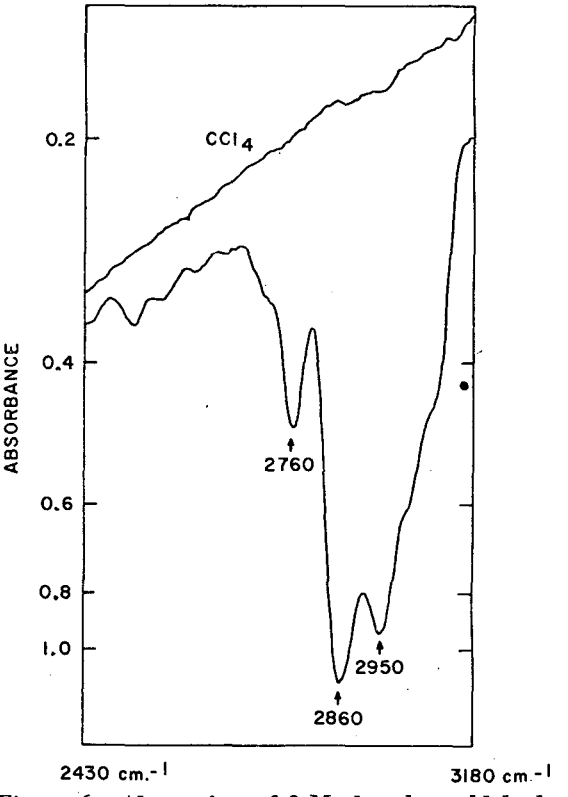


Figure 6. Absorption of 2-Methoxybenzaldehyde in C—H Region

Cell thickness. 2 mm.
Concentration. 0.023 gram plus 1 ml. of CCl_4

The fact that *o*-nitrophenol shows a decrease (*24*) in the O—H stretching frequency, because of hydrogen bonding, while in *o*-nitrobenzaldehyde an increase in the C—H frequency is observed, is therefore not amazing.

The lower C—H frequency of the ortho-substituted 2-hydroxy-3-methoxybenzaldehyde (No. 14) may be the result of the formation of a (chelated) structure which leaves the C—H group free.

Besides chloroform (*19*) and its analogs (*8*), the only recorded cases in which (intermolecular) C—H. . .R bonding was observed seem to be hydrogen cyanide (*27*, p. 294) and acetaldehyde (*1*).

The observed photoisomerization of *o*-nitrobenzaldehyde to *o*-nitrosobenzoic acid (*22*) is in good agreement with the assumption of the existence of such a hydrogen bond in *o*-nitrobenzaldehyde.

The second characteristic band of the aldehydic group at about 2820 cm.$^{-1}$ also changes its usual frequency in the aldehydes of the second group. Here again the orthoaldehydes show higher frequencies and absorb at 2860 to 2890 cm.$^{-1}$ as compared with 2820 to 2865 cm.$^{-1}$ in the case of the first group. This rise can again be attributed to the effect of a hydrogen bonding in the members of the second group.

Figures 4 to 7 show some typical spectra of absorption in the C—H region.

It is thus possible to determine with safety from its infrared absorption spectrum whether or not a group such as the nitro

group or an alkoxy group is situated in a position ortho to the formyl group in an unknown aldehyde.

Various Aldehydes. Although chloral shows a weak band at about 2680 cm.$^{-1}$, it seems to have its C—H stretching band at about 2850 cm.$^{-1}$; this is also where it absorbs strongly in the Raman spectrum (*6*), while there is no Raman band at about 2680 cm.$^{-1}$ (such a band was, however, observed doubtfully in the case of bromal). Judging from its weak intensity, the band at 2680 cm.$^{-1}$ seems to be due to some overtone or combination vibration (possibly the overtone of the Raman band observed at about 1350 cm.$^{-1}$).

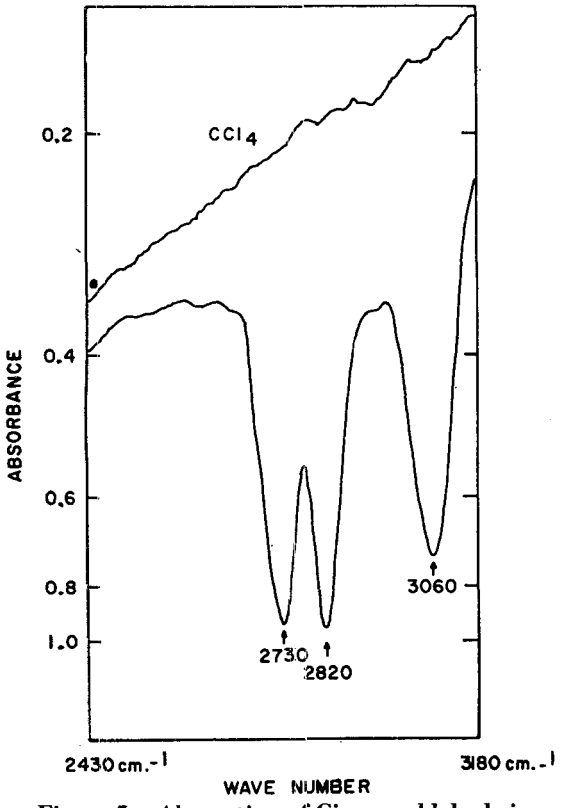


Figure 7. Absorption of Cinnamaldehyde in C—H Region

Cell thickness. 2 mm.
Concentration. 0.029 gram plus 1 ml. of CCl_4

The very high frequency of the C—H stretching in chloral, probably, cannot be explained only by the electronic effect of the chlorine atoms (which suppresses the polarization of the carbonyl double bond), as this effect brings about only a rise of about 40 cm.$^{-1}$ in the C=O stretching frequency [Cheng (*6*) reports a Raman frequency of about 1770 cm.$^{-1}$ in the case of chloral; Grove and Willis (*14*) report a C=O band at about 1730 cm.$^{-1}$ in the case of aliphatic aldehydes]. The fact that perfluoroacetaldehyde, which from the point of view of the inductive effect of its fluorine atoms is similar (the inductive effect of the three fluorine atoms being probably even stronger than that of the chlorine atoms) to chloral, shows this band at 2800 cm.$^{-1}$ also suggests that the high value of 2850 cm.$^{-1}$ must be attributed in part to some other effect. Since the C—H stretching direction in chloral is at a very acute angle to the line joining the center of the hydrogen atom to that of the neighboring chlorine atom (whatever is the structure of the chloral molecule), it is tempting to assume that here also a hydrogen bond between these atoms is responsible for this extra rise in frequency. The very similar high frequency of the corresponding Raman band of dichloroacetaldehyde (*6*) is in accordance with such an assumption.

The probable lack of such bonding in the case of perfluoroacetaldehyde may be explained by the smaller radius of the fluorine atom, which does not allow an intimate interaction with the hydrogen atom. A similar phenomenon is to be found in the case of *o*-fluorophenol, which shows the smallest shift, of the OH frequency, due to hydrogen bonding of all the *o*-halophenols (*24*); the ethylene halohydrins also behave analogously (kindly pointed out by Abbott Pozefsky).

Tiglaldehyde, although α,β-unsaturated, shows the characteristics of the aliphatic aldehydes: two strong bands at about 2710 and 2790 cm.$^{-1}$ Similarly, *n*-amylcinnamaldehyde also displays these bands at about 2710 and 2830 cm.$^{-1}$; cinnamaldehyde, however, shows the first band at 2730 cm.$^{-1}$ as in the case of the benzaldehydes. It seems that the alkyl substituent in the alpha position to the formyl group in tiglaldehyde and amylcinnamaldehyde interferes with the conjugation of the double bond or the aromatic ring with the aldehydic group and therefore these aldehydes absorb at 2710 cm.$^{-1}$ rather than 2730 cm.$^{-1}$ These results show that even in extended conjugation, such as exists in cinnamaldehyde, the frequency of the aldehydic C—H stretching does not change appreciably. The case of 2-ethoxy-1-naphthaldehyde can best be considered with that of the anomalous orthoaldehydes mentioned above. However, its 2745-cm.$^{-1}$ shoulder may be due to a C—H band occurring at about 2730 cm.$^{-1}$, which might be expected of a possible free form in equilibrium with the hydrogen-bonded structure.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Alexander, E. A., and Lambert, J. D., *Trans. Faraday Soc.*, 37, 421 (1941).
(2) Amstutz, E. D., Hunsberger, I. M., and Chessick, J. J., *J. Am. Chem. Soc.*, 73, 1220 (1951).
(3) Barnes, R. B., Gore, R. C., Liddel, U., and Williams, V. Z., "Infrared Spectroscopy," p. 79, New York, Reinhold Publishing Corp., 1944.
(4) Bergmann, E. D., and Pinchas, S., *J. chim. phys.*, 49, 537 (1952).
(5) Bergmann, E. D., and Pinchas, S., *J. Org. Chem.*, 15, 1184 (1950).
(6) Cheng, M., *Z. physik. Chem.*, **B26**, 288 (1934).
(7) Colthup, N. B., *J. Opt. Soc. Amer.*, 40, 397 (1950).
(8) Earp, D. P., and Glasstone, S., *J. Chem. Soc.*, **1935**, 1709.
(9) Fieser, L. F., Hartwell, J. L., and James, J. E., *Org. Syntheses*, 20, 11 (1940).
(10) Fox, J. J., and Martin, A. E., *Proc. Roy. Soc. (London)*, **A175**, 208 (1940).
(11) Freymann, R., and Guéron, J., *Compt. rend.*, **205**, 859 (1937).
(12) Fyfe, W. S., *J. Chem. Phys.*, **21**, 2 (1953).
(13) Gaydon, A. G., *Trans. Faraday Soc.*, 43, 36 (1947).
(14) Grove, J. F., and Willis, H. A., *J. Chem. Soc.*, **1951**, 877.
(15) Hass, H. B., and Bender, M. L., *Org. Syntheses*, 30, 99 (1950).
(16) Hunsberger, I. M., *J. Am. Chem. Soc.*, 72, 5626 (1950).
(17) Husted, D. R., and Ahlbrecht, A. H., *Ibid.*, 74, 5422 (1952).
(18) Institute of Petroleum, Research Group, *J. Inst. Petroleum*, 37, 119 (1951).
(19) Jones, L. H., and Badger, R. M., *J. Am. Chem. Soc.*, 73, 3132 (1951).
(20) Kadesh, R. G., and Weller, S. W., *Ibid.*, 63, 1310 (1941).
(21) Kohlrausch, K. W. F., and Koeppl, F., *Z. physik. Chem.*, **B24**, 370 (1934).
(22) Leighton, P. A., and Lucy, F. A., *J. Chem. Phys.*, 2, 756 (1934).
(23) Linnett, J. W., *Trans. Faraday Soc.*, 41, 228 (1945).
(24) Luettke, W., and Mecke, R., *Z. physik. Chem.*, **196**, 56 (1950).
(25) Morris, J. C., *J. Chem. Phys.*, 11, 230 (1943).
(26) Nielsen, H. H., *Phys. Rev.*, 46, 117 (1934).
(27) Pauling, L., "The Nature of the Chemical Bond," p. 327 Ithaca, N. Y., Cornell University Press, 1940.
(28) Pozefsky, A., and Coggeshall, N. D., ANAL. CHEM., 23, 1611 (1951).
(29) Rodebush, W. H., and Buswell, A. M., *J. Phys. Chem.*, 43, 219 (1939).
(30) Thompson, H. W., and Harris, G. P., *Trans. Faraday Soc.*, 38, 37 (1942).
(31) Vergnoux, A. M., and Dadillon, R., *Compt. rend.*, **213**, 166 (1941).
(32) Walsh, A. D., *Trans. Faraday Soc.*, 43, 60 (1947).
(33) Wilson, E. B., and Wells, A. J., *J. Chem. Phys.*, 9, 319 (1941).

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Application of Infrared Spectrophotometry to Quantitative Analysis in the Solid Phase

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The pressed potassium bromide pellet technique has been utilized as an aid in the quantitative determination, by infrared spectrophotometry, of two closely related alkaloids, atropine and scopolamine. Difficulties encountered in the use of the technique are discussed, as well as the precision to be expected from the method of application described. Recoveries from standard samples are satisfactory. Reasons for moderate accuracy of the assay in the case of a complex commercial mixture are discussed. Refinements of the method and further work are planned.

THE simplicity and elegance of a recently introduced method (*22, 23, 25*) which makes use of pressed potassium bromide pellets as supporting media for the observation of infrared spectra suggested its potential value as an aid in the quantitative determination of two closely related alkaloids, atropine and scopolamine, in a complex mixture. Present chemical methods for the determination of small quantities of these alkaloids, while sensitive, are not specific.

The principles underlying the use of photometric methods for quantitative analysis apply with equal vigor to all spectral regions (*26*). Measurements in the infrared, however, are hampered by the introduction of certain practical problems not so evident in the visible or ultraviolet. The nature of energy absorption in the infrared connotes rather narrow spectral bands, while at the same time, sources of infrared radiation are of relatively low intensity. As a consequence, instruments designed to pass a sufficiently narrow band of frequencies are complex.

The effect of the relatively broad band pass in present infrared spectrophotometers on absorption measurements has been studied at some length. Ramsay (*20*) has made calculations, utilizing an assumed triangular function of energy over the slit width, which indicate that for a ratio of slit width to band width (n) of 0.5, the indicated absorptivities are of the order of 20% below the true values. He suggests a method of integrating absorption over the band to give useful results. Philpotts, Thain, and Smith (*18*) have examined the same problem, using a transmittance function assumed constant between the limiting wave lengths, and have come to the conclusion that when n is equal to 1, Beer's law will be obeyed. Observed values will be 15% below true values and a 10% variation in slit width will cause a 3% variation in observed calibration coefficient. While Ramsay (*20*) feels that slit widths are of the same order of magnitude as band widths, Philpotts, Thain, and Smith (*18*) suggest that n is often greater than 1.

Robinson (*21*) has studied the same problem and come to a similar conclusion; if n is equal to or less than 1, then Beer's law will apparently hold for observed values. In addition, he has examined the effects of errors in the 0 and 100% lines (largely owing to noise and stray light) and suggested that errors from these sources are minimized if readings are made between 20 and 60% transmittance.

Since, as stated, infrared spectrophotometers are relatively complex instruments, attention has been drawn to their ability to yield constant, reproducible values. Fortunately, the instrument used for the work described in this paper, a Perkin-Elmer Model 21, has been discussed in detail. Bowman and Tarpley (*3*) examined the reproducibility of readings obtained with their instrument and examined some of the sources of error. They concluded that the instrument was capable of reproducing absorbance values, over a period of several days, to approximately 4% (2σ limits), but note that the average absorbance level shifts to a degree necessitating regular running of reference samples. They feel that difficulty in obtaining a 0% transmittance line free of pen drift is a major source of absorbance level variations. Hausdorff, Sternglanz, and Williams (*9*) feel that with the amplifier in a condition of "stable unbalance" transmittance reproducibility is within the manufacturers specification of $\pm 0.25\%$ for day to day averages. Neither paper explicitly mentions variations in slit width but Bowman and Tarpley (*3*) note that the use of a wire screen for a sample, instead of a polystyrene film, did not effect precision. Childers and Struthers (*5*) have observed a long-term standard deviation of 1.85% for the same instrument.

Despite these difficulties, infrared spectrophotometry has successfully been applied to a variety of analytical problems. While the petroleum industry has undoubtedly been most vigorous in the use of the technique, nevertheless a variety of specific applications in other fields exists. Parke, Ribley, Kennedy, and Hilty (*17*) describe a method for the analysis of aspirin, phenacetin, and caffeine in tablets. A differential method of assay, which has a potential application to the technique here reported, has been described by Hammer and Roe (*7*), who claim an accuracy and precision of $\pm 0.1\%$.

Of particular interest is the report by Hausdorff (*8*) on the work of Schiedt, describing the quantitative results obtained with amino acids by making use of pressed potassium bromide pellets. More recently, Jensen (*12*) has demonstrated that this technique is suitable for the quantitative analysis of sodium benzyl penicillin.

The potassium bromide pellet technique, which has been described in detail (*8, 22, 23, 25*), has certain unique advantages, among which is the relative freedom from background absorption.

Because of the unique advantages of this method of sample preparation and the specificity of infrared absorption analysis it was decided to attempt to apply the method to the quantitative determination of the alkaloids atropine and scopolamine (hyoscine) as found in a commercial antinausea tablet. While these two compounds can be separated and determined by chemical means (*27*), the quantities present in the tablet are small, and the most suitable analytical method, which makes use of the Vitali-Morin reaction, does not distinguish between the two alkaloids. This reaction has been described a number of times (*1, 2, 4, 6*) and its nature is clearly understood (*11*). Briefly, the alkaloid bases are nitrated with fuming nitric acid; an acetone solution of the nitrated material is then treated with strong base to yield a transient purple color. Since it is the tropic acid portion of the molecule which is nitrated, the reaction is clearly not specific.

The infrared spectra of some of the alkaloids have been described (*13*), with recommendations for quantitative assay (*19*), and Washburn (*28*) has described a quantitative method for either atropine or scopolamine in ointments. His method, which makes use of a solution of the ointment in carbon tetrachloride, does not apply to the simultaneous determination of both the alkaloids in a mixture. No photometric method of this nature has been found in the literature.

INSTRUMENTS AND APPARATUS

Spectrophotometer. A Perkin-Elmer Model 21 recording infrared spectrophotometer, Serial 106, was used throughout the course of the work. The following instrument settings were maintained: slit auto; resolution 927; gain 6; response 1; Source amperes 2.8 to 3.0; speed 3; suppression 0.

Pellet Die. The die, the design of which was suggested by G. B. Hess of Chas. Pfizer and Co., was made of hardened tool steel. A modification has been described by Merritt and Wiberley (*15*). The dimensions of the main block are approximately 5 × 5 × 7.5 cm. wide. The dimensions of the pellets produced are 5 × 20 mm.

Pellet Holder. The holder is a brass unit designed to fit in the sample beam aperture of the spectrophotometer.

Hydraulic Press. A Carver Laboratory press, Serial 7733-5, 10-ton capacity, was used to press the pellets.

EXPERIMENTAL

Reagents. Reagents used were atropine alkaloid, Mallinckrodt U.S.P. XIII; scopolamine hydrobromide, Merck U.S.P. powder; chloroform, Mallinckrodt C.P. reagent; and potassium bromide, Baker and Adamson reagent, ACS (except as noted).

Procedure. PREPARATION OF POTASSIUM BROMIDE PELLETS. Investigation was first directed toward the production of satisfactory pellets of potassium bromide alone. Baker's reagent potassium bromide was used for this phase of the work.

Reproducible pellets should be of the same thickness and weight. Potassium bromide, ground fine in a porcelain mortar and dried in a muffle at 500° C., was weighed out in 100-mg. portions. The die was then inverted with the plunger extending upward through the body. A spacer was inserted between the plunger base and the die body in order to maintain position. The weighed potassium bromide was placed in the cavity above the plunger and smoothed with a glass rod. The anvil was then placed in position and the entire assembly was reinverted and placed in the hydraulic press. Pressure was applied with the spacer removed. At the end of the assigned time period the pressure was released, spacing blocks were placed under the die body, and the pellet was gently pressed out.

A "fill and smooth" technique was adopted. The die was filled with the potassium bromide containing the compound and the excess was removed with a spatula. Pressing time was standardized at 10 minutes and the pressure at 100,000 pounds per square inch for the rest of the work, since these conditions gave a reproducible pellet with average weight (sixteen observations) of 0.1289 gram, mean deviation of ±0.0105 gram, variance 0.000167 gram, standard deviation ±0.0129 gram, and standard error of the mean ±0.0032.

A crucial point in a determination of this nature is the measurement of the quantity of material in the sample beam. With the die used, this is perhaps best accomplished by measuring the thickness of the pellets. Many of these pellets were tapered, however. The effect of the taper on the measured absorbance has been evaluated (*24*) and the error is small, but difficulty in making the measurement remains.

PREPARATION OF CALIBRATION CURVES. It appeared that the absorptivities of the alkaloid bases could most properly be determined by running several different concentrations at varying thicknesses. Therefore, an experimental program calling for the measurement of pellets made from three concentrations (approximately 0.3, 0.6, and 0.9%) of each of the alkaloid bases in potassium bromide at each of three thicknesses, was followed as nearly as possible.

A 10.11% concentration of atropine base in the potassium bromide for the pelleting tests was prepared by agitating the alkaloid with the ground salt. This was diluted by adding more salt to form mixtures containing 0.302, 0.603, and 1.06%, respectively. Pellets were prepared from these and were inserted in the pellet holder. The holder was inserted in the light path after the 0 and 100% transmittance levels had been checked. The spectra were then scanned from 2 to 15 microns.

There was no decrease in energy transmittance when the pellet holder was inserted in the beam. A fiducial mark was established on the holder and instrument. Rotation of the empty holder within several degrees of this mark in either direction had no observable effect on transmission.

Absorbance values at various wave lengths for each of the three atropine systems were plotted against the thickness of the respective pellets in a manner similar to that adopted for the graphs. The plot for the 0.302% system indicated that Lambert's law was obeyed. The 0.603 and 1.06% systems showed deviations of increasing magnitude. These deviations were ascribed to the excessive size of the atropine particles and a resultant loss of energy by scattering, which would increase with concentration. For this reason a portion of the 1.06% system was sieved through bolting silk (200 mesh) and re-examined. The increase in absorptivity was marked.

Because of the interest in the effect of particle size, a new series of atropine systems was prepared by dilution of a 4.914% concentrate and screening through 200-mesh bolting silk after grinding.

The potassium bromide used in these preparations was ground in a glass mortar and sieved through a 230-mesh standard sieve. Subsequently it has been stored at 105° C. and has shown a gradual decrease in the amount of contained water, judging by the decrease in the intensity of the bands at 3 and 6 microns. (A trace taken from this material shortly after its preparation may be found in Figure 1.)

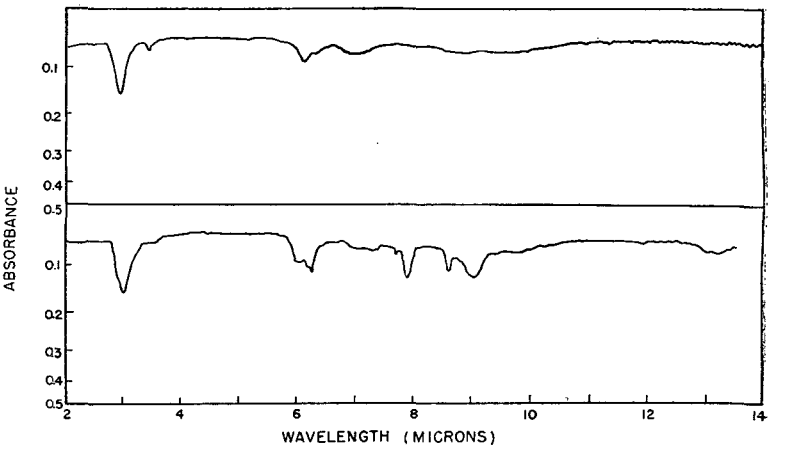

Figure 1. Infrared Spectra

Top. KBr pellet
Bottom. Extract of antinausea components, without the two alkaloids, in KBr pellet

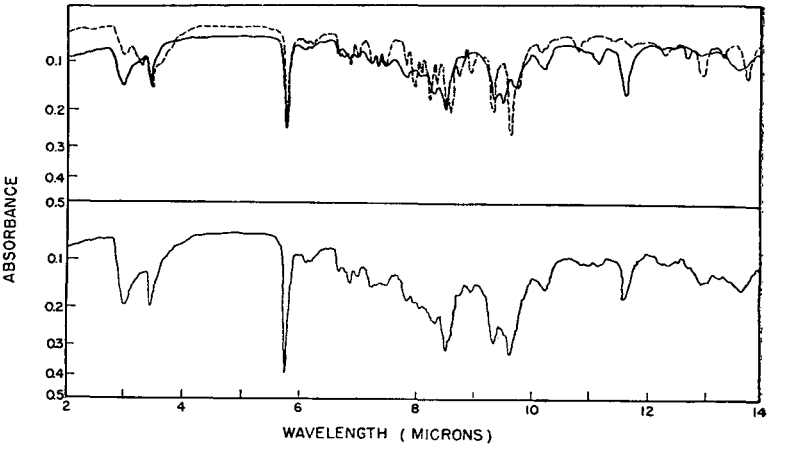

Figure 2. Infrared Spectra

Top. —— Scopolamine, 0.3% in KBr
– – – Atropine, 0.3% in KBr
Bottom. Absorbance curve of 50% w./w. mixture of atropine and scopolamine, ≈0.3% in KBr

A second set of curves was prepared from these new dilutions. The results appeared to be in reasonable conformity with Lambert's law.

Meanwhile a scopolamine concentrate had been prepared in the following way: Scopolamine hydrobromide (0.1444 gram, equivalent to 0.1000 gram of base) was weighed out and transferred to a separatory funnel. Water (10 ml.) and 10% sodium carbonate solution (2 ml.) were added and the suspension was swirled. This was extracted with 4-, 3-, and 2-ml. portions of chloroform. The pooled chloroform was diluted to 10.0 ml. and dried with anhydrous sodium sulfate. In a slightly warmed mortar, 1.5077 grams of potassium bromide were flattened out and 8.0 ml. of the chloroform solution were added. The chloroform was partially removed in a vacuum desiccator and the mixture was ground until dry and warmed at 60° C. under 50-mm. vacuum for several hours. It was then stored in a dry bottle. The mixture was not dry, as scopolamine base is a liquid, but it was not difficult to handle.

This 5.039% concentrate was diluted with the powdered potassium bromide to 0.314, 0.648, and 0.912% scopolamine by weight. Pellets and charts were prepared from this material according to the procedure described.

Superposed absorbance curves for atropine and scopolamine may be found in Figure 2, which also illustrates the absorbance curve of a 50% mixture. It was decided to confine attention to the 5.80-, 11.66-, and 13.00-micron bands. The latter two are reasonably discrete and should serve as appropriate points of measurement for the quantitative determination of the alkaloids in a mixture. The 5.80-micron band is a strong band, common to both alkaloids, and measurements at this wave length might help to serve as checks on the validity of results. Washburn (*28*) made use of a band at 11.21 microns for measurement of scopolamine concentration and of a band at 8.56 microns for the determination of the atropine. No reason for his choice is given, other than that these were convenient wave lengths. It is clear that he did not attempt to assay mixtures.

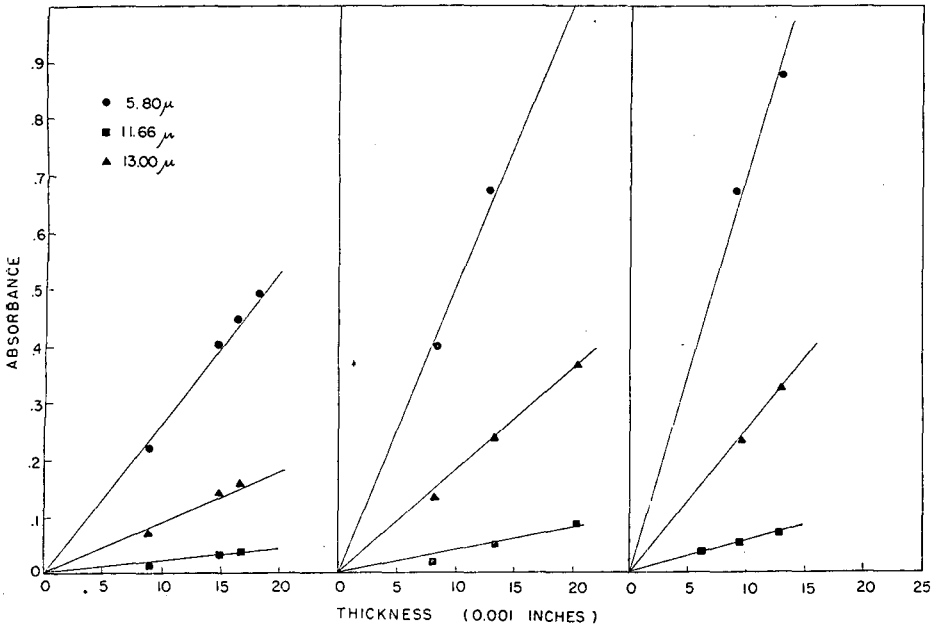

Figure 3. Absorbance of 0.3, 0.6, and 0.9% Atropine in Potassium Bromide Pellets at Indicated Wave Lengths

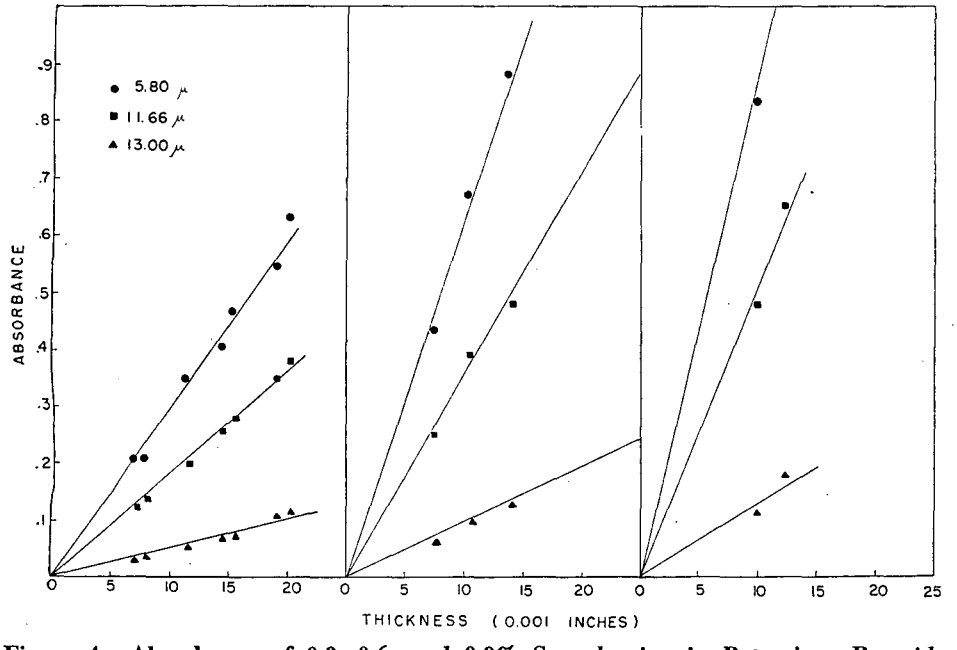

Figure 4. Absorbance of 0.3, 0.6, and 0.9% Scopolamine in Potassium Bromide Pellets at Indicated Wave Lengths

As it had become apparent that the transfer of the alkaloid from the sample under examination to the potassium bromide was going to involve solvent evaporation, it was decided to regrind some of the 0.6% atropine system with a few milliliters of chloroform and retest it. Again the absorptivities changed markedly, although to a lesser degree. For this reason the remaining 4.914% atropine concentrate was ground with chloroform in a glass mortar and the mixture, after drying at 100° C. for a few hours, was diluted with the powdered salt to form 0.308, 0.624, and 0.914% systems. These were run again.

Carefully obtained calibration data for scopolamine yielded indicated absorptivities of 3.69 ± 0.16 (5.80 microns), 2.21 ± 0.09 (11.66 microns), and 0.58 ± 0.06 (13.00 microns), and for atropine, 3.46 ± 0.25 (5.80 microns), 0.241 ± 0.036 (11.66 microns), 1.14 ± 0.06 (13.00 microns).

To check the reproducibility of this method of preparing the atropine dilutions, portions of atropine were weighed on a microbalance into warmed mortars. The appropriate quantities of potassium bromide and chloroform were added and the mixtures were ground until dry

and then dried in the oven at 100° C. for about half an hour. Four samples, of 0.610, 0.621, 0.565, and 0.708% concentration, were prepared in this way. Two pellets were prepared from each of the four systems. Reproducibility was not unreasonable.

The corrections applied to the observed absorbances were based on the observation that the "absorbance" of the blank potassium bromide pellets was a relative constant, independent of the thickness of the pellet. This phenomenon, which has been noted elsewhere (*22*, *23*), makes correction a relatively simple matter, if it is assumed that pellets made of the potassium bromide–alkaloid systems behave in the same way.

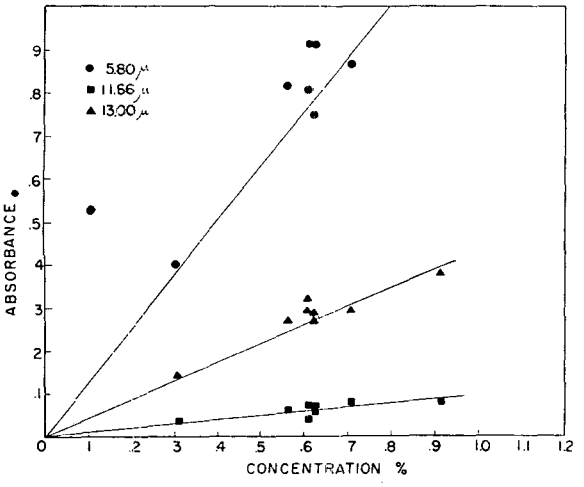

Figure 5. Composite Beer's Law Plot for Atropine

Plots of the corrected absorbance values for each alkaloid at three concentration levels are shown in Figures 3 and 4. From these, composite Beer's law plots for the two alkaloids were obtained, as shown in Figures 5 and 6. For convenience, the data were taken at an arbitrary thickness of 0.015 inch. The relative distribution of the points on the Beer's law plots is not affected by this selection.

Simultaneous equations for the absorptivity of the system at 13.00 and 11.66 microns were set up and solved in the usual way (*16*), using the average values for indicated absorptivity (to distinguish it from true absorptivity) obtained from the collected data.

The solutions to these equations follow:

$$C_{\text{scopolamine}} = \frac{4.75\ A_{11.66\mu} - A_{13.00\mu}}{9.9\ b} \quad (1)$$

$$C_{\text{atropine}} = \frac{3.81\ A_{13.00\mu} - A_{11.66\mu}}{4.11\ b} \quad (2)$$

where C = concentration of alkaloid, milligrams per gram; b = pellet thickness, centimeters; and A = corrected absorbance at the indicated wave length. For notation see (*10*).

ANALYSIS OF KNOWN MIXTURES AND ANTINAUSEA TABLETS. Two known mixtures of the two alkaloids were prepared from the concentrates used to prepare the standards. Two pellets were pressed from each system and the results were calculated on the basis of Equations 1 and 2. The data show a mean recovery of 104% atropine and 98.2% scopolamine.

A mixture of the ingredients of the antinausea tablet with the exception of the alkaloids was prepared to assist in testing extraction procedures. In addition to scopolamine hydrobromide and atropine sulfate the tablets contain vitamin B_2, vitamin B_6, niacinamide, Benzocaine, Luminal, and excipients, including starch, lactose, magnesium stearate, and oil of peppermint. To prepare the blank the oil of peppermint was ground in a mortar with a portion of the lactose, adding more lactose while grinding until the mixture was dry enough to sieve. To this was added the remaining lactose and other ingredients, with grinding. The entire mixture, after thorough grinding, was transferred to a bottle and well shaken.

After several attempts to devise a procedure which would extract the alkaloids from the powder but eliminate interfering substances, the following method was adopted.

Weigh accurately about 0.7 gram of ground tablet mixture and transfer to a stoppered centrifuge tube. Add 10 ml. of water and agitate vigorously for 5 minutes, then centrifuge. Decant through a small No. 1 Whatman filter paper into a separatory funnel. (A vitamin B_1 separatory reaction vessel is ideal.) Wash the filter paper with a few milliliters of water. (The solution should at this time be slightly acid.) Wash twice with 2 ml. of chloroform, discarding each wash. Add 1 ml. of 0.1*N* sodium hydroxide and extract the solution with 3.0 ml., then 1.0 ml. of chloroform. Pool the chloroform and wash with 10 ml. of water. After washing the chloroform, transfer it to a centrifuge tube, dry it by adding anhydrous sodium sulfate, and centrifuge. Take 3.0 ml. for the assay.

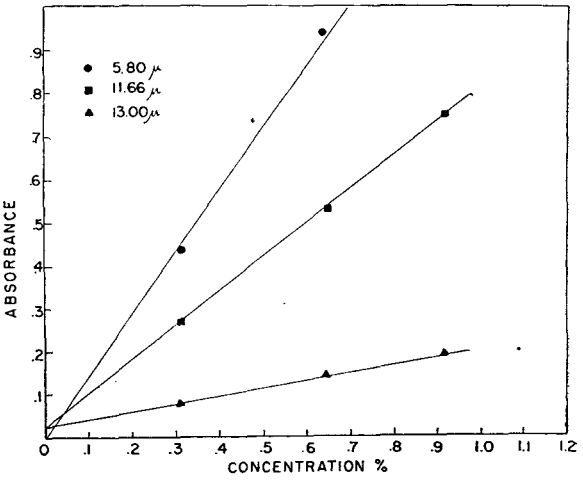

Figure 6. Composite Beer's Law Plot for Scopolamine

The absorbance curve of a pellet prepared in this way from the blank mixture is shown in Figure 1. The chloroform solution was handled much as described for the preparation of the scopolamine standards: transferring the aliquot to a warmed glass mortar containing a known weight of powdered potassium bromide (in this case, about 0.12 gram), removing the bulk of the chloroform in a vacuum desiccator, grinding the remaining solvent with the potassium bromide until dry, then drying for a short while in a 100° C. oven.

Two commercial lots of tablets supplied by W. C. MacLennan of Winthrop-Stearns, Inc., were examined in the same way. Pellet traces appear in Figure 7. Results are as follows:

	γ/Tablet		
	Found	Found	Claimed
Scopolamine	117	113	138
Atropine	132	117	84
Total	249	230	222

Data are collected in Table I.

DISCUSSION

An examination of the results shows the relative error to be high. Some potential sources of error have been noted, including

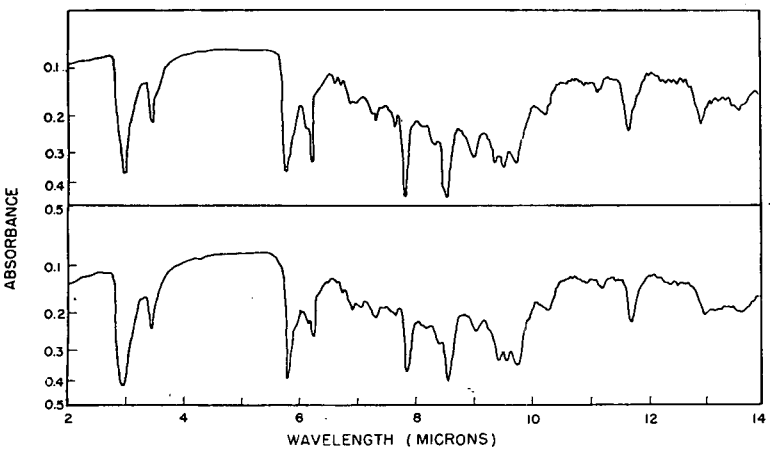

Figure 7. Traces of Extracts from Commercial Antinausea Tablets

the following: slit width variation, instrument reproducibility, pellet taper, particle distribution, and particle size. Others arise in connection with the preparation of the sample—for example, incomplete extraction of the alkaloid, loss on glassware in grinding (this applies also to the standards), erroneous blank corrections, interference patterns, and possible unknown factors. The possibility of interaction between the alkaloid and the potassium bromide is felt to be small, as is the possibility of damage while pressing.

Slit width variation was felt to be insignificant in this case. Variation in slit width was checked by observation of the slit counter during operation. Values were observed to vary by less than 1 micron, or less than ±1% at 5.80 microns. Even during a short period when a noisy detector amplifier necessitated slightly lower resolution to compensate for increased noise the variation did not exceed ±1.5% of the average at 5.80 microns. This is well under the 10% limit, which, it was noted, would cause an error of 3%.

Table I. Assay of Atropine-Scopolamine Tablets

	Tablet A	Tablet B
Sample wt., g.	0.7034	0.7370
KBr, g.	0.1474	0.1600
Pellet thickness, inch	0.0170	0.0202
Absorbance		
11.66μ		
Obsvd.	0.213	0.230
Corr.[a]	0.163	0.180
13.00μ		
Obsvd.	0.198	0.207
Corr.[a]	0.123	0.132

[a] Corrections were applied on the basis of the absorbance curve of Figure 1, blank tablet mixture.

Instrument reproducibility leaves room for improvement but generally is a minor source of error (*3, 5, 9*).

Pellet taper, already noted, is not highly significant in itself (*24*), except that it impairs the accuracy of thickness measurement.

Measurements on a number of pellets pressed under the same conditions were examined to test the possibility that pellet weight might be a better measure of sample in the beam than is the thickness. The average deviation of the mean of the ratio was nearly ±3%, a value judged to be somewhat higher than that which could be accounted for by error in measurement of thickness. To further test this point, absorptivities have been calculated on the basis of pellet weight for a group of scopolamine pellets. Analysis of the data yield an average deviation of the mean of ±5.8%, in contrast to ±4.3% for the same group on the basis of thickness.

A potential source of error in this method is the presence of interference patterns in the trace (see Figure 1, top). This phenomenon has been noted and utilized for the measurement of cell thickness. It remains to choose the appropriate pellet thickness to eliminate the difficulty.

Particle distribution should not be a significant feature in this case, as Lambert's law is obeyed fairly well for the standards (*14*).

The effect of particle size, or, more accurately, control of particle size, has not adequately been investigated in this work. While this should not be a significant factor with the liquid scopolamine, it is planned to measure the particle sizes of the group of atropine samples responsible for the bad scatter on the composite (Beer's law) plot, and in addition, measure, by chemical means, the alkaloid concentrations of the same systems.

Testing the recovery of known amounts of alkaloid from a blank should be deferred until the previous suggestions have been examined, but will, of course, be undertaken when other conditions have been shown to be reproducible.

LITERATURE CITED

(1) Allport, N. L., and Jones, N. R., *Quart. J. Pharm. and Pharmacol.*, **15**, 238 (1942).
(2) Allport, N. L., and Wilson, E. S., *Ibid.*, **12**, 399 (1939).
(3) Bowman, H. M., and Tarpley, W. B., *Appl. Spectroscopy*, **7**, 57 (1953).
(4) Brunzell, A., Canbäck, T., and Seydlitz, H., *Farm. Revy*, **46**, 729 (1947); reviewed in *Quart. J. Pharm. and Pharmacol.*, **21**, 72 (1948).
(5) Childers, E., and Struthers, G. W., ANAL. CHEM., **25**, 1311 (1953).
(6) Colby, A. B., and Beal, J. L., *J. Am. Pharm. Assoc.*, **41**, 351 (1952).
(7) Hamner, C. F., and Roe, H. R., ANAL. CHEM., **25**, 668 (1953).
(8) Hausdorff, H., *Appl. Spectroscopy*, **7**, 75 (1953).
(9) Hausdorff, H., Sternglanz, H., and Williams, V. Z., *Ibid.*, **7**, 63 (1953).
(10) Hughes, H. K., *et al.*, ANAL. CHEM., **24**, 1349 (1952).
(11) James, W. O., and Roberts, M., *Quart. J. Pharm. and Pharmacol.*, **18**, 29 (1945).
(12) Jensen, J. B., *Acta Chem. Scand.*, **8**, 393 (1954).
(13) Marion, L., Ramsay, D. A., and Jones, R. N., *J. Am. Chem. Soc.*, **73**, 305 (1951).
(14) Mellon, M. G., ed., "Analytical Absorption Spectroscopy," New York, John Wiley & Sons, 1950.
(15) Merritt, P. E., Ph.D. thesis, Rensselaer Polytechnic Institute, 1954.
(16) Nachod, F. C., Zippin, C., and Hinkel, E. T., *J. Am. Pharm. Assoc.*, **38**, 173 (1949).
(17) Parke, T. V., Ribley, A. M., Kennedy, E. E., and Hilty, W. W., ANAL. CHEM., **23**, 953 (1951).
(18) Philpotts, A. R., Thain, W., and Smith, P. G., *Ibid.*, **23**, 268 (1951).
(19) Pleat, G. B., Harley, J. H., and Wiberley, S. E., *J. Am. Pharm. Assoc.*, **40**, 107 (1951).
(20) Ramsay, D. A., *J. Am. Chem. Soc.*, **74**, 73 (1952).
(21) Robinson, D. Z., ANAL. CHEM., **23**, 273 (1951).
(22) Schiedt, U., *Z. Naturforsch.*, **8b**, 66 (1953).
(23) Schiedt, U., and Reinwein, H., *Ibid.*, **7b**, 270 (1952).
(24) Spell, A., and Rector, H. E., paper presented at Pittsburgh Conference on Applied Spectroscopy and Analytical Chemistry, Pittsburgh, Pa., March 4, 1954.
(25) Stimson, M. M., and O'Donnell, M. J., *J. Am. Chem. Soc.*, **74**, 1805 (1952).
(26) Strong, F. C., ANAL. CHEM., **24**, 338 (1952).
(27) Trautner, E. M., Neufeld, O. E., and Rodwell, C. N., *Australian Chem. Inst. J. & Proc.*, **15**, 55 (1948); reviewed in *Analyst*, **74**, 55 (1949).
(28) Washburn, W. H., *J. Am. Pharm. Assoc.*, **41**, 603 (1952).

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Quantitative Infrared Determination of Trace Impurities in Solids Using Fractional Crystallization Technique

Determination of Catechol and Resorcinol in Hydroquinone

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An analytical procedure was needed to detect the possible isomeric impurities, catechol and resorcinol, in photographic hydroquinone at the 0.1% level. The combination of a fractional crystallization technique plus the differential infrared spectroscopic method was applied successfully to this problem. Using acetonitrile as the solvent, the isomers were quantitatively determined at the 0.05% level and the limit of detectability was calculated to be 0.01% for both isomers. The fractional crystallization technique is applicable to a wide variety of infrared analyses. It permits concentrating of impurities relative to the major constituent and thereby increases the sensitivity of infrared spectroscopic analyses.

INFRARED spectrophotometric determinations of small concentrations of impurities in solids by examination of the sample dissolved in a suitable solvent usually have a detectability limit of about 1 mole %. This limit is governed by the weakness of the absorption bands of the impurities in the presence of the absorption bands of the major constituent. One method of extending this detectability limit is to concentrate the impurity relative to the main constituent by fractional crystallization. In general, it is possible to effect a manifold increase in the relative concentration of the impurities in the solution remaining after fractional crystallization of the major constituent.

Analysis by the fractional crystallization technique is applicable to most solid materials provided the following requirements can be fulfilled:

The solubility of the main constituent in the chosen solvent at room temperature or below should be of the same order of magnitude as that of the impurity.

The solubility of the main constituent must increase with temperature.

No large portion of the impurity should be occluded by the main constituent during crystallization.

The absorption spectrum of each constituent to be measured must have a band which is not interfered with materially by the absorption of the solvent, the main constituent, or any other impurity.

This fractional crystallization technique is being used in a number of routine analyses in this laboratory; however, only a single example has been chosen to illustrate the general technique.

As a part of the examination of photographic grade hydroquinone, it became necessary to determine quantitatively small amounts of resorcinol and catechol (in the order of 0,1%) in hydroquinone. These isomers are possible contaminants in some hydroquinone manufacturing methods. After experimenting with a number of solvents, the relative solubility of the isomers in acetonitrile was found to be favorable. At room temperature about 1.1 grams of hydroquinone, 6.5 grams of resorcinol, and 1.3 grams of catechol, each would dissolve in 10 ml. of acetonitrile. A comparison of the infrared absorption spectra of these acetonitrile solutions and acetonitrile itself (Figure 1) showed that the absorption bands of the solvent, catechol, or hydroquinone, do not interfere materially with the absorption band of resorcinol at 10.4 microns. At 7.85 microns the catechol absorption band is nearly free from interference by resorcinol and acetonitrile, but hydroquinone at the saturated solution concentration in acetonitrile does contribute a sizable absorption. In spite of the latter difficulty, the analytical method has a calculated detectability limit of 0.01% by weight of either impurity in hydroquinone.

EXPERIMENTAL

Hydroquinone, 12.5 grams, was placed in a 50-ml. Erlenmeyer flask to which 15 ml. of acetonitrile were added. Solution was obtained by heating the mixture on a hot plate and breaking up the larger lumps with a stirring rod. The hot flask was then stoppered and the solution cooled rapidly under cold water with swirling. The precipitated hydroquinone (11.5 grams) was filtered through a 50-mm. fritted disk, Büchner-type funnel into a 50-ml. suction filter flask using a water aspirator. The precipitate was then washed with two 2.5-ml. portions of acetonitrile.

The filtrate containing about 1 gram of hydroquinone was evaporated on a hot plate with the aid of a glass bead until the hydroquinone began to crystallize, at which time 0.5 ml. of acetonitrile was quickly added to the flask to prevent the evaporation from going to complete dryness. The hot flask was then stoppered and cooled rapidly under cold water. The precipitated hydroquinone was filtered, using a water aspirator, through a 15-mm. fritted disk, Büchner-type funnel into a 15-ml. graduated centrifuge tube which was contained in a filtering flask. The precipitate was then washed with three 0.5-ml. portions of a cool, saturated solution of hydroquinone in acetonitrile. The resulting filtrate volume (slightly greater than 2 ml.) was adjusted to 2 ± 0.05 ml. in the centrifuge tube by evaporating with a stream of dry nitrogen.

Infrared absorption measurements on the filtrate were then made in a 0.1-mm. liquid absorption cell using a Baird recording spectrophotometer. Compensation was provided by a saturated solution of hydroquinone in acetonitrile in a 0.1-mm. liquid cell prepared in exactly the same manner as the sample. The measurements for catechol were made at the 7.85-micron peak and those for resorcinol were obtained at the 10.4-micron peak.

To eliminate the differences between the cells and also to increase the analytical sensitivity, the differential method was used (*1*). This required that the absorption (maximum absorbance columns in Tables I and II) obtained with the sample solution in the sample cell and the compensating solution in the reference cell be diminished by the absorption (minimum absorbance columns in Tables I and II) obtained with the solutions exchanged but not the cells. To do this the 100% transmittance line was adjusted to about 56%. The slits were opened slightly for measurements at 7.85 microns. Caution was exercised during the absorbance measurements to ensure that the number of additions and withdrawals of solutions to and from each cell was equal.

Calibrating solutions were prepared by adding various known quantities of the isomers to the standard sample (12.5 grams) of hydroquinone and carrying out the processing and absorbance measurements using the above technique.

In order to determine the maximum recovery—that is, the percentages of catechol and resorcinol which remain in the filtrate after following the fractional crystallization technique described—the procedure was: A sample was prepared containing 12.5 mg. of each isomer in a 2-ml. saturated solution of hydroquinone in acetonitrile, and absorbance measurements were made on these samples using the differential method. A comparison of these absorbance measurements with those made on calibrating solutions subjected to the fractional crystallization technique, indicated that the method allowed for a maximum recovery of catechol and resorcinol of 82 and 84%, respectively, at the 0.1% level.

The hydroquinone, resorcinol, and catechol chemicals used in this investigation were Eastman Kodak Co., white label grade.

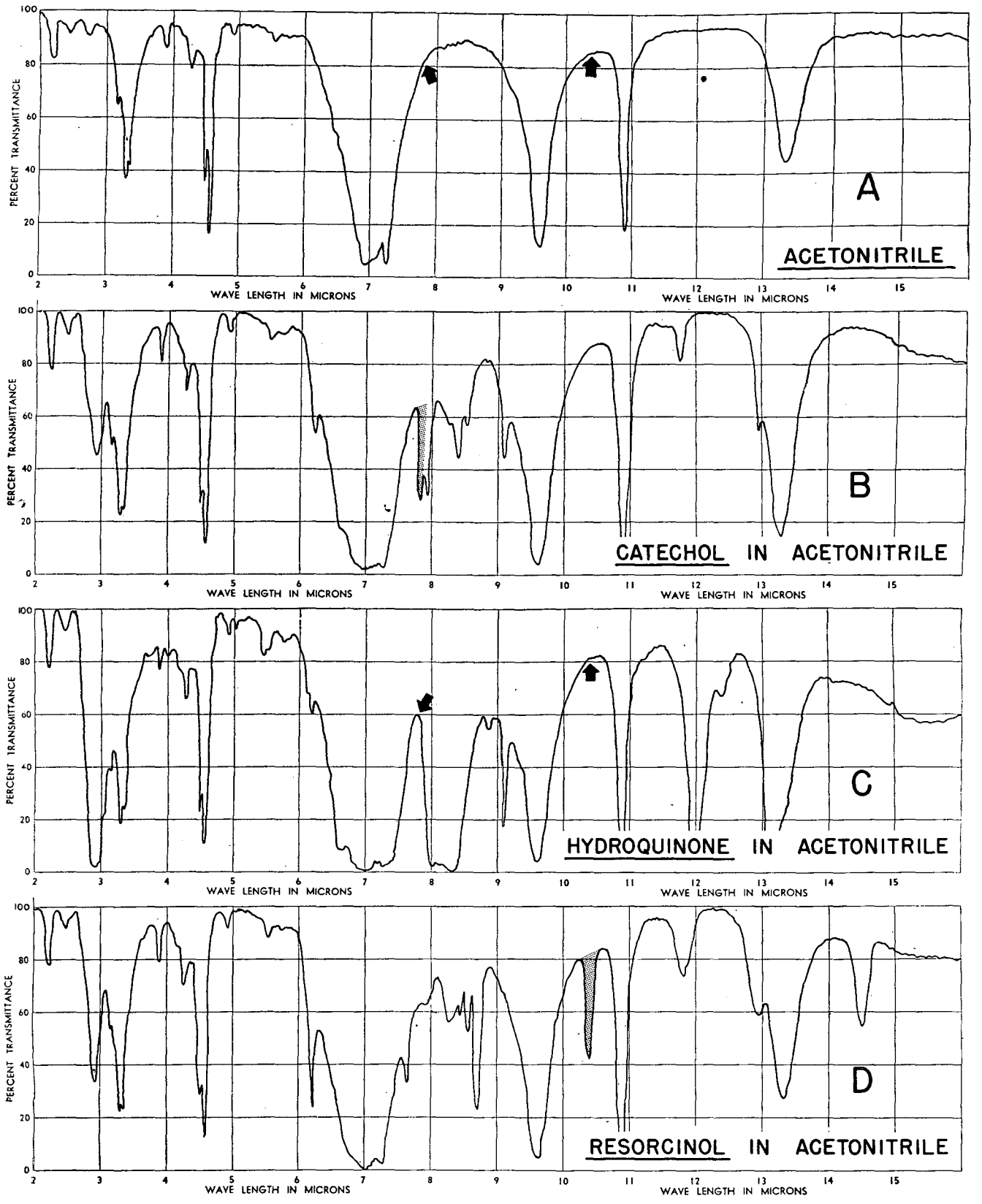

Figure 1. Infrared Absorption of Acetonitrile Solutions

A. 0.1-mm. path length
B. 0.1-mm. path length, 20 mg. per ml.
C. 0.1-mm. path length, saturated solution
D. 0.1-mm. path length, 20 mg. per ml.

Table I. Data Used to Calculate Formulas for Determination of Catechol and Resorcinol in Hydroquinone

Added to HQ		A, 7.85 μ		
% catechol	% resorcinol	Max.	Min.	Δ
		Catechol Determinations		
0.00	0.10	0.270	0.230	0.040
0.05	0.05	0.339	0.167	0.172
0.10	0.00	0.409	0.101	0.308
0.10	0.10	0.428	0.089	0.339
0.15	0.15	0.530	0.018	0.512
		A, 10.4 μ		
		Max.	Min.	Δ
		Resorcinol Determinations		
0.10	0.00	0.262	0.231	0.031
0.05	0.05	0.297	0.196	0.101
0.00	0.10	0.342	0.161	0.181
0.10	0.10	0.350	0.149	0.201
0.15	0.15	0.409	0.112	0.297

Table II. Calculation of Analytical Tolerance, 2σ Limits, at 0.1% Level

Sample	A, 7.85 μ Max.	Min.	Δ	A, 10.4 μ Max.	Min.	Δ
1	0.432	0.097	0.335	0.357	0.161	0.196
2	0.432	0.089	0.343	0.347	0.140	0.207
3	0.420	0.092	0.328	0.347	0.149	0.198
4	0.426	0.078	0.348	0.347	0.146	0.201
Average			0.339			0.201
Std. dev., σ			0.0076			0.0042
2σ			0.015			0.008

RESULTS AND DISCUSSION

Table I lists the results obtained from a series of calibrating solutions. Measurements were made according to the procedure outlined in the experimental section. Figure 2 shows typical absorption curves from which the data in Table I were obtained. All the values listed in Table I are averages of duplicate analyses except those obtained from solutions containing 0.1% of both isomers which were averages of four analyses. Table I shows that there is a noticeable effect caused by resorcinol on the catechol determination at the 0.1% level (0.040 absorbance unit) and a similar effect of catechol on the resorcinol determination at the same level (0.031 absorbance unit). This effect is significant and can be shown by the ±2σ limits which are appended to the analytical data in Table II.

Table III. Comparison of Absorbance, A, between Saturated Hydroquinone Solution and Saturated Processed Hydroquinone Solution both in Acetonitrile

Sample	A, 7.85 μ	A, 10.4 μ
Saturated, unprocessed, HQ in CH_3CN	0.009	0.000
Processed HQ in CH_3CN	0.040	0.004

Table II lists the absorbances obtained from the analyses of four different samples containing 0.1% resorcinol and catechol in hydroquinone (averages are listed in Table I). The standard deviation (σ) of each set of absorbances was calculated. Two standard deviation limits were found to be ±0.015 for catechol and ±0.008 for resorcinol at the 0.1% level of each isomer. Using these tolerances the absorbances listed should be reported to the nearest hundredth of a density unit. It is assumed that the tolerances associated with the absorbances at the 0.05 and the 0.15% levels are the same as those determined for the absorbances at the 0.1% level.

Table III shows the absorbance effect of hydroquinone decomposition products formed during an analysis. This effect is significant but is also sufficiently reproducible to be canceled out in the regular procedure. All measurements were made in 0.1-mm. liquid cells with saturated unprocessed hydroquinone dissolved in acetonitrile used as compensation.

Because each isomer interferes in the analysis of the other, an extension of Beer's law was used rather than empirical working curves for determining the concentrations of the isomers in an unknown sample of hydroquinone. Beer's law for a two-component system becomes:

$$A_\lambda = a_{1\lambda}c_1 + a_{2\lambda}c_2 \quad (1)$$

where A_λ is the total absorbance of the mixture of two materials at a particular wave length and $a_{1\lambda}$, $a_{2\lambda}$, c_1, c_2 are the absorptivities and concentrations of the corresponding components at the same wave length. The analytical technique used to obtain the calibrating data in Table I provided for complete compensation of hydroquinone absorption, and absorption due to hydroquinone decomposition between the liquid absorption cells used in the analysis. Therefore, the absorbances actually measured were only those of catechol and resorcinol.

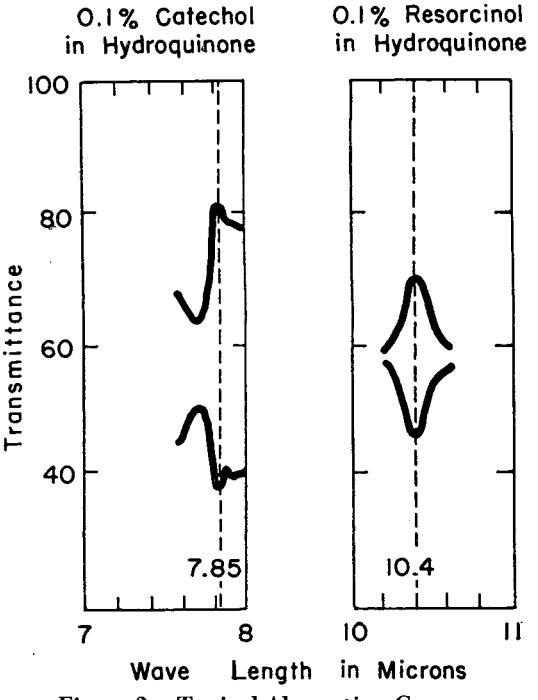

Figure 2. Typical Absorption Curves
Used in calibrating data in Table I

Equation 1 can now be restated according to the authors' analytical conditions as follows:

$$A_{7.85} = a_{R7.85}c_R + a_{C7.85}c_C \quad (2)$$

$$A_{10.4} = a_{R10.4}c_R + a_{C10.4}c_C \quad (3)$$

Each of the values of the four absorptivities in Equations 2 and 3 were calculated from two independent sets of data (Table I) at the 0.1% level, using the measurements from solutions containing 0.1% catechol and 0% resorcinol in one case, and 0% catechol and 0.1% resorcinol in the other case. The results of these calculations are shown in Table IV. These absorptivities were used to calculate the absorbances expected at the 0.05% and 0.15% levels of resorcinol and catechol if the isomers obeyed Beer's law. A comparison of these calculated values, listed in Table V, with the corresponding values experimentally obtained (Table I) shows them to be the same within the experimental error (2σ limits) of ±0.02 for catechol and ±0.01 for resorcinol as calculated previously. It can be concluded that, in the concentration range between 0.05 and 0.15%, resorcinol and catechol in hydroquinone obey Beer's law.

To determine the percentages of catechol and resorcinol in a sample of hydroquinone of unknown purity, the absorbances are obtained according to the described procedure. These absorbances and the absorptivities as calculated above are substituted in Equations 2 and 3, which are then solved for concentrations of catechol and resorcinol. It may be well to redetermine the absorptivities of catechol and resorcinol periodically, since they may vary with instrumental fluctuations.

There is one interference in the analysis for which the authors have compensated in the procedure and which should be understood so as to prevent needless difficulties. During the absorbance measurements on a sample, a decomposition product(s) of hydroquinone is deposited on the sodium chloride plates of the liquid cells. As long as the number of contacts of these solutions with each cell is kept the same, the above effect is eliminated. This effect, though small, can become troublesome if it has not been compensated for.

The estimated limit of detectability of the catechol and resorcinol of hydroquinone using this method of analysis can be determined by calculating the concentrations of both isomers when the tolerances ($\pm 2\sigma$ limits) are substituted in Equations 2 and 3. This calculation gives a 0.01% limit for each isomer. In other words, when this method of analysis is used, 0.01% by weight of either isomer should be detected in hydroquinone. One way to obtain a lower limit of detectability would be to increase the initial sample size. This, however, would probably require an additional crystallization in order to obtain a final 2-ml. working volume. Another limiting factor would be the greater quantity of decomposition products formed during the analysis when a larger initial sample is used.

Table IV. Calculated Absorptivities from Equations 2 and 3 and Table I

Method	7.85 Microns		10.4 Microns	
	$a_{R7.85}$	$a_{C7.85}$	$a_{R10.4}$	$a_{C10.4}$
1	0.30	3.00	1.69	0.20
2	0.40	3.10	1.80	0.31
Average	0.35	3.05	1.75	0.25

Table V. Comparison of Calculated Absorbances

(From average absorptivities of Table IV and Equations 2 and 3 with observed values from Table I)

% Catechol in HQ	% Resorcinol in HQ	A, 7.85 μ		A, 10.4 μ	
		Calcd.	Obsd.	Calcd.	Obsd.
0.05	0.05	0.170	0.172	0.100	0.101
0.15	0.15	0.510	0.512	0.300	0.297

The method of analysis presented here would not in any way eliminate the effects of other impurities which might be present in a sample of hydroquinone (of unknown purity). If impurities other than catechol or resorcinol were present, their effects would have to be determined at the wave lengths in question and compensation would then be required for these effects.

LITERATURE CITED

(1) Robinson, D. Z., ANAL. CHEM., 24, 619 (1952).

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Spectrographic Analysis of Petroleum Products and Related Materials

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A new method for the preparation of samples in the spectrographic determination of relatively large quantities of metals and phosphorus in various materials is described. A small amount of sample is burned on a relatively large bed of graphite powder containing copper oxide as additional buffer and internal standard. After mixing, portions of this powder are tamped into a shallow cratered graphite electrode and arced for 60 seconds in a direct current arc. Analytical curves are constructed using the same technique on samples of known composition. Used and unused lubricating oils, additive concentrates, greases, sludges, and deposits can be analyzed using this technique.

SEVERAL spectrographic methods have been described for the determination of metallic elements and phosphorus in lubricating oils. Calkins and White (*1*) used red-hot graphite electrodes immersed in the oil and spark excitation of the oil-impregnated electrodes. For more accurate results, Clark and others (*3*) have found that this technique should be used only for the analysis of unused oils and that the standards and samples should be of a similar nature. A porous cup method was used by Gassmann and O'Neill (*5*) for the determination of additive metals and phosphorus in unused oils. Later, they found (*4*) that this method cannot be used for determining the total amount of an element in used oils containing suspended solids because of filtering action of the electrodes. Pagliassotti and Porsche (*8*) utilized a disk-shaped graphite electrode rotating through the oil sample for the determination of phosphorus in unused oils. Gambrill, Gassmann, and O'Neill (*4*) have used the same type of rotating electrode for determining iron, lead, and additives in used lubricating oils with good results. Hansen, Skiba, and Hodgkins (*6*) described a method for iron in used oils in which the sample is ashed directly in a specially designed electrode.

In order to analyze used oils containing insoluble materials, a graphite powder bed method developed by the authors is deemed more satisfactory and flexible than the methods mentioned. In addition, other types of samples such as greases, additive concentrates, sludges, and deposits can be analyzed with the same general technique. The method is described for the determination of relatively large quantities of metals present in used or unused oils.

APPARATUS

A Baird Associates 3-meter grating spectrograph and an Applied Research Laboratories Multisource unit were used with the settings given in Table I.

For the densitometry of plates, an Applied Research Laboratories microphotometer was employed. The photographic plates used were Kodak spectrum analysis No. 1 plates developed, washed, and dried in Applied Research Laboratories processing equipment. Electrodes were made from $^1/_4$-inch high purity graphite rods supplied by The National Carbon Co. The sample electrode had a crater at one end $^3/_{32}$ inch deep and $^3/_{16}$ inch in diameter. The upper counter electrode was machined to a tip $^1/_8$ inch in diameter.

Table I. Spectrograph and Source Unit Settings

Baird Spectrograph

Spectral position	2180–3600 A. (1st order)
Grating aperture	13 mm.
Slit width	50 microns
Quartz condensing lens	25 cm. focal length to focus electrodes on grating
Filters	10% transmittance filter in radiation beam and 50% filters over 3345 A. Zn line and 2663.2 A. Pb line

Multisource Unit

Capacitance	60 microfarads
Inductance	400 microhenries
Resistance	22 ohms
Discharge *vs.* charge	0°
Output amperage	10 amperes
Exposure	60 seconds
Analytical gap	3 mm.
Sample electrode	Positive

PRELIMINARY EXPERIMENTS

At first, the oil sample was burned in a small graphite crucible. This was fashioned from graphite rods 0.5-inch in diameter. It had very thin walls and a height of 0.5 inch. It weighed about 0.7 gram and had two small holes opposite each other near the top so that it could be suspended by means of a fine wire during ignition. After weighing the oil into the crucible, a microburner was used to ignite and burn the oil. After burning, the crucible plus residue were then made up to a definite weight (0.900 gram) with graphite, and copper oxide was added as internal standard. The crucible was transferred to a mortar, crushed carefully, and mixed thoroughly. Cratered electrodes were tamped in this mixture and spectrograms recorded by means of a direct current arc. Since the crushing and grinding of the graphite crucible were time-consuming and tedious, other techniques were tried.

Table II. Wave Lengths of Analysis Lines

Element	Wave Length, A.
Copper (intern. std.)	3108.6
Magnesium	2779.8
Zinc	2800.9, 3345.0
Barium	2335.3, 2347.6
Tin	2429.5
Lead	2823.2, 2663.2
Phosphorus	2535.7, 2553.3
Calcium	3158.9
Sodium	3302.3

Small crucibles were made of thin aluminum foil. The foil was shaped into a small cup and the sample weighed directly into it. With oil samples, a few tenths of a gram were burned and the aluminum foil cup was then rolled into a ball, placed in a cratered graphite electrode, and subjected to direct current arc excitation. In general the sensitivity was not adequate and the aluminum ball did not burn satisfactorily in the arc.

The graphite powder bed method described below was developed to overcome these disadvantages.

PROCEDURE FOR GRAPHITE POWDER BED METHOD

Preparation of Standards. For the analysis of oils, base oil stocks and additive materials are blended and chemically analyzed to make standards in the range desired. From these, powder standards are prepared as described in the analysis of samples. Usually only one or two oil standards are needed, since by varying the weight of the oil burned, any number of powder standards can be made covering the desired range. From the percentage of each element in the oil standard and the weight burned, the milligrams of each element in the powder standard are calculated. These powder standards are then used to prepare analytical curves. The milligrams of element per gram of powder standard versus intensity ratios of element line to internal standard line are plotted on log-log graph paper. Several typical analytical curves are shown in Figure 1.

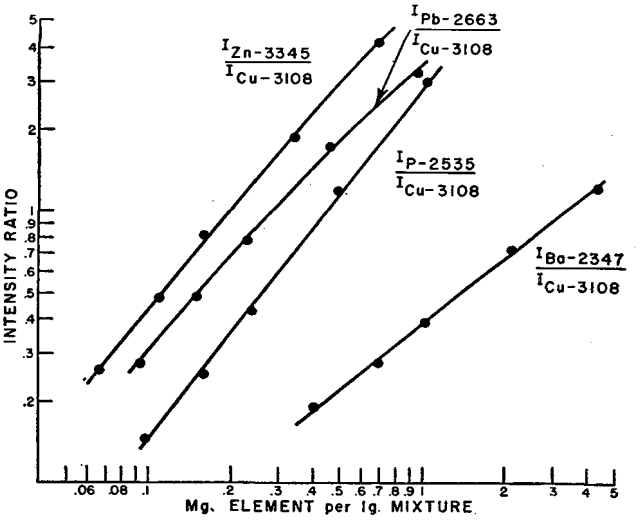

Figure 1. Typical Analytical Curves

Table III. Comparison of Spectrographic and Chemical Results

(Values given are percentages)

Sample Type	Method	Elements				
		Mg	Zn			
Used oil	Spec.	0.050	0.078			
	Chem.	0.048	0.072			
Used oil	Spec.	0.040	0.054			
	Chem.	0.035	0.056			
Used oil	Spec.	0.041	0.060			
	Chem.	0.043	0.059			
Concentrate	Spec.	0.40	0.88			
	Chem.	0.45	0.91			
		P	Ca	Ba	Sn	Pb
Unused oil	Spec.	0.056	...	0.11	0.063	...
	Chem.	0.053	...	0.11	0.065	...
Unused oil	Spec.	0.036	...	0.16	0.028	...
	Chem.	0.037	...	0.16	0.030	...
Used oil	Spec.	0.12	...	0.12	0.042	...
	Chem.	0.12	...	0.12	0.037	...
Used oil	Spec.	...	0.16	0.21	...	0.19
	Chem.	...	0.17	0.20	...	0.20
Used oil	Spec.	...	0.18	0.027	...	0.22
	Chem.	...	0.17	0.029	...	0.20
Used oil	Spec.	...	...	0.038	...	0.18
	Chem.	...	...	0.040	...	0.21
Unused oil	Spec.	0.043	0.051	0.060	...	...
	Chem.	0.044	0.051	0.066	...	...
Used oil	Spec.	0.063	0.066	0.13	...	...
	Chem.	0.059	0.065	0.15	...	...
		Na	Ca	Pb		
Grease	Spec.	1.2	0.16			
	Chem.	1.1	0.17			
Grease	Spec.	0.85	0.12			
	Chem.	0.70	0.12			
Grease	Spec.	...		0.39		
	Chem.	...		0.38		
Grease	Spec.	...		4.3		
	Chem.	...		4.7		
		P	Pb	Ba	Zn	Sn
Engine deposit	Spec.	2.7	25	4.5		
	Chem.	2.3	25	4.1		
Engine deposit	Spec.	1.8	25	2.4	0.82	
	Chem.	1.6	27	2.5	0.80	
Engine deposit	Spec.		54			
	Chem.		52			
Engine deposit	Spec.		51			
	Chem.		52			
Engine deposit	Spec.		57			7.8
	Chem.		52			8.0

Table IV. Repeatability of Spectrographic Method

(Analysis of blended oil standard)

Detn. No.	Ba			P			Pb			Zn		
	Blended, %	Found, %	Diff.	Blended, %	Found, %	Diff.	Blended, %	Found, %	Diff.	Blended, %	Found, %	Diff.
1	0.30	0.28	−0.02	0.042	0.044	+0.002	0.076	0.077	+0.001	0.036	0.040	+0.004
2		0.29	−0.01		0.046	+0.004		0.077	+0.001		0.036	0
3		0.29	−0.01		0.046	+0.004		0.072	−0.004		0.035	−0.001
4		0.27	−0.03		0.041	−0.001		0.075	−0.001		0.038	+0.002
5		0.28	−0.02		0.039	−0.003		0.075	−0.001		0.034	−0.002
	Av.	0.28			0.043			0.075			0.037	

For standards difficult to prepare by blending, such as additives or greases, one or two chemically analyzed samples are sufficient to prepare the powder standards as described.

Preparation of Graphite–Copper Oxide Mixture. As suggested by Jaycox (*7*), copper oxide is used as internal standard. A graphite–copper oxide mixture to serve as a matrix material is prepared as follows:

To 100-mesh graphite powder, enough 20% C.P. copper nitrate solution is added to yield 75 mg. of copper oxide per gram of final dry mixture. This is stirred well, dried first at 210° F., and then muffled at 1000° F. for 15 minutes to convert the copper nitrate to the oxide. This mixture after thorough mixing is ready for use. For convenience approximately 200 grams at a time are prepared and kept dry. The actual amount of copper oxide in this mixture is not critical, since the same graphite mixture is used in preparing both the standards and samples.

Analysis of Samples. One gram of the graphite–copper oxide mixture is weighed into a small porcelain crucible (00 tall-form Coors). It is packed slightly by tapping the crucible and a small indentation is made in the center of the mixture with the bottom of a small test tube. After the oil sample is thoroughly mixed and the crucible containing the graphite–copper oxide mixture weighed, a suitably sized sample (usually 0.1 to 0.6 gram) is transferred by means of an eye dropper to the graphite bed in the crucible and weighed to the nearest milligram. The crucible is then heated slowly and the oil is ignited and allowed to burn. The temperature is kept just high enough to burn off all the oil. The resulting dry mixture is then thoroughly mixed in the crucible, or it may be transferred to a dental amalgamator and mixed in a matter of seconds. Portions of this mixture are packed into the cratered electrodes by tamping the electrodes into the mixture and arced for 60 seconds in a direct current arc. A metallized quartz neutral filter having a transmittance value of 10% is placed in the radiation path and 50% transmittance filters are placed in the cassette immediately in front of the plate in the 2663.2 and 3345.0 A. regions. These latter two filters serve to reduce further the densities of the lead line and the zinc line and background. Usually three exposures of standards and samples are made.

The exposed plate is developed for 4 minutes at 70° F. in Eastman Kodak developer D-19, placed in an acetic acid stop bath for about 20 seconds, and fixed for 5 minutes in Kodak rapid liquid x-ray fixer.

The transmittance values of the lines listed in Table II are determined.

No corrections for background are made since it is extremely low. The per cent transmittance values are converted to relative intensities by means of an emulsion characteristic curve. A rotating 6-step sector (ratio 1 to 2) and a preliminary curve suggested by Churchill (*2*) are used to obtain this curve.

From the intensity ratio of the element to copper and the proper analytical curve, the milligrams of the element of interest are determined. This value multiplied by 100 and divided by the sample weight in milligrams gives the percentage of the element in the sample.

DISCUSSION

The advantage of this technique in the preparation of samples of lubricating oils is that the relatively high temperatures encountered when an oil is taken to an ash in a muffle are avoided. This minimizes the loss of the more volatile elements and precludes the fusion of any material to the crucible as may occur in the ashing of some samples. In addition, less handling and transferring of the sample are required than if the ash technique is used.

Although this method has been used primarily for the analysis of oils, the same general technique has been applied to the analysis of other materials—for example, additive concentrates, greases, engine deposits, and sludges. In each case, the amount of sample taken for analysis is governed by the concentration of the element sought. With additive concentrates, greases, and deposits, 2 to 20 mg. are usually sufficient. When analyzing deposits, the sample is ground to a fine powder first and weighed directly into the 1-gram mixture of graphite and copper oxide. This is then mixed thoroughly and portions are arced. When working with such small samples, extreme care should be exercised to ensure homogeneity.

In order to increase the sensitivity, 0.5-gram portions of the graphite–copper oxide mixture have been used in the preparation of samples and standards with satisfactory results.

ACCURACY AND PRECISION

Table III gives the data obtained on a variety of samples analyzed both by this method and chemical means and indicates the accuracy of the spectrographic procedure. From the 48 determinations, the average error is about ±10% of the element present, with one determination showing a difference of 21% from the chemical result, one determination differing from the chemical result by 17%, and three differing by 14%.

Table IV lists the results obtained on analyzing a blended oil standard five times with this spectrographic technique and is indicative of the repeatability of the method.

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LITERATURE CITED

(1) Calkins, L. E., and White, M. M., *Natl. Petroleum News*, **38**, No. 27, R519 (1946).
(2) Churchill, J. R., IND. ENG. CHEM., ANAL. ED., **16**, 653 (1944).
(3) Clark, R. O., *et al.*, ANAL. CHEM., **23**, 1348 (1951).
(4) Gambrill, C. M., Gassmann, A. G., and O'Neill, W. R., *Ibid.*, **23**, 1365 (1951).
(5) Gassmann, A. G., and O'Neill, W. R., *Ibid.*, **21**, 417 (1949).
(6) Hansen, J., Skiba, P., and Hodgkins, C. R., *Ibid.*, **23**, 1362 (1951).
(7) Jaycox, E. K., *Ibid.*, **22**, 1115 (1950).
(8) Pagliassotti, J. P., and Porsche, F. W., *Ibid.*, **23**, 198 (1951).

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Flame Photometric Determination of Strontium in Sea Water

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The purpose of the present investigation was to develop an accurate and relatively simple flame photometric procedure for the analysis of strontium in sea water and marine products. Factors which affect the intensity of the light emitted by strontium were determined. The band-width and radiation interferences of the major constituents of sea water were studied. Calcium ions were the only ones which showed band-width interference. The chloride, sulfate, and magnesium ions showed a negative radiation interference, whereas a positive interference was given by the sodium, calcium, and potassium ions. The "internal standards" technique was developed to eliminate the interferences caused by the fluctuation of the physical and chemical properties of the solution. Sea water samples collected from various oceans were analyzed for strontium. The average strontium-chlorinity ratio was found to be 0.0048 ± 0.0002 where the strontium is expressed as milligram-atoms.

THE presence of strontium in sea water was first reported by Sonstadt (*11*), who mistook the spectral lines of calcium and strontium for those of rubidium and cesium but later corrected the observational error (*12*). Dittmar (*3*) detected the element spectroscopically in sea water, marine organisms, and boiler scales. Makin (*4*) and Coppock (*1*) could not detect the element in sea water. The first quantitative data on strontium was given by Desgrez and Meunier (*2*) who reported 0.154 mg.-atom per liter for the waters of the English Channel. Thompson and Robinson (*13*) cite the data of Thomas and Thompson, who obtained a value of 0.145 mg.-atom per kg., calculated for 19% chlorinity for waters of San Juan Channel in the state of Washington. Later results by Ramage (*9*) showed 0.456 to 0.470, by Noll (*6*) 0.080 to 0.090, and by Vinogradov (*14–16*) 0.091 to 0.114 mg.-atom per liter. Miyake (*5*) using the nitrate extraction method found 0.164 mg.-atom per liter for the waters of the Japan Sea. Smales (*10*) using a combination of flame photometric and radioactive tracer technique, reported 0.103 to 0.126 mg.-atom per liter for five samples from the Atlantic Ocean. Odum (*7, 8*) separated strontium and calcium from other salts in the sea waters, previous to the arc and flame spectroscopic analysis, and obtained from the analyses of 160 samples of Atlantic Ocean water an average of 0.093 mg.-atom per liter strontium calculated for waters of 19.38‰ chlorinity. Odum's data thus showed a strontium-chlorinity ratio of 0.0048.

CHEMICALS AND EQUIPMENT

All chemicals used in the present investigation were of analytical grade and tested for traces of strontium. A stock strontium solution of 6.00 mg.-atom per liter was prepared by dissolving 0.886 gram of strontium carbonate in a limited volume of hydrochloric acid, and then diluting to 1 liter. From such stock solutions suitable aliquots were taken and diluted for strontium standards. Other standard solutions of sodium chloride, magnesium chloride, calcium chloride, potassium chloride, ammonium chloride, and ammonium sulfate were prepared. Polyethylene containers were used for the storage of standard solutions for short periods of time in order to avoid possible contamination from glassware. Tanks of hydrogen and oxygen were used as the fuel.

EXPERIMENTAL

The intensity of light emitted by strontium was measured with a Beckman DU spectrophotometer fitted with the multiplier phototube (No. 4300) and the modified flame attachments (No. 9220). With the use of the multiplier phototube the response of the spectrophotometer to a given light signal will be increased at least 100-fold as compared to the ordinary blue-sensitive phototube.

The strontium spectral line of 460.7 mμ was chosen as the wave length for the emission intensity measurement. Owing to the continuous emission of the solvent, the flame background must be subtracted from all strontium readings. The background intensity of the wave length of 454 mμ was equal to that of 460.7 mμ and was unaffected by changing strontium concentration. The difference in reading obtained at these two wave lengths was used as a measurement of the light intensity emitted by strontium. The sensitivity of the instrument was operated at the counterclockwise position, so that the maximum detectibility of the weak strontium line could be obtained. It also permitted the use of a 0.02-mm. slit width which reduced the flame background intensity and thus minimized the possible band-width interference of other constituents. A warm-up period of 15 minutes was employed before measurements were made in order to attain the maximum stability in operation. The atomizer-burner was thoroughly rinsed with distilled water between each sample change. The stability of the instrument was checked with standard strontium solutions at the beginning and the end of each series of measurements.

A linear relation between the emission intensity and the strontium concentration was obtained under the following operating conditions of the spectrophotometer:

Wave length	Strontium spectral line, 460.7 mμ Flame background, 454 mμ
Selector	0.1
Phototube	Multiplier phototube
Resistor	22 megohms
Slit width	0.02 mm.
Oxygen	15 lb. per sq. inch
Hydrogen	4 lb. per sq. inch

Table I. Band-Width Interference of Major Constituents in Sea Water

Interfering Ions		Emission Intensity at 460.7 mμ	Flame Background at 454 mμ	Net Emission Intensity
Constituents	Concn., mg.-atom/l.			
Chloride	833	3.0	3.0	0.0
Sodium	333	77.5	74.7	2.8
	167	39.0	37.8	1.2
	83	20.8	20.0	0.8
	42	11.8	11.2	0.6
Sulfate	100	2.5	2.5	0.0
Magnesium	110	2.5	2.5	0.0
Calcium	20	13.7	3.0	10.7
	16	11.7	3.0	8.7
	12	9.4	3.0	6.4
	8	7.4	3.0	4.4
	4	5.2	3.0	2.2
Potassium	16.7	10.9	10.9	0.0
	8.4	6.8	6.8	0.0
Strontium	0.18	98.1	2.5	95.6
	0.09	49.6	2.5	47.1

BAND-WIDTH INTERFERENCE

Studies were made to determine the extent of band-width interference that might be caused by the presence of the major constituents of sea water (chloride, sodium, sulfate, magnesium, calcium, and potassium). Individual standard solutions containing one of these ions were prepared and the emission intensity of each was measured at wave lengths of 460.7 and 454 mμ. The data obtained are shown in Table I.

As indicated in Table I, the chloride, sulfate, magnesium, and potassium ions did not emit net light intensity at the wave length

of 460.7 mμ. Light was emitted by sodium ions at this wave length, but the interference could be corrected by subtracting the flame background at 454 mμ. Calcium ions were the only ones to show band-width interference at the wave length of 460.7 mμ.

RADIATION INTERFERENCE

The effect of radiation interference on the strontium determination by the presence of the various ions in the sea water was studied. The emission intensities of the solutions containing known quantities of strontium together with other ions were measured and the amount of strontium recovered was calculated. The data are given in Table II. The magnesium ions greatly suppressed the strontium recovery, the deviation being proportional to the concentration of magnesium. The presence of sulfate ions gave low results and the effect was apparently independent of the concentration. A slight deviation was observed for the chloride ions. The sodium ions caused the strontium to emit more light, giving a high strontium recovery. The calcium ions produced positive interferences on the strontium analysis, the effect being directly proportional to the calcium concentration. A slight interference was given by potassium ions.

Table II. Radiation Interference of Major Constituents in Sea Water

Interfering Ions		Strontium, Mg.-Atom/L.	
Constituents	Concn., mg.-atom/l.	Added	Found
Chloride	0.0	0.060	0.060
	49.0	0.060	0.059
	98.0	0.060	0.058
	146.0	0.060	0.058
	192.0	0.060	0.058
	550.0[a]	0.060	0.055
Sodium	0.0	0.200	0.200
	52.0	0.200	0.222
	104.0	0.200	0.224
	208.0	0.200	0.230
	312.0	0.200	0.240
	416.0[a]	0.200	0.240
Sulfate	0.0	0.150	0.150
	37.5[a]	0.150	0.129
	75.0	0.150	0.129
	150.0	0.150	0.129
	225.0	0.150	0.129
	300.0	0.150	0.129
Magnesium	0.0	0.200	0.200
	9.2	0.200	0.184
	18.3	0.200	0.168
	36.7	0.200	0.150
	55.0[a]	0.200	0.134
	73.4	0.200	0.122
	91.7	0.200	0.118
	110.0	0.200	0.118
Calcium	0.0	0.120	0.120
	1.0	0.120	0.122
	2.0	0.120	0.124
	4.0	0.120	0.126
	6.0	0.120	0.128
	8.0	0.120	0.131
	10.0[a]	0.120	0.132
Potassium	0.0	0.120	0.120
	2.5	0.120	0.121
	5.0	0.120	0.122
	10.0[a]	0.120	0.123
	15.0	0.120	0.123
	20.0	0.120	0.123

[a] Denotes approximate concentrations in sea waters.

CALIBRATION GRAPHS FOR STRONTIUM DETERMINATION IN SEA WATER

A synthetic sea water, free of strontium, containing all of the major constituents in the proportions found in sea water and having a chlorinity of 38.00‰, was prepared by dissolving strontium-free chemicals in the following quantities in a liter of distilled water:

	Grams
NaCl	46.96
$MgCl_2.6H_2O$	21.30
$Na_2SO_4.10H_2O$	17.80
$CaCl_2$	2.20
KCl	1.46
$NaHCO_3$	0.38

Aliquot parts of this water were diluted with distilled water in order to secure solutions with different ranges in chlorinity. Known quantities of strontium were then added in different amounts and the net emission intensities determined at the wave length of 460.7 mμ. The data are given in Table III and plotted in Figure 1. Deviations in the slopes of the graphs demonstrate that the emission intensity is not only a function of the strontium concentration but also the concentration of other constituents of the solution. Thus, calibration graphs would have to be constructed for waters of varying chlorinities when making strontium determinations on samples of sea water. Such graphs would have to be frequently prepared during the analyses of a series of samples because of the fluctuations in the characteristics of the flame photometer.

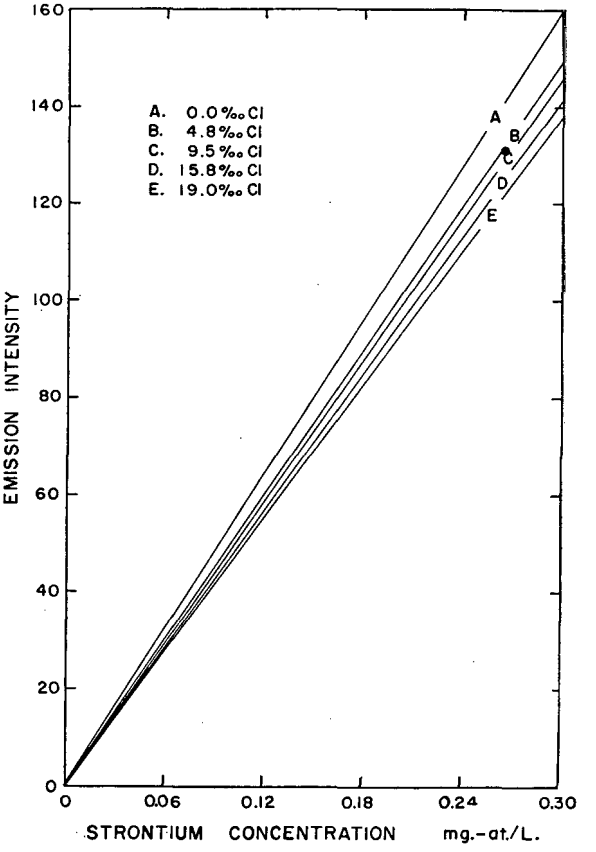

Figure 1. Calibration Graphs of Strontium in Synthetic Sea Water

In order to secure the most consistent results and to make the determination independent of the chemical and physical conditions of the solutions being analyzed, the use of "internal standards" was devised. The method was as follows: Equal volumes of the sample of sea water to be analyzed for strontium were added to a series of standard solutions containing different known quantities of strontium. The emission intensity of the resulting solutions was then determined at the wave length of 460.7 and 454 mμ. The resulting net intensity of the light was emitted by the strontium in the standard plus the strontium in the unknown. If there were any interfering substances existing in the solution, they would affect equally the emission of light resulting from the standard and the unknown. The resulting net intensities were plotted linearly against the concentration of the standard strontium solutions which had been added to the unknown. The line intersecting the ordinate indicates the emission intensity of the

Table III. Calibration Graph Data on Strontium

Strontium Concn., Mg.-Atom/L.	Chlorinity, ‰	Net Emission Intensity at 460.7 mμ
0.00	0.0	0.0
0.03	0.0	16.0
0.06	0.0	32.5
0.09	0.0	47.1
0.12	0.0	63.7
0.15	0.0	79.2
0.18	0.0	95.6
0.24	0.0	129
0.30	0.0	160
0.00	4.8	0.0
0.06	4.8	30.1
0.12	4.8	60.2
0.18	4.8	91.0
0.24	4.8	119
0.30	4.8	150
0.00	9.5	0.0
0.06	9.5	29.0
0.12	9.5	60.0
0.18	9.5	87.0
0.24	9.5	116
0.30	9.5	146
0.00	15.8	0.0
0.06	15.8	30.0
0.12	15.8	58.0
0.18	15.8	85.0
0.24	15.8	113
0.30	15.8	142
0.00	19.0	0.0
0.03	19.0	13.0
0.06	19.0	27.0
0.09	19.0	40.0
0.12	19.0	55.0
0.15	19.0	69.0
0.18	19.0	83.0
0.24	19.0	112
0.30	19.0	139

Table IV. Results of Strontium Analysis

Interfering Ions, Mg.-Atom/L.			Strontium, Mg.-Atom/L.	
Mg	SO_4	Na	Added	Found
0.0	0.0	0.0	0.090	0.089
0.0	50.0	500.0	0.090	0.088
91.8	91.8	500.0	0.090	0.087
0.0	0.0	0.0	0.120	0.118
91.8	91.8	0.0	0.120	0.120
0.0	50.0	0.0	0.120	0.122

strontium in the unknown. An example is illustrated in Figure 2. Since the graph in Figure 2 is a straight line, $Y = a + bx$, the unknown strontium concentration, x, is given by the ratio of Y to slope of the line, when $a = 0$.

The validity of the internal standards technique for elimination of interference from some of the major constituents of sea water was tested with solutions of known concentrations. The results are given in Table IV.

DETERMINATION OF STRONTIUM IN SEA WATER

The method of internal standards technique was applied to the analysis of samples of sea water collected at various depths at a station in each of three oceans, the Arctic, Pacific, and Atlantic. Table V shows the locations of the stations, depths at which samples were obtained, temperature of the waters *in situ*, and the chlorinity values determined shortly after collection of samples. Polyethylene bottles were used to preserve the samples on which strontium determinations were to be made. Considerable time elapsed before the analyses for strontium were made. During this period slight evaporation occurred as is evidenced by the two chlorinity values, one designated as 1952 being made shortly after collection, and the other, 1954, determined when the strontium values were obtained. The evaporation does not affect the validity of the ionic ratio, milligram-atom strontium per kilogram of water to parts per mille chlorinity, but the strontium concentrations determined may be from 0.001 to 0.002 mg.-atom too high. Therefore, the actual strontium content of the waters *in situ* for the Arctic and the Pacific were calculated for the several

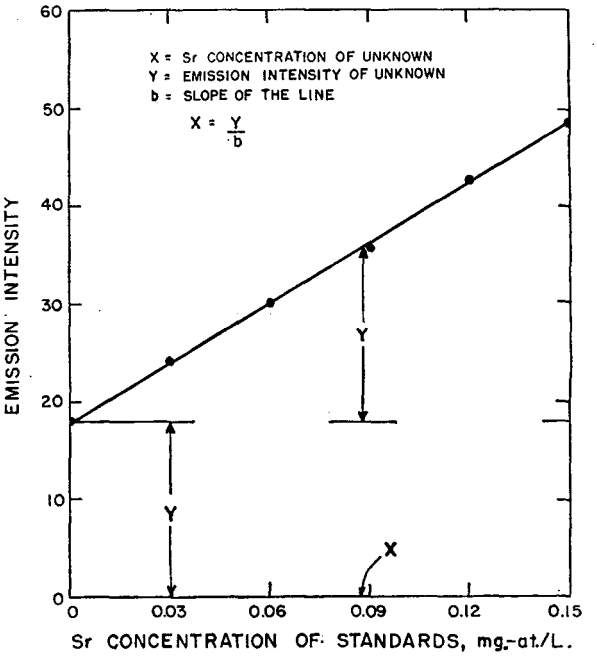

Figure 2. Determination of Strontium by Internal Standards Method

Table V. Strontium Content of Sea Water from Various Depths in Several Oceans

Location	Depth, Meter	Temp., ° C.	‰ Cl (1952)	Calcd. Sr, Mg.-Atom/Kg.	‰ Cl (1954)	Sr, Mg.-Atom/Kg.	Sr / ‰ Cl
Beaufort Sea, Arctic Ocean	0	−1.36	14.52	0.070	14.85	0.071	0.0047_8
Lat. 72° 14.4′ N	10	−1.42	15.25	0.076	15.62	0.078	0.0049_9
Long. 155° 00′ W	20	−1.02	16.44	0.083	16.79	0.085	0.0050_6
Sept. 14, 1952	30	−1.18	17.12	0.081	17.37	0.082	0.0047_2
	50	−0.82	17.55	0.084	17.92	0.086	0.0048_0
	75	−0.20	17.88	0.084	18.31	0.086	0.0047_0
	200	−0.94	18.84	0.090	19.35	0.092	0.0047_5
	500	+0.58	19.30	0.097	19.50	0.098	0.0050_2
	1000	−0.02	19.33	0.095	19.61	0.096	0.0048_9
	2000	−0.42	19.35	0.090	19.75	0.092	0.0046_6
						Av.	0.0048_4
Pacific Ocean	0	10.14	18.03	0.088	18.13	0.088	0.0048_5
Lat. 49° 30′ N	5	10.12	18.06	0.089	18.15	0.089	0.0049_0
Long. 138° 14.5′ W	50	6.41	18.06	0.084	18.27	0.085	0.0046_6
July 7, 1952	200	5.27	18.73	0.091	18.93	0.092	0.0048_6
	300	4.33	18.73	0.089	19.16	0.091	0.0047_5
	750	3.74	19.00	0.091	19.35	0.093	0.0048_1
	1000	2.90	19.01	0.086	19.35	0.088	0.0045_4
	2000	2.03	19.15	0.094	19.49	0.096	0.0049_2
						Av.	0.0047_9
Atlantic Ocean	0	23.61	...	...	19.53	0.093	0.0047_6
Lat. 38° 57′ N	9	...	...	...	19.68	0.095	0.0048_2
Long. 71° 01.5′ W	18	23.51	...	...	19.57	0.095	0.0048_5
Sept. 24, 1952	27	23.49	...	...	19.56	0.093	0.0047_6
	91	14.28	...	...	19.95	0.099	0.0049_6
	183	9.92	...	...	19.53	0.095	0.0048_7
	462	5.54	...	...	19.41	0.093	0.0048_9
	838	4.17	...	...	19.29	0.095	0.0049_2
	1028	3.96	...	...	19.37	0.095	0.0049_0
	1413	3.65	...	...	19.37	0.095	0.0049_0
						Av.	0.0048_6

samples by multiplying the chlorinity obtained at the approximate time of sampling by the ionic ratio.

The data show that strontium exists in the oceans in constant proportions to the chlorinity expressible by the ratio 0.0048 ± 0.0002. The lowest ratio was obtained on the sample taken at 1000 meters in the Pacific Ocean. This depth is in the zone of minimum oxygen and slight anomalies for other constituents have been observed.

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LITERATURE CITED

(1) Coppock, J. B., *Chem. News*, **96**, 212 (1907).
(2) Desgrez, M. A., and Meunier, Jean, *Compt. rend.*, **183**, 689 (1926).
(3) Dittmar, W., *Challenger Reports, Phys. and Chem.*, **1**, 9 (1884).
(4) Makin, J. S., *Chem. News*, **77**, 155 (1898).
(5) Miyake, Yasuo, *Bull. Chem. Soc. Japan*, **14**, 55 (1939).
(6) Noll, W., *Chem. Erde*, **8**, 507 (1934).
(7) Odum, H. T., *Science*, **114**, 211 (1951).
(8) *Ibid.*, p. 407.
(9) Ramage, Hugh, *South Eastern Naturalist and Antiquary*, **38**, 54 (1933).
(10) Smales, A. A., *Analyst*, **76**, 348 (1951).
(11) Sonstadt, E., *Chem. News*, **22**, 25 (1870).
(12) *Ibid.*, p. 44.
(13) Thompson, T. G., and Robinson, R. J., *Bull. Natl. Research Council (U. S.)*, No. **85**, 174 (1932).
(14) Vinogradov, A. P., *Trav. lab. biogéochim. acad. sci. U.S.S.R.*, **3**, 63 (1935).
(15) Vinogradov, A. P., *Uspekhi Khim.*, **7**, 639 (1938).
(16) *Ibid.*, **13**, 3 (1944).

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Spectrophotometric Determination of 1-Naphthol in 2-Naphthol Utilizing Differences in Reaction Rates

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A spectrophotometric method for the determination of 1-naphthol has been used in the range of 0.00 to 0.50% 1-naphthol in 2-naphthol. It depends on the faster rate of reaction of 1-naphthol than of 2-naphthol with a diazonium salt. In the procedure a separation of the two is made by precipitating the 2-naphthol from alkaline solution by addition of acid. The filtrate, which contains the 1-naphthol and a small amount of 2-naphthol, is treated with diazotized 2-naphthylamine-5,7-disulfonic acid at a controlled temperature. The absorbancy of the reddish color formed is measured at 485 mμ after an exact time of reaction. With the conditions of time, temperature, and acidity chosen, only a slight amount of 2-naphthol reacted. The standard deviation was ±0.004% of 1-naphthol for samples containing 0.07 to 0.35% 1-naphthol.

A REVIEW of the literature indicated a number of tests proposed for the detection or estimation of 1-naphthol in the presence of 2-naphthol, but none appeared entirely satisfactory for the quantitative determination of quantities of the order of 0.1%. Callan (*1*) has compared the sensitivities of a number of tests. He found of most interest as a quantitative method in the range of 0.1 to 0.5% 1-naphthol, a method proposed by Liebmann (*4*), later modified by Prochazka (*5*) and finally by Callan (*1*). These methods are based on the fact that 1- and 2-naphthols couple with diazotized *p*-nitroaniline to form azo compounds. These can be separated by the solubility of the alpha compound in sodium hydroxide. The beta compound is insoluble. However, these methods have certain disadvantages in that they involve a tedious extraction or evaporation and the colored product has limited stability in sodium hydroxide solution.

This work was begun by studying a series of diazotized substituted aromatic amines to find a compound which would preferentially couple with 1- and not with 2-naphthol in a high acidity medium. The following compounds were studied: *o*-nitroaniline, 2,6-dimethylaniline, 2-chloro-4-nitroaniline, 2,4-dinitroaniline, *m*-nitroaniline, 4-methoxy-2-nitroaniline, 1-sulfo-4-nitroaniline, 2-methoxy-4-nitroaniline, 3-nitro-4-methoxyaniline, 2-naphthylamine-5,7-disulfonic acid, 1-naphthylamine-2-sulfonic acid, 2-naphthylamine-1-methyl sulfonic ester, and 2-naphthylamine-1-azobenzene. Of these none appeared specific for 1-naphthol; however, diazotized 2-napthylamine-5,7-disulfonic acid was found to be the most promising coupling agent for further study, as it reacted faster with 1- than with 2-naphthol in acid medium. Furthermore, the azo compounds were completely water soluble, which was advantageous for colorimetric work. Although 1-naphthol couples faster than 2-naphthol, it was found necessary to reduce the amount of 2-naphthol present for a feasible colorimetric method. This was done by dissolving the sample in sodium hydroxide and then precipitating most of the 2-naphthol with acid, thus leaving some of the beta and all of the alpha compound in the filtrate. Strongly acid conditions were chosen for carrying out the coupling reaction so as to decrease the rate of coupling of 2-naphthol. Since only a small amount of the 1-naphthol present in the solution actually reacts with the diazonium salt, the reaction is rate controlled. Hence, the color produced is a function of time and temperature as well as of concentration of 1-naphthol. By controlling the acidity, temperature, and time of the diazo reaction, a reproducible method for 1-naphthol was obtained.

Examples of the use of differences in reaction rate for providing new analytical procedures were reported recently by Lee and Kolthoff (*3*).

APPARATUS AND REAGENTS

The Coleman Model 11 Universal spectrophotometer, the Beckman Model B, or other direct-reading instruments are recommended, as the measurements must be made while watching a stop watch. The Coleman Model 11 was used except where otherwise noted.

Diazotized 2-Naphthylamine-5,7-disulfonic Acid. Technical grade monosodium salt of 2-naphthylamine-5,7-disulfonic acid is recrystallized from water several times with the addition of decolorizing carbon until the product is no more than faintly yellow. The reagent, dried in a steam oven, is then titrated

with 0.1N sodium nitrite to an external starch-iodide end point so as to establish the amount of nitrite necessary for preparing the reagent solution. It is undesirable to have an excess of nitrite present in the reagent solution. An amount of reagent equivalent to 0.325 gram of the monosodium salt of 2-naphthylamine-5,7-disulfonic acid is dissolved in 25 ml. of 1 to 1 hydrochloric acid. The solution is cooled to at least 10° C. and placed in an ice bath; 10 ml. of 0.1N sodium nitrite are added slowly with stirring. The solution is then diluted to 50 ml. with ice water in a volumetric flask and kept in an ice bath. This reagent should be made up fresh daily.

2-Naphthol. 2-Naphthol was purified by precipitating technical material from sodium hydroxide with acid several times and then precipitating from benzene by the addition of heptane. The purified 2-naphthol used in this work had a freezing point of 121.40° C., with an estimated cryoscopic purity of 99.9 mole %.

1-Naphthol. 1-Naphthol was purified by recrystallizing technical 1-naphthol first from benzene and then from carbon tetrachloride to a constant melting point. The material used in this work had a freezing point of 95.2° C., with an estimated cryoscopic purity of 99.7 mole %.

PROCEDURE

Weigh 1.00 gram of the 2-naphthol sample into a 125-ml. Erlenmeyer flask and dissolve with 10 ml. (pipet) of 1N sodium hydroxide. Warm gently if necessary to dissolve, but do not approach the boiling point. Then add 50 ml. of water and heat to 70° to 80° C. Add slowly from a pipet 9.0 ml. of 1 to 1 hydrochloric acid with constant swirling of the flask. Then cool the mixture in an ice bath to 2° C. Allow to stand for 10 minutes, remove the flask from the ice bath, and filter at once into a 250-ml. suction flask on a 6.5-cm. Büchner funnel through a 5.5-cm. No. 201 Reeve-Angel filter paper. Wash out the Erlenmeyer flask with 5 ml. of ice water, cool the washings in the Erlenmeyer flask in an ice bath, and when the filter cake is practically free of liquid, add the cold washings to the cake and suck through.

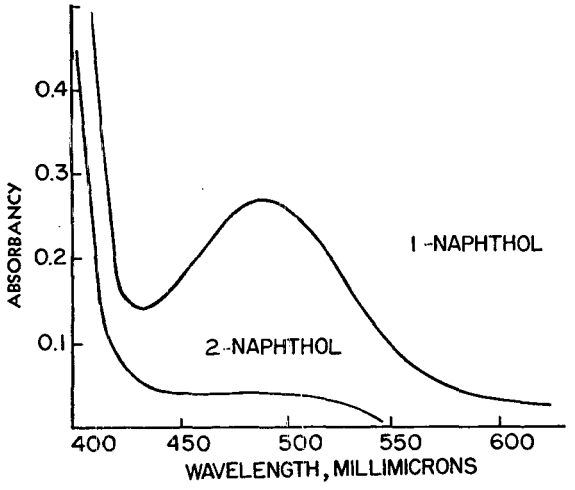

Figure 1. Spectrophotometric Curves

Dilute the contents of the filter flask to 100 ml. and transfer to a clean 125-ml. Erlenmeyer flask. Place the stoppered flask in a bath maintained at 23° to 24° C. and when the solution in the flask comes to the bath temperature, add 4 ml. of diazotized 2-naphthylamine-5,7-disulfonic acid from a 5-ml. Mohr pipet, starting a stop watch as soon as the flow of diazo begins. Shake the flask occasionally in the bath but do not expose the flask to direct sunlight or other strong light. Remove the flask from the bath after 13 minutes and transfer a portion to a 2 × 4 cm. cell. Place the cell in a Coleman Model 11 Universal spectrophotometer containing a PC-4 light filter so that the light path is 2 cm. Using distilled water as the reference liquid, at exactly 15 ± 0.1 minutes from the time the diazo reagent begins to flow from the pipet read the per cent transmittancy on the galvanometer deflection scale with the wave-length scale adjusted to 485 mμ. The per cent 1-naphthol is read from a calibration curve.

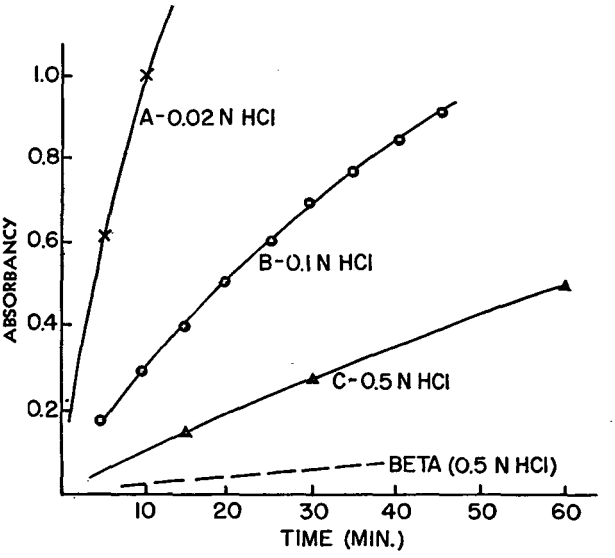

Figure 2. Rate of Color Development

Preparation of Calibration Curve. A solution is prepared containing 1 mg. of purified 1-naphthol per milliliter by dissolving 1.00 gram of 1-naphthol in 25 ml. of 3A alcohol, pouring into a large volume of water, and finally diluting to 1 liter. Then 1-, 2-, 3-, and 4-ml. aliquots of the 1-naphthol solution are added, respectively, to 1-gram samples of 2-naphthol and the samples are carried through the regular procedure. The per cent transmittancy is then plotted against per cent 1-naphthol on semilogarithmic graph paper. This serves as the working curve. A new calibration curve should be prepared whenever a new 2-naphthylamine-5,7-disulfonic acid is used.

DISCUSSION

Color Reaction. The upper spectrophotometric curve shown in Figure 1 represents the reaction product of 1-naphthol and diazotized 2-naphthylamine-5,7-disulfonic acid after a 15-minute reaction in 0.5N hydrochloric acid. The lower curve represents the reaction product of 2-naphthol—that is, the small amount in solution not removed by precipitating with acid—and the diazo reagent along with any color caused by the reagent solution. This really amounts to a blank. These curves were obtained by means of the General Electric recording spectrophotometer using a high scanning speed. The spectrophotometric readings for the method were taken at the absorption maximum of 485 mμ. Measurements made at 485 mμ on various concentrations of 1-naphthol which had reacted with the reagent for 15 and 30 minutes conformed to Beer's law. 1-Naphthol separated from plant 2-naphthol by the recommended procedure gave a curve similar to that of Figure 1 with an absorption maximum of 485 mμ.

Acid Concentration. The marked effect of acidity on the rate of reaction of 1-naphthol with the reagent is shown by the curves in Figure 2. These curves are for solutions containing 0.02, 0.1, and 0.5N hydrochloric acid and 1 mg. of 1-naphthol present per 50 ml. Curve A shows that time has a greater effect on the absorbancy with 0.02N hydrochloric acid than at the higher acidity values. The effect of 2-naphthol left in solution because of solubility in 25 ml. of 1N hydrochloric acid (diluted to 50 ml. before adding the diazo reagent) is shown by the dashed line in Figure 2. The reaction of the beta compound is about the same in 0.1N as in 0.5N hydrochloric acid but is much more appreciable in 0.02N hydrochloric acid. Figure 3 illustrates the effect of acidity on absorbancy for a given time period. Absorbancy is plotted versus hydrochloric acid concentration for solutions containing 1 mg. of 1-naphthol per 50 ml. of 0.02, 0.1, and 0.5N hydrochloric acid which were reacted with 2 ml. of reagent

for 15 minutes. The absorbancy attributable to the 2-naphthol reaction is shown by the dashed line in Figure 3. A 0.5N hydrochloric acid concentration was chosen for the method, since the effect of small changes in acidity on the absorbancy would be less critical and it would be easier to adjust the solution to this hydrochloric acid concentration. Furthermore, 2-naphthol interference and the effect of time should be less at the higher acidity.

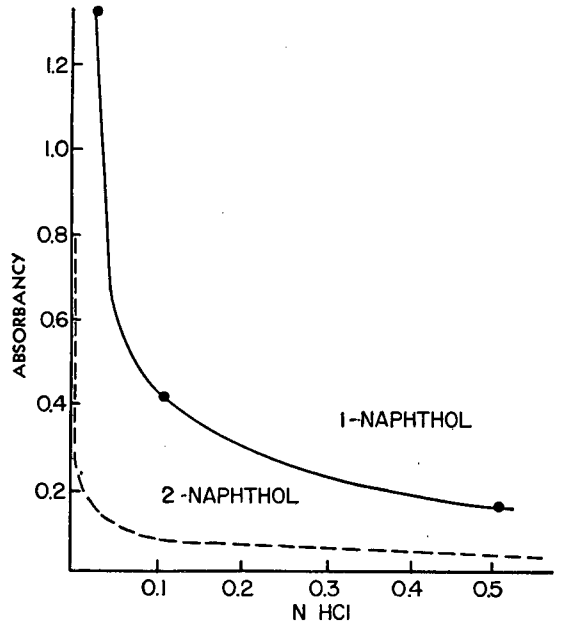

Figure 3. Effect of Acidity

Time. Time periods of color development of 15 and 30 minutes were used, as the absorbancy readings at these time periods at 485 mμ for various concentrations of 1-naphthol would be within the range of absorbancies corresponding to the alpha content expected in the 2-naphthol samples of interest. Moreover, a 15-second error in time for a 15-minute period could be made without significance. Although a somewhat higher sensitivity could be obtained at 30 minutes of reaction time, the 15-minute period was chosen for the control method because of the saving in time.

Table I. Effect of Temperature

Temp., ° C.	Transmittancy, %, at 485 Mμ
14–15	62.5, 63.0
23–24	52.0, 52.5
30–31	41.0, 41.5

Table II. Synthetic Samples

1-Naphthol, Mg.	Transmittancy, %, at 485 Mμ	Mg Found (Corr. for 2-)	Error, Mg.	1-Naphthol Recovered, %
0.00	92	...	...	..
1.00	81	0.80	−0.20	80
3.00	62	2.64	−0.36	88
4.00	53.5	3.68	−0.32	92
10.00	26	9.0	−1.0	90

Temperature. The effect of temperature on the rate of color development is shown by the data in Table I. Values are given for the reaction of 2 mg. of 1-naphthol in 50 ml. of 0.5N hydrochloric acid for 15 minutes with 2 ml. of 0.02M reagent at the temperatures indicated in column 1.

Interpolations of these data indicate that the temperature must be controlled to ±0.5° C. in order for the temperature error to be less than 0.1 mg. of 1-naphthol (equivalent to 0.01% for the described procedure).

Reagent. The reagent concentration was kept constant at $7.7 \times 10^{-4}M$. This represents a 2.2-fold excess of reagent over the stoichiometric requirement for 5 mg. of 1-naphthol. It is desirable to keep the excess of reagent at this value to prevent 2-naphthol interference. Actually, only a small fraction of the total 1-naphthol present reacts with the reagent in the 15- or 30-minute period.

Separation of 1- from 2-Naphthol. Although 1-naphthol couples faster than 2-naphthol in the acid solution, it was necessary to reduce the concentration of beta to obtain the desired sensitivity for alpha. This was done by dissolving a 1-gram sample of 2-naphthol in sodium hydroxide and precipitating most of it by adding acid as described in the procedure. Results on synthetic samples prepared from pure 2- and 1-naphthols are presented in Table II. A 1.00-gram sample of pure 2-naphthol was taken. The alpha values in column 3 were corrected for the absorbance at 485 mμ due to 2-naphthol (or blank) before reading the milligrams found from a pure 1-naphthol calibration curve.

Data in column 5 indicate an average recovery of 88%. On dissolving and reprecipitating the 2-naphthol which was filtered off from the synthetic sample containing 3 mg. of 1-naphthol according to the procedure, the recovery is raised to nearly 100%. It was demonstrated by successive reprecipitations that a plant sample containing 0.3% alpha gave essentially complete separation after two precipitations. It was decided to prepare the calibration curve from synthetic samples using one precipitation so that a correction for the unrecovered 1-naphthol and for absorption due to beta would be unnecessary. A plot of absorbancy against percentage 1-naphthol is linear up to 0.5% 1-naphthol. The plot does not intersect the origin, since the 2-naphthol correction is not made.

Effect of Heat. Heating (70° to 80° C.) in sodium hydroxide before precipitation with acid indicated no decomposition of 1-naphthol. However, weak 1-naphthol solutions which have been standing in caustic solution for several days develop a coloration. Any prolonged heating at 70° to 80° C. should be avoided.

Table III. Effect of Light

Conditions	1-Naphthol, %
Lab. light	0.35, 0.35
Dark	0.32
Daylight lamp	0.32
Sunlight	0.31, 0.30

Table IV. Reaction Time

Sample	1-Naphthol, %	
	15-min. period	30-min. period
1	0.21, 0.21	0.21
2	0.30, 0.30	0.29, 0.29
3	0.35, 0.34	0.35, 0.34

Light Stability. Light stability studies using the conditions indicated in Table III and with the analysis run on a plant sample by the recommended procedure showed that sunlight and the other variations from normal laboratory lighting give slightly lower results, but these differences are not significantly large. Cis-trans isomerism is possible with azo compounds and light is known (*2*) to affect the inner conversion of the cis-trans isomeric forms of the azo compounds which may also have different absorption spectra.

Effect of Impurities. 2-Thionaphthol and β,β'-dinaphthyl disulfide if present in technical 2-naphthol up to 1% will not cause interference. A further indication that only 1-naphthol

Table V. Analysis of 2-Naphthol Samples for 1-Naphthol

Sample	1-Naphthol, %
1	0.212, 0.213
2	0.297, 0.300
3	0.352, 0.345
4	0.310, 0.310
After purification of sample 4	<0.01
5	0.307, 0.308
6	0.333, 0.334
7	0.070, 0.069
8	0.098, 0.092
9	0.217, 0.228
10	0.257, 0.261
11	0.347, 0.348
12	0.207, 0.218
13	0.165, 0.155
14	0.261, 0.262

is coupling is shown by the good agreement for plant samples where the measurement was made at two different times as shown in Table IV.

It is unlikely that other substances which might be present would couple at the same rate as 1-naphthol. Because the 1-naphthol reaction with the diazo depends upon the rate of reaction, any catalytic effect would be serious. However, no anomalous effects have been encountered during the past 3 years that the method has been in daily use.

PRECISION AND ACCURACY

The results presented in Table V represent a variety of different samples of 2-naphthol from this laboratory as well as a number of other sources. The standard deviation calculated for 14 samples run in duplicate was ±0.004% 1-naphthol (absolute). Some of the duplicate determinations were made on different days. There is no evidence to indicate the occurrence of systematic errors.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Callan, T., *J. Soc. Chem. Ind.* (*London*), **44**, 125T (1925).
(2) Hartley, G. S., *J. Chem. Soc.*, **1938**, 633–42.
(3) Lee, T. S., and Kolthoff, I. M., *Ann. N. Y. Acad. Sci.*, **53**, 1093–107 (1951).
(4) Liebmann, A., *J. Soc. Chem. Ind.* (*London*), **16**, 294 (1897).
(5) Prochazka, J., *Ind. Eng. Chem.*, **15**, 944 (1923).

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Spectrophotometric Determination of Bismuth with Sodium Diethyldithiocarbamate

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A simple sensitive procedure has been developed for the spectrophotometric determination of bismuth in the presence of other metals by using a mixture of ethylenediaminetetraacetic acid, cyanide, and ammonium hydroxide. Such a mixture prevents the metals other than bismuth from forming colored complexes with sodium diethyldithiocarbamate. The maximum absorption of the bismuth diethyldithiocarbamate complex in carbon tetrachloride is at 370 mμ. This wave length is the most sensitive, but large amounts of mercury and lead interfere. However, the wave length of 400 mμ, which is less sensitive than 370 mμ, is specific for the bismuth complex. The proposed method should prove useful in determining bismuth in alloys.

SEVERAL methods for the determination of bismuth have been reported (*2, 3, 5, 6*). However, none is satisfactory without separation of the interfering substances. Tompsett (*8*) and LaCoste *et al.* (*3*) have reported that bismuth diethyldithiocarbamate can be extracted into ethyl ether or chloroform and that many elements interfere. Recently, Šedivec and Vašák (*7*), Přibil (*4*), and Cheng and Bray (*1*) reported that when sodium diethyldithiocarbamate and ethylenediaminetetraacetic acid were used for the colorimetric determination of copper, only bismuth interfered and the copper diethyldithiocarbamate complex was destroyed by the addition of cyanide while the bismuth diethyldithiocarbamate was not. No practical application of sodium diethyldithiocarbamate for the quantitative determination of bismuth in the presence of other metals was found in the literature.

This investigation was initiated to develop a sensitive and specific procedure for the quantitative determination of bismuth in the presence of other metals without separation. This method requires the extraction of stable bismuth diethyldithiocarbamate complex with carbon tetrachloride by adding a mixture of ethylenediaminetetraacetic acid, cyanide, and ammonium hydroxide to complex the interfering metals. The maximum absorption of the bismuth diethyldithiocarbamate complex in carbon tetrachloride is at 370 mμ. This wave length is the most sensitive to the bismuth complex, but large amounts of mercury and lead interfere. However, a wave length of 400 mμ, which is less sensitive than 370 mμ, is specific for the bismuth complex. Since this method is sensitive to 1 p.p.m. of bismuth in carbon tetrachloride, it may be useful for the determination of bismuth in alloys and biological material.

REAGENTS

Standard Bismuth Solution, 20.00 mg. of bismuth per liter in 1 to 100 nitric acid. Prepare by dilution of a 1.0000 gram per liter bismuth solution obtained by dissolving 1.0000 gram of pure bismuth metal in 10 ml. of nitric acid and diluting to 1 liter with water.

Sodium Diethyldithiocarbamate Solution, 0.2% in water, stored in a brown bottle (obtained from Eastman Kodak Co.).

Complexing Mixture. Fifty grams of disodium dihydrogenethylenediaminetetraacetic acid (Versenate) and 50 grams of sodium cyanide are dissolved in 1 liter of 1.5*M* ammonium hydroxide (1 part of water to 10 parts of concentrated ammonium hydroxide, by volume).

Carbon Tetrachloride, reagent grade.

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PROCEDURE

Preparation of Standard Curve. Transfer amounts of the standard bismuth solution containing 0.00, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mg. of bismuth, respectively, to 125-ml. separatory funnels. Add 10 ml. of the complexing mixture, 1 ml. of sodium diethyldithiocarbamate solution, and then exactly 10 ml. of carbon tetrachloride. Stopper and shake for 30 seconds. Filter the separated carbon tetrachloride through Whatman No. 1 filter paper and measure the absorbance within 30 minutes with a spectrophotometer at 370 or 400 mμ.

Application to Samples. If bismuth is not in soluble form, the sample is digested with nitric acid or a mixture of nitric acid and hydrochloric acid (*5*, *6*). After digestion, the solution is treated in the manner as for preparation of standard curve. The amounts of ethylenediaminetetraacetic acid, cyanide, and ammonium hydroxide added depend upon the amounts of interfering metals present in the sample. Because excess amounts of complexing agents do not affect the bismuth determination, an excess of 10 to 20 ml. of the complexing mixture may be present.

RESULTS

The data for a calibration curve according to the above procedure are given in Figure 1. The absorbance measurements were made at 370, 400, 420, and 440 mμ, respectively, and obeyed Beer's law.

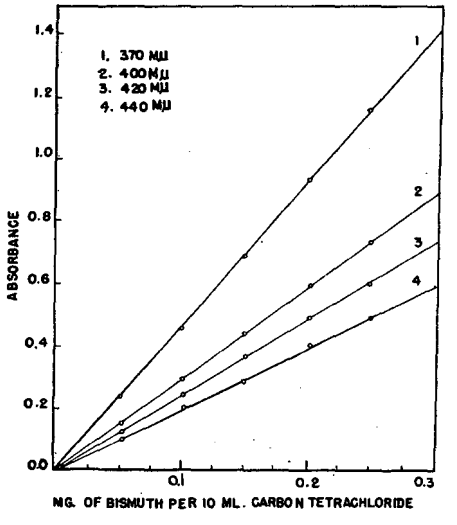

Figure 1. Beer's Law Curves Measured Different Wave Lengths

The absorption curves for bismuth, mercury, lead, and silver, determined by the proposed procedure, are shown in Figure 2. The results given in Table I are an indication of specificity and reproducibility of the method. The value for the lead-base bearing metal is in good agreement with that certified by the National Bureau of Standards.

DISCUSSION

Specificity. Previous publications (*1*, *4*, *7*) have indicated that in the presence of ethylenediaminetetraacetic acid, cyanide, and ammonium hydroxide, only bismuth forms a yellowish coloration with sodium diethyldithiocarbamate in certain organic solvents. This is a specific qualitative test for bismuth. LaCoste *et al.* (*3*) suggested measuring the intensity of the bismuth complex at 370 mμ, the maximum absorption wave length of the complex, but pointed out that the interfering metals must be removed. The curves in Figure 2 illustrate that the measurement at 370 mμ for bismuth shows interference by mercury and lead, and that the measurement at 400 mμ is specific for bismuth but less sensitive than at 370 mμ. If excess amounts of the complexing mixture were added to the solution containing metals other than bismuth, no yellowish coloration was found in the carbon tetrachloride extract. However, mercury and lead were not prevented from forming colorless complexes with sodium diethyldithiocarbamate by adding ethylenediaminetetraacetic acid, cyanide, and ammonium hydroxide. The colorless complexes were extracted into the organic solvents. The curves in Figure 2 indicated their absorption at wave lengths below 400 mμ. Therefore, the selection of a wave length for measuring the absorbance of the bismuth complex depends upon the amounts of mercury or lead present (Table I). The addition of tartaric acid prevents the precipitation of hydroxides of antimony and beryllium.

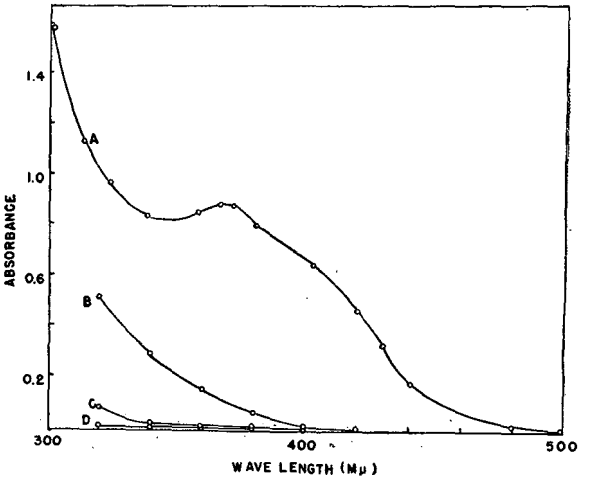

Figure 2. Absorption Spectra of Metal Diethyldithiocarbamates

A. Bismuth(III), 0.20 mg.
B. Mercury(II), 0.50 mg.
C. Lead(II), 1.00 mg.
D. Silver(I), 1.00 mg.

pH. The solution being analyzed should be adjusted to pH 7 to 10 in order to complex effectively the interfering metals, such as iron, nickel, copper, manganese, cobalt, and zinc.

Organic Solvents. Carbon tetrachloride, chloroform, ethyl acetate, and isoamyl alcohol were employed. These solvents were found to be good for the bismuth diethyldithiocarbamate complex; 10 ml. of carbon tetrachloride were sufficient to extract the diethyldithiocarbamate complex containing up to 0.3 mg. of bismuth.

Table I. Determination of Bismuth in Presence of Foreign Metals

(Bi, 0.115 mg. in 10 ml. of CCl_4)

Foreign Metal Added	Amount, Mg.	Absorbance 370 mμ	Absorbance 400 mμ
None	0.0	0.537	0.344
Cadmium	1.0	0.538	0.345
Mercury	1.0	0.652	0.342
Copper	5.0	0.545	0.345
Silver	1.0	0.538	0.346
Lead	1.0	0.538	0.344
Lead	100.0	1.730	0.348

Amount of Reagent. One milliliter of 0.2% sodium diethyldithiocarbamate solution was sufficient for reacting with less than 0.5 mg. of bismuth. When more sodium diethyldithiocarbamate was added to the solution containing large amounts of mercury or lead, a heavy precipitate was formed. More diethyldithiocarbamate was not necessary, even though large amounts of other metals were present. This is due to the fact

that the bismuth diethyldithiocarbamate complex is more stable than other metal diethyldithiocarbamate complexes.

Anions. No interference was found from nitrate, sulfate, chloride, acetate, perchlorate, phosphate, tartrate, and citrate for the amounts of bismuth determined.

Stability of Color. The yellowish coloration of the bismuth diethyldithiocarbamate complex in organic solvents—such as chloroform, carbon tetrachloride, and ethyl acetate—was found not to be very stable, especially with respect to light. The intensity of color gradually decreased with time of standing (Figure 3). When the carbon tetrachloride extract was allowed to stand at room temperature for more than 1 hour, it became turbid. Therefore, it is recommended that the absorbance be measured as quickly as possible and that the unknown sample be determined at the same time as the standard bismuth solutions.

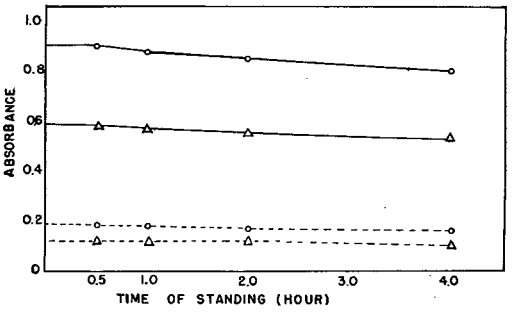

Figure. 3. Stability of Bismuth Complex

——— Bismuth, 0.20 mg. per 10 ml. of CCl_4
— — — — Bismuth, 0.04 mg. per 10 ml. of CCl_4
○ Wave length, 370 mμ
△ Wave length, 400 mμ

DETERMINATION OF BISMUTH IN LEAD-BASE ALLOY

Lead is one of the metals which commonly interfere with the determination of bismuth by other methods (*2*, *6*). In order to test the reliability of the proposed method, the lead-base alloy was selected.

Alloy. The lead-base bearing metal sample (NBS 53c, containing 10.20% antimony, 5.16% tin, 0.214% copper, 0.044% arsenic, 0.0023% nickel, 0.0017% iron, and 0.093% bismuth) was treated in the following manner:

A 1.0000-gram sample was dissolved in 20 ml. of 20% nitric acid by warming on the steam bath. After cooling, 3 grams of ethylenediaminetetraacetic acid and 10 grams of tartaric acid were added, followed by concentrated ammonium hydroxide (about 10 ml.) to adjust the solution to pH 7 to 8. The solution was then transferred to a 100-ml. volumetric flask and diluted to volume with water. The solution was sometimes cloudy. After being mixed thoroughly, an aliquot of 10 to 25 ml. of the solution was pipetted into a separatory funnel, followed by 10 ml. of water, 2 ml. of 5% sodium cyanide, 1 ml. of 0.2% sodium diethyldithiocarbamate, and 10 ml. of carbon tetrachloride. The mixture was shaken for 30 to 60 seconds, and the organic layer was filtered through a filter paper. The absorbance of the extract was measured at 400 mμ, and the concentration of bismuth was read from a calibration curve obtained in a similar manner by the extraction of known amounts of bismuth.

The amount of bismuth in the alloy was found to be 0.093 and 0.095%. These two values are in agreement with the average value of 0.093% as indicated by the National Bureau of Standards.

LITERATURE CITED

(1) Cheng, K. L., and Bray, R. H., ANAL. CHEM., **25**, 655 (1953).
(2) Etheridge, A. T., *Analyst*, **75**, 279 (1950).
(3) LaCoste, R. J., Earing, M. H., and Wiberley, S. E., ANAL. CHEM., **23**, 871 (1951).
(4) Přibil, R., *Collection Czechoslov. Chem. Communs.*, **16**, 542 (1953).
(5) Sandell, E. B., "Colorimetric Determination of Traces of Metals," 2nd ed., p. 210, Interscience Publishers, New York, 1950.
(6) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., Vol. 1, pp. 150-61, Van Nostrand, New York, 1939.
(7) Šedivec, V., and Vašák, V., *Collection Czechoslov. Chem. Communs.*, 15, 260 (1950).
(8) Tompsett, S. L., *Analyst*, **63**, 250 (1938).

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Simultaneous Spectrophotometric Determination of Iron(II) and Total Iron with 1,10-Phenanthroline

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When 1,10-phenanthroline is added to a solution containing both iron(II) and iron(III), a reddish orange iron(II) complex and a yellow iron(III) complex form immediately. The iron(II) complex has an absorbance maximum at 512 mμ, at which wave length there is little absorption by the iron(III) complex. The two complexes have identical absorbance coefficients at 396 mμ. A method is presented for the determination of iron(II) and total iron in the same solution by simultaneous measurements of absorbance at 396 mμ and at 512 mμ. The limiting concentrations for interference by 22 cations and 14 anions are reported.

THE 1,10-phenanthroline complex with iron(II) was first discovered by Blau (*1*). Walden, Hammett, and Chapman (*4*) used the complex as an internal indicator in the oxidimetric titration of iron with the ceric ion. A spectrophotometric determination of iron dependent on the formation of the iron(II)–1,10-phenanthroline complex was developed by Fortune and Mellon (*2*).

The iron(II)–1,10-phenanthroline complex, which is reddish orange in color, may be oxidized to a blue complex. Upon standing, this blue complex changes to a yellow complex, which also may be produced by complexing iron(III) and 1,10-phenanthroline directly. Harvey and Manning (*3*) showed that this yellow complex has a maximum absorbance at 360 mμ and a small but measurable absorbance at 512 mμ. Above 380 mμ the absorption by 1,10-phenanthroline is negligible.

In the method for the simultaneous determination of iron(II) and total iron reported in the present paper, advantage is taken of the difference in the absorption spectra of the reddish orange iron(II) and the yellow iron(III) complexes which are formed instantly on the addition of 1,10-phenanthroline to a solution containing these ions.

EXPERIMENTAL

Instruments. A Beckman quartz spectrophotometer, Model DU, with 10-mm. silica or Corex absorption cells, was used for

spectrophotometric measurements. All measurements were made at maximum sensitivity of the instrument.

A Beckman Model G pH meter was used to check the pH of all buffer solutions.

Reagent Solutions. Reagent grade 1,10-phenanthroline monohydrate was obtained from G. Frederick Smith Chemical Co. The reagent was dissolved in distilled water which had been heated just to boiling. After it had cooled, the solution was diluted to the desired volume. A 0.1% reagent solution was used in forming the complexes for obtaining the complete absorption curves. A 0.3% solution of the reagent assured a sufficient excess in preparing the standard concentration curves and analyzing unknowns.

Standard Iron Solutions. The standard iron(II) and iron(III) solutions were prepared by dissolving 7.0213 grams of reagent grade ferrous ammonium sulfate hexahydrate, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, and 8.6337 grams of reagent grade ferric ammonium sulfate dodecahydrate, $NH_4Fe(SO_4)_2 \cdot 12H_2O$, respectively, in freshly distilled water containing 3 ml. of concentrated sulfuric acid and diluting to a liter.

The concentration of iron in each solution was determined gravimetrically as ferric oxide, Fe_2O_3, and was found to be 1.000 mg. per ml. The concentration of iron(II) in the former solution was determined periodically with ceric sulfate to avoid the use of the solution after any oxidation had occurred. Working standard solutions were prepared by dilution of these stock solutions.

Buffer Solution. A 0.2*M* solution of potassium biphthalate gave a buffer of pH 3.98.

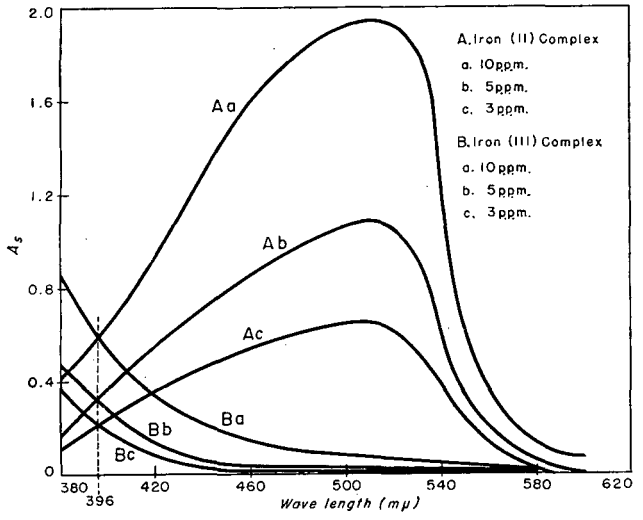

Figure 1. Absorption Curves of Iron–1,10-Phenanthroline Complexes

Absorption Curves. The absorption curves for the iron(II) and for the iron(III) complexes were determined at several concentrations of iron between 2 and 10 p.p.m. Absorbance values were read at intervals of 5 mμ in the wave-length range from 380 to 600 mμ. All of the solutions for absorbance measurements were prepared by putting a suitable aliquot of the proper standard solution into a 25-ml. volumetric flask, adding 10 ml. of 0.1% reagent solution and 5 ml. of potassium biphthalate buffer, and diluting to the mark with distilled water.

Figure 1 presents the absorption curves for the iron(II) and for the iron(III) complexes at iron concentrations of 3, 5, and 10 p.p.m. The iron(II) complex absorption curves show a maximum absorbance at 512 mμ, at which wave length there is little absorption by the iron(III) complex. The intersection at 396 mμ of all curves for equal concentrations of iron(II) and iron(III) indicates identical absorbance coefficients for the two complexes at this wave length.

These curves suggest the possibility of determining iron(II) from the absorbance at 512 mμ and total iron from the absorbance at 396 mμ. Iron(III) then could be determined by difference.

Studies of the Effect of pH. The method of Fortune and Mellon (*2*) gives a range from pH 2 to 9 for the determination of iron(II). Harvey and Manning (*3*) showed that the iron(III) complex was independent of pH over a range from 3 to 8. However, a study of the effect of change of pH on the wave length of intersection of the absorption curves of the iron(II) and iron(III) complexes was desirable.

Two series of solutions were prepared containing, respectively, 6 p.p.m. of iron(II) and 6 p.p.m. of iron(III). The pH in each series was varied in intervals of 0.5 units from pH 2.8 to 5.5. Absorbance values for each solution were measured over the wave-length range from 390 to 399 mμ.

Table I shows that the wave length of intersection remained constant at 396 mμ for all solutions within the pH range from 3 to 5. The middle of this range, pH 4, was used in establishing standard concentration curves for the determination of iron(II) and total iron.

Table I. Effect of pH on Wave Length of Intersection

pH	Wave Length of Intersection, Mμ
2.8	395
3.0	396
3.5	396
4.0	396
4.5	396
5.0	396
5.2	397
5.5	398

Standard Concentration Curves. Solutions of the iron(II) complex and of the iron(III) complex were prepared by adding to aliquots of the desired stock solution, 10 ml. of 0.3% reagent solution, and 5 ml. of buffer solution, and diluting to 25 ml. with distilled water. The concentration of iron in each of the two series of solutions ranged, in increments of 2 p.p.m., from 1 to 15 p.p.m. The absorbance of each solution was measured at both 396 and 512 mμ. The absorbance at 512 mμ of the iron(II) complex in concentrations greater than 10 p.p.m. was too intense to be read in 10-mm. cells. The absorbance *vs.* concentration curves at 512 mμ for iron(II) and iron(III), and a single concentration curve at 396 mμ for both the iron(II) and iron(III) were all linear over the concentration ranges measured. The slopes of these three lines were 0.196, 0.004, and 0.054 p.p.m.$^{-1}$, respectively.

To establish the fact that the absorbances of the two complexes are additive at 396 mμ, two other series of solutions were prepared. In the first series, the total iron concentration was held constant at 10 p.p.m. The concentrations of iron(II) and of iron(III) were X and $10 - X$, respectively. The value of X was varied, in increments of 1 p.p.m., from 0 to 10 p.p.m. In the second series, the iron(II) concentration was held constant at 5 p.p.m. while the concentration of the iron(III) was varied from 0 to 10 p.p.m. in 1-p.p.m. increments. The absorbance of each of these solutions was read at 396 mμ and at 512 mμ. In the first series, the absorbance at 396 mμ was constant and was identical to the absorbance at 10 p.p.m. read from the standard concentration curve for 396 mμ. The absorbance values at 512 mμ, when corrected for the iron(III) interference at that wave length, duplicated the iron(II) concentration curve at 512 mμ.

In the second series, the absorbance values at 396 mμ checked with those from the corresponding standard concentration curve over the concentration range from 5 to 15 p.p.m., which was the range of total iron concentration. Moreover, the readings at 512 mμ checked with the value from the standard concentration curve for iron(II) at 5 p.p.m. when suitable corrections were made for iron(III) interference. Standard curves at 512 mμ should be prepared for both the iron(II) complex and the iron(III) complex. The standard solution of iron(II) used for this purpose may contain an excess of hydroxylamine hydrochloride. Because the absorbances are additive, the standard curve at 396 mμ may be obtained at the same time from the solutions of either complex.

Stability of Iron(III) Complex. After a short time the concentration of the iron(II) complex increased in solutions which contained both complexes with excess 1,10-phenanthroline.

Table II. Limiting Concentrations of Diverse Ions with 2 P.P.M. of Iron(III)

Ion	Concn., P.P.M.	Apparent Concn. of Iron(III), P.P.M.
Cations		
Aluminum	500	2.00
Ammonium	500	2.00
Arsenic(III)	100	2.00
Cadmium	50	1.80
	25	2.00
Calcium	500	1.81
	250	2.00
Cobalt(II)	10	2.55
	5	2.00
Copper(II)	10	2.00
Lithium	1,000	2.00
Magnesium	500	2.00
Manganese(II)	500	2.92
	15	2.00
Mercury(I)	10	2.00
Mercury(II)	1	2.00
Nickel	2	2.00
Potassium	1,000	2.00
Strontium	500	2.00
Uranium	100	2.00
Zinc	50	2.00
Zirconium(IV)	100	0.25
	2	2.00
Anions		
Acetate	500	1.05
	1	2.00
Borate	500	2.00
Bromide	500	2.00
Carbonate	500	0.71
	5	2.00
Chlorate	500	2.00
Chloride	1,000	2.00
Citrate	500	1.05
	20	2.00
Fluoride	500	0.20
	25	2.00
Nitrate	500	2.00
Nitrite	500	1.62
	25	2.00
Oxalate	500	0.42
	1	2.00
Sulfate	500	2.00

Even solutions of the iron(III) complex acquired a reddish tinge upon prolonged standing. To study the rate of the shift, two solutions were prepared. One contained 10 p.p.m. of complexed iron(III), and the other 5 p.p.m. of complexed iron(II) and 5 p.p.m. of complexed iron(III). The absorbances of both of these solutions were measured at 512 mμ at frequent intervals over an extended period of time. No appreciable change in absorbance occurred for 30 minutes. After that time, the change in readings was marked. The increase in absorbance at 512 mμ was greater for the mixture than for the iron(III) complex alone. After 15 days, the two solutions had approximately the same absorbance, indicating that an equilibrium between the two oxidation states had been reached. Further study of this shift will be undertaken.

Interference of Diverse Ions with the Iron(III) Complex. A study was made of the effect of 22 cations and 14 anions on the absorbance of the iron(III) complex at 396 mμ. The cations were present in solution as the chlorides or nitrates while the anion solutions were of the sodium or potassium salts. The concentration of iron(III) was held constant at 2 p.p.m. Aliquots of iron(III) solution and of the solution of the ion to be tested were placed in a 25-ml. volumetric flask, complexed with 10 ml. of 0.3% reagent solution, buffered with 5 ml. of buffer solution, and diluted to the mark with distilled water. The absorbance then was measured at 396 mμ and the apparent concentration of iron was read from the standard concentration curve. The results are presented in Table II. The higher concentration of each diverse ion, where two concentrations are shown in Table II, or the single value given is the same as the limiting concentration found by Fortune and Mellon (*2*) in their study of interferences with the iron(II)–1,10-phenanthroline complex. Barium and lead sulfates were precipitated by the standard iron solution. A precipitate formed in solutions containing molybdate ions when the complexing agent was added. Precipitation also occurred upon the addition of the buffer to solutions containing thorium(IV) ions. Reducing agents such as iodide, thiosulfate, and tin(II) reduced the iron to form the iron(II)–1,10-phenanthroline complex.

The limiting concentrations of cadmium, calcium, and cobalt with the iron(III) complex are one half of the values reported (*2*) with the iron(II) complex. Interference from manganese and from zirconium are considerably greater for the higher-valence complex. Because ferric ions complex more readily with anions than do ferrous ions, the anion interference in many cases is more pronounced with the iron(III)–1,10-phenanthroline complex. Thus, the limiting concentrations for acetate, carbonate, citrate, fluoride, nitrite, and oxalate are much lower than the corresponding limiting concentrations with the lower-valence iron. For all other cations and anions tested, the limiting concentrations are at least as great as the concentrations permissible in the determination of iron(II).

ANALYSIS OF UNKNOWN SAMPLES

Solid mixtures of ferrous ammonium sulfate and ferric ammonium sulfate were prepared by weighing each salt and grinding them together in a mortar. The samples were sealed under an atmosphere of nitrogen to prevent oxidation of the iron(II), and were issued to one of the authors as unknowns. The proportions of iron(II) to iron(III) could not be calculated from the weights of the components because oxidation and loss of water of crystallization during grinding could not be prevented entirely. However, the concentrations of iron(II) and iron(III) in each sample were determined independently of the method under investigation as follows: After oxidation of an aliquot of each sample with nitric acid, total iron was determined spectrophotometrically by the method of Yoe and Jones (*5*) using the red complex of Tiron and iron(III). The iron(III) in another aliquot was determined by reduction with an excess of potassium iodide and titration of the resulting iodine with a standard solution of sodium thiosulfate.

Procedure. Weigh out a 300.0-mg. sample and dissolve it in distilled water slightly acidified with sulfuric acid. Dilute to 250 ml. in a volumetric flask. Each sample then should be analyzed immediately without interruption.

Withdraw three 1-ml. aliquots and place each in a separate 25-ml. volumetric flask. Add 10 ml. of 0.3% 1,10-phenanthroline solution, buffer with 5 ml. of 0.2*M* potassium biphthalate solution, and dilute to the mark with distilled water. Read the absorbance of each solution at 396 mμ and 512 mμ as soon as possible and not later than 30 minutes after the complexes are formed.

Calculation of Results. Determine the concentration of total iron and the approximate concentration of iron(II) from standard concentration curves at 396 and 512 mμ, respectively. Obtain the approximate concentration of iron(III) by difference. Find the absorbance value corresponding to this approximate concentration from the standard curve for iron(III) at 512 mμ. Subtract this value from the observed absorbance at 512 mμ to obtain

Table III. Analyses of Prepared Samples

Sample No.	Constituent	Found[a] Parts per million[b] Method A[c]	Method B[d]	Diff. B − A	% of Sample Method A[c]	Method B[d]	Diff. B − A
1	Fe(total)	6.56	6.60	0.04	13.67	13.74	0.07
	Fe(II)	5.96	5.94	−0.02	12.42	12.37	−0.05
	Fe(III)	0.60	0.66	0.06	1.25	1.37	0.12
2	Fe(total)	5.89	6.02	0.13	12.27	12.54	0.27
	Fe(II)	1.29	1.26	−0.03	2.69	2.62	−0.07
	Fe(III)	4.60	4.76	0.16	9.58	9.92	0.34
3	Fe(total)	6.61	6.48	−0.13	13.77	13.50	−0.27
	Fe(II)	3.83	3.66	−0.17	7.98	7.63	−0.35
	Fe(III)	2.78	2.82	0.04	5.79	5.87	0.08
4	Fe(total)	6.08	6.12	0.04	12.67	12.75	0.08
	Fe(II)	1.69	1.66	−0.03	3.52	3.46	−0.06
	Fe(III)	4.39	4.46	0.07	9.15	9.29	0.14
5	Fe(total)	6.95	7.00	0.05	14.48	14.58	0.10
	Fe(II)	6.53	6.52	−0.01	13.61	13.58	−0.03
	Fe(III)	0.42	0.48	0.06	0.87	1.00	0.13

[a] Average values from 3 aliquots of solution of sample.
[b] Parts per million in 25 ml. of solution containing 1.2 mg. of sample.
[c] Method A. Total iron and iron(II) determined with 1,10-phenanthroline. Iron(III) determined by difference.
[d] Method B. Total iron determined with Tiron and iron(III) by iodide-thiosulfate procedure. Iron(II) determined by difference.

the corrected concentration of iron(II) from the appropriate standard curve. For the correct concentration of iron(III), subtract the corrected concentration of iron(II) from the concentration of total iron already determined. The results are not changed appreciably by a second approximation.

In Table III, the analyses of 5 prepared samples determined by the simultaneous spectrophotometric method with 1,10-phenanthroline are compared with analyses obtained by determining total iron with Tiron and iron(III) with the iodide-thiosulfate procedure.

In columns 3 and 4, results are expressed as parts per million in a 25-ml. solution containing a $^1/_{250}$ aliquot of 300 mg. of the sample. In columns 6 and 7, the compositions of the samples are given in percentages. In method B, the values given for parts per million of iron(III) were calculated from data obtained from the iodide-thiosulfate procedure using 50-ml. aliquots of a 250 ml. solution containing 300 mg. of sample. Values for iron(III) and iron(II) were gotten by difference in methods A and B, respectively. Values for parts per million of total iron and iron(II) in method A and for total iron in method B were obtained directly from absorbance measurements.

The precision of the 1,10-phenanthroline method was determined by measuring the absorbances of solutions prepared with 0.5-, 1.0-, and 1.5-ml. aliquots of a solution of each sample. Duplicate determinations were made for each dilution. The average precision was 1.5% for total iron and 2.3% for iron(II). The maximum deviations were 5.4 and 5.3%, respectively.

Discussion. Table III illustrates that results of analyses by the 1,10-phenanthroline method are in good agreement with results obtained independently by a method involving accepted procedures of high accuracy. Comparison of results from these methods is particularly advantageous because in one case iron(II) is determined directly and iron(III) gotten by difference, whereas in the other case the determination of iron(III) is direct and that of iron(II) is by difference.

The concentrations of total iron determined with 1,10-phenanthroline are in close agreement with values obtained with Tiron. This indicates that, although the absorbance coefficient of the 1,10-phenanthroline complexes at 396 mμ is relatively small, absorbance measurements at this wave length give satisfactory results for total iron.

The method presented in this paper is to be recommended for its simplicity. Two simultaneous spectrophotometric measurements on the same solution are sufficient for an analysis. No preliminary steps such as reduction, oxidation, or extraction of the sample are necessary.

LITERATURE CITED

(1) Blau, F., *Monatsh. Chem.*, **19**, 647–89 (1898).
(2) Fortune, W. B., and Mellon, M. G., IND. ENG. CHEM., ANAL. ED., **10**, 60–3 (1938).
(3) Harvey, A. E., Jr., and Manning, D. L., *J. Am. Chem. Soc.*, **74**, 4744–46 (1952).
(4) Walden, G. H., Hammett, L. P., and Chapman, R. P., *Ibid.*, **55**, 2649–54 (1933).
(5) Yoe, J. H., and Jones, A. L., IND. ENG. CHEM., ANAL. ED., **16**, 111–15 (1944).

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Automatic Recording pH Instrumentation

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Titration at constant pH offers great potentialities for following enzymatic activity, but because of lack of suitable instrumentation, it has been employed only for studies on certain hydrolytic enzymes. The apparatus described permits fully automatic determination of both ionization constants and volume of titrating fluid added as a function of time at constant pH. Use of the instrument in enzyme research is illustrated with acetyl esterase and lactic dehydrogenase. Automatic, recording pH instrumentation is particularly useful for studying metal chelation and for following the course of many other chemical reactions, both catalyzed and uncatalyzed.

THE pH meter is probably the most fundamental and useful electronic instrument to be found in the biochemical laboratory. It also finds wide application in bacteriological and chemical laboratories as well as in plant industrial processes.

Apart from simple measurements of the hydrogen ion concentration of solutions, two research applications of the pH meter are of paramount importance: the determination of the neutral equivalent and the ionization constants (pK_a values) of unknown compounds and the measurement of volume of titrating fluid that must be added over a certain time interval in order to maintain a certain fixed pH. When the first of these techniques is applied to an unknown compound, information is obtained on the minimum molecular weight, the purity, and the possible structural features of the substance. The second application provides the data necessary for a kinetic analysis of a vast number of reactions, of both the catalyzed and uncatalyzed variety.

Both types of operation may, of course, be carried out manually, and in fact virtually all of such work has been so performed. The use of automatic recording devices, such as those described in this paper, greatly reduces the required experimental time, increases the sensitivity of the methods, avoids the human error, and makes it possible to follow reactions that are too rapid for manual methods.

The instrument described is based on principles used by Lingane (*6*) and Jacobsen and Léonis (*2*). However, certain modifications have been made in the design of the titration cell and in the method of adding the titrating fluid. The utility of the instrument in specialized biochemical problems, such as the study of enzymes, has been demonstrated.

VARIABLE pH TITRATION

Apparatus. Figure 1 shows the titration cell in detail as well as an outline of the other components of the system.

A $^1/_{150}$-hp. synchronous Bodine motor is used to drive the buret plunger. The slow shaft speed of this motor is 6 r.p.m.

The gear box and clutch assembly are shown in Figure 2. The slow-speed shaft of the motor is attached via a Lovejoy coupling to the fast-speed transmission shaft. The latter runs through a hollow shaft which bears a 16-tooth and a 32-tooth gear. The two shafts are connected with a keyway and the outside shaft may be slid back and forth over the inside shaft in order to position either the 16- or 32-tooth gear. The slow-speed transmission shaft carries a 120-tooth and a 100-tooth gear. One end of this shaft protrudes through the wall of the gear box and is attached directly to the clutch, which consists of a set of half nuts held by spring tension to the threaded buret drive shaft. When the motor turns, the fast and slow transmission shafts and the half nuts of the clutch are rotated. The clutch then pushes forward the threaded buret drive shaft, which cannot rotate. When the

16- and 120-tooth gears are engaged, the half nuts rotate at 0.80 r.p.m. and when the 36- and 100-tooth gears are engaged the half nuts rotate at 2.16 r.p.m. These two speeds deliver 1*N* titrating fluid at the rate of 50 and 135 microequivalents in 2 minutes. These quantities of titrating fluid provide, in 2 minutes, a complete titration curve for substances with molecular weights of 200 or 70, respectively. A sample size of 10 mg. represents a quantity which can be conveniently weighed on the analytical balance and added directly to the titration cell. At the chart speed used, a 2-minute interval is sufficient to give a 1.33-inch vertical trace of the pen.

The buret is a 1.0-ml. Gilmont ultramicroburet from which the manual crank has been removed and replaced by the automatic drive described above. The buret may be rinsed and filled after the clutch has been disengaged, by moving the threaded shaft manually back and forth.

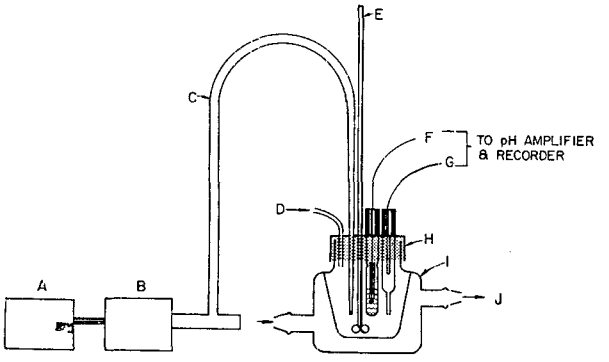

Figure 1. Cell and Experimental Arrangement for Variable pH Titration

A. Motor
B. Gear box
C. Buret
D. Nitrogen inlet
E. Stirrer
F. Glass electrode
G. Reference electrode
H. Plastic cover
I. Cell
J. Constant-temperature water

The cell shown in Figure 1 is made by fusing together, by means of a ring seal, two borosilicate glass cylinders, one 3.0 cm. and the other 5.0 cm. in diameter. An inlet and outlet tube is placed in the outside compartment of the cell in order to provide for the circulation of water from a thermostat. The cell is fitted with a plastic cover which is permanently mounted to a ring stand. A platform which can be raised or lowered vertically holds the cell in place. During a titration nitrogen is swept through the cell (not under the surface of the liquid) and the sample is agitated vigorously with a variable-speed mechanical stirrer. A sample volume of 5.0 ml. is just sufficient to cover the tips of the buret and electrodes. The glass and reference electrodes are Beckman microelectrodes Nos. 4990–29 and 4970–29, respectively.

The detecting device is a Beckman Model R pH meter–amplifier with a temperature compensation element immersed in the thermostat. The pH is recorded with a Minneapolis-Honeywell recorder Model 153 XIIV-W5-28, using strip chart 5429-N.

Application. Figure 3 shows the titration curve for pyridoxine hydrochloride. The apparent ionization constants calculated from this curve by published methods (*9, 11*) are $pK_{a1}' = 5.00$ and $pK_{a2}' = 8.96$. Both of these dissociation constants are "spectrophotometrically operable" and have been identified as the phenolic and pyridinium ionizations, respectively (*1, 11*).

In the coenzyme, pyridoxal phosphate, the corresponding constants have been found to be 4.14 and 8.69 (*11*). Therefore, at physiological pH the heterocyclic ring nitrogen atom of this important coenzyme bears essentially a full positive charge.

The variation in the pK_a' values calculated from duplicate runs by accepted methods (*9*) is ±0.02 pH unit. Neutral equivalents calculated from the type of curve shown in Figure 3 agree within ±2% of the theoretical value.

CONSTANT pH TITRATION

Apparatus. The instrument described in this paper can be used for either the variable or constant pH type of titration. The

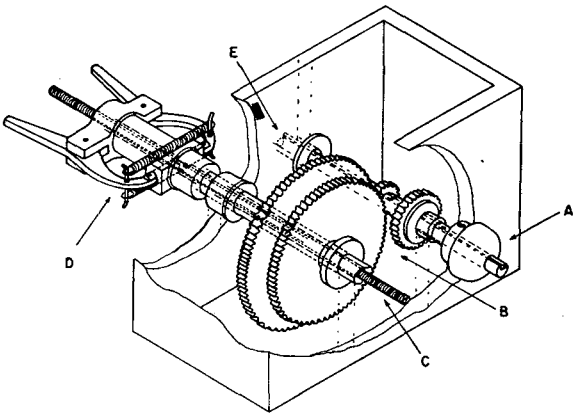

Figure 2. Transmission and Clutch Assembly for Variable pH Titration Unit

A. Fast-speed transmission shaft and gears
B. Slow-speed transmission shaft and gears
C. Buret drive shaft
D. Clutch
E. Connection to motor drive shaft

conversion from one type of instrument to the other is made in 1 or 2 minutes by throwing one switch, changing the chart paper, and exchanging the electrode connections.

The design of the authors' instrument follows that described by Jacobsen and Léonis (*2*) in employing a milliammeter equipped with contacts as the essential element controlling the addition of reagent, which in both is added from a motor-driven micrometer syringe. In the instrument of Jacobsen and Léonis the syringe drive is connected mechanically to the pencil which traces the curve, whereas in the instrument described, syringe movement is translated into a change in electrical potential which is suitable to operate a standard potentiometric recorder.

For greater convenience and more efficient use of desk space the equipment is assembled in two units (Figure 4). Those elements to the left of the dashed line in Figure 4 belong to the syringe drive unit; those to the right and below the dashed line pertain to the control unit. The titration cell is exactly the same as that shown in Figure 1, except that an extra port is provided in the plastic cover in order to permit the addition of reagents while an experiment is in progress.

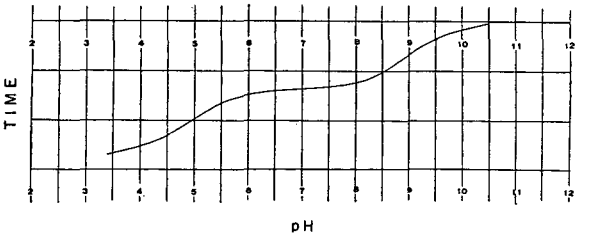

Figure 3. Titration Curve of 50 Micromoles of Pyridoxine Hydrochloride in 0.15*M* Sodium Chloride at 25° C.

Buret speed, 25 microequivalents of 1*N* NaOH per minute
Chart speed, 0.66 inch per minute

The rotation of the motor, M_1, is transmitted to the micrometer of a 0.5-ml. Agla syringe by means of a yoke which permits the necessary axial travel of the micrometer head. This yoke is geared (5 to 1) to a 10-turn helipot, R_1, which in turn is geared to a cam that operates limit switches set to turn off the motor at either end of helipot rotation. With this arrangement 10 turns of the helipot equal 50 turns of the micrometer, or full syringe travel.

In parallel with the motor-driven helipot is connected another identical helipot. This is needed to obtain use of the full width of the chart (electrical zero of the recorder is not at the end of the recorder scale), and to make it possible to reset the pen to zero

without reversing (and refilling) the syringe—a great convenience and timesaver. Potential across these two helipots is obtained from a 1.5-volt battery through a potentiometer, R_3, and a range switch, S_3. R_3 serves to make the potential correspond with the range of the recorder and to adjust for changes in battery voltage. The range switch, S_3, varies the ratio of pen movement to syringe travel (amount of reagent added). For example, if potentiometer R_3 is adjusted so that 50 revolutions of the micrometer move the recorder pen all the way across the scale with S_3 in position 5, turning S_3 to position 4 gives full scale movement for 25 revolutions; or to position 1, for 5 revolutions. A similar effect may be obtained by varying the concentration of reagent, but this is not easily done after a reaction is under way, while the range switch may be shifted at any time that it seems desirable to alter the slope of the curve.

The micrometer syringe which delivers reagent to the reaction vessel is driven by a two-phase induction motor (Minneapolis-Honeywell recorder pen-drive motor). This motor has certain characteristics which make it particularly suitable for this use: It is reversible; its speed can be varied from less than 4 to 27 r.p.m. (full speed) by a rheostat located as indicated in the circuit diagram; and it can be stopped without any appreciable coast by shorting the series-capacitor winding (n to m, Figure 4). The syringe drive motor is controlled through a three-position lever switch, S_5. In the reverse position, circuit c of this switch shunts the rheostat so the motor will reverse at full speed without disturbing the forward speed setting on the rheostat. Circuit a turns on the stirrer and circuit d the recorder chart drive when S_5 is in the forward position.

The reverse limit switch, S_9, opens one winding of the syringe motor, whereas the forward limit switch turns off not only the syringe drive motor but the contact relay meter control circuit, the stirrer, and the recorder chart drive as well. This permits leaving the apparatus unattended overnight with slow reactions, as all the critical elements will be off when the reagent is exhausted.

The contact meter relay, *CMR*, is a moving coil galvanometer (milliammeter), bearing one contact on the indicating pointer, and on either side of the indicating pointer an adjustable contact which can be moved manually across the scale so as to set the control point at any pH. It differs from that described by Jacobsen and Léonis (*2*) by having, in addition to the signal winding, a locking winding in series with the meter contacts and connected to an external power supply. Thus, as soon as the contacts touch lightly, current commences to flow in the locking winding, augmenting pointer movement and closing the contacts firmly. The adjustable contact is spring mounted, so that the pointer is pulled in sharply one or two degrees on the make and kicked back perhaps twice this distance when the locking circuit is broken.

The normally closed contacts of relay Y_1 serve to break the locking circuit automatically and release the pointer. If the signal (pH) has not changed so as to cause the pointer to recede from the control point, the contacts will continue to lock in and be released. The normally open contacts of Y_1 operate relay Y_2, which controls the addition of reagent by controlling the syringe drive motor through making or breaking the connection n to m. (The actual circuit is more roundabout, but tracing the connections will show that this is what is accomplished.)

A meter relay is essentially a two-position device. However, the speed with which the pointer returns to the control point, and consequently the rate of pull-in and release, are markedly affected by whether the signal is just at the controlling magni-

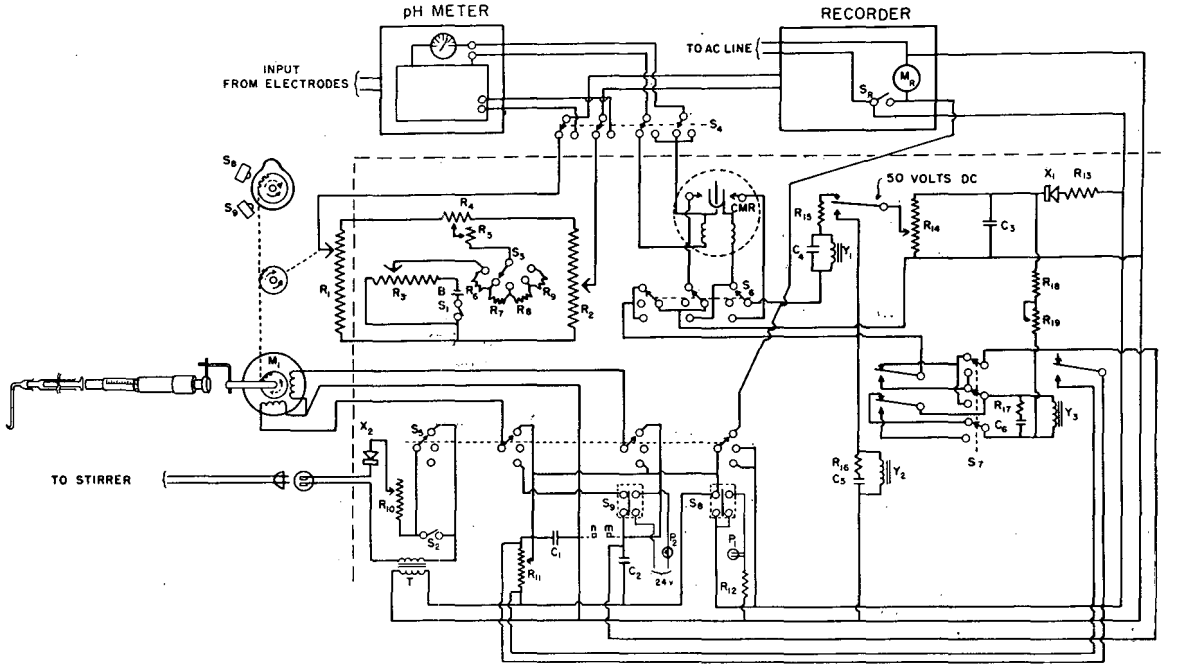

Figure 4. Circuit Diagram of Assembly for Constant pH Titration

B. 1.5-volt dry cell
CMR. Contact meter relay (Symplytrol, Assembly Products, Inc., Chagrin Falls, Ohio) 0–1 ma., with double adjustable contacts, isolated signal and locking windings [no electrical connection between signal (left) and locking (right) windings]
C_1. 2-μfd., oil-filled
C_2. 1-μfd., oil-filled
C_3, C_5. 20-μfd., electrolytic
C_4. 60-μfd., electrolytic
C_6. 120-μfd., electrolytic
M_1. Syringe drive motor (Minneapolis-Honeywell recorder pen drive motor)
M_R. Recorder chart drive motor
M_S. Stirrer (Lindsay 12-volt d.c. model train motor)
P_1. Neon pilot (lit when syringe drive has reached forward limit)
P_2. 28-volt filament pilot (lit when syringe drive is fully reversed), green jewel
R_1, R_2, R_3. 100-ohm, 10-turn helipots
R_4, R_5. 5-ohm wire-wound compensating potentiometers
R_6, R_7. 50-ohm, 1-watt precision (1%)
R_8. 100-ohm, 1-watt precision (1%)
R_9. 250-ohm, 1-watt precision (1%)
R_{10}. 25-ohm, 25-watt wire-wound rheostat
R_{11}. 10,000-ohm, 10-watt wire-wound rheostat
R_{12}. 50,000-ohm, 1/2-watt
R_{13}. 50-ohm, 10-watt wire-wound
R_{14}. 3000-ohm, 10-watt wire-wound, adjustable center tap
R_{15}. 5000-ohm, 2-watt
R_{16}, R_{17} 1000-ohm, 2-watt
R_{18}. 6800-ohm, 2-watt
R_{19}. 5000-ohm, 5-watt wire-wound potentiometer
S_R. Recorder chart drive switch
S_1, S_2. Toggle switches, SPST
S_3. Range switch, 1-circuit, 5-position, rotary action
S_4. Selector switch, 4-circuit, 2-position rotary action (located on recorder case)
S_5. Syringe motor switch, 4-circuit, 3-position lever action
S_6. CMR switch, 3-circuit, 3-position lever action
S_7. 3-circuit, 2-position lever switch
S_8. Forward limit switch, General Electric Switchette, snap action, 2-circuit, one NO, other NC
S_9. Reverse limit switch (same as S_8)
T. Transformer, 117 to 24 volts
X_1. Selenium rectifier, 100 ma., 117 volts
X_2. Selenium rectifier, 500 ma., 26 volts
Y_1. Relay, SPDT, 3000-ohm coil
Y_2. Relay, DPDT, 10,000-ohm coil
Y_3. Relay, SPDT 12,000-ohm coil, with roller-spring snap action contacts, giving wide differential between pull-in and drop-out (Assembly Products)

tude or somewhat greater. The circuit shown in Figure 4 takes advantage of this change of rate of cycling to achieve a degree of proportional control. This is done by choosing values for C_4 and C_5 so that relay Y_2 has a slower break than relay Y_1. Thus as the cycling of the meter contacts and Y_1 becomes faster, Y_2 is in the operated position a greater percentage of the time, until it finally remains constantly in the operated position. In the present instrument this is when the pH exceeds the control value by about 0.05 pH unit.

A further refinement is achieved by relay Y_3, which operates if relay Y_2 remains closed and speeds up the motor by shunting R_{11}. Relay Y_3 is made "slow operate" (by R_{18}, R_{19}, and C_6) and serves to distinguish between the pulsed and steady states of Y_2. This is accomplished by the second set of contacts on Y_2, which shunt the capacitor, C_6, every time Y_2 passes from one position to the other. R_{19} provides a means of adjusting the interval between the time Y_2 reaches a steady state and Y_3 operates.

In terms of the syringe drive motor (with switches set as in Figure 4) this means that as the pH reaches and then exceeds the control point, the motor which at first runs intermittently will gradually turn a larger percentage of the time until it runs continuously. If this continuous addition of reagent is not sufficient to return the pH to the control point, then relay Y_3 will operate, shunting R_{11}, and bringing the motor up to full speed.

Switch S_6 selects which of the adjustable contacts (upper or lower) of the contact meter relay is to be used for control. In its center position it disconnects the contact meter relay contacts, and isolates relay Y_2 from the motor so that motor control passes entirely to switch S_5.

Switch S_7 selects whether the syringe drive motor will turn with relay Y_2 in the operated position (as set in Figure 4) or in the unoperated position. With switches S_6 and S_7 it is possible to set up the following situations:

A. pH decreasing (reaction liberating H^+ ions) and syringe adding alkali
   1. Lower contact of *CMR* controlling. Switch S_7 set so relay Y_2 permits motor to turn (adds alkali) in operated position. Alkali will be added every time indicating pointer moves down scale to control point and locks in. Figure 5 obtained with this setting.
   2. Upper contact of *CMR* controlling. Switch S_7 set so relay Y_2 permits motor to turn (adds alkali) in unoperated position. Alkali will be added continuously until indicating pointer moves up scale to control point and locks in. Figure 6 obtained with this setting.

B. pH increasing (reaction absorbing H^+ ions) and syringe adding acid.
   1. Lower contact of *CMR* controlling. Switch S_7 set so relay Y_2 permits motor to turn (adds acid) in unoperated position. Acid will be added continuously until indicating pointer moves down scale to control point and locks in.
   2. Upper contact of *CMR* controlling. Switch S_7 set so relay Y_2 permits motor to turn (adds acid) in operated position. Acid will be added every time indicating pointer moves up scale to control point and locks in.

Situations A2 and B1 are most suitable for fast reactions, while A1 and B2 are better for slow reactions, because they avoid danger of overtitration at the start. The latter positions also avoid wear on the *CMR*, as it operates only when the reaction causes the pH to pass the control point, whereas in situations A2 and B1 the *CMR* is cycling at all times except when the pH moves the indicator away from the control point.

The variable pH titrations are recorded on Minneapolis-Honeywell strip chart No. 5401-N.

Application. Figure 5 shows a recording of the hydrolysis of triacetin by the enzyme acetyl esterase (*4*) using 0.02*N* sodium hydroxide as titrating fluid at pH 7.00 and 25°.

The enzyme solution is prepared by grinding orange peel in a meat grinder and expressing the juice. The press juice is clarified by filtration through Filter-Cel and adjusted to pH 7.05.

The titration cell is charged with 2.5 ml. of 10% triacetin, 0.5 ml. of 2.5*M* sodium chloride, and distilled water to make a final volume of 5.0 ml. Allowance is made for the volume of enzyme solution to be added later. The contents of the cell are adjusted to pH 7.05 with the 0.02*N* sodium hydroxide in the syringe, the nitrogen flow is started, and the enzyme solution is added through the port in the cell cover.

Curves 1 and 2 in Figure 5, which are for 0.5 and 1.0 ml. of press juice, respectively, show that the reaction is zero order throughout its entire course. Furthermore, it is apparent that the rate is exactly proportional to the enzyme concentration. Blank experiments with triacetin alone and with the enzyme alone show zero uptake of alkali over the experimental period.

In order to check the versatility of the instrument, an experiment was carried out with a much more rapid reaction—i.e., the reduction of diphosphopyridine nucleotide with lactate in the presence of the enzyme heart lactic acid dehydrogenase. In this case the reaction L(+)-lactate + diphosphopyridine nucleotide$_{ox}$ $\rightleftharpoons$ pyruvate + diphosphopyridine nucleotide$_{red.}$ + H^+ also produces acid, since the strongly basic quaternary nitrogen atom in the coenzyme is converted to a weakly basic tertiary nitrogen atom. The titration fluid is again 0.02*N* sodium hydroxide, the temperature is 25° C., and a pH of 9.50 is used as the control point.

Lactic dehydrogenase is crystallized from beef-heart muscle by the method of Straub (*7, 8, 10*). A $2 \times 10^{-5}M$ stock solution of the enzyme is prepared in $10^{-2}M$ phosphate buffer at pH 7.0.

The titration cell is charged with 1.0 ml. of 1.0*M* DL-sodium lactate, 0.5 ml. of $10^{-2}M$ sodium diphosphopyridine nucleotide$_{ox.}$, and water to make a final volume of 5.0 ml. The nitrogen flow is started, the cell contents are adjusted to pH 9.55 as before, and the enzyme solution is added with a micropipet. The results are shown in Figure 6.

The curve shown in Figure 6 is for $2 \times 10^{-8}M$ lactic dehydrogenase. It is apparent that a zero-order course is followed only in the initial phase, after which the reaction reverts to one of higher order. From the equilibrium constant, 0.3×10^{-11} (*7*), it can be calculated that the reaction should cease after the addition of 0.15 ml. of 0.02*N* sodium hydroxide. This volume of titrating fluid corresponds to a scale deflection of 70 divisions under the conditions used in the experiment shown in Figure 6.

DISCUSSION

The instrument described delivers titrating fluid from a buret for the variable pH and from a syringe for the constant pH titrations. The Gilmont buret is used because it can be readily adapted for automatic drive. A syringe is used for the constant pH titrations, because it is not desirable to allow mercury to come in contact with a solution that is to be added to an enzyme-catalyzed reaction.

The concentration of an enzyme in natural material cannot be measured directly. However, from a quantitative measurement

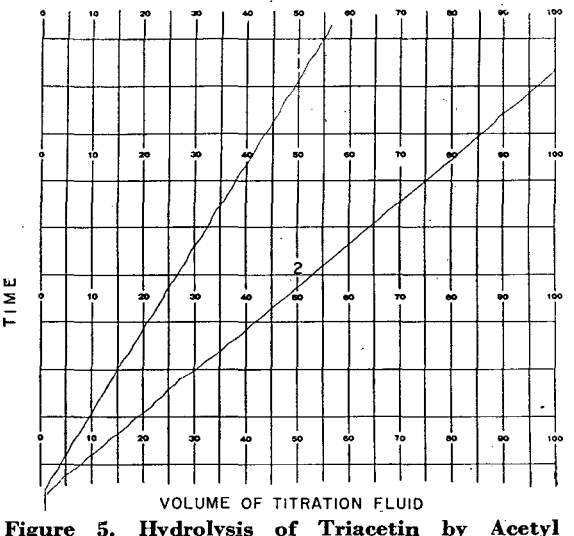

Figure 5. Hydrolysis of Triacetin by Acetyl Esterase of Orange Peel at pH 7.00 and 25° C.

Full scale (100 divisions of the chart) is 0.22 ml. of 0.02*N* NaOH. Curve 1 obtained with 0.5 ml. and curve 2 obtained with 1.0 ml. of enzyme solution. Chart speed is 0.66 inch per minute

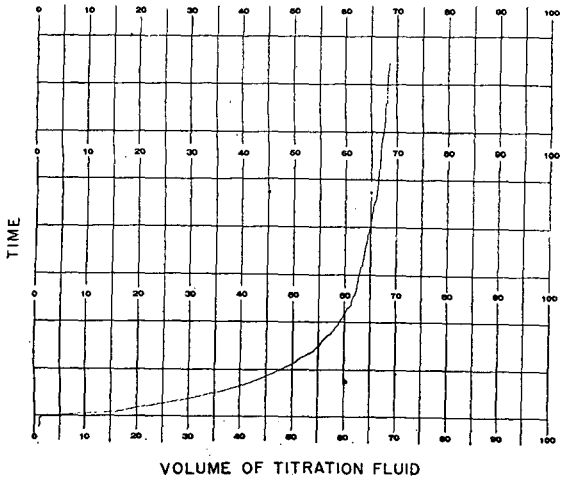

Figure 6. Reduction of Diphosphopyridine Nucleotide by Lactic Dehydrogenase of Heart at pH 9.50 and 25° C.

Initial concentrations. Sodium DL-lactate, 0.2*M*; diphosphopyridine nucleotide, $10^{-3}M$; crystalline heart lactic dehydrogenase, $2 \times 10^{-6}M$. Other conditions same as for experiment described in Figure 5

of its activity, a figure proportional to the enzyme concentration can be obtained. A spectrophotometric method, when applicable, is undoubtedly the most convenient, sensitive, and accurate way to assay an enzyme. For example, all pyridine nucleotide–requiring enzymes, including the lactic dehydrogenase used in the above experiments, are so assayed (*8*). Generally speaking, however, it is not possible to devise a spectrophotometric assay for the vast number of enzymes which catalyze hydrolytic reactions. Acetyl esterase (described above) and such important enzymes as acetylcholine esterase fall into the latter category. The use of constant pH titration as a means of assaying lactic dehydrogenase has been demonstrated here only to emphasize that this technique is not restricted to hydrolytic enzymes.

The amino acid decarboxylases have been hitherto studied almost exclusively with cumbersome manometric methods, but there is no reason why such enzymes cannot also be assayed by titration (*3*). For instance, the decarboxylation of glutamic acid in the region of pH 5 will consume acid, since the glutamate with a net negative charge is converted to the net uncharged aminobutyrate and carbonic acid. In this case advantage is taken of the fact that the alpha-carboxyl group of the amino acid is a much stronger acid than carbonic. Thus, if the pH of the experiment is carefully selected, the types of enzyme-catalyzed reactions which can be investigated by constant pH titration are almost unlimited.

Titration at constant pH may be applied for a variety of uncatalyzed reactions. Amino groups may be determined, inasmuch as a stoichiometric amount of acid is generated when these groups are benzylated with reagents such as dinitrofluorobenzene (*5*). The alkaline decomposition of nucleoproteins such as tobacco mosaic virus may also be followed quantitatively, since this reaction liberates acid. The data in Figure 6 show that the apparatus is useful for determining completeness of reaction and equilibria as well as for rate measurements.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Harris, S. A., Webb, T. J., and Folkers, K., *J. Am. Chem. Soc.*, 62, 3198 (1940).
(2) Jacobsen, C. F., and Léonis, J., *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, 27, 333 (1951).
(3) Jang, R., and Axelrod, B., personal communication.
(4) Jansen, E. F., Nutting, M. D. F., and Balls, A. K., *J. Biol. Chem.*, 175, 975 (1948).
(5) Levy, A. L., personal communication.
(6) Lingane, J. J., "Electroanalytical Chemistry," Interscience Publishers, New York, 1953.
(7) Neilands, J. B., *J. Biol. Chem.*, 199, 373 (1952).
(8) Neilands, J. B., in "Methods in Enzymology," ed. by S. P. Colowick and N. O. Kaplan, Academic Press, New York, in press.
(9) Parke, T. V., and Davis, W. W., ANAL. CHEM., 26, 642 (1954).
(10) Straub, F. B., *Biochem. J.*, 34, 483 (1940).
(11) Williams, V. R., and Neilands, J. B., *Arch. Biochem. Biophys.*, in press.

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Determination of Easily Hydrolyzable Fructose Units in Dextran Preparations

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Characterization of dextran preparations required analytical methods for small amounts of free and combined fructose, present as impurities or minor constituent-units in the glucose polymers. A qualitative procedure was developed to detect as little as 0.01% free or easily liberated fructose in dextran samples. The two quantitative colorimetric methods which were developed measure, in addition, at least part of the fructose in such compounds as melezitose and leucrose, from which it is difficultly liberated. The method using a modified anthrone reagent also is particularly suitable for quantitative paper chromatography of fructose. In both this and a modified resorcinol method the color-forming power of glucose is limited to about 1/240th that of fructose. These qualitative and quantitative methods should prove useful for obtaining information on the amount and relative ease of hydrolysis of fructose units in many natural and enzymically synthesized products.

THE glucose polymers known as dextrans are formed from sucrose by the action of microorganisms such as *Leuconostoc mesenteroides* or of enzyme solutions derived from cultures of the organisms (*4*). The dextran preparations may contain fructose in any of several forms. Thus, levan, an easily acid-hydrolyzed polymer of fructose, often is formed along with dextran (*8*) and may remain as a contaminant during isolation of the dextran. Free fructose or sucrose in the medium may be carried down with the dextran. Because the disaccharide leucrose (5-D-glucopy-

ranosyl-D-fructose) has been obtained in the enzymic synthesis of dextran (*14*), this and other difficultly hydrolyzed compounds of fructose also are potential contaminants of dextran preparations. Finally, fructose units may be present in small amounts as structural parts of dextran molecules—for example, as easily hydrolyzed terminal fructosyl units (*6*) or as difficultly liberated units carrying glucosidic linkages on one or more of the hydroxyl groups. For fundamental studies of dextrans and their structures it is essential, therefore, that analytical evidence be obtained on the presence of fructose in dextran samples.

To fill the need for methods of fructose determination adapted to the research on dextran, one qualitative and two quantitative methods were developed. These methods were devised with particular emphasis on the ability to measure amounts of fructose below 1% of the dextran sample. They are based, in general, on known procedures and principles, with modifications as necessary to meet the specific requirements of their proposed use. It was particularly important to avoid interference from glucose and its polymers.

QUALITATIVE CHROMATOGRAPHY PROCEDURE FOR FREE OR EASILY LIBERATED FRUCTOSE

The qualitative procedure was developed specifically to detect free fructose and its easily hydrolyzed combinations, such as levan or sucrose, in contrast to fructose in more difficultly hydrolyzed combinations such as leucrose (5-D-glucopyranosyl-D-fructose, *14*), melezitose, and possibly some dextran molecules. The procedure provides for chromatographic identification of the liberated fructose and gives evidence of the ease of hydrolysis. In addition, quantitative determinations have been performed by an extension of the method.

The detection of easily liberated and free fructose in dextran preparations is based on paper chromatography of the ethyl alcohol–soluble sugars formed by limited acid hydrolysis of the sample. When free fructose only is to be detected, the hydrolysis step is omitted. Under the conditions selected for hydrolysis (0.2*N* sulfuric acid at 70° C. for 1 hour) the recoveries of fructose from sucrose and levan samples were over 90%, as measured by quantitative paper chromatography. The hydrolysis of dextran, however, amounted to only about 0.2 to 0.9%, as measured by reducing power expressed as glucose equivalent. Even so, suitable developing solvents and a selective spray reagent must be used to avoid interference from glucose in the chromatography step, especially when dextran samples containing only a small amount of fructose—e.g., 0.5% or less—are being studied.

An improved spray reagent for the selective detection of fructose in the presence of aldoses on the paper chromatograms was obtained by replacing the hydrochloric acid in the urea–hydrochloric acid reagent of Hough *et al.* (*7*) with phosphoric acid, as suggested for several other reagents by Bryson and Mitchell (*2*). The urea–phosphoric acid reagent provides a better color differentiation between fructose and glucose and excellent freedom from background color on the paper, as compared with the urea–hydrochloric acid reagent. Fructose gives a characteristic blue-gray color, in sharp contrast to the light brown color given by much higher concentrations of glucose. On standing overnight and longer, the fructose spot becomes gray and then gray-brown, but otherwise is stable. About 25 to 50 times as much glucose is required to give as intense a spot as fructose if the heating period is not too long. It is possible, after development of the chromatogram, to detect as little as 4 γ of fructose in the presence of at least 100 times as much glucose.

After the limited hydrolysis of the dextran sample, careful control of the pH of the solutions is advisable to avoid alkaline rearrangement of the glucose present to fructose. The use of bromothymol blue as indicator together with neutralization of the sulfuric acid with barium hydroxide permitted the pH to be held easily at about 6.1 to 6.6. Earlier trials involving neutralization with an excess of barium carbonate gave much less satisfactory control of pH (see also *11*). The indicator does not interfere with paper chromatography of the sugars, as its R_f value is nearly unity in the developing solvent used.

Apparatus and Reagents. Thermoregulated water bath at 70° C.

Apparatus for paper chromatography (*3*, *9*).

Solution of 0.2*N* sulfuric acid.

Approximately 0.4*N* barium hydroxide.

Bromothymol blue indicator.

Urea spray reagent made up as follows: To 100 ml. of 1*M* phosphoric acid in water-saturated butanol (about 80% butanol by weight) add 3 grams of urea, followed by about 5 ml. of ethyl alcohol to eliminate the water phase which forms when the urea dissolves. The reagent is stable for several months.

Procedure. To 5 grams, or less, of dextran sample add enough 0.2*N* sulfuric acid to give a 5% carbohydrate concentration and heat the mixture at 70° C. for 1 hour. Neutralize the cooled solution with approximately 0.4*N* barium hydroxide, using bromothymol blue as an indicator. Add, with stirring, enough absolute ethyl alcohol to give a solution which contains 85% alcohol by volume. Remove the barium salts together with the precipitated dextran by decanting and centrifuging the solution. Evaporate the supernatant solution in vacuo to dryness. When low concentrations of combined fructose—e.g., below about 1% of the sample—are involved, a second alcohol precipitation from a smaller aqueous volume may be necessary to reduce further the amount of partially degraded dextran, the presence of which can cause elongation and streaking of the sugar spots on the chromotogram. In extreme cases, where spot elongation still has not been avoided because of remaining dextran or salts, the fructose-containing area of a duplicate chromatogram can be eluted with water (*3*), the eluate evaporated to dryness, and the residue rechromatographed.

Dissolve the dried solubles from the precipitation step in a drop or two of water and transfer the solution with an ultramicroburet to a paper chromatogram, using patterns of contiguous spots (*3*). Develop the chromatogram once or twice with the butanol-pyridine-water (6 to 4 to 3) solvent mixture (*9*) and then dry and spray with the urea–phosphoric acid reagent. After drying the sprayed paper at room temperature, heat it in an oven for several minutes at 100° to 110° C. The presence of fructose is indicated by a blue-gray spot whose position corresponds to that of a known sample of fructose on the same chromatogram.

For quantitative paper chromatography, perform the spotting and eluting of strips containing the unknown as previously described (*3*) and measure the fructose by the alcoholic anthrone method described below. If the amount of fructose is small—e.g., 50 to 100 γ per strip—collect the eluate directly in weighed test tubes, dilute with water to a weight of 2.00 grams and use the entire quantity for one anthrone determination.

QUANTITATIVE FRUCTOSE METHOD USING ALCOHOLIC ANTHRONE

The fact that fructose reacts more rapidly than glucose in the color-forming reaction of the anthrone determination of total carbohydrate had been noted in this laboratory (*13*) as well as by Koehler (*10*). This observation, together with experience previously gained (*3*) in the use of the anthrone reaction, prompted studies leading to the present modification of the anthrone reaction for the determination of fructose in free and most combined forms.

The fructose procedure differs from the total carbohydrate method in the use of a lower temperature (50°) and a lower concentration of acid in the reaction mixture, so that incomplete reaction of fructose and very limited reaction of glucose occur. Dilution of the anthrone–sulfuric acid reagent before use avoids heat of mixing which would result in uncontrolled color formation. The use of ethyl alcohol, instead of water, as diluent provides a twofold advantage of a more intense color and avoidance of precipitation of anthrone at the lower concentration of sulfuric acid.

The alcoholic anthrone–sulfuric acid reagent is advantageous in several respects. The small sample size required makes the reagent suitable for the measurement of fructose by quantitative paper chromatography, since 25 to 100 γ of fructose suffices for a determination. In the analysis of dextran samples, the small sample size permits analysis of dextrans which give hazy solutions without need for the corrective steps described for the resorcinol procedure below. For known mixtures the results were

accurate within ±2% for concentrations of fructose in dextran at least as low as 0.04% (see Table I). In addition, the precision of the method was very good, as shown by the fact that each of the values under column 3 of Table I is the average of duplicates having an average deviation within 0.0003 or 0.0004 mg. of fructose.

Apparatus and Reagents. Spectrophotometer—e.g., Coleman Model 11—adapted, if necessary, for use of the reaction tubes in the cuvette carrier.

Borosilicate glass tubes, 18 × 150 mm., selected for uniformity in spectrophotometric measurements.

Thermoregulated water bath at 50° ± 0.5° C. and ice bath, with a suitable basket or rack for the test tubes.

Alcoholic anthrone reagent. To 60 ml. of absolute ethyl alcohol add slowly, with cooling, 100 ml. of concentrated sulfuric acid. When the mixture is at room temperature, add 200 mg. of anthrone. The reagent is ready for use immediately and can be kept in a refrigerator (about 6° C.) for at least 3 weeks.

Procedure. Weigh samples of dextran up to 60 mg. (or transfer up to 2.0 ml. of solution) containing the equivalent of 25 to 100 γ of fructose or levan into the tubes. Add 2 ml. of water or sufficient water to give a total of 2 ml. if a solution was used. Include a fructose standard (60 γ) and a reagent blank in each run. In addition, if the fructose content is below 10%, have a control for the dextrans consisting of a high purity sample of the same type of dextran at approximately the same concentration.

To each of the solutions, cooled in ice water, layer in 8 ml. of the cold alcoholic anthrone reagent. Transfer the well-stirred cold mixtures to the 50° C. water bath. After 20 ± 0.1 minutes, return the tubes to the ice bath for 1 minute to cool the solutions to a little below room temperature. Measure the absorbance promptly at 620 mμ against the reagent blank. As the color formation still is continuing slowly, the tubes should be read in order from the first to the last, then from the last to the first, and the average of the two readings used. In making these readings a uniform rate schedule should be maintained.

Calculation of Results. From the absorbance of the fructose standard, calculate the factor for converting absorbance to weight of fructose:

$$K = \frac{\text{mg. of fructose}}{\text{absorbance}}$$

This factor will vary somewhat from run to run, mainly because of variations in the time required to read the tubes and also because of other variations in conditions. Therefore, a fructose standard is included in each set of tubes to be heated.

Calculate the weight of the fructose in the dextran sample using the absorbances, A, of the "unknown" dextran and the control dextran sample:

$$\text{Weight of fructose} = K\ (A \text{ unknown} - A \text{ control})$$

SAMPLE CALCULATION.

Absorbances.

0.060 mg. of fructose	0.524
10 mg. of unknown dextran	0.599
10 mg. of control dextran	0.182

Calculations.

$$K = \frac{0.060}{0.524} = 0.1145$$

Fructose in unknown sample = 0.1145 (0.599 − 0.182)
= 0.048 mg. or 0.48%

Expressed as levan = 0.048 × 0.9 = 0.043 mg. or 0.43%

The K value under a given set of conditions is constant over a wide range of absorbances in conformance to Beer's law, as shown in Figure 1 for the alcoholic anthrone reagent. A similar linear relationship was observed with the resorcinol–hydrochloric acid method described below.

QUANTITATIVE FRUCTOSE METHOD USING RESORCINOL

Initial studies on the determination of fructose in dextran samples were directed toward adaptation of the colorimetric Seliwanoff reaction using resorcinol in the presence of hydrochloric acid, for which several detailed reports had appeared (*1*, *5*, *12*). The modified method which was developed is described briefly here because of its apparent usefulness, in combination with the alcoholic anthrone method, for getting a partial differentiation between easily and difficultly liberated forms of fructose in a sample.

Table I. Typical Results on Known Mixtures[a] and Fructose-Containing Sugars

Combinations	Total Weight Sample, Mg.	Fructose Found Mg.	Fructose Found %	Recovery, % of Theory
Alcoholic Anthrone Method				
Dextran + fructose	16.0	0.0480	0.300	100
Dextran + fructose	16.0	0.0060	0.038	100
Dextran + levan	0.5	0.0508	10.2	99
Dextran + levan	10.0	0.0507	0.507	98
Raffinose.$5H_2O$	0.165	0.0498	30.2	100
Sucrose	0.095	0.0502	52.8	100
Melezitose.$2H_2O$	0.150	0.0520	34.7	104
Leucrose	0.095	0.0097	10.2	19
Resorcinol Method				
Dextran + fructose	203.0	0.377	0.186	94
Dextran + fructose	204.0	0.045	0.022	90
Dextran + levan	5.0	0.486	9.72	98
Dextran + levan	100.0	0.465	0.465	94
Raffinose.$5H_2O$	1.65	0.492	29.8	98
Sucrose	0.95	0.492	51.8	98
Melezitose.$2H_2O$	1.50	0.178	11.9	36
Leucrose	0.95	0.010	1.0	2

[a] In all cases the dextran was a highly purified sample from *Leuconostoc mesenteroides* NRRL B-512 prepared as described (*8*).

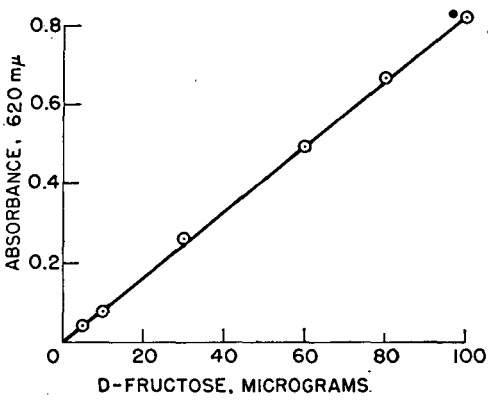

Figure 1. Absorbance Curve, Alcoholic Anthrone Method

The procedure described by Gray (*5*) was used as a basis for the present method. Ferric chloride was added to the hydrochloric acid, as done by Bacon and Bell (*1*) in order to intensify the color produced and minimize the effect of possible traces of iron in the hydrochloric acid. The temperature at which the color-forming reaction was conducted was lowered from 80° C., used by Gray, to 50° C. This lowering of the reacting temperature, together with a reaction time of 20 minutes, allowed a greater differentiation between fructose and glucose in mixtures of the two. The selectivity thus was increased threefold—i.e., 240 parts instead of 80 parts (*5*) of glucose were required to give the same absorbence as 1 part of fructose. Ice-bath cooling of the dextran solution and reagents before mixing was adopted to eliminate erratic results attributed to variable amounts of reaction before the timed period of heating.

Application of the procedure to known mixtures containing as little as 0.02% of fructose, sucrose, or levan in dextran gave recoveries of fructose ranging from 90 to 100 ± 2%. The decrease in accuracy with the lower percentages of fructose, shown in Table I, tentatively is attributed to a lowering of the effective acidity of the reaction mixture by the larger weights of dextran sample required for fructose contents below about 1%.

Apparatus and Reagents. The spectrophotometer, borosilicate glass test tubes, and the water and ice baths are the same as for the alcoholic anthrone method.

Hydrochloric acid, concentrated, specific gravity 1.18 to 1.19, to which has been added 0.0124 gram of ferric chloride hexahydrate per liter.

Resorcinol reagent, consisting of a 0.1% solution of resorcinol in absolute ethyl alcohol.

Procedure. The procedure is essentially the same as for the alcoholic anthrone method, except that the sample consists of up to 500 mg. of dextran (or up to 2.0 ml. of solution) containing the equivalent of 200 to 800 γ of fructose or levan, while the fructose standard is 500 γ. To each sample in 2.0 ml. of cold aqueous solution is added 5 ml. of the cold hydrochloric acid followed by 2 ml. of cold resorcinol reagent. The well-mixed solutions are heated and cooled, and their absorbances measured as for the alcoholic anthrone method, except that a wave length of 505 mμ is used.

The calculation of results is similar to that for the alcoholic anthrone method. If any of the solutions are hazy, haze blanks must be prepared of those dextrans and the control dextran. For a haze blank, the 2 ml. of resorcinol reagent is replaced with an equal volume of cold absolute ethyl alcohol. The absorbances of these solutions then are used as a correction as follows:

Weight of fructose = K (A unknown $-$ A unknown haze blank $-$ A control $+$ A control haze blank).

DISCUSSION

Glucose gives the same color reaction as fructose with the anthrone or resorcinol methods but requires longer heating to attain a similar absorbance. Under the conditions of limited reaction described here, for either method, 1 mg. of fructose is equivalent in absorbance to about 240 mg. of glucose.

This very favorable ratio of color formation from fructose and glucose is achieved by restricting the extent of reaction. Thus, heating for more than 20 minutes or at higher temperatures than 50° C. gives a higher absorbance per unit weight of either sugar. However, the increase for glucose is relatively greater than for fructose, so that more interference is obtained from glucose in mixtures under such conditions. The incompleteness of the color-forming reaction, together with the low reaction temperature of 50° C., results in a slow continuation of reaction at room temperature during the spectrophotometric measurements. Since the absorbances of the reaction mixtures in either method are increasing at a rate of about 0.4% per minute at room temperature, a set of tubes must be read twice and in a uniform manner from first to last and then from last to first if highest accuracy is desired. Any attempt to wait until the change in absorbance decreases or ceases will result in greater interference from glucose and its polymers.

Both colorimetric methods measure at least part of the fructose in combinations less easily hydrolyzed than sucrose or levan, as shown by the results with melezitose and leucrose (5-D-glucopyranosyl-D-fructose) (*14*), Table I, neither of which yields fructose under the hydrolytic conditions of the qualitative chromatographic procedure. The two methods differ significantly in the extent to which they measure such forms of fructose, the alcoholic anthrone reagent being the more effective. This difference probably results, at least in part, from the greater hydrolyzing power of the anthrone–sulfuric acid reagent, as has been indicated by preliminary estimates of the extents of hydrolysis of dextran by the two reagents under the conditions of the fructose determination. Advantage can be taken of this difference by using the resorcinol method where the measurement is to be limited more nearly to the easily hydrolyzed fructose units and employing the alcoholic anthrone method for obtaining more nearly a "total fructose" value. Even with the latter method, however, only a minor part of the fructose units may be measured in structures reacting like leucrose, whether present in impurities or as part of the dextran molecule.

The use of a control dextran in the colorimetric analysis of samples containing low concentrations of fructose is required to compensate for color formation from glucose liberated from the dextran sample by the hydrolytic action of the reagent. The measurement of fructose content, therefore, is relative rather than absolute, in so far as the control sample may be more easily hydrolyzed (liberation of glucose) or may contain free or combined fructose. The effect of differences in rate of liberation of glucose, which probably would result from differences in the proportion and kind of non-1,6′-glucosidic linkages, must remain well below the equivalent of about 0.4% fructose in the sample, since this is the difference in color formation between glucose itself (equivalent to immediate complete hydrolysis) and the blank (equivalent to no hydrolysis of dextran).

The possible presence of fructose units in the control is a potential source of greater difficulty. Free or easily hydrolyzed fructose can be detected and measured by the chromatographic procedure. Some indication of the presence of difficultly hydrolyzed fructose units can be obtained from the total color formation in the reaction of the control. The B-512 dextran used as a control in the present studies gave absorbances equivalent to about 0.06 and 0.25% fructose in the sample by the resorcinol and anthrone methods, respectively. Preliminary estimates of the extent of hydrolysis of this dextran sample to glucose by these two reagents have suggested that approximately half of this apparent fructose may arise from sources other than glucose liberation. This dextran, however, contained not over 0.02% fructose in free or easily hydrolyzed form, as shown by application of the chromatographic procedure described and comparison with known amounts of fructose on the chromatograms.

The possibility thus suggested that difficultly hydrolyzed fructose units, or sugar units giving comparable color reactions, may be present in dextrans is given further support by observations on other dextran samples. Some highly purified, partially degraded dextrans, for example, have given apparent fructose contents, measured against controls, as high as 0.3%, although no easily hydrolyzed fructose units could be detected by the qualitative procedure. The interpretation of such data must await the results of further studies of the chemical structure of dextrans. These observations emphasize, however, that samples of dextran to be used as controls should be selected on the basis not only of tests by the qualitative procedure but also of determinations of the actual amount of color, compared with reagent blanks, produced in the quantitative procedures.

The extent to which other sugars may interfere in the determination of fructose was investigated. Only the ketohexose sorbose gave an appreciable amount of color. The absorbance developed in the resorcinol and anthrone methods was 52 and 74%, respectively, of that given by an equal weight of fructose. Xylose gave only 0.4 and 1.8%, respectively. For either method, densities below 0.5% were obtained with mannose, arabinose, and glucuronic acid.

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LITERATURE CITED

(1) Bacon, J. S. D., and Bell, D. J., *Biochem J. (London)*, **42**, 397 (1948).
(2) Bryson, J. L., and Mitchell, T. J., *Nature*, **167**, 864 (1951).
(3) Dimler, R. J., Schaefer, W. C., Wise, C. S., and Rist, C. E., ANAL. CHEM., **24**, 1411 (1952).
(4) Evans, T. H., and Hibbert, H. "Advances in Carbohydrate Chemistry," Vol. 2, p. 203, Academic Press, New York, 1946.
(5) Gray, D. J. S., *Analyst*, **75**, 314 (1950).
(6) Hehre, E. J., private communication.
(7) Hough, L., Jones, J. K. N., and Wadman, W. H., *J. Chem. Soc.*, **1950**, 1702.
(8) Jeanes, A., Wilham, C. A., and Miers, J. C., *J. Biol. Chem.*, **176**, 603 (1948).
(9) Jeanes, A., Wise, C. S., and Dimler, R. J., ANAL. CHEM., **23**, 415 (1951).
(10) Koehler, L. H., *Ibid.*, **24**, 1576 (1952).
(11) Laidlaw, R. A., and Reid, S. G., *J. Sci. Food Agr.*, **3**, 19 (1952).
(12) Roe, J. H., *J. Biol. Chem.*, **107**, 15 (1934).
(13) Scott, T. A., and Melvin, E. H., ANAL. CHEM., **25**, 1656 (1953).
(14) Stodola, F. H., Koepsell, H. J., and Sharpe, E. S., *J. Am. Chem. Soc.*, **74**, 3202 (1952).

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Use of Empirically Derived Correction Factors

Polarographic Determination of Free Cyanide in Presence of Sulfides

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A rapid polarographic method is presented for the determination of free cyanide in the presence of complex metallocyanides and large amounts of sulfides to show how empirically derived empirical factors can be employed to correct systematic errors. This procedure is based upon measurement of the anodic wave produced by the cyanide ion at the dropping mercury electrode. The bulk of the sodium sulfide, which produces an earlier wave and would interfere with the cyanide determination, is removed by treatment with a slightly less than stoichiometrical amount of silver nitrate. The cyanide ion losses, incurred by the presence of large amounts of the silver sulfide precipitate, can be corrected by a series of correction factors developed in this study.

IN CERTAIN cases when no accurate methods are available for carrying out a specified determination, it may be necessary to employ a procedure which is known to contain inherent systematic errors. In such a case, where the error can be mathematically defined and predicted from a knowledge of the sample, the use of correction factors may prove useful—for example, the determination of free cyanide in petroleum refinery units producing or fractionating light catalytic gases. This determination is important, for the free cyanide is thought to be related to a type of corrosion known as "hydrogen blistering" (*1*). Systematically low results were observed when 61 synthetic samples, containing various combinations of concentrations of contaminants, were analyzed for free cyanide using a polarographic method. These results were used as a basis to prepare a series of correction factors. The application of statistical techniques to the corrected values indicated that the use of correction factors would be satisfactory for practical purposes.

The complicating factors found in the determination of free cyanide in these types of refinery samples are the possible presence of high concentrations of soluble sulfides and complex metallocyanides (the free cyanide must be differentiated from the complex cyanides because the complex cyanides are not thought to cause hydrogen blistering).

The presence of large amounts of sulfides affects the accuracy of the determination. In many methods of determining the cyanide ion (*5, 7, 9, 11*) large concentrations of sulfide interfere and if the sulfide ion is removed by precipitation as a heavy metal sulfide (*4*), appreciable amounts of free cyanide will be occluded on the precipitate and hence low results will be obtained. The presence of the complex metallocyanide prevents the use of a precision acidic distillation technique—i.e., those of Fisher and Brown (*3*) and Serfass *et al.* (*10*)—for separating the free cyanide ion from a heavy metal sulfide because the metallocyanide complexes (as the ferrocyanide ion, known to be present in some of these samples) decompose to hydrocyanic acid on being heated in acidic solutions (*3, 7*).

Recent work of Dodge and Zabban (*2*), in studying the volatility of hydrocyanic acid, showed that at pH 6.5 simple cyanides could be volatilized as hydrocyanic acid; at this pH complex metallocyanides would not yield hydrocyanic acid. This work indicates that development of an analytical method for determining free cyanide ion may be possible.

In the absence of a developed procedure for analyzing these types of samples by distillation, it was decided to determine if the free cyanide could be determined with reasonable accuracy by employing a correction factor to compensate for the cyanide lost by occlusion on the heavy metal precipitate.

DEVELOPMENT OF ANALYTICAL PROCEDURE

The polarographic method, although not as sensitive as a colorimetric method, was employed for actually determining the free cyanide because it offered one advantage—namely, that the cyanide ion could be determined anodically in presence of moderate concentrations of sulfide ion. In preliminary experiments the sulfide ion concentration, which could be tolerated, was found to be about ten times the cyanide ion concentration, providing the sulfide ion concentration in the polarographic cell did not exceed $5 \times 10^{-4} M$. For this reason complete removal of the sulfide was unnecessary and the possible loss of cyanide ions by complexation with excess metal ion, used in precipitating the sulfide ion, was avoided.

The partial removal of the sulfides was effected by a potentiometric titration of an aliquot sample with silver nitrate solution. The titration was stopped just short of the end point, so as to avoid an excess of silver ions in the solution which may complex with the cyanide ions. Although this procedure proved satisfactory for samples containing small amounts of sulfides or large amounts of cyanides, low results were always obtained on synthetic samples when a large precipitate of silver sulfide was present in the treated portion and if the cyanide concentration was low. This dependence of the recovery of the cyanide ion on both the concentration of the cyanide and the weight of the silver sulfide present suggested that the cyanide loss was the result of a Freundlich-type adsorption. Although preliminary work indicated that with pure solutions Freundlich isotherm constants could be obtained over a limited range, subsequent work on plant samples indicated that this was not the only factor responsible for the losses. For example, sodium thiosulfate and sodium bisulfite, which could form by oxidation of the sodium sulfide present, could interfere with the polarographic cyanide wave. Furthermore, other oxidation products of sodium sulfide are elemental sulfur and sodium polysulfide which can react with the cyanide to form a thiocyanate. Because the rate of oxidation of the sodium sulfide to the thiosulfate, bisulfite, or polysulfide is related to the hydroxide ion, the $[OH^-]$ concentration of the original sample also becomes a variable that could influence the accuracy of the method.

CORRECTION FACTORS

To determine the magnitude of these errors, a series of 61 synthetic samples, containing varying amounts of sodium cyanide, sodium thiosulfate, sodium hydroxide, and sodium sulfide, was analyzed for sodium cyanide. (Sodium ferrocyanide was not included in the study because it has no effect upon the polarographic determination of the cyanide ion.) This series of samples was run in a $3 \times 3 \times 3 \times 2$ completely randomized factorial design so as to yield pertinent statistical information. The statistical examination of these raw data reveals that the largest effect was apparently due to the interaction of the sulfide ion and the cyanide ion concentrations, and that the remaining interactions were small. Since the sulfide ion could be readily approximated, an attempt was made to correct for the losses of cyanide ion due to the presence of sulfide ion. Therefore, another series of synthetic samples was prepared containing various sulfide and

cyanide ion ratios to simulate conditions in given aliquots of plant samples. From these values a series of correction curves was obtained and these correction factors were applied to the original 61 synthetic samples.

In preparing the synthetic samples sodium sulfide was prepared from hydrogen sulfide and sodium hydroxide because available commercial grades of sodium sulfide were contaminated with small quantities of sodium thiosulfate. Although the samples were blanketed with inert gas, no synthetic sample was allowed to remain longer than 48 hours before being analyzed.

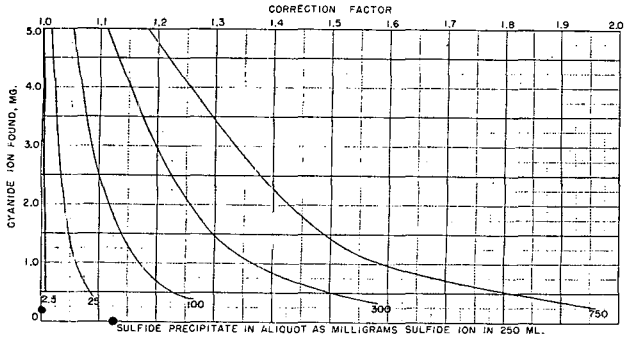

Figure 1. Cyanide Ion Correction Graph

The lower limit of detectability of the method using synthetic samples is about 0.00125 gram of cyanide ion per liter in the absence of appreciable quantities of sulfide. If the sulfide content is 30.0 grams per liter, the detectability of the method is reduced to 0.02 gram per liter, as the sample size has to be limited because of the excessive sulfide concentration.

The series of samples used for the preparation of correction factors was analyzed in a 5 × 5 complete factorial design using 0.5, 1.0, 2.0, 3.0, and 5.0 mg. of cyanide ion and 2.5, 25.0, 50.0, 100.0, and 750.0 mg. of sulfide ion. Two to five replicates were analyzed on each sample with a total of 70 determinations. The cyanide concentration of each sample was obtained according to the proposed procedure. To obtain the fraction of cyanide recovered, the average of the replicates of each sample was divided by the amount of cyanide present. The reciprocal of the fraction recovered represents the correction factor or that value by which the amount of cyanide found has to be multiplied in order to obtain the amount of cyanide originally present in the aliquot.

The calibration curve was prepared by plotting the correction factors against the amount of cyanide ion found for a given amount of sulfide in the aliquot. Figure 1 shows the correction curves for 2.5, 25, 100, 300, and 750 mg. of sulfide ion. (Extrapolations may be made for intermediate amounts of sulfide present.)

DISCUSSION OF POLAROGRAPHIC VARIABLES

The polarographic determination of cyanides is based upon the fact that, when an increasing positive voltage is applied to the dropping mercury electrode, an anodic wave is produced by the oxidation of the mercury. In ions forming complexes or insoluble compounds with the mercury, the anodic waves are shifted to more negative potentials depending upon the solubility product of the compounds or the dissociation constants of the complexes. The anodic current, produced by the oxidation of the mercury, is governed by the rate of diffusion of the anion to the electrode surface and hence is proportional to the anion concentration in the body of the solution.

While the half-wave potential of the cyanide ion is largely fixed by the solubility product of mercuric cyanide, a further variation in the half wave is due to the concentration effect. Kolthoff and Miller (*6*) have found that the following equation is applicable for predicting the shift in half wave due to concentrations for anions of this type:

$$E_{1/2} = \text{constant} - \frac{0.0591}{2} \log \text{concn. of anion} \qquad (1)$$

Figure 2, *A* and *B* are idealized polarograms of sodium sulfide and sodium cyanide, in 0.1*N* sodium borate solution (*adjusted to* pH 10.8) as the supporting electrolyte. For concentrations of $5 \times 10^{-4}M$, the half waves are about −0.63 and −0.26 *vs.* the saturated calomel electrode. Therefore the oxidation waves were spaced sufficiently far apart to allow the formation of well-defined waves. However, there were certain limits to the amount of sulfide ion that could be present and to the ratio of the sulfide to the cyanide ion. If these limits were exceeded the sulfide wave would merge into the cyanide one. For this reason it was necessary to study the effect of sulfide concentration upon the accuracy of the cyanide determination and the changes in the location of the waves produced by the concentration. The effect of the hydroxyl ion concentration was likewise considered.

During the studies with synthetic sodium sulfide samples the amount of sodium thiosulfate that was present as a result of the sulfide oxidation was found to be sufficiently high to interfere with the accurate cyanide determination. Sodium thiosulfate has a half wave of approximately −0.10 (Figure 2, *C*) which falls between the cyanide (Figure 2, *B*) and the hydroxide waves and is close enough to the cyanide wave to cause trouble if it is present in sufficient quantities.

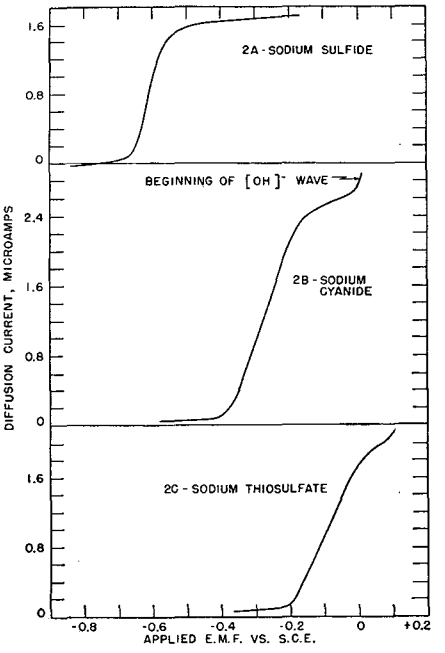

Figure 2. Anode Waves Given by Sulfide, Cyanide, and Thiosulfate Ions with Dropping Mercury Electrode

Anion concn., $5 \times 10^{-4}M$
Supporting electrolyte, sodium hydroxide and boric acid, adjusted to pH 10.8

Effect of Sulfide Ion Concentration. A well-defined sulfide wave can be obtained at about −0.63 volt when the concentration is about $5 \times 10^{-4}M$. At concentrations significantly above this value, the wave becomes irregular in shape and the apparent half wave shifts appreciably to more positive voltages. The irregular shape of the wave causes it to merge with the cyanide wave. The erratic behavior of the sulfide is presumably due to the coating of the mercury drops with a film of mercuric sulfide.

Because a cell concentration of sulfide in excess of $5 \times 10^{-4}M$ is undesirable, and the cyanide concentration in many samples is several hundred times smaller than that of the sulfide, some of the sulfide must be removed because the amount of cyanide in samples containing the appropriately low concentrations of the sulfide would not be detected on the polarograph. This removal

can be facilitated by selecting an adequate size sample, so as to yield a cyanide concentration in the cell of at least $10^{-5}M$, and potentiometrically titrating with $0.25M$ silver nitrate solution. The titration is stopped just short of the inflection point, leaving a small amount of sulfide in the sample which, being below $5 \times 10^{-4}M$, will not interfere with the subsequent cyanide wave.

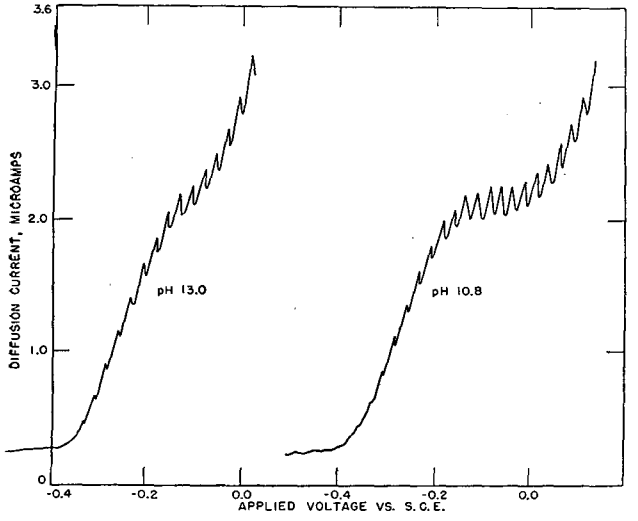

Figure 3. Effect of Hydroxyl Ion on Cyanide Determination

Effect of Hydroxyl Ion Concentration. Figure, 2, *B*, shows the beginning of the oxidation wave of the hydroxyl ion which is present in the electrolyte. From Equation 1 it may be observed that for every tenfold increase in the hydroxyl ion concentration, the half-wave potential of that ion shifts approximately 0.03 volt in a more negative direction; and conversely, for every tenfold decrease in the cyanide ion concentration there is a 0.03-volt shift of its half wave in a more positive direction. Thus, for samples of low cyanide concentration and high pH, there is a possibility that the two waves may approach each other sufficiently closely to make the measurement of the cyanide wave difficult. For this reason it was decided to reduce the hydroxyl ion concentration to as low a value as possible, so as to thrust its wave in a positive direction. However, too low a pH is undesirable because of the ease with which sodium cyanide can be hydrolyzed to hydrocyanic acid and lost to the solution during the bubbling operation to remove dissolved oxygen. Calculation of the appropriate pH of the equivalence point of sodium hydroxide and hydrocyanic acid to yield a $0.01N$ solution of the salt revealed that this value was 10.6. Therefore, some value slightly above this pH was selected. In Figure 3 the effect of the hydroxyl ion on the cyanide wave shows how the two waves tend to merge if the pH is too high.

To achieve the desired pH of 10.8 ± 0.2, the excess sodium hydroxide in the aliquot is neutralized with a saturated solution of boric acid beyond the equivalence point (pH 11.3 for $0.1M$ sodium orthoborate) to the desired pH.

Effect of Thiosulfate. In using a synthetic sample of sodium sulfide and sodium cyanide with the pH of the solution reduced to 10.8, the hydroxyl ion oxidation wave seemed to begin much too early and appeared on the shoulder of the cyanide wave. This type of poorly defined cyanide wave was also found in some plant samples. Upon investigation sodium thiosulfate was found to be formed by oxidation of the sodium sulfide in presence of water:

$$2Na_2S + 2O_2 + H_2O \rightarrow Na_2S_2O_3 + 2NaOH$$

The half-wave potential of a $5 \times 10^{-4}M$ solution of sodium thiosulfate is shown in Figure 2, *C*, as being −0.10 volt *vs.* the saturated calomel electrode.

To study the effect of thiosulfate upon the cyanide determination a series of synthetic blends was prepared containing known amounts of sodium cyanide and sodium thiosulfate. The results are given in Table I. When the thiosulfate ion concentration in the cell is less than or approximately equal to $5.0 \times 10^{-4}M$, the accuracy is satisfactory. Higher concentrations of thiosulfate produce lower cyanide results, or entirely prevent its measurement by merging with the cyanide wave. Hence the presence of more than $2.0 \times 10^{-3}M$ (cell concentration) thiosulfate forms one of the limitations of the method. This restriction, however, is not too serious, as this would correspond to a large amount of thiosulfate in the original sample, and usually the sample can be diluted so that the thiosulfate will be below the critical cell concentration. In the event the thiosulfate is unusually high and merges with the cyanide wave, the analysis is of no value because of the alteration in the position and shape of the polarographic wave.

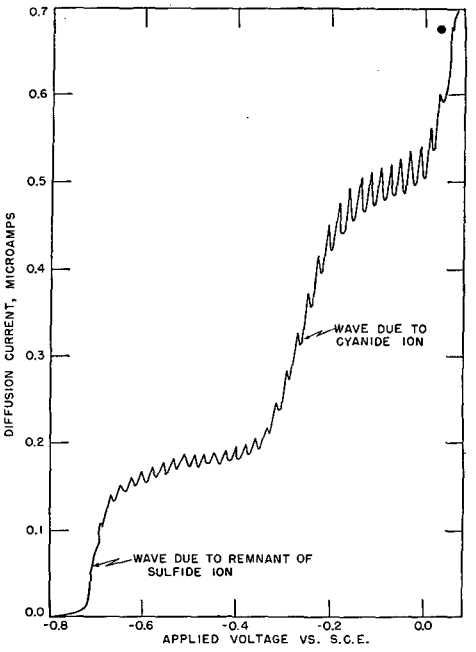

Figure 4. Polarogram of Typical Plant Sample

Table I. Effect of Thiosulfate on Cyanide Determination

$S_2O_3^{--}$ Added, Cell Concn., M	CN^-, Cell Concn., M		CN^- Recovery, %
	Added	Found	
0.0	5.29×10^{-4}	5.33×10^{-4}	+ 0.8
2.60×10^{-4}	1.06×10^{-3}	1.04×10^{-3}	− 2.0
5.20×10^{-4}	1.06×10^{-3}	1.05×10^{-3}	− 1.0
1.04×10^{-3}	1.06×10^{-3}	1.00×10^{-3}	− 6.0
1.04×10^{-3}	5.29×10^{-4}	4.80×10^{-4}	− 9.3
2.08×10^{-3}	5.29×10^{-4}	4.60×10^{-4}	−13.0
5.20×10^{-3}	5.29×10^{-4}	...[a]	
1.04×10^{-2}	5.29×10^{-4}	...[a]	

[a] Cyanide wave could not be measured, as it merged with thiosulfate wave.

ANALYTICAL PROCEDURE

Apparatus. POLAROGRAPH, Sargent, Model XXI.

H-TYPE POLAROGRAPHIC CELL, fitted with calomel half cell (*8*).

TITRATOR, such as the Fisher Titrimeter, equipped with stirring motor and stand.

ELECTRODES FOR TITRATOR. Calomel electrode, Beckman #4970; silver wire electrode, Beckman #1281-5; and glass elec-

trode, Beckman Type E, for sodium solutions in range of pH 9 to 14.

NITROGEN GAS, CYLINDER, AND REGULATOR, oxygen-free, for bubbling sample to remove dissolved oxygen.

Solutions. Sodium hydroxide, 0.5*N*.

Boric acid, 0.4*M*. Dissolve 12.36 grams of boric acid in about 250 of nearly boiling water. Make up volume to 500 ml. with cool water. Adjust to volume when solution reaches room temperature.

Silver nitrate, 0.25*N*.

Sodium cyanide stock solution, 0.2500 gram per liter of CN^-. Dissolve 0.2466 gram of 95.5 weight % sodium cyanide in 0.5*N* sodium hydroxide solution, and make up to 500 ml. with the alkaline solution. The true cyanide content of each batch of sodium cyanide should be determined by an argentimetric titration (*5*).

Procedure. CALIBRATION. Prepare sodium cyanide stock solution containing 0.2500 gram per liter of CN^-. Pipet 10 ml. of this solution into a 250-ml. beaker containing about 25 ml. of 0.5*N* sodium hydroxide solution. Add about 100 ml. of water; adjust to an end point of pH 10.8 with boric acid; make up volume to 250 ml. with water. Transfer a portion of the solution to the polarographic cell and obtain polarogram. Use this standard to calculate concentration of sample.

SAMPLE MANIPULATION. Add 25 ml. of 0.5*N* sodium hydroxide to a 250-ml. beaker, and pipet a portion of the sample into this solution. The amount of sample selected should be governed by the cyanide and the sulfide content—the object being to use an amount of sample that contains a minimum of sulfide and yet contains 0.25 to 10.0 mg. of cyanide.

If the sulfide content cannot be estimated at this time, an arbitrary amount of sample may be selected and this step repeated after the sulfide content has been approximated. Add distilled water to bring volume to about 100 ml. and prepare to titrate potentiometrically with 0.25*N* silver nitrate solution (or any convenient concentration) using a silver sulfide and a calomel electrode. Prepare silver sulfide electrode by immersing silver electrode in a solution of sodium sulfide of any convenient concentration—such as, 5 grams of sodium sulfide per liter—until a uniform black coating of silver sulfide is obtained. Insert electrodes and stirring device into solution and connect electrodes to a potentiometer, such as the Fisher Titrimeter (or the Beckman Model G pH meter). Add silver nitrate solution slowly to remove the bulk of the sulfide present. Plot the voltage readings against volume of silver nitrate added after the addition of each incremental portion and stop the titration at the beginning of the first break in the curve. This will leave a small amount of sulfide ion in the solution which does not interfere with the polarographic cyanide wave; an excess of silver, however, would cause low results. After the technique has become familiar, an actual plot will not be necessary for the approach of the sulfide end point will be indicated by the increasingly large changes in voltage per addition of a unit volume of titrant. If the product of the volume of silver nitrate, in milliliters, times its normality exceeds 46.8, it is advisable to discard that portion of the sample and to select a smaller aliquot of the sample so that the silver nitrate titer times its normality does not exceed that value. Disconnect the silver sulfide electrode from the instrument and remove from beaker. Replace it with the glass electrode. (If instrument is a Beckman Model G pH meter, throw switch on instrument so that dial will read pH.) Add 0.4*M* boric acid solution until pH reads 10.8. Allow bulk of precipitate of silver sulfide to settle, and transfer solution to a 250-ml. volumetric flask. Wash the precipitate remaining in the beaker with at least three changes of water. The solution in the volumetric flask does not have to be perfectly clear, for small amounts of silver sulfide have no effect on the results. Make up volume to 250 ml. with water and mix. Transfer a portion of this solution of an H-type polarographic cell having a calomel half cell built in the cell and connected to the sample-containing portion by a conventional agar plug. Insert dropping mercury electrode into the solution and then bubble the solution 5 minutes with nitrogen to remove dissolved oxygen. Set dials on polarograph, so that an anodic wave can be recorded, with the initial voltage being set at about −0.8 volt and the applied voltage across the cell increasing in a positive direction. (For a Sargent Model XXI polarograph, flip the switches so that they are on opposed, positive, and 3-volt span, and manipulate the dials, so that voltmeters read 2.0 volts on bridge, and 0.8-volt initial voltage.) Obtain polarogram over the range of −0.8 volt to about −0.1, which is the top of the cyanide wave. Measure the height of the cyanide wave (Figure 4) which appears between −0.4 and −0.2 volt. (The position of the half wave shifts slightly as the cyanide concentration varies.) The earlier wave beginning at about −0.8 is due to the sulfide which was allowed to remain in sample. If too much sulfide has been left in the sample from the silver nitrate titration, this wave will be irregular and large enough to merge with the cyanide wave. In such a case, repeat the determination, making certain that more of the sulfide is removed. On the other hand when analyzing sulfide-bearing samples, if no sulfide wave is present, it may mean that too much silver nitrate was added and hence the cyanide results may be low. In such a case, repeat the determination.

Table II. Cyanide Concentrations in Synthetic Samples after Application of Empirical Correction

		S^{--}, 0.1 G./L.				S^{--}, 3.0 G./L.				S^{--}, 30 G./L.			
		NaOH, G./L.											
		1.0		25.0		1.0		25.0		1.0		25.0	
		CN^- Found											
$S_2O_3^{--}$, G./L.	CN^-, G./L.	G./L.	%	G./L.	%	G./L.	%	G./L.	%	G./L.	%	G./L.	%
0.0	0.02	0.019	95	0.02	100	0.015	75	0.18	90	0.020	100	0.024	120
	0.10	0.101	101	0.101	101	0.100	100	0.086	86	0.101	101	0.096	96
										0.125	125		
	1.0	1.02	102	0.99	99	0.95	95	1.07	107	0.98	98	0.93	93
0.04	0.02	0.018	90	0.020	100	0.021	105	0.020	100	0.023	115	0.021	105
				0.021	105								
	0.10	0.099	99	0.097	97	0.106	106	0.095	95	0.100	100	0.090	90
		0.101	101										
	1.0	1.03	103	1.02	102	1.02	102	1.05	105	1.12	112	1.11	111
		1.03	103										
0.15	0.02	0.016	80	0.015	75	0.020	100	0.020	100	0.021	105	0.020	100
												0.018	90
	0.10	0.094	94	0.088	88	0.090	90	0.099	99	0.080	80	0.09	90
						1.101	101	0.095	95				
	1.0	1.00	100	0.96	96	1.00	100	1.02	102	1.06	106	1.01	101

Table III. Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	*F*
CN	11.14659804	2	5.57329902	4098.28[a]
S	0.00098193	2	0.00049096	N.S.[b]
OH	0.00003585	1	0.00003585	N.S.
S_2O_3	0.00568248	2	0.00284124	N.S.
CN × S	0.00211851	4	0.00052963	N.S.
CN × OH	0.00003448	2	0.00001724	N.S.
CN × S_2O_3	0.00956430	4	0.00239108	N.S.
S × OH	0.00244904	2	0.00122452	N.S.
S × S_2O_3	0.00416474	4	0.00104118	N.S.
OH × S_2O_3	0.00021515	2	0.00010758	N.S.
CN × S × OH	0.00543963	4	0.00135991	5.23[c]
CN × S × S_2O_3	0.00946082	8	0.00118260	4.55[c]
CN × OH × S_2O_3	0.00100252	4	0.00025063	N.S.
S × OH × S_2O_3	0.00079363	4	0.00019841	N.S.
CN × S × OH × S_2O_3	0.00207970	8	0.00025996	5.04[c]
Error	0.00036100	7	0.00005157	
Total	11.19062082	53		

[a] Statistical significance at 0.1% level.
[b] Not significant.
[c] Statistical significance at 5% level.

Calculation. From the silver nitrate potentiometric titration approximate the amount of sulfide precipitate in the sample aliquot, using the following equation:

$$[S]^{--} \text{ in aliquot, mg.} = \text{vol. of } AgNO_3\text{, ml.} \times \text{normality of } AgNO_3 \times 16 \quad (2)$$

Calculate weight of cyanide ion found in sample aliquot:

$[CN]^-$ found, mg. uncorrected = current produced by sample × volume to which sample portion diluted × wt. of $[CN]^-$ present in standard, mg./current produced by standard × volume to which standard was diluted (3)

The volume to which the sample was diluted and volume to which standard was diluted cancel, as the procedure recommends that in both cases the volume be made up to 250 ml.

Knowing the amount of the sulfide precipitate and the amount of the cyanide ion found, use the correction graph, Figure 1, to find correction factor.

Calculate concentration of $[CN]^-$ in sample:

Table IV. Summary of Accuracy and Reproducibility

(Concentration of principal components, grams per liter)

	Cyanide Ion								
	0.02			0.10			1.00		
	Sulfide Ion								
	0.10	3.00	30.0	0.10	3.0	30.0	0.10	3.00	30.0
Av. CN^- found, g./l.	0.018	0.019	0.021	0.097	0.096	0.097	1.01	1.01	1.04
St. dev. (single det.), ±	0.0022	0.0022	0.0020	0.0047	0.0064	0.0141	0.0256	0.0407	0.0750
St. dev. from true value, ±	0.0025	0.0026	0.0023	0.0057	0.0073	0.0143	0.0261	0.0430	0.0840
% error	12.5	13.0	11.5	5.7	7.3	14.3	2.61	4.3	8.4

Concn. of $[CN]^-$ in original sample, g./l. = uncorrected $[CN]^-$ found, mg. × correction factor/sample aliquot, ml. (4)

EVALUATION OF RESULTS

Statistical Data on Synthetic Samples. The results of each of the 61 samples (which contained all possible combinations of three levels each of cyanide concentrations, thiosulfate concentration, and sulfide concentration, and two levels of sodium hydroxide concentration) were multiplied by the appropriate correction factor based upon the amount of cyanide and sulfide found in the aliquot. The corrected values are reported in Table II.

The analysis of variance (*12*), given in Table III, and the corrected data in Table II show that the $[CN]^-$ variation is now the only significant one. The 5% level of significance of [CN] × [S] × [OH], the [CN] × [S] × $[S_2O_3]$, and the [CN] × [S] × [OH] × $[S_2O_3]$ interactions, indicate that these interactions are statistically, but probably not chemically, significant.

A summary of the results showing the accuracy and reproducibility of the over-all method, using the correction factors, is presented in Table IV.

Plant Samples. In the absence of any plant samples of known cyanide ion content, the method was evaluated by adding known amounts of sodium cyanide to plant samples. The results of such a series obtained on 12 different samples from six sample points are shown in Table V. Reasonable recoveries of the computed amounts of cyanide ion were obtained. Some results, however, are low by as much as 17%. This procedure is believed to be actually better than this method of evaluation indicates, because certain plant samples contain some contaminant which reacts with the cyanide ion. Because this reaction is not instantaneous but is partially dependent upon the concentration of the cyanide ion, any increase in the cyanide ion concentration would result in an equilibrium shift.

A sample of this type, in which some reactive sulfur types may be present, is continually changing. To illustrate this instability a typical sample of this type was selected and allowed to stand in the laboratory in a screw-top glass bottle for 25 days. Free cyanide determinations were carried out periodically. The data presented in Table VI show that the free cyanide content had fallen to about one third of the original concentration in 25 days. Also, the sulfide ion concentration decreases. The sulfide was thought to be slowly oxidated by air to a polysulfide which, in turn, reacted with the cyanide to form a thiocyanate. Qualitative tests for both the polysulfide and the thiocyanate have been obtained on these samples which have been allowed to oxidize. This emphasizes the importance of protecting samples from oxidation and analyzing them as soon as possible.

Table V. Analyses of Plant Samples

Sample			Grams per Liter				
			Sulfide,	CN^-		Tot. CN^-,	Rec.,
Description	Point	No.	found	Found	Added	found	Wt. %
Cat. cracker condensate accumulator water	D-3-1	1	2.32	0.036	0.037	0.076	104.0
	D-3-1	2	2.68	0.046	0.187	0.230	98.6
	D-22-1	3	8.2	0.15	0.46	0.63	97.0
	D-22-1	4	7.5	0.17	0.28	0.46	102.1
	D-2-2	5	10.4	0.81	0.38	1.07	83.0
	D-2-2	5′[a]	10.4	0.81	0.77	1.45	91.8
	B.S.	6	2.3	0.0	0.18	0.17	94.5
	B.S.	7	4.2	0.0	0.037	0.035	94.6
	B.S.	8	4.6	0.0	0.037	0.040	108.0
Depentanizer overhead accumulator water	D-23-1	9	10.0	0.41	0.93	1.15	86.0
	D-23-1	10	9.0	0.37	0.37	0.65	87.9
Depropanizer overhead accumulator water	D-3-2	11	10.1	0.82	0.38	1.17	97.5
	D-3-2	12	10.9	0.91	0.46	1.22	89.1

[a] No. 5′ is the same as 5 except a different amount of NaCN was added.

Table VI. Loss of Free Cyanide on Storage

(In plant sample containing sulfide ion)

Days of Storage[a]	Cyanide Ion, G./L.	Sulfide Ion, G./L.
0	1.17	10.6
3	1.15	9.8
7	0.94	8.4
25	0.36	4.4

[a] Stored in screw-top glass bottle.

Although the use of these correction factors does not yield results as precise as may be desired, the over-all method is sufficiently accurate and precise to be usefully employed in plant control for many practical purposes.

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LITERATURE CITED

(1) Bonner, W. A., Bornham, H. O., Conradi, J. J., and Skei, T., "Prevention of Hydrogen Attack on Steel in Refinery Equipment," American Petroleum Institute, Division of Refining, New York, N. Y., May 12, 1953.
(2) Dodge, B. F., and Zabbon, W., *Plating*, 39, 1133–9, 1235–41 (1952).
(3) Fisher, F. B., and Brown, J. S., ANAL. CHEM., 24, 1440 (1952).
(4) Karchmer, J. H., and Dunahoe, J. W., *Ibid.*, 20, 911 (1948).
(5) Kolthoff, I. M., and Furman, M. H., "Potentiometric Titrations," 2nd ed., p. 159, Wiley, New York, 1931.
(6) Kolthoff, I. M., and Miller, C. S., *J. Am. Chem. Soc.*, 63, 1405, 2732 (1941).
(7) Kruse, J. M., and Mellon, M. G., ANAL. CHEM., 25, 446 (1953).
(8) Lingane, J. J., and Laitinen, H. A., IND. ENG. CHEM., ANAL. ED., 11, 504 (1939).
(9) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., p. 661, ed. by H. Furman, Van Nostrand, New York, 1949.
(10) Serfass, E. J., Freeman, R. B., Dodge, B. F., and Zabbon, W., *Plating*, 39, 267–73 (1952).
(11) Smith, R. G., *J. Am. Chem. Soc.*, 51, 1171–4 (1929).
(12) Youden, W. J., "Statistical Methods for Chemists," Wiley, New York, 1951.

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Flame Photometric Study of Boron

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This study was undertaken to develop a flame photometric method for boron and, in particular, to adapt it to the Beckman DU spectrophotometer with the Model 9220 flame attachment and photomultiplier unit. The effects of acid and methanol concentration and of various anions and cations commonly associated with boron upon the flame emission of boron in 1 to 1 methanol-water solution were studied for the three prominent oxide band systems: 492, 518, and 546 mμ. The interference of many elements was of sufficient magnitude to necessitate making a pseudo-background correction by measuring the luminosity at the minima or troughs between the overlapping band systems of boron. Compensation by this means rendered interference effects by many elements negligible. The flame photometric method is more rapid than existing chemical methods and is comparable in accuracy and precision to them. Optimum range of applicability is 50 to 200 p.p.m. of boron. Sensitivity is within 1 to 3 p.p.m., depending upon particular phototube response.

THIS investigation describes the application of the flame photometer to the rapid, routine determination of boron. The method should be of particular interest to those laboratories that are concerned with borohydrides and other boron-containing compounds. The flame photometric procedure offers a rapid instrumental means of determining boron compared to conventional distillation methods and is particularly applicable to the processing of a large number of samples. The boron-containing compound can be dissolved in an appropriate solvent and the resulting solution aspirated directly into the flame. Thus, all preliminary decompositions, either by wet or dry methods, are no longer necessary. A considerable saving in time results and there is no longer any danger that part of the sample will be lost, through either incomplete digestion or volatilization during the preliminary decomposition step.

Determinations of boron by means of the green flame coloration are discussed by Stahl (*13*) and applied to agricultural materials by McHargue (*9*), McHargue and Calfee (*10*), Calfee and McHargue (*3*), and Weber and Jacobson (*14*). These workers used either visual methods or photographic recording in conjunction with a spectrograph. It would be desirable to have a method available which would permit the use of modern flame photometers.

GENERAL EXPERIMENTAL WORK

Apparatus. A Beckman Model DU spectrophotometer with Model 9220 flame attachment and photomultiplier unit was used. The spectrophotometer has been described (*4*).

An all-metal atomizer-burner unit, supplied with the flame attachment, was used as the excitation source. The gases chosen were oxygen and acetylene, largely because of availability. However, the higher excitation energy available from the oxy-acetylene flame, as compared with an oxygen-hydrogen flame, was a prominent consideration.

Reagents. A standard solution of boron, 1.00 ml. equivalent to 1.00 p.p.m. as boron, was prepared by dissolving 5.715 grams of fresh crystals of reagent grade boric acid in demineralized water and diluting to 1 liter.

A typical flame photometric standard solution, containing 100 p.p.m. of boron, was prepared by pipetting out 10.0 ml. of the first solution, adding (by pipet) 50.0 ml. of drum grade methanol, and diluting to volume in a 100-ml. volumetric flask with demineralized water. For storage, all solutions should be transferred to polyethylene containers.

Demineralized water, used exclusively in preparing all solutions and samples, was prepared by passing ordinary distilled water through a bed of Amberlite MB-3 resin.

Spectrophotometer Settings. The instrument settings used for measuring the boron flame emission were as follows:

Sensitivity control	5 to 6 turns from clockwise limit
Selector switch	0.1
Phototube resistor	22 megohms
Slit	0.030 mm.
Acetylene	5 pounds per square inch
Oxygen	8 to 16 pounds per square inch, depending upon burner used

Individual operators should be aware that different burners, even though of similar construction, will not necessarily reproduce the tabulated luminosities for the operating conditions used in this work. In particular, obstructions in or around the oxygen orifice, often due to accumulating carbon deposits, will affect not only the flow of oxygen but also the rate of aspiration of the solution under examination. These factors will alter flame temperature and therefore will affect both the flame background and the boron luminescence. Consequently, the burner should be cleaned frequently.

Table I. Oxide Band Systems of Boron in Flames

Wave Length of Band Maxima, Mμ	Rel. Intensity of Band System, Min. Quantity Easily Detectable, P.P.M. of Boron[a]
345	200
452/454	30
471/473	10
492	5
518	3
545/548	3
577/580	10
603	. . .[b]
620	. . .[b]
639	. . .[b]

[a] For slit width of 0.03 mm.
[b] Relative intensities not reported by Singh (*12*), but found to be very low in this work.

Table II. Relative Emissivity of Boron from Methanol-Water Solutions[a]

Methanol present, ml./100 ml. total soln.	0	20	50	75	90
Emission intensity, transmittance scale units	5	12	34	53	85

[a] Boron concentration 100 p.p.m. in all solutions.

Characteristics of Boron in the Flame. Certain molecules which can exist in the oxyacetylene flame may be excited to emit band spectra, also known as molecular spectra. Such a molecule is B_xO_y. The general characteristics of band systems are that they arise from transitions between a few of the lowest electronic levels of the molecule concerned. With each electronic level is associated a suite of vibrational levels, and with each vibrational level is associated a suite of rotational levels. These latter two transitions cause the emitted radiation to be spread over a portion of the spectrum rather than being concentrated in a discrete line. The radiation is centered about the wave length associated with the electronic transition, with the energy of the bands degraded either toward the red or the blue portion of the spectrum. Consequently, the bands are not symmetrical about any center. The fine structure is not observable with the dispersion obtained from the optics of the Beckman spectrophotometer; rather only the envelope is observed.

Because the excitation potential of B_xO_y falls within the exitation range of the oxyacetylene flame, the emission of narrow bands attributable to the B_xO_y molecule is observed when boron is introduced into the flame. These boron flame bands belong to molecules which are electrically neutral but are not stable in the chemical sense (*11*). Gilbert and associates (*7*) reported six oxide bands, and from photographic studies Singh (*12*) has reported four additional band systems. The wave length of the band head of these oxide bands together with the relative intensities of each are listed in Table I. Strangely, Lundegårdh (*8*) reported no flame spectra for boron in his pioneering investigations.

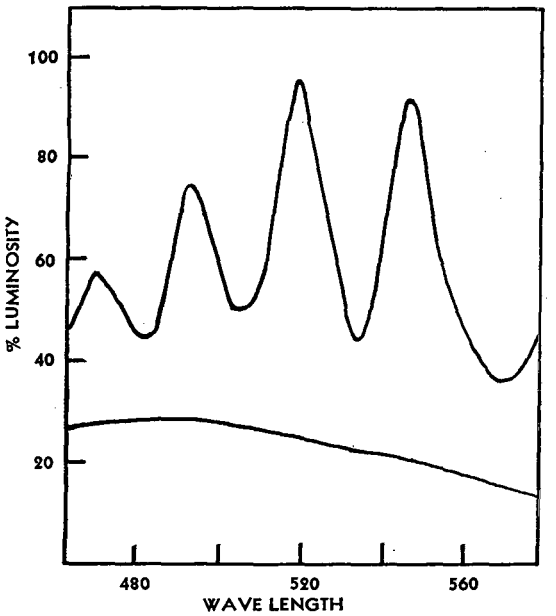

Figure 1. Emission Spectrum of Boron in 1 to 1 Methanol-Water Solution

Present. 200 p.p.m. of boron
Slit width, 0.030 mm.
Lower line is background of solvent alone

Most of the boron band systems overlap each other. In Figure 1 is given the flame emission spectrum of boron in the region suitable for the flame photometric determination of boron. The more intense flame emissions occur from the band groupings centered around 492, 518, and 546 mμ.

The intensity of the boron emission is influenced strongly by the nature of the solvent. Introduction of methanol causes a marked increase in the intensity of the boron bands. A continual increase is experienced as the methanol concentration is increased. Table II gives the relative emissivities for a series of methanol-water solutions. The effect may be due in part to the formation of methyl borate, but more probably is caused by the lowered surface tension of the solution undergoing aspiration into the flame. Other alcohols also enhance the emission characteristics of the boron oxide bands but to a lesser degree. Ethanol and 2-propanol, for example, are roughly 75% as effective as methanol, volume for volume. The effect of acetone and dioxane on the relative emission has been reported (*1*). Unfortunately, this report was called to the authors' attention toward the conclusion of this work. Apparently a slight advantage would be gained from the use of either of these two solvents in place of methanol. It is further apparent that any advantage accruing from the use of any of the aforementioned solvents must be due to injection of larger amounts of sample into the flame because of lowered surface tension of the aspirated solution, rather than the formation of a more volatile boron compound involving the solvent. The recent paper by Caton and Bremner (*5*) should be consulted. Curtis *et al.* (*6*) found that greater intensity and sensitivity may be obtained by atomization from certain hydrocarbon and non-hydrocarbon solvents than by solubilizing and atomizing from aqueous solutions.

All of the data reported subsequently in this paper were obtained from solutions and standards which were composited from aqueous solutions and an added amount of methanol equal to 50% of container volume employed for the final dilution. To ensure uniformity, the methanol was added before diluting to volume, so that additional demineralized water could be added to care for the volume contraction which occurs upon mixing aqueous solutions with methanol.

Calibration Curve. The overlapping band systems of boron presented a problem not often encountered in flame analyses: how to choose a general background reference wave length. Usually one refers to the general background reading in the vicinity of the band head or emission line in order to determine the correction to be applied to the observed emission at the wave length of the band head or emission line. Because the band systems of boron overlap in the useful region, this procedure is not possible. Fortunately, the minimum intensity in the troughs between the band heads will serve the same purpose as a normal background reading.

The calibration curve for boron is strictly linear up to at least 300 p.p.m. of boron. The luminosity reading, given by the minimum in an adjacent trough, subtracted from the luminosity reading of the neighboring band head, gave a net relative luminosity which was plotted against the concentration of boron present in the respective standard solution. Calibration curves were constructed from a series of standards each time a set of samples was analyzed.

Table III. Consistency of Proposed Method of Calculation

Fuel Pressure, Lb./Sq. Inch			Emission in Scale Divisions				Ratio of Emission at 518 mμ − Background to Difference
Acetylene	Oxygen	Slit Width, Mm.	Wave length, 518 mμ	Wave length, 505 mμ	Difference, 518 mμ − 505 mμ	Boron Present, P.P.M.	
4	16	0.03	27	29	−2	0	...
			42.5	35	7.5	50	1.63
			55	40	15	100	1.66
			69	45	24	150	1.61
			82	49	33	200	1.57
			107	58	49	300	1.57
5	16	0.03	36	33	3	0	...
			44	36	8	50	1.60
			52	39	13	100	1.60
			62	42.5	19.5	150	1.57
			70	45.5	24.5	200	1.58
			88	52	36	300	1.58
5	16	0.06	10	11	−1	0	...
			24	14	10	25	1.27
			32	18.5	13.5	50	1.51
			42	22	18	75	1.58
			50	24.5	25.5	100	1.51
						Av.	1.58 ± 0.04

Table III illustrates the consistency of this method of calculation for different operating conditions. The data were obtained from measurement of the emission intensities at the boron oxide band system maximum located at 518 mμ and from the minimum point in the valley or trough at 505 mμ, which lies between the band heads at 492 and 518 mμ. The other band heads and troughs could also have been chosen and would further verify the conclusions. In columns 4 and 5 of Table III are listed the emission intensities observed as instrument scale divisions for the respective concentrations of boron given in column 7. Normally a calibration curve is graphed simply by taking the observed emission reading (column 4), subtracting from it the background reading observed for the solvent blank alone (the first line across

for no boron present), and plotting these difference values against the respective concentrations (column 7). Instead, the emission reading obtained at 505 mμ (column 5), the minimum in the trough between the 492 and 518 band heads, is subtracted from the emission readings at 518 mμ (column 4), and these differences are plotted against the respective boron concentrations (column 7). If this alternative method of graphing the data is valid, the ratio of the differences obtained in each case should be constant. That these are constant within experimental uncertainties is shown by the agreement of the values in column 8. The background emission is not the same at the two wave lengths and this necessitates a corrective term to be added to the difference values: 518 mμ − 505 mμ. Other pairs of wave lengths equally applicable are the 492-mμ band head and the 482-mμ trough minimum and the 546-mμ band head and the 536-mμ trough minimum.

Influence of Various Elements. EFFECT OF CATIONS. A major part of the experimental work in this research was concerned with determining the radiation interference, if any, caused by the various cations generally associated with boron. For each substance tested for interference, a series of methanol-water solutions was prepared containing several known concentrations of the test substance and generally 100 p.p.m. of boron. Table IV shows the effect of various cations on the determination of boron. For much of the earlier work the boron band head at 518 mμ was used with the background taken at 505 mμ. However, because of serious interference from certain cations at this pair of wave lengths, the study was extended to the 492- and 546-mμ band heads, with background readings taken at 482 and 536 mμ, respectively. As a further check on interference effects, the emission characteristics of each cation individually were determined in the methanol-water solution in the absence of boron. These results for the more important interfering cations are shown as Figures 2, 3, and 4. On these plots the little arrows indicate the pairs of wave lengths used when measuring the boron flame emissions: the 492-mμ peak and 482-mμ background, 518-mμ peak and 505-mμ background, and the 546-mμ peak and 535-mμ background.

From these studies the cations investigated can be grouped roughly into four categories:

1. Elements which offer no interference and whose emission spectrum in methanol-water is indistinguishable from that of the solvent blank. These are ammonium, cadmium, copper, lead, silver, and zinc ions.
2. Elements exhibiting general background radiation. This occurs with most of the cations when present in relatively high concentrations. Note the emission spectrum of potassium in Figure 2. This type of radiation, to a great extent, is compensated by the method of measurement employed. Very large changes in general background radiation were without effect upon the recovery of boron.
3. Coincidences or near coincidences, such as the enhancement of the boron 518-mμ band head by magnesium 518 or the overlap of a weak potassium band with the 505-mμ reference trough (Figure 2). Sometimes the latter type of interference can be mitigated by the use of narrow slits, as with potassium, but with a corresponding loss in sensitivity for boron. Enhancement from calcium will be significant unless small slit widths, 0.03 mm. or less, are used, since the boron 518-mμ band is adjacent to the short wave-length side of the prominent calcium 553-mμ band (Figure 3).
4. General interference at all of the prominent boron band systems and their intervening trough minimums is observed for chromium, manganese, iron, cobalt, and nickel.

Effect of Anions. The effect of various anions was next de-

Table IV. Effect of Cations in Determination of Boron by Flame Photometry

(100 p.p.m. of boron present in all cases)

Cation Tested	Concn., P.P.M.	Boron Found, P.P.M. at 492 mμ	518 mμ	546 mμ
Aluminum	3000	...	...[a]	96
	2000	...	...	98
	1000	133	130	98
	500	113	112	100
Ammonium	3000	100	100	100
	1000	100	100	100
Cadmium	3000	...	96	...
	2000	...	94	...
	1000	...	94	...
	500	...	96	...
Calcium	2000	94	127	...
	1000	96	107	...
	500	97	102	...
	100	100	100	...
Chromium	3000	...	136	97
	2000	...	...	106
	1000	...	136	99
	500	...	118	100
	100	...	100	99
Cobalt	400	...	110	90
	300	...	106	...
	200	...	103	95
Copper	1000	152	100	80
	500	126	100	90
Iron	500	...	127	...[a]
	300	110	118	...
	100	102	104	...
Lead	1000	100	103	100
	400	100	100	100
Lithium	3000	78	84	90
	2000	97	95	96
	1000	100	95	96
	500	105	100	99
Magnesium	3000	...	...	106
	2000	...	...	109
	1000	...	...	96
	400	142	142	102
	100	109	108	102
Manganese	200	...	127	...
	100	...	101	...
Nickel	500	...	111	92
	100	...	103	99
Potassium	3000	110	88	37
	2000	103	100	58
	1000	103	100	79
	500	100	100	89
	100	...	100	98
Silver	3000	...	100	...
Sodium	3000	...	103	...
	2000	103	103	130
	1000	103	103	112
	500	100	100	108
Strontium	2000	...	103	100
	1000	...	103	100
Zinc	3000	...	98	...
	2000	...	98	...
	1000	...	100	...

[a] A strong band or line is emitted by the test cation which coincides with or overlaps the boron band head or adjacent trough minimum.

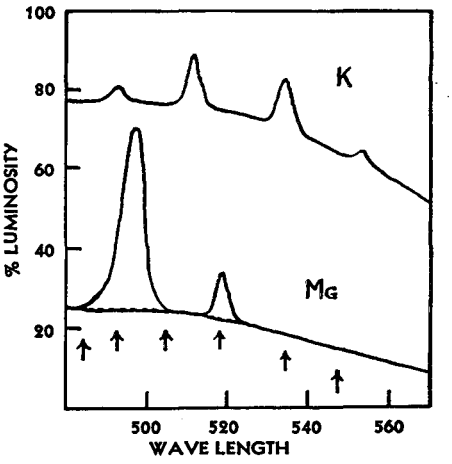

Figure 2. Emission Spectrum of Potassium and Magnesium

Present. 2000 p.p.m. of potassium (upper curve) and 1000 p.p.m. of magnesium (lower curve), each in 1 to 1 methanol-water solution
Slit width, 0.030 mm.
Base line is background of solvent alone

termined, chiefly those which were used for the solution of inorganic samples or which might be associated with boron in its coordination compounds. A series of several concentrations of each of the anions as their sodium salts was prepared with 100 p.p.m. of boron. The results obtained are given in Table V. Acetate ion caused a small error. Nitrate and chloride ions are recommended when acid anions must be introduced.

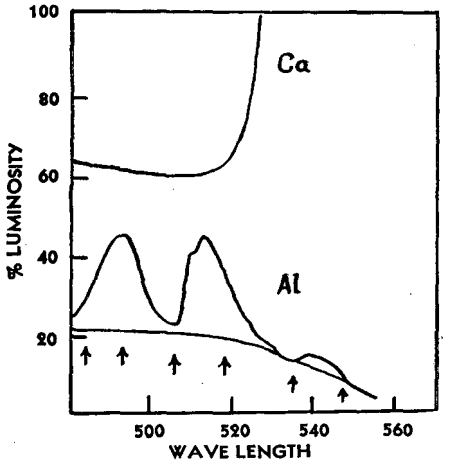

Figure 3. Emission Spectrum of Calcium and Aluminum

Present. 2000 p.p.m. each of calcium (upper curve) and aluminum (lower curve)
Base line is background of solvent alone
Slit width, 0.030 mm.

Table V. Effect of Anions on Boron

(Added as their sodium salts)

Boron Present, P.P.M.	Anion Added	Anion Present, P.P.M.	Boron Found, P.P.M. at 492 Mμ	518 Mμ	546 Mμ
100	Acetate	3000	106	111	116
		2000	106	108	110
		1000	104	106	103
		500	103	104	108
		100	100	98	90
100	Nitrate	3000	103	104	100
		2000	103	106	108
		1000	103	102	103
		500	96	102	104
		100	...	100	96
100	Sulfate	3000	97	98	118
		2000	95	102	103
		1000	100	104	100
		500	97	102	107
		100	97	102	105
100	Chloride	3000	103	103	130
		2000	103	102	112
		1000	100	100	108
100	Bromide	1000	...	102	...
100	Iodide	1000	...	102	...
100	Fluoride	1000	...	102	...

Influence of Hydrogen Ion Concentration. The influence of hydrogen ion concentration on the flame emission of boron is of interest to those dealing with the analysis of gas streams containing high concentrations of hydrochloric acid and hydrolyzable boron halides. Further, acids are used for the solution of inorganic residues. Variation in the concentration of hydrochloric acid, 0.5*M* and less, had no effect on the determination of boron. Higher concentrations of acid exerted a depressing effect on the boron emission. The results obtained are shown in Table VI.

Table VI. Influence of Hydrogen Ion Concentration

HCl Present, Moles/Liter	Boron Found,[a] P.P.M.
0.01	100
0.05	100
0.10	100
0.50	100
1.0	91
2.5	83
4.0	74

[a] 100 p.p.m. of boron present in each case. Measurements taken at 518- to 505-mμ pair of wave lengths.

DISCUSSION OF RESULTS

Table VII lists the flame photometric results obtained on mineral samples supplied by the Pacific Coast Borax Co. In the absence of absolute standards, the accuracy of the flame photometric method can only be discussed in relative terms and compared with values obtained by other chemical methods. The volumetric results were obtained by the procedure described by Brunisholz and Bonnet (*2*). These authors removed all metallic ions from the sample solution, adjusted to pH 1 to 5, by passage through Amberlite IR-120, a strong cation exchange resin in the hydrogen form. Boric acid is displaced and is eluted with 200

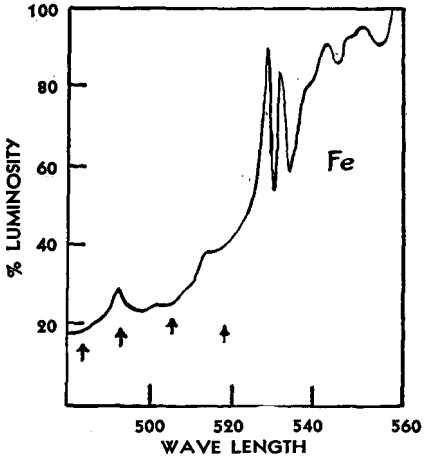

Figure 4. Emission Spectrum of Iron

Present. 3000 p.p.m. of iron in 1 to 1 methanol-water solution
Slit width, 0.030 mm.

Table VII. Comparison of Flame Photometric and Chemical Results on Mineral Samples

Sample[a]	Boron, %, Volumetric	Boron, %, by Flame Photometric Analysis At 492 mμ	At 518 mμ
1 G	11.30	11.45	11.40
	11.30	11.30	11.65
2 G	11.28	11.50	11.25
	11.28	11.15	11.30
	...	11.30	11.30
	...	11.15	11.15
Av. value and associated std. dev.	11.29 ± 0.01	11.31 ± 0.15	11.34 ± 0.17
1C	12.28	12.10	12.70
	12.32	12.25	12.42
	...	...	12.62
	...	...	12.70
	...	...	12.25
2 C	12.38	12.50	12.60
	12.29	12.00	12.28
	...	...	12.25
	...	...	12.30
Av. value and associated std. dev.	12.32 ± 0.05	12.21 ± 0.22	12.46 ± 0.20

[a] Samples G, Gerstley borate, contained: 16% CaO, 5% Na_2O, 1% Al_2O_3, 4% MgO, and 10% SiO_2; samples C, colemanite, contained 26% CaO, 2% Al_2O_3, 1% MgO, and 5% SiO_2.

ml. of distilled water. The eluate was then titrated with boron- and carbonate-free sodium hydroxide, using bromocresyl purple indicator; then mannitol was added and the titration continued until the pink color of phenolphthalein indicator was observed. The amount of base consumed between the bromocresyl purple and phenolphthalein indicator changes is equivalent to the boric acid present in the aliquot sample. This ion exchange and subsequent volumetric titration method requires approximately 1 hour's time in comparison with the rapid method of analysis employing the flame photometer, without considering any extra time which would be consumed in decomposing certain boron-containing compounds prior to their titrimetric determination.

The reproducibility of the flame photometric determinations was very good. The standard deviation from the mean of replicate samples was approximately 2.0%. The difference between the flame photometric and chemical results was within 1.0%. This accuracy and precision are probably somewhat fortuitous as flame photometric methods generally are not credited with accuracies exceeding ±3–5% of the amount present. The sensitivity of the flame photometer used in this work limited the determination of boron to the nearest 1 to 3 p.p.m., depending upon the particular sensitivity of the phototube employed with the photomultiplier attachment. The concentration of boron in the aliquot taken for flame photometric determination was adjusted to lie in the range between 50 and 200 p.p.m.

The use of a photomultiplier attachment with the flame photometer is imperative. Without it slit widths larger than 0.03 mm. must be employed to achieve any reasonable sensitivity. With such large slit widths, serious interference will be encountered from many elements. More important is the excessive overlapping of the boron band systems which precludes the use of the intervening troughs between the band heads as a pseudo-background reference point.

Methods for Circumventing Radiation Interferences. Several elements frequently present in materials containing boron offer serious interference in the flame photometric method for boron. In ore samples, iron, aluminum, calcium, and magnesium cause results to be high when they are present in amounts exceeding 100 p.p.m. in most cases. Even the relatively innocuous elements, when they are present in very large amounts, will cause the results for boron to be high. Methods have been suggested for overcoming the interference of one element upon the flame emission of another.

Disturbing effects often can be eliminated by working at sufficiently high dilutions. However, this step is undesirable, since the boron band intensities are not particularly high and sensitivity then suffers. Self-compensating standards can be prepared—that is, standard solutions containing all the important constituents of the unknown in approximately the correct concentrations can be used. The approach is particularly feasible when a large number of samples of similar constitution are undergoing analysis. Compensation for disturbing factors and elements usually is much more complete than with any other correction method.

Rather than prepare self-compensating standards, one could simply determine the concentration of an interfering element by another method or at another wave length and its luminosity at the boron wave lengths. Difficulties with magnesium and aluminum at both the 492- and the 518-mμ band regions can be resolved in this manner.

When working with samples that are not of a routine nature and when interference effects are suspected or feared, a simple and practical procedure has been suggested by Gilbert *et al.* (*7*). The apparent concentration of the element in question in the undiluted sample is compared with that in a portion diluted to half its original concentration, using a suitable pair of standards having a concentration ratio of 2 to 1. In the absence of interference, the second value will be exactly half the first. If this is not the case, a first approximation to the corrected reading on the second sample will be twice the second reading minus half the first.

This method of correction is useful when it is necessary to operate with strong acid solution or with the hydrolyzates of nonmetallic halides. As shown in Table VI, a hydrogen ion concentration in excess of 0.5*M* causes a marked depression in the flame emission of boron. However, this depressant effect is roughly proportional to the acid concentration, when it exceeds 0.5*M*, which permits this type of correction to be employed. When applied to known samples, the results found for boron are in fair agreement with the amounts present, even for solutions containing 5*M* acid.

ACKNOWLEDGMENT

The effort of Morton Salkind on the early phases of this investigation is greatly appreciated. Thanks are due the Pacific Coast Borax Co. for kindly supplying samples of colemanite and Gerstley borate.

LITERATURE CITED

(1) Bricker, C. E., Dippel, W. A., and Furman, N. H., U. S. Atomic Energy Commission, *Rept.* NYO 794 (Dec. 31, 1951).
(2) Brunisholz, G., and Bonnet, J., *Helv. Chim. Acta*, 34, 2074 (1951).
(3) Calfee, R. K., and McHargue, J. S., IND. ENG. CHEM., ANAL. ED., 9, 288 (1937).
(4) Cary, H. H., and Beckman, A. O., *J. Opt. Soc. Amer.*, 31, 682 (1941).
(5) Caton, R. D., and Bremner, R. W., ANAL. CHEM., 26, 805 (1954).
(6) Curtis, G. W., Knauer, H. E., and Hunter, L. E., Am. Soc. Testing Materials, *Tech. publ.* 116, 67 (1952).
(7) Gilbert, P. T., Hawes, R. C., and Beckman, A. O., ANAL. CHEM., 22, 772 (1950).
(8) Lundegårdh, H., "Die Quantitative Spektralanalyse der Elemente," Jena, G. Fischer, Part I, 1929; Part II, 1934.
(9) McHargue, J. S., *J. Assoc. Offic. Agr. Chemists*, 16, 465 (1933).
(10) McHargue, J. S., and Calfee, R. K., IND. ENG. CHEM., ANAL. ED., 4, 385 (1932).
(11) Pearse, R. W. B., and Gaydon, A. G., "Identification of Molecular Spectra," 2nd ed. rev., Wiley, New York, 1950.
(12) Singh, N. L., *Current Sci. (India)*, 11, 276 (1942).
(13) Stahl, W., *Z. anal. Chem.*, 83, 268, 340 (1931).
(14) Weber, H. C., and Jacobson, R. D., IND. ENG. CHEM., ANAL. ED., 10, 273 (1938).

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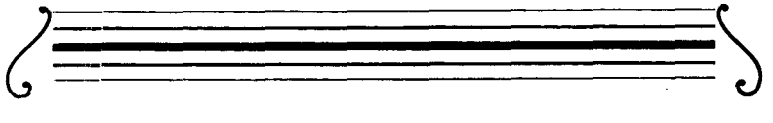

Potentiometric Titration of Very Weak Acids

Titration with Hydroxides in Nonaqueous Media Using Glass–Calomel Electrode System

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The glass–calomel electrode system used in conjunction with alcoholic hydroxide titrants has been successfully applied to the potentiometric titration of very weak acids using ethylenediamine and dimethyl formamide as solvents. This approach is more convenient for routine use than previously published methods employing less common electrodes and alkoxide titrants and has equivalent or somewhat greater scope. Ethylenediamine is used as the solvent to distinguish very weak acids such as hydroxybenzene from stronger acids. Mineral acids and carboxylic acids are not differentiated from each other in ethylenediamine. Dimethyl formamide is used as the solvent to differentiate all three types of acid. The method has been applied to a large number of materials including simple phenols, dihydric phenols, polyphenolic compounds, and dialkyl phosphites. It has been applied with satisfactory precision to a number of mixtures of phenol and stronger acids.

CONDUCTOMETRIC (*3, 23*), amperometric (*8*), and high-frequency (*24*) titration procedures have been applied to the determination of very weak acidity, but most of these do not apply to water-insoluble samples. Potentiometric acid-base titration procedures for phenols and other very weak acids have not been generally successful in aqueous solutions nor in many nonaqueous solvents.

The influence of the solvent on the relative strength of acids and bases (*6, 9, 15, 18, 36*) and the value of titration in particular nonaqueous solutions have been recognized for many years (*21, 22, 26, 29, 31*); however, this information was apparently first applied successfully to the determination of very weak acidity by Moss, Elliott, and Hall (*25*). They chose a basic solvent, ethylenediamine (EDA), and a strongly basic titrant, sodium aminoethylate, with the hope that phenol would behave as a weak acid and carboxylic acids would behave as strong acids as suggested earlier by Hammett (*14*). This procedure is analogous to the widely used titration of very weak bases in glacial acetic acid proposed earlier by Conant and Hall (*6*).

Moss (*25*) obtained promising results for amino acids, carboxylic acid–phenol mixtures, and dihydric phenols such as resorcinol when titrating in carbon dioxide–free atmosphere using an antimony indicating electrode with a second antimony electrode inserted in the titrant stream to act as a reference. He also successfully employed a hydrogen indicating electrode with the antimony reference electrode. A calomel reference electrode inserted in the titration beaker was found to work but was considered less convenient than antimony in the titrant stream. A glass electrode did not respond to changes in acidity in this system.

The procedure of Moss *et al.* (*25*) was investigated further by Gran and Althin (*12*), who used the platinum indicating electrode with an improved calomel electrode prepared with ethylenediamine. Later, Katz and Glenn (*19*) used the glass electrode as a reference with antimony or platinum as indicating electrode. Part of their work was done using a recorder which provided graphs of either the derivative or direct titration curve. They measured the acidity of 18 very weak acids, including hindered phenols and di- and trihydric phenols, as well as the phenolic acidity in coal hydrogenation oils.

A bromometric titration procedure for phenols was reported by Tomicek and Dolezal (*34*) who used a platinum–calomel electrode system and glacial acetic acid as solvent. Higuchi, Zuck, Concha, and Kuramoto (*16, 17*) were able to titrate phenols and other very weak acids by a different approach, using lithium aluminum hydride and lithium aluminum amides as reagents. Fritz, Lisicki, Vespe, and Keen (*10, 11, 35*) have described the visual titration of very weak acids in butylamine, ethylenediamine, and dimethyl formamide (DMF) solvents, using *o*-nitroaniline, thymol blue, and *p*-nitrobenzeneazoresorcinol as indicators.

Kirrmann and Daune-Dubois (*20*) have titrated phthalic, oleic, and succinic acids in dimethyl formamide using aqueous potassium hydroxide as titrant with the platinum–calomel electrode system. They developed this method for use with resin samples. Derivative as well as conventional titration curves were obtained. Pifer, Wollish, and Schmall (*27*) state that weak acids may be titrated with increased sensitivity by dissolving the sample in a small amount of solvent such as ethylenediamine or dimethyl formamide diluted with an excess of nonpolar solvent such as benzene. Sprengling (*32*) has used ethylenediamine and benzene–isopropyl alcohol solvents to study hydrogen bonding in phenolic resins such as the novolacs and relates his findings to the physical properties of these materials. He reported difficulty in obtaining reproducible titration curves with the antimony indicating electrode.

There are several disadvantages to the procedures so far reported for the potentiometric titration of very weak acids. The indicating electrodes, antimony and platinum, tend to give nonreproducible potentials, and sodium alkoxide titrants are relatively inconvenient to prepare, store, and dispense in a sufficiently anhydrous state to prevent them from turning to hydroxide. Although very weak acids such as phenol can be distinguished from stronger acids in ethylenediamine, mineral acids cannot be distinguished from carboxylic acids in this solvent.

In an attempt to find a simple and convenient means of determining very weak acids in a wide variety of materials, a titration method has been developed which appears more adaptable to routine use than those presented in the literature heretofore and which has equivalent or somewhat greater scope. Either ethylenediamine or dimethyl formamide are used as solvents, the choice depending principally upon the type of sample to be titrated. The solvent properties of the two are somewhat different (*2*). Simple phenols, carboxylic acids, and mineral acids can be determined simultaneously in dimethyl formamide. The dimethyl formamide, however, appears to be somewhat unstable in the presence of excess base and therefore may be less desirable than ethylenediamine under some circumstances.

The glass electrode is insensitive in ethylenediamine solutions when titrants containing sodium are used, possibly because it responds to sodium ions as well as to hydrogen ions in strongly alkaline solutions. It is so insensitive that it has been used as a reference electrode (*19*). Use of potassium hydroxide or quaternary ammonium hydroxides as the titrant enables the glass electrode to function satisfactorily as an indicating electrode, resulting in cell potentials which are very reproducible. The

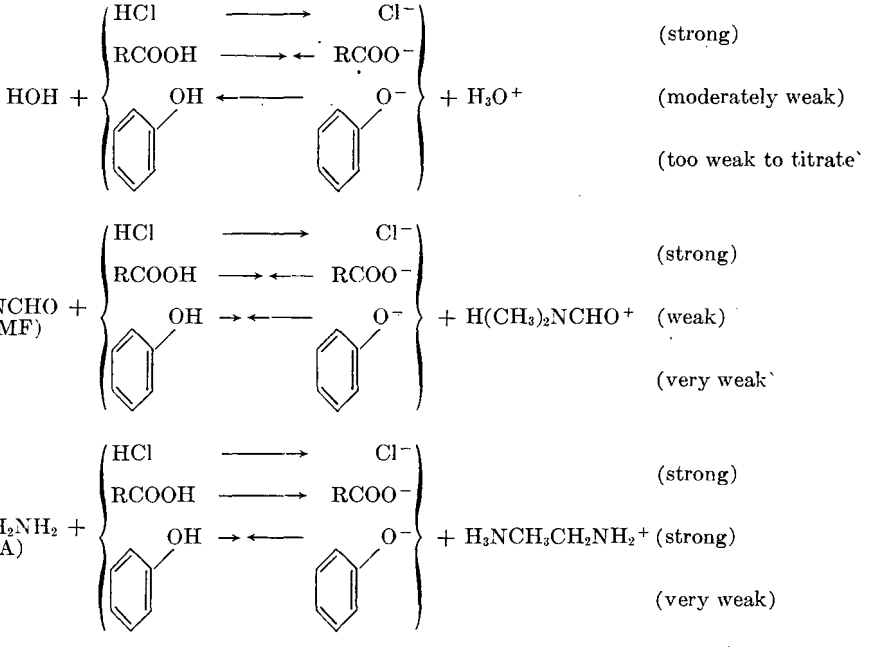
sleeve-type calomel electrode is satisfactory as a reference. The fiber-tip calomel electrode should not be used for nonaqueous titrations because the precipitation of potassium salts on the small fiber destroys the liquid junction. Potassium hydroxide in isopropyl alcohol was found to be a sufficiently strong base and is already available in many laboratories for the determination of acidity in lubricating oils (*1*, *22*). Tetrabutylammonium hydroxide (TBAOH) is substituted for the potassium hydroxide in certain cases in which a precipitate, formed with the latter, results in a rough, uncertain titration curve.

The qualitative terms of acid strength used in this paper are not rigidly defined but were chosen only to illustrate relative acidity in the following discussion of solvent effect. The term "very weak acidity" is used to indicate those compounds which appear on titration to be similar in strength to phenol. Some phenolic compounds will appear stronger than phenol and some compounds other than phenols will appear as very weak acids.

SOLVENT EFFECT

With either potassium hydroxide or tetrabutylammonium hydroxide as titrant, phenol is ordinarily too weak to titrate in water, but it titrates as a very weak acid in both ethylenediamine and dimethyl formamide. Carboxylic acids, on the other hand, titrate as moderately weak acids in dimethyl formamide and as strong acids in ethylenediamine. Mineral acids such as hydrochloric acid will titrate as strong acids in any of the three solvents. The difference in applicability of the solvents is readily apparent. All three acid strengths may be distinguished from each other in dimethyl formamide. Mineral acids and carboxylic acids cannot be distinguished from each other in ethylenediamine but they are readily distinguishable from phenol in this solvent. In water, mineral acids may be distinguished from carboxylic acids. The effect of the solvent in titration of acidity in ethylenediamine, dimethyl formamide, and water is illustrated in the following diagram and curves are compared in Figure 1 for a mixture of hydrochloric acid, acetic acid, and phenol titrated in these three solvents.

Solvolysis (reaction with the solvent):



Titration of the solvated proton:

METHOD FOR DETERMINATION OF VERY WEAK ACIDITY

Apparatus. The apparatus consisted of a meter, glass electrode (Beckman general purpose, No. 4990-80), calomel reference electrode (Beckman Sleeve-Type, No. 4970-71) and stand, prepared, maintained, and tested as described by Lykken *et al.* (*22*), and The American Society for Testing Materials (*1*). The cell potential may be read on any electronic voltmeter or potentiometer which meets the ASTM requirements (*1*)—that is, any instrument which has a low grid current of less than 5×10^{-12} amperes and which covers a range of +800 to −800 mv. The Precision Dual AC Titrometer is well suited for nonaqueous titrations because it has a range of +1650 to −1650 mv. and a meter of high sensitivity, 250 mv. full scale. A buret (5-ml. microburet), graduated in 0.02-ml. increments and supplied with a small Ascarite absorber tube at the top was used. A magnetic stirrer with glass-covered stirring bars about 4 mm. in diameter and 18 mm. long and a titration beaker, which was a special reduced-scale beaker marked at the 20-ml. volume with a Bakelite cover (see Figure 2) were used. Alternatively, a Precision-Dow Recordomatic Titrator equipped with a syringe 1/5 the diameter of the standard syringe may be used with the reduced-scale beaker and cover.

Reagents. Ethylenediamine (95 to 100%, Eastman Organic Chemicals) and dimethyl formamide (E. I. du Pont de Nemours

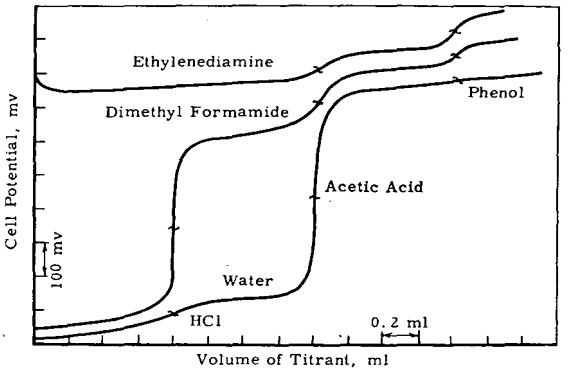


Figure 1. Comparison of Curves for an Acid Mixture Titrated with 0.1*N* Potassium Hydroxide in Three Solvents

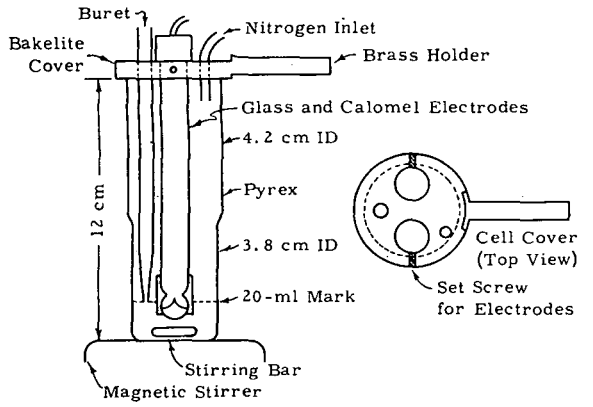


Figure 2. Reduced-Scale Titration Cell

& Co.) are dispensed from the original container in such a way that the solvents are protected from atmospheric carbon dioxide and water by an absorber tube containing Ascarite and indicating Drierite. Potassium hydroxide solution, 0.1 and 0.2*N* alcoholic, was prepared as described by ASTM (*1*) and Lykken (*22*). The use of barium hydroxide to precipitate carbonate is not necessary, since potassium carbonate is essentially insoluble in the alcohol. Tetrabutylammonium hydroxide solution, 0.1*N* alcoholic, was prepared by dilution of 1.0*M* aqueous hydroxide (Southwestern Analytical Chemicals, Austin, Tex.) with anhydrous isopropyl alcohol. The nitrogen used was essentially free of carbon dioxide.

Procedure. SAMPLE TITRATION. Into a special reduced-scale titration beaker, introduce directly or by aliquot a quantity of sample weighed to 0.3 mg. which will give a titration of 1 or 2 ml. Dispense the solvent rapidly into the sample beaker to the 20-ml. mark. Keep a slow stream of nitrogen flowing into the beaker while adding the solvent. Without delay, place in position under clean electrodes wiped nearly dry with tissue. Keep the titration beaker essentially airtight during titration to reduce to a minimum the absorption of carbon dioxide and water. Start the magnetic stirrer and continue stirring throughout the titration at a rate sufficient to produce vigorous agitation without spattering.

Fill the 5-ml. buret with potassium hydroxide or tetrabutylammonium hydroxide titrant and cover the buret top with a small Ascarite absorber tube. Place in position in the titration assembly. Record the initial buret and meter (cell potential) readings. Add carefully 0.02- to 0.05-ml. portions of the titrant and, after a constant potential has been established, record the buret and meter readings. Consider the cell potential constant if the meter reading changes less than 2 mv. in 30 seconds in either direction. Make the next addition of titrant without delay. If the cell potential reaches a maximum and starts to drop after addition of titrant, record the maximum and add more titrant immediately. Continue the titration until the cell potential approaches that of the completed blank titration and remains relatively constant or begins to drop steadily. After each titration clean the electrodes and wash the buret tip and undersurface of the beaker cover carefully with water, including also any metal parts which may have been exposed to spattering or fuming of the solvent. Keep any ethylenediamine or dimethyl formamide which is in open containers under an efficient fume hood. Pour waste solutions into a sink under a fume hood and wash away with water.

BLANK TITRATION. Make a blank titration of the solvent used for the sample titration including any solvent added as an aliquot.

CALCULATION. Prepare a titration curve by plotting added volume of titrant against the corresponding meter readings. To make these comparable to curves from macrotitrations (100-ml. cell solutions), plot the volume scale expanded five times as shown in Figure 3 (see also the section on experimental details).

When the sample shows two inflections in ethylenediamine solvent, use the difference between the first and second inflection points as the volume of standard titrant equivalent to the very weak acid.

When only one inflection point appears in ethylenediamine solvent, it may be that a large excess of very weak acid has obscured the strong acid end point. Determine whether the inflection represents titration of strong or very weak acidity by comparison with a curve for pure phenol. When there is no inflection for very weak acidity, consider its titration to be less than 0.03 ml. If the inflection appears to represent very weak acidity, use the difference between the blank titration and the sample titration to calculate the volume of standard titrant equivalent to the very weak acid in the sample.

When the sample shows one or more inflections in dimethyl formamide solvent, use the difference between successive inflection points to calculate the volume of standard titrant equivalent to the acids represented. Correct for the blank titration where necessary. If it is not clear whether any of these inflections represent very weak acidity, compare with a titration curve for pure phenol made under the same conditions as the sample curve (same electrodes, solvent, etc.). Where there is no inflection representing very weak acidity, consider its titration to be less than 0.03 ml.

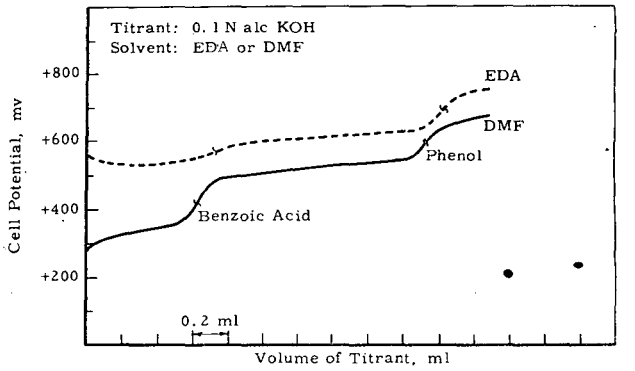


Figure 3. Comparison of Titration Curves for Mixed Acids in Ethylenediamine and Dimethyl Formamide

Calculate the acidity as follows:

$$\text{Very weak acidity, equivalents/100 g.} = \frac{(A)(N)}{(10)(W)}$$

$$\text{Known acid, weight \%} = \frac{(A)(N)(E)}{(10)(W)}$$

where A = volume of standard base, milliliters, used in titrating the very weak acid
N = normality of base used in titrating the very weak acid
E = equivalent weight of a compound which the very weak acidity is known to represent
W = weight of sample, grams

TITRATION METHOD APPLIED TO KNOWN ACIDS

Distinction between phenol and many stronger acids can be made in either ethylenediamine or dimethyl formamide, but it is probably best done in dimethyl formamide where the stronger acid inflections are more clearly defined. The strongly basic solvent, ethylenediamine, tends to compress the range of cell potentials, so that an entire titration covers only about 200 mv. In contrast, the range of cell potentials obtainable in dimethyl formamide is approximately 900 mv. One of the principal disadvantages in titrating very weak acids in ethylenediamine has been the difficulty in choosing precisely the first inflection point which represents absorbed carbon dioxide in the solvent, or other acidity, stronger than phenol, in the sample. The first inflection in ethylenediamine is small and often is preceded by a dip in the curve, resulting in some uncertainty in the choice of end point (Figures 3, 7, and 8).

Mixtures of Phenol with Stronger Acids. An example of the distinction between phenol and a stronger acid, benzoic acid, in both ethylenediamine and dimethyl formamide solvents is given in Figure 3. Titration is quantitative for both components in either solvent, but the inflections are more definitive in dimethyl formamide. Distinction among three acids (phenol, acetic acid, and hydrochloric acid) in dimethyl formamide solvent is shown in Figure 4 on titration with both potassium hydroxide and tetrabutylammonium hydroxide.

When the ratio of stronger acid to phenol is large and the

optimum total titration of 1 to 2 ml. is maintained, the relative precision of the phenol determination is necessarily poor. Increasing the sample size to increase the volume of the phenol titration was found to be satisfactory in increasing the relative precision when the stronger acid was hydrochloric acid. When the stronger acid was benzoic or acetic acid, however, the sharpness of the first inflection was seriously impaired, so that the resulting relative precision of the phenol titration was not improved. This effect was found to be independent of the alcohol introduced with the titrant. The effect of sample size on the sharpness of acetic and hydrochloric acid inflections is shown in Figure 5 for a 6 to 1 molar ratio of the stronger acid to the phenol, in ethylenediamine solvent. At a 15 to 1 ratio of hydrochloric acid to phenol, good distinction is still obtained between the two acids; however, benzoic and acetic acid inflections begin to blend with the phenol inflection at a 6 to 1 ratio.

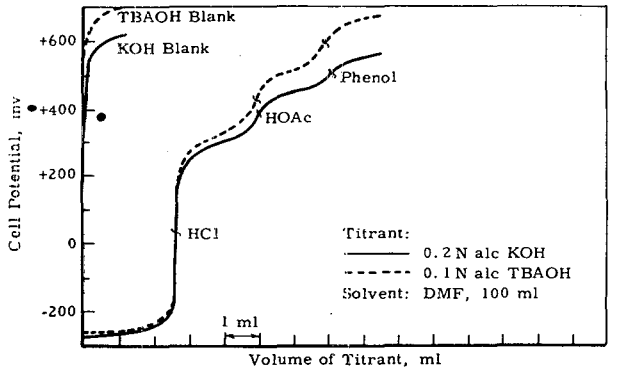


Figure 4. Acid Mixtures Titrated in Dimethyl Formamide Using Potassium Hydroxide and Tetrabutylammonium Hydroxide Titrants

When phenol is the major acidic component of the mixture, the sample size may be adjusted to give a reasonable phenol titration without exceeding the limit of a 1- to 2-ml. total titration. If phenol exceeds the stronger acid content sufficiently, the volume of titrant equivalent to the stronger acid will be too small to be read accurately. In this case the stronger acid may be determined in a separate titration using a larger sample size. The error resulting from choice of the end point in some of these mixtures is given in Table I for a series of mixtures titrated within a few days of each other on a modified Precision-Dow Recordomatic titrator. The error of difference between amounts of added and recovered acid in the mixtures was based on a comparison with values obtained for the various components when titrated separately. The standard deviation of the final end points is 0.03 ml.

Alkyl Phenols. Titration curves for two phenols with *tert*-butyl groups in the ortho position are shown in Figure 6, *B* and *C*. Sample *B* (2,6-di-*tert*-butyl-4-methylphenol), though unextractable from hydrocarbons by aqueous sodium hydroxide, can be titrated as an acid in these solvents.

Dihydric Phenols. Titration of catechol (1,2-dihydroxybenzene) in dimethyl formamide shown in Figure 6, *E*, gave separate inflections for each of the two hydroxyl groups, the first inflection being very small and almost indistinguishable. Titration of hydroquinone (1,4-dihydroxybenzene) in dimethyl formamide is not illustrated but also gives separate inflections for each of the two hydroxyl groups, both inflections being small. Titration of hydroquinone with potassium hydroxide in ethylenediamine was not successful. The first inflection represented titration of one hydroxyl group, but was too small to be reliable. The second inflection was sufficiently large, but did not represent quantitative recovery of the sample, probably because of the precipitation which took place after titration of the first hydroxyl group.

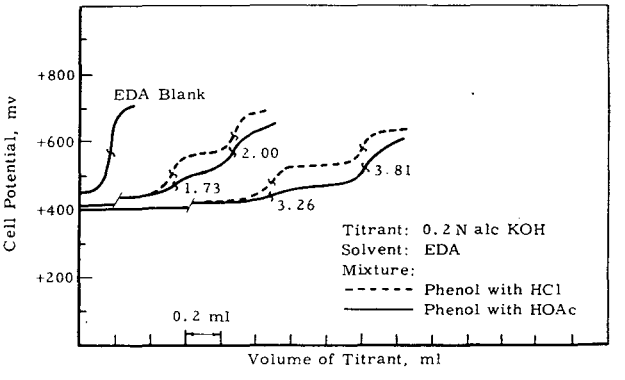


Figure 5. Effect of Sample Size in Titration of 6 to 1 Mixtures of Stronger Acid with Phenol in Ethylenediamine

Isopropyl alcohol content of all cell solutions was 5% at start of titration. Figures given at inflection point are milliliters of titrant

Table I. Titration of Phenol-Stronger Acid Mixtures in Ethylenediamine

(Isopropyl alcohol content of the cell solution was 5% vol. at start of titration for all examples given below)

Ratio of Stronger Acid to Phenol	HCl, Ml.[a]			Acetic Acid, Ml.[a]			Phenol, Ml.[a]			Total, Ml.[a]		
	Added	Found	Diff.	Added	Found	Diff.	Added	Found	Diff.	Added	Found	Diff.
0.1–1	0.11	0.07	−0.04	..	..	..	1.05	1.07	+0.02	1.16	1.14	−0.02
	..	..	..	0.10	0.08	−0.02	1.05	1.05	0.00	1.15	1.13	−0.02
0.5–1	0.53	0.52	−0.01	..	..	..	1.05	1.06	+0.01	1.58	1.58	0.00
	..	..	..	0.52	0.50	−0.02	1.05	1.07	+0.02	1.57	1.57	0.00
1–1	1.59	1.54	−0.05	..	..	..	1.57	1.66	+0.09	3.16	3.20	+0.04
	1.59	1.55	−0.04	..	..	..	1.57	1.66	+0.09	3.16	3.21	+0.05
	1.06	1.05	−0.01	..	..	..	1.05	1.05	0.00	2.11	2.10	−0.01
	..	..	..	1.56	1.55	−0.01	1.57	1.63	+0.06	3.13	3.18	+0.05
	..	..	..	1.04	1.03	−0.01	1.05	1.06	+0.01	2.09	2.09	0.00
3–1	2.23	2.18	−0.05	..	..	..	0.73	0.77	+0.04	2.96	2.95	−0.01
	1.59	1.61	+0.02	..	..	..	0.52	0.52	0.00	2.11	2.13	+0.02
	..	..	..	1.56	1.57	+0.01	0.52	0.55	+0.03	2.08	2.12	+0.04
6–1	3.18	3.14	−0.04	..	..	..	0.52	0.52	0.00	3.70	3.66	−0.04
	1.59	1.57	−0.02	..	..	..	0.26	0.27	+0.01	1.85	1.84	−0.01
	..	..	..	3.12	3.10	−0.02	0.52	0.55	+0.03	3.64	3.65	+0.01
	..	..	..	1.56	1.57	+0.01	0.26	0.27	+0.01	1.82	1.84	+0.02
10–1	3.18	3.15	−0.03	..	..	..	0.31	0.34	+0.03	3.49	3.49	0.00
	1.59	1.55	−0.04	..	..	..	0.16	0.17	+0.01	1.75	1.72	−0.03
	..	..	..	3.12	3.16	+0.04	0.31	0.31	0.00	3.43	3.47	+0.04
	..	..	..	1.56	1.53	−0.03	0.21	0.17	−0.04	1.77	1.70	−0.07
15–1	3.18	3.13	−0.05	..	..	..	0.21	0.22	+0.01	3.39	3.35	−0.04
	1.59	1.54	−0.05	..	..	..	0.11	0.11	0.00	1.70	1.65	−0.05

[a] Ml. refers to milliliters of titrant corresponding to designated acid.

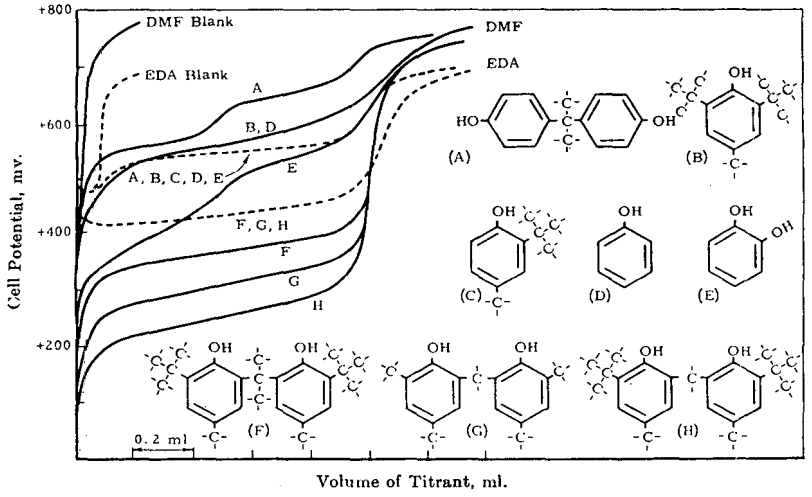


Figure 6. Phenolic Compounds Titrated in Ethylenediamine and Dimethyl Formamide

Titrant. Alcoholic potassium hydroxide for titrations in ethylenediamine; alcoholic tetrabutylammonium hydroxide for titrations in dimethyl formamide. Solvent. Ethylenediamine or dimethyl formamide

Polyphenolic Compounds. Compounds which contain two or more phenolic groups linked through a single carbon atom in the ortho position, as in *o,o'*-dihydroxydiphenylmethane, show a marked increase in acid strength over simple phenols owing to interaction between the hydroxyl groups. Titration curves for three bisphenol compounds of this type in ethylenediamine and dimethyl formamide solvents are shown in Figure 6, *F*, *G*, and *H*. It is evident that the titrations in dimethyl formamide provide more information as to the relative acid strength of these compounds. Only one hydrogen is titrated in each of these cases because of hydrogen bonding (*32*). The titration curves for a pentaphenolic compound of this type are shown in Figure 7. Three inflections are obtained in dimethyl formamide, the first possibly representing some strong acid impurity in the sample. The second and third seem to represent two and three hydroxyl groups, respectively. The totals are the same in both solvents when corrected for the blank.

Compounds linked through the para position as in *p,p'*-dihydroxydiphenyldimethylmethane (Figure 8) show a definite interaction between the hydroxyl groups when titrated in dimethyl formamide but the effect is less marked than for the ortho-linked compounds. Thus both hydroxyl groups are titrated but exhibit different acid strengths, resulting in two definite inflections in the titration curve. No interaction between hydroxyl groups is shown in ethylenediamine with this type of compound; thus, both hydroxyls are titrated but only one inflection occurs in the titration curve.

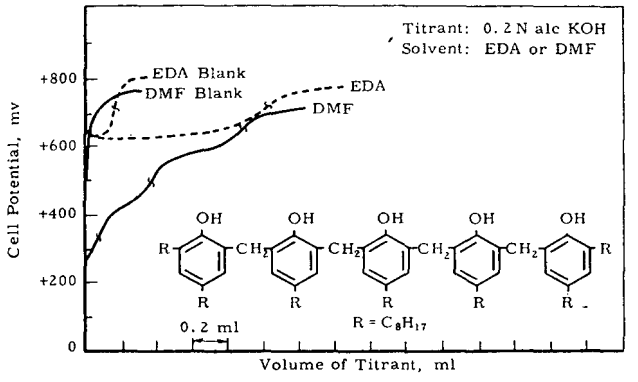


Figure 7. Comparison of Titration Curves for a Polyphenolic Compound in Ethylenediamine and Dimethyl Formamide Solvents

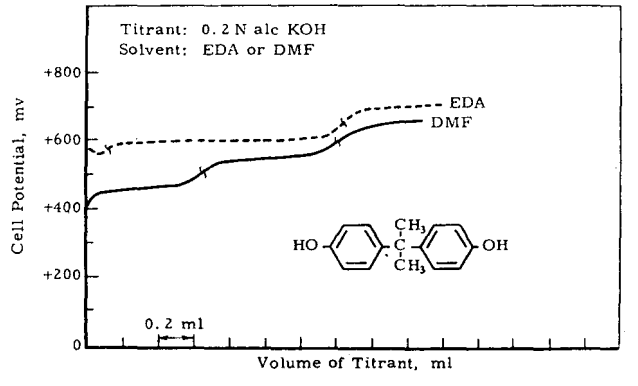


Figure 8. Comparison of Titration Curves for a *p,p'*-Dihydroxyphenylmethane in Ethylenediamine and Dimethyl Formamide Solvents

Phthalic Acids. Some interesting titration curves (Figure 9) were obtained for the three isomers of phthalic acid titrated in dimethyl formamide. Two inflections were obtained for each isomer when tetrabutylammonium hydroxide was used as titrant. A precipitate formed in some cases on titration with potassium hydroxide.

Dialkyl Phosphites. The method has been applied to a number of di- and trialkyl phosphites separately and in mixtures. The behavior of these compounds in ethylenediamine and dimethyl formamide is not thoroughly understood. Direct titration in benzene–isopropyl alcohol and in methanol indicated a small amount of acidity, probably representing traces of monoalkyl phosphite or orthophosphorus acid; however, the dialkyl phosphite is too weak an acid to titrate in these solvents. Hydrolysis was rapid (Figure 10) in the alcoholic solvents and slower in

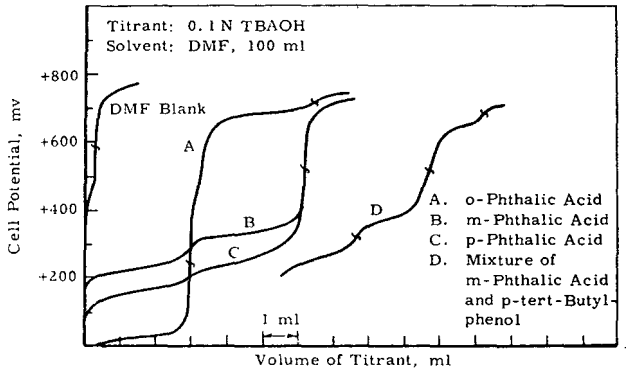


Figure 9. Phthalic Acid Isomers Titrated in Dimethyl Formamide

ethylenediamine. In order to obtain consistent results in ethylenediamine, these samples were always titrated as rapidly as possible, taking the maximum scale reading after each addition of titrant. The meter needle would often rise to a maximum positive potential and then drop back, indicating that one of the ester groups was reacting with the basic solvent (solvolysis). Although the total titration does not seem to vary (Figure 11)

the titration to the first inflection increases with titration time. The same phenomenon is noted when water has been allowed to react with diethyl phosphite solutions for varying lengths of time (Figure 12). The solvent in these tests was added just prior to titration and after reaction (under nitrogen) of the sample with water. Titration in ethylenediamine indicated that at 30 minutes of reaction time about half of the dialkyl phosphite had been converted to some stronger acid, probably either the monoalkyl ester or free phosphorus acid. Ethylenediamine solvent does not distinguish between the two. Results on pure materials were usually 2 to 10% above theoretical in ethylenediamine. The probable reactions are given in the following equations, assuming the structure of dialkyl phosphite to be as shown.

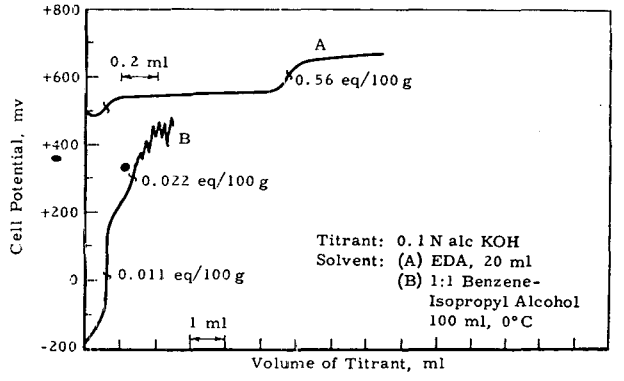
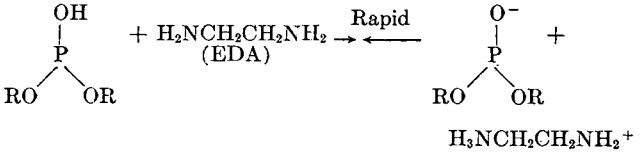


Figure 10. Dibutyl Phosphite Titrated in Ethylenediamine and Benzene–Isopropyl Alcohol (ASTM D664)

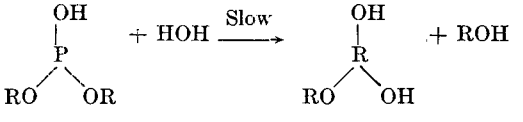
Solvolysis of the very weak acid in ethylenediamine:



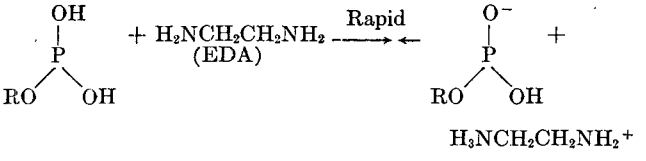
Titration of the solvolyzed phosphite-ethylenediamine:

$$H_3NCH_2CH_2NH_2^+ + OH^- \longrightarrow HOH + H_2NCH_2CH_2NH_2$$

Hydrolysis of an ester group:



Solvolysis of the stronger acid in ethylenediamine:



In the monoalkyl phosphite formed on slow hydrolysis, one hydroxyl group is stronger than the original and one is weaker (too weak to be titrated in ethylenediamine). Because of this the sum of the dialkyl phosphite which has hydrolyzed and that which has not still gives the same total acidity as the original dialkyl phosphite.

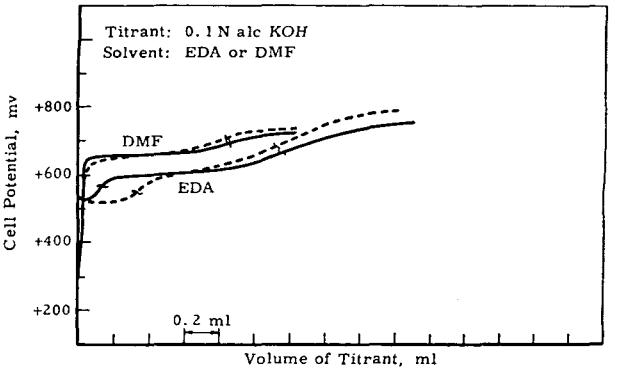


Figure 11. Change in Apparent Diethyl Phosphite (Very Weak Acidity) with Titration Time

Minutes Titration to Last End Point	Very Weak Acid, Calcd. as Diethyl Phosphite, Wt. %	
	Ethylenediamine	Dimethyl formamide
—— 5	98	79
------ 14	86	75, 76

Comparable titrations in dimethyl formamide (Figure 11) show little change in the very weak acidity with changes in titration time, although results on pure materials were usually 10 to 15% below theoretical.

Trialkyl Phosphites. Pure trialkyl phosphites should not give a titration for acidity. All the trialkyl phosphites tested behaved in this manner except trimethyl phosphite. A sample of trimethyl phosphite which appeared to contain 3% dimethyl phosphite by rapid titration in ethylenediamine showed 11% in a slower titration (20 minutes to end point). The same sample showed about 90% conversion to some titratable form when left 20 minutes under nitrogen in contact with water prior to addition of the ethylenediamine. By comparing curve plateaus (not shown) for the trimethyl phosphite samples with those for diethyl phosphite the titratable material in the trimethyl phosphite appears to be of much stronger acidity than that found in the diethyl phosphite samples. This suggests that dimethyl phosphite is a stronger acid than diethyl phosphite, but no pure di-

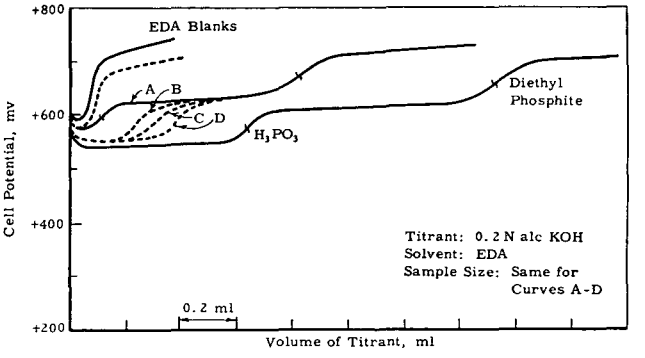


Figure 12. Effect of Water on Apparent Diethyl Phosphite (Very Weak Acidity) Titrated in Ethylenediamine

Cell solutions indicated by dotted curves contain 1/4 ml. water added prior to titration

	Calcd. as Diethyl Phosphite, Wt. %		
	Very weak acid	Total acid	Remarks
A	107	110	Titrated immed. (no water added)
B	75	99	Titrated immed.
C	65	100	10-min. reaction before starting
D	59	105	30-min. reaction before starting

methyl phosphite was available to test this point. Instability of the trimethyl phosphites in ethylenediamine appears to be so much greater than that noted for the higher alkyl phosphites that this method is not considered suitable for determination of acidity in the presence of trimethyl phosphite. Behavior of this material in dimethyl formamide was not studied.

Phosphorus Acid and Phosphoric Acid. Orthophosphorus acid (Figure 12) gives one inflection in ethylenediamine, representing two hydrogens. Two good inflections appear in dimethyl formamide, representing each of the first two hydrogens. There is some tendency for the phosphorus acid salt to precipitate in dimethyl formamide on titration with potassium hydroxide and a smoother titration was obtained by substituting tetrabutylammonium hydroxide as titrant. Although the first inflection using the latter titrant is not sharp, the second inflection is very distinct and represents titration of two hydrogens.

Orthophosphoric acid (not shown) formed a gel in ethylenediamine and could not be determined quantitatively. Two inflections were obtained in dimethyl formamide but titration was slow because of precipitation of the potassium salt. Substitution of tetrabutylammonium hydroxide for potassium hydroxide largely eliminated this problem, although a small amount of precipitate was still observed. Recovery was not determined, but two inflections at about equal volumes were obtained with either titrant in dimethyl formamide and the total appeared to represent titration of two equivalents of phosphoric acid.

EXPERIMENTAL DETAILS

Preparation of Reduced-Scale Titration Curve. Because of the limited amount of sample often encountered and the toxicity of the solvents, reduced-scale (20 ml. of solvent) titrations have been used for the determination of very weak acidity. It has been the custom in these laboratories to plot the potential-volume curves from potentiometric titrations in a specified manner, so that regardless of the solution volume, any curves are readily comparable and easily understood. Thus, on the usual macro scale the sample size is selected where possible to give a 5- to 10-ml. titration and the titration is carried out in 100 ml. of solvent. The curve is plotted on graph paper so that 1 ml. of titrant (horizontal axis) and 100 mv. (vertical axis) are represented by an equal number of scale divisions. When a reduced-scale titration is made the sample size and solvent volume are reduced to one fifth the usual amount, resulting in a solvent volume of 20 ml. and a titration end point of 1 to 2 ml. To plot this titration curve in a manner which will be comparable to that from the usual titration in 100 ml. of solvent, the volume scale must be expanded five times. Thus, 0.2 ml. of titrant and 100 mv. are represented by an equal number of scale divisions on the graph paper.

Likewise, the titration curve for acid-base titrations is plotted to allow immediate perception of the nature of the sample. Thus, in titration of acidity, the curve always proceeds upward on the graph paper, while in titration of basicity the curve proceeds downward.

Sample Size. Titration volumes greater than 1 or 2 ml. are seldom practical in the reduced-scale titration because of the tendency for longer titrations to produce unsharp, sloping inflections; however, choice of the optimum sample size depends somewhat upon the nature of the sample. For many resins a 1- or 2-ml. titration results in poor inflections and a considerably smaller sample may have to be taken. In these instances it is necessary to consider for the particular situation whether the error in reading a small-volume steeper inflection would be less than the error from uncertain choice of a large-volume poor inflection.

Choice of Titrant. The strength of titrant may be either 0.1 or 0.2*N*, with very little difference in the appearance of the curves. For difficultly soluble samples it would seem more practical to use 0.1*N* titrant in order to obtain the maximum titration with the least sample. For certain applications tetrabutylammonium hydroxide has been found superior to potassium hydroxide. In particular, it is useful for titration in dimethyl formamide solvent of dibasic or polybasic acids which normally precipitate before titration with potassium hydroxide is complete —e.g., *p*-phthalic acid. It has a greater apparent basic strength than the anhydrous potassium hydroxide (Figure 4), even though it contains 10% water.

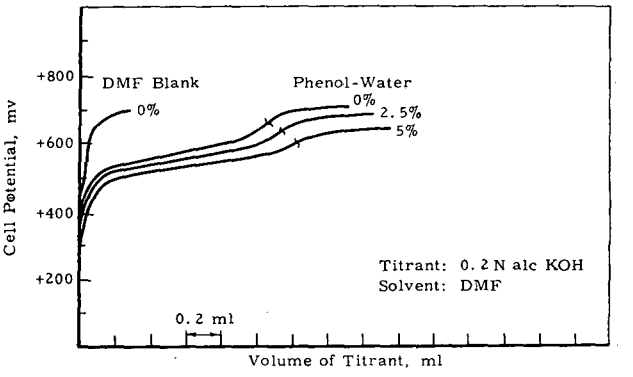


Figure 13. Effect of Water on Phenol Curves Titrated in Dimethyl Formamide

Numbers show per cent of cell volume for water added to dimethyl formamide

Choice of Solvent. Although the solvent properties of ethylenediamine and dimethyl formamide and their reactivity with the sample may enter into the decision, the choice of solvent will depend principally upon the information to be gained from the titration curve. In general, dimethyl formamide appears to be preferable because it facilitates reading of the strong acid inflection and does not have a large blank correction. The behavior of dimethyl formamide as a solvent is not fully understood as yet, but satisfactory titrations of acidity are obtained if the titration is made rapidly (10 to 15 minutes). Titrations for basicity are not recommended in this solvent because of apparent solvent decomposition. When there is some advantage in titrating at ice temperatures—e.g., to reduce the rate of some undesired side reaction—dimethyl formamide is preferable because difficulty may be encountered from freezing of the ethylenediamine-sample solution. The freezing point of dimethyl formamide is at −61° C. while that of ethylenediamine is above zero (*5, 30*). Pure ethylenediamine freezes at about 11° C. while 89% ethylenediamine in water freezes at about 2° C.

The commercial dimethyl formamide used in these experiments reacts slowly with strong base or contains some impurities which do. Strong base added to the dimethyl formamide is converted slowly to a weak base resembling sodium formate in basic strength. It is thought that either the dimethyl formamide itself or possibly small amounts of formamide or monomethyl formamide in it hydrolyze at a moderate rate to form ammonia, methylamine, and formic acid in the presence of water or strong base. Fritz and Keen (*10*) in titrating very weak acidity in dimethyl formamide using *o*-nitroaniline as a color indicator, report that the end point tends to fade. This may be the result of the same reaction.

Titration of 100 ml. of dimethyl formamide with alcoholic potassium hydroxide shows the presence of a small amount of weak acid (less than 0.02 meq. per 100 ml.). Titration with alcoholic hydrochloric acid shows a small amount of weak base to be present (less than 0.04 meq. per 100 ml).

Purification of Solvent. Since the presence of water in the titration cell has been shown to be detrimental to the determination of very weak acidity, the removal of water from the solvents

may improve the sharpness of the inflections. Neither ethylenediamine nor dimethyl formamide was purified for the work reported here, but a number of suggestions appear in the literature. One of the earliest (*28*) suggests digestion of the ethylenediamine over sodium hydroxide, refluxing over sodium, and finally, fractionation (boiling point, 116.2° C.). Treatment with activated alumina prior to distillation was reported by Bromley and Luder (*4*). Others recommend vacuum distillation (*33*) and azeotropic distillation with benzene (*7*, *19*).

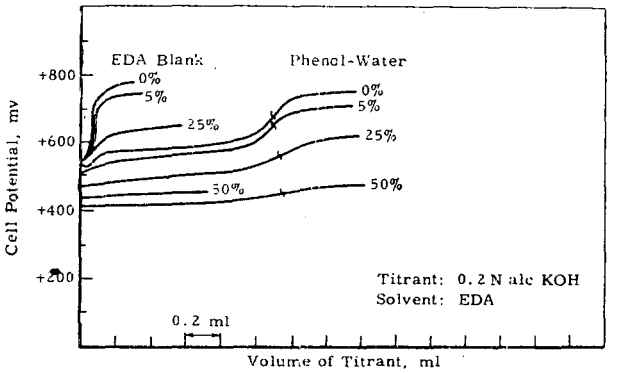


Figure 14. Effect of Water on Phenol Curves Titrated in Ethylenediamine

Numbers show per cent of cell volume for water added to ethylenediamine

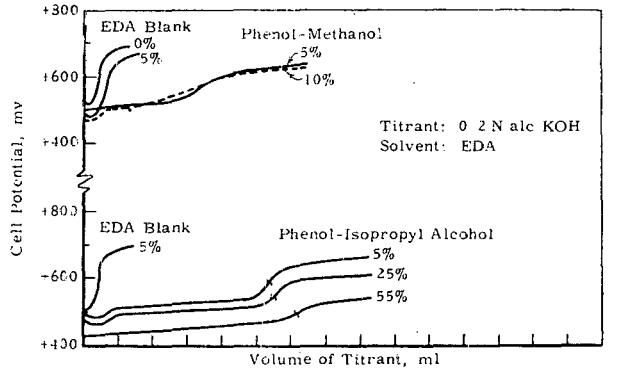


Figure 15. Effect of Alcohol on Phenol Curves Titrated in Ethylenediamine

Numbers show per cent of cell volume for alcohol added to ethylenediamine

Apparently, dimethyl formamide can be purified by distillation (boiling point, 153° C.). It forms maximum boiling azeotropes (*13*, *30*) with formic acid: (1) 85° C. at 50 mm. (67% dimethyl formamide, 33% formic acid), and (2) 153.2° C. at 757 mm. (97.4% dimethyl formamide, 1.2% formic acid). While it is relatively stable to hydrolysis even at 100° C., an excess of hot concentrated alkali or acid will cause decomposition to dimethylamine and formic acid.

Effect of Exposure of Solvent to Air. The ethylenediamine absorbs carbon dioxide so rapidly and efficiently that it has been proposed as an absorbing agent for quantitative carbon dioxide recovery (*33*). The amine carbonate titrates as a strong acid in ethylenediamine and accounts for most of the blank correction found in the ethylenediamine solvent. Titrations in ethylenediamine must be carried out rapidly, preferably under a blanket of nitrogen, and the solvent must be stored with protection from the air.

Since dimethyl formamide is somewhat hygroscopic (*13*), it too should be stored with protection from the air. Although the presence of water in the titration cell was shown to be serious (Figure 13), absorption of water from the air did not appear to be sufficiently rapid to cause difficulty when titrations were carried out in an open beaker.

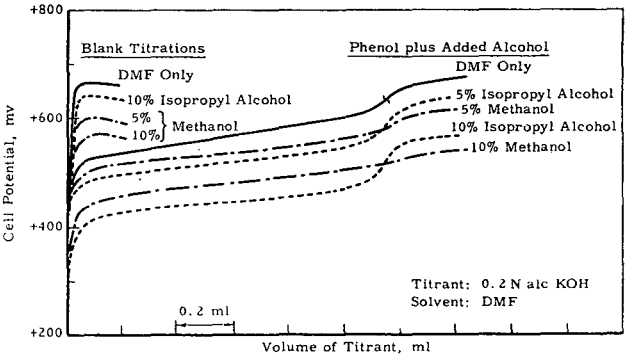


Figure 16. Effect of Methanol and Isopropyl Alcohol on Phenol Curves in Dimethyl Formamide

Numbers show per cent of cell volume for various solvents added to dimethyl formamide

Promising results have been obtained with the Precision-Dow Recordomatic Titrator using both ethylenediamine and dimethyl formamide in titrating phenol–stronger acid mixtures and dialkyl phosphites. The rapidity with which titrations may be made virtually eliminates any difficulty from absorption of atmospheric carbon dioxide or water or decomposition of the dimethyl formamide solvent. Transfer of the titrant directly from the storage container to the cell solution is an additional advantage of this instrument, preventing any contamination of the titrant which might come through the use of a conventional buret.

Effect of Sample Diluents on Phenol Inflection. Since samples frequently must be aliquoted before titration, a study of the effect of various diluents on the phenol inflection was made. Water appears to be considerably more serious in dimethyl formamide titrations than in ethylenediamine (Figures 13 and 14) and should be kept below 1% in dimethyl formamide or 5% in ethylenediamine cell solutions. Tests were made assuming the water content of the commercial ethylenediamine to be constant. The effect of alcohols is shown in Figures 15 and 16. Methanol seriously obscures the phenol inflection in either solvent and should not be used. The effect of isopropyl alcohol is less serious and up to 10% may be present in the cell solution with no apparent ill effect. Benzene up to 10% does not appear to disturb the phenol inflection in either solvent. Because of the difficulty of excluding carbon dioxide, ethylenediamine is never used for aliquoting.

Electrode Stability in Ethylenediamine and Dimethyl Formamide. It has been the authors' experience that neither ethylenediamine nor dimethyl formamide have a deleterious effect upon the glass and calomel electrodes, although certain electrode pairs are customarily kept specifically for use in these solvents and the saturated potassium chloride solution is replaced frequently, usually once a day, in the calomel electrode. Moss (*25*) reports the glass electrode to be unstable when sodium aminoethylate is used as titrant in anhydrous ethylenediamine solvent. Under these circumstances, the glass–calomel electrode system in the present study was found to be insensitive to changes in acidity; however, with substitution of alcoholic potassium hydroxide and commercial 95 to 100% ethylenediamine, sensitivity was restored and stable readings were obtained.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, "ASTM Standards on Petroleum Products and Lubricants," D 664, p. 253, Philadelphia, 1951.
(2) Audrieth, L. F., and Kleinberg, Jacob, "Nonaqueous Solvents," New York, John Wiley & Sons, 1953.
(3) Britton, H. T. S., "Conductometric Analysis," New York, D. Van Nostrand Co., 1934.
(4) Bromley, W. H., Jr., and Luder, W. F., *J. Am. Chem. Soc.*, **66**, 107 (1944).
(5) Carbide & Carbon Chemicals Corp., New York, "Organic Nitrogen Compounds," July 31, 1946.
(6) Conant, J. B., and Hall, N. F., *J. Am. Chem. Soc.*, **49**, 3047 (1927).
(7) Creamer, R. M., and Chambers, D. H., *J. Electrochem. Soc.*, **101**, 162 (1954).
(8) Elofson, R. M., and Mecherly, P. A., ANAL. CHEM., **21**, 565 (1949).
(9) Folin, Otto, and Flanders, F. F., *J. Am. Chem. Soc.*, **34**, 774 (1912).
(10) Fritz, J. S., and Keen, R. T., ANAL. CHEM., **25**, 179 (1953).
(11) Fritz, J. S., and Lisicki, N. M., *Ibid.*, **23**, 589 (1951).
(12) Gran, Gunnar, and Althin, Bengt, *Acta Chem. Scand.*, **4**, 967 (1950).
(13) Grasselli Chemicals Dept., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del., "Dimethyl Formamide Product Information."
(14) Hammett, L. P., "Physical Organic Chemistry," p. 261, New York, McGraw-Hill Book Co., 1940.
(15) Hantzsch, A., and Voigt, W., *Ber.*, **62B**, 975 (1929).
(16) Higuchi, Takeru, Concha, Jesusa, and Kuramoto, Roy, ANAL. CHEM., **24**, 685 (1952).
(17) Higuchi, Takeru, and Zuck, D. A., *J. Am. Chem. Soc.*, **73**, 2676 (1951).
(18) Izmailov, N. A., *Zhur. Anal. Khim.*, **4**, 267, 275 (1949).
(19) Katz, Max, and Glenn, R. A., ANAL. CHEM., **24**, 1157 (1952).
(20) Kirrmann, Albert, and Daune-Dubois, Nelly, *Compt. rend.*, **236**, 1361 (1953).
(21) Lavine, T. F., and Toennies, G., *Am. J. Med. Sci.*, **185**, 302 (1933).
(22) Lykken, L. L., Porter, P. E., Ruliffson, H. D., and Tuemmler, F. D., IND. ENG. CHEM., ANAL. ED., **16**, 219 (1944).
(23) Maron, S. H., Ulevitch, I. N., and Elder, M. E., ANAL. CHEM., **21**, 691 (1949).
(24) Masui, Masaichiro, and Kamura, Yuichi, *J. Pharm. Soc. Japan*, **71**, 1194 (1951).
(25) Moss, M. L., Elliott, J. H., and Hall, R. T., ANAL. CHEM., **20**, 784 (1948).
(26) Palit, S. R., IND. ENG. CHEM., ANAL. ED., **18**, 246 (1946).
(27) Pifer, C. W., Wollish, E. G., and Schmall, Morton, ANAL. CHEM., **25**, 310 (1953).
(28) Putnam, G. L., and Kobe, K. A., *Trans. Electrochem. Soc.*, **74**, 609 (1938).
(29) Ruehle, A. E., IND. ENG. CHEM., ANAL. ED., **10**, 130 (1938).
(30) Ruhoff, J. R., and Reid, E. E., *J. Am. Chem. Soc.*, **59**, 401 (1937).
(31) Seltz, Harry, and Silverman, Louis, IND. ENG. CHEM., ANAL. ED., **2**, 1 (1930).
(32) Sprengling, G. R., *J. Am. Chem. Soc.*, **76**, 1190 (1954).
(33) Swick, R. W., Buchanan, D. L., and Nakao, Akira, ANAL. CHEM., **24**, 2000 (1952).
(34) Tomicek, O., and Dolezal, J., *Chem. Listy*, **43**, 193 (1949).
(35) Vespe, Vincent, and Fritz, J. S., *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 197 (1952).
(36) Wolff, J. P., *Anal. Chim. Acta*, **1**, 90 (1947).

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Determination of Active Hydrogen Atoms

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This work was done in an endeavor to obtain satisfactory analytical data on certain alkaloids. In Zerewitinoff determinations it was found that the influence of temperature is probably often restricted to promoting solution and liberating occluded gases, that the influence of solvent is relatively unimportant, and that the activity of the hydrogen atoms of active methylene groups varies considerably with environment. The role of heat and of solvent in the estimation and the activity of active hydrogen atoms were determined.

IN THE Zerewitinoff determination of active hydrogen atoms in various *Mitragyna* alkaloids, it was found that the values increased considerably with increase in temperature. Little attempt has hitherto been made to investigate the influence of either temperature or solvent on the results of Zerewitinoff determinations. The main work is that of Lieff, Wright, and Hibbert (*9*), who examined 23 substances in amyl ether, dioxane, and pyridine. Little attempt has been made to discuss the reasons for anomalous results. Fuchs, Ishler, and Sandhoff (*2*) attribute the low values for certain acids to insolubility, and Lehman and Basch (*8*) suggest that a further contributory factor may be that the reaction takes place only at the surface. Kohler, Stone, and Fuson (*6*) mention three possible difficulties—relative insolubility of the substance, insolubility of intermediate products, and occurrence of successive reactions.

Except for these comments such contradictory results as those for resorcinol (see Table I) seem to have passed unchallenged.

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Table I. Literature Values for Resorcinol

Solvent for Reagent	Solvent for Resorcinol	Temp., ° C.	No. of Active H Atoms	Ref.
Butyl ether	Isoamyl ether	90	1	(*2*)
None	Pyridine	90	1	(*8*)
Ethyl ether	None	0	1.5	(*7*)
Amyl ether	Amyl ether	140	2	(*3*)
Pyridine	Amyl ether	Room	2	(*11*)

EXPERIMENTAL

Solvents. The pyridine was stored over solid potassium hydroxide and distilled before use. The ethyl ether was dried overnight over sodium wire and then distilled. The other solvents were first refluxed for 5 hours with sodium wire and stored over the wire overnight. Next they were distilled, first from sodium and then from phosphorus pentoxide. Since the butyl ether gave a peroxide test with vanadic acid, the peroxide was destroyed by treatment with alkaline silver nitrate.

Analytical Specimens. 3-Nitrosalicylic acid, 2-nitroresorcinol, 3-nitro-*o*-cresol, 1-phenyl-3-methyl-5-pyrazolone, purpurogallin, and the various fluorene derivatives and dibasic esters used were synthesized by standard methods. The other substances were obtained from reputable sources. All were carefully purified before use.

Analytical Procedure. The Grignard reagent was prepared in the usual way. To prevent excess methyl iodide producing methane at higher temperatures (see Table II), an excess of magnesium was always used. With every batch of solvent and reagent used, control experiments were done at both room temperature and at 170° C. The standard procedures for the active determinations were all found satisfactory (*1, 4, 10, 11*). In this investigation the substance was dissolved in 2.0 ml. of solvent and about 2 ml. of reagent were added. In cases of low solubility, 4.0 ml. of solvent were used. At room temperature the

Table II. Number of Active Hydrogen Atoms Found

Acid	No. of Active Hydrogen Atoms Found: Room temp.	50–60° C.	100° C.	170° C.
Carboxylic Acids[a]				
Benzoic	1.08	1.08	1.08	1.10
Phthalic[b, c]	0.32	0.33	0.49	2.10
Isophthalic[b, c]	0.55	0.55	0.78	1.20
Terephthalic[b, c]	1.27	1.33	1.67	2.04
Salicylic[b, c]	1.67	1.80	2.02	2.02
4-Hydroxybenzoic[b, c]	0.29	0.39	0.66	2.00
3-Nitrosalicylic, H_2O	2.99	..	..	..
2,4-Dihydroxybenzoic[d]	0.00	0.00	0.00	1.73
2,5-Dihydroxybenzoic[d]	0.00	0.00	0.00	1.87
2,6-Dihydroxybenzoic[c]	2.23	2.60	3.29	3.66
2,6-Dimethoxybenzoic	0.97	0.99	..	..
2-Aminobenzoic[b, c]	1.21	1.86	2.64	2.83
4-Aminobenzoic[b, c]	1.56	1.72	3.04	..
Benzilic[d]	0.71	0.71	0.77	1.92
Mandelic[d]	0.57	0.75	1.30	1.95
Chloroacetic[c]	0.48	0.63	0.97	0.98
Phenols				
OH only				
Catechol	1.73	1.73	1.82	1.82
Resorcinol[c]	1.79	1.83	1.87	1.92
OH COOH				
Salicylic[b, c]	1.67	1.80	2.02	2.02
4-Hydroxybenzoic[b, c]	0.29	0.39	0.66	2.00
3-Nitrosalicylic, H_2O	2.99	..	..	..
2,4-Dihydroxybenzoic[d]	0.00	0.00	0.00	1.73
2,5-Dihydroxybenzoic[d]	0.00	0.00	0.00	1.87
2,6-Dihydroxybenzoic[c]	2.23	2.60	3.29	3.66
OH COOR				
Ethyl salicylate	1.03	..	..	..
Methyl 4-hydroxybenzoate[c]	1.04	..	..	..
OH CHO				
Salicylaldehyde	1.06	..	..	..
4-Hydroxybenzaldehyde[b, c]	0.41	0.48	0.55	0.87
OH COR				
2-Hydroxyacetophenone	1.06	..	..	..
3-Hydroxyacetophenone[c]	0.66	0.83	1.00	..
1-Hydroxyanthraquinone[b, c]	0.76	1.03	..	..
Purpurogallin[b]	1.37	1.81	2.57	3.98
OH NO_2				
2-Nitrophenol	1.01	..	..	..
4-Nitrophenol	0.97	..	..	..
3-Nitro-*o*-cresol	1.00	..	..	..
2-Nitroresorcinol[c]	2.00	..	..	..
2,4,6-Trinitroresorcinol[c]	2.00	..	..	..
OH Cl				
2-Chlorophenol	1.04	..	..	..
4-Chlorophenol	1.10	..	..	..
Heterocyclic				
8-Quinolinol[c]	0.77	0.77	0.77	0.79
Amines[e]				
NH_2 only				
Aniline	1.29	1.32	1.45	2.02
N-Methylaniline	1.08	..	..	..
Cyclohexylamine	1.23	1.35	1.39	2.03
NH_2 Me				
o-Toluidine[c]	1.23	1.50	1.62	1.82
m-Toluidine	1.21	1.45	2.01	..
p-Toluidine[c]	1.61	1.66	1.73	1.97
Two Activating Groups (*Continued from col. 1, p. 57*)				
$COCH_2CN$				
Ethyl cyanoacetate[c]	0.87	0.91	0.95	0.97
$COCH_2$ Phenyl				
Ethyl phenyl acetate	0.04	0.04	0.04	0.05
Dibenzyl ketone	0.56	0.63	0.74	0.83
Phenyl CH=				
Indole	1.01	1.30	..	1.97
	1.04	1.12	..	1.80
COCH=				
Dypnone	0.04	0.04	0.07	0.08
Phenyl CH_2CN				
Phenylacetonitrile	0.36	0.36	0.49	0.59
Phenyl CH_2 phenyl				
diphenylmethane	0.00	0.00	0.09	0.17
Fluorene derivatives				
Fluorene	0.40	0.61	0.66	1.43
2,7-Dichlorofluorene	0.00	0.00	0.05	0.18
2,7-Dibromofluorene[b, c]	0.27	0.87	0.97	1.87
Three Activating Groups				
COCH Phenyl CO				
Diethyl phenylmalonate	0.91	0.98	..	..
Ethyl 2,4-dinitrophenyl acetoacetate[c]	0.99	..	..	..
Phenyl $_3C$				
Triphenylmethane	0.00	0.05	0.05	0.05
9-Substituted fluorenes				
Fluorene-9-carboxylic acid[b]	1.75	1.99	..	..
2,7-Dichlorofluorene-9-carboxylic acid	0.96	1.03	1.06	2.00
9-Carbomethoxyfluorene	0.97	..	..	..
2,7-Dichloro-9-carbomethoxyfluorene	0.98	..	..	..
2,7-Dibromo-9-carbomethoxyfluorene[c]	0.96	0.96	0.98	1.03
Nitro Compounds				
2-Nitrophenol	1.01	..	..	..
4-Nitrophenol	0.97	..	..	..
3-Nitro-*o*-cresol	1.00	..	..	..
2-Nitroresorcinol[c]	2.00	..	..	..
2,4,6-Trinitroresorcinol[c]	2.00	..	..	..
3-Nitrosalicylic acid, H_2O[b, c]	2.99	..	..	..
2-Nitroaniline[f]	1.77	..	..	2.33
3-Nitroaniline[c]	0.98	1.01	1.05	1.75
4-Nitroaniline[c]	0.56	0.66	0.71	..
2-Nitro-4-methoxyaniline[c]	1.40	1.40	1.53	..
Halogen Compounds[g]				
Methyl iodide	0.00	0.02	1.00	..
3-Chloropropanol[c]	0.51	0.66	0.97	0.98
Chloroacetic acid[c]	0.48	0.63	0.97	0.98
2-Chloroaniline	1.30	1.48	2.08	..
4-Chloroaniline[c]	1.24	1.26	1.63	2.01
s-Tribromoaniline	2.05	..	..	..
Reagent and Substance in Dibutyl Ether (Final Values at 140° C.)				
Phthalic acid[b]	0.87	1.78	..	..
	0.11	0.25	0.39	1.97
	2.02	..	..	..
Isophthalic acid[b]	0.31	0.71	1.14	1.54
Terephthalic acid[b]	0.65	0.65	0.67	1.86
Resorcinol[c]	1.98	..	..	..
	2.01	..	..	..

NH_2 CO				
2-Aminobenzoic acid[b,c]	1.21	1.86	2.64	2.83
4-Aminobenzoic acid[b,c]	1.56	1.72	3.04	..
1-Aminoanthraquinone	0.49	0.53	1.27	1.68
NH_2 halogen				
2-Chloroaniline	1.30	1.48	2.08	..
4-Chloroaniline[c]	1.24	1.26	1.63	2.01
2,4,6-Tribromoaniline	2.05	..	..	..
NH_2 NO_2				
2-Nitroaniline[f]	1.77	..	..	2.33
3-Nitroaniline[c]	0.98	1.01	1.04	1.15
4-Nitroaniline[c]	0.56	0.66	0.71	..
2-Nitro-4-methoxyaniline[c]	1.40	1.40	1.53	..
Heterocyclic				
Piperidine[c]	0.62	0.79	1.11	..
Indole	1.01	1.30	..	1.97
	1.04	1.12	..	1.80
Skatole	1.00	1.00	1.00	1.00
Carbazole	0.91	0.95	0.95	0.96
Pyridine	0.00	0.00	0.00	0.00
α-Picoline	0.00	0.00	0.00	0.08
γ-Picoline	0.02	0.06	0.06	0.07
s-Collidine	0.00	0.00	0.00	0.00
Isoquinoline	0.07	0.08	0.08	0.08
8-Quinolinol	0.77	0.77	0.77	0.79
1-Phenyl-3-methyl-5-pyrazolone[b,c]	0.07	0.15	0.57	1.02
ALKALOIDS				
Atropine[b]	0.00	0.00	0.02	0.18
Brucine	0.05	0.06	0.18	0.51
Calycanthine[b,c]	2.19	2.34	3.08	3.79
Cinchonine	0.86	0.96	1.17	1.66
Mitragynine[b,c]	1.89	2.26	2.84	2.97
Morphine[b]	0.10	0.37	0.72	2.00
Narcotine	0.00	0.00	0.00	0.00
Rhynchophylline[b,c]	0.87	1.53	1.81	3.08
Strychnine[b,c]	0.36	0.36	0.45	1.02
Yohimbine hydrochloride[b,c]	1.38	1.60	1.86	2.00
COMPOUNDS CONTAINING ACTIVE METHYLENE GROUPS				
One Activating Group				
CH_2CO				
Methyl ethyl ketone	0.04	0.06	0.06	0.08
Diethyl succinate	0.03	0.03	0.04	0.05
Diethyl adipate	0.05	0.06	0.06	0.06
1-Phenyl-3-methyl-5-pyrazolone[b,c]	0.07	0.15	0.57	1.02
Phenyl CH_2				
Dibenzyl	0.00	0.00	0.05	0.06
CH_2 NO_2				
Nitromethane	0.01	0.02	0.02	0.02
Me—C=N				
α-Picoline	0.00	0.00	0.00	0.08
γ-Picoline	0.02	0.06	0.06	0.07
s-Collidine	0.00	0.00	0.00	0.00
Two Activating Groups				
$COCH_2CO$				
Diethyl malonate	1.71	1.71	1.71	2.05
2-Carbomethoxycyclopentanone	1.02	..	..	..
Ethyl acetoacetate	0.73	0.73	0.83	0.93
Acetylacetone	0.59	0.59	0.61	0.90
Malonamide[b,c]	0.15	0.46	0.46	1.30
COCHRCO				
Diethyl *n*-propyl malonate	0.99	..	..	..
Diethyl isopropyl malonate	1.00	..	..	..
Diethyl *n*-butyl malonate	0.98	..	..	..
Ethyl methyl acetoacetate	0.79	0.89	0.96	1.02
Hydroquinone[b,h]	0.50	0.61	1.41	2.00
	1.92	..	..	..
Phloroglucinol[b,c]	2.23	2.37	2.99	..
Indole[c]	0.78	0.78	0.85	1.07
	1.09	1.14	1.25	1.44
Diethyl malonate[c]	0.90	0.96	1.26	1.34
	1.28	1.36	1.77	..
Ethyl acetoacetate	1.03	1.06	1.13	..

[a] Fluorene-carboxylic acids are given in section on compounds containing active methylene groups.
[b] Substance insoluble.
[c] Reaction product insoluble.
[d] Determination by Rachel Gourley.
[e] Four amides showed similar results.
[f] This result is typical of several done on different specimens of the compound.
[g] Certain halogeno-fluorenes are given in section on compounds containing active methylene groups.
[h] In the first estimation with hydroquinone, since a large amount was taken, it was only partly dissolved. This is a good illustration of the influence of solubility.

Table III. Data on Substances in Various Solvents and Reagent in Phenetole

Substance	Solvent	No. of Active H Atoms Found: Room temp.	50–60° C.	100° C.	170° C.
Phthalic acid	Ether[a,b]	1.93	..	..	..
	Amyl ether[a]	0.32	0.33	0.49	2.14
Resorcinol	Anisole	1.98	..	..	..
	Ether[b]	1.98	..	..	..
	Amyl ether	1.45	1.78	1.81	1.84
	Pyrine[b]	0.05	0.03	..	..
		0.13	0.13	..	..
		0.05	0.02	0.10	..
Hydroquinone	Anisole	1.40	1.55	1.79	..
	Ether	2.05	..	..	..
	Amyl ether[b]	0.47	0.49	0.60	1.55
	Pyridine	1.99	..	..	..
Phloroglucinol	Ether[b]	2.99	..	..	..
Fluorene	Anisole	0.00	0.00	0.00	..

[a] Substance insoluble.
[b] Reaction product insoluble.

Table IV. Values in Various Solvents at Room Temperature[a]

Substance	Solvents: Phenetole	Anisole	Amyl ether	Ether	Pyridine	Butyl ether	Ether	Values of Ref. (2)
Catechol	1.73	..	..	..	..	..	..	1.99
	1.82[b]	1.98	..	..	..	..	..	..
Resorcinol	1.79	1.98	1.45	1.98	0.10	2.01	2.02	0.98
	1.92[c]	..	1.84[c]	..	1.13[b]	..	..	..
Hydroquinone	1.02	1.40	0.47	2.05	1.99	2.09	0.81	0.00
	1.95[c]	1.79[c]	1.55[c]	..	..	..	..	..
Phloroglucinol	..	..	..	2.99	..	2.23	2.93	0.00
	..	..	..	..	..	2.99[c]	..	..
Phthalic acid	0.32	..	0.32	1.93	..	2.02	1.95	0.00
	2.10[c]	..	2.14[c]	..	..	..	..	..
Isophthalic acid	0.55	..	..	..	..	0.31	0.00	0.00
	1.20[c]	..	..	..	..	1.54[c]	..	..
Terephthalic acid	1.27	..	..	..	..	0.65	..	0.00
	2.04[c]	..	..	..	..	1.66[c]	..	..
Diethyl malonate	1.71	..	..	..	..	1.28	..	0.66[d]
	2.05[c]	..	..	..	..	1.77[c]	..	..
Ethyl acetoacetate	0.73	..	..	..	..	1.03	..	0.99
	0.93[c]	..	..	..	..	1.13[c]	..	..
Fluorene	0.40	0.00	..	..	..	0.15	..	..
Indole	1.01	..	..	..	..	1.09	..	..
	1.97[c]	..	..	..	..	1.44[c]	..	..

[a] In the first five columns the reagent is in phenetole, and in the next two, in butyl ether.
[b] At 100° C.
[c] At reflux temperature.
[d] Miscalculated by Fuchs, Ishler, and Sandhoff (2) as 1.00.

mixture was let stand until no more methane was evolved—i.e., until there was no difference in two readings of the buret taken 10 minutes apart. At higher temperatures the time allowed was 5 minutes. If the substance and reagent solutions, before mixing, were allowed to stand in the apparatus until oxygen uptake was complete, it was found unnecessary to work in an inert atmosphere.

RESULTS OBTAINED

Data are tabulated in Tables II, III, and IV. For convenience in comparing related compounds some substances are mentioned twice—e.g., salicylic acid is listed as both an acid and a phenol.

DISCUSSION

Influence of Temperature. Temperature plays a dual role. In certain cases, particularly with amino, amido, and active methylene groups, activity increases with increase in temperature. In the other cases, as long as the substance and the reaction product are in solution, the expected values are obtained at room temperature. In cases of insolubility the effect of heating may be threefold—substances insoluble in the cold may dissolve in the hot solvent; agitation, especially in a refluxing mixture, may help to bring the substance into closer contact with the reagent; and gases occluded in a gelatinous reaction product may be expelled.

With the exception of 2-nitroaniline, none of the 78 compounds examined at 170° C. showed any abnormally high value. On the other hand, although 100° C. is often regarded as the upper temperature limit in Zerewitinoff determinations, many substances failed to show the full amount of active hydrogen until refluxed. Examples are phthalic and 4-hydroxybenzoic acids, purpurogallin, aniline, and cyclohexylamine.

Since chelated acids are more readily decarboxylated, the high value (3.66) for 2,6-dihydroxybenzoic acid at 170° C. is due to the partial decarboxylation at higher temperatures of this highly chelated acid. Similar high values would also be expected of 2,6-diamino- and 2,4,6-trihydroxybenzoic acids. This decarboxylation effect is not, however, shown by the monochelated acids—e.g., 2-amino- and -hydroxy-, and 2,4- and 2,5-dihydroxybenzoic acids—nor by the α-hydroxy acids benzilic and mandelic acids.

Influence of Solvents. With the mixed solvent of *n*-butyl ether and isoamyl ether, Fuchs, Ishler, and Sandhoff (*2*) obtained very low values for the various phthalic acids and dihydroxybenzenes and for phloroglucinol and diethyl malonate. Since most of these gave satisfactory results in phenetole, a series of comparative determinations was done with the reagent in either phenetole or dibutyl ether and the substance in one of a variety of solvents. As is shown by the data in Table IV, as long as the substance is soluble the influence of solvent is negligible.

Influence of Groupings and Structural Considerations. Indolic Compounds. That the extra active hydrogen atom shown by indole at higher temperatures occurs in neither skatole (3-methylindole) nor carbazole suggests some activation of the beta position in the pyrrole nucleus by the aromatic nucleus on the one side and the double bond of the heterocyclic ring on the other. This is paralleled of course by the well-known ease of substitution of indole in the beta position.

Nitro Compounds. Although Jurecek (*5*) asserts that nitro compounds show abnormal results, an examination of Table II shows that 2-nitroaniline is the only nitro compound to show any abnormality.

Amines and Amides. The results in Table II support the attention in the literature that in the amino group the second hydrogen is active only at high temperatures. It is interesting that *N*-methylaniline is fully active at room temperature.

Halogen Compounds. From the results available it seems that if the chlorine atom has no activating influence. On the other hand, comparison of the values for aniline and 2,4,6-tribromoaniline and for fluorene and 2,7-dibromofluorene suggests that the bromine atom does activate.

Chelation. That intermolecular hydrogen bonding has no deactivating influence on active hydrogen atoms is obvious from the readiness with which so many acids, alcohols, and phenols show their activity. That the intramolecular bond may have a deactivating influence is suggested by the low values for the 2,*x*-dihydroxybenzoic acids. This suggestion is contradicted, however, by the results with salicylic acid, ethyl salicylate, salicylaldehyde, 2-hydroxyacetophenone, 2-nitrophenol, and the very strongly chelated 2-nitroresorcinol. The absence of any activating influence is shown by the normal behavior of the 2-amino- compounds. As already mentioned, the facility with which the hydrogen atoms of the weakly chelated 2,4,6-tribromoaniline react is probably due to halogen activation.

One chelation effect does exist. In that intramolecularly bonded phenols and acids are more soluble than their isomers in organic solvents, chelation may be said to decrease inactivity because of insolubility.

Steric Hindrance. The absence of steric hindrance is shown by the complete activity of such compounds as 2-nitroresorcinol, 2,6-dimethoxybenzoic acid, and 2,4,6-trinitroresorcinol.

Active Methylene Groups. *One Activating Group.* Although the γ-hydrogen atom in esters, nitro compounds, etc., enters into a variety of reactions—e.g., the Claisen and Dieckmann condensations of esters and the reaction of primary and secondary nitro compounds and of methyl ethyl ketone with nitrous acid—one activating group is insufficient to cause the production of methane.

Two Activating Groups. With a methylene group between two activating groups both hydrogen atoms may be active—e.g., in diethyl malonate. Often however, as in acetylacetone, and in cyan- and acetoacetates, even at higher temperatures only one atom is active. Thus the —COMe group is less of an activator than is the —COOEt group. That the phenyl group is even weaker is shown by the negligible activity of di- and even triphenylmethane.

The result with malonamide is frankly puzzling.

As far as the fluorenes are concerned three facts are significant: In fluorene itself, although there is not complete activity, nevertheless it is greater than that of di- or even triphenylmethane. Ortho bridging between the two phenyl groups has greatly increased the activity. Introduction of a 9-carboxy or -carbomethoxy group greatly increases the activity. Although 2,7-dichloro- compounds usually show less activity, the introduction of two bromine atoms in these positions increases activity.

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LITERATURE CITED

(1) Braude, E. A., and Stern, E. S., *J. Chem. Soc.*, **1946**, 404.
(2) Fuchs, W., Ishler, N. H., and Sandhoff, A. G., Ind. Eng. Chem., Anal. Ed., **12**, 507 (1940).
(3) Hibbert, H. H., and Sudborough, J. J., *J. Chem. Soc.*, **1904**, 933.
(4) Houben-Weil, "Methoden der organischen Chemie," p. 317, "Anal. Methoden," Phreme, Stuttgart, 1952.
(5) Jurecek, M., *Chem. Listy*, **40**, 239 (1946); *Brit. Abstr.*, A II, 913 (1950).
(6) Kohler, E. P., Stone, J. F., and Fuson, R. C., *J. Am. Chem. Soc.*, **49**, 3181 (1927).
(7) Krnitsky, J. A., Johnson, J. L., and Carhart, H. W., *Ibid.*, **70**, 486 (1948).
(8) Lehman, R. A., and Basch, H., Ind. Eng. Chem., Anal. Ed., **17**, 428 (1945).
(9) Lieff, M., Wright, G. F., and Hibbert, H., *J. Am. Chem. Soc.*, **61**, 865 (1939).
(10) Wild, F., "Estimation of Organic Compounds," p. 55, Cambridge University Press, Cambridge, Eng., 1953.
(11) Zerewitinoff, T., *Ber.*, **40**, 2023 (1907).

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Radiochemical Determination of Cerium in Fission

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The work reported was undertaken to develop a simple and rapid radiochemical procedure for the determination of cerium activity produced in nuclear fission. A method was developed based on solvent extraction of cerium(IV) with methyl isobutyl ketone. Good separation from the large number of elements encountered in fission product sources is afforded by the extraction procedure as well as a considerable saving in time and effort over the ceric iodate precipitation method in previous use. Further applications of the new procedure are the preparation of carrier-free radioactive cerium and activation analysis for small amounts of cerium.

THE procedure currently employed for the radiochemical determination of cerium activity in fission (*1, 3*) is based on the insolubility of ceric iodate and involves a series of separations by precipitation. This procedure is consequently somewhat lengthy and tedious. It would be of considerable advantage to the analyst faced with many determinations or with the isolation of short-lived cerium activities to have a more rapid procedure without sacrificing accuracy or radiochemical purity. In developing such a procedure the solvent extraction of tetravalent cerium seemed most attractive. Extraction of cerium(IV) by ether from nitric acid solution was first studied by Imre (*6*), and also employed by Gryder and Dodson (*5*) and Bock and Meyer (*2*). Although cerium(IV) can be extracted by this method, the attack of ether by strong nitric acid and the oxidizing agents required to oxidize cerium(III) to cerium(IV) is a serious drawback. Warf (*11*) found that cerium(IV) is extracted by tri-*n*-butyl phosphate and that this solvent is satisfactorily resistant to nitric acid and strong oxidants. The disadvantage in this case, however, is that the separation from trivalent rare earths is not adequate (*7*). Rothschild *et al.* (*10*) observed that thorium nitrate is extracted by methyl isobutyl ketone (hexone). A patent has been issued to Pitzer (*9*) for a procedure in which cerium in trace concentration is extracted by methyl isobutyl ketone from aqueous media containing dichromate as the oxidizing agent. In the present investigation the method of Pitzer was found to be unsuccessful with cerium in macro concentrations. Good extraction of cerium by methyl isobutyl ketone was obtained in both trace and macro concentrations, however, from strong nitric acid solution using sodium (or potassium) bromate as oxidant, and a procedure was developed for the radiochemical determination of cerium based on this method. In the new procedure cerium(III) carrier is oxidized to cerium(IV) by sodium bromate in 9*M* nitric acid solution, extracted into methyl isobutyl ketone, back-extracted into water containing hydrogen peroxide to reduce cerium(IV) to cerium(III), and precipitated as cerium oxalate for gravimetric determination of yield and activity measurements. The extraction step replaces seven precipitations used in the older procedure and results in considerable saving of time and effort. The cerium is obtained in high yield and radiochemical purity.

EXPERIMENTAL

Extraction of Cerium. For extraction tests both technical grade and redistilled methyl isobutyl ketone were used. Although the latter was somewhat superior with respect to reactivity with nitric acid and oxidizing agents, the technical grade solvent was found to be satisfactory for extraction of cerium, provided it is first equilibrated with nitric acid containing sodium bromate to remove reducing substances. The optimal nitric acid concentration for the extraction was determined both for carrier-free cerium-144 tracer and for cerium at a concentration of about 1 mg. per ml. The results are shown in Figure 1, where the distribution of cerium obtained by counting equal aliquots of the organic and aqueous phases is plotted as a function of nitric acid concentration. Cerium is well extracted at nitric acid concentrations above 7*M*, the optimal range being 8 to 10*M*. The aqueous phase should be relatively free of chloride ion (not greater than 0.1*M*) to avoid reduction of bromate and cerium(IV). Sulfate ion also lowers the efficiency of extraction, although concentrations up to 0.5*M* can be tolerated without serious loss in yield.

Separation from Other Elements. The elements with radioisotopes formed in appreciable yield in fission and likely to be extracted by methyl isobutyl ketone are zirconium, niobium, and ruthenium. Other interfering elements which may be encountered in fission product sources are thorium, uranium, and neptunium. The cerium procedure (as described) was tested for coseparation of these elements by using the following radioactive tracers: zirconium-95-niobium-95 (equilibrium mixture), ruthenium-106, normal uranium with UX_1 (thorium-234) in equilibrium, and neptunium-237. The results of these experiments are summarized in Table I. These data indicate that the separation from zirconium, niobium, and ruthenium is entirely adequate for fission product mixtures generally encountered. Separation from trivalent rare earths was shown to be excellent, not more than 0.1% of trivalent cerium-144 being extracted. For the remaining elements in Table I, however, modifications of the procedure may be required under certain conditions. For example, in the slow neutron-induced fission of uranium-235 in normal uranium the neptunium-239 activity formed by neutron capture in uranium-238 exceeds that of fission-produced cerium for 2 to 3 weeks after irradiation. During this time the separation of cerium from neptunium (Table I) would be inadequate.

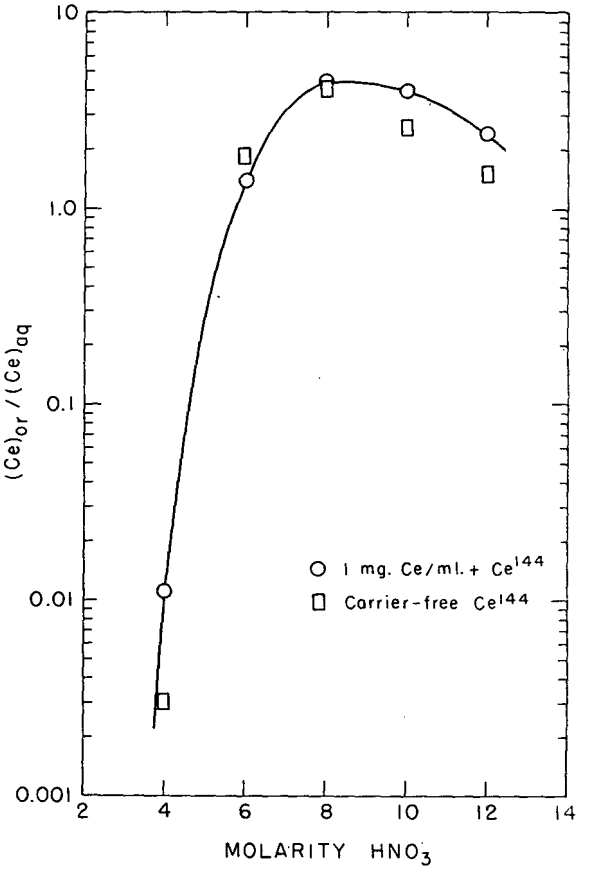

Figure 1. Effect of Nitric Acid Concentration on Extraction of Cerium by Methyl Isobutyl Ketone

Table I. Coseparation of Various Elements with Cerium

Element	% Extracted	% in Back-Extract	% in Cerium Oxalate	
			First	Second
Zr-Nb	25	7	0.5	0.03
Ru	..	1	0.05	0.002
Th	55	26	...	20
U	86	6	...	0.2
Np	96	7	...	1

The separation from uranium and thorium would also be insufficient if cerium activity in low intensity were to be isolated from large quantities of normal uranium. In this case the cerium would be contaminated both with weighable amounts of uranium (causing an error in the gravimetric determination of yield) and with the natural radioactivity of thorium-234. The additional separation from uranium, neptunium, and thorium required for such samples is readily obtained by a simple modification of the procedure to include extraction of these elements before oxidation and extraction of cerium. This is accomplished by adjusting the sample taken for analysis (containing cerium carrier) to a nitric acid concentration of 10*M* and a volume of about 10 ml., and extracting with 50 ml. of methyl isobutyl ketone for removal of uranium and neptunium, or with 50 ml. of tri-*n*-butyl phosphate for removal of thorium (*8, 11*). The solvents should be equilibrated with 10*M* nitric acid before use. The methyl isobutyl ketone extraction removes 86% of the uranium and 96% of the neptunium, and the butyl phosphate extraction removes more than 99% of the thorium with only a few per cent loss of cerium (*8*). Repeated extractions may be made if further purification is desired. The aqueous phase is then treated with 2 ml. of 2*M* sodium bromate solution to oxidize cerium(III) to cerium(IV), and the cerium is separated by the regular procedure.

Reaction of Methyl Isobutyl Ketone with Nitric Acid. In extractions of strong nitric acid solutions (6 to 12*M*) with methyl isobutyl ketone considerable amounts of nitric acid pass into the organic phase. It was observed that such solutions of nitric acid in methyl isobutyl ketone are unstable and will undergo a violent reaction after standing for a few hours. The methyl isobutyl ketone phases remaining after back-extraction with 5 ml. of water were observed to react similarly but only after standing for about 3 days. It is recommended, therefore, that the methyl isobutyl ketone not be equilibrated with nitric acid until just before use and that it be washed thoroughly with water (three times with an equal volume) soon after use. It is also recommended that nitric acid solutions which have been in contact with methyl isobutyl ketone be neutralized with ammonium hydroxide before storing or discarding. By observing these simple precautions no difficulties with reactivity have been encountered.

REAGENTS

Methyl isobutyl ketone (hexone)
Tri-*n*-butyl phosphate
Nitric acid, 6*M*, 9*M*, and concentrated
Sodium bromate, 2*M*
Hydrogen peroxide, 30%
Ammonium hydroxide, concentrated
Oxalic acid, saturated
Ethyl alcohol, absolute
Ethyl ether

Cerium Carrier Solution (cerium, 10 mg. per ml.). Dissolve 23 grams of C.P. cerous nitrate in 1 liter of water. Standardize as follows: Pipet 5-ml. aliquots into 50-ml. centrifuge tubes. To each add 1 ml. of 6*M* nitric acid and 15 ml. of water. Heat just to boiling and add 15 ml. of saturated oxalic acid with stirring. Cool in an ice bath for 10 minutes with occasional stirring. Filter on a weighed sintered-glass crucible with suction, transferring and washing with three 5-ml. portions of water. Wash three times with 5 ml. of ethyl alcohol, three times with 5 ml. of ether, and place in a vacuum desiccator. Evacuate for 2 minutes, release, and evacuate again for 5 minutes. Weigh as $Ce_2(C_2O_4)_3 \cdot 10H_2O$.

PROCEDURE

To the aliquot (1 to 5 ml.) taken for analysis add 1 ml. (10 mg.) of standardized cerium carrier, 2 ml. of 2*M* sodium bromate, and sufficient concentrated nitric acid to make the solution 8 to 10*M* in nitric acid. Transfer to a separatory funnel containing 50 ml. of methyl isobutyl ketone (which has just been equilibrated with 10 ml. of 9*M* nitric acid containing 2 ml. of 2*M* sodium bromate) and shake for 15 to 30 seconds. Withdraw the aqueous phase and wash the methyl isobutyl ketone phase twice with 10 ml. of 9*M* nitric acid containing a few drops of 2*M* sodium bromate. (Caution. Combine the aqueous phase and washings, and neutralize with ammonium hydroxide before discarding.) Back-extract the cerium by shaking the methyl isobutyl ketone phase with 5 ml. of water containing 2 drops of 30% hydrogen peroxide. (Caution. Wash the methyl isobutyl ketone three times with 50 ml. of water before discarding.) Neutralize the aqueous phase by adding concentrated ammonium hydroxide (3 to 5 ml.) until a precipitate just appears, and acidify with 1.5 ml. of 6*M* nitric acid. Dilute the solution to a volume of 15 ml. with water, heat just to boiling, and add 15 ml. of saturated oxalic acid. Cool for 2 to 3 minutes with running water (or ice bath), centrifuge, decant, and wash the precipitate with 10 ml. of water. Dissolve the precipitate in 1 ml. of 6*M* nitric acid (warming if necessary), and dilute with water to 15-ml. volume. Repeat the oxalate precipitation, centrifuge, and filter with suction on a weighed filter paper disk in a small funnel, transferring and washing with three 5-ml. portions of water. Wash three times with 5 ml. of ethyl alcohol, three times with 5 ml. of ether, and place in a vacuum desiccator. Evacuate for 2 minutes, release, and evacuate again for 5 minutes. Weigh the cerium oxalate to determine the yield through the procedure, and mount for measurement of radioactivity.

The yield of cerium through the above procedure is usually about 80%, and the time required is approximately 1 hour.

RESULTS AND DISCUSSION

The methyl isobutyl ketone extraction procedure was compared with the ceric iodate precipitation procedure (*3*) by analysis for 282-day cerium-144 in 21 different samples of neutron-irradiated uranium. The results obtained by the two procedures agreed to within 2% for every sample. The extraction procedure was also employed for isolation of short-lived cerium activities from uranium fission. The separations were made shortly after irradiation, and the procedure was modified to include the pre-extraction with methyl isobutyl ketone for additional removal of neptunium-239. Decay curves showed the presence of the known 15-minute cerium-146, 33-hour cerium-143, and 33-day cerium-141 and confirmed the recent observations of Caretto and Katcoff (*4*) that no 1.8-hour cerium → 4.5-hour praseodymium chain exists in fission.

Extraction with methyl isobutyl ketone is also well suited for convenient isolation of carrier-free cerium activity from fission products. For this purpose, however, the extraction cycle should be repeated to obtain good separation from zirconium, niobium, and ruthenium (Table I). In the course of another investigation carrier-free cerium-144 of high purity was prepared by this method and examined in a mass spectrometer. No trace of other rare earths was observed.

Another application for which the new procedure should be highly satisfactory is the determination of small amounts of cerium by radioactivation. The sensitivity of this method is proportional to the neutron flux and to the cross section for activation. In the case of cerium as little as 10^{-7} gram may be determined with an accuracy of about 5% by counting the 33-hour cerium-143 produced in a 1-day irradiation at a thermal neutron flux of 2×10^{12} per sq. cm. per second (generally available in nuclear reactors).

LITERATURE CITED

(1) Ballou, N. E., "Radiochemical Studies: The Fission Products," C. D. Coryell and N. Sugarman, eds., National Nuclear Energy Series, Div. IV, Vol. 9, p. 1673, New York, McGraw-Hill Book Co., 1951.
(2) Bock, R., and Meyer, K. H., *Chem. Ing. Tech.*, **25**, 141 (1953).
(3) Boldridge, W. F., and Hume, D. N., "Radiochemical Studies: The Fission Products," C. D. Coryell and N. Sugarman, eds., National Nuclear Energy Series, Div. IV, Vol. 9, p. 1693, New York, McGraw-Hill Book Co., 1951.
(4) Caretto, A. A., and Katcoff, S., *Phys. Rev.*, **89**, 1267 (1953).
(5) Gryder, J. W., and Dodson, R. W., *J. Am. Chem. Soc.*, **73**, 2890 (1951).
(6) Imre, L., *Z. anorg. u. allgem. Chem.*, **104**, 214 (1927).
(7) Peppard, D. F., Faris, J. P., Gray, P. R., and Mason, G. W., *J. Phys. Chem.*, **57**, 294 (1953).
(8) Peppard, D. F., private communication.
(9) Pitzer, E. C., U. S. Patent **2,615,798** (Oct. 28, 1952).
(10) Rothschild, B. F., Templeton, C. C., and Hall, N. F., *J. Phys. & Colloid Chem.*, **52**, 1006 (1948).
(11) Warf, J. C., *J. Am. Chem. Soc.*, **71**, 3257 (1949).

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Determination of Organic Soda in Aluminate Solutions by an Ion Exchange Method

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A fast and accurate method was needed for the determination of the organic soda content of aluminate solutions. Fluorides and phosphates are removed with alumina by a carbon dioxide precipitation. Subsequent treatment with a cation exchanger removes the remaining cations, molybdate, and most of the vanadate. Organic acids plus the sulfuric and hydrochloric acids are titrated with standard sodium hydroxide in the effluent. After sulfate and chloride have been determined in the titrated solution, the sodium hydroxide equivalents are calculated and subtracted from the titration which represents the organic soda content of the sample. Five determinations may be completed in one day. Increased information on the various forms of alkali present in aluminate solutions is made available, which is of interest for the control of the Bayer process for obtaining alumina.

WHEN bauxite is submitted to the Bayer process for extracting alumina, part of the small amount of humic matter present is degraded and oxidizes to acids which remain in the aluminate solution in the form of sodium salts. The fate of starch, sometimes used as a filter aid, is similar, so that this addition also contributes to the organic content of aluminate solutions. The amount of sodium thus bound to organic acids and expressed as sodium carbonate is called organic soda. Because this form of alkali is not directly accessible to the usual acidimetric determination of total soda, a method of determination is desirable. Until recently the organic soda content of aluminate solutions was obtained by determining the total sodium content of the sample by the classical zinc uranyl acetate method and expressing the result as sodium carbonate. The total sodium carbonate by titration and the sodium carbonate equivalents of the sodium sulfate and sodium chloride present were subtracted and the difference was called organic soda. Corrections for silicate, vanadate, phosphate, and fluoride were neglected. This rather unsatisfactory system was used because a thorough literature search had failed to reveal any method for determining organically bound soda in aluminate solutions.

A more direct and accurate method has now been worked out, using an ion exchange. The principle of this method is as follows: If the alumina is removed from a suitable aliquot of the sample by means of a carbon dioxide precipitation and the filtrate from this separation submitted to exchange in a cation exchanging column, the effluent contains all the acidic constituents in the free state and thus they are accessible to alkalimetric titration after boiling out the carbon dioxide. This titration, corrected for the soda equivalents of the sodium sulfate and chloride present, is a direct measurement of the organic soda. In practice, the following minor constituents of Bayer solutions have to be considered.

Fluoride, Phosphate, and Silicate. Fluoride and phosphate could be expected to be quantitatively recovered by ion exchange and the silicate more or less completely. The presence of these acids in the effluent would require correction. Fortunately, they have been found to precipitate with the alumina during the carbon dioxide precipitation and thus have been eliminated from the system.

Molybdates. The molybdate content of aluminate solutions is generally low. Because molybdate is reduced by the ion exchange resin (*1*), it is removed at that point and does not appear in the effluent.

Table I. Recovery of Sodium Oxalate, Acetate, and Formate as Organic Na_2CO_3

Salt	Taken: Equiv. organic soda, mg.	Found, Organic Soda, Mg.
$Na_2C_2O_4$	52.7	52.4
	78.9	78.6, 78.8, 78.4
CH_3COONa	131.1	130.9
HCOONa	131.1	130.9

Vanadate. The case of this compound, which is always present in Bayer solutions, is slightly more complicated for small amounts find their way into the column effluent. In an Amberlite IR-120 column, 60 to 80% of the vanadate has been found to be reduced and retained. The soda equivalent of the amount remaining in the effluent is very small; tests carried out on synthetic samples have indicated that this error is compensated by a small loss of organic acids by adsorption on the precipitated alumina.

Chloride and Sulfate. These follow the organic acids throughout the procedure and the resulting hydrochloric and sulfuric acids are titrated at the same time as the organic acids. These two constituents must be determined in the titrated solution in order to make the proper corrections. Sulfate is easily determined by the usual barium sulfate precipitation method. The chloride determination is generally complicated by the presence of traces of iodide and measurable amounts of thiocyanate. No appreciable error is introduced by counting the former as chloride, but the latter must be eliminated before determining the chloride. For this purpose, the destruction of thiocyanate by boiling with hydrogen peroxide in alkaline solution (*2*) has been found most satisfactory.

EXPERIMENTAL EQUIPMENT AND REAGENTS

Ion Exchange Resin. Three cation exchanging resins have been tried with synthetic solutions containing organic sodium salts known or suspected to be present in actual Bayer solutions. A carboxylic type was entirely unsuitable, whereas Amberlite IR-120 was the best of the two sulfonated hydrocarbon types tried. Typical results obtained with sodium oxalate, acetate, and formate are summarized in Table I.

Tests carried out with sodium salts of other organic acids gave similar recoveries of 100 ± 0.2%. The same applies to other organic compounds actually extracted from Bayer aluminate solutions.

Ion Exchange Column. This simply consists of a 22-mm.-inside diameter glass tube, 550 mm. long, closed at the bottom by a one-hole rubber stopper through which passes a 5-mm.-inside diameter piece of glass tubing. To this tubing, is attached a piece of rubber tubing provided with a screw clamp which permits starting, stopping, and the regulating of the flow. The resin is supported by a wad of glass wool which effectively prevents any resin particles from passing into the effluent. This column is filled with Amberlite IR-120 cation exchange resin to a height of 330 mm.

Before using, water should be passed through the column until a 25-ml. portion does not require more than 1 drop of 0.1*N* sodium hydroxide to give a strong alkaline reaction to phenolphthalein. After using, the column is easily regenerated by passing through slowly 100 ml. of 4*N* hydrochloric acid and washing until acid-free with distilled water.

PROCEDURE

Determination of Total Acids. Pipet a 50-ml. sample of the aluminate solution into a 500-ml. volumetric flask, and make up

to the mark with water at 20° C. Mix thoroughly and pipet a 25-ml. aliquot into a 250-ml. Phillips beaker. Add 50-ml. of hot water, heat to 80° C. on a water bath, and pass in a brisk current of carbon dioxide for 20 minutes.

Set an 11-cm. Whatman No. 52 paper in an ordinary funnel placed on a Fisher Filtrator. Completely fill the paper with 2*N* sodium hydroxide, allow to soak for about 1 minute, and wash thoroughly with hot water using light suction. Discard washings and replace receiver by a 250-ml. alkali-resistant beaker. This prewash removes alkali-extractable compounds from the paper and ensures constant corrections.

Filter the precipitated alumina under light suction and wash 4 to 6 times with hot water. Police the precipitation flask but do not attempt to recover all strongly adhering alumina. Concentrate the filtrate to 30 to 40 ml. and cool to room temperature. Reserve as filtrate I.

Open the paper containing the precipitate on a flat surface (glass or plastic) and by using a spatula, transfer the precipitate back into the precipitation flask. Add 5 ml. of 1*N* sodium hydroxide and a little hot water; digest to dissolve the precipitate. Dilute to 75 ml. with hot water and reprecipitate, filter, and wash as above. Concentrate filtrate to 30 to 40 ml. and reserve as filtrate II.

Cautiously introduce filtrate I into the ion exchange column which has been washed with water to the point where a 25-ml. portion of water run through will not require more than 1 drop of 0.1*N* sodium hydroxide to react strongly alkaline to phenolphthalein. A brisk evolution of carbon dioxide takes place; when this slows down, open the screw clamp allowing the sample to slowly displace the water in the column. Close the screw clamp and allow to stand for 5 minutes. Replace receiver by a 300-ml. Erlenmeyer flask provided with a ground joint.

During the standing period, large carbon dioxide bubbles form and create gas pockets in the column. The escape of this gas will be greatly helped by tapping or vibrating the column.

After the 5 minutes have elapsed, add 25 ml. of water to the column and regulate the screw clamp, so as to obtain a flow of 25 ml. per minute (a slower rate does no harm). As soon as the liquid level reaches the top of the resin layer, add another 25-ml. portion of water and repeat this until eight 25-ml. portions of wash water have been used. Close the screw clamp and remove the receiver. Add 2 to 3 glass beads, and attach the flask to a reflux condenser by a ground joint. Bring to boiling and boil briskly for 5 minutes. Remove the heat, detach the condenser, and immediately cover the mouth of the flask with a small watch glass. Cool in running water. When sufficiently cool to handle comfortably, add 8 to 10 drops of 0.1% phenolphthalein indicator solution and titrate with 0.1*N* carbonate-free sodium hydroxide solution. Deduct 0.2 ml. and record titration I. Reserve titrated solution for determining chloride and sulfate.

After regenerating the column (or using another ready one) proceed in the same manner with filtrate II. Titrate, deduct 0.2 ml., and record titration II. Discard titrated solution which is sterile in chloride and sulfate.

Determination of Sodium Sulfate. Introduce reserved solution I into a 250-ml. volumetric flask and adjust to the mark with water at 20° C. Mix well, and pipet a 100-ml. aliquot into a 250-ml. beaker. Add 2 drops of methyl orange indicator solution and acidify with 12*N* hydrochloric acid, adding 0.5 ml. in excess. Bring to boiling, and from a pipet introduce 3 ml. of 1*N* barium chloride solution while stirring. Boil for 5 to 10 minutes, cover, and digest at 80° to 90° C. for 2.5 to 3 hours. Remove from heat, add 1 drop of S & S ash-free anticreep reagent without stirring. Filter on a 9-cm. (S & S no. 589) blue band paper. Decant all the clear supernatant liquid before transferring precipitate to the paper. Wash 5 times with hot water containing 1 ml. of anticreep fluid per 500 ml., once with alcohol or acetone, and 5 additional times with hot water containing anticreep fluid. Place the paper in a tared 15-ml. platinum crucible, dry at 120° to 130° C. in oven for 0.5 hour; ignite at 1000° C. for 0.5 hour. Cool in a desiccator and weigh.

Determination of Sodium Chloride. Pipet a second 100-ml. aliquot of the titrated solution into a 250-ml. Phillips beaker, and add 12.5 ml. of 2*N* sodium hydroxide followed by 10 ml. of Superoxol (30% hydrogen peroxide). Bring to boiling and boil for 15 minutes. Cool, add another 5 ml. of Superoxol, and boil again for 10 minutes. Cool and transfer to a 250-ml. beaker. Acidify with 2 ml. of concentrated nitric acid and stir in 6 to 8 ml. of 0.1*N* silver nitrate. Heat to boiling, stirring occasionally, to coagulate the precipitated silver chloride. Cover beaker and place in the dark. When the precipitate has settled, filter on a tared fritted filtering crucible and wash 3 to 4 times with cold 0.1*N* nitric acid. Dry at 125° to 130° C. for 2 hours and weigh.

If so desired, the chloride may also be titrated after the peroxide treatment.

Calculations. The sum of titrations I and II multiplied by 2.12 represents the sodium carbonate equivalent of all acids titrated, expressed in grams per liter.

With the aliquoting system used above, 100/250 of the titrated solution represents 1.0 ml. of the original aluminate solution, so that each milligram of barium sulfate and silver chloride actually represents 1 gram per liter. The sodium sulfate and chloride values and their sodium carbonate equivalents are obtained as follows:

$$(\text{Mg. } BaSO_4) \times 0.6086 = \text{g./l. } Na_2SO_4 \text{ and } (\text{mg. } BaSO_4) \times 0.4541 = \text{g./l. } Na_2CO_3$$

$$(\text{Mg. AgCl}) \times 0.4078 = \text{g./l. NaCl and (mg. AgCl)} \times 0.370 = \text{g./l. } NaCO_3$$

and finally:

(Na_2CO_3 equivalent of total acids) − (Na_2CO_3 equivalent of Na_2SO_4 plus NaCl) = organic Na_2CO_3 content in g./l.

If other sample or aliquot sizes are taken, the calculations should be changed accordingly.

This procedure applies to solutions containing approximately 20 to 80 grams per liter of alumina and 230 to 270 grams per liter of total soda. For other aluminate solutions the sampling and aliquoting may be changed to provide suitable titrations. For practical reasons, however, the amount of sodium carbonate introduced into the exchange column should not exceed 0.6 to 0.7 gram. For weak solutions or wash solutions containing phosphate and/or fluoride, it may be advisable to raise the alumina content by adding a measured amount of pure sodium aluminate (reagent grade) solution; this is to ensure complete removal of fluoride and phosphate. Excessive frothing in the column may be controlled by adding one drop of ether or a very thin spray of Corning antifoam; the use of the former is preferred because the latter may in time coat the resin with very hard-to-remove silicone compound. Aluminate solutions should not be introduced into the exchange column without first removing the bulk of the alumina by a carbon dioxide precipitation; if this is not done aluminium hydroxide will precipitate on the resin in the column and cause loss in efficiency and heavy adsorption losses. Such precipitated aluminum hydroxide is also difficult to remove entirely.

Adding some resin to the sample before pouring into the exchange column was not found satisfactory. The operation was messy and led to uncertainties regarding the quantitative washing out of the liberated acids.

ANALYTICAL RESULTS

Table II shows the results obtained by applying the method to a number of Bayer aluminate solutions.

Table II. Reproducibility of Organic Soda Determinations in Bayer Solutions

(Expressed as grams per liter of Na_2CO_3)

	Sample No.										
	1	2	3	4	5	6	7	8	9	10	11
	32.6	44.1	15.7	21.9	6.8	9.2	3.6	4.8	25.1	33.5	46.5
	32.7	43.7	15.5	21.7	6.3	9.3	3.8	4.3	24.6	33.5	46.9
	32.4	43.7	15.7	22.0	6.5	9.2	3.8	4.6	..	33.1	..
	32.5	43.8	15.6	20.8	6.2	9.0	..	4.2	..	..	..
	32.6	43.6	16.0	21.8	..	9.1	..	4.6	..	..	..
	32.3	44.2	..	..	..	9.2	..	..	..	..	..
	..	43.4	..	..	..	..	..	..	..	..	..
	..	43.6	..	..	..	..	..	..	..	..	..
Av.	32.5	43.8	15.7	21.6	6.4	9.2	3.7	4.5	24.8	33.3	46.7
Std. dev.	0.1	0.3	0.2	0.2	0.2	0.1	0.1	0.2		0.2	

The reproducibility is satisfactory and the accuracy is estimated as being of the same order with a slight negative bias owing to unavoidable small losses of organic acids by adsorption on the precipitated alumina hydrate. According to the experience acquired in this work an analyst can easily operate five columns at the same time and from one to five samples may be analyzed in one 8-hour day.

LITERATURE CITED

(1) Samuelson, O., "Ion Exchangers in Analytical Chemistry," pp. 74, 136, New York, John Wiley & Sons, 1953.
(2) Schulek, E., *Z. anal. Chem.*, **112**, 415 (1938).

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Separation of Mixtures of Tritium and Hydrogen Using Hertz Pumps

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Tritium of a purity in excess of 99.9% has been prepared from hydrogen-tritium mixtures by means of a 12-Hertz-pump system and a 16-pump system employing continuous gas flow. Satisfactory separations were accomplished at pressures greatly in excess of those described by other workers.

HYDROGEN-3, or tritium, has in recent years become available for experimental purposes. This paper describes a method suitable for obtaining isotopically pure tritium in quantities satisfactory for many laboratory purposes. Performance data pertinent to the operation of the two systems are given.

In the analysis of tritium-hydrogen mixtures it was found that the equilibration of such mixtures, according to the equation

$$H_2 + T_2 \rightleftharpoons 2HT$$

posed a problem. This reaction proceeds at room temperature with a measurable velocity, and its course can be followed by the thermal conductivity method of analysis. Therefore, in order to obtain unambiguous analyses it was necessary to charge the analysis cells only with gas mixtures which had already undergone equilibration. This reaction probably occurs via a free-radical mechanism, induced by the radioactivity of tritium. It is known that the analogous equilibration of hydrogen and deuterium can be accelerated by exposure to external radiation (*3*). Figure 1 illustrates the practical effect of this self-equilibration on analysis by thermal conductivity. For this figure, the self-equilibrated points were obtained by allowing a gas mixture to stand at 35° C. until several successive thermal conductivity analyses became constant. This equilibrated mixture was reacted with cold, finely divided uranium, and then re-evolved by pumping at low pressure on the warm (approximately 175°) hydride. Fractionation of the mixture was avoided by evolution and analysis of the entire gas sample. A change in the analyses after this treatment might be expected because of equilibration at different temperatures, but none was observed, possibly because the time required for evolution was sufficiently long for self-equilibration by β-particle action to mask a small change.

This self-equilibration also means that the separation of a hydrogen-tritium mixture containing little hydrogen is primarily a separation of tritium and HT molecules, so that the differences in physical properties favorable to separation are reduced. Even so, the mass ratio of 6 to 4 is still considerably more favorable than one ordinarily encounters in isotope separations.

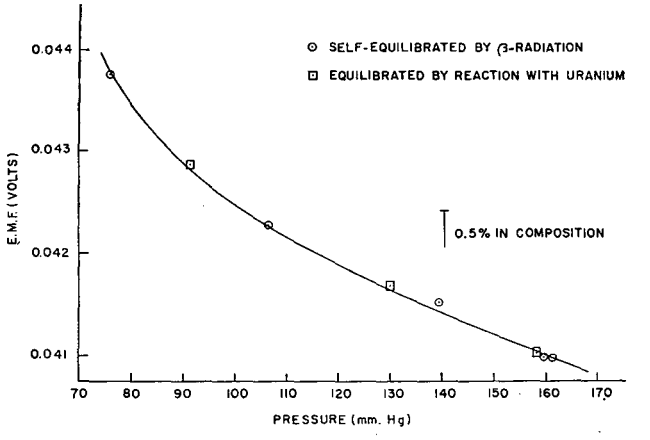

Figure 1. E.M.F. *vs.* Pressure with Fixed Composition of Hydrogen-Tritium

Showing pressure dependence of e.m.f. and apparent self-equilibration

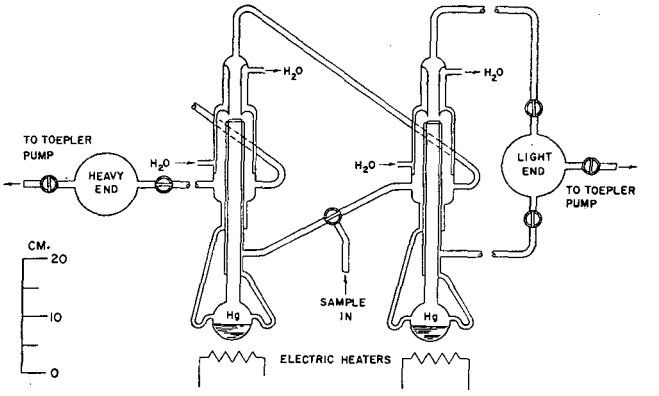

Figure 2. Hertz Pump Separation Line

Another difficulty attributable to the β-decay of tritium is that the helium-3 produced in uranium tritide is removed completely only with difficulty. For this reason, freshly prepared uranium tritide was used to minimize helium content.

EXPERIMENTAL PROCEDURE

Apparatus. The diffusion pumps employed (Figure 2) were described by Sherr (*4*, *5*), who modified Hertz's earlier pumps (*2*). In the static separation line, 12 such pumps were connected in series, the first and last pumps being connected also to bulbs of 400-ml. volume (later increased to 1 liter). Provision was made for the introduction and removal of gas at either end and at the center of the line. The initial charge for the line was obtained from uranium hydride storage furnaces and transferred by automatic Toepler pumps. The operation of these pumps was controlled by an *RF* oscillator circuit, in order to eliminate glass to metal seals. Coils wrapped around the inlet and outlet arms of the Toepler pumps changed in inductance as mercury filled these arms, interrupting the oscillation and thereby operating the appropriate solenoid valves. In the event of an accident, these controls would not provide the spark necessary to ignite a hydrogen-air mixture. When the line and end volumes contained gas at the desired pressure, the pumps were started. After equilibration was established, the end volumes were isolated from the rest of the line by stopcocks and their contents transferred to the analysis system, using Toepler pumps. The line was then ready for refilling.

In the 16-pump, continuous-flow system these interruptions were unnecessary. Feed gas was continuously supplied by a uranium furnace maintained at a temperature sufficiently high to supply gas at the desired pressure. The end volumes were absent, having been replaced by ceramic variable leaks. The back pressure in these controlled leaks was maintained at 20 to 50 microns by Toepler pumps, which transferred the enriched or depleted gas into storage uranium furnaces. When mixtures richer than 95% tritium were being further purified, the light-end controlled leak was ordinarily closed entirely and feed gas was introduced at this end. The lighter gases were simply allowed to accumulate in the end pumps and in the feed uranium furnace for the duration of the run.

Analysis. Two analytical procedures were employed in these experiments: thermal conductivity and mass spectrometry. For the thermal conductivity measurements, Leeds and Northrup thermal conductivity bridges, with the cells modified to reduce their volume to 10 ml., were used. An improvement in this apparatus was the addition of a photoelectric servo-mechanism, which made precise control of the bridge current possible (*1*). With this control, analyses were reproducible to $\pm 0.02\%$.

To test the behavior of the separation and analysis systems, several experiments were made with hydrogen-deuterium mixtures. As a result of these experiments, it was believed that if 90 to 95% tritium were cycled through the line several times, the heavy-end product would be essentially pure tritium. Using pure tritium obtained in this manner, mixtures of tritium-hydrogen of accurately known composition were prepared and used to calibrate the thermal conductivity cells. For convenience, curves of e.m.f. against composition and e.m.f. against pressure at fixed composition were constructed, so as to simplify the interpolation. In general, the pressures employed for analysis were approximately 140 mm. Figures 1 and 3 are calibration curves constructed in this manner.

The fact that correct results were obtained by this procedure was subsequently indicated by comparison with mass spectrometer analyses (thermal conductivity 99.44 ± 0.02% tritium, mass spectrometer 99.44 ± 0.05% tritium). Another independent check was made with the cooperation of Louis Rosen, who measured proton-proton scattering of a sample using the Los Alamos cyclotron (thermal conductivity 98.60 ± 0.02% tritium; proton-proton 98.8 ± 0.2% tritium).

The agreement among the three methods of analysis led to the belief that in the thermal conductivity method interpolation was possible even at the higher tritium concentration end of the curve. Since this is the case, the tritium whose thermal conductivity was unaffected by subsequent reprocessing in the Hertz pump line was substantially pure.

The mass spectrometer employed was a Consolidated-Nier Model 201. For these analyses, samples were withdrawn from the systems and transported to the mass spectrometer in sample bulbs fitted with greased pressure stopcocks. Although the authors' experience indicated that it was advantageous to minimize the time during which highly purified tritium was exposed to stopcock grease (Apiezon type N), because of the radioactivity-induced exchange, this method of analysis was frequently used for samples obtained from the continuous-flow line. Because comparatively small gas samples were required, the operation of this line could then be more easily controlled.

RESULTS

The literature dealing with Hertz pump separations has invariably stressed the necessity of operating such pumps at low pressures (*2*, *4*, *5*). This leads to low over-all separation rates, because the volume of gas obtained at the completion of a run is small. Consequently, once the lines were known to perform satisfactorily at low pressures (5 to 8 mm. operating pressure), a series of experiments on the static line was undertaken to determine the highest pressure at which a useful separation rate was to be obtained. Surprisingly, it was found that a much higher running pressure could be used with moderately longer running times. Subsequent static runs were therefore made at operating pressures of 20 to 28 mm., with running times of 2.5 to 3 hours. Runs could not be made at pressures above 35 mm. because enough heat could not be provided to obtain mercury vapor jets. (Operating pump pressures were those measured while the pumps were running. The difference between "operating" and "initial"—i.e., room temperature—pressures was greater than could be attributed solely to temperature change of the gas. The discrepancy was attributed to the fact that, in operation, the stream of mercury vapor in the individual pumps efficiently expelled the gas above the liquid mercury surface, largely compressing it into the lines between the pumps.)

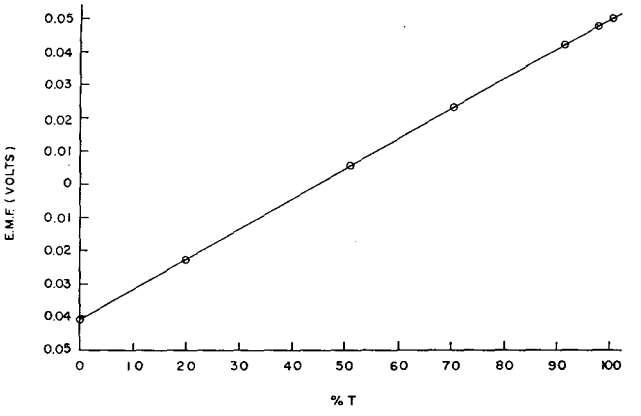

Figure 3. E.M.F. *vs.* Tritium Concentration
P – 140 mm. Hg

The following conditions were found to be satisfactory for the operation of the static line when 1-liter end bulbs were installed:

Time, hours	2.5–3
Operating pressure (measured in center of line), mm.	20–25
Heat input (pumps insulated), watts/pump	300

Under these conditions, it was possible to obtain 25 ml. (standard temperature and pressure) of enriched tritium per run. The average rate of gas removal from the continuous-flow line was 150 ml. (standard temperature and pressure) per day, compared with 50 ml. per day with the manual line, even though the operating pressure was considerably lower in the former. This advantage results in part from the fact that the former was run continuously and from the relatively few interruptions necessary to recharge or remove gas from the uranium furnaces, as compared to removal from the glass end volumes of the static line. Tables I, II, and III contain examples of the separations obtained in a number of runs under varying conditions of operation. Separation factors are not included in these tables because conditions were adjusted to obtain satisfactory separation rates. As a result, the separation factors vary.

Table I. Typical Separation Data for Hydrogen-Deuterium in Static Line

Time, min.	30
End volumes, ml.	400 × 2 (total line volume = 4000 ml.)
Operating pressure, mm.	8
Initial mixture, %	55 D, 45 H
Light end analysis, %	99.5 H
Heavy end analysis, %	99.0 D

Table II. Typical Separation Data for Tritium-Hydrogen in Static Line

(End volumes, each 1000 ml.)

Running Time, Hr.	Pressure, Mm.	Tritium, % Starting material	Heavy end	Light end
2.5	18.5	44	75	10
2.5	29.7	85	99	41
5.0	20.5	96.5	99.8	65
12.0	22.0	97	99.5	Not removed
47.5	13.6	99.4	99.9+	Not removed

Table III. Separation Data for Tritium-Hydrogen in Continuous-Flow Line

Pressure, Mm.	Flow Rate, Ml./Min.	Tritium, % Initial gas	Heavy end	Light end
9.8	0.064	95.85	99.75	Not removed
8.3	0.053	99.1	99.7	Not removed
8.4	0.120	97.9	99.44	89.5
5	0.015	98.9	99.56	Not removed
3	0.015	98.9	99.65	Not removed
8	0.052	98.9	99.61	Not removed
9	0.072	99.2	99.87	Not removed

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Dunn, F. J., Mann, J. B., and Mosley, J. R., ANAL. CHEM., in press.
(2) Hertz, G., *Z. Physik*, **91**, 810 (1934).
(3) Mund, W., Lories, R., and Huyskens, P., *Bull. soc. chim. belges*, **57**, 469 (1948).
(4) Sherr, R., *J. Chem. Phys.*, **6**, 251 (1938).
(5) Sherr, R., and Bleakney, W., *Phys. Rev.*, **49**, 882 (1936).

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Colorimetric Determination of *o*-Hydroxyphenylacetic Acid in Samples from Penicillin Fermentations

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Estimation of the amount of *o*-hydroxyphenylacetic acid in penicillin samples is necessary in the purification of penicillin. Most of the known methods for determining phenols cannot be used for this purpose in the analysis of penicillin fermentations, as it is difficult to differentiate the acid from nonphenolic interfering substances contained in the fermentation medium. A colorimetric method based on Stoughton's nitrosation method for phenols was developed in which the interfering substances produced a color intensity so low that it affected the analytical result negligibly. The procedure described is simpler and more sensitive than Stoughton's original method and can determine as little as 3 γ of *o*-hydroxyphenylacetic acid in a penicillin sample.

THE occurrence of *o*-hydroxyphenylacetic acid in the mother liquors left after the crystallization of potassium penicillin has been reported by King and Hambly (*4*) and by Nishikida (*7*). It is apparently a product of phenylacetic acid metabolism during penicillin fermentations, because these two compounds are closely related in structure and phenylacetic acid is widely used as a precursor for the biosynthesis of penicillin G (*1*, *9*). Since the occurrence of *o*-hydroxyphenylacetic acid creates a purification problem in penicillin manufacture, a method for its estimation in samples of fermentation media or crude penicillin has been urgently needed.

o-Hydroxyphenylacetic acid is phenolic in nature and can apparently be determined by a number of known methods (*6*). Preliminary tests revealed, however, that most of these methods cannot be adapted to the analysis of samples from penicillin fermentations, inasmuch as there is no simple way of differentiating *o*-hydroxyphenylacetic acid from nonphenolic interfering substances contained in the fermentation medium. After some experimentation, a method based on the same principle as that of the method proposed by Stoughton (*11*) for phenols was developed and was found to be satisfactory. The color intensity developed from the interfering substances in a penicillin fermentation medium was so low that it affected the analytical result to an almost negligible extent.

Stoughton's original method (*11*) involves the conversion of phenols in an acetic acid solution into *p*-nitrosophenol (quinone monoxime), which exhibits a bright yellow color when the solution is neutralized with excess alcoholic ammonia. Different modifications have been proposed (*5*, *10*, *12*) and the method has been applied to the analyses of a number of different materials, including petroleum products (*5*, *12*) and phenolic resins (*2*). As it is known that the nitrosation of phenols can be easily carried out in dilute aqueous acid solutions (*3*), attempts were made to modify the method by carrying out the reactions entirely in an aqueous medium. As a result of such a modification, it was shown that not only can the procedure be simplified, but the method can be made somewhat more sensitive. As little as 3 γ of *o*-hydroxyphenylacetic acid can be accurately determined by the procedure described.

EXPERIMENTAL

Test Solutions and Reagents. STANDARD *o*-HYDROXYPHENYLACETIC ACID SOLUTION. The acid was dissolved in water to a concentration of 1 mg. per ml. This stock solution was further diluted to 40 γ per ml. with water.

SODIUM NITRITE SOLUTION. 4 grams in 100 ml. of water.

AMYL ACETATE. Amyl acetate from Eastman Organic Chemicals Division was used without further purification.

Calibration Curve. After a few preliminary tests, the following procedure was developed for completing the color reaction.

Transfer by means of pipets duplicate aliquots of standard *o*-hydroxyphenylacetic acid solution, representing 0, 4, 8, 16, 24, 32, and 40 γ to absorption tubes for the Evelyn photoelectric colorimeter. Make up the volume to 1.0 ml. with water in each case. Add 1.0 ml. of 2*N* sulfuric acid, followed by 0.1 ml. of the sodium nitrite solution. Keep the tubes loosely corked and heat in a boiling water bath for 10 minutes. Cool, and add 5 ml. of 1 to 3 diluted ammonium hydroxide solution (approximately 5*N*). Read the per cent transmittance in the Evelyn colorimeter equipped with a 420-mμ filter. Set the tube containing only the reagents at 100% transmittance.

A plot of per cent transmittance against the amount of *o*-hydroxyphenylacetic acid used is shown in Figure 1. The yellow color follows Beer's law up to a concentration of 30 γ per 7 ml. of the reaction mixture. There is a deviation from the straight line at *o*-hydroxyphenylacetic acid concentrations higher than 30 γ per 7 ml. of reaction mixture. but the error introduced through this deviation amounts to less than 5% up to 40 γ of *o*-hydroxyphenylacetic acid.

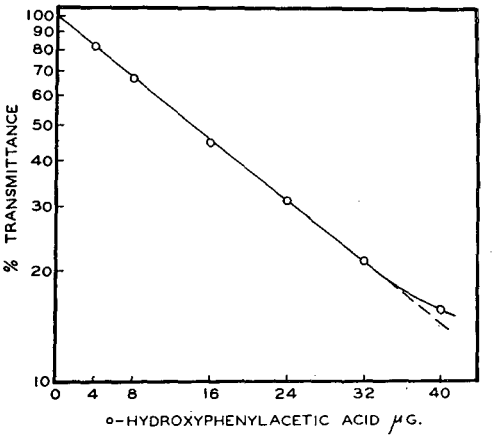

Figure 1. Calibration Curve for Determination of *o*-Hydroxyphenylacetic Acid

Per cent transmittance determined with a 420-mμ filter

Each step in the foregoing procedure was studied in some detail. For maximum color production, 10 minutes' heating in a boiling water bath was shown to be necessary. When the reaction was allowed to proceed at room temperature, a reaction time of 60 minutes produced a far lower color intensity than was obtained after heating. The color intensity increased with increase in sulfuric acid concentration, reaching a plateau at 1*N*. Sodium nitrite concentrations lower than that specified lowered the color intensity slightly while higher concentrations tended to destroy the colored complex. Other alkalies could be used in place of ammonium hydroxide, but the calibration curves obtained were not as satisfactory. In agreement with Stoughton's result (*11*), the color developed was exceedingly stable. Identical transmittance readings were obtained before and after the colored solution was allowed to stand overnight at room temperature.

Specificity of the Color Reaction. Phenylacetic acid and penicillin G, both nonphenolic in nature, were found to yield ab-

solutely no color in this test. This is, of course, of great importance for applying the method to the analyses of samples from penicillin fermentations, as phenylacetic acid and penicillin G are invariably present in these samples.

In agreement with earlier reports (*5*, *11*), different phenolic compounds produced different color intensities. Of about a dozen phenolic substances tested, however, none was found to produce as high a color intensity as *o*-hydroxyphenylacetic acid. The color intensity produced by phenol itself amounted to 66% and that by *p*-hydroxyphenylacetic acid, the side chain acid of penicillin X, amounted to 25% of that produced by *o*-hydroxyphenylacetic acid on an equal molar basis. Phenolic substances which would interfere with the analysis are apparently present to only a negligible extent in a cornsteep medium either before or after fermentation by penicillin-producing fungi (see below).

Table I. Extraction[a] of *o*-Hydroxyphenylacetic Acid and Interfering Substances by Amyl Acetate

Samples	Init. *o*-Hydroxyphenylacetic Acid Concn., γ/Ml.	*o*-Hydroxyphenylacetic Acid Found, γ/Ml.	Extraction[b] Efficiency, %
Water	50	42	84.0
Water	250	216	86.2
Water	500	448	89.0
Unfermented cornsteep medium I[c]	...	14–18	...
Unfermented cornsteep medium II[d]	...	27–35	...
Fermented cornsteep medium[e]	...	17.0	...

[a] A 1-ml. sample was mixed with 1 ml. of 1*N* H_2SO_4 and extracted with 5 ml. of amyl acetate.
[b] Extraction efficiency denotes percentage of *o*-hydroxyphenylacetic acid extracted after shaking once with solvent.
[c] The medium contained 2% cornsteep solids, 2% lactose, and 1% $CaCO_3$.
[d] The medium contained 3.5% cornsteep solids, 3.5% lactose, and 1% $CaCO_3$.
[e] Cornsteep medium II fermented with *P. chrysogenum* strain Wis. 49-133 for 96 hours. No phenylacetic acid was added as precursor during the fermentation.

Extraction of *o*-Hydroxyphenylacetic Acid. In order to separate the *o*-hydroxyphenylacetic acid from other ingredients of a cornsteep liquor medium, extraction by a suitable solvent was apparently a feasible means. Preliminary tests showed that a number of solvents could be used for this purpose. Among all the solvents tested, amyl acetate was chosen because it extracted the least amount of interfering substances contained in an unfermented cornsteep medium.

The procedure finally adopted for extracting the *o*-hydroxyphenylacetic acid from samples of fermentation media is as follows:

Transfer by means of a pipet a 1-ml. sample of cell-free filtrate into a 5 × 5/8 inch test tube. Add 1 ml. of 1*N* sulfuric acid and 5 ml. of amyl acetate. Shake the tubes vigorously for 30 seconds, using thumb as stopper. [The same general extraction procedure as used in similar extraction methods developed in this laboratory (*8*) was followed.] Other containers—e.g., volumetric flasks equipped with ground glass stoppers—can also be used, but the use of rubber stoppers or corks must be avoided. Pour the contents into 5 × 5/8 inch test tubes after shaking, in case other containers are used. Centrifuge the tubes for 5 minutes to secure a satisfactory separation of the two layers. Pipet an aliquot of the amyl acetate extract containing 3 to 30 γ of *o*-hydroxyphenylacetic acid into an absorption tube adapted for use in the Evelyn photoelectric colorimeter, add 0.1 ml. of 0.1*N* sodium hydroxide solution, and evaporate the whole contents to dryness in a 50° to 70° C. water bath under a gentle current of air. Dissolve the dried residue in 1 ml. of water and then follow the procedure as described in the section on the calibration curve.

Instead of obtaining complete extraction of *o*-hydroxyphenylacetic acid by repeated shaking with solvent, a procedure considered too long and involved, the distribution coefficient was carefully determined. For convenience, the percentage of *o*-hydroxyphenylacetic acid extracted after shaking once with the solvent was used to express the distribution coefficient and is termed extraction efficiency in this report.

Data collected in determining the extraction efficiency for pure *o*-hydroxyphenylacetic acid in an aqueous solution and in estimating the amount of interfering substances which were extracted from samples of penicillin fermentation media are summarized in Table I. With 2.5 volumes of amyl acetate per volume of aqueous solution, the extraction efficiency varied somewhat with the initial concentration of the *o*-hydroxyphenylacetic acid. However, within the range of 50 to 500 γ per ml., the extraction efficiency varied between 84 and 89%. An average value of 86% could obviously be used as a correction factor and the errors introduced would be within ±3%.

Data summarized in Table I also showed that when an unfermented cornsteep liquor medium was extracted and tested in the same way, the color intensity produced depended upon the amount of cornsteep liquor used in the medium. However, even with 3.5 grams of cornsteep solids per 100 ml. of medium, a color intensity equivalent to less than 35 γ of *o*-hydroxyphenylacetic acid per ml. was observed. When such a medium was fermented with *Penicillium chrysogenum* (Wis. strain No. 49-133), for 4 days and tested again, the color intensity was even lower (17 γ per ml.). Therefore, when analyzed with this method, substances in a cornsteep medium which behaved like *o*-hydroxyphenylacetic acid amounted to an almost negligible value either before or after penicillin fermentation. This is again an essential requirement in order to make the method applicable to the analysis of fermentation samples.

The extraction efficiency could be increased to 90% or higher by saturating the aqueous phase with anhydrous sodium sulfate and using a larger volume of amyl acetate. However, the color intensity from interfering substances was also greatly increased by such a treatment. This disadvantage overbalanced any gain in the extraction efficiency.

Recoveries. Recovery of *o*-hydroxyphenylacetic acid added to samples of unfermented and fermented cornsteep media was studied with the extraction procedure described. The results are summarized in Table II. The recovery values, calculated on the assumption that the extraction efficiency was 86% in every

Table II. Recovery of *o*-Hydroxyphenylacetic Acid Added to Penicillin Fermentation Media

Sample[a]	Apparent *o*-Hydroxyphenylacetic Acid in Sample[b], γ/Ml.	*o*-Hydroxyphenylacetic Acid Added, γ/Ml.	*o*-Hydroxyphenylacetic Acid Found, γ/Ml.	*o*-Hydroxyphenylacetic Acid Recovered[c], γ/Ml.	Recovery[d], %
Unfermented cornsteep medium I	20.9	100	117.4	96.5	96.5
	20.9	400	421.0	400.1	100.0
Unfermented cornsteep medium II	31.4	100	131.6	100.2	100.2
	31.4	400	437.4	406.0	101.5
Cornsteep medium I fermented 72 hours	16.3	100	109.4	93.1	93.1
	16.3	400	412.5	396.2	99.1
Cornsteep medium II fermented 120 hours	19.7	100	113.0	93.3	93.3
	19.7	400	418.7	399.0	99.8

[a] Same samples as described in footnotes of Table I.
[b] A correction factor for extraction efficiency, 86%, has been applied in calculation to obtain these values.
[c] Values obtained by subtracting column 1 from column 3.
[d] Values obtained here by $\frac{\text{column 4}}{\text{column 2}} \times 100$.

case, ranged from 93.1 to 101.5%. This was considered satisfactory.

When the apparent *o*-hydroxyphenylacetic acid in the samples (column 1, Table II), which represents the interfering substances, is *not* subtracted from the *o*-hydroxyphenylacetic acid found (column 3, Table II), as is true in the analysis of unknown samples, the analytical results obtained are necessarily higher than the true *o*-hydroxyphenylacetic acid content. The effect of these unavoidable errors naturally depends upon the *o*-hydroxyphenylacetic acid content. When this value is 400 γ per ml. or higher, the error introduced is less than 5% and can be considered negligible for most practical purposes. As has been mentioned, when the samples are tested with other known methods for phenols—e.g., the use of Folin's phenol reagent (*6*)—the values for the interfering substances are so high as to invalidate the significance of the analytical results.

Analysis of Potassium Penicillin Samples. Since crystalline potassium benzyl penicillin is prepared by amyl acetate extraction of fermentation broth, the extraction procedure as described for fermentation samples can obviously be omitted. Direct application of the procedure as described in the section on the calibration curve to samples of potassium penicillin has been shown to be entirely satisfactory.

DISCUSSION

It is obvious from the nitrosation procedure described that the present method is much simpler than the original method proposed by Stoughton (*11*). When *o*-hydroxyphenylacetic acid was tested with Stoughton's method according to the procedure described by Savitt *et al.* (*10*), the color intensity produced was equal to approximately two thirds of that obtained with the present method. Since different phenols produce different color intensities, it seems entirely possible that the present method works especially well with *o*-hydroxyphenylacetic acid. For other phenols, Stoughton's original method or other modifications (*5*, *10*) might be preferred.

The present method has been developed on the basis that the interfering substances contained in samples from fermentations of cornsteep medium by penicillin-producing fungi amount to an almost negligible value. This, of course, might not be true with samples of an entirely different nature—e.g., blood, urine, etc.—it is not known whether the present method can be applied to these samples.

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LITERATURE CITED

(1) Florey, H. W., *et al.*, "Antibiotics," Vol. II, Chap. 18, Oxford University Press, London, 1949.
(2) Haslam, J., Whettem, S. M. A., and Newlands, G., *Analyst*, **78**, 340 (1953).
(3) Hodgson, H. H., and Moore, F. H., *J. Chem. Soc.*, **123**, 2499 (1923).
(4) King, N. K., and Hambly, A. N., *Roy. Australian Chem. Inst. J. & Proc.*, **17**, 403 (1950).
(5) Lykken, L., and Treseder, R. S., and Zahn, V., IND. ENG. CHEM., ANAL. ED., **18**, 103 (1946).
(6) Milton, R. F., and Waters, W. A., "Methods of Quantitative Microanalysis," Edward Arnold & Co., London, 1949.
(7) Nishikida, T., *J. Antibiotics (Japan)*, **4**, 299 (1951).
(8) Pan, S.-C., and Perlman, D., ANAL. CHEM., **26**, 1432 (1954).
(9) Perlman, D., *Botan. Rev.*, **16**, 449 (1950).
(10) Savitt, S. A., Goldberg, A. M., and Othmer, D. F., ANAL. CHEM., **21**, 516 (1949).
(11) Stoughton, R. W., *J. Biol. Chem.*, **115**, 293 (1936).
(12) Wetlaufer, L. A., Van Natta, F. J., and Quattlebaum, H. B., IND. ENG. CHEM., ANAL. ED., **11**, 438 (1939).

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Use of Mixed Adsorbents in Chromatographic Separation of Organic Compounds

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A number of organic compounds representing several different classes have been adsorbed on binary mixtures of commonly used adsorbents. Measured R_f values indicate that adsorption is either representative of the mixture, in which case the R_f value varies with percentage composition, or, adsorption is characteristic of one component of the mixture while the second component serves as diluent. With mixtures of boron oxide and silicic acid an unusual interaction took place resulting in an adsorption strength higher than either of the pure components and selective toward amines.

THERE is an abundance of information available regarding the characteristics of pure adsorbents and the manner in which adsorption takes place on some of them. Cassidy (*1*) and Strain (*6*) give a thorough discussion of this material.

Smith and LeRosen (*5*) utilized two adsorbents for the indirect determination of R_f values. They employed the technique of placing one adsorbent in its pure form on the bottom of the column, then packing a second pure adsorbent directly above it. An adsorptive was chromatographed on this column and the apparent R_f value on the lower adsorbent noted. From the difference between the apparent R_f value on the lower adsorbent and that obtained on the lower adsorbent in its pure form, the R_f value can be calculated on the upper adsorbent. The method has been found useful for colored adsorbents, such as charcoal, on which zones cannot be detected by the direct application of streak reagents.

The present study was made in order to determine the manner in which selected organic compounds behave on adsorbent mixtures as evidenced by their R_f values on the mixtures. Further, the data obtained from this investigation should afford the practical chromatographer a variety of adsorbents from which to choose in undertaking a particular separation.

In using mixed adsorbents adsorption would be expected to follow one of two general patterns. Adsorption strength would be characteristic of one or the other of the two adsorbents in the mixture and would be of about the same magnitude as that of the pure adsorbents. The other adsorbent would act as diluent for the active one. Alternatively, there would be a competition between the two adsorbents in which the resultant adsorption would be characteristic of the mixture and would vary with percentage composition of the mixture. This could be predicted

for a certain few adsorptives on adsorbents of nearly the same donor and acceptor strengths.

REAGENTS

Benzene, Merck reagent grade, was used as developer and as solvent in preparing 0.01*M* solutions of the various adsorptives.

Adsorptives. Ethylaniline, dimethylaniline, aniline, methyl amyl ketone, methyl *p*-tolyl ketone, butyraldehyde, benzaldehyde, *n*-amyl alcohol, nitrobenzene, and *n*-decylamine were purified by distillation. Phenol, *Mallinckrodt, analytical* reagent, was used without further purification.

Adsorbents. Silicic acid, Merck, reagent grade
Florisil, Floridin Co.
Calcium carbonate, Merck, reagent grade
Boron oxide, Eastman Kodak Co., practical
Magnesium oxide, C.P., J. T. Baker Chemical Co.
Calcium hydroxide, C.P., J. T. Baker Chemical Co.
*Alu*minum oxide, Merck, reagent grade

Table I. R_f Values of Different Adsorptives on Mixed Adsorbents

Mixture	Proportions by Weight 0/100	20/80	40/60	60/40	80/20	100/0	Streak[a] Reagent
Methyl amyl ketone							
Alumina/Florisil	0.10	0.22	0.28	0.33	0.39	0.41	1
MgO/silicic acid	0.00	1.00	1.00	1.00	1.00	1.00	2
B_2O_3/silicic acid	0.00	0.00	0.00	0.00	0.00	1.00	1
B_2O_3/Florisil	0.10	0.17	0.25	0.28	0.34	1.00	1
B_2O_3/Alumina	0.41	0.52	0.53	0.67	0.71	1.00	1
Silicic acid/Florisil	0.10	0.00	0.00	0.00	0.00	0.00	1
Silicic acid/alumina	0.41	0.39	0.33	0.13	0.00	0.00	1
$CaCO_3$/silicic acid	0.00	0.00	0.00	0.00	0.00	1.00	2
$Ca(OH)_2$/silicic acid	0.00	0.00	0.00	0.00	0.00	1.00	2
ZnO/silicic acid	0.00	0.00	0.00	0.00	0.00	1.00	2
$CaCO_3$/MgO	1.00	1.00	1.00	1.00	1.00	1.00	2
$Ca(OH)_2$/MgO	1.00	1.00	1.00	1.00	1.00	1.00	2
$CaCO_3$/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
$CaCO_3$/Florisil	0.10	0.12	0.14	0.14	0.14	1.00	2
$Ca(OH)_2$/Florisil	0.10	0.12	0.12	0.12	0.12	1.00	2
MgO/Florisil	0.10	0.12	0.12	0.12	0.14	1.00	2
Dimethylaniline							
Silicic acid/alumina	0.76	0.81	0.73	0.71	0.67	0.56	2, 3, 4
Silicic acid/$CaCO_3$	1.00	0.97	0.93	0.80	0.75	0.56	2
Silicic acid/MgO	1.00	1.00	1.00	0.79	0.77	0.56	2
Silicic acid/B_2O_3	1.00	0.00	0.00	0.00	0.00	0.56	3
Florisil/MgO	1.00	0.96	0.80	0.64	0.54	0.50	2
Florisil/B_2O_3	1.00	0.00	0.00	0.00	0.00	0.50	3
Florisil/$CaCO_3$	1.00	0.70	0.58	0.55	0.53	0.50	2
Florisil/$Ca(OH)_2$	1.00	0.94	0.70	0.62	0.54	0.50	2
B_2O_3/alumina	0.76	0.24	0.30	0.40	0.46	1.00	2
MgO/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
MgO/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	2
$CaCO_3$/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
Silicic acid/Florisil	0.50	0.61	0.61	0.64	0.65	0.56	2, 3, 4
B_2O_3/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	2
B_2O_3/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
Phenol							
Silicic acid/B_2O_3	1.00	0.46	0.40	0.34	0.37	0.31	5, 2
Silicic acid/alumina	0.00	0.00	0.00	0.11	0.15	0.31	2
Silicic acid/MgO	1.00	0.44	0.42	0.36	0.33	0.31	2
Silicic acid/$CaCO_3$	0.75	0.59	0.51	0.49	0.44	0.31	2
Silicic acid/$Ca(OH)_2$	0.93	0.45	0.44	0.37	0.33	0.31	2
Silicic acid/Florisil	0.33	0.31	0.33	0.31	0.32	0.31	5, 2
Florisil/alumina	0.00	0.00	0.00	0.00	0.14	0.33	2
Florisil/B_2O_3	1.00	0.78	0.65	0.62	0.56	0.33	2
Florisil/MgO	1.00	0.44	0.42	0.36	0.34	0.33	2
Florisil/$Ca(OH)_2$	0.93	0.43	0.40	0.41	0.35	0.33	2
Florisil/$CaCO_3$	0.75	0.54	0.47	0.44	0.40	0.33	2
$CaCO_3$/$Ca(OH)_2$	0.93	0.82	0.79	0.82	0.84	0.75	2
MgO/$Ca(OH)_2$	0.93	0.86	0.85	0.85	0.88	1.00	2
MgO/alumina	0.00	0.00	0.00	0.00	0.14	1.00	2
Alumina/B_2O_3	1.00	0.15	0.00	0.00	0.00	0.00	2
Alumina/$Ca(OH)_2$	0.93	0.14	0.00	0.00	0.00	0.00	2
Benzaldehyde							
Florisil/silicic acid	0.72	0.72	0.72	0.72	0.72	0.71	1
Florisil/MgO	1.00	1.00	1.00	1.00	1.00	0.71	2
Florisil/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	0.71	2
Florisil/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	0.71	2
Florisil/alumina	0.68	0.69	0.70	0.71	0.71	0.71	1
Alumina/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	0.68	2
Alumina/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	0.68	2
Alumina/MgO	1.00	1.00	1.00	1.00	1.00	0.68	2
Alumina/B_2O_3	1.00	0.81	0.76	0.83	0.72	0.68	1
Florisil/B_2O_3	1.00	0.77	0.74	0.76	0.77	0.71	1
Silicic acid/B_2O_3	1.00	0.83	0.83	0.80	0.81	0.72	1
Silicic acid/MgO	1.00	1.00	1.00	1.00	1.00	0.72	2
Silicic acid/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	0.72	2
Silicic acid/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	0.72	2
MgO/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
MgO/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	2
Silicic acid/alumina	0.68	0.75	0.74	0.75	0.74	0.72	2
***n*-Amyl alcohol**							
Silicic acid/Florisil	0.41	0.35	0.35	0.25	0.32	0.17	6
Silicic acid/$Ca(OH)_2$	0.69	0.50	0.39	0.31	0.24	0.17	6
Silicic acid/$CaCO_3$	0.88	0.41	0.36	0.29	0.21	0.17	6
Silicic acid/MgO	0.60	0.40	0.35	0.28	0.25	0.17	6
Silicic acid/alumina	0.09	0.12	0.13	0.12	0.15	0.17	7
Florisil/alumina	0.09	0.18	0.22	0.24	0.37	0.41	6
Florisil/$CaCO_3$	0.88	0.51	0.51	0.47	0.41	0.41	6
Florisil/$Ca(OH)_2$	0.69	0.66	0.63	0.48	0.38	0.41	6
Florisil/MgO	0.60	0.56	0.55	0.49	0.42	0.41	6
Alumina/MgO	0.60	0.35	0.24	0.20	0.13	0.09	6
Alumina/$Ca(OH)_2$	0.69	0.37	0.24	0.14	0.10	0.09	6
Alumina/$CaCO_3$	0.88	0.35	0.19	0.16	0.12	0.09	6
$CaCO_3$/$Ca(OH)_2$	0.69	0.93	0.92	0.90	0.90	0.92	6
$CaCO_3$/MgO	0.60	0.88	0.91	0.88	0.91	0.88	6
Florisil/B_2O_3	..	0.37	0.34	0.34	0.36	0.41	6
Silicic acid/B_2O_3	..	0.18	0.16	0.17	0.13	0.17	6
$CaCO_3$/B_2O_3	..	0.80	0.88	0.88	0.90	0.88	
Methyl-*p*-tolyl ketone							
Silicic acid/$CaCO_3$	1.00	0.80	0.74	0.65	0.62	0.59	2
Silicic acid/$Ca(OH)_2$	1.00	0.88	0.80	0.63	0.58	0.59	2
Silicic acid/MgO	1.00	0.95	0.81	0.71	0.59	0.59	2
Silicic acid/alumina	0.51	0.51	0.53	0.55	0.53	0.59	1
Silicic acid/Florisil	0.64	0.58	0.53	0.59	0.60	0.59	1
Florisil/alumina	0.51	0.50	0.50	0.52	0.53	0.64	1
Florisil/MgO	1.00	0.85	0.77	0.73	0.55	0.64	2
Florisil/$Ca(OH)_2$	1.00	0.85	0.77	0.70	0.59	0.64	2
Florisil/$CaCO_3$	1.00	0.81	0.70	0.69	0.64	0.64	2
Alumina/MgO	1.00	0.85	0.75	0.57	0.56	0.51	2
Alumina/$Ca(OH)_2$	1.00	0.74	0.70	0.62	0.54	0.51	2
Alumina/$CaCO_3$	1.00	0.78	0.64	0.56	0.51	0.51	2
Alumina/B_2O_3	1.00	0.66	0.63	0.53	0.52	0.51	1
Silicic acid/B_2O_3	1.00	0.71	0.68	0.65	0.62	0.59	1
Florisil/B_2O_3	1.00	0.70	0.68	0.66	0.56	0.64	1
B_2O_3/MgO	1.00	1.00	1.00	1.00	1.00	1.00	2
MgO/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	2
$CaCO_3$/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
Butyraldehyde							
Silicic acid/Florisil	0.67	0.67	0.67	0.63	0.56	0.56	1, 2
Silicic acid/alumina	0.48	0.49	0.49	0.53	0.52	0.56	1, 2
Silicic acid/$CaCO_3$	1.00	0.90	0.76	0.67	0.61	0.56	2
Silicic acid/$Ca(OH)_2$	1.00	0.91	0.81	0.72	0.64	0.56	2
Silicic acid/MgO	1.00	0.90	0.75	0.63	0.56	0.56	2
Silicic acid/B_2O_3	1.00	0.72	0.65	0.59	0.57	0.56	1, 2
Florisil/alumina	0.48	0.57	0.59	0.63	0.67	0.67	1, 2
Florisil/$CaCO_3$	1.00	0.87	0.83	0.83	0.78	0.67	2
Florisil/$Ca(OH)_2$	1.00	0.91	0.86	0.76	0.72	0.67	2
Florisil/MgO	1.00	0.91	0.83	0.75	0.71	0.67	2
Florisil/B_2O_3	1.00	0.91	0.77	0.69	0.66	0.67	1, 2
Alumina/$CaCO_3$	1.00	0.90	0.79	0.58	0.52	0.48	2
Alumina/$Ca(OH)_2$	1.00	0.96	0.91	0.70	0.57	0.48	2
Alumina/MgO	1.00	0.96	0.87	0.72	0.60	0.48	2
Alumina/B_2O_3	1.00	0.76	0.70	0.66	0.54	0.48	1, 2
$CaCO_3$/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
$CaCO_3$/MgO	1.00	1.00	1.00	1.00	1.00	1.00	2
MgO/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	2
$CaCO_3$/B_2O_3	1.00	1.00	1.00	1.00	1.00	1.00	2
Nitrobenzene							
Silicic acid/Florisil	0.94	1.00	1.00	1.00	1.00	1.00	7, 8
Silicic acid/MgO	1.00	1.00	1.00	1.00	1.00	1.00	7, 8
Silicic acid/$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	7, 8
Silicic acid/B_2O_3	1.00	1.00	1.00	1.00	1.00	1.00	7, 8
Florisil/alumina	0.93	0.98	0.95	0.95	0.94	0.94	7, 8
Florisil/$CaCO_3$	1.00	1.00	1.00	1.00	0.98	0.94	7, 8
Florisil/MgO	1.00	1.00	1.00	1.00	1.00	0.94	7, 8
Florisil/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	0.94	7, 8
Florisil/B_2O_3	1.00	1.00	1.00	0.95	0.96	0.94	7, 8
Alumina/silicic acid	1.00	0.93	0.95	0.93	0.97	0.93	7, 8
Alumina/B_2O_3	1.00	0.99	1.00	0.95	0.98	0.93	7, 8
Alumina/MgO	1.00	1.00	1.00	1.00	1.00	0.93	7, 8
Alumina/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	0.93	7, 8
Alumina/$CaCO_3$	1.00	1.00	1.00	1.00	0.96	0.93	7, 8
MgO/B_2O_3	1.00	1.00	1.00	1.00	1.00	1.00	7, 8
$CaCO_3$/$Ca(OH)_2$	1.00	1.00	1.00	1.00	1.00	1.00	7, 8
***n*-Decylamine**							
Silicic acid/Florisil	0.00	0.00	0.00	0.00	0.00	0.00	9, 2
Silicic acid/alumina	1.00	0.00	0.00	0.00	0.00	0.00	2
Silicic acid/$CaCO_3$	..	..	..	..	..	..	2
Silicic acid/B_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	2
Florisil/$Ca(OH)_2$	1.00	1.00	..	..	..	0.00	2
Florisil/alumina	1.00	0.00	0.00	0.00	0.00	0.00	2
Alumina/$Ca(OH)_2$	1.00	..	1.00	1.00	1.00	1.00	2
$Ca(OH)_2$/$CaCO_3$	1.00	..	1.00	1.00	1.00	1.00	2
$CaCO_3$/MgO	1.00	..	1.00	1.00	1.00	1.00	2

[a] Streak reagents.
1. 2,4-Dinitrophenylhydrazine in HCl (*2*).
2. Alkaline permanganate (*3*).
3. Chloranil in dioxane (*4*).
4. Bismuth iodide complex (*4*).
5. Ceric nitrate (*4*).
6. Permanganate in concd. NaOH (0.0075*M* in 25% NaOH).
7. Ammonium chloride–zinc dust (*4*).
8. Zinc dust–alcoholic NaOH (*3*).
9. Dimethylamidoazobenzene, 3% in benzene.

Boric acid, Merck, reagent grade
Zinc oxide, U.S.P., Matheson, Coleman and Bell

EXPERIMENTAL

Number one chromatographic tubes, Scientific Glass Apparatus Co., were packed with the adsorbent mixture to a height of 77 ± 2 mm. Three to five drops of 0.01*M* (in benzene) solutions of the various adsorptives were introduced into the column and developed with benzene under the full vacuum provided by a Cenco Hyvac vacuum pump.

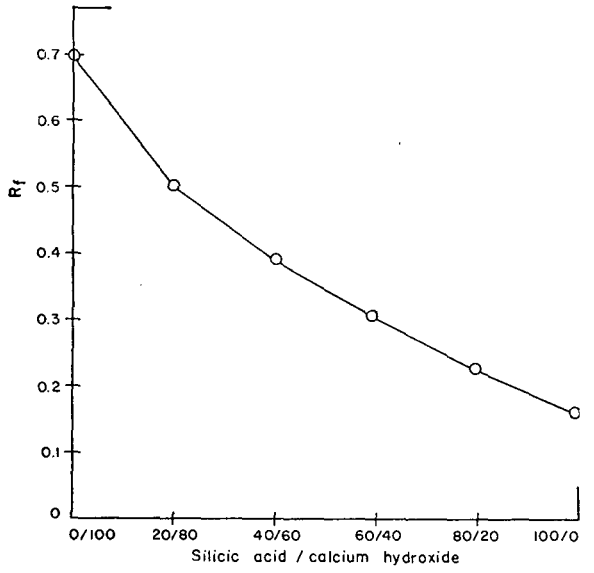

Figure 1. Adsorption of *n*-Amyl Alcohol from Benzene on Silicic Acid–Calcium Hydroxide Mixtures

The mixed adsorbents were packed in the following proportions by weight: 0/100, 20/80, 40/60, 60/40, 80/20, and 100/0. Weighed quantities of the pure adsorbents were transferred to a mortar and ground until an intimate mixture resulted with an approximate mesh size of 250 to 350. In order to ensure efficient mixing, not more than 10 grams of the mixture was treated in this manner at one time; this was then mixed with previously ground portions and transferred to the column.

The streak reagents employed to detect zones on the surface of the extruded column are listed in Table I. A previously unreported streak reagent, dimethylamidoazobenzene (butter yellow), when prepared as a 3% solution in benzene, was found to give a red zone against a yellow background with organic acids on silicic acid columns.

Results are recorded in Table I. Reported R_f values represent at least duplicate determinations with an average deviation of less than 0.02. Blank spaces appear in the table where zones could not be detected. On a mixture of boron oxide and silicic acid, dimethylaniline was found not to move, whereas on boron oxide alone it moves with an R_f value of 1.0 and on silicic acid it moves with an R_f value of 0.56. This unusual interaction was also observed with mixtures of boron oxide and Florisil, and to a lesser extent with alumina–boron oxide mixtures. Because the boron oxide was a technical grade and was known to contain indefinite quantities of boric acid, the latter was substituted for boron oxide in the mixture with the same results. Aniline and ethylaniline behave in a manner similar to dimethylaniline on boron oxide–silicic acid and boron oxide–Florisil mixtures. The strength of the boron oxide–silicic acid mixture as an adsorbent is best seen by considering the following comparison of silicic acid with 40% boron oxide–60% silicic acid in adsorbing aniline developed with several different solvents:

	R_f Values		
Developer	40% boron oxide–60% silicic acid	Silicic acid	Boron oxide
Benzene	0.00	0.34	1.0
Chloroform	0.06	0.47	1.0
Nitrobenzene	0.12	0.59	1.0
Ethyl ether	0.76	1.00	1.0
Ethyl alcohol	1.00	1.00	1.0

Magnesium oxide–calcium hydroxide mixtures show a higher affinity for phenol than do either of the pure adsorbents. On the other hand, dimethylaniline is adsorbed less strongly on silicic acid–Florisil mixtures than on either pure adsorbent, and benzaldehyde behaves similarly on silicic acid–alumina mixtures. These anomalies are not so marked as the behavior of aromatic amines on certain mixtures containing boron oxide.

DISCUSSION

The data presented in the table show that mixed adsorbents generally behave as one of the two adsorbents behaves in the pure form, while the other serves simply as diluent, or, adsorption is shared between the two adsorbents, in which case adsorption varies almost linearly with percentage composition of the mixture. In the former case there is little or no deviation from the R_f value exhibited by the adsorptive on the stronger of the two adsorbents Typical plots are found in Figures 1 and 2.

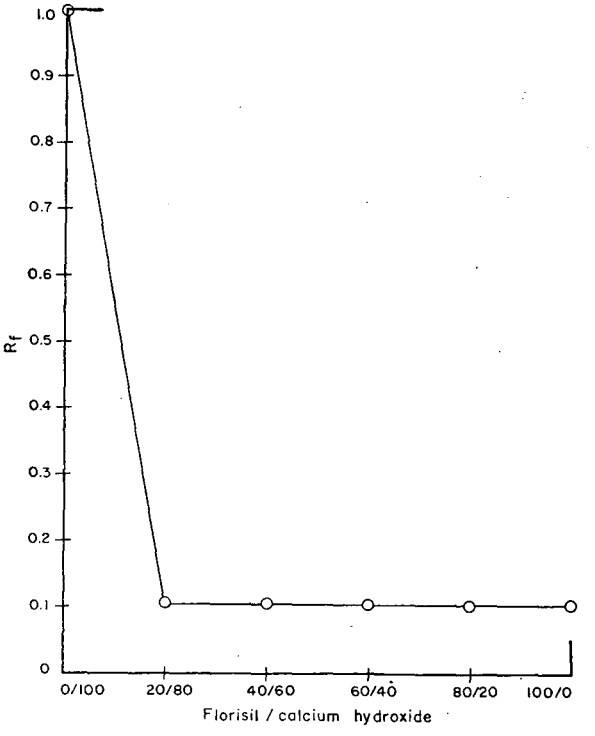

Figure 2. Adsorption of Methyl Amyl Ketone from Benzene on Florisil–Calcium Hydroxide Mixtures

Mixtures of boron oxide and silicic acid, boron oxide and Florisil, and boron oxide and alumina were found to interact in such a way as to adsorb certain amines more strongly than they were adsorbed by either of the pure adsorbents. This phenomenon was observed when aniline, ethylaniline, and dimethylaniline were developed with benzene on columns of the above mixtures. It is peculiar that this higher adsorption strength for the mixture should be selective toward amines. If boron oxide were dehydrating the silicic acid, thus activating it as Trueblood and Malmberg (7) have done by heating it, then phenol, benzaldehyde, and acetophenone should also have lower R_f values on the

mixture than on pure silicic acid. Actually, R_f values for these compounds on the various mixtures fell between R_f values measured on the pure components.

Evidently, the acidic nature of one of the two absorbents is enhanced by the presence of the other. The nature of the interaction which brings about increased acidity has not been determined. Use can be made of this characteristic, however, to separate aliphatic amines, certain *N*-substituted and ring substituted aromatic amines. The following general method is offered for the separation: Sample size and column length will have to be adjusted to the relative concentrations of the individual constituents.

The sample is first dissolved in benzene and applied to a silicic acid column employing benzene as developer. *N*-Alkyl-substituted aromatic amines and certain ring-substituted aromatic amines pass into the filtrate while the aliphatic amines are held firmly near the top of the column. The filtrate is then concentrated by evaporation and introduced into a column of 50% by weight of boron oxide–silicic acid where the *N*-alkyl substituted aromatic amines remain at the top of the column and the ring substituted aromatic amines pass into the filtrate. The amines to which this method has been applied are:

N-alkyl-substituted aromatic amines—methylaniline, ethylaniline, dimethylaniline, and diethylaniline.

Ring-substituted aromatic amines—*o*- and *p*-nitroaniline, *o*- and *p*-chloroaniline, and *o*- and *p*-methylaniline.

Aliphatic amines—butylamine, amylamine, and decylamine.

Those mixed adsorbents which share the adsorption of organic molecules, thus exhibiting R_f values intermediate between those measured on the pure adsorbents, should afford the practical chromatographer a wider choice of adsorbents.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Cassidy, H. G., "Technique of Organic Chemistry: Adsorption and Chromatography," Vol. V, pp. 28–39, 188–206, Interscience Publishers, Inc., New York, 1951.
(2) Iddles, H. A., Low, A. W., Rosen, B. D., and Hart, R. I., IND. ENG. CHEM., ANAL. ED., **11**, 102 (1939).
(3) LeRosen, A. L., Monaghan, P. H., Rivet, C. A., Smith, E. D., and Suter, H. A., ANAL. CHEM., **22**, 809 (1950).
(4) LeRosen, A. L., Moravek, R. T., and Carlton, J. K., *Ibid.*, **24**, 1335 (1952).
(5) Smith, E. D., and LeRosen, A. L., *Ibid.*, **26**, 928 (1954).
(6) Strain, H. H., "Chromatographic Adsorption Analysis," pp. 47–63, Interscience Publishers, Inc., New York, 1942.
(7) Trueblood, K. N., and Malmberg, E. W., ANAL. CHEM., **21**, 1055 (1949).

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Separation of Protactinium and Niobium by Liquid-Liquid Extraction

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The extraction behavior of protactinium and niobium in several liquid-liquid systems is described. Niobium carrier in oxalic acid markedly inhibits the extraction of protactinium; a method of circumventing this difficulty is given. Sulfuric acid enhances the extractability into diisobutylcarbinol of protactinium from dilute hydrochloric acid and of niobium from dilute hydrochloric or hydrofluoric acid. A rapid and effective separation of protactinium from niobium is described; niobium is extracted into diisobutylcarbinol from a dilute hydrofluoric acid–sulfuric acid solution and protactinium remains in the aqueous phase.

THE purpose of this investigation was to develop a method for the separation of protactinium from niobium. The chemistry of these two elements is very similar and it has been found that protactinium follows through the standard radiochemical (*3*) method for the determination of niobium.

Solvent extraction was considered as a separation technique because a rapid method was needed that could be adapted to remote control, if necessary. The extraction of protactinium from aqueous solutions into organic solvents has been investigated by several atomic energy project workers.

Hyde and Wolf (*4*) and Kraus and Van Winkle (*5*) used diisopropyl ketone to extract protactinium from aqueous nitrate solutions. Kraus and Van Winkle (*6*) found diisopropylcarbinol to be a more efficient extractant than diisopropyl ketone for protactinium; also, they demonstrated that protactinium readily extracted from hydrochloric acid solution into diisopropylcarbinol. They found that protactinium in macro concentrations tended to hydrolyze to a nonextractable polymer in nitric acid solution and that hydrochloric acid prevented the hydrolysis. Overholt and Steahly (*8*) and Hudgens, Warren, and Moore (*2*) employed diisopropylcarbinol to extract protactinium tracer from aqueous nitrate solutions. Gresky and Brandt (*1*) found diisobutylcarbinol superior to diisopropylcarbinol for the extraction of protactinium from aqueous nitrate solutions. The superiority of the hydrochloric acid system over the nitric acid system was verified by Reynolds, who devised an analytical method (*7*) for the extraction and determination of protactinium. It was found that niobium extracted to some extent, thus interfering in the determination of protactinium. Because of the obvious advantage of the hydrochloric acid system, it was decided to investigate the extraction behavior of protactinium and niobium in hydrochloric acid solutions.

EXTRACTION OF PROTACTINIUM AND NIOBIUM FROM HYDROCHLORIC ACID SOLUTIONS

In order to find an effective organic extractant for protactinium, several liquid-liquid systems that are used occasionally at the Oak Ridge National Laboratory were compared in the following manner. Equal volumes (6 ml.) of the aqueous phase that contained protactinium-233 and of the organic phase were mixed for 15 minutes. The total amount of protactinium-233

Table I. Extraction of Protactinium-233 Tracer from Aqueous Solutions of Acids into Various Organic Reagents

Aqueous Phase	Organic Phase	Pa^{233} Activity Extracted, %
6*M* HCl	5% MDOA[a]-xylene	95.0
6*M* HCl	5% MDOA[a]-chloroform	88.5
6*M* HCl	0.5*M* TTA[b]-xylene	88.5
2*M* HCl	0.5*M* TTA[b]-xylene	95.8
6*M* HNO_3	Diisopropylcarbinol (saturated with 6*M* HCl)	89.5
6*M* HCl	Diisopropylcarbinol (saturated with 6*M* HCl)	99.6
6*M* HCl	Diisobutylcarbinol (saturated with 6*M* HCl)	99.9

[a] Di-*n*-octylmethylamine.
[b] Thenoyltrifluoroacetone.

radioactivity originally in the aqueous phase was 9.9×10^5 gamma counts per minute. Aliquots of each phase were counted for gamma radioactivity by use of a scintillation counter having a sodium iodide crystal (thallium activated). The results of the preliminary tests are shown in Table I. Each value is the average of the results of at least two determinations.

Because diisobutylcarbinol was indicated to be the best extractant and is readily available in high purity, it was selected for further study.

From exploratory experiments (Table II), it was observed that the extraction of protactinium-233 tracer from 6*M* hydrochloric acid into various organic reagents was inhibited markedly by the presence of niobium carrier. Equal volumes (6 ml.) of the aqueous and the organic phases were mixed for 15 minutes. The total amount of protactinium-233 radioactivity originally in the aqueous phase was 5.7×10^6 gamma counts per minute. The data of Table II show that oxalic acid, in a solution of which niobium carrier is usually prepared, does not inhibit the extraction of protactinium-233 tracer from 6*M* hydrochloric acid solution into diisopropylcarbinol or diisobutylcarbinol, but that niobium carrier in oxalic acid does inhibit greatly the extraction of protactinium-233 into these solvents and into 0.5*M* thenoyltrifluoroacetone xylene. The same general effect was observed when 6*M* nitric acid was substituted for 6*M* hydrochloric acid.

When 10 mg. of zirconium carrier (in dilute nitric acid solution) was substituted for the niobium (in oxalic acid solution), it was possible to extract the protactinium-233 quantitatively. Also, when the niobium carrier was introduced into the system in hydrochloric acid solution (no oxalic acid in the system), it was possible to extract 80 to 90% of the protactinium-233 tracer into diisobutylcarbinol from an aqueous phase that contained 10 mg. of niobium carrier in 6*M* hydrochloric acid. However, approximately 15% of the niobium was extracted also.

The addition of oxalate-complexing metals, such as zirconium or aluminum, to the system under the conditions given in Table II (experiment 6) was observed to increase the amount of protactinium-233 tracer extracted to approximately 38%. A similar increase in the amount of protactinium-233 tracer extracted was possible when the aqueous phase was made 10*M* in hydrochloric acid. Protactinium complexing anions, such as fluoride, sulfate, or phosphate, were not detected in the reagents.

Table II. Effect of Niobium Carrier on the Extraction of Protactinium-233 Tracer in Several Systems

Expt. No.	Aqueous Phase HCl, *M*	Aqueous Phase $(COOH)_2$, *M*	Aqueous Phase Nb, mg.	Organic Phase	Pa^{233} Activity Extracted, %
1	6	...	...	Diisopropylcarbinol (satd. with 6*M* HCl)	99.6
2	6	0.075	...	Diisopropylcarbinol (satd. with 6*M* HCl)	99.6
3	6	0.037	10	Diisopropylcarbinol (satd. with 6*M* HCl)	0.8
4	6	...	...	Diisobutylcarbinol (satd. with 6*M* HCl)	99.5
5	6	0.19	...	Diisobutylcarbinol (satd. with 6*M* HCl)	98.6
6	6	0.037	10	Diisobutylcarbinol (satd. with 6*M* HCl)	5.2
7	6	...	...	0.5*M* TTA[a]-xylene	86.0
8	6	0.037	10	0.5*M* TTA[a]-xylene	5.7

[a] Thenoyltrifluoroacetone.

Table III. Effect of Sulfuric Acid Concentration of Aqueous Phase on Extraction of Protactinium-233 Tracer from Niobium–Oxalic Acid–Hydrochloric Acid System into Diisobutylcarbinol

Aqueous Phase[a] H_2SO_4, *M*	Pa^{233} Activity Extracted, %
0	7.3
2.3	85.0
3.1	92.8
3.7	98.4
4.0	99.1

[a] Each aqueous phase also contained 12.9 mg. of niobium and was 0.03*M* in oxalic acid and 6*M* in hydrochloric acid.

Table IV. Effect of Sulfuric Acid on Extraction of Protactinium-233 Tracer from Dilute Hydrochloric Acid Solutions into Diisobutylcarbinol

Aqueous Phase HCl, *M*	Aqueous Phase H_2SO_4, *M*	Pa^{233} Activity Extracted, %
1.2	...	0.8
1.2	7	67.3
2.4	...	9.4
2.4	2	30.4
2.4	4	91.0
2.4	8	94.5

Table V. Extraction of Protactinium-233 Tracer from Aqueous Solutions of Sulfuric Acid into Diisobutylcarbinol

Aqueous Phase, H_2SO_4, *M*	Pa^{233} Activity Extracted, %
2.0	0
3.1	0
5.0	2.8
7.0	28.2
8.8	27.7

Table VI. Effect of Sulfuric Acid on Extraction of Niobium from Aqueous Solutions of Hydrochloric Acid into Diisobutylcarbinol

Aqueous Phase HCl, *M*	Aqueous Phase H_2SO_4, *M*	Nb Extracted, %
4.1	2.0	9.0
4.2	3.3	24.8
4.0	4.0	50.0
5.4	...	12.3
5.4	4.0	76.7
6.1	...	14.6
6.1	4.1	83.1

In view of these observations, it is thought that a nonextractable complex of niobium-protactinium oxalate is formed in the aqueous phase. Direct proof of the existence of such a complex is beyond the scope of this work.

The addition of sulfuric acid to the aqueous phase that contained niobium carrier and oxalic acid under the conditions given in Table II (experiment 6) was found to render the protactinium-233 tracer extractable into diisobutylcarbinol.

The data of Table III show the effect of the sulfuric acid concentration of the aqueous phase on the extraction of protactinium-233 tracer from the niobium–oxalic acid–hydrochloric acid system into diisobutylcarbinol. Equal volumes (6 ml.) of the aqueous and the organic phases were mixed for 15 minutes. The aqueous phase contained a total of 12.9 mg. of niobium carrier in a solution that was 0.03*M* in oxalic acid and 6*M* in hydrochloric acid; the sulfuric acid concentration of the aqueous phase was varied as indicated. The organic phase was diisobutylcarbinol saturated with 6*M* hydrochloric acid.

It was found that a 5-minute extraction period was sufficient to recover more than 99% of the protactinium-233 tracer.

The results in Table III suggest that sulfuric acid is very effective in destroying the postulated niobium-protactinium oxalate complex.

EFFECT OF SULFURIC ACID ON EXTRACTION OF PROTACTINIUM AND NIOBIUM FROM HYDROCHLORIC ACID SOLUTIONS INTO DIISOBUTYLCARBINOL

From additional experiments, it was observed that sulfuric acid enhanced the extraction of protactinium-233 tracer into diisobutylcarbinol. In Table IV, evidence of this effect is shown. Aqueous phases that contained protactinium-233 tracer (1.3×10^7 gamma counts per minute) and that varied in concentrations of hydrochloric and sulfuric acids were mixed for 5 minutes with an equal volume of diisobutylcarbinol that was saturated with 6*M* hydrochloric acid.

The results of the extraction of protactinium-233 tracer from sulfuric acid solutions that contained no hydrochloric acid are given in Table V. Protactinium-233 tracer (4.7×10^5 gamma

counts per minute) solutions of various concentrations of sulfuric acid were extracted once for 5 minutes with equal volumes of diisobutylcarbinol (untreated). The results suggest that negligible quantities of sulfate species of protactinium extract from solutions of sulfuric acid concentration less than $5M$.

The very marked enhancement by sulfuric acid of the extraction of protactinium-233 tracer from dilute hydrochloric acid solutions suggested that the extraction of niobium might be affected similarly. Aqueous phases (5 ml.) that were $0.05M$ oxalic acid and that contained a total niobium-95 radioactivity of 2×10^5 gamma counts per minute, 9.4 mg. of niobium carrier, and various concentrations of hydrochloric and sulfuric acids were extracted for 5 minutes with equal volumes of diisobutylcarbinol that was saturated with $6M$ hydrochloric acid. The results given in Table VI show that sulfuric acid also enhances the extraction of niobium from hydrochloric acid solutions into diisobutylcarbinol.

Thus, although sulfuric acid renders the protactinium-233 tracer readily extractable into diisobutylcarbinol from the niobium–oxalic acid–hydrochloric acid system, it also enhances the extraction of niobium and causes a poor separation of the two elements. Attempts to use the system $6M$ nitric acid–$4M$ sulfuric acid resulted in poor extractions of protactinium-233 tracer.

SEPARATION OF PROTACTINIUM AND NIOBIUM IN THE HYDROFLUORIC ACID–SULFURIC ACID SYSTEM

The results given in Tables IV and VI led to a search for another acid system. At this time, Stevenson and Hicks (*9*) reported the separation of tantalum and niobium by the extraction of tantalum into diisopropyl ketone from a mineral acid–hydrofluoric acid aqueous solution of the two elements. Also, they reported a 90% extraction of niobium into an equal volume of diisopropyl ketone from an aqueous phase that was $6M$ in sulfuric acid and $9M$ in hydrofluoric acid. The behavior of protactinium in this system had not been studied.

Table VII. Extraction of Protactinium and Niobium from Aqueous Hydrofluoric Acid–Sulfuric Acid Solutions into Diisobutylcarbinol

Aqueous Phase[a], HF, M	Extracted, % Pa	Nb
0	...	<0.1
0.5	<0.01	87.8
1.0	<0.02	92.5
2.0	<0.02	96.8
4.0	<0.02	98.4
6.0	<0.01	98.2[b]

[a] Each aqueous phase was $6M$ in sulfuric acid.
[b] A second extraction left no detectable niobium in the aqueous phase.

Table VIII. Effect of Time on Extraction of Niobium from $6M$ Hydrofluoric Acid–$6M$ Sulfuric Acid Solution into Diisobutylcarbinol

Time, Min.	Nb Extracted, %
0.5	97.3
1	98.0
3	98.2
5	98.6

Because experience at the Oak Ridge National Laboratory had shown diisobutylcarbinol to be more effective than diisopropyl ketone for the extraction of protactinium from nitric acid or hydrochloric acid solutions, some experiments were performed to determine whether diisobutylcarbinol could be employed to separate niobium from protactinium in aqueous solutions of dilute hydrofluoric acid. Preliminary results indicated that protactinium-233 tracer did not extract from a hydrofluoric acid–sulfuric acid solution, whereas niobium readily extracted into diisobutylcarbinol from hydrofluoric acid–sulfuric acid solution. Table VII shows the results of the extraction of niobium into diisobutylcarbinol from hydrofluoric acid–sulfuric acid solutions that contained protactinium. The conditions were as follows: Polyethylene bottles were used in all the extractions. The original aqueous phases contained 1 mg. of niobium carrier (dissolved in $0.18M$ oxalic acid) per milliliter and a total protactinium-233 radioactivity of 1.46×10^6 gamma counts per minute or niobium-95 radioactivity of 5.4×10^4 gamma counts per minute. Separate extractions were done under the same conditions for protactinium-233 tracer and niobium-95 tracer. Three-minute extractions were performed with equal volumes (9 ml.) of diisobutylcarbinol that had been pretreated for 3 minutes with a hydrofluoric acid solution of the same concentration as the original aqueous phase. The organic phases were separated, centrifuged, and washed for 1 minute with an equal volume of a solution of the same hydrofluoric acid–sulfuric acid concentration as the original aqueous phase.

The extraction of niobium from $6M$ hydrofluoric acid–$6M$ sulfuric acid as a function of time is shown in Table VIII. Conditions not given in the table were as stated for Table VII.

The diisobutylcarbinol extraction of niobium was found to be more effective from hydrofluoric acid–sulfuric acid or from hydrofluoric acid–hydrochloric acid aqueous solutions than from hydrofluoric acid–nitric acid aqueous solutions. The hydrofluoric acid–sulfuric acid system was selected in this work because exploratory experiments indicated that few elements other than niobium extract from this system into diisobutylcarbinol. Table IX gives data to show that the efficiency of the extraction of niobium from aqueous solutions of hydrofluoric acid into diisobutylcarbinol is dependent on the sulfuric acid concentration of the aqueous phase. Conditions not indicated in the table were as stated for Table VII.

Table IX. Effect of Sulfuric Acid Concentration on Extraction of Niobium from Aqueous Hydrofluoric Acid Solutions into Diisobutylcarbinol

Aqueous Phase HF, M	Aqueous Phase H_2SO_4, M	Extraction Time, Min.	Nb Extracted, %
2	3	3	24.6
2	6	3	96.8
6	0	1	0
6	3	1	64.4
6	6	1	98.0

The niobium readily "stripped" from the diisobutylcarbinol into distilled water; a 1-minute re-extraction of the diisobutylcarbinol with an equal volume of distilled water was found to remove the niobium essential quantitatively from the organic phase.

The use of hydrofluoric acid for the separation of niobium and protactinium affords the obvious advantage of avoiding the hydrolysis usually encountered in work with these elements.

LITERATURE CITED

(1) Gresky, A. T., Bennett, M. R., Brandt, S. S., McDuffee, W. T., and Savolainen, J. E., U. S. Atomic Energy Commission Secret Report, **ORNL-1367** (Jan. 2, 1953).
(2) Hudgens, J. E., Warren, B., and Moore, F. L., U. S. Atomic Energy Commission Declassified Report, **Mon-N-234** (Sept. 9, 1947).
(3) Hume, D. N., Ballou, N. E., and Glendenin, L. E., U. S. Atomic Energy Commission Secret Report, **CN-2815** (June 30, 1945).
(4) Hyde, W. H., and Wolf, M. J., *Ibid.*, **CC-2636** by Katzin, L. I., 8 (Dec. 15, 1944).
(5) Kraus, K. A., and Van Winkle, Q., *Ibid.*, **CC-3365** (June 1945).
(6) Kraus, K. A., and Van Winkle, Q., *Ibid.*, **ORNL-239** (February 1949).
(7) Reynolds, S. A., *Ibid.*, **ORNL-715**, by D. E. Ferguson and E. L. Nicholson, 105 (July 1950).
(8) Steahly, F. L., and Associates, *Ibid.*, **Mon-N-125** (July 1946).
(9) Stevenson, P. C., and Hicks, H. G., ANAL. CHEM., **25**, 1517–19 (1953).

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Calorimetric Determination of Purity

Design and Operation of a Small Adiabatic Calorimeter

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An adiabatic calorimeter has been developed for determination of the purity of the reference compounds required for spectroscopic investigations. Two interchangeable calorimeters with volumes of 0.5 and 5 ml. are used. A complete determination, including calculations, usually requires less than 4 hours. Although the purity determination is based on the measurement of the melting point depression, this method, unlike conventional melting point methods, does not require a previous determination of the heat of fusion and the melting point of the pure compound. Instead, the method determines these quantities as part of the analysis. The calorimeter is used to measure the equilibrium temperature of the sample as a function of heat input as it passes from the solid state to the liquid state. Analysis of the data gives the heat of fusion, the melting point of the sample, and the melting point of the pure compound. The error in the determination of the purity of samples with a purity above 99.8 mole % is less than 0.05 mole %. For less pure samples the error increases with the amount of impurity.

THE reliability of an observed physical property of a compound is frequently questionable because of the lack of any information regarding the purity of the particular sample used for the measurement. The present paper is concerned with the design and operation of an apparatus for determining the purity of small samples of reference compounds such as are required for spectroscopic and mass spectrometric methods.

The purity of a sample may be used as a basis for deciding that the sample is adequately pure for some particular purpose, for applying a correction to the observed measurements for the effect of the impurities, or as a basis for planning further purification.

Methods for determining the purity may be based on a direct determination of the percentage of either the major component or the sum of the impurities. For either approach the errors are apt to be roughly proportional to the magnitude of the quantity being measured. Consequently, in a direct determination of the major component, the error will tend to be independent of the purity and a very accurate method will be required for proper evaluation of a pure sample. On the other hand, a direct determination of the sum of the impurities tends to give a very accurate measure of the purity of a pure sample and less accurate results for impure samples. The latter approach seems to be the most consistent with the purposes of a purity determination as stated, except, possibly, for applying a correction for the effects of the impurities. This case is of limited interest, as there is often considerable question as to the validity of the corrected value obtained from an impure sample regardless of the accuracy of the purity determination.

If the errors are approximately proportional to the magnitude of the quantity being measured, then the error in the determination of the sum of the impurities can represent a relatively large fraction of the total impurity without significantly affecting the usefulness of the determination. Considerable advantage has been taken of this fact in designing the apparatus described in this paper.

The melting point depression (the lowering of the melting point of a pure substance due to the presence of the impurity) is for most substances directly proportional to the mole fraction of impurity and thus is an ideal basis for a method of determining purity. Rossini and coworkers (*5*) have developed a precise method of measuring melting points for this purpose. Unfortunately, the melting point of the sample is useful only as it can be compared to the melting point of the pure compound to give the melting point depression. In addition, the value of the cryoscopic constant or the heat of fusion is required for calculating the purity from the melting point depression. Although the method described by Rossini includes a procedure for extrapolating the observed data to obtain a value for the melting point for zero impurity and for obtaining the heat of fusion, this complicates the method considerably. Also methods based on a comparison of the melting point of the sample with the previously determined melting point of the pure compound have the disadvantage of requiring a high degree of absolute accuracy in the temperature measurement. The 40-ml. sample required for the Rossini method further limits its applicability.

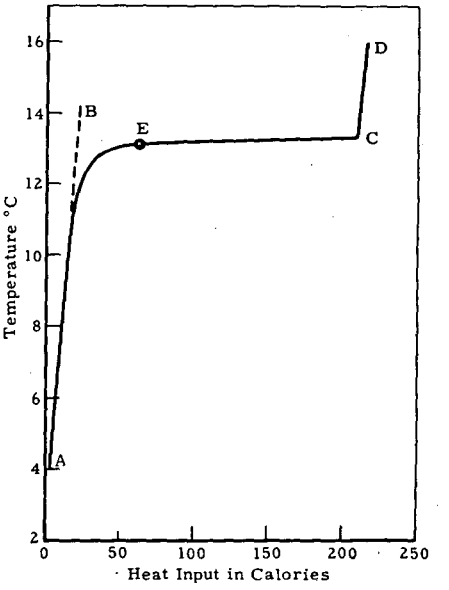


Figure 1. Calculated Melting Curve

p-Xylene, 5 grams, 99 mole % pure

Most of the disadvantages of the usual melting point method can be avoided by determining the equilibrium temperature of the sample in a calorimeter as a function of heat input as the sample is melted. Analysis of these data yields the desired melting point depression and the heat of fusion without reference to any other measurement.

THEORY

Although the calculation of the purity of a sample from its melting curve has been described in varying degrees of detail by a number of workers (*2, 4, 6–11*), the principles of the method do not seem to be generally known; consequently, it seems pertinent to discuss the theory briefly.

The usual simplified equation (5) relating the mole fraction of impurity in a sample to the melting point is as follows:

$$N_2 = \frac{\Delta H_f(T_0 - T_1)}{RT_0^2} \quad (1)$$

where

N_2 = mole fraction of impurity
ΔH_f = molar heat of fusion of the major component in the pure state at T_0
T_0 = melting point of the pure compound, ° K.
T_1 = melting point of the sample, ° K.
R = the gas constant per mole

This relation assumes that the impurities are insoluble in the solid phase and form an ideal solution in the liquid phase during the melting process.

Calculations for Very Pure Samples. A typical melting curve of a sample of high purity is shown in Figure 1. The slopes of the straight lines AB and CD represent the heat capacities of the solid and liquid phases, respectively. The horizontal distance, at a temperature near the melting point, between the lines AB and CD is, for practical purposes, equal to the heat required to melt the sample. The heat required to melt the portion which is liquid at any point, E, is equal to the horizontal distance between AB and E. The fraction of the sample melted at E is then the ratio of the heat required to melt this portion to the heat required to melt the entire sample.

Assuming that the impurity is insoluble in the solid phase, then the concentration of impurity in the liquid phase at any temperature during the melting process is given by

$$N_2' = \frac{1}{F} N_2 \quad (2)$$

where N_2 = mole fraction of impurity in the entire sample, N_2' = mole fraction of impurity in the liquid phase at the temperature, T, and F = fraction of sample melted at the temperature, T.

Since, from Equation 1 the melting point depression is directly related to the mole fraction of impurity, then

$$T_0 - T = \frac{1}{F}(T_0 - T_1) \quad (3)$$

It is apparent from Equation 3 that a plot of the reciprocals of the fractions melted against the corresponding equilibrium temperatures gives a straight line. Extrapolation of this line to $1/F = 1$ and $1/F = 0$ gives the melting point of the sample, T_1, the melting point of the pure compound, T_0, respectively, and the slope of the line gives the desired melting point depression.

Calculations for Impure Samples. Although the determination of the purity of an impure sample has been of minor interest in this investigation, some consideration has been given to the calculation of the purity of such samples.

A typical melting curve of an impure sample is shown in Figure 2. In this case a significant fraction of the sample is already melted at the beginning of the determination and the sample continues to melt at a significant rate as the temperature is increased. Consequently, the observed slope of the curve near the beginning of the determination does not give an adequate measure of the heat capacity of the solid phase. The method used for estimating the purity in this case is based on the use of successive approximations. Since the heat capacity of the solid phase of an impure sample cannot be readily determined from measurements near the melting point, it is assumed that the heat capacity of the solid phase is equal to the heat capacity of the liquid phase. Accordingly, line AB is drawn from A, the starting point in the determination, parallel to CD. Values of ΔH_f, T_0, and T_1 are then calculated as described for a pure sample. Although the plot of $1/F$ *vs.* T is slightly curved in this case, an approximate extrapolation is usually possible.

The relation between the calculated heat of fusion and the true heat of fusion is given approximately by

$$\Delta H_f \text{ (observed)} = (1 - F_s)\Delta H_f \quad (4)$$

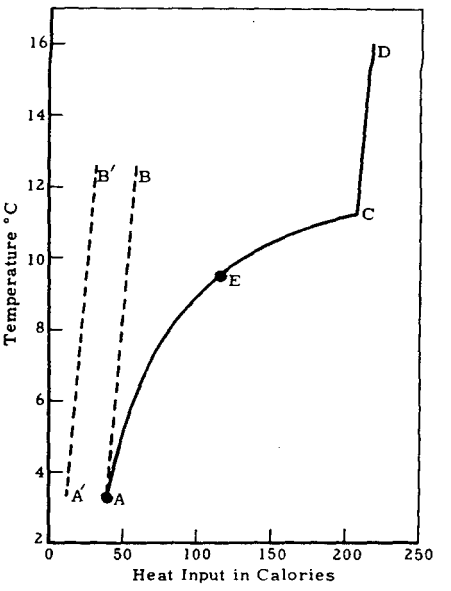


Figure 2. Calculated Melting Curve

p-Xylene, 5 grams, 95 mole % pure

where F_s = fraction of the total sample melted at the temperature, T_s, corresponding to the beginning of the determination. Then, combining Equations 3 and 4

$$\Delta H_f = \frac{T_0 - T_s}{T_1 - T_s}\Delta H_f \text{ (observed)} \quad (5)$$

The approximate values of T_0 and T_1 obtained are substituted in Equation 5 to obtain a more accurate value for the heat of fusion. A new line $A'B'$, is drawn parallel to AB such that the distance between $A'B'$ and CD is equal to the corrected heat of fusion. This gives new values for the fraction melted at each point, which in turn yields more accurate values for T_0 and T_1, and so on.

Although the successive approximations give a significant improvement in accuracy over the value obtained in the first calculation, the results do not converge as rapidly as desired. For example, successive calculation of the purity from the calculated curve shown in Figure 2 gave the following results: 97.3, 96.2, 95.8, 95.6, and 95.5 mole % (true value 95.0 mole %).

Effect of Solid Solutions. All previous considerations are based on the assumption that the impurity is not soluble in the solid phase. If the impurity does form a solid solution with the major component, then the concentration of impurity in the liquid phase will be less than that predicted by Equation 2 and the corresponding equilibrium temperatures will be too high, consequently the plot of $1/F$ *vs.* T will be curved concave upward. Such curvature is considered as evidence of the formation of solid solution. Although a linear plot of $1/F$ *vs.* T for values of F throughout the entire melting of the sample is usually considered as evidence of the absence of solid solution, this does not eliminate the possibility of a solid solution of a special type.

Calculation of Purity from Premelting Heat Capacities. The apparent heat capacity of a sample just below the melting point is always greater than the true heat capacity of the solid phase because of incipient melting. This apparent increase in the heat capacity has been used as a basis for calculating the purity (*2*, *10*). This method is considered to be superior for determining the purity of very pure samples because of greater sensitivity. Aston and coworkers (*2*) point out that agreement between the purity calculated from premelting data and the value obtained from a plot of $1/F$ *vs.* T is evidence of the absence of solid solution.

The calculation of purity from premelting data according to the method described by Weissberger (*10*) is rather laborious.

An easier but approximately equivalent way of handling data from the premelting region is to include it in the plot of $1/F$ *vs.* T. The linearity of this plot over the entire region gives essentially the same criterion as to the absence of solid solution as the method recommended by Aston. This method also gives high sensitivity in the calculation of the purity of very pure samples.

DESCRIPTION OF APPARATUS

The calorimetric method of determining purity has been used principally by workers whose primary interest lay in the accurate determination of the thermodynamic properties of various substances. These investigations required very precise calorimeters which were much too complex to be adaptable to the comparatively simple task of determining purity. The large calorimeter also required too much sample. Aston and coworkers (*3*) designed a simplified calorimeter for the dual purpose of determining purity and heat capacity. Even this apparatus is more complicated than desirable, requires 15 ml. of sample, and is not readily adaptable to the analysis of solids. Clarke and coworkers (*4*) described a simple apparatus requiring only 3 to 4 ml. of sample, but this apparatus also cannot be used for the analysis of solids. The apparatus described in this paper was designed specifically for the routine determination of the purity of small samples of either solids or liquids. The design has been greatly simplified to take advantage of the fact that it is unnecessary to determine the amount of impurity with high accuracy. Several features considered as an essential part of an accurate calorimeter have been omitted in favor of simplicity and ease of operation.

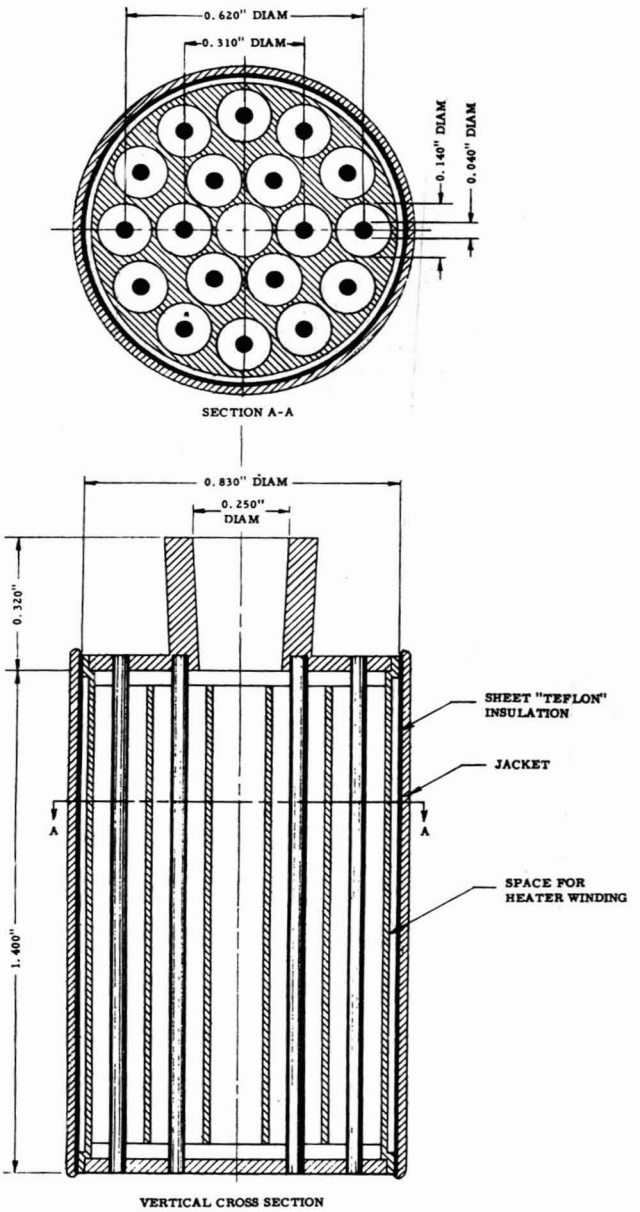


Figure 3. Diagram of 5-Ml. Calorimeter

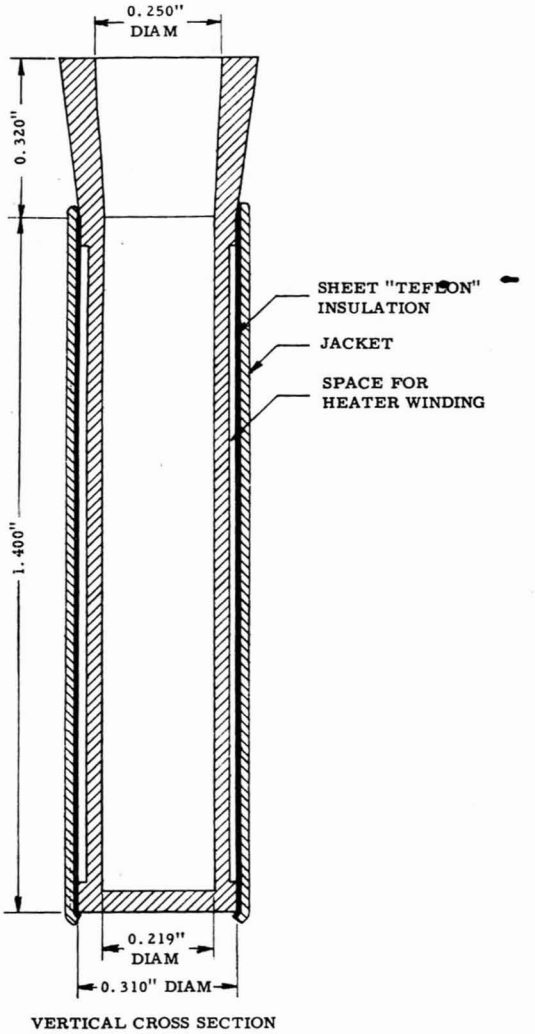


Figure 4. Diagram of 0.5-Ml. Calorimeter

Calorimeter and Radiation Shield Assembly. The two princi pal parts of an adiabatic calorimeter are the calorimeter and th radiation shield. The calorimeter contains the sample and i provided with a sensitive temperature-measuring device and a electrical heater for adding measured quantities of electrica energy. The radiation shield surrounds the calorimeter and maintained at the same temperature as the calorimeter in orde to avoid heat flow between the calorimeter and its surroundings

Two calorimeters with sample volumes of 5 and 0.5 ml. are use interchangeably. Both calorimeters are constructed of a 90% gold–10% copper alloy for high heat conductivity and for co rosion resistance. The design of the 5-ml. calorimeter is show in Figure 3. It consists of a gold cylinder containing 19 close spaced holes drilled parallel to the axis of the cylinder and co nected together at the top and the bottom. A removable therm couple well fits into the center hole. Gold wires are placed dov the center of each of the other holes and soldered to the top a bottom of the calorimeter. The sample occupies the 0.050-in

(1.3-mm.) annular space between the sides of the holes and the wires.

The electrical heater (No. 36 B. and S. gage Manganin wire) is wound around the outside of the cylinder; one end is attached to the body of the calorimeter and the other end to a jacket which covers the winding. The jacket is electrically insulated from the body of the calorimeter with sheet Teflon. As described later, this arrangement facilitates making the electrical connection to the calorimeter.

The 0.5-ml. calorimeter, as shown in Figure 4, is similar in principle but has only the one central hole. The sample occupies the 0.040-inch (1.0-mm.) annular space between the sides of this hole and the thermocouple well. The heater is again wound around the outside and covered by an insulated jacket.

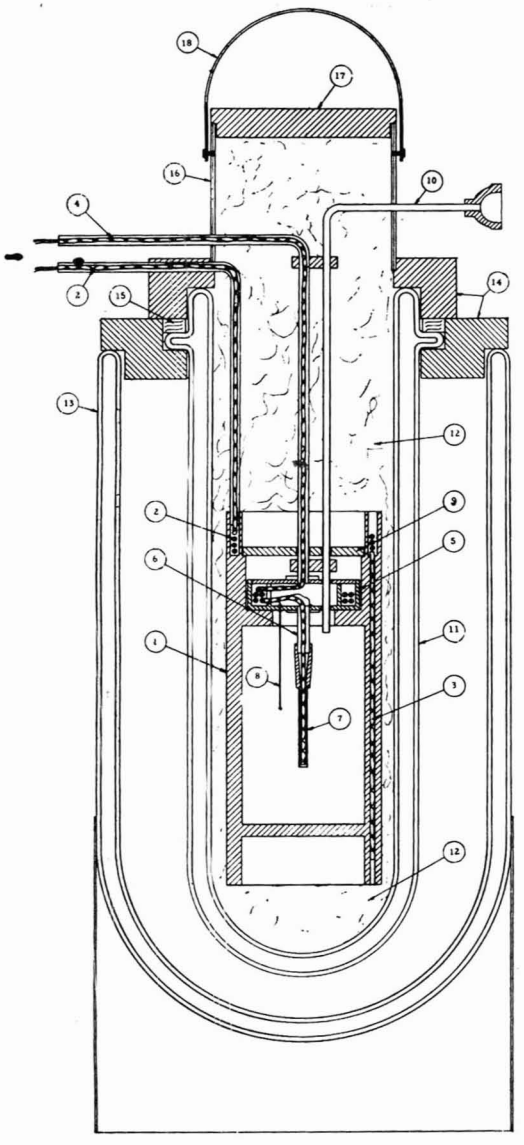


Figure 5. Diagram of Radiation Shield Assembly

1. Radiation shield
2. Radiation shield thermocouple and heater leads
3. Radiation shield thermocouple junctions
4. Calorimeter thermocouple and heater leads
5. Inner cover containing coil of thermocouple leads
6. Stainless steel tube
7. Gold calorimeter thermocouple well
8. Spring contact to calorimeter heater
9. Outer cover
10. Stainless steel tube for liquid nitrogen cooling
11. Glass Dewar (evacuated and lightly silvered)
12. Glass wool
13. One-gallon wide-mouthed Dewar
14. Bakelite rings
15. Cork ring
16. Removable Bakelite tube
17. Removable Bakelite cover
18. Bail for removing Bakelite tube

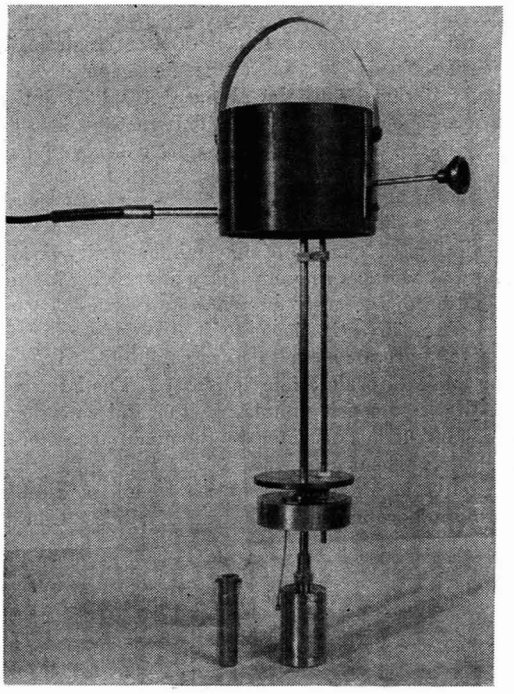


Figure 6. Inner Assembly of Radiation Shield with Calorimeters

The radiation shield assembly is shown in Figure 5. The radiation shield itself is constructed from a section of copper tubing with $^1/_4$-inch walls. A thermocouple is located in the wall and an electrical heater is wound around the outside. It is padded with glass wool and placed in an evacuated and lightly silvered glass Dewar which is supported in a cooling bath. The temperature of the radiation shield is controlled by balancing the constant heat loss through the Dewar against the adjustable heat output of the electrical heater.

The temperature of the radiation shield and the calorimeter is measured by means of 10-junction copper-constantan thermocouples. The thermocouple wires, the electrical heaters, and the leads are all glass-insulated and silicone-varnished to permit operation at elevated temperatures. The thermocouple leads and associated heater leads are brought in through stainless steel tubes. A coil of about 18 inches of the leads of both thermocouples is in direct thermal contact with the radiation shield in order to reduce heat conduction down the leads to the junctions.

The calorimeter is attached to the gold thermocouple well by means of a friction fit on the taper joint. As shown in Figure 5, the thermocouple well is separated from the inner cover by a short section of stainless steel tubing of low heat conductivity. Two pairs of calorimeter heater leads are used. One pair supplies the current to the calorimeter; the other pair is used for measuring the potential drop across the heater. One lead from each pair is soldered inside the inner cover to the stainless steel tube supporting the calorimeter. The other leads from each pair are likewise soldered inside the cover to a short length of small diameter stainless steel tubing which is attached to the lower side of the cover and touches the outside wall of the calorimeter. Both of these stainless steel tubes are electrically insulated from the cover. This arrangement provides a convenient means of connecting the calorimeter heater to the heater leads. Because of the reduced diameter of the 0.5-ml. calorimeter, a small connecting link is required between the jacket and the small stainless steel tube.

Another stainless steel tube extends from outside the radiation shield down into the cavity containing the calorimeter for the introduction of liquid nitrogen for rapid cooling of the calorimeter and the radiation shield.

All the inner parts of the radiation shield assembly, consisting of the calorimeter thermocouple well, the inner and outer covers, the tube carrying the calorimeter thermocouple leads, and the coolant tube are supported from a short section of Bakelite tubing which rests on top of the inner Dewar. Thus it is possible to lift out this entire inner assembly, including the calorimeter, as a unit. This ready accessibility of the calorimeter is of considerable advantage, particularly when analyzing solid samples. A photograph of this section of the apparatus with the 5-ml.

calorimeter attached and the 0.5-ml. calorimeter standing to the left is shown in Figure 6. Figure 7 shows a photograph of the complete apparatus with the calorimeter in place.

Power Supplies. The direct current for the calorimeter heater is obtained from a regulated power supply for which the circuit is shown in Figure 8. The calorimeter current switch, S_2, as well as the current selector switch, S_1, are all wired so as to keep a constant load on the power supply at all times. Likewise when using the 0.5-ml. calorimeter with its lower resistance in the heater, additional resistance can be placed in the heater circuit by means of S_{12}. The maximum output of this power supply is about 65 ma. The stability of the power supply is such that the current and voltage usually vary less than 0.1% during a run.

Alternating current is not satisfactory for the radiation shield heater because of excessive pickup in the thermocouples. The direct current power supply for this purpose is also shown in Figure 8. The maximum output of this power supply is about 1 ampere.

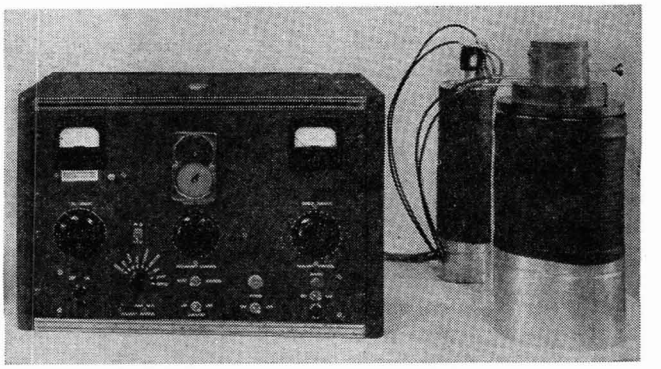
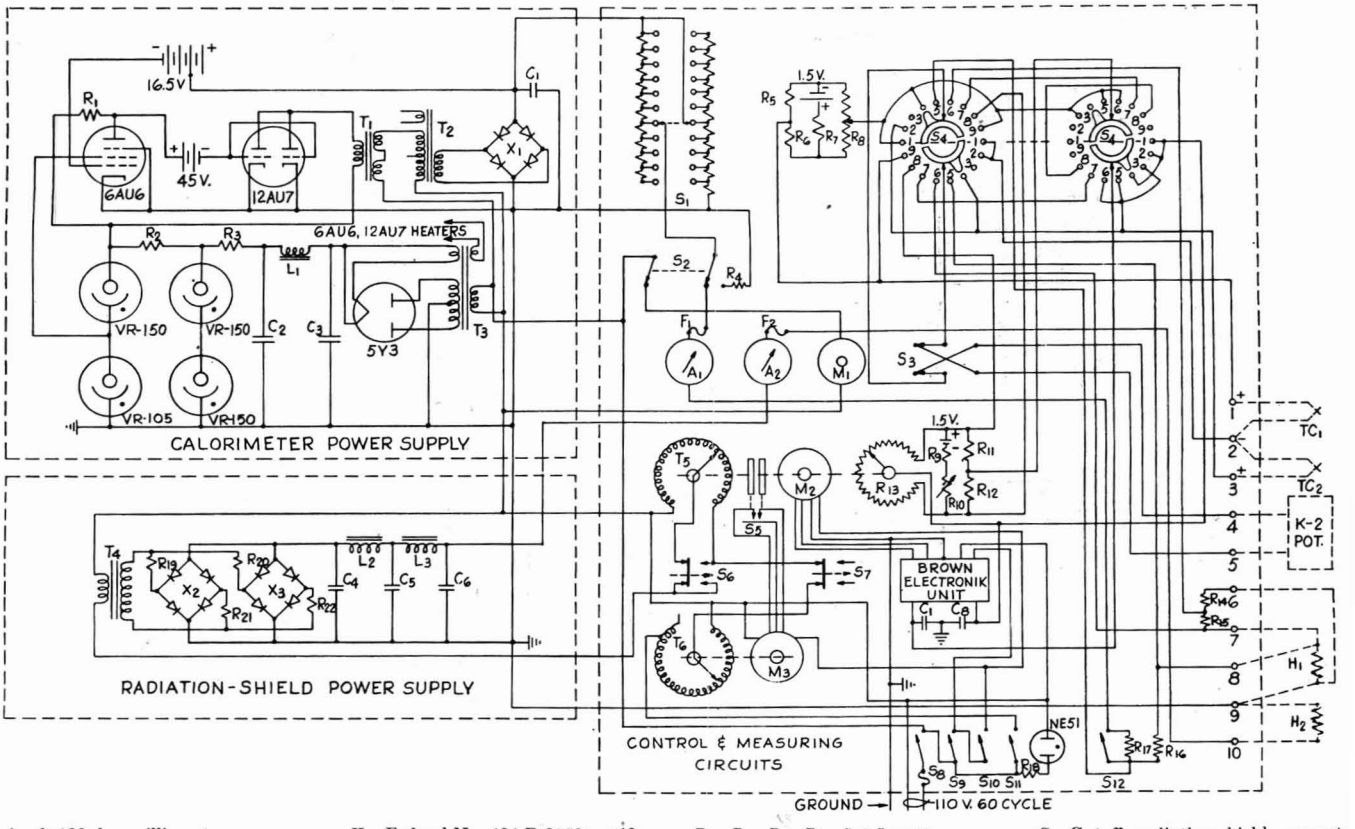


Figure 7. Control Chassis and Radiation Shield Assembly

Control and Measuring Circuits. The arrangement of the control and measuring circuits is shown in Figure 8. The selector switch, S_4, provides a means of connecting the radiation shield and calorimeter thermocouples to a Leeds and Northrup Type K-2 potentiometer. The same selector switch provides for measuring the calorimeter heater current by observing the voltage drop across a 1-ohm resistor in series with the heater and for measuring the voltage drop across the heater through a voltage divider with a ratio of 1000 to 1. The total time the current has passed through the heater is measured with an ordinary electric interval timer.

The current through the radiation shield heater is controlled by two variable transformers in series with the input to the power supply. The position of these variable transformers is



A_1. 0–100 d.c. milliameter
A_2. 0–1000 d.c. milliameter
C_1. 6000 mfd. 25 volts
C_2, C_3. 40 mfd. 450 volts
C_4, C_5, C_6. 20 mfd. 450 volts
C_7, C_8. 2 mfd.
F_1. 1/8 ampere fuse
F_2. 1 1/4 ampere fuse
H_1. Calorimeter heater
H_2. Radiation-shield heater, 100 ohms
L_1. Choke 8 hy. 40 ma.
L_2, L_3. UTC S-37 choke 20 hy. 500 ma.
M_1. Precision Time-it electric stopwatch, modified for panel mount
M_2. Brown No. 76750-3 motor
M_3. Barber-Coleman bYAe 451 motor
X_1. Federal No. 404 D 3552 rectifier
X_2, X_3. Bridge-connected Sylvania NJ5 rectifiers
R_1. 330 K Ω 1 watt
R_2. 2000 Ω 5 watt
R_3. 2000 Ω 10 watt
R_4. 200 Ω 5 watt
R_5, R_6. 100 Ω 1watt 1% WW
R_7. 180 K Ω 1watt
R_8. 200 Ω Type A Helipot
R_9. 200 K Ω 1watt
R_{10}. 100 K Type A Helipot
R_{11}, R_{12}. 25 Ω 1watt 1% WW
R_{13}. 50 Ω Type JZ spc. Helipot
R_{14}. 250 K Ω 1watt 1% WW
R_{15}. 250 Ω 1watt 1% WW
R_{16}. 1 Ω 1watt 1% WW
R_{17}. 133 Ω 5 watt
R_{18}. 120 K Ω 0.5 watt
R_{19}, R_{20}, R_{21}, R_{22}. 5 Ω 5 watt
S_1. Calorimeter current selector switch
S_2. Calorimeter current switch
S_3. Polarity reversing switch
S_4. Communication Products selector switch w/2 "E" decks
1. Radiation-shield thermocouple
2. Calorimeter thermocouple
3. Automatic temp. control
4. Thermocouple differential
5. Calorimeter current
6. Calorimeter voltage
7. Test signal to Brown unit
8. E.m.f. across R_{13}
9. Correction voltage

S_5. Cam-operated NO type microswitches
S_6. Increase radiation-shield current
S_7. Cutoff radiation-shield current
S_8. Main power
S_9. Brown Electronik unit
S_{10}. Power to automatic control unit
S_{11}. Radiation-shield current
S_{12}. Load resistor for 0.5-ml. calorimeter
T_1. GE 66G418 sat. reactor
T_2. Triad No. 6473 110 v/220 v. ct.
T_3. Stancor P-4076
T_4. 115 volt/260 volt/100 watt transformer
T_5. Primary variable transformer
T_6. Secondary variable transformer
TC_1. Radiation-shield thermocouple
TC_2. Calorimeter thermocouple

Figure 8. Wiring Diagram of Power Supplies and Control and Measuring Circuits

automatically adjusted so as to keep the temperature of the radiation shield as close as possible to the temperature of the calorimeter. This is accomplished by connecting the radiation shield and calorimeter thermocouples in series opposition to a Brown Electronik continuous balance unit (No. 351921-1). Any unbalance in the signals from these two thermocouples causes a motor to turn quickly the first (primary) variable transformer in such a direction as to tend to reduce the temperature difference. The primary transformer is mechanically coupled to a potentiometer which is connected in series with the thermocouples. The point of zero voltage output from the potentiometer corresponds to a position on the variable transformer near the center of its adjustment. Any unbalance between the radiation shield and calorimeter thermocouples causes a change in the radiation shield current and the magnitude of this change is related to the size of the unbalance signal.

The second (secondary) variable transformer is connected to a separate motor which is controlled by cams and microswitches on each side of the balance point of the potentiometer. Whenever the potentiometer is on either side of the balance point, the secondary transformer is slowly adjusted in such a direction as to tend to reduce the temperature difference. The purpose of this arrangement is to adjust the position of the secondary transformer so that the control point on the primary transformer will be close to the balance point of the potentiometer. Both variable transformer drives are equipped with clutches to avoid damage when the transformer reaches the end of its travel. This provision also allows manual adjustment when desired. The motor speed and associated gear trains are such that 2 seconds and 3 minutes are required to drive the primary and secondary transformers, respectively, from one end of their travel to the other.

An additional potentiometer is located in the radiation shield thermocouple circuit for the purpose of introducing a correction to the output of this thermocouple as may be required to obtain a more nearly adiabatic environment for the calorimeter. Conditions which may require such correction are differences between the response of the radiation shield and the calorimeter thermocouples, heat leaks down the thermocouple leads to the calorimeter, and induced electromotive force in the thermocouples. This potentiometer will be referred to as the correction potentiometer.

Table I. Properties of the Two Calorimeters

	5-Ml. Calorimeter	0.5-Ml. Calorimeter
Weight, g.	110.4	20.8
Internal vol.[a], ml.	5.1	0.48
Effective heat capacity, g.-cal./° C.	5.06	1.58
Heater resistance, ohms	205	72

[a] Volume with thermocouple well in place.

Provision is also made for manual control of the radiation shield current, if desired, by manually adjusting the same two variable transformers. Pushbutton switches permit increasing or decreasing the radiation shield rapidly without disturbing the adjustment of the variable transformers.

Instrument Performance. The heater resistance, weight, and effective heat capacity of the two calorimeters are given in Table I. Although the heat capacities of these calorimeters might be considered excessive, the rather massive construction was chosen deliberately in order to minimize the time required for thermal equilibrium through the calorimeter and the sample. This high heat capacity does not seem to be a significant disadvantage in the determination of purity. The rate of heat leak from the radiation shield to the calorimeter for a given difference in temperature was determined from the heat capacity of the calorimeter and the rate of change of the calorimeter temperature for a constant temperature difference of 0.2° C. The calculated rate of heat leak for the 5- and 0.5-ml. calorimeters are, respectively, 20 and 13 calories per hour for a temperature differential of 1° C.

The automatic temperature controller for the radiation shield works fairly satisfactorily. The Brown amplifier has a sensitivity of about 5 μv. (about 0.01° C.), provided there is no alternating current pickup in the thermocouples. Elimination of the alternating current pickup has given considerable trouble. Careful filtering of the radiation shield heater current has eliminated most of this trouble. Residual effects are believed to be due to too close proximity of the thermocouples and associated circuits to the alternating current components in the power supply. A complete separation of the alternating current and direct current sections of the circuits would probably give better performance.

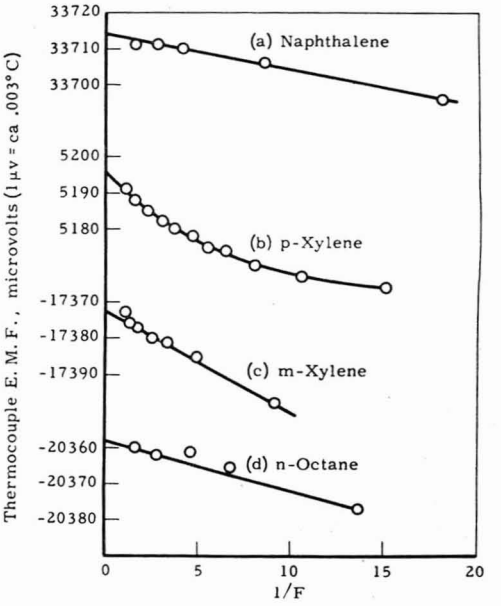


Figure 9. Typical Results Obtained Using 5-Ml. Calorimeter

The correction potentiometer has been only partially successful. Although the potentiometer can be adjusted so as to reduce the rate of the drift in temperature of the calorimeter to about 0.5 μv. (0.001° C.) per minute, the setting required for zero temperature drift does not seem to be reproducible. The cause of this effect is unknown. It may be due to induced voltages in the radiation shield thermocouple circuit. In the analysis of the samples described in a later section the correction was adjusted to +50 μv. in all cases. The current practice is to adjust the correction voltage at the beginning of each determination to a value which gives a negligible drift.

PROCEDURE

Preparation of Apparatus. The calorimeter containing a weighed amount of sample is attached to the thermocouple well, then the calorimeter and supporting structure are lowered into place in the radiation shield and the space above the outer cover is packed loosely with glass wool for insulation. The outer Dewar is filled with liquid nitrogen for samples with a melting point below 75° C., with water when the melting point is in the range from 75° to 150° C., and left empty if the melting point is above 150° C. Again, depending upon the melting point of the sample, the calorimeter is heated or cooled until both the calorimeter and the radiation shield are at a temperature 10° to 20° C. below the estimated melting point of the sample. The cooling

is accomplished by forcing liquid nitrogen down the tube provided for this purpose. The radiation shield temperature is then put on automatic control. The calorimeter heater current selector switch is adjusted to a current which will raise the temperature of the calorimeter by about 2° C. for a heating time of 100 seconds.

Making the Run. The run consists of adding electrical energy to the calorimeter in a series of measured increments and then recording the calorimeter temperature after each increment as soon as it becomes constant. (A drift of less than 1 μv. per minute is considered to be adequately constant when the sample is melting. A more rapid drift is acceptable when measuring the heat capacity of the solid or liquid phases.) The course of the run is followed by calculating the rate of temperature rise expressed as microvolts per second of heating time. The duration of the heating periods is chosen so as to give at least two consecutive intervals with a constant heating rate on the solid and liquid portions of the melting curve and from five to eight points on the flat. The radiation shield heater is left on automatic control at all times except when reading the temperature of the calorimeter, which is done as quickly as possible since the control circuit is shut off during this period. The calorimeter current and the voltage drop across the calorimeter are measured during heating periods near the beginning, the middle, and the end of the run.

Calculations. The data from the run are plotted directly in terms of the calorimeter thermocouple reading in microvolts against the corresponding heating time in seconds. Straight lines are drawn representing the heat capacity of the solid and liquid phases plus the heat capacity of the calorimeter and then the fusion time in seconds is determined from the distance between these lines. This time is converted into calories per mole by means of the expression

$$\Delta H_f = \frac{E\,i\,t\,M}{4.184\,g} \qquad (6)$$

where

E = average voltage drop across the calorimeter
i = average calorimeter current in amperes
t = fusion time in seconds
M = molecular weight
g = sample weight in grams

The temperatures (in microvolts) of points obtained on the flat are plotted against the reciprocal of the fraction melted and the straight line so obtained is extrapolated to $1/F = 0$ and $1/F = 1$ to give T_0 and T_1, respectively. The thermocouple voltages so obtained are converted to actual temperatures and then the impurity in the sample is calculated from Equation 1.

DISCUSSION

The apparatus and method have been checked by determining the purity of eight standard samples from the American Petroleum Institute, Carnegie Institute of Technology, Pittsburgh, Pa., and four synthetic samples prepared by adding known amounts of *m*-xylene to an API sample of *p*-xylene. At least two determinations were made on each sample with each calorimeter. In order to conserve the sample, the duplicate runs were made on the same weighed portion of the sample. The results of these determinations are shown in Tables II and III. Although there is no significant difference between the purities as determined in the two calorimeters, there does seem to be a tendency towards too high a purity for most of the samples. For the very pure samples the error is small and of unknown origin. In the case of the 98.5 and 95.9 mole % *p*-xylene samples the errors are much larger. At least part of these errors are probably due to the fact that the results are based on calculations carried only through the second approximation stage described in the section on the analysis of impure samples.

Some typical plots of $1/F$ *vs.* thermocouple electromotive force for runs listed in Table II are shown in Figures 9 and 10. These curves illustrate the uncertainty often encountered in extrapolating to T_0 when a nonlinear relation between $1/F$ and the temperature is observed. A good example is run *b* for *p*-xylene using the 5-ml. calorimeter (Figure 9). The curvature observed is taken as evidence of solid solution formation. Extrapolation of this curve gives a value for the impurity in agreement with the API value. However, the more extreme curvature shown in run *f* for the same sample run in the 0.5-ml. calorimeter (Figure 10) does not permit of any reasonable extrapolation. At least part of the difference in the data obtained from the two calorimeters is attributed to the fact that occasionally points near the end of the flat (small values of $1/F$) tend to be slightly high. Although the reason for this is not definitely known, it is probably caused by the last of the solid falling down to the bottom of the calorimeter so that equilibrium temperatures are virtually impossible to obtain. Although this effect is observed in both calorimeters, it is somewhat greater in the 0.5-ml. calorimeter where it tends to increase the curvature.

The cause of the relatively large discrepancy between the observed values for the impurity in naphthalene and the API value is unknown. The essentially linear relation between $1/F$

Table II. Analysis of Pure API Standard Samples

			API Values (*1*)		5-Ml. Calorimeter					0.5-Ml. Calorimeter				
							Heat of Fusion					Heat of Fusion		
Compound	API Sample No.	Approx. M.P., ° C.	Heat of fusion, kcal./ mole	Impurity, mole %	Sample wt., g.	Figure 9 curve	Found, kcal./ mole	Error, %	Impurity, mole %	Sample wt., g.	Figure 10 curve	Found, kcal./ mole	Error, %	Impurity, mole %
Naphthalene	577	80.3	4.52[a]	0.04 ± 0.03	4.194	a	4.63	+2.4	0.004[b]	0.360	e	5.05	+11.7	0.008[b]
							4.62	+2.2	0.004[b]			5.08	+12.4	0.006[b]
2-Methyl-naphthalene	579	34.6	2.84[a]	0.09 ± 0.06	3.724		2.88	+1.4	0.06	0.401		3.19	+12.3	0.07
							2.90	+2.1	0.05			3.10	+9.2	0.04
p-Xylene	215e	13.3	4.090	0.04 ± 0.02	4.293	b	4.18	+2.2	0.026	0.353		4.17	+2.0	...[c]
							4.14	+1.2	0.033			4.38	+7.1	...[c]
n-Dodecane	559	− 9.6	8.803	0.031 ± 0.025	3.464		8.87	+0.8	0.008	0.347		9.18	+4.3	0.014
							8.89	+1.0	0.003			9.24	+5.0	0.009
o-Xylene	213b	−25.2	3.250	0.005 ± 0.004	3.838		3.27	+0.6	0.002	0.355		3.52	+8.3	0.001
							3.12	−4.0	0.003			3.47	+6.8	0.003
m-Xylene	214b	−47.9	2.765	0.07 ± 0.03	3.856	c	2.79	+0.9	0.024	0.371	g	2.94	+6.3	0.038
							2.81	+1.6	0.023			2.96	+7.1	0.047
n-Octane	230	−56.8	4.957	0.06 ± 0.04	3.164	d	5.02	+1.3	0.013	0.328	h	5.29	+6.7	0.040
							4.98	+0.5	0.023			5.23	+5.5	0.034
n-Heptane	216a	−90.6	3.354	0.01 ± 0.01	2.712		3.36	+0.2	0.010	0.300		3.59	+7.0	0.012
							3.31	−1.3	0.011			3.49	+4.1	0.016

[a] Unpublished values from American Petroleum Research Project 44, Carnegie Institute of Technology, Pittsburgh, Pa.
[b] Cause of discrepancy between observed value and API value is unknown.
[c] See text for comments on failure to secure value using 0.5-ml. calorimeter.

Table III. Analysis of Synthetic *p*-Xylene Samples

	5-Ml. Calorimeter					0.5-Ml. Calorimeter				
		Purity		Heat of Fusion			Purity		Heat of Fusion	
Actual Purity, Mole %	Sample wt., g.	Found, mole %	Error, mole %	Found, kcal./ mole	Error,[a] %	Sample wt., g.	Found, mole %	Error, mole %	Found, kcal./ mole	Error,[a] %
99.78	4.323	99.79	+0.01	4.09	0.0	0.3591	99.81	+0.03	4.35	+6.4
		99.83	+0.05	4.06	−0.7		99.76	−0.02	4.34	+6.1
99.30	4.200	99.44	+0.14	4.01	−2.0	0.3632	99.32	+0.02	4.07	−0.5
		99.47	+0.17	4.05	−1.0		99.39	+0.09	4.15	+1.5
98.5	4.284	98.8	+0.3	3.95	−3.4	0.3430	98.6	+0.1	4.38	+7.1
		98.8	+0.3	3.95	−3.4		98.2	−0.3	4.19	+2.4
							98.7	+0.2	4.02	−1.7
95.9	4.287	97.2	+1.3	3.86	−5.6	0.3316	97.1	+1.2	3.76	−8.1
		96.9	+1.0	3.78	−7.6		95.8	−0.1	4.46	+9.1
							96.5	+0.6	4.06	−0.7

[a] Based on API value of 4.090 kcal./mole.

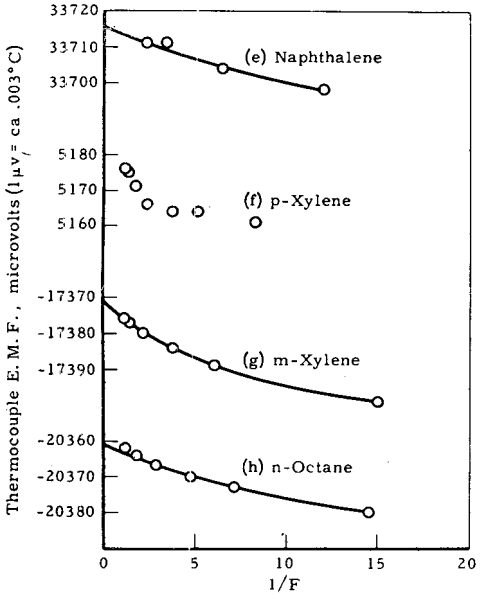


Figure 10. Typical Results Obtained Using 0.5-Ml. Calorimeter

and temperature shown in Figures 9 and 10 for runs *a* and *e* are typical of all four determinations.

Although the two calorimeters give equivalent results in the determination of purity, the 5-ml. calorimeter gives somewhat more reproducible values for the heats of fusion. The positive systematic error observed in the heats of fusion obtained in the 0.5-ml. calorimeter is assumed to be due to improper setting of the correction voltage in the radiation shield thermocouple circuit. A very small heat leak between the radiation shield and the calorimeter will, of course, cause a rather large error in the determination of the heat of fusion of such a small sample. Although some of these errors appear to be rather large, they do not have a significant effect on the determination of the purity, which is the primary function of this apparatus.

This apparatus is being used with considerable success for the routine determination of the purity of spectroscopic standards. The lowest melting compound examined is *n*-heptane (melting point, −90.6° C.) and the highest melting is anthracene (melting point 217° C.); the upper range of operation is about 250° C. Measurements at temperatures below −100° C. are impossible because the Dewar surrounding the radiation shield does not permit a sufficient rate of heat transfer to the liquid nitrogen in the outer Dewar to offset the heat leaks through the top. Much lower operating temperatures could be obtained by using a more lightly silvered Dewar.

As there is no provision for evacuating the calorimeter, the melting points observed in this apparatus are not triple points but melting points in air under a pressure of 1 atmosphere. The lack of a tight seal may make it difficult to obtain good data for a sample which is volatile at its melting point.

Less than 4 hours is required for a complete determination including calculations and cleaning the calorimeter, provided the sample crystallizes readily on cooling and the approximate melting point is known before beginning the determination. Some samples give considerable trouble by excessive supercooling. Table IV gives the average number of heating periods, the time required for equilibrium, and similar statistics for the determinations listed in Tables II and III.

Table IV. Average Operating Conditions for Analysis of Samples Listed in Tables II and III

	5-Ml. Calorimeter	0.5-Ml. Calorimeter
Heater current, ampere	0.063	0.048
No. of heating periods	15	16
Total heating time, seconds	1490	1140
Total time for run.[a] minutes	96	90
Av. time for temp. equilibrium, minutes	4.7	4.4

[a] Time from beginning of first heating period to measurement of temperature at end of last heating period.

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LITERATURE CITED

(1) American Petroleum Institute Research Project 44, "Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds," Pittsburgh, Carnegie Press, 1953.
(2) Aston, J. G., Cines, M. R., and Fink, H. L., *J. Am. Chem. Soc.*, **69**, 1532 (1947).
(3) Aston, J. G., Fink, H. L., Tooke, J. W., and Cines, M. R., ANAL. CHEM., **19**, 218 (1947).
(4) Clarke, J. T., Johnston, H. L., and DeSorbo, W., *Ibid.*, **25**, 1156 (1953).
(5) Glasgow, A. R., Jr., Streiff, A. J., and Rossini, F. D., *J. Research Natl. Bur. Standards*, **35**, 355 (1945).
(6) Pitzer, K. S., and Scott, D. W., *J. Am. Chem. Soc.*, **63**, 2419 (1941).
(7) Rossini, "Chemical Thermodynamics," p. 455, New York, John Wiley & Sons, 1950.
(8) Scott, R. B., Meyers, C. H., Rands, R. D., Jr., Brickwedde, F. G., and Bekkedahl, N., *J. Research Natl. Bur. Standards*, **35**, 39 (1945).
(9) Todd, S. S., Oliver, G. D., and Huffman, H. M., *J. Am. Chem. Soc.*, **69**, 1519 (1947).
(10) Weissberger, A., ed., "Physical Methods of Organic Chemistry," 1st ed., Vol. I, p. 350, New York, Interscience Publishers, 1945.
(11) White, W. P., *J. Phys. Chem.*, **24**, 393 (1920).

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Use of Fluoboric Acid for the Direct Determination of Potassium

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A practical analytical method is proposed for the direct determination of potassium by precipitation as potassium fluoborate from an ice-cold solution. Solutions containing 20 to 250 mg. of potassium chloride in the presence of up to 500 mg. of sodium chloride have been analyzed, with a relative error in potassium chloride of less than 1% and with good reproducibility. Moderate to comparatively high ratios of the chlorides of copper(II), zinc, cadmium, cobalt, nickel, manganese(II), iron, aluminum, chromium, calcium, lithium, or magnesium to potassium do not interfere. Combinations of these chlorides are permissible; however, aluminum and calcium must not be present in the same solution with potassium. The ammonium, barium, and sulfate ions interfere.

OF THE various methods that are available today for the determination of potassium (*7*), the two most often used are the chloroplatinate and perchlorate methods (*5*). The chloroplatinate method generally is considered to be the most accurate for determining potassium in the presence of sodium, but considerable time is required to regenerate the expensive chloroplatinic acid. The perchlorate method, which requires a much less expensive reagent, has been adopted by many laboratories and found to give good results, but it requires more attention to details and involves more steps in the procedure. Considerable interest has been stimulated recently in more rapid and more specific methods for potassium—especially the use of sodium tetraphenylboron (*2–4*), but the reagent is expensive.

In this paper an inexpensive and simple routine method is proposed for the rapid direct determination of potassium in the presence of the mixed chlorides of the alkali metals (except cesium and rubidium), with a prepared alcoholic solution of fluoboric acid as the precipitating agent.

The preparation of potassium fluoborate dates back to the early 1820's when it was made by mixing fluoboric acid with a solution of a potassium salt, such as the carbonate, nitrate, or chloride (*1*). In 1915, Mathers *et al.* (*6*) developed a qualitative test for the presence of sodium in potassium, lithium, and magnesium mixtures. During the course of their work the potassium was removed as potassium fluoborate. However, apparently no one has utilized the insolubility of potassium fluoborate as a means for the quantitative determination of potassium in the presence of the other common alkalies, as well as many other elements. This is a report of the development of a workable method based on the insolubility of potassium fluoborate.

EXPERIMENTAL

Reagents. Throughout the investigation reagent-grade salts were used. Solutions of the following specifications were used: 48 to 50% purified Baker and Adamson fluoboric acid; absolute methanol, analytical reagent, 99.5% assay; and 95% ethyl alcohol.

Preparation of Precipitating Solution. A 0.50-gram sample of sodium chloride is dissolved in 40 ml. of distilled water. To it are added 250 ml. of fluoboric acid, 500 ml. of 95% ethyl alcohol, and 500 ml. of methanol. The mixture is placed in a chipped ice bath until the temperature of the solution is about 3° C. and then filtered by suction through an asbestos-padded porcelain Gooch crucible until the filtrate is clear. The prepared solution is stored in polyethylene bottles.

Procedure. To a 100-ml. borosilicate glass beaker containing 10 ml. of a cold solution of the mixed chlorides of the alkali metals (except cesium and rubidium) are added 30 ml. of the prepared alcoholic solution of fluoboric acid. The beaker is kept in an ice bath until the solution has reached a constant temperature of about 3° C., from 45 minutes to an hour. The supernatant liquid is decanted, with suction applied, through a fritted-glass Gooch-type crucible of medium porosity, which has been inserted into a soft-rubber holder and placed in a funnel and surrounded by chips of ice. After the decantation process, the beaker is returned to the ice-water bath until the filtrate has passed through the crucible. The outside of the beaker is wiped free of water and the bulk of the precipitate is added carefully to the crucible and allowed to drain free of liquid. The precipitate remaining in the beaker is washed quantitatively into the crucible with a fine stream of ice-cold (3° C.) 1 to 1 methanol—ethyl alcohol solution from a wash bottle.

The crucible and contents are washed with three or four 10-ml. portions of the alcohol solution and dried in an oven at 105° to 110° C. for 30 minutes. The crucible is cooled to room temperature and weighed. The potassium content is calculated by multiplying the weight of potassium fluoborate by the stoichiometric factor, 0.3105. In a mixture in which more than 0.50 gram of sodium chloride is expected in the mixed chlorides, the precipitate should be rewashed with 30 ml. of 1 to 1 methanol—ethyl alcohol solution, redried, and reweighed until a change in weight no greater than 0.5 mg. is reached between successive weighings.

The drying time may be reduced considerably by washing the excess alcohol from the precipitate with ice-cold ether and drawing air through the precipitate until the ether odor is gone.

The Gooch crucibles can be cleaned by suction filtering about 100 ml. of hot water through them after the bulk of the precipitate has been removed. After numerous determinations only very slight weight changes and etching of the glass have been noticed.

Alcohol to Sample Solution Ratio. In order to establish the correct conditions for precipitating potassium fluoborate, the ratio of alcohol to reagent to sample solution was studied. Varying amounts of 95% ethyl alcohol and fluoboric acid were added to 5-ml. portions of a potassium chloride solution (5 ml. equivalent to 0.0200 gram potassium chloride) at room temperature. It was found that a greater than 1 to 1 ratio of alcohol to sample solution gave quite reproducible but low results. The amount of fluoboric acid added (2 to 10 ml.) likewise had no effect on the reproducibility of the determination at this alcohol concentration.

Temperature of Precipitation. It was suspected that the low results were due to the effect of temperature on the solubility of the potassium fluoborate precipitate. Therefore, the precipitations were made in an ice-water bath. Table I shows that the precipitation is more nearly quantitative when carried out at about 3° C.

Table I. Effect of Temperature on Precipitation of Potassium Fluoborate

[Precipitating solution. 5 ml. KCl solution (0.0200 gram KCl), 10 ml. HBF_4, and 25 ml. C_2H_5OH]

KCl Recovered, G.	
At room temp.	At 3° C. (ice bath)
0.0191	0.0200
0.0192	0.0197
0.0192	0.0201
0.0192	0.0199
0.0194	0.0200

Effect of Sodium Chloride. An important requirement of any method for potassium is that the procedure be applicable in the presence of large amounts of the most common alkali metal, sodium. Therefore, increments of sodium chloride (0.10 to 0.50 gram) were added to several standard potassium solutions and the solutions were analyzed.

In all cases, consistently high results (15 mg. per 10 ml. of fluoboric acid reagent used) were obtained. A blank on the reagent plus sodium chloride showed that the high values were due to impurities in the fluoboric acid in combination with

the sodium chloride. However, this difficulty was eliminated by pretreating the fluoric acid reagent as follows: A stock solution containing 0.5 gram of sodium chloride dissolved in 5 ml. of water, 30 ml. of fluoboric acid, and 125 ml. of ethyl alcohol was cooled in ice (~ 3° C.) and filtered through an asbestos-padded, porcelain Gooch crucible. The minimum amount of sodium chloride required to remove the impurity will vary with each batch of fluoboric acid reagent; however, 0.5 gram of sodium chloride was sufficient to purify a stock solution containing up to 250 ml. fluoboric acid.

The impurities removed by the pretreatment of fluoboric acid with sodium chloride are probably sodium fluosilicate and sodium sulfate.

Effect of Other Cations. A series of acidified solutions containing moderate to excessive proportions of other salts to potassium chloride were analyzed for potassium with good results. For each 10 ml. of sample solution 30 ml. of the stock solution were used. Table II shows that 2 grams each of cadmium, lithium, zinc, magnesium, manganese(II), iron, cobalt, calcium, aluminum, and copper(II) salts show little interference in the determination of 0.04 gram of potassium chloride. Good potassium recoveries were obtained from potassium chloride solutions containing combinations of 0.2 gram each, of the chlorides of cadmium, lithium, cobalt, sodium, magnesium, zinc, manganese(II), chromium, nickel, aluminum, and iron.

Table II. Effect of Foreign Cations on Potassium Analysis with Fluoboric Acid

(Precipitating solution. 0.5 gram NaCl, 40 ml. H_2O, 250 ml. HBF_4, and 1000 ml. C_2H_5OH; compound dissolved in 10 ml. stock solution containing 0.0400 gram KCl)

Compound, 2 G.	Recovered KCl, G.	Compound, 2 G.	Recovered KCl, G.
$CdCl_2.2.5\ H_2O$	0.0401	$CoCl_2.6\ H_2O$	0.0406
$ZnCl_2$	0.0400	$AlCl_3.6\ H_2O$	0.0400
$MgCl_2.6\ H_2O$	0.0402	$Ca(NO_3)_2.4\ H_2O$	0.0403
$MnCl_2.4\ H_2O$	0.0404	$Cu(NO_3)_2.3\ H_2O$	0.0401
$FeCl_3.6\ H_2O$	0.0400	LiCl	0.0401

Table III. Effect of Foreign Anions on the Determination of Potassium with Fluoboric Acid

(Precipitating solution. 0.5 gram NaCl, 40 ml. H_2O, 250 ml. HBF_4, 500 ml. C_2H_5OH, and 500 ml. CH_3OH)

Ratio of Soln.: Pptg. Soln.	Acid, 1 Ml.	NaCl, G.	KCl, G. Added	KCl, G. Recovered
11:30	H_3PO_4	..	0.0200	0.0206
	H_3PO_4	0.25	0.0200	0.0208
	H_3PO_4	0.25	0.2500	0.2490
	HNO_3	0.25	0.0200	0.0199
	HNO_3	0.25	0.2500	0.2492
12:30	HNO_3 + H_3PO_4	..	0.0200	0.0196

When fluoboric acid was added to an alcoholic calcium chloride solution, a white cloudiness would sometimes result, but when the alcohol was added to an aqueous calcium chloride solution containing fluoboric acid, no cloudiness was encountered. Acidification of the calcium chloride—alcohol mixture with 1 ml. of hydrochloric acid before the addition of the fluoboric acid prevented the cloudiness without affecting the solubility of the potassium fluoborate. If calcium salts are known to be present, the solution should be acidified before the precipitating solution is added.

It was found that the addition of an alcoholic fluoboric acid mixture to a solution containing both aluminum and calcium chlorides produced a precipitate if the alcohol solution ratio was slightly greater than 1 to 1. Numerous other solvents were tried (1-butyl alcohol, acetone, methanol, ethyl acetate, chloroform, ether, etc.), but precipitation still occurred.

The ammonium ion interferes with the determination because of coprecipitation and solubility effects and its removal by ignition of the mixed chlorides is required.

Effect of Other Anions. The insolubility of the sodium and potassium sulfates in alcohol necessitates the absence of the sulfate ion but the addition of 1 ml. each of phosphoric and nitric acids to the chlorides had no effect on the determination of potassium under the conditions presented here (Table III).

Although this point was not investigated, it is probable that the fluoride ion should not be present with the alkalies, for it has been reported (*3*) that when potassium fluoride reacts with fluoboric acid, a potassium fluoborate other than KBF_4 is formed.

Table IV. Effect of Methanol on Determination of Potassium with Fluoboric Acid

(Precipitating solution. 1 gram NaCl, 40 ml. H_2O, 250 ml. HBF_4, and 1000 ml. CH_3OH)

Ratio of Soln.: Pptg. Soln.	NaCl, G.	KCl, G. Added	KCl, G. Recovered
10:30	...	0.0200	0.0194
	...	0.0400	0.0392
	...	0.1000	0.0974
	...	0.2500	0.2485
20:40	0.5	0.0400	0.0395
20:60	1.0	0.0400	0.0390
	2.0	0.0400	0.0391

Table V. Effect of Ethyl Alcohol plus Methanol on Analysis of Potassium with Fluoboric Acid

(Precipitating solution. 0.5 gram NaCl, 40 ml. H_2O, 250 ml. HBF_4, 500 ml. C_2H_5OH, and 500 ml. CH_3OH)

Ratio of Soln.: Pptg. Soln.	Chloride Added to Pptg. Soln.	G.	KCl, G. Added	KCl, G. Recovered
10:30	...	..	0.0200	0.0199
	...	..	0.0400	0.0402, 0.0397
	...	..	0.1000	0.0999, 0.0991
	...	..	0.2500	0.2496, 0.2497
	NaCl	0.10	0.0400	0.0399
	NaCl	0.20	0.0400	0.0403
	NaCl	0.25	0.0200	0.0202, 0.0197
	NaCl	0.25	0.2500	0.2499
	NaCl	0.25	0.2900	0.2904
	NaCl	0.50	0.0200	0.0200
	NaCl	0.50	0.0400	0.0409, 0.0406
	NaCl	0.50	0.2500	0.2492
11:30	$CaCl_2$	0.25	0.2500	0.2495
	$CaCl_2$	1.0	0.0200	0.0198
	$CaCl_2$	1.0	0.2500	0.2501
	$CaCl_2$ + NaCl	1.0 + 0.25	0.2500	0.2502
	$CaCl_2$	2.0	0.0200	0.0200
	$CaCl_2$ + NaCl	2.0 + 0.25	0.0200	0.0206
	$CaCl_2$ + NaCl	2.0 + 0.25	0.2500	0.2499
15:45	$NiCl_2.6\ H_2O$ + $CrCl_3.6\ H_2O$	2.0 + 2.0	0.0200	0.0198, 0.0202
	$NiCl_2.6\ H_2O$	2.0	0.2500	0.2499
	$CrCl_3.6\ H_2O$ + NaCl	2.0 + 0.25		

Ethyl Alcohol versus Methanol. Ethyl alcohol was used initially as a solvent in this investigation and it proved to be satisfactory for the determination of as much as 0.25 gram of potassium chloride in the presence of 0.25 gram of sodium chloride. Since both sodium and potassium chloride are more soluble in absolute methanol, the effect of methanol was studied. It was found that although larger amounts of sodium chloride could be tolerated in the presence of potassium chloride, the results were slightly low (Table IV). A 1 to 1 mixture of the alcohols was used next in the precipitating solution as well as the wash liquid, and acceptable results were obtained in the presence of at least 0.50 gram of sodium chloride. Table V shows that 20 to 250 mg. of potassium chloride can be determined in the presence of up to 500 mg. of sodium chloride with a relative error of less than 1% and that moderate amounts of calcium, nickel, and chromium chlorides do not interfere in the determination of potassium. Thus, a 1 to 1 mixture of ethyl alcohol–methanol seems superior to ethyl alcohol alone and it appears that a higher concentration of methanol to ethyl alcohol also will give good results in the presence of even greater amounts of sodium chloride. The 1 to 1 mixture proved much more efficient than the ethyl

alcohol alone for washing the potassium fluoborate precipitate free of occluded salts; only when high amounts of sodium chloride or other salts were present was more than one washing found to be necessary. The precipitate was considered free of impurities when washing with 30 ml. of the 1 to 1 ice-cold solution caused no greater than a 0.5-mg. change in weight.

When ethyl alcohol or the 1 to 1 mixture of ethyl alcohol–methanol is used for the determination of potassium fluoborate, the precipitate has a white, almost gelatinous appearance, but when methanol alone is used, the potassium fluoborate appears to be very fine, granular, and almost transparent. The precipitate from methanol requires more careful handling during transfer to the crucible, and technique difficulties may be the reason for the slightly lower results.

DISCUSSION

Except for the difficulty encountered when calcium and aluminum are present in the same solution, the determination of potassium as potassium fluoborate in many materials is simple by this rapid method in the presence of the common alkalies and other salts. The method has its advantages over the perchlorate and chloroplatinate methods in speed and costs, respectively, and should be ideal for routine work.

The necessity of having to work with ice-cold solutions is a slight disadvantage but when more is known about the solubility of potassium fluoborate in other organic solvents, it is conceivable that a method could be developed for precipitating potassium fluoborate quantitatively at room temperature.

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LITERATURE CITED

(1) Booth, H. S., and Martin, D. R., "Boron Trifluoride and Its Derivatives," pp. 99–106, New York, John Wiley & Sons, 1949.
(2) Flaschka, H., and Amin, A. M., *Chemist Analyst*, **42**, No. 4, 78 (1953).
(3) *Ibid.*, **43**, No. 1, 4 (1954).
(4) Gloss, G. H., *Ibid.*, **42**, No. 3, 50 (1953).
(5) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," New York, N. Y., John Wiley & Sons, Inc., 1929.
(6) Mathers, F. C., Stewart, C. O., Housemann, H. V., and Lee, I. E., *J. Am. Chem. Soc.*, **37**, 1515–17 (1915).
(7) Robinson, J. W., *Chem. Age (London)*, **66**, 447–50, 467–9, 507–10, 573–7 (1952).

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Semiautomatic Gas Separation Equipment

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With manual operation of apparatus for gas separation, the chance of introducing errors is great. A simultaneous-sampling, double automatic gas buret apparatus was developed whereby one operator could handle the equipment with a greater degree of accuracy because the timing and pressure control are automatic. Experimental data with the described instrument agreed well with data obtained from the manually operated apparatus and less time was required per determination.

FROM 1829, when Graham (*4*) carried out his initial work on gas separation, which resulted in the statement of Graham's law, until 1945, gaseous phase separation was only a laboratory phenomenon. The first and foremost important application of gas separation, using a porous membrane, was in the separation of uranium isotopes.

When a membrane or barrier, whether it be plastic, porous glass or ceramic, or metal is considered for use as a separating membrane, two characteristics should be determined: the rate of gas flow for a given pressure drop across the membrane, and the amount of enrichment in one or more of the components of the gas as it permeates through the membrane.

The theory and the literature of the gaseous phase separation have been well covered in previous publications (*1*, *2*, *5*, *7–9*). The membranes under consideration will permit one or more of the three types of flow which are normally encountered when gases flow through membranes: molecular streaming or Knudsen flow, viscous or Poiseuille flow, and a mixture of molecular streaming and viscous flow. Recent publications (*3*, *5*) have emphasized the fact that another flow phenomenon must be considered when vapor flow through microporous membranes is included—that is, the occurrence of adsorbed (or condensed or surface) flow, resulting from presumable formation of a sorbed liquid phase in the microporous structure of the membrane.

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TYPES OF MEMBRANES

Membranes available for separation are essentially of two types: plastic films and porous bodies. While a plastic membrane undoubtedly possesses a porous structure, it has been found helpful to differentiate between plastic films on one hand and microporous membranes on the other hand. In general, microporous membranes are considered to have pores with diameters of about the mean free path size of gases, while the much smaller porous structure in the plastic membranes is caused by the spacing between the molecules of the plastic.

Microporous membranes are actually capillary systems with interconnected pores. Such membranes can be prepared by two methods—producing micropores by removing an interdispersed phase or component or by reducing large holes which already exist in the membrane. A good example of the removal of a dispersed phase is the preparation of porous glass (*6*). The technique of reducing the size of existing holes in a membrane would be represented by ceramic practice and powder metallurgy.

It was shown (*1*) that the test gas mixture hydrogen–carbon dioxide may behave very differently when plastic membranes are used than when microporous membranes are used. The carbon dioxide is often selectively enriched when the gas permeates through many of the plastic membranes. This selective enrichment is considered to be caused by solubility phenomena (*1*). Separations in microporous membranes essentially obey Graham's diffusion law, which in its simplest form states that the separation is a function of the square root of the inverse ratio of the molecular weights. Under certain conditions the phenomenon of condensed flow will be encountered even with such a gaslike substance as carbon dioxide.

THEORETICAL CONSIDERATIONS

From the rate equations for gas diffusion and a material balance, Weller and Steiner (*8*, *9*) developed the equations for the binary system, providing a method for predicting separation results for two somewhat different cases of flow conditions. The

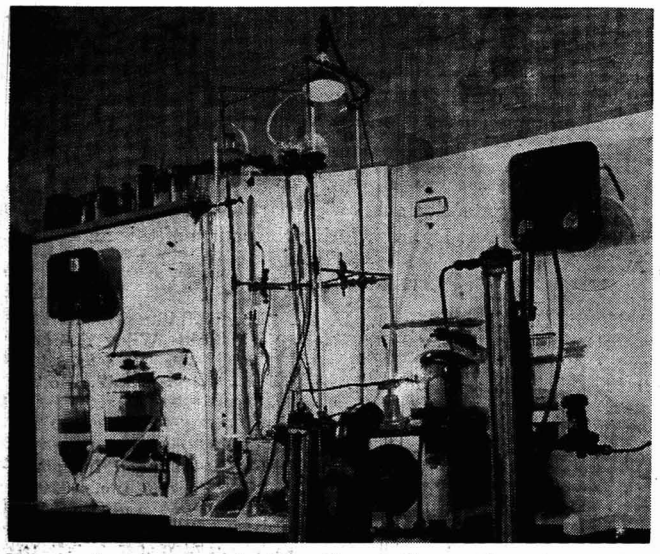

Figure 1. Automatic Gas Buret Separation Apparatus

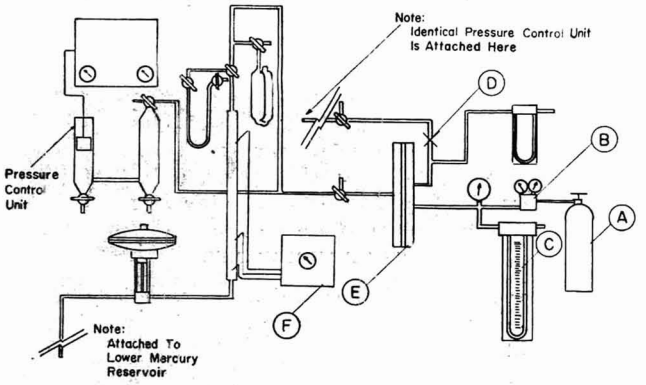

Figure 2. Diagram of Automatic Gas Buret Separation Apparatus

A. Test gas cylinder
B. Test gas regulator
C. High pressure manometer
D. Purge valve assembly
E. Diffusion cell
F. Electric timer

amount of gas permeated divided by the amount of gas fed to the separation cell is defined as F factor. Permeability corresponds to an F factor of 1.00. The F factor may be calculated from either flow rate data or composition data.

EQUIPMENT

Most of the types of apparatus for gas separation experiments which have been reported to date require a large number of manual operations. If a simultaneous-sampling unit for the permeated and the purged gas is to be used, two operators are needed to handle the apparatus during the sampling operations. In collecting gas samples, two leveling bottles have to be lowered at such a rate that constant pressure (usually atmospheric) is maintained in the gas burets at all times. In addition, multiple timing operations have to be performed. While manual operation is possible and has been used extensively, the chance of introducing errors is great. With an automatic gas buret system one operator could easily operate the equipment with an improved degree of experimental accuracy, because the timing and pressure control are automatic. The need for such an apparatus is evident; therefore, a simultaneous-sampling, double automatic gas buret apparatus was developed.

This apparatus is designed so that any diffusion cell can be tested. Any system of gases or noncondensable vapors can be used as a test mixture.

The measurements to be made with this apparatus are the determination of the flow rates of the permeated and purged streams and the accurate composition analysis of the two streams; the operating temperature and pressure must also be recorded. The flow rate is determined by allowing the test gas to displace a liquid in calibrated gas burets. The time to displace a standard volume can be determined by two different timing methods—the electrode mercury contact method and the photoelectric cell method.

DESCRIPTION OF APPARATUS

The simultaneous-sampling, double gas buret system consists of three main parts: the separation cell, the two different flow rate measuring systems, and the pressure control system, Figure 1. There are two identical pressure control systems, one for each of the streams of gas which are measured and analyzed—i.e., the purged stream and the permeated stream. The feed gas flow need not be measured, as it is represented by the sum of permeated and purged streams, provided it is certain that no leaks exist in the system. The feed gas composition is determined by analyzing the feed gas reservoir before the membrane testing is started. Figure 2 presents a diagram of the apparatus.

A separation cell must have at least three gas connections—one for high pressure gas inlet, one for low pressure gas outlet, and one for high pressure purge outlet. In the high pressure purge outlet line, there must be a valve for controlling the F factor. There must be some means to seal the membrane so that none of the high pressure gas can diffuse around and thus bypass the membrane. It may be advisable that there be a large enough gas space in front of the membrane—i.e., the high pressure side—to allow good mixing of the feed and purge gases.

Circuit diagrams of the arrangement used to measure the flow rate by means of the electrode mercury contact method are shown in Figures 3 and 4. Two tungsten electrodes are located in a gas buret at different heights, thus defining a known volume.

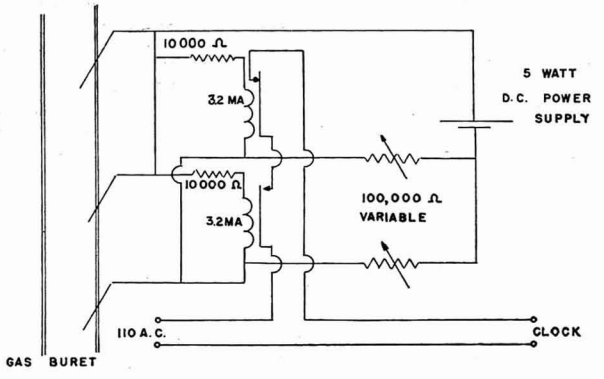

Figure 3. Stop-Clock Starting and Stopping Circuit

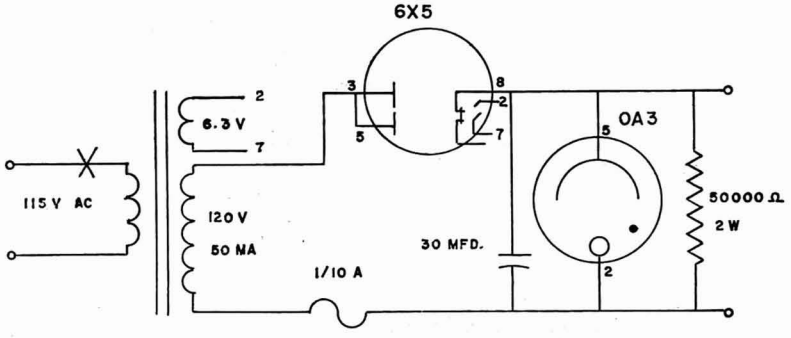

Figure 4. Five-Watt Direct Current Power Supply

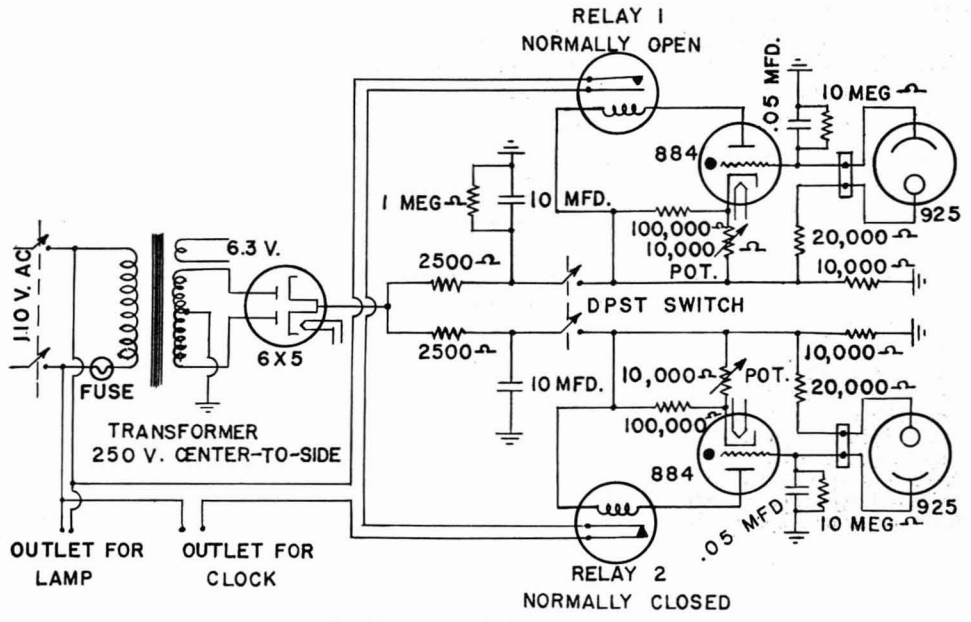

Figure 5. Diagram of Photoelectric Relay

that the gas burets have to be cleaned daily and refilled with clean mercury, and (2) the toxic danger from mercury vapor poisoning if any of the mercury is spilled. A disadvantage of the photoelectric cell method is the possibility of different amounts of gas being absorbed in the saturated salt solution.

The pressure-control system actuates the valve at the bottom of the gas buret, which opens and closes at such a rate that the gas in the buret is collected under practically atmospheric pressure conditions. The gas stream from the separation cell is split into two lines: one connecting to the gas buret and one connecting to the pressure controller which is vented to the atmosphere. To maintain atmospheric conditions in the collection gas buret, the methods of electrical control and pneumatic control were tried.

As it takes a few seconds for the instrument to gain control, the timing electrodes are installed at 25 and 75 cc. when a 100-cc. gas buret is used. The principle of the electric timer system is that the electrical resistance of the mercury (used as confining liquid for the gas) is much less than that of the relay circuit, Figure 3. The relay circuit for the top electrode is normally open, while the lower relay circuit is normally closed. One side of the 110-volt alternating current line which operates the electric timer is connected to two relay circuits; the other side is connected directly to the electric timer. A 5-watt, 75-volt direct current power supply operates the coils of the relay, Figure 4.

The device used to measure rate of flow by means of the photoelectric cell method consists of two independent units which operate on 110-volt alternating current. Each unit includes an accurately calibrated gas buret and two photocell assemblies spaced at a standard distance apart on the tube, usually 50 cc. The photocell assemblies are connected to an amplifier unit (Figure 5) which in turn controls an electric timer. Dyed salt solution is used in the buret as confining liquid.

Two disadvantages of the electrode mercury contact method are: (1) when a gas test mixture is used with a high oxygen content (about 50%) the mercury oxidizes at such a rapid rate

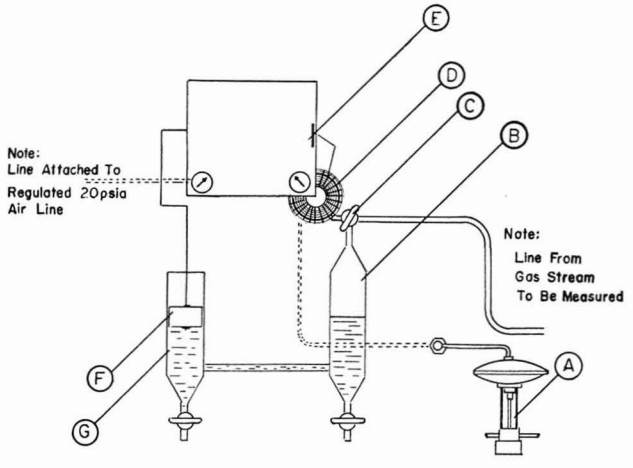

Figure 6. Diagram of Pressure Control System

A. Pneumatic motor valve
B. Pressure vessel
C. Controlling stopcock
D. Pressure recording chart
E. Level-trol (Fisher Model No. 2504)
F. Float
G. Atmospheric vessel

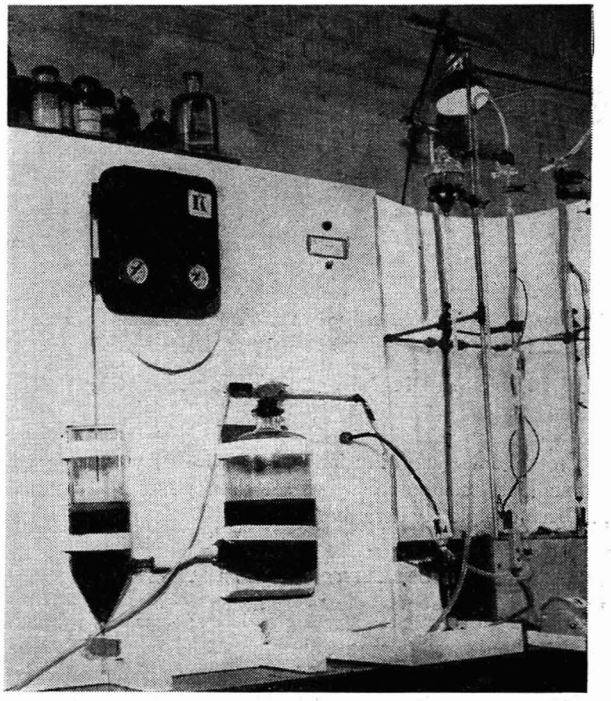

Figure 7. Pressure Control System

When electrical control, consisting of an off-on pressure cell and solenoid valve, was used, it resulted in too much fluctuating or cycling control. Therefore, a proportional band pressure controller and a pneumatic pressure motor valve were installed to take its place. The electrical control failed to give suitable service because it superimposed a pulsating pressure differential on the low pressure side of the membrane when in operation. Therefore, the equilibrium in the separation cell was continually being upset.

The pneumatic pressure control system (Figures 6 and 7) was therefore developed. It is patterned after a liquid-level controller. The system consists of two glass vessels which are partially filled with water and connected below the water line. One vessel is closed to the atmosphere and connects with a three-way stopcock bypass arrangement to the pressure tap of either the permeated or purged downstream gas line. The other vessel is open to the atmosphere and is equipped with a float. For greater

sensitivity the closed vessel may be larger than the vessel open to the atmosphere. The float arm is attached to a level controller (Model 2504 Fisher Controller Level-trol). The instrument works on reverse action. Connected to the float arm is a pen which records movement of the float. Thus a permanent record of the pressure variations of either the permeated or the purged stream is obtained. By adjusting the motor valve loading, the pressure during the collecting period is regulated within $\pm 1/32$ inch of water, above or below atmospheric pressure.

TYPES OF SEPARATION CELLS

There are many types of separation cells in use today.

One type used for plastic or sheet materials is the flanged separation cell (Figure 8), constructed from two disks of stainless steel with six bolts. Inside the ring of bolts is a Teflon gasket. The capillary holes for the feed and purge stream are spaced on one of the diameters of the high pressure side flange. The capillary outlet hole for the permeated stream is in the center of the low pressure flange. For efficient operation, the flanged cell must be free of all grease, oil, or dirt. The cell gasket must also be clean. The membrane is cut the size of the outside diameter of the flange gasket. The backing (filter paper or other suitable porous material) which is used to fill the space between the membrane and the flange should be clean, as a small piece or particle of dirt may cut the membrane and a leak can develop. When all of the parts of the separation cell have been correctly assembled and the nuts tightened with a torsion wrench, the cell is connected to the apparatus.

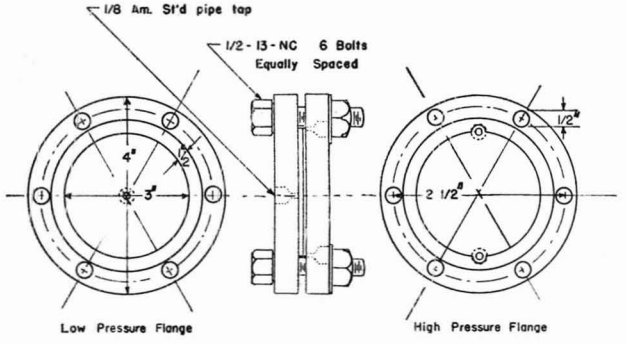

Figure 8. Diagram of Flanged Separation Cell

The thimble-type diffusion cell (Figure 9) was designed for porous glass membranes which are shaped like a test tube. It has been completely described (*5*, *7*).

OPERATION OF APPARATUS

Prior to the start of the testing period, the gas flow is regulated to give the desired flow rate through the membrane. After the flow rate has been determined, the purge needle valve is set, which determines the *F* factor, and the system is allowed to reach equilibrium conditions by letting the purged and permeated gases escape to the atmosphere for a sufficient period of time, usually a number of hours.

Equilibrium conditions were determined by checking either the flow rates or the composition of permeated streams at half-hour intervals. This was continued until three consecutive readings were constant and this condition was taken as flow equilibrium. The purge needle valve control setting determines the *F* factor. To maintain the equilibrium conditions during the testing period, the samples of permeated and purged gases must be collected under atmospheric conditions.

When equilibrium has been established, the membrane is ready to be tested. Before readings are taken, all of the electrical and pneumatic equipment is started and tested. The metering liquid is raised manually to the top of the gas burets. The pneumatic controls are set correctly to correpond to the flow rate. The control stopcock at the top of the pressure vessel is set manually, open to the pressure vessel and closed to the atmosphere, and the liquid in the buret is allowed to fall to a reservoir at such a rate that the gas in the buret is kept under atmospheric pressure $\pm 1/32$ inch of water head.

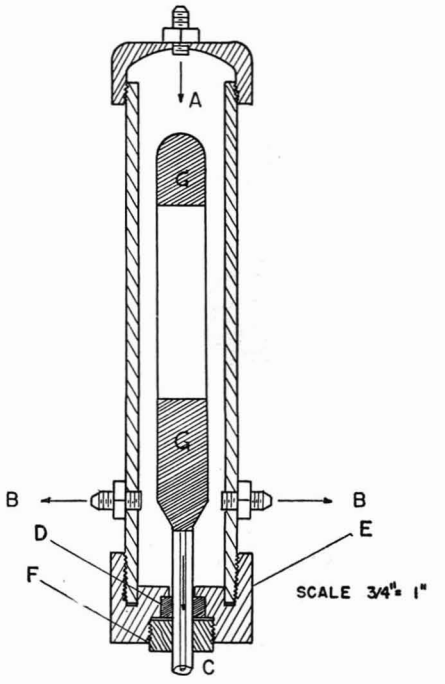

Figure 9. Thimble Type of Diffusion Cell

A. Feed gas
B. Purge gas
C. Permeated gas
D. Rubber gasket
E. Screw cap
F. Packing nut
G. Areas blanked with nonporous material

When the run is finished, the stopcock on top of the pressure vessel is opened manually to the atmosphere; thus the controller returns to its normally closed position which closes the valve at the bottom of the gas buret and liquid stops draining from the buret. Simultaneously with the opening of the pressure vessel

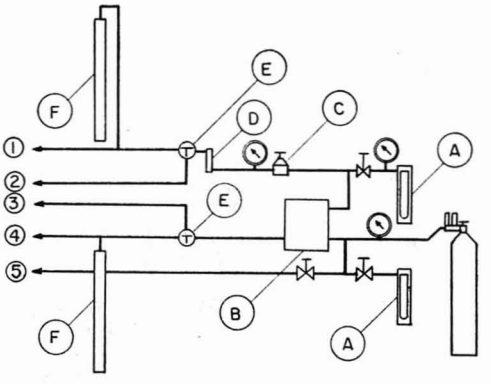

Figure 10. Flow Diagram of Gases

A. Manometer
B. Diffusion cell
C. Pressure regulator
D. Bourdon valve (Foxboro Part U-101-BC)
E. Controlling stopcock
F. Gas burets
1. Purge stream to pressure controller
2. Purge stream to oxygen analyzer
3. Permeated stream to pressure controller
4. Permeated stream to oxygen analyzer
5. Feed stream to oxygen analyzer

stopcock, the gas buret is disconnected from the separation apparatus and is connected to the analyzing unit by means of a bypass stopcock system.

With noncorrosive oxygen gas mixtures the Beckman oxygen analyzer was used exclusively and thus stream compositions could be obtained without upsetting the flow of gases or causing back pressures or surges. Therefore, in this situation the composition was determined first and then flow rates were determined as described.

ANALYSIS OF THE GAS STREAMS

The correct analysis of the gas streams is as important as the determination of the flow rates of the various gas streams. Any mixture of gases or noncondensable vapors can be used in the separation apparatus. A helium-oxygen mixture was used because of the relatively large molecular weight difference of the gases and convenience in analyzing such a mixture. Also, the mixture was safe to handle, as the gases were not toxic, flammable, or explosive.

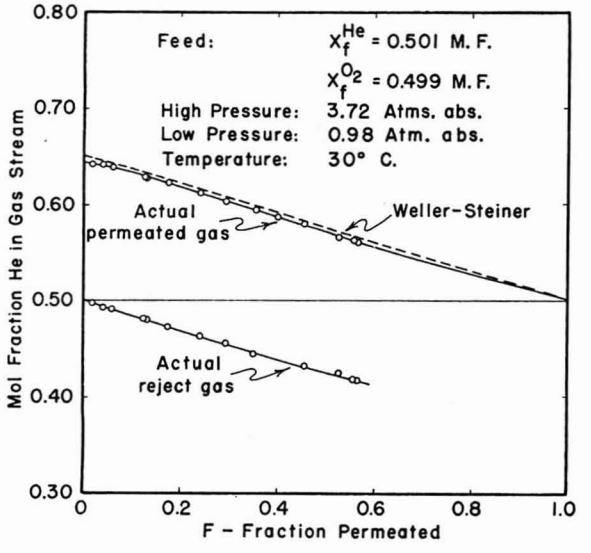

Figure 11. Separation of Helium-Oxygen Mixture with Porous Glass

Once the gas sample has been collected, it can be analyzed by chemical absorption or instrumental analysis.

A Beckman Model E-2 oxygen analyzer was incorporated in the experimental equipment. When this instrument was used, it was connected directly to the three gas streams with a selective manifold-type connecting system so that a continuous analysis could be obtained. Figure 10 shows the flow of gases through the apparatus to the oxygen analyzer. The Beckman oxygen analyzer operated very quickly and with greater accuracy than the chemical absorption systems.

EXPERIMENTAL DATA

The experimenta data taken with this apparatus agreed very well with the data obtained from the manually operated apparatus. The data could be obtained with much greater ease of operation and with less time required per determination. The results also may be more accurate, because the equilibrium of the cell is not likely to be upset as much as with manual operation.

Separation results with a mixture of helium and oxygen are presented in Figure 11. The membrane used was porous glass. The operating conditions were as follows:

Composition of feed mixture, $x_f^{He} = 0.501$ mole fraction
$x_f^{O_2} = 0.499$ mole fraction
Pressure on high side, $\Pi = 3.72$ atmospheres absolute
Pressure on low side, $p = 0.98$ atmosphere absolute
Permeability ratio, $\frac{P_{He}}{P_{O_2}}$, $\alpha = 2.28$
Calculated composition (*2*) in permeated gas stream at $F = 0$ is $x_p^{He} = 0.648$ mole fraction

The solid curve corresponds to the experimental data and the dashed curve represents values calculated according to the Weller and Steiner equation, Case I (*7–9*). The slight deviation at $F = 0$ has been observed in many cases but has not as yet been explained in a satisfactory manner. The progressive deviation with increasing F values is due to a cell efficiency effect which becomes more pronounced as the flow type changes from turbulent to laminar.

Results are shown only to an F factor of about 0.6 because the limited degree of enrichment at higher F values is usually not of much interest. Furthermore, operation at $F = 0.5$ would usually be preferred, as it permits easy balancing of cells in multistage units.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Brubaker, D. W., and Kammermeyer, K., *Ind. Eng. Chem.*, **44**, 1465 (1952).
(2) *Ibid.*, **46**, 733–9 (1954).
(3) Carman, P. C., and Malherbe, P. le R., *Proc. Roy. Soc. (London)*, **A203**, 165–78 (1950).
(4) Graham, T., *Trans. Roy. Soc. (London)*, **153**, 385 (1863).
(5) Hagerbaumer, D. H., and Kammermeyer, K., *Chem. Eng. Progr., Symposium Ser.*, No. **10**; *Collected Research Papers*, **50**, 25–44 (1954).
(6) Hood, H. P., and Nordberg, M. E., U. S. Patent 2,106,744 (1938).
(7) Huckins, H. E., and Kammermeyer, K., *Chem. Eng. Progr.*, **49**, 180–4, 294–8 (1953).
(8) Weller, S., and Steiner, W. A., *Ibid.*, **46**, 585 (1950).
(9) Weller, S., and Steiner, W. A., *J. Appl. Phys.*, **21**, 279 (1950).

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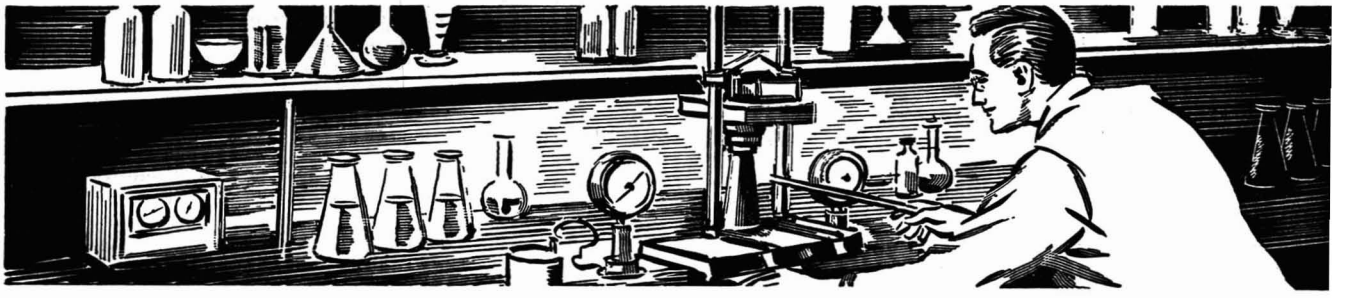

New Techniques for Analyzing Mixtures of Trace Metals

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The Atlantic Refining Co., Philadelphia, Pa.

The accurate determination of trace amounts of metals in mixtures is difficult owing to interferences. Many of the existing spectrophotometric methods are inadequate as they do not provide a means for eliminating these interferences. Methods of analysis for nine trace metals are given here, wherein most of the interferences have been eliminated. The metals included are vanadium, iron, nickel, manganese, titanium, lead, copper, chromium, and platinum. The procedures are particularly adapted to the analysis of used petroleum catalysts and to the metallic residues from petroleum. All techniques involve spectrophotometric methods.

TRACE amounts of foreign metals on catalysts used in petroleum processing are harmful. These trace metals can cause increased carbon deposit on cracking catalysts and the loss of activity of both cracking and reforming catalysts. To relate these metals to their effects, accurate determinations of the amounts of trace metals present in oils and on catalysts are necessary.

These trace metals occur as mixtures on the catalysts and in the inorganic residues from oil. The existing colorimetric methods will usually tolerate the presence of only a few foreign ions. The interferences in the methods are often mentioned but an exact procedure is not given for their removal. A notable example of this is in the determination of chromium by the diphenylcarbazide method (*11*). Iron, which frequently occurs with chromium, seriously interferes and there is no easy method for the quantitative separation of iron and chromium in micro quantities. Some of the other methods of analysis for trace metals are unsatisfactory because of nonreproducibility and unknown interfering conditions. The colorimetric method for nickel using dimethylglyoxime (*11*) is an example of this. In order to obtain any acceptable accuracy such methods had to be modified or new methods devised.

Some new techniques of colorimetric analysis have been developed. The procedures used for the determination of nine metals commonly occurring together are given here. These procedures have been found to be satisfactory for used catalysts and inorganic residues from oil. All methods involve spectrophotometric techniques.

The instruments used in the development of the methods were the Beckman Model B and DU spectrophotometers and the Cenco Photelometer. All reagents used were of highest purity and the special reagents described are all available commercially.

PREPARATION OF SAMPLE

Catalyst. The size of the sample taken depends on the concentration of the metals and the number of determinations to be made. A procedure is given for the preparation of a 1-gram sample.

Approximately 10 grams of thoroughly mixed silica-alumina catalyst are ground to a fine powder and dried in a muffle at 550° C. for 1 hour. A 1-gram sample is accurately weighed into a platinum dish or crucible and moistened with a few drops of water. Three to four milliliters of concentrated sulfuric acid are added, followed by 5 ml. of hydrofluoric acid. The mixture is heated gently on a hot plate until all the silica is removed, and a little more hydrofluoric acid is added if necessary. The solution is evaporated to sulfur trioxide fumes and cooled, and 5 to 10 ml. of water are added. Heating is continued until solution is complete.

Oil. The preparation of an oil sample for the analysis of metal content involves the reduction of the oil to an inorganic residue. This can be done by several appropriate procedures, but care should be exercised in the proper choice of one so as to prevent any loss of metals (*9*). This metallic residue is put into solution as was the 1-gram catalyst sample.

DETERMINATION OF METALS

Vanadium. Vanadium is commonly determined colorimetrically as the yellow phosphotungstovanadic acid (*11*, *14*). This method has been studied and found to be very accurate with only a few modifications. As stated by Wright and Mellon (*14*), ammonium ions cause precipitation of the phosphotungstate. The common method of oxidation of vanadium with ammonium persulfate has therefore been replaced. Acid and alkaline bromine oxidation methods were compared to the ammonium persulfate technique and all were found to oxidize the vanadium quantitatively. Since the alkaline bromine oxidation method is the more rapid of the two bromine methods, it was adopted.

The elimination of the interference attributable to iron is accomplished by mercury cathodic deposition as suggested by Sandell (*11*). Table I shows the degree of this interference if iron is not removed.

An additional step has been introduced into the original procedure by extracting the colored complex with isobutyl alcohol. This accomplishes two things: it eliminates the necessity of removing all the bromine by boiling and it gives a slight gain in sensitivity. The data in Table II show that the bromine color is eliminated by reaction with the isobutyl alcohol.

A comparison of the approximate molecular extinction coefficients for three conditions is shown in Table III.

PROCEDURE. An aliquot of the prepared solution containing 20 to 300γ of vanadium is electrolyzed with a mercury cathode to remove interfering elements. This solution should contain 1 ml. of sulfuric acid per 50 ml. At a potential of 4 volts and a current of 1 ampere several hours will be required for the complete removal of interfering metals (*6*). The solution is then made just alkaline with 1*N* sodium hydroxide, a few drops of saturated bromine water are added, and the solution is heated to near boiling for 5 minutes. Just enough dilute sulfuric acid is added to make the solution acidic and the heating is continued until most of the bromine is expelled. The cooled solution is transferred to a separatory funnel and diluted to about 70 ml.; then 15 ml. of 5*N* sulfuric acid, 10 ml. of 5*M* phosphoric acid, and 5 ml. of 0.5*M* sodium tungstate solution are added and the funnel is shaken. The yellow color is extracted with 20-ml. portions of isobutyl alcohol until completely removed. The alcohol extracts are diluted to a 100-ml. volume and the color is compared in a spectrophotometer against a reagent blank at 400 mμ, using 5-cm. cells. The

Table I. Effect of Iron in Vanadium Determination with Phosphotungstate

Soln. Composition		V Found, γ/Ml., at 400 Mμ
V, γ/ml.	Fe, γ/ml.	
1.32		1.34
1.32	4.85	1.73

Table II. Interference of Bromine in Vanadium Determination with Phosphotungstate

	Transmittancy, %, at 400 Mμ (5-Cm. Cells)	
V, γ/Ml.	Br_2 expelled	Br_2 present
0.90	63.8	63.7
1.87	41.0	41.0
3.6	16.9	17.0

Table III. Molecular Extinction Coefficients of Phosphotungstovanadic Acid

Conditions Used	Extinction Coef.
Filter photometer (aq.), green filter	907
Spectrophotometer (aq.), 400 mμ	1890
Spectrophotometer (isobutyl alc.), 400 mμ	2190

concentration is read from a calibration curve prepared from known solutions made from vanadium pentoxide or the metal as the source of vanadium.

Iron. Smith, McCurdy, and Diehl (*13*) reported a reagent which is specific for iron. This reagent, 4,7-diphenyl-1,10-phenanthroline, (Batho-phenanthroline) has been adopted for the colorimetric determination of iron. The authors (*13*) state that many cations do not seriously interfere; it was found in this work that ratios of foreign ions to iron in the order of 10 are tolerated. However, very large excesses can cause very weak color development.

The procedure as given here differs slightly from the original in that the reagent is more concentrated to ensure excess when other reactive foreign ions are present. Also the final solution is diluted entirely with isobutyl alcohol and not ethyl alcohol as originally prescribed. This has no effect on the intensity of the color.

PROCEDURE. An aliquot of sample solution containing 10 to 70γ of iron is measured into a separatory funnel. One milliliter of 10% hydroxylamine hydrochloride is added, followed by 2 ml. of 10% sodium acetate solution and 2 ml. of the reagent solution. (The reagent solution contains 0.2 gram of 4,7-diphenyl-1,10-phenanthroline dissolved in 70 ml. of ethyl alcohol and diluted to 100 ml. with water.) The volume is adjusted to about 30 ml. with water. The red color of the iron complex is extracted with 10 to 15 ml. of isobutyl alcohol. After separating the two phases more reagent is added to the aqueous layer and any further color is extracted with isobutyl alcohol. The alcohol extracts are finally diluted to 50 ml. and the color is compared against isobutyl alcohol using 1-cm. cells at 530 mμ. Electrolytic iron solutions were used in preparing the calibration curve.

Nickel. The most widely used method for the determination of nickel is the dimethylglyoxime procedure. As shown (*11*) it has been found difficult to obtain reproducible colors because of slight variations in pH, complexing reagents, and other metallic ions. To overcome these difficulties another method for the determination of nickel has been developed.

Sandell (*11*) shows that nickel is extracted from an ammoniacal solution by dithizone in carbon tetrachloride. It was decided to investigate this as a possible means for the quantitative determination of nickel. A faintly ammoniacal aqueous solution of nickel was extracted with purified dithizone in carbon tetrachloride. Chloroform was used to dilute to a definite volume, as carbon tetrachloride alone occasionally caused precipitation.

The nickel dithizonate exhibits three absorption bands in the visible region. Using the described extraction procedure the results were somewhat nonreproducible at any of these wave lengths. To locate the cause of nonreproducibility, extractions were made with the concentration of ammonia in the aqueous solution being varied from 0.02 to 2.00*N*. The data obtained showed that a 1*N* ammonia solution gave good reproducibility. This procedure is undesirable, however, owing to the inherent instability of most metal dithizonates in either strong base or acid. Extractions were then made using a chloroform solution of dithizone as the extracting medium. The effect of variations in the ammonia concentration upon this extraction procedure is shown by the data in Table IV.

These data show that less ammonia is now needed to obtain complete and reproducible extractions. The reproducibility using all chloroform as the extraction medium and a 0.3*N* ammonia solution for varying amounts of nickel is shown in Table V.

The final conditions selected were a 0.006% dithizone solution in chloroform, extraction from a 0.3*N* ammonia solution, and final dilution with chloroform. The wave length chosen was 665 mμ which, although less sensitive, eliminates some of the interferences attributable to other metals extracted from ammoniacal solution. Table VI shows the interference of these metals.

The data in Table VI indicate that it is necessary to remove all the copper and cobalt before nickel can be determined accurately.

Table IV. Effect of Ammonia in Nickel Determination with Dithizone

Soln. Composition		Transmittancy, %, (1-Cm. Cell)		
Ni, γ/ml.	NH_4OH, *N*	480 mμ	550 mμ	665 mμ
0.252	0.02	82.8	83.5	88.2
0.252	0.10	75.7	78.0	81.9
0.252	0.20	75.8	78.1	82.0
0.252	1.00	76.0	78.4	82.0

Table V. Reproducibility of Nickel Determination with Dithizone

Soln. Composition		Transmittancy, %, (1-Cm. Cell)		
NH_4OH, *N*	Ni, γ/ml.	480 mμ	550 mμ	665 mμ
0.3	0.252	75.8	78.1	82.0
		75.7	78.0	81.9
		76.2	78.5	82.5
0.3	0.63	52.3	57.4	62.7
		51.8	58.0	62.3
0.3	1.26	26.4	32.0	38.0
		26.6	32.2	38.0

Table VI. Effect of Certain Metals in Nickel Determination with Dithizone

Soln. Composition		Transmittancy, %, at 665 Mμ (1-Cm. Cells)
Metal	Concn., γ/ml.	
Zn	2.0	98.5
Pb	2.0	99.0
Cu	0.1	97.5
Co	0.1	97.0

The copper is extracted by dithizone in carbon tetrachloride after adjusting the aqueous pH to 5.0 with hydrochloric acid. No nickel is extracted at this pH. The nickel is separated from cobalt by forming the nickel dimethylglyoxime complex in an ammoniacal solution and extracting it with chloroform (*11*). The chloroform is removed and the nickel placed in aqueous solution for the final dithizone extraction.

PROCEDURE. Five milliliters of 10% sodium citrate solution are added to an aliquot of the sample solution containing 20 to 120γ of nickel, followed by dilute sodium hydroxide until neutral. The pH is then adjusted to 5.0 with dilute hydrochloric acid. The aqueous solution is placed in a separatory funnel and extracted with a 0.006% solution of dithizone in carbon tetrachloride. These extractions are continued until no more color develops in the carbon tetrachloride. The extracts contain the copper and are discarded. The aqueous layer is made slightly ammoniacal and 2 ml. of 1.0% dimethylglyoxime in ethyl alcohol are added. The nickel complex is extracted with chloroform. The chloroform extract is evaporated to dryness, the final organic residue being digested with a few drops of perchloric and nitric acid. Water (25 ml.) is added to effect solution. The solution is then made 0.3*N* in ammonia, placed in a separatory funnel, and extracted with successive portions of a 0.006% dithizone solution in chloroform until no further color change is noted. The extracts are washed with separate portions of a 0.5*N* ammonium hydroxide solution until the aqueous layer remains colorless. The chloroform layer is diluted to 100 ml. and compared against chloroform at 665 mμ in 1-cm. cells. A wave length of 480 mμ can be used if no other metal dithizonates are present. The calibration curve was prepared from a solution of a nickel salt which was standardized gravimetrically.

Manganese. Manganese is commonly determined colorimetrically as the permanganate ion in an acid solution. By using a basic solution and a wave length of 525 mμ the interference of chromium is eliminated. The spectrum of the permanganate ion in acid and in slightly alkaline solution is the same; however the dichromate ion spectrum is shifted to that of the chromate ion by a basic medium. It was found that 20 γ per ml. of acid dichromate in a 5-cm. cell gave a per cent transmittancy of 82 at the permanganate absorption peak, whereas 500 γ per ml. of chromate in a basic medium gave a per cent transmittancy of 99.

The presence of iron and copper would interfere with the permanganate color, so they are removed by extraction of their cupferrates with chloroform. Any metals such as aluminum will form a precipitate in the basic medium but the permanganate solution can be centrifuged clear with no loss in color.

The manganese is oxidized to permanganate in an acid medium with periodate. The presence of phosphoric acid has been found to increase the rate of oxidation markedly. The phosphoric acid does not change the absorption of the chromium and manganese at the 525-mμ wave length.

PROCEDURE. An aliquot of the solution containing 10 to 150 γ of manganese is made 2*N* in sulfuric acid and placed in a separatory funnel. A few milliliters of an aqueous cupferron solution are added and then the solution is extracted with chloroform to remove the interfering metal cupferrates such as iron, copper, etc. The aqueous layer is evaporated to dryness and the organic residue destroyed with a few drops of perchloric acid. The residue is dissolved in 2*N* sulfuric acid. Five milliliters of 5*M* phosphoric acid are added and then 0.2 gram of potassium periodate. The solution is heated to near boiling for 10 minutes, cooled in an ice bath, and then made just alkaline with 2*N* sodium hydroxide. The solution is diluted to exactly 50 ml. and if there are insoluble hydroxides present such as aluminum, the solution is poured into a centrifuge tube. After centrifuging, the clear solution is drawn off and compared against a blank at 525 mμ using 5-cm. cells. A standard permanganate solution can be used for calibration purposes.

Titanium. Titanium can be determined colorimetrically by means of several reagents. The two most promising reagents appeared to be L-ascorbic acid (*4*) and tiron (*15*). The L-ascorbic acid method of Boltz and Hines (*4*) was found to give good reproducibility using pure solutions. The effect of vanadium was then investigated as it remains with titanium after mercury cathodic deposition.

The data in Table VII show that vanadium causes serious interference. Because of this the tiron method (*15*) was then investigated as to reproducibility and interferences. The method as given was somewhat unsatisfactory because of slowly developing colors. Solutions in the present study were made from titanium dioxide, and since the original authors had used hydrogen peroxide in the preparation of standards, trace amounts of peroxide were added. The results indicated that hydrogen peroxide is necessary to obtain a rapid reaction. The results in Table VIII show the effects of various concentrations of peroxide with respect to time. These results show that a concentration of peroxide in the order of 0.0006% gives the most color after 2 to 3 hours. This amount of peroxide was tried with varying amounts of titanium and found to be satisfactory.

Yoe and Armstrong (*15*) indicate that vanadium will interfere with the tiron method. Vanadium is left with the titanium in solution after electrolysis with the mercury cathode (*6*) to remove other interferences. The effect of vanadium has been investigated and its interference eliminated. The data in Table IX show the intensity of color developed by various amounts of vanadium when the reagents are added in the order: buffer, peroxide, and tiron. The color is compared against distilled water.

This shows that the vanadium does not react with tiron under these conditions and the color is due entirely to its reaction with the buffer and peroxide. It was found necessary to increase the peroxide concentration in order to have enough to react with the vanadium and also form the titanium tiron color. This introduced other difficulties by causing a much weaker color to be formed with the titanium. The data in Table X show the effect of varying conditions of solution composition. Each solution is compared against a specific blank containing all the same constituents except the tiron.

These data show that by increasing the amount of buffer solution and reagent good sensitivity and stability can be attained. The final conditions selected of 0.02% hydrogen peroxide, 20% buffer solution, and 1.6% tiron were tried for varying concentrations of titanium and vanadium. The results are shown in Table XI where each reference solution contains everything except the tiron.

These data show that with vanadium concentrations up to about 20 γ per ml. of final solution, very little error will be introduced into the method after 2 to 3 hours.

PROCEDURE. An aliquot of the solution containing 10 to 100 γ of titanium is electrolyzed with a mercury cathode as in the vanadium determination. To the electrolyzed solution are added 10 ml. of buffer solution (1 to 1 mixture of 1*M* sodium acetate and 1*M* acetic acid), 1 ml. of 1% hydrogen peroxide, and 2.0 ml. of 40% aqueous tiron in this order. The volume is adjusted to 50 ml. and after 2.5 hours the color is compared against an equal aliquot of solution containing all reagents except tiron at 380 mμ in 1-cm. cells. A calibration curve is prepared from a standard solution of titanium oxide.

Lead. Lead in trace quantities is usually determined as the dithizonate (*11*). This method, although sensitive, is unsatisfactory because of instability of the color and the inherent error

Table X. Effect of Buffer, Vanadium, and Reagent in Titanium Determination with Tiron

Solution Composition					Transmittancy, %, at 380 Mμ (1-Cm. Cell)						
H_2O_2, %	Ti, γ/ml.	V, γ/ml.	Buffer Soln., %	Tiron, %	Immed.	10 min.	30 min.	1 hr.	2 hr.	3 hr.	24 hr.
0.02	0.96	9.0	10	0	100.0	..	..	..	..	..	..
0.02	0.96	9.0	10	0.4	81.0	79.2	78.4	78.0	77.3	..	..
0.02	0.96	0.0	10	0.4	59.8	57.8	57.2	57.0	57.0	..	..
0.02	0.96	9.0	20	0.4	67.0	63.5	63.0	62.7	62.4	..	..
0.02	0.96	9.0	20	0.8	59.5	56.8	56.2	56.0	56.0	..	..
0.02	0.96	9.0	20	1.6	56.0	54.5	53.5	53.3	53.5	..	53.0
0.02	0.96	0.0	20	1.6	53.5	52.0	51.9	52.0	52.0	52.0	51.5

Table XI. Reproducibility of Titanium Determination in Presence of Vanadium Using Tiron

Soln. Composition		Transmittancy, %, at 380 Mμ (1-Cm. Cell)							
Ti, γ/ml.	V, γ/ml.	Immed.	10 min.	20 min.	30 min.	1 hr.	2 hr.	3 hr.	72 hr.
0.48	9.0	75.5	74.3	74.0	73.6	72.7	72.3	72.3	71.5
0.96	9.0	53.5	52.8	52.7	52.7	52.0	52.0	52.0	52.3
1.92	9.0	29.5	28.0	27.6	27.5	27.2	27.2	27.2	27.4
2.89	9.0	16.0	14.5	14.2	14.2	14.0	14.0	14.0	14.0
0.48	18.0	87.3	82.8	81.0	79.0	75.7	73.5	72.5	73.3
0.96	18.0	70.5	64.0	61.5	59.3	55.8	52.8	52.5	53.2
1.92	18.0	41.5	34.0	32.7	30.7	29.2	28.0	27.7	28.4
2.89	18.0	25.0	18.2	17.0	16.0	15.0	14.2	14.2	14.4

Table VII. Effect of Vanadium in Titanium Determination with L-Ascorbic Acid

Soln. Composition		Transmittancy, %, at 360 Mμ (1-Cm. Cell)
Ti, γ/ml.	Concn. of V, γ/ml.	
2.41	0.0	62.0
2.41	10.0	60.5
2.41	20.0	53.5
2.41	100.0	31.5
0.00	20.0	81.0

Table VIII. Effect of Varying Peroxide on Titanium Determination with Tiron

Soln. Composition		Transmittancy, %, at 380 Mμ (1-Cm. Cell)						
Ti, γ/ml.	H_2O_2, %	Immed.	15 min.	30 min.	1 hr.	2 hr.	3 hr.	24 hr.
0.96	0.0	88.0	77.0	..	64.0	58.0	54.8	50.5
0.96	0.06	79.0	70.0	..	69.0	69.0	69.0	69.0
0.96	0.006	65.5	53.2	53.3	53.4	..	..	53.0
0.96	0.0006	67.5	57.8	55.2	53.3	52.0	52.0	..
0.96	0.001	75.0	64.8	62.2	56.7	54.7	53.7	..

Table IX. Reaction of Vanadium with Buffer, Peroxide, and Tiron

	Transmittancy, %, at 380 Mμ (1-Cm. Cell)	
V, γ/Ml.	With tiron	Without tiron
3.6	95.3	95.5
9.0	86.0	85.7
45.0	50.5	49.8

found in destroying the excess dithizone or using a mixed color technique (*7*). DeSesa and Rogers (*5*) indicated that several metals had absorption bands in the ultraviolet region owing to their complex chlorides. Merrit, Hershenson, and Rogers (*8*) have given a quantitative procedure for the determination of lead. The reproducibility of absorption of various concentrations of lead solutions in 1 to 1 hydrochloric acid is shown in Table XII where the reference solution is 1 to 1 acid. (More recently, the iodide complex of lead has been investigated and found suitable for the determination of lead. A solution of lead which is 1*M* in potassium iodide and containing a trace of sodium thiosulfate has the same absorption at 355 mμ as the same amount of lead in 1 to 1 hydrochloric acid at 270 mμ. In addition 2.5 γ per ml. of iron and 25 γ per ml. of copper do not interfere. Platinum, bismuth, and antimony do interfere, however.)

Table XII. Reproducibility of Lead Determination as Chloride

Pb, γ/Ml.	Transmittancy, %, at 270 Mμ (1-Cm. Cells) (30 Min.)
0.828	92.5, 92.5
1.24	88.0, 88.0
2.07	77.0, 78.0
4.14	58.5, 59.0
8.28	33.0, 33.5
16.56	12.0, 12.0

The interference of other metals was then considered and it was found that several metals such as copper, iron, etc., will interfere. The copper can be eliminated by a preliminary extraction from acid solution with dithizone in carbon tetrachloride. To eliminate other interferences the lead can then be extracted from an ammoniacal solution with dithizone in chloroform. Bismuth is the only metal now present with the lead that has any appreciable absorption at 270 mμ in a hydrochloric acid solution. Bismuth can be tolerated up to 10 γ per ml. in the final acid solution without causing interference.

PROCEDURE. For the determination of lead the original solution of the sample should be made using perchloric acid in place of the sulfuric acid. An aliquot of solution containing 100 to 1500γ of lead is adjusted to pH 3 with dilute hydrochloric acid and extracted with a carbon tetrachloride solution of dithizone. The carbon tetrachloride extracts are discarded and the aqueous layer is made ammoniacal and extracted with the chloroform dithizone solution. The chloroform extract is evaporated to dryness, the organic residue being completely destroyed with perchloric acid. The moist residue is dissolved in water and transferred to a 100-ml. volumetric flask and the volume is adjusted to about 40 ml. with water. Concentrated hydrochloric acid (50 ml.) is added, the flask is cooled, and the volume is adjusted to 100 ml. with water. This solution is compared after 30 minutes against 1 to 1 hydrochloric acid at 270 mμ using 1-cm. cells. A standardized solution of lead nitrate can be used for calibration.

Copper. Copper can be determined by forming a colored complex with neo-cuproine and extracting into isobutyl alcohol. This method has been described as a general procedure by Smith and McCurdy (*12*). An exact procedure follows.

PROCEDURE. An aliquot of the sample solution containing 20 to 400γ of copper is taken and 2 ml. of 10% aqueous hydroxylamine hydrochloride are added. Then 10 ml. of 10% sodium acetate are added, the solution is diluted to about 30 ml. with water, and 2 ml. of 0.2% neo-cuproine in 50% ethyl alcohol are added. The solution is extracted with 15-ml. portions of isobutyl alcohol until the alcohol no longer is colored. The alcohol extracts are diluted to 50 ml. and compared against a blank at 450 mμ using 1-cm. cells. A standard solution of copper can be prepared by dissolving a known amount of electrolytic copper.

Chromium. A sensitive method for the determination of chromium is the diphenylcarbazide method (*11*). This method was therefore selected for study, and since the chromium must be in the oxidized state for reaction, various methods of oxidation were tried. The results of several methods are shown in Table XIII.

Table XIII. Oxidation of Chromium

Cr, γ/Ml.	Transmittancy, %, at 540 Mμ (1-Cm. Cell) Acid $(NH_4)_2S_2O_8$	Acid, Ag^+ $(NH_4)_2S_2O_8$	Alk. Br_2	H_2SO_4 $HClO_4$
0.22	77.8 (fades)	78.3	78.3	84.6
0.22	78.3 (fades)	78.3	78.3	79.9
0.22	78.9 (fades)	78.3	..	82.0
0.44	..	61.5	61.5	..
0.44	..	61.5	61.6	..
1.11	36.0 (fades)	31.3	31.3	44.1
1.11	32.5 (fades)	31.3	31.5	37.9
1.11	..	31.3	..	40.5

Table XIV. Effect of Sodium Sulfate in Chromium Determination with Diphenylcarbazide

Soln. Composition Cr, γ/Ml.	Na_2SO_4, %	Transmittancy, %, at 540 Mμ (1-Cm. Cells)
0.44	0.0	61.5[a]
0.44	2.0	64.6[b]
0.44	2.0	68.0[c]

[a] Stable.
[b] Fades to 73.8 in 8 minutes.
[c] Fades to yellow in 20 minutes.

The data show that the persulfate alone is poor because of the fading of the diphenylcarbazide chromium color. This is due to the incomplete removal of the persulfate on boiling; the unremoved persulfate reacts with the color, destorying it. The presence of silver ions eliminates this interference but causes further trouble in that manganese, if present, is oxidized to permanganate which will interfere. The permanganate cannot be reduced completely with hydrochloric acid or other common reducing agents without the risk of some reduction of the dichromate. For these reasons the persulfate method was abandoned. The perchloric acid oxidation method shows very erratic results and is therefore unsatisfactory. The alkaline bromine oxidation seemed to be the best as far as reproducibility and completeness.

Another factor which was found to be critical in the diphenylcarbazide method was the salt content of the solution. The data shown in Table XIV indicate that care should be exercised to keep the salt content very low.

Table XV. Effect of Iron in Chromium Determination with Diphenylcarbazide

Fe, γ/Ml.	Chromium Found, γ/Ml.		
0.0	0.22	0.44	1.11
0.24	0.21	0.43	1.06
0.61	0.19	0.42	1.04
1.21	0.18	0.41	1.04
2.42	0.20	..	1.01
6.05	0.20	0.39	1.01
12.1	..	0.38	0.94

Table XVI. Separation of Iron and Chromium by Cupferron Precipitation

Soln. Composition Cr, γ	Fe, γ	Cr Found, γ
44.0	0.0	44.0
44.0	24.0	39.0, 35.0
44.0	121.0	21.0, 32.5
44.0	605.0	8.0, 17.5

From previous work (*11*) it was known that iron would cause some interference in this method. The degree of interference is shown by the data in Table XV. From this it is seen that amounts of iron equal to the chromium cause some slight interference but larger amounts cause serious interference. The solutions also tended to fade, depending on the amount of iron present. Since iron occurs with chromium in most samples encountered, and in much greater quantities, its removal is impera-

tive. A few common methods were used to try to separate small amounts of chromium from larger amounts of iron. The data and conclusions from several techniques follow.

The set of data in Table XVI shows the results of the precipitation of the iron cupferrate away from the chromic ion as suggested by Sandell (*11*). The chromium was then oxidized and the color developed. From this it would appear that the chromium is being held onto the iron cupferrate in the filtration step. In an effort to eliminate this possibility the iron cupferrate was extracted with chloroform. The chloroform remaining in the aqueous layer was removed by evaporating and digesting with nitric and sulfuric acids. The results were still unsatisfactory.

The next separation tried was the extraction of the iron thiocyanate complex with ether. This was eliminated when it was found that the ammonium thiocyanate left in the aqueous layer prevented the proper formation of the chromium color with diphenylcarbazide. Even fuming the aqueous layer with perchloric acid failed to destroy the thiocyanate completely. Also, an actual extraction of iron showed error in addition to that caused by the ammonium thiocyanate.

The next method tried was the commonly used procedure of precipitating the iron as the hydroxide from the chromium as chromate. The results are shown in Table XVII and again there is a considerable loss of chromium.

Table XVII. Separation of Iron and Chromium by Hydroxide Precipitation

Soln. Composition		
Cr, γ	Fe, γ	Cr Found, γ
	Filtering through Filter Paper	
44.0	0.0	44.0
44.0	48.0	7.0, 8.0
44.0	480.0	3.0, 3.0
	Filtering through Sintered Glass	
44.0	48.0	39.0, 40.0
44.0	480.0	34.5

Separation by means of adsorption on an alumina column was tried next. It was found that chromium in the oxidized state was not absorbed at all, whereas iron as the thiocyanate complex was completely absorbed. Potassium thiocyanate was used for complexing as it does not interfere with the color formation with diphenylcarbazide as did ammonium thiocyanate. The results were unsuccessful, for apparently the thiocyanate in the presence of alumina causes the reduction of chromium to the III state which is adsorbed by the alumina.

Ion exchange techniques were then tried. First a cation exchange resin (Dowex 50) was conditioned with hydrochloric acid and then water washed. The chromium was oxidized so as not to be retained in the column. The results were likewise very poor as the data showed that some dichromate is retained in the presence of iron. Another cation resin (IR-112) was tried with nitric acid conditioning with no better results. An anion exchange resin (Nalcite SAR) was tried and was conditioned with sodium hydroxide followed by water washing. Chromium was adsorbed as the dichromate from acid solution but it was impossible to elute all the chromium even with strong caustic.

Saltzman (*10*) stated that the interference of iron in the diphenylcarbazide method for chromium could be reduced by means of sodium dihydrogen phosphate. The results of several experiments are shown in Table XVIII where it is seen that the interference is not eliminated by this means as the amount of iron present will determine the error in the reading. This is not a very desirable situation but could be tolerated if nothing better was available.

Another means for determining chromium was sought after being unable to easily eliminate the iron interference in the diphenylcarbazide method. The color of the dichromate ion was finally used although it is a much less sensitive method. Other colored ions which absorb at the same wave length should be absent. Vanadate ions will interfere in more than equal amounts so the chromium is deposited in a mercury cathode. The mercury is distilled away and the chromium residue dissolved (*6*). Any iron present will remain with chromium but its light absorption is shifted to shorter wave lengths in the presence of phosphoric acid. The data in Table XIX show the degree of absorption of iron at several wave lengths. The solutions are 0.4*M* in phosphoric acid and 0.25*N* in sulfuric acid.

Table XVIII. Effect of Sodium Dihydrogen Phosphate on Interference of Iron in Determination of Chromium by Diphenylcarbazide

Soln. Composition		Transmittancy, %, at 540 Mμ (1-Cm. Cell) Observed						
Cr, γ	Fe, γ	Immed.	5 min.	10 min.	15 min.	20 min.	1 hr.	Theoretical
	5 Ml. of 4*M* NaH_2PO_4 Added before Reagent							
28.3	0.0	62.0	46.5	46.5	46.5	46.5	..	46.2
28.3	500.0	58.0	49.0	49.2	49.2	49.3	..	46.2
	5 Ml. of 4*M* NaH_2PO_4 Added after Reagent							
28.3	0.0	47.8	45.2	45.4	45.5	45.5	46.2	46.2
28.3	500.0	51.3	51.3	51.5	51.7	51.8	53.5	46.2

A wave length of 440 mμ was selected as chromium has an absorption peak here, at this acid concentration, and most of the iron interference is eliminated. The data in Table XX show results using various chromium and iron concentrations. The phosphoric and sulfuric acid concentrations are as described for Table XIX.

Table XIX. Absorption of Iron in Phosphoric–Sulfuric Acid Solution

Wave Length, Mμ	Transmittancy, % (5-Cm. Cell) Fe present, γ/ml. 2010	4020
430	93.0	84.0
440	97.2	92.5
450	98.2	94.8
460	98.2	95.0

Table XX. Effect of Iron on Absorption of Dichromate

Cr, γ/Ml.	Transmittancy, %, at 440 Mμ (5-Cm. Cell) No Fe present	2010γ/Ml. Fe Present 30 min.	1½ hr.	2 hr.
3.6	85.5	...	...	...
5.5	78.0	75.0	77.2	77.2
7.2	74.0	...	...	...
11.0	62.0	60.0	62.0	62.0
17.7	47.6	...	...	...
27.7	31.0	28.2	30.0	30.0

Although the sensitivity of the dichromate method is poor it was adopted because of the elimination of interferences, particularly that of iron.

Procedure. A portion of the sample solution containing 60 to 700γ of chromium is electrolyzed as in the vanadium determination. The mercury cathode is drained into a porcelain boat and the mercury distilled as described by Furman (*6*). The residue from the distillation is dissolved in a few milliliters of dilute sulfuric acid and the solution transferred to a beaker. The solution is made alkaline with 1*N* caustic, bromine water is added, and the solution is heated to near boiling for 5 minutes. The solution is made acid with sulfuric acid and then boiled until all the bromine is expelled. After cooling, 1.5 ml. of phosphoric acid are added and the volume is adjusted to 25 ml. The color is compared against a reagent blank at 440 mμ in 5-cm. cells after 1.25 hours.

Platinum. Platinum is usually determined on catalyst samples so the procedure will specifically refer to a catalyst. It has been

found necessary to reduce all the platinum to the metal in order to destroy any complexes which might interfere in the formation of the tin chloride colored complex. This reduction can be accomplished by heating the sample at 600° C. in the presence of hydrogen. The catalyst sample is then treated with aqua regia and after all the platinum has dissolved the solution is evaporated to a moist residue. This residue is treated with water and the solution is filtered away from the insoluble portion.

The platinum is determined as the colored complex formed by treating a hydrochloric acid solution of platinum with stannous chloride. The changes necessary in the original method (*11*) have been confirmed by the work of Ayers (*2, 3*).

Table XXI. Relative Minimum Quantitative Sensitivity of Metals Determined

Metal	Min. Sensitivity, 1-Cm. Cells, P.P.M. in Final Soln.
V	1.0
Fe	0.2
Ni	0.2
Mn	1.0
Ti	0.2
Pb	1.0
Cu	0.4
Cr	12.0
Pt	1.0

Table XXII. Standard Deviations for Various Methods

Metal	Std. Dev., Rel. %
V	5.1
Fe	2.2
Ni	3.9
Mn	2.6
Ti	3.7
Pb	7.1
Cu	2.5
Cr	5.0
Pt.	1.2

PROCEDURE. An aliquot of the solution containing 20 to 400γ of platinum is placed in a 100-ml. volumetric flask. Four milliliters of concentrated hydrochloric acid and 4 ml. of a 10% stannous chloride solution in concentrated hydrochloric acid are added. The volume is adjusted to 100 ml. and the color compared at 400 mμ against a blank using 5-cm. cells. A standard solution for calibration can be prepared by dissolving platinum wire in aqua regia.

DISCUSSION

The procedures given here have been found to be applicable to used catalyst samples and to the inorganic residues from petroleum. Most of the methods have eliminated the interferences commonly encountered in these types of samples.

The minimum amount of each metal which can be quantitatively determined is listed in Table XXI. A 90% transmittancy was selected as the minimum quantitative reading on the spectrophotometer. Of course, the precision at this reading will vary slightly for each metal as pointed out by Ayers (*1*). For uniformity of presentation the concentrations given in Table XXI are based on 1-cm. cell lengths and are expressed as parts per million of the metal in the final solution. Several of the methods used longer cells for increased sensitivity and of course this could be applied to all of them. For example, lead has been determined using 10-cm. cells down to 0.1 p.p.m. with excellent reproducibility.

The standard deviations for the various methods have been calculated and the values are given in Table XXII. These values were calculated from replicate determinations of several different samples having varying levels of metal concentration.

LITERATURE CITED

(1) Ayers, G. H., ANAL. CHEM., **21**, 652 (1949).
(2) *Ibid.*, **25**, 1622 (1953).
(3) Ayers, G. H., and Meyer, A. S., Jr., *Ibid.*, **23**, 299 (1951).
(4) Boltz, D. F., and Hines, Edward, *Ibid.*, **24**, 947 (1952).
(5) DeSesa, M. A., and Rogers, L. B., *Anal. Chim. Acta*, **6**, 534 (1952).
(6) Furman, N. H., Bricker, C. E., and McDuffie, Bruce, *J. Wash. Acad. Sci.*, **38**, 159–66 (1948).
(7) Irving, H. M., and Butler, E. J., *Analyst*, **78**, 571 (1953).
(8) Merrit, Charles, Jr., Hershenson, H. M., and Rogers, L. B., ANAL. CHEM., **25**, 572 (1953).
(9) Middleton, G., and Stuckey, R. E., *Analyst*, **78**, 532 (1953).
(10) Saltzman, B. E., ANAL CHEM., **24**, 1016 (1952).
(11) Sandell, E. B., "Colorimetric Determination of Traces of Metals," New York, Interscience Publishers, Inc., 1944.
(12) Smith, G. F., and McGurdy, W. H., Jr., ANAL. CHEM., **24**, 371 (1952).
(13) Smith, G. F., McCurdy, W. H., Jr., and Diehl, Harvey, *Analyst*, **77**, 418 (1952).
(14) Wright, E. R., and Mellon, M. G., IND. ENG. CHEM., ANAL. ED., **9**, 251 (1937).
(15) Yoe, J. H., and Armstrong, A. R., ANAL. CHEM., **19**, 100 (1947).

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Detection of Certain Ions in 10^{-10}- to 10^{-15}-Gram Particles

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The modified spot-test methods described in this paper are extensions of a technique previously described by the author for the identification of micron and submicron chloride particles. The technique depended upon microscopic observation of chemical reactions in the surface of a gelatin medium sensitized with a mercurous salt. This paper discusses in detail similar procedures for the identification of copper, cobalt, nickel, ferrous and ferric ions, sodium, and potassium, as well as iodides and carbonates in particulate matter. The sensitivity of the method permits the identification of masses four to six orders of magnitude smaller than is possible by conventional spot-test procedures as reported in the literature. The methods have been used primarily for the study of atmospheric particles but they may be applied to other fields of study.

THIS investigation was suggested by the need for methods of identifying certain constituents in the atmosphere that occur in the form of solid particles or in solution in droplets. The technique discussed permits identification of individual particles in the presence of thousands of other particles of different composition and it is not limited to aerosol particles. The need for micromanipulation of the particles is obviated, and in most cases, a permanent record results.

Relatively little chemical work has been done on single particles as found in collections of atmospheric particulate matter. In studies of the Los Angeles smog, Cadle, Rubin, Glassbrook, and Magill (*2*) developed some useful microchemical techniques for the identification of smog constituents. Winckelmann (*8*) described various techniques employing gelatin-glycerol and dry gelatin films as a medium for spot-test reactions with ions in solution. Feigl (*4*) has published procedures for excellent and

sensitive spot tests as carried out on various media for the detection of a large number of different cations and anions. Welcher's (*7*) compendium provides an extensive source of information concerning the application of organic reagents to inorganic analysis.

The methods discussed are essentially an extension of spot-test reactions, modified and applied to the identification of individual particles in the mass range of 10^{-10} to 10^{-15} gram.

GENERAL METHOD

Particles to be examined are collected by impaction or by other means upon the surface of a gelatin-glycerol medium and are made to react with a specific reagent to form a characteristic color or halo that can be identified under the microscope. Two procedures of chemical treatment have been employed.

Procedure 1. The essential feature here is that the reagent is dissolved in the gelatin-glycerol medium as previously described (*6*). Without further treatment, soluble particles brought in contact with the sensitized medium will immediately react to form a halo. A sensitized film is prepared by mixing 9 parts of the warm gelatin-glycerol medium with 1 part of the reagent to be used in a specific test. While still fluid, the mixture is applied to glass or plastic microscope slides as a uniform film approximately 0.3 mm. in thickness.

In this and the following procedure, insoluble particles may be made to react with a specific reagent by adding a solvent to the gelatin-glycerol medium or by covering the particles with a plastic film, according to Procedure 2, and exposing the particles to the dissolving action of the vapors of appropriate acids or ammonium hydroxide.

Procedure 2. The reagents are applied to the particles by diffusion through a plastic film covering the sample, collected on the surface of an unsensitized gelatin medium. This procedure permits the use of reagents that are unstable when mixed in the warm gelatin-glycerol medium. After the particles are brought into contact with the gelatin-glycerol film, they are covered with a thin plastic film by flowing a 1% solution of Saran resin X121 (Dow Chemical Co., Midland, Mich.) in ethylene chloride over the surface of the sample and permitting it to dry. A drop of the reagent, dissolved to saturation in glycerol or a glycerol-water mixture, is placed on the plastic film directly above the sample and allowed to diffuse through the plastic and contact the particles. The glycerol prevents evaporation of the drop and subsequent crystallization of the dissolved reagent, thus permitting the reagent to contact the particles for the period of time necessary for a reaction to take place. If desired, the reagent may be rinsed away gently with a few drops of distilled water and a new reagent applied. A number of successive tests may be made in this manner. The plastic film holds the particles firmly in place during the application of the reagents; it aids in preventing contamination of the sample by particles from other sources and permits examination of the particles with the oil immersion objective without damage to the sample.

Two classes of halos are formed in Procedures 1 and 2. The first class consists of characteristic precipitates, and the second consists of transparent color reactions produced by the formation of soluble complexes adsorbed by the gelatin. In some instances the particle may be identified by a change in color on part or on all of its surface when in contact with a specific reagent.

SPECIFIC PROCEDURES

Iodides. Soluble iodides may be identified by the same method used for chlorides (*6*). Mercurous fluosilicate is mixed with the gelatin-glycerol medium and applied to plastic microscope slides according to Procedure 1. A soluble iodide particle contacting the sensitized gelatin surface forms a strong yellow halo composed of very small crystals of mercurous iodide. The yellow color of the halo is determined by microscopic examination using light-field illumination.

If the reacting particle contains sodium or potassium, a highly refractive nucleus of sodium or potassium fluosilicate forms in the center of the halo.

The identification limit for iodide is approximately 10^{-10} gram. The strong yellow color is not evident in the halo formed by an iodide particle of mass less than 10^{-10} gram and it may be confused with the chloride halo (*6*).

Copper, Cobalt, and Nickel. Copper, cobalt, and nickel ions may be identified by the formation of halos of insoluble, characteristically colored complexes with rubeanic acid (*7*). The color of the complexes may be determined with the microscope using light-field illumination. Procedure 1 is followed, using a saturated solution of rubeanic acid and ammonium acetate in ethylene glycol monoethyl ether for the reagent.

Copper forms an intense green or green-black halo, cobalt a yellow or yellow-brown halo, nickel an intense blue or blue-violet halo. The identification limit for particles containing copper is approximately 10^{-15} gram; this limit was determined by bringing a very fine dust of copper oxide into contact with the sensitized film and estimating the diameter of the smallest particles that formed a recognizable halo. The identification limit for particles containing cobalt or nickel is approximately 10^{-14} gram.

Each of the three metallic ions may be detected in the presence of several thousand parts of the other two ions, owing to the difference in diffusion velocities. Cobalt, having a higher diffusion rate than copper, will form a yellow concentric ring about the green copper halo; nickel, having the highest diffusion rate, forms a blue halo about the cobalt halo, or about the copper halo, if cobalt is absent.

In order to dissolve the sample sufficiently to give a reaction with the rubeanic acid, insoluble particles containing copper, cobalt, or nickel may be covered with the plastic film as in Procedure 2 and exposed to the vapors of concentrated nitric, hydrochloric, or hydrofluoric acids. In many instances, several minutes' exposure to the vapor of concentrated ammonium hydroxide will dissolve enough of the particle to produce a characteristic halo.

Ferrous Iron. The ferrous ion forms a deep red, soluble complex with 2,2′-bipyridine. This makes possible the detection of very small quantities of ferrous ions in the presence of large quantities of ferric ions. The 2,2′-bipyridine is dissolved to saturation in ethylene glycol monoethyl ether and used as described in Procedure 1.

When soluble particles containing ferrous iron contact the sensitized gelatin film, a deep red, transparent halo is formed, which is easily identified under the microscope using light-field illumination. Particles of moderate solubility containing ferrous iron—e.g., siderite or other carbonates containing ferrous iron—are stained various shades of red over part or all of their surfaces; this staining evidently depends upon the degree of solubility, the ferrous iron concentration, and the distribution of the ferrous iron in the particle.

Highly insoluble particles containing ferrous iron—e.g., magnetite and ferrous oxide in silicates—may form a red halo if the particles are first covered with the plastic film as in Procedure 2 and exposed for approximately 5 minutes to the vapors of a mixture of equal parts of concentrated hydrofluoric and hydrochloric acids. Longer exposure to the acid vapors liquefies the gelatin-glycerol medium and destroys the plastic film. The hydrofluoric acid aids in dissolving the particle and, if ferric iron is present, aids in detection of the red bipyridine complex by suppressing the yellow color of the ferric ion.

The identification limit for moderately soluble particles containing ferrous iron is approximately 10^{-10} gram, whereas the

limit for soluble particles forming a halo is approximately 10^{-12} gram. The difference in identification limits is due to the difficulty of confirming the presence of the red color on the surface of the moderately soluble particles less than 4 or 5 microns in diameter.

Potassium ferricyanide dissolved to saturation in glycerol also may be used for the detection of ferrous iron by following Procedure 2. A halo of Turnbull's blue forms about the particle if ferrous iron is present.

Ferric Iron. The ferric ion may be detected by its reaction with potassium ferrocyanide to form Prussian blue; the blue color is determined by microscopic examination using light-field illumination. A saturated aqueous solution of potassium ferrocyanide is used according to Procedure 1.

Soluble particles containing ferric iron form an intense blue halo without additional treatment. The limit of identification is approximately 10^{-13} gram as determined by observing and measuring the smallest particle that would form a recognizable blue halo.

Insoluble particles—e.g., hematite or pyrite—covered with the plastic film as in Procedure 2, may be exposed to acid vapors so that sufficient decomposition will take place to develop the blue halo. Particles of hematite, for example, will partially decompose and react if exposed for approximately 5 minutes to the vapor of a mixture of equal parts of concentrated hydrofluoric and hydrochloric acids.

Relatively insoluble particles will be stained blue in some instances and will not form a halo, if not treated with an acid. The failure to form a halo has been observed with some of the naturally occurring carbonates that have slight solubility and contain some iron as a contaminant. The limit of identification for such particles containing ferric iron is approximately 10^{-10} gram.

Sodium and Potassium. Sodium and potassium form highly refractive, characteristic crystals with fluosilicic acid; the structure of the crystals may be determined easily with dark-field illumination. Concentrated fluosilicic acid is mixed with the gelatin-glycerol medium in a plastic beaker and applied to plastic microscope slides, according to Procedure 1.

Particles containing sodium form a halo composed of feathery crystals of sodium fluosilicate. At times, individual six-pointed rosettes may be observed immediately adjacent to the halo. Particles containing potassium form a halo composed of small cubic crystals of potassium fluosilicate.

The low solubility of these fluosilicates in the sensitized gelatin-glycerol film makes possible a limit of identification of approximately 10^{-10} gram for particles containing sodium or potassium.

Carbonates. Carbonates—e.g., calcite and aragonite—may be detected by their reaction when in contact with a nickel dimethylglyoxime equilibrium solution. Red nickel dimethylglyoxime is precipitated on or near the carbonate particle, because the balance of the equilibrium solution is disturbed, owing to the change in hydrogen ion concentration.

An equilibrium solution, based upon that described by Feigl (*4*), is prepared by dissolving 0.47 gram of dimethylglyoxime in the least amount of warm glycerol and mixing with a 50-ml. solution of glycerol containing 0.4 gram of nickel nitrate. After approximately 24 hours the suspension is filtered and the solution is ready for use. Glycerol was used as a solvent instead of water and alcohol to retard the evaporation of the reagent during the time required for diffusion through the plastic film. An added advantage in using glycerol is that a higher concentration of the nickel dimethylglyoxime could be obtained.

The equilibrium solution is applied to the carbonate particles by following Procedure 2. The formation of the red needles of nickel dimethylglyoxime may be observed under the microscope using dark-field illumination.

Gentle washing of the plastic surface with a few drops of distilled water may halt the action of the equilibrium solution before the field under the microscope becomes obscured by the continued growth of the red crystals. This does not disturb the particles and they can be re-examined at higher magnifications if necessary. The identification limit for individual carbonate particles is approximately 10^{-10} gram.

The equilibrium solution will react with all materials which lower the hydrogen ion concentration; this includes particles other than carbonates, such as acid-decomposable silicates, hydroxides, and oxides which may be found in collections of airborne particles. Silicates, hydroxides, and oxides do not react for several hours, however, under the conditions of the test, because of their relative insolubility in the glycerol solution of the reagent. Carbonates, which have relatively greater solubilities, react within 10 or 15 minutes, because of the formation of the bicarbonate ion.

Carbonate particles also may be detected by following Procedure 2, "inverting" the sample over a small drop of concentrated hydrochloric acid placed in the depression of a hanging-drop slide. Observation under the microscope will show small bubbles of carbon dioxide forming about the carbonates under the plastic film. With continued exposure to the acid vapor, the reaction usually proceeds until the particles are completely dissolved.

APPLICATIONS

The methods described in this paper have provided a means of identifying and counting various particles in impactor collections of atmospheric particles.

In addition, the technique for the detection of copper has been used in an experiment designed to study the effect of sunlight on the action of silver iodide particles as sublimation nuclei. An aerosol of copper sulfate was dispersed into a large outdoor tank containing a smoke of silver iodide, the relative concentrations of the two aerosols were determined at frequent intervals before and after exposure to sunlight, and the decay rate of the photolytic silver iodide was compared with the decay rate of the nonphotolytic copper sulfate (*5*). Early experiments using aerosols of copper compounds dispersed into the atmosphere were instrumental in the development of an improved method for tagging and tracing air parcels (*1*). The procedures for the detection of cobalt, nickel, and iron have been used in an attempt to identify particles of supposed metallic meteoritic material on the assumption that, if all three metals were found in a given particle, the particle probably was of meteoritic origin (*3*).

LITERATURE CITED

(1) Braham, R. R., Seely, B. K., and Crozier, W. D., *Trans. Am. Geophys. Union*, **33**, 825 (1952).
(2) Cadle, R. D., Rubin, Sylvan, Glassbrook, C. I., and Magill, P. L., *Arch. Ind. Hyg. and Occupational Med.*, **2**, 698 (1950).
(3) Crozier, W. D., and Seely, B. K., *Proc. Natl. Air Pollution Symposium, 1st Symposium, Pasadena, Calif.*, **1949**, 45.
(4) Feigl, F., "Qualitative Analysis by Spot Tests," New York, Elsevier Publishing Co., 1946.
(5) Reynolds, S. E., Hume, W., 2nd, and McWhirter, Max, *Bull. Am. Meteorol. Soc.*, **33**, 26 (1952).
(6) Seely, B. K., ANAL. CHEM., **24**, 576 (1952).
(7) Welcher, F. J., "Organic Analytical Reagents," Vol. 4, New York, D. Van Nostrand Co., 1948.
(8) Winckelmann, J., *Mikrochemie*, **10**, 437 (1931); **16**, 203 (1936).

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Rapid Spot Tests for Identification of Biphenyl, *o*-, *m*-, and *p*-Terphenyl, and Certain Other Polyphenyls

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The formaldehyde–sulfuric acid reagent was investigated as a means of identifying polyphenyls, such as biphenyl and the three isomeric terphenyls, individually or after separation by filter paper chromatography. When such polyphenyls are dissolved in cyclohexane, as little as 3 γ of biphenyl or 2 γ of terphenyl can be detected. Moreover biphenyl, *o*-terphenyl, *m*-terphenyl, and *p*-terphenyl have specific color reactions when dissolved in cyclohexane. Biphenyl, from 9 to 33% by weight, can be detected in the presence of *p*-terphenyl; from 9 to 50% biphenyl can be detected in the presence of *m*-terphenyl. Higher polyphenyls such as quaterphenyl, quinquephenyl, and sexaphenyl can also be detected. The test can be used in conjunction with ultraviolet spectroscopy to follow the separation by paper chromatography, or by other means, of mixtures of polyphenyls. The rapid spot test offers quick location of the separated polyphenyls by the R_f value and gives clues to their nature. Such analysis may be supplemented by ultraviolet absorption tests to verify identification and provide a quantitative analysis of the original sample.

FORMALDEHYDE–sulfuric acid reagent was used by Jacobs (*2*) for the detection of small amounts of benzene in air. Jacobs (*2*) and Ficklen (*1*) extended the method so that the degree of color development was compared with a color standard to make a quantitative estimate of the amount of aromatic hydrocarbon. Morris and coworkers (*5*) showed that they could determine 0.1 mg. of hydrocarbon within 10% of its value. Snell and Snell (*8*) found their method applicable to benzene, toluene, styrene, and related hydrocarbons if the hydrocarbon present had been previously identified. These authors suggested determining biphenyl by ultraviolet absorption.

Le Rosen and coworkers (*3*) employed a chromatographic column of silicic acid and used specific reagents to streak the chromatographic column in order to identify organic chemical compounds. Biphenyl and 1,3,5-triphenylbenzene are shown to give blue and blue-green colors, respectively, with formaldehyde–sulfuric acid reagent, but no other polyphenyls are mentioned in their reports.

Paper chromatography has a facile and economic advantage over column chromatography when it is necessary not only to identify a material as with the Le Rosen streak test but also to recover the material and use it for absorption tests or for spot tests. This is made possible by dissolving the material from the paper and using the solution for absorption measurements or spot tests, or both.

This investigation was instituted to ascertain if the various polyphenyls could be identified by spot tests, what the sensitivity might be, and perhaps what selectivity is possible. Spot tests as well as absorption spectroscopy tests (*7*, *8*) were performed after preliminary paper chromatography separations, cutting the papers and then dissolving the isolated polyphenyls.

In addition to studying the separation of polyphenyls by chromatography, the method was used to confirm results obtained by absorption spectroscopy tests of other means of separation, such as distillation (*7*) and differential solubility (*6*).

Table I. Color Reaction of Various Solvents with Formaldehyde–Sulfuric Acid Reagent

Solvent[a]	Reaction[b]
Benzene	Instant development of red color
Xylene	Instant development of dark-brown color
Phenylcyclohexane	Dark brownish red
Hexane	Instant development of red color
Heptane	Faint yellow with time
Carbon tetrachloride	Faint yellow with time
Chloroform	Faint yellow with time
Cyclohexane	Faint yellow with time
Bicyclohexyl	Dark brownish red
Decalin	Dark brownish red

[a] One milliliter of solvent.
[b] Ten drops of formaldehyde–sulfuric acid reagent.

EXPERIMENTAL

Apparatus and Chemicals.

Beckman spectrophotometer, Model DU.
Centrifuge tubes, graduated, with cone point, 15 ml.
Pipets, Kirk transfer type, 10 to 750 μl.
Volumetric flasks, 5, 10, and 25 ml.
Filter paper, Whatman No. 1.
Formaldehyde–sulfuric acid reagent. Prepare fresh each day by pipetting 0.2 ml. of 37% formaldehyde into 10 ml. of concentrated sulfuric acid.
Sulfuric acid, C.P., specific gravity 1.84.
Formaldehyde, C.P., 36.5%.
Phenylcyclohexane, $C_6H_5CH(CH_2)_4CH_2$, industrial sample.
n-Hexane.
n-Heptane.
Carbon tetrachloride, analyzed reagent.
Chloroform, analyzed reagent.
Cyclohexane, spectrophotometric grade.
Bicyclohexyl, industrial sample.
Decalin (decahydronaphthalene), purified.
Biphenyl, best grade available.
o-, *m*-, and *p*-Terphenyl, best grade available.
p-Quaterphenyl, purified.
p-Quinquephenyl, purified.

Procedure. STANDARD SOLUTIONS were prepared in two strengths, 1.00 and 0.10 mg. of sample polyphenyl per milliliter of solvent. Solution was accomplished by slight warming, when necessary. Several solvents were used.

COLOR DEVELOPMENT. A suitable aliquot of the standard solution ranging from 0.5 γ to 0.5 mg. was pipetted into a 15-ml. centrifuge tube with cone point. The total volume of the organic solution was kept below 1 ml. to give a stronger color reaction. Ten drops of formaldehyde–sulfuric acid reagent were added. The centrifuge tube was capped with a rubber stopper encased in aluminum foil and the contents were mixed by vigorous shaking. The mixture was allowed to stand up to 1 hour to ensure complete color development. The color and its intensity were noted and recorded.

UNKNOWN POLYPHENYL SAMPLES were prepared in concentrations of 0.1 to 1 mg. per ml. when received in the solid form. If the sample was received in the form of a dilute solution, the solvent was slowly evaporated to 1 ml. or less by heating in a 50° C. water bath. The unknown was then treated with formaldehyde–sulfuric acid as described above and compared with standards of similar nature and concentration.

If the unknown organic sample was first separated chromatographically on filter paper, 20 ml. of the developer solution containing polyphenyl dissolved in cyclohexane or chloroform were placed in a 50-ml. beaker. A glass cylinder was set vertically in the beaker. A 12-inch strip of Whatman No. 1 filter paper was inserted into the glass cylinder, so weighted and supported that it was immersed approximately 1 inch in the developer solution and remained vertical. The immersion depth was noted (for R_f calculation) and the open end of the cylinder was stoppered. The system was allowed to stand until the solvent front had

approached the upper end of the filter strip, approximately 4 hours. Then the filter paper was removed, allowed to dry, and cut up into small predetermined sizes. These pieces were placed in 15-ml. centrifuge tubes and extracted with 1 ml. of cyclohexane, by heating the tube in a water bath at 50° C. for 2 hours. At the end of this time the cyclohexane was decanted into another centrifuge tube and analyzed in the manner described.

THE REACTIONS OF VARIOUS SOLVENTS with formaldehyde–sulfuric acid reagent were observed (Table I). Those solvents with negative reactions, carbon tetrachloride, chloroform, *n*-heptane, and cyclohexane, were studied further as solvents for the polyphenyls (Tables II, III, IV, and V). The lower limits of detection of biphenyl, *o*-, *m*-, and *p*-terphenyl in cyclohexane and chloroform (Tables II and V) were determined. The reactions of higher polyphenyls such as *p*-quaterphenyl and *p*-quinquephenyl were noted.

INTERFERENCES between biphenyl and *p*-terphenyl were studied (Table VI). The factors involved are the weight ratios of the two polyphenyls, the total quantity of biphenyl, and the total volume of solvent. The reactions of various percentages of biphenyl in *p*-terphenyl from zero to 17% by weight (0 to 20 γ) were studied in a total volume of approximately 0.5 ml.; the reactions of 13 to 50% by weight biphenyl (15 to 50 γ) in a total solvent volume of 1 to 2 ml. were also studied. For purposes of comparison, pure biphenyl solutions and pure *p*-terphenyl solutions in concentrations equal to those of the synthetic mixtures were run simultaneously.

Interferences between biphenyl and *m*-terphenyl were studied in a similar fashion. The color reactions of 0 to 37% by weight biphenyl in *m*-terphenyl in a total volume of about 0.5 ml. were studied. In the higher range, the color reactions of 17 to 18% by weight of biphenyl in *m*-terphenyl were noted. The synthetic mixtures were compared with similar dilutions of the pure biphenyl and the pure *m*-terphenyl.

Table II. Color Reactions of Polyphenyls in Chloroform with Formaldehyde–Sulfuric Acid Reagent

Compound	Weight, Mg.	Volume, Ml.	Reaction
Biphenyl	0.5	1.0	Bright blue
	0.1	1.0	Faint blue
	0.05	0.5	Very faint blue
	0.025	0.25	No color
o-Terphenyl	0.5	1.0	Faint blue
	0.2	0.4	Very faint blue
	0.1	1.0	No color
	0.05	0.5	No color
m-Terphenyl	0.5	1.0	Faint blue
	0.2	0.4	Faint blue
	0.1	1.0	No color
	0.05	0.5	No color
p-Terphenyl	0.5	1.0	Faint blue
	0.2	0.4	Faint blue
	0.1	1.0	No color
	0.05	0.5	No color

Table III. Color Reactions of Polyphenyls in Heptane with Formaldehyde–Sulfuric Acid Reagent

Compound	Weight, Mg.	Volume, Ml.	Reaction
Biphenyl	0.5	0.5	Greenish blue
	0.1	1.0	Blue ring at interface
	0.05	0.5	Blue ring at interface
o-Terphenyl	0.5	0.5	Very light greenish blue
	0.1	1.0	Slight brown
	0.05	0.5	Slight brown
m-Terphenyl	0.5	0.5	Violet blue
	0.1	1.0	Brown ring at interface
	0.05	0.5	Brown ring at interface
p-Terphenyl	0.5	0.5	Violet at interface, pink below
	0.1	1.0	Slight brown
	0.05	0.5	Slight brown

Table IV. Color Reactions of Polyphenyls in Cyclohexane with Formaldehyde–Sulfuric Acid Reagent

Compound	Weight, Mg.	Volume, Ml.	Reaction
Biphenyl	0.5	0.5	Greenish blue
	0.1	1.0	Brown ring
	0.05	1.0	Brown ring
	0.01	0.2	Yellow-brownish tinge
o-Terphenyl	0.5	0.5	Light green
	0.1	1.0	Light green
	0.05	0.5	Yellowish green
	0.01	0.1	Yellowish green
m-Terphenyl	0.5	0.5	Dark violet
	0.1	1.0	Violet
	0.05	0.25	Violet
	0.01	0.05	Violet
p-Terphenyl	0.5	0.5	Magenta ring with pink below
	0.1	1.0	Magenta
	0.05	0.5	Magenta ring
	0.01	0.1	Pinkish

Table V. Lower Limits of Detection of Biphenyl and Terphenyls in Cyclohexane Using Formaldehyde–Sulfuric Acid Reagent

Compound	Weight, γ	Volume of Sample, μl.	Reaction
Biphenyl	10	100	Yellow, brownish tinge
	5	50	Chartreuse
	4	40	Chartreuse
	3	30	Chartreuse
	2	20	Very pale yellow
	1	10	Colorless
	0.5	5	Colorless
o-Terphenyl	10	100	Green
	5	50	Green
	3	30	Green
	2	20	Green
	1	10	Colorless
m-Terpheny	10	100	Dark violet
	5	50	Dark violet
	4	40	Violet
	3	30	Very faint violet
	2	20	Very faint violet
	1	10	Colorless
p-Terphenyl	10	100	Magenta
	5	50	Magenta
	2	20	Magenta
	1	10	Colorless

RESULTS AND DISCUSSION

Effect of Solvent. First, dry crystals of the polyphenyls were tested on a spot plate for their reaction with the formaldehyde–sulfuric acid reagent. Only the lower polyphenyls such as biphenyl and *o*-terphenyl gave good color tests. The addition of a few drops of solvent such as cyclohexane or chloroform greatly enhanced the color development.

The results of the investigation into the color reactions of possible solvents with the formaldehyde-sulfuric reagent are shown in Table I. This reagent is an indicator for aromatic hydrocarbons (*3*) and solvents such as benzene, xylene, and phenylcyclohexane give color reactions ranging from red to reddish brown. Some aliphatics, including hexane, bicyclohexyl, and decalin, give intense color reactions with the reagent. Heptane, which was used by Le Rosen (*3*) and coworkers, gives only a faint yellow tinge after the mixture has stood for an hour. Other solvents which give nearly negative tests are chloroform, carbon tetrachloride, and cyclohexane. These latter compounds were investigated further as solvents for the polyphenyls.

When the lower polyphenyls are dissolved in chloroform, a blue color is obtained with biphenyl and with the three isomers of the terphenyls upon the addition of the formaldehyde–sulfuric acid reagent. Biphenyl produces a more intense blue than any of the terphenyls; 0.05 mg. of biphenyl produces approximately the same color intensity as 0.5 mg. of *o*-, *m*-, or *p*-terphenyl. The minimum amount of biphenyl which may be detected is

Table VI. Reaction of *p*-Terphenyl in Cyclohexane with Formaldehyde–Sulfuric Acid Reagent in Presence of Small Amounts of Biphenyl

Biphenyl, γ	*p*-Terphenyl, γ	Biphenyl in Total Weight of Solute, %	Total Volume of Solution, Ml.	Color Reaction	Interpretation
0.0	50.0	0	0.5	Magenta	*p*-Terphenyl
2.0	50.0	4	0.5	Magenta with slight yellow below	*p*-Terphenyl
5.0	100.0	4	0.5	Magenta with slight yellow below	*p*-Terphenyl
3.0	50.0	6	0.5	Magenta with slight yellow below	*p*-Terphenyl
4.0	50.0	7	0.5	Magenta with slight yellow below	*p*-Terphenyl
5.0	50.0	9	0.5	Magenta with slight chartreuse below	*p*-Terphenyl plus biphenyl
10.0	100.0	9	0.5	Brownish magenta	*p*-Terphenyl plus biphenyl
15.0	85.0	15	0.5	Brownish magenta, slight yellow below	*p*-Terphenyl plus biphenyl
10.0	50.0	17	0.6	Brown plus magenta, slight yellow below	*p*-Terphenyl plus biphenyl
20.0	100.0	17	0.7	Brown with yellow below	Biphenyl
15.0	100.0	13	1.1	Brown plus magenta, slight yellow below	*p*-Terphenyl plus biphenyl
20.0	100.0	17	1.2	Brown plus magenta, slight yellow below	*p*-Terphenyl plus biphenyl
30.0	100.0	23	0.9	Brown plus magenta, yellow below	*p*-Terphenyl plus biphenyl
40.0	100.0	28	1.0	Brown plus magenta, yellow below	*p*-Terphenyl plus biphenyl
50.0	100.0	33	1.5	Magenta with some blue	*p*-Terphenyl plus biphenyl
30.0	50.0	37	1.0	Brown with yellow	Biphenyl
60.0	100.0	37	2.1	Brown with yellow	Biphenyl
70.0	100.0	41	1.8	Brown with yellow	Biphenyl
40.0	50.0	44	0.9	Brown with yellow	Biphenyl
50.0	50.0	50	1.0	Brown with yellow	Biphenyl

0.05 mg. (Table II); the minimum amount of the terphenyls which is detectable is 0.2 mg. in chloroform solution.

When the polyphenyls are dissolved in carbon tetrachloride, a blue color is obtained with biphenyl and with each of the three isomers of the terphenyls. The limits of detection were not investigated.

When polyphenyls are dissolved in heptane, colors obtained with the formaldehyde–sulfuric acid reagent vary with the type of polyphenyl (Table III) but there is no distinction in the colors of the three terphenyl isomers when the amounts are 0.1 mg. or less.

Much more decisive colors are obtained when the polyphenyls are dissolved in cyclohexane, and the limits of detection are lower (Table IV). The more concentrated samples give intense colors in the formaldehyde–sulfuric acid phase resembling precipitates; a tint of the same color might be observed in the solvent phase above. When more dilute samples are used, the color is present as a ring occurring at the interface between the solvent and the formaldehyde–sulfuric acid phase.

The color of the biphenyl in cyclohexane produced with the formaldehyde–sulfuric acid reagent varies from an intense greenish blue in the more concentrated samples, through a brown, to a greenish-yellow color in dilute samples. *m*-Terphenyl produces various intensities of violet. *p*-Terphenyl, depending upon the concentration of the samples, produces various intensities of magenta, described by Maerz and Paul (*4*) as a bluish-red shade of a nonspectral color, the optical complement of which absorbs at 496 mμ. Since in the preliminary study the use of cyclohexane as solvent produced characteristic and more sensitive tests, only cyclohexane was used in the following work.

Cyclohexane was used as a solvent for higher polyphenyls; *p*-quaterphenyl and *p*-quinquephenyl give magenta colors with formaldehyde–sulfuric acid resembling the reaction of *p*-terphenyl but the intensities are low. Higher polyphenyls produce slight colorations varying from violet to brown. Chromatographic fractions identified by ultraviolet absorption (*7*) as 3,4′-(4″,4‴-dixenyl) biphenyl (a sexaphenyl with a single meta linkage) give faint colors varying from magenta to violet. Other higher molecular weight polyphenyls which are detectable by ultraviolet absorption spectroscopy give no color reaction with the sulfuric acid–formaldehyde reagent. These latter polyphenyls were separated from the low molecular weight polyphenyls by paper chromatography; their exact nature is unknown.

Time for Color Development. The time of color development also varies with the isomer. The color development with biphenyl is almost immediate. With *p*-terphenyl, at least 15 minutes is required for color development. With *m*- and *o*-terphenyl the time is intermediate. The higher polyphenyls require at least 1 hour.

Complete color development is ensured when the samples are allowed to stand for an hour. The color thereafter is stable for several hours, but tends to darken and become brown if the samples are permitted to stand overnight.

Lower Limits of Detection. The lower limits of detection of biphenyl and the terphenyls in a total solvent volume of 100 μl. or less are shown in Table V. The lowest detectable amount of biphenyl is 2 γ but 3 γ gives a more decisive test. Below 5 γ, biphenyl produces a greenish yellow (chartreuse) color. In larger volumes (from 100 μl. to 1 ml.) larger quantities of biphenyl (10 to 100 γ) produce a brown ring (Table IV). Above 500 γ a characteristic greenish blue color is produced.

The lowest detectable amount of *o*-terphenyl is 2 γ. The color produced by *o*-terphenyl in cyclohexane with formaldehyde–

Table VII. Reaction of *m*-Terphenyl in Cyclohexane with Formaldehyde–Sulfuric Acid Reagent in Presence of Small Amounts of Biphenyl

Biphenyl, γ	*m*-Terphenyl, γ	Biphenyl in Total Weight of Solute, %	Total Volume of Solution, Ml.	Color Reaction	Interpretation
0.0	50.0	0	0.5	Violet ring, slight yellow below	*m*-Terphenyl
5.0	200.0	2	0.5	Violet ring, yellow below	*m*-Terphenyl
2.0	50.0	4	0.5	Violet ring, slight yellow below	*m*-Terphenyl
7.5	200.0	4	0.5	Violet ring, slight yellow below	*m*-Terphenyl
10.0	200.0	5	0.3	Violet blue, yellow below	*m*-Terphenyl plus biphenyl
5.0	50.0	9	0.55	Bluish violet ring, slight yellow below	*m*-Terphenyl plus biphenyl
20.0	200.0	9	0.4	Violet blue, yellow below	*m*-Terphenyl plus biphenyl
15.0	85.0	15	0.5	Violet-brown ring, chartreuse below	*m*-Terphenyl plus biphenyl
100.0	500.0	17	0.6	Heavy violet ring, chartreuse below	*m*-Terphenyl plus biphenyl
20.0	50.0	29	0.7	Violet ring, yellow below	*m*-Terphenyl plus biphenyl
250.0	500.0	33	0.5	Violet ring, yellow below	*m*-Terphenyl plus biphenyl
30.0	50.0	37	0.8	Violet ring, yellow below	*m*-Terphenyl plus biphenyl
20.0	100.0	17	1.2	Brown ring, yellow below	Biphenyl
50.0	100.0	33	1.5	Violet ring, yellow below	*m*-Terphenyl plus biphenyl
40.0	50.0	44	0.9	Violet ring, chartreuse below	*m*-Terphenyl plus biphenyl
50.0	50.0	50	1.0	Violet ring, chartreuse below	*m*-Terphenyl plus biphenyl
60.0	40.0	60	1.0	Brownish ring, yellow below	Biphenyl
70.0	30.0	70	1.0	Brownish ring, yellow below	Biphenyl
80.0	20.0	80	1.0	Brownish ring, yellow below	Biphenyl

sulfuric acid is green throughout the entire range of concentrations tested (from 2 to 500 γ); the color varies only in intensity.

The smallest amount of *m*-terphenyl detectable is 2 γ; the color is violet regardless of concentration and varies only in intensity. The lowest amount of *p*-terphenyl which is detectable in cyclohexane solution is 2 γ; the color is magenta regardless of concentration.

Interferences. Interferences between biphenyl and *p*-terphenyl are shown in Table VI. Three factors have an effect on the color reaction: the ratio of biphenyl to *p*-terphenyl (per cent by weight of biphenyl), the total quantity of biphenyl, and the total volume of the solution.

When the total solvent volume is approximately 0.5 ml., 9 to 17% biphenyl (5 to 10 γ of biphenyl per 50 γ of *p*-terphenyl) can be detected. On the other hand, should the quantity of biphenyl exceed 10 γ (as in 20 γ per 100 γ of *p*-terphenyl), then the *p*-terphenyl color would become obscured by the more intense biphenyl color.

When the total solvent volume is 1 to 2 ml., 13 to 33% biphenyl (15 to 50 γ of biphenyl per 100 γ of *p*-terphenyl) can be detected. When the biphenyl concentration reaches 37% or more its color masks that of *p*-terphenyl regardless of dilution, so that small amounts of *p*-terphenyl could not be detected in greater amounts of biphenyl.

Interferences between biphenyl and *m*-terphenyl are shown in Table VII. The total quantity of biphenyl and the total volume of solvent are not so critical as in the detection of biphenyl in *p*-terphenyl.

When the volume of solvent is approximately 0.5 ml., 9 to 37% biphenyl (10 to 60 γ of biphenyl per 100 γ of *m*-terphenyl) can be detected. When the total solvent volume is 1 ml. or more, 17 to 50% biphenyl (20 to 100 γ of biphenyl per 100 γ of *m*-terphenyl) can be detected. Above 60% the biphenyl color masks that of the *m*-terphenyl.

The work was not extended to mixtures of biphenyl and *o*-terphenyl.

CONCLUSION

The value of this test lies in the fact that it may be used in conjunction with ultraviolet spectroscopy to follow the separation of polyphenyls by chromatographic or by other means. When two parallel chromatographic separations are made on paper, one of the strips can be analyzed with the formaldehyde–sulfuric acid reagent after extraction into cyclohexane. Such analyses offer quick location of the separated materials by the R_f value and give clues to their nature. Ultraviolet analysis of the cyclohexane extracts of the other parallel strip serve to identify the separated components further and provide a quantitative analysis of the original sample.

LITERATURE CITED

(1) Ficklen, J. B., "Manual of Industrial Health Hazards," p. 43, Lancaster, Pa., Science Press Printing Co., 1940.
(2) Jacobs, M. B., "Analytical Chemistry of Industrial Poisons, Hazards and Solvents," pp. 521–55, Interscience Publishers, New York, 1941.
(3) Le Rosen, R. L., Moravek, R. T., and Carlton, J. K., ANAL. CHEM., 24, 1335–6 (1952).
(4) Maerz, A., and Paul, R., "A Dictionary of Color," McGraw-Hill Book Co., New York, 1950.
(5) Morris, H. E., Stiles, R. B., and Lane, W. B., ANAL. CHEM., 18, 294–5 (1946).
(6) Silverman, L., and Shideler, M., North American Aviation, *Spec. Rept.* 1153.
(7) Silverman, L., Trego, K., Houk, W., and Shideler, M., unpublished North American Aviation report.
(8) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Vol. III, p. 22, D. Van Nostrand Co., New York, 1953.

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Determination of Chloride by Automatic Titration

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A procedure is described for application of the Beckman automatic titrator to the determination of chloride in organic compounds.

IT HAS been the practice in the control laboratories to complete the determination of chlorine in organic compounds by a Volhard titration of the chloride obtained either by oxidation or by sodium reduction. Because of the large number of samples received, it appeared desirable to mechanize these titrations. The silver–silver chloride electrode pair frequently applied to argentometric titrations is relatively unstable in regard to potential and it is therefore preferable to apply a differential method, independent of absolute potential, involving the change in potential (or current) near the end point. The differential method could not be used with the Beckman automatic titrator available in this laboratory, because this instrument can only be set to titrate to a selected potential. It was necessary to develop conditions of sufficient stability to permit the use of a preset end point. The application of the Beckman titrator has resulted in a marked saving in time by eliminating the end-point difficulties of the Volhard titration, and in improved precision and accuracy of routine determinations.

EXPERIMENTAL

Apparatus. Beckman automatic titrator, Model K, with 50-ml. buret. Silver electrode, Beckman No. 1281-5. Silver–silver chloride electrode ( ).

Solutions. Standard 0.1*N* silver nitrate solution was used throughout the investigation. A 1 to 1 solution of nitric acid was employed for the acidification of sample solutions to thymol blue before boiling. After boiling and cooling, final pH adjustment was made on a Beckman pH meter using 1 to 3 nitric acid or 1 to 1 ammonium hydroxide. The preparation of the silver–silver chloride electrode solution has been described (*3*).

Sample Preparation. Organic chlorine is converted to chloride by the conventional macro Parr bomb procedure (*2*) or the sodium reduction method. The Parr bomb sample solutions, in 400-ml. beakers, are acidified just to the red phase of thymol blue with 1 to 1 nitric acid and boiled for 5 minutes; after cooling, the solution is diluted to approximately 300 ml., and the pH is adjusted to 2 ± 0.2 using a pH meter. Sample solutions from the sodium reduction method are treated similarly, except that boiling is unnecessary. Ionizable chlorine samples are weighed directly into titration beakers and the pH is adjusted as above.

Titration. The instrument is operated in general as described in the Beckman manual (*1*). The silver electrode is set so that it is approximately 1 cm. to the right, 0.5 cm. to the front, and at the same height as the stirrer blade. The buret tip is placed approximately 2 cm. in back of the tip of the silver electrode. The position of the silver–silver chloride electrode is not critical. The anticipation dial, which controls the speed of the titration near the end point, is usually set at 3, but this may vary with the instrument. The rate of flow of silver nitrate solution is adjusted to 25 ml. per minute by means of a screw clamp on the tubing connecting the buret and diaphragm valve. Before each determination, the electrodes are rinsed with water and the silver electrode is wiped with cotton moistened with 1 to 1 ammonium hydroxide, and rinsed again.

A set of 6 to 30 samples is usually titrated in series. The first sample is titrated using the manual control, and the volume of titrant and potential obtained after each addition of titrant is

Table I. Results of Determination of Chlorine by Automatic Titration

Sample	Chlorine, Theoretical, %	Decomposition Method	No. of Detns.	Chlorine Found, Av. %	Standard Deviation, %
Monochlorobenzene (technical)	31.50	Parr bomb	3	31.56	0.09
Benzylthiopseudourea HCl (Eastman Kodak 2124)	17.49	Parr bomb	8	17.47	0.02
Dichlorodiphenyltrichloroethane, technical, recrystallized	50.01	Parr bomb	4	49.87	0.08
Dichlorodiphenyltrichloroethane, technical, recrystallized	50.01	Sodium reduction	2	49.96	0.10
Dichlorodiphenyldichloroethane, technical, recrystallized	44.31	Sodium reduction	2	43.88	0.10

recorded. From these data a titration curve is plotted and the potential of the end point selected. The pH-MV dial of the instrument is set to the preselected end point, the acid-base dial is set at "base," and subsequent titrations are made automatically in the usual way.

DISCUSSION

In preliminary experiments, the button type of silver chloride electrode provided with the instrument was found to lack sensitivity and stability, but the electrode devised by Yeck (*3*) provided a very satisfactory reference electrode.

The stability and reproducibility of the end point was found to be dependent on complete removal of peroxide, maintenance of a reasonably constant pH, and salt concentration. The peroxides are removed by boiling with acid, and the optimum pH was found to be 2.0. The end-point potential increases slightly with salt concentration, but this variation is not significantly large for a given type of determination. Despite the precautions, the end-point potential of a given electrode pair drifts from day to day, but the drift is inconsequential if the end point is determined as described, on the first sample of a series, by plotting a titration curve. The stability is improved if the silver electrode is cleaned between determinations.

Silver nitrate solutions are standardized against 0.1*N* sodium chloride, employing a manual titration for the first solution to determine the end point. Because of the low salt content, the end point potential is approximately 25 mv. lower than that of sample solutions.

As usual for automatic titrations of this type, the sensitivity of the titration and any tendency to overtitrate are influenced by the geometry of the titration assembly and the rate of flow of titrant. This is so because the response of the titrator is controlled by the relatively slow dissolution of the increment of titrant rather than by the electrical characteristics of the instrument. The conditions described are considered optimum for the purposes of this laboratory; greater speed of titration could be obtained but at the expense of precision. The average titration requires about 2 minutes; 30 samples can be carried through the pH adjustment and titration stages in 1.5 hours. The titrator operates with a standard deviation of less than 0.1 ml. and the over-all coefficient of variation of a series of Parr bomb determinations of chlorine in standard samples of organic pesticides (10 to 50% of chlorine), run over a period of several weeks, was 0.003. The accuracy was within these limits. Some results on typical compounds are listed in Table I.

LITERATURE CITED

(1) Beckman Instruments, Inc., South Pasadena, Calif., *Beckman Bull.* 239.
(2) Parr Instrument Co., Moline, Ill., *Parr Manual* **121.**
(3) Yeck, R. P., and Kissin, G. H., ANAL. CHEM., **17**, 692 (1945).

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Detection of Some Antioxidants in Vulcanized Rubber Stocks

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Elution chromatography has been used with ultraviolet spectroscopic methods to study the acetone-extractable material from vulcanized rubber stocks, with particular attention to the separation and identification of Santoflex B, B-L-E, *N,N*-diphenyl-*p*-phenylenediamine, and *N*-phenyl-2-naphthylamine. The procedure permits the isolation of sufficient amounts for melting point determinations, or a few milligrams for the measurement of ultraviolet adsorption spectra. Sulfur, pine tar, paraffin, and the acetone extract from smoked sheet have been examined for possible interference.

THE absence of a suitable procedure for the separation and identification of antioxidants in vulcanized rubber stocks was the primary reason for the experimental work described in this paper. Numerous authors have explored this subject; but for various reasons, their work was considered inadequate.

Color tests have been the sole means of identification in most of the previous procedures (*4–6, 8–14, 16–18*). These reactions are specific for classes of antioxidants and should be employed with caution as a final proof of identification. Craig (*7*) used aqueous sodium hydroxide extraction, steam distillation, benzene or petroleum ether extraction, and hydrochloride precipitation to isolate amine antioxidants. Final proof was based on melting point data.

The use of chromatography to effect a partial separation of acetone-extractable compounding ingredients has been of great value to other investigators. Parker and Berriman (*22*) used silica gel as the adsorbent; the final identification was made by the use of streak tests and column position. Bellamy and coworkers (*1, 2*) separated the antioxidants, mineral oil, and waxes from the rest of the acetone extract. They then proceeded to identify the antioxidants by streak or color tests, or in some cases by melting point determinations. Mann (*19*) used Bellamy's separation technique, but offered infrared spectroscopy as the means of identification.

Most of the procedures used previously have dealt with antioxidants alone or with relatively simple vulcanizates that contain only one oxidation inhibitor. Streak reagents may provide useful information at times; but melting point data (*2*) and infrared spectra (*19*) are a much more conclusive means of identification. Infrared spectroscopy suffers from lack of good solvents and low sensitivity when compared with ultraviolet.

The present paper offers an improved method for the isolation of antioxidants from vulcanizates using alumina of two activities and employing three chromatographic schemes. Identification was accomplished by the use of melting point determinations, ultraviolet spectra, chromatographic fraction number, and infrared spectral data. Satisfactory separation of the antioxidants from other compounding ingredients was achieved; and in most cases

the antioxidants were separated from one another. The one exception was the incomplete separation of *N*-phenyl-2-naphthylamine from Santoflex B.

APPARATUS AND MATERIALS

Apparatus. A Cary Recording spectrophotometer, Model 11, was used for all ultraviolet work.

The chromatographic column was 23 mm. in diameter by 40 cm. in length. All connections were ground glass and were used without lubricant to prevent contamination of the samples. A diagram is shown in Figure 1.

Alumina. The adsorbent was alumina, activated chromatographic powdered catalyst grade, Al-0109P (90% Al_2O_3) from Harshaw Scientific, Division of Harshaw Chemical Co., Cleveland 6, Ohio. Two activities were used.

Adsorbent A. No extra preparation was required for the alumina as received from Harshaw. As soon as a fresh sample was opened, it was stored in small glass-stoppered bottles in a desiccator over calcium chloride.

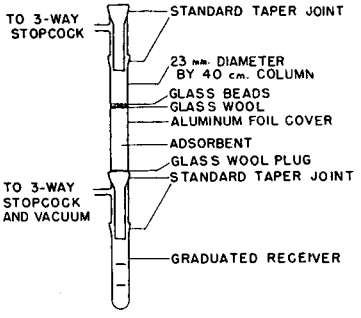

Figure 1. Chromatographic Apparatus

Adsorbent B. About 500 grams of Harshaw alumina was deactivated by allowing the adsorbent to stand on a metal tray in an air-conditioned room maintained at 77° F. and 50% humidity for 6 days. At this time the alumina was stored in a bottle in the same room.

Solvents. Absolute ethyl alcohol was used without further purification for both chromatography and ultraviolet spectroscopy.

Benzene was shaken with calcium oxide, filtered, and distilled, the middle 80% of the distillate was reserved for use.

Carbon tetrachloride was purified by shaking with distilled water. The solvent was separated, dried with calcium oxide, and filtered. The first and last 10% of the distillate were discarded.

Compounding ingredients. 2,2,4-Trimethyl-6-phenyl-1,2-dihydroquinoline (TPDQ), the principal ingredient of Santoflex B, was purified by vacuum distillation of the crude commercial product. The distillate was crystallized three times from absolute ethyl alcohol, melting point, 102° C. (corrected).

N,N'-Diphenyl-*p*-phenylenediamine (DPPD) was crystallized twice from aniline and was extracted three times with absolute ethyl alcohol, melting point, 154° C. (corrected).

N-Phenyl-2-naphthylamine (PBNA) was crystallized three times from acetic acid and twice from a benzene-hexane mixture, melting point, 110° C. (corrected).

B-L-E was used as received from the Naugatuck Chemical Division, U. S. Rubber Co. It is reported to be a high temperature reaction product of diphenylamine and acetone.

Santoflex-B was obtained from the Monsanto Chemical Co.

2-Mercaptobenzothiazole (MBT) was converted to the sodium salt, which was decolorized with carbon and the 2-mercaptobenzothiazole regenerated. The recovered 2-mercaptobenzothiazole was crystallized from an acetone-water mixture and from benzene; melting point 182° C. (corrected).

Smoked sheet rubber was extracted with acetone for 16 hours. The solvent was evaporated; and the dried extract, which amounted to 2.86% of the rubber, was used for the investigations.

Pine tar from Russell Farley & Co. was used as such.

Rubbermaker's sulfur was obtained from Stauffer Chemical Co.

Refined paraffin, 123–25°, was received from the Standard Oil Co.

Stearic acid was purchased from General Mills, Inc.

PROCEDURE

Preparation of Column. A small plug of borosilicate glass wool was placed in the bottom of the column. Then 110 grams of adsorbent *B* or 103 grams of adsorbent *A* were added in small portions and packed by gently tapping the bottom of the column on a pad of paper. A very small plug of the glass wool was placed on top of the alumina and tamped with a wooden dowel. A half inch of small glass beads was added on top of the glass wool. A sheet of aluminum was cut of such length that the glass beads were above the top of the sheet when it was wrapped around the column. The aluminum sheet was needed to minimize the decomposition by light. Figure 1 is a diagram of the apparatus.

Preparation of the Sample. The cured stock was first milled to facilitate extraction. About 75 grams of the crumbled vulcanizate were extracted for 16 hours with acetone. The solvent was evaporated on a low temperature hot plate and the residue was chromatographed according to the procedures described below.

Chromatographic Separations. PROCEDURE A. A dried extract was dissolved in 40 ml. of benzene, using heat to aid in the solution. After cooling to room temperature, the solution was poured on a column containing adsorbent *B*, and a gentle suction was applied to the base of the column such that the flow rate was approximately 5 ml. per minute. The separation was accomplished by the addition of the following amounts of solvent mixtures in the order given: 205 ml. of benzene, 205 ml. of 0.25% ethyl alcohol plus 99.75% benzene, 205 ml. of 1% ethyl alcohol plus 99% benzene, 205 ml. of 5% ethyl alcohol plus 95% benzene, and enough ethyl alcohol to make 26 40-ml. fractions. The fractions were numbered consecutively in the order of elution starting with the first drop of liquid to emerge from the column. All solvent mixtures were expressed in volume per cent.

PROCEDURE B. Fractions 1 to 6, inclusive, from Procedure A were combined; the solvent was evaporated; and the residue was dissolved in 40 ml. of carbon tetrachloride. The solution was chromatographed on a column of adsorbent A using the same technique as above except that the solvents were as follows: 205 ml. of 5% benzene plus 95% carbon tetrachloride, 205 ml. of 20% benzene plus 80% carbon tetrachloride, 205 ml. of benzene, 205 ml. of 1% ethyl alcohol plus 99% benzene, and enough 5% ethyl alcohol plus 95% benzene to make 26 fractions.

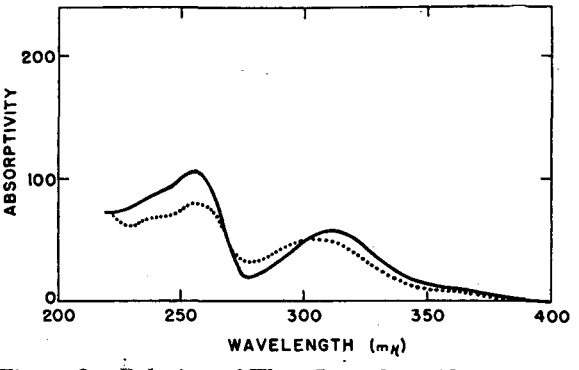

Figure 2. Relation of Wave Length to Absorptivity

———Pure 2,2,4-trimethyl-6-phenyl-1,2-dihydroquinoline
. . . . . Fraction 12 isolated from vulcanizate by Procedure B

PROCEDURE C. Fractions 22 to 26, inclusive, from Procedure B were combined; the solvent was evaporated; and the residue was dissolved in 40 ml. of carbon tetrachloride. The solution was chromatographed on adsorbent *B*, and elution was accomplished by the use of the following solvent mixtures: 205 ml. of carbon tetrachloride, 205 ml. of 1% benzene plus 99% carbon tetrachloride, 205 ml. of 5% benzene plus 95% carbon tetrachloride, 205 ml. of 25% benzene plus 75% carbon tetrachloride, and enough benzene to make 26 fractions.

Treatment of Chromatographic Fractions. Each of the fractions was transferred to a separate tared beaker. After the solvent was evaporated, the weight of the residue was obtained. An inspection of the weights of all of the fractions revealed that maxima occur. Each fraction in which a maximum occurred was dissolved in absolute ethyl alcohol and the concentration was adjusted to 0.0100 gram per liter. The ultraviolet spectrum was taken of the solution using a 1-cm. cell. A comparison with the spectra of known antioxidants tentatively identified the compound. Final confirmation was made by recovering the antioxidant from the solution and recrystallizing from an appropriate solvent. The melting point and mixed melting point were then observed together with the ultraviolet spectrum of the purified sample.

RESULTS

Santoflex B. Approximately 80% of the commercial material was found to be 2,2,4-trimethyl-6-phenyl-1,2-dihydroquinoline.

Fractions 11, 12, and 13 from Procedure B contained the bulk of this antioxidant. Purification was made from *n*-hexane or absolute ethyl alcohol. The melting point was found to be 102° C. (corrected).

N,N'-Diphenyl-*p*-phenylenediamine. The purified material was eluted in fractions 12, 13, and 14 of Procedure C. The antioxidant was crystallized from carbon tetrachloride and melted at 154° C. (corrected).

N-Phenyl-2-naphthylamine. The commercial antioxidant was 97% or better *N*-phenyl-2-naphthylamine. Elution occurred in fractions 15, 16, and 17 of Procedure B when an appreciable amount of Santoflex B was present; otherwise elution took place in fractions 11, 12, and 13 of the same procedure. Commercial *n*-hexane was used as the solvent for purification, yielding a crystalline solid with a melting point of 109° C. (corrected).

B-L-E. This material is a complex mixture resulting from a high temperature reaction between acetone and diphenylamine. The fractions of most value in detecting this antioxidant were 7 and 8 of Procedure B. Ultraviolet and infrared spectra were the sole means used to show the presence of this antioxidant since the fractions were liquid. Some diphenylamine separated occasionally as a solid, but this was not common.

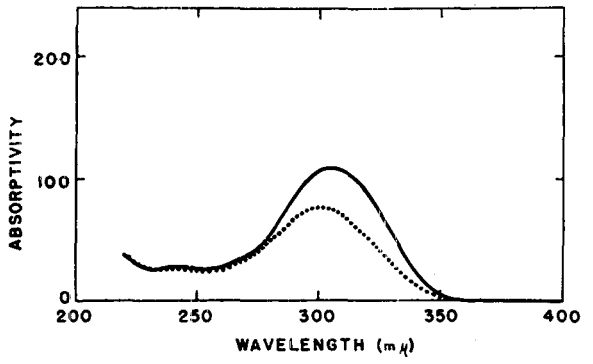

Figure 3. Relation of Wave Length to Absorptivity

——— Purified *N,N'*-diphenyl-*p*-phenylenediamine
..... Fraction 12 isolated from vulcanizate by Procedure C

Application to Vulcanizates Containing Mixtures of Antioxidants. Figures 2 to 5 show the ultraviolet spectra of the antioxidants studied as well as spectra of appropriate fractions isolated from a natural rubber tread stock which contained all four of the compounds.

The procedure has been applied to several other vulcanizates of complex composition. The ultraviolet spectra of the isolated antioxidants after purification were taken in most cases. The curves were found to be nearly identical with that of the purified known materials. In one instance 2,2,4-trimethyl-6-phenyl-1,2-dihydroquinoline, *N,N'*-diphenyl-*p*-phenylenediamine, and *N*-phenyl-2-naphthylamine were separated from one vulcanizate in sufficient amounts to permit identification of all three by melting point data.

DISCUSSION

The usual equation was used for the ultraviolet curves

$$\log_{10}\frac{I_0}{I} = A = acl,$$

where

A = absorbance
a = absorptivity
c = concentration in grams per liter
l = cell thickness in centimeters

The concentration and cell thickness were kept constant in all measurements at 0.0100 gram per liter and 1.000 cm., respectively. A very useful relationship exists for this special condition, $a = 100\ A$; and since Cary spectra are recorded as absorbance *vs.* wave length, the absorptivity may be read directly from the Cary graph. The superimposition of the unknown curve over that for the known made possible a rapid, accurate means of comparision. The use of the concentration and cell thickness proposed here is of value for compounds having absorptivity maxima in the vicinity of 100.

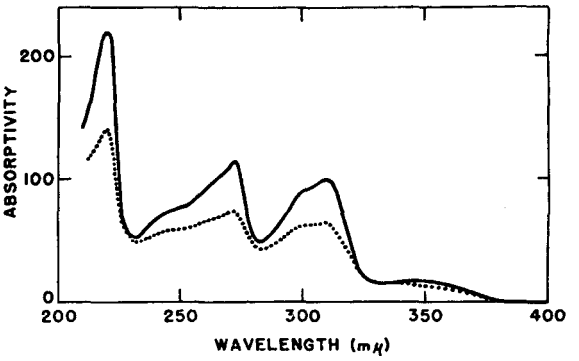

Figure 4. Relation of Wave Length to Absorptivity

——— Purified *N*-phenyl-2-naphthylamine
..... Fraction 17 isolated from vulcanizate by Procedure B

The curves of 2,2,4-trimethyl-6-phenyl-1,2-dihydroquinoline and *N*-phenyl-2-naphthylamine show considerable detail, and are very valuable for the identification of these two antioxidants. The DPPD and B-L-E spectra are not as characteristic, and one should be careful when using ultraviolet as the only means of identification. The fraction in which elution takes place is also a characteristic property; thus the use of chromatographic fraction number and ultraviolet spectrum would make a more convincing argument. The isolated antioxidant was recovered after spectrophotometric analysis and was purified for final proof. Thus one separation sufficed for both the preliminary survey and the final confirmation.

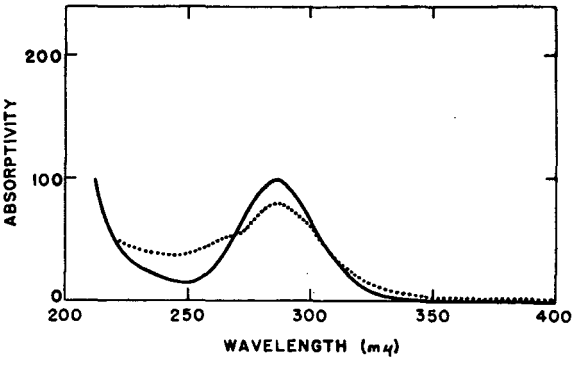

Figure 5. Relation of Wave Length to Absorptivity

——— Fraction 8 isolated from B.L.E. by Procedure B
..... Fraction 8 isolated from vulcanizate by Procedure B

Although positive proof of identity could not be obtained on the crude chromatographic fractions by ultraviolet absorption curves, it was possible and convenient to use the spectroscopic data of the purified antioxidant from the chromatographic fraction for confirmatory purposes. Melting points were used for final identification of all antioxidants except B-L-E. Infrared and ultraviolet spectra were considered sufficient for this antioxidant.

Most of the methods listed in the literature for the preparation of a standardized alumina (*3, 20, 21, 23*) were found to be unsatisfactory. The standardization could be accomplished without difficulty, but the preparation was a hit or miss affair. Two

factors seemed to be most important, the moisture content and the temperature. Accordingly, alumina of two different activities was prepared by controlling the humidity and temperature of the atmosphere surrounding it. The temperature was maintained at 77° F. in both cases; but the relative humidity of the air-conditioned room was kept at 50% for one preparation while the second system used a desiccator containing anhydrous calcium chloride. After standing for 6 days, the alumina was found to have a constant activity. The absorbent equilibrated at the lower humidity had the higher activity. Alumina prepared in this manner had a remarkably constant activity which could be reproduced without need of standardization. Ewing, Sharpe, and Bird (*15*) used the same idea by employing a constant humidity produced with sulfuric acid solutions.

Most of the variables affecting columnar chromatography, as treated by Weil-Malherbe (*23*), were carefully controlled. The initial volume of the solvent was larger than that usually employed, but this amount was found necessary in order to ensure proper solution of the sample.

The principal ingredient of each of the antioxidants studied was eluted in the first six fractions of Procedure *A*. As the method was designed for the isolation and identification of the accelerator, 2-mercaptobenzothiazole, as well as the antioxidants in the acetone extract, this procedure was necessary. In a few cases, a direct separation of antioxidants employing Procedure B without prior use of Procedure A produced the same results as were obtained when both procedures were used. Hence, it would seem that the preliminary separation by Procedure A is not necessary for the isolation of antioxidants.

Other compounding ingredients may hasten or retard elutions. Even though elutions were usually in the same fractions, it was found to be advisable to inspect two or three fractions on either side of the one ordinarily examined before an antioxidant was stated as absent.

2-Mercaptobenzothiazole, pine tar, sulfur, paraffin, stearic acid, and the extracts from HAF carbon black and smoked sheet were examined. Pine tar interfered somewhat with the ultraviolet spectrum of B-L-E; but no difficulty has been experienced in detecting this antioxidant in the vulcanizates studied, some of which contained pine tar. There is a fraction of the acetone extract from smoked sheet eluted in the fractions containing *N*-phenyl-2-naphthylamine and 2,2,4-trimethyl-6-phenyl-1,2-dihydroquinoline but the ultraviolet absorption was less than an absorptivity of two for the entire range from 220 to 400 mμ. The rest of the compounding ingredients do not interfere.

LITERATURE CITED

(1) Bellamy, L. J., Lawrie, J. H., and Press, E. W. S., *Trans. Inst. Rubber Ind.*, **22**, 308 (1947).
(2) *Ibid.*, **23**, 15 (1947).
(3) Brockmann, H., and Schodder, H., *Ber.*, **74B**, 73 (1941).
(4) Budig, K. H., *Kautschuck u. Gummi*, **1**, 305 (1948).
(5) Burchfield, H. P., and Judy, J. N., ANAL. CHEM., **19**, 786 (1947).
(6) Burmistrov, S. I., *Zavodskaya Lab.*, **14**, 787 (1948).
(7) Craig, D., IND. ENG. CHEM., ANAL. ED., **9**, 56 (1937).
(8) Deal, A. J. A., *Trans. Inst. Rubber Ind.*, **23**, 148 (1947).
(9) Endô, H., *J. Soc. Chem. Ind., Japan*, **38**, Suppl. binding, 618 (1935).
(10) *Ibid.*, **39**, Suppl. binding, 11 (1936).
(11) *Ibid.*, p. 12.
(12) *Ibid.*, p. 17.
(13) *Ibid.*, p. 52.
(14) *Ibid.*, p. 146.
(15) Ewing, D. T., Sharpe, L. H., and Bird, O. D., ANAL. CHEM., **25**, 599 (1953).
(16) Howland, L. H., and Hart, E. J., IND. ENG. CHEM., ANAL. ED., **12**, 81 (1940).
(17) Koide, T., Kubota, T., Furuhashi, M., and Sato, T., *J. Soc. Rubber Ind. Japan*, **22**, 108 (1949).
(18) Koide, T., Kubota, T., and Sato, T., *Ibid.*, **22**, 256 (1949).
(19) Mann, J., *Trans. Inst. Rubber Ind.*, **27**, 232 (1951).
(20) Muller, P. B., *Helv. Chim. Acta*, **26**, 1945 (1943).
(21) *Ibid.*, **27**, 404 (1944).
(22) Parker, C. A., and Berriman, J. M., *Trans. Inst. Rubber Ind.*, **28**, 279 (1952).
(23) Weil-Malherbe, H., *J. Chem. Soc.*, 303 (1943).

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Halogen–Cobalt(II) Acetate–Acetic Acid Solutions in Organic Qualitative Analysis

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The presence of active unsaturation is indicated by testing the substance with a solution of halogen and cobalt(II) acetate in acetic acid to give a blue color. Other groups, in general, fail to give any color or give different colors which can assist in their identification as a class.

THE use of bromine solutions to spot-test for active unsaturation is standard procedure in organic qualitative analysis. In dealing with minute samples, however, the test has its limitations, as the disappearance of the color may result from dissolution of the bromine as well as bromination by substitution. Other spot tests usually serve to supplement the bromine test.

In order to provide an additional supplementary test for groups that halogenate easily, the writer performed rather interesting tests which provide information which could be useful in qualitative analysis. The work is by no means exhaustive, but merely a starting point, so that others might evaluate its potential usefulness.

Various organic compounds were halogenated with bromine and with chlorine in the presence of cobalt(II) acetate tetrahydrate, with the result that a very sensitive color test was developed, primarily to provide experimental proof for the existence of an ethylene bond, or an easily substituted hydrogen. Further observations indicate a possible extension to oxygenated compounds, but exceptions were apparent.

Tables I and II contain the observations made when the test was applied to a variety of compound.

DISCUSSION

The formation of a blue color indicates the conversion of cobalt(II) acetate to either cobalt(II) chloride or cobalt(II) chloride-acetate. At the same time chloroacetoxylation of the unsaturated linkage occurs.

This view agrees well with experience, for it is known that ethylene will form β-chloroethyl acetate when it is chlorinated in the presence of sodium acetate (*1*). The blue color formed by bubbling dry hydrochloric acid into an acetic acid solution of

cobalt(II) acetate was about the same as observed when the writer performed the test herein described.

The colors resulting from oxygenated compounds present a more complex problem, and it is suggested that some sort of coordination complex forms involving the unshared pair of electrons present on the oxygen. Ether responds to give a green color. Ether and cobalt(II) acetate alone do not form a color, which proves the necessity for halogen in the test. The fact that ether and dioxane can form onium salts may account for the green color produced in the test. The colors resulting from aldehydes cannot be correlated with the failure of acetone to give a rapid color formation. Both types—namely, aldehydes and ketones—however, do give colors, as would be expected from their α-hydrogen activity.

Table I. Color Formation Resulting from Bromination of Organic Compounds

Compound	Color on Reaction	Color after 2 Hours
Blank	Yellow	Yellow
Pyridine	Lavender	Lavender
Monoethanolamine	Lavender	Lavender
Dicyandiamide	Green	Green
Heptaldehyde	Green-blue	Olive green
Benzaldehyde	Olive green	Green
Acetaldol	Straw	Straw
Acetone	Yellow	Blue
n-Butanol	Green	Blue-green
Glycerol	Yellow	Yellow
Ethylene glycol	Yellow	Yellow
Hexamethylene glycol	Yellow	Green
Methanol	Yellow	Yellow
Ethyl ether	Green	Blue-green
Dioxane	Green	Green
Methyl ricinoleate	Blue	Blue
Linoleic acids	Blue-green	Olive green
Oleic acid	Blue	Light blue
Butyl oleate	Blue	Blue green
Methyl undecylenate	Blue	Faint lavender
α-Pinene	Blue	Faint lavender
Benzene	Yellow	Yellow
Toluene	Yellow	Green
Fumaric acid	Yellow	Yellow
Dibutyl sebacate	Yellow	Yellow
Petroleum ether	Blue	Blue
Chloroform	Yellow	Yellow
Ethyl acetate	Yellow	Blue
2-Naphthol	Green	Dark green

Nitrogen compounds were quick to respond to color formation. Aniline derivatives gave very intense colors which could not be correlated.

Compounds known to be resistant to halogenation—e.g., dibutyl sebacate—resisted color formation and merely diluted the test solution. Compounds of low molecular weight—e.g., ethyl acetate—were evidently not resistant to halogen.

Like many qualitative tests, there are exceptions, but from a knowledge of the physical properties (molecular weight) and the general type of chemical, its purity as regards unsaturation in very small concentration may be qualitatively shown to be present or absent.

As in other analytical tests, there are interfering substances, but the application of the test described can be used to advantage in specific cases. The degree of hydrogenation, for example, can be followed by spot testing with the cobalt acetate–bromine reagent, the color intensity being the guide. Halogenation may similarly be followed qualitatively by the test procedure employed for following hydrogenation.

EXPERIMENTAL

Preparation of Bromine–Cobalt Acetate–Acetic Acid Solution Three liters of reagent may be prepared by dissolving 2.5 grams of cobalt(II) acetate tetrahydrate in 1 liter of glacial acetic acid. To this are added 8.1 grams of bromine in about 1 liter of glacial acetic acid. The reagent is then diluted to the 3-liter mark. The reagent is stored in an amber stock bottle.

Table II. Color Formation Resulting from the Chlorination of Organic Compounds

Compound	Color on Reaction	Color after 2 Hours
Pyridine	Purple	Purple
Aniline	Dark brown	Dark brown
Dimethylaniline	Dark green	Very dark green
Monoethanolamine	Deep blue	Deep blue
2,4-Dinitrophenylhydrazine	Green	...
Dicyandiamide	Blue	...
Acetone	No change	...
Heptaldehyde	Medium green	...
Benzaldehyde	Light olive green	...
Isophorone	Blue	...
Phenol	Blue green	...
Phloroglucinol	Blue green	...
2-Naphthol	Blue green	...
Methanol	No change	...
Isopropyl alcohol	Blue	...
1-Butanol	Light green	...
Methyl isobutyl carbinol	Green	...
Glycerol	No change	...
Ethylene glycol	No change	...
Hexamethylene glycol	No change	...
Dioxane	Green	...
Ether	Green	...
Benzene	No change	...
Toluene	Light green	...
Xylene	Blue	...
p-Toluenesulfonic acid	No change	...
α-Pinene	Blue	...
Oleic acid	Blue	...
Butyl oleate	Blue	...
Methyl ricinoleate	Blue	...
Methyl undecylenate	Blue	...
Ethyl acetate	Blue	...
Petroleum ether	Blue	...
Chloroform	No change	...
Dibutyl sebacate	No change	...
Iso-octane	No change	...
Starch	No change	...
Fumaric acid	No change	...

Preparation of Chlorine–Cobalt Acetate–Acetic Acid Solution. A solution of 2.5 grams of cobalt(II) acetate tetrahydrate in 1 liter of glacial acetic acid is saturated with chlorine gas. The solution must be used freshly for testing organic compounds for color formation, as the chlorine is gradually lost to the atmosphere. A modified procedure consists in preparing the solution in a funnel and maintaining a slow but steady flow of chlorine through the solution, taking the necessary steps to have an exhaust lead-off out of the work area.

Running the Color Test. The mixture of almost any quantity of sample with an equal amount of halogen reagent is sufficient for color development to take place. Samples as small as 1 mg. mixed with about 0.5 ml. of reagent give unmistakable colors.

For the purpose of this paper, 0.5 cc. of sample was mixed with about 1.0 cc. of reagent. The ratio of one to the other determined the depth of color as well as hue.

In applying the test to follow hydrogenation or halogenation, a standard amount of sample and reagent must be used to develop a comparative series of colors. The presence of other metallic ions may affect the color, and their effect should be determined independently of the test reported herein.

The analyst, in running this test, should look for instantaneous color formation as a positive indication of reactive groups. When a blue color is observed, isolated double bonds and compounds of low molecular weight are usually present. The latter type, of course, are generally known to be present if they are extremely volatile. Resonating structures, like benzene and fumaric acid, do not respond. This type of unsaturated group may be suspected when a negative test results.

Oxygenated compounds like aldehydes, ethers, and ketones tend to give various shades of green. Such structures should be suspected when a positive green color results either immediately or on standing.

LITERATURE CITED

(1) Weber, F. C., Hennion, G. F., and Vogt, R. R., *J. Am. Chem. Soc.*, 61, 1457–8 (1939).

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Direct Method for the Determination of Methoxy Group in the Presence of Borohydrides

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A quantitative method for the determination of methoxy group in borohydrides is based on oxidation of methanol to formic acid with a standard solution of ceric nitrate. A precision within 0.5% of the methoxy group present in borohydrides is obtained.

SODIUM methylate and methoxy borohydrides are formed during the preparation of sodium borohydride; thus, it is of value to know the trace amounts of methoxy-group contaminants present in the final product. Such a determination is also applicable in analyzing the by-product, sodium methylate. Analysis of methoxy borohydrides for methoxy content is useful, for the present assays are based on boron analysis. Further, a methoxy determination gives a more accurate value for the per cent of oxygen present in borohydrides.

The several methods tried previous to the direct method of methoxy determination proved unsatisfactory. When a water-methanol mixture is distilled from a solution of sodium methylate, varying amounts of boron are carried over. This makes it impossible to compare the specific gravity and/or the index of refraction of the distillate against standard solutions. A micro-method, using a modified Zeisel procedure, also is unsatisfactory. The method has several disadvantages, inasmuch as sodium compounds present tend to interfere, and more specialized micro equipment is necessary (*2*). A direct method of determining methoxy by sodium content, and a subsequent correction for sodium hydroxide, cannot be applied because of the presence of sodium hydride and other sodium compounds (*4*).

The principles of the current method were first developed and applied during an investigation of the reactions of quadrivalent cerium with aliphatic alcohols in nitric acid solutions (*3*). In the present application, the assumption is made that methoxy group is present as methoxy borohydrides, sodium methylate, methyl borate, methanol, or similar compounds formed during the production of borohydrides. The proposed method gives an accuracy within ±0.5% in the presence of such contaminants as sodium borohydride, sodium hydride, sodium borate, and carbon. Sodium methylate of analytical purity was unavailable at the time, so the accuracy is best shown by a comparison with carbon analyses. This report shows that the methoxy group is determined quantitatively by oxidation to formic acid with quadrivalent cerium.

REAGENTS

Standard ceric nitrate. The approximate 0.1*N* solution is prepared by dissolving 540 grams of ceric ammonium nitrate in 5 liters of water containing 810 ml. of nitric acid, and then diluting to 9 liters. (The nitric acid is boiled until colorless to remove oxides of nitrogen.) A 9-liter carboy is used to store the solution. This solution is standardized against standard arsenite solution as follows. About 40 ml. of ceric ammonium nitrate solution are measured into a flask. Ten milliliters of 6*N* sulfuric acid, 4 drops of 0.01*M* osmium tetroxide, and 1 drop of 0.025*M* ferroin are added, and the reagent is titrated with standard arsenite until the first faint pink color.

Standard 0.1*N* arsenious oxide solution (*1*). Pure arsenious oxide, 2.4725 grams, is weighed and dissolved in 20 ml. of 1*N* sodium hydroxide. Sulfuric acid, 1*N*, is added to the solution until reaction is slightly acid to litmus. The solution is transferred to a 500-ml. volumetric flask and made up to the mark. For larger volumes of arsenious oxide, the solution may be standardized against a normal iodine solution.

Osmium tetroxide solution, 0.01*M*. Ordered as perosmic acid crystals, Merck; Howe & French suppliers.

Nitric acid, 8*N*, a 1 to 1 solution.

Sulfuric acid, 6*N*, 5 volumes of water to 1 volume of concentrated sulfuric acid.

Standard methanol solution, Fisher Scientific Co.

PROCEDURE

A sample containing approximately 50 mg. of methoxy group is cautiously dissolved in 10 ml. of 8*N* nitric acid (the solution being kept cool with an ice bath to prevent loss of methanol), and the solution is filtered (No. 42 Whatman) into a 500-ml. Florence-type reflux flask. (A larger sample may be taken and appropriate aliquots used.) The sample is refluxed in a bath of boiling water for 10 minutes and then cooled to room temperature; 100.00 ml. of 0.1*N* ceric ammonium nitrate are added, and the mixture is refluxed for an additional 10 minutes. After cooling, 20 ml. of 6*N* sulfuric acid, 3 drops of osmium tetroxide (catalyst), and 1 drop of *o*-phenanthroline ferrous sulfate (ferroin) indicator are added. The excess quadrivalent cerium is then back-titrated with standard arsenite solution to the first trace of light pink.

CALCULATIONS

The weight of methoxy group is calculated by means of the following formula:

$$\text{Mg. of } CH_3O^- = (A \times N - B \times R) \times 0.007758$$

where

A = total milliliters of quadrivalent cerium added in excess
N = normality of quadrivalent cerium
B = milliliters of arsenious oxide solution used for back-titration
R = equivalence of quadrivalent cerium to arsenious oxide: $\frac{\text{ml. of } Ce^{+4}}{\text{ml. of } As_2O_3}$

EXPERIMENTAL

A quantitative recovery of methanol, in the absence of other constituents, was established. Measured amounts of methanol were refluxed with standard ceric ammonium nitrate solution. Table I shows the recovery of methanol and verifies that methanol can be quantitatively analyzed by this procedure (*3*).

Table I. Recovery of Methanol

Methanol Present, Gram	Methanol Recovered, Gram		Recovery, %
0.02382	0.02383		100.04
0.02382	0.02383		100.04
0.02382	0.02384		100.08
0.03970	0.03979		100.23
0.03970	0.03976		100.14
0.03970	0.03976		100.14
		Average	100.13
		Std. dev.	±0.07

Sodium methylate hydrolyzes into stoichiometric amounts of sodium hydroxide and methanol; thus, per cent recovery of methanol in the presence of sodium hydroxide is established.

$$NaOCH_3 + H_2O \longrightarrow NaOH + HOCH_3$$

Table II shows the per cent methanol recovered under these conditions.

Table II. Recovery of Methanol in Presence of Sodium Hydroxide

Methanol Present, Gram	Ceric Ammonium Nitrate, Ml.	Methanol Recovered, Gram	Recovery, %
0.03970	100.00	0.03976	100.14
0.03970	100.00	0.03993	100.58
0.03970	100.00	0.03976	100.14
		Average	100.29
		Std. dev.	±0.22

Table III. Comparison between Analyses of Sodium Methylate by Carbon Combustion and Direct Oxidation with Quadrivalent Cerium

Sample No.	Carbon, %	Carbon as Methoxy, %	Methoxy with Quadrivalent Cerium Oxidation, %
I-a	21.39	55.25	55.42
II-a	21.59	55.79	54.84
II-b	21.16	54.78	55.16
II-c	21.17	54.71	55.25
III-a	21.77	56.25	55.13
III-b	20.52	53.02	55.50
Average	21.27 ± 0.27	54.97 ± 1.27	55.22 ± 0.29

Table IV. Determination of Methoxy Group in Simulated Commercial By-product Material

Sample No.	Sodium Methylate, Wt. %	Sodium Borohydride, %	Sodium Hydride, %	Sodium Borate, %	Methoxy, Gram: Theoretical	Methoxy, Gram: Recovered	Recovery, %
4-1	52.79	21.95	22.81	2.46	0.06395	0.06406	100.11
4-1	66.19	21.95	10.55	2.02	0.08523	0.08485	99.55
4-1	92.36	2.4	2.23	2.96	0.05403	0.05414	100.20
4-1	74.94	9.03	15.13	10.90	0.07078	0.07125	99.34
4-3	89.93	5.36	0.70	1.01	0.07215	0.07330	98.83
						Average	99.61
						Std. dev.	±0.38

The total carbon content of sodium methylate was assumed to be present as the methoxy group. A sample of material was obtained (Matheson, Coleman, and Bell), and further investigations were made, using this material. Analyses by carbon combustion in comparison with the present method were made on three separately sampled portions.

The results in Table III show an agreement of better than 0.4% of total methoxy present when the material was analyzed by two different methods (direct and indirect). Of the two methods, better precision was obtained by the direct-oxidation method.

Synthetic mixtures containing sodium methylate, sodium borohydride, sodium hydride, and sodium borate were made, these compounds being the chief constituents in the commercial product. Table IV shows the recovery of methoxy when it was present with varying amounts of other compounds.

Poor precision during early analyses of methoxy in commercial material was probably due to the presence of "suspended" and/or colloidal carbon (some organic decomposition occurring during the production of sodium borohydride). When colloidal and amorphous carbon were refluxed with quadrivalent cerium, there was only a partial recovery of the ceric solution. Thus, this consumption of quadrivalent cerium gave erroneously high results.

The contaminating effect of suspended carbon was eliminated by filtering it off from the sample. Experimentation showed that colloidal carbon was oxidized completely by refluxing with nitric acid. After refluxing a mixture of colloidal carbon, methanol, and nitric acid, a measured amount of quadrivalent cerium was added. The per cent recovery of methanol on three determinations was 99.55 ± 0.17.

Table V shows the methoxy determination of sodium trimethoxyborohydride and compares the results with those that were obtained by boron analyses. Table V shows that filtering off carbon and refluxing with nitric acid previous to refluxing with quadrivalent cerium resulted in more accurate and precise work.

The results and precision obtained when commercial material was analyzed for methoxy are shown in Table VI. The precision increased several fold when the samples were first refluxed with nitric acid.

DISCUSSION OF METHODS AND RESULTS

The basic chemistry of the method involved the oxidation of methanol to formic acid with excess standard ceric nitrate and the subsequent quantitative back-titration of the latter with standard arsenite solution.

$$CH_3OH + 4Ce^{++++} + H_2O = 4Ce^{+++} + HCOOH + 4H^+$$

With the removal of interfering carbon from commercial material, the precision of results was improved from about ±0.8 to ±0.1%. The per cent recovery of methoxy group which was obtained from simulated by-product mixtures is ±0.45%. It is felt that methoxy group can be accurately determined by a fairly

Table V. Methoxy Group in Sodium Trimethoxyborohydride

Sample No.	Methoxy Group (Theoretical from Boron Analysis), %	Methoxy Group Recovered, %	Average % Recovery of Methoxy Group	Standard Deviation	Remarks
5-1	68.86	69.83	100.48	±1.09	Carbon not filtered off (three determinations)
5-2	69.24	69.29	100.07	±0.34	Carbon filtered off; refluxed with HNO_3 (two determinations)

Table VI. Methoxy Group in Commercial By-products

Sample No.	Sample Wt., Gram	Ceric Ammonium Nitrate, Ml.	Methoxy Group, Gram	Methoxy Group, %	Average	Remarks
6-1	0.0918	39.69	0.0319	34.75	34.04 ± 0.72	No reflux with HNO_3
	0.1743	70.18	0.05813	33.32		
6-2	0.1333	54.18	0.04864	36.49	37.20 ± 0.71	No reflux with HNO_3
	0.1460	66.99	0.05535	37.91		
6-3	0.2331	66.80	0.05599	23.98	23.45 ± 0.53	No reflux with HNO_3
	0.1252	36.74	0.02945	22.91		
6-4	0.1524	32.00	0.03388	22.23	22.36 ± 0.12	Reflux with HNO_3
	0.2046	56.11	0.04600	22.48		
6-5	0.1659	58.04	0.04857	29.28	29.39 ± 0.11	Reflux
	0.1699	61.49	0.05062	29.50		
6-6	0.1263	82.57	0.04383	34.59	34.65 ± 0.06	Reflux
	0.1987	58.58	0.06875	34.70		
6-7	0.1284	49.98	0.1284	31.81	31.77 ± 0.04	Reflux
	0.1265	49.30	0.1265	31.73		

simple method in the presence of expected interfering compounds and carbon.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," 3rd ed., New York, Macmillan Co., 1952.
(2) "Scott's Standard Methods of Chemical Analysis," 5th ed., Vol. II, p. 2527, New York, D. Van Nostrand Co., 1925.
(3) Skogg, D. A., and Sister Monica, ANAL. CHEM., 25, 822 (1953).
(4) U. S. Industrial Chemicals Co., New York, "Laboratory Procedure. Purity of Sodium Methylate," 1954.

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Purity of *n*-Butane, Isobutane, Isobutene, and *n*-Octane from Freezing Points

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Measurements were made of the lowering of the freezing point of *n*-butane, isobutane, isobutene, and *n*-octane on the addition of known amounts of probable impurities. The data, covering the range 100 to 92 mole % of the major component, show that these systems follow the ideal solution laws in this respect.

ONE of the most important general methods for evaluating the purity of chemical substances is to compare the freezing point of the actual sample with the value for zero impurity. The theoretical principles have been fully described (*4*, *5*). The method depends, however, upon the physical assumptions that the impurity forms an ideal solution with the major component, and that it is solid-insoluble. It is of considerable importance to test these assumptions for a wide variety of systems to see for what mixtures the ideal solution law is obeyed, and to extend the range of substances for which this criterion of purity can be used with certainty.

This has been done for *n*-butane, isobutane (2-methylpropane), isobutene (2-methylpropene), and *n*-octane by measuring the freezing points of these substances with known amounts of known impurities added. The impurities were chosen to be of type similar to those expected to be present in the highly purified samples.

The mixtures of the volatile substances, *n*-butane, isobutane, and isobutene were made by the method previously described (*3*, *6*). For *n*-octane, the mixtures were made up by weight using a stoppered bottle. The apparatus and experimental procedure for measuring the freezing points have been described (*4*, *6*). All C_4 hydrocarbons used in this investigation were research grade hydrocarbons from the Phillips Petroleum Co. All other compounds were highly purified samples from the American Petroleum Institute Research Project 6.

Table I. Lowering of the Freezing Point of *n*-Butane, Isobutane, Isobutene, and *n*-Octane

System			
Major component	Solute	Mole % of Solute	Lowering of Freezing Point,[a] °C.
n-Butane	Isobutane	3.717	1.236 ± 0.003
		7.195	2.412 ± 0.004
Isobutane	*n*-Butane	3.605	0.901 ± 0.005
		6.692	1.666 ± 0.016
Isobutene	Isobutane	3.457	0.915 ± 0.003
		6.853	1.711 ± 0.004
	1-Butene	4.034	1.015 ± 0.002
		6.820	1.761 ± 0.004
	cis-2-Butene	4.239	1.069 ± 0.006
		8.691	2.222 ± 0.004
	trans-2-Butene	3.597	0.936 ± 0.005
		7.626	1.961 ± 0.000
n-Octane	2,2,4-Trimethylpentane	7.461	1.486 ± 0.004
	2,4-Dimethylhexane	7.746	1.572 ± 0.003
	1-Methyl-3-ethylcyclopentane (*cis* + *trans*)	8.531	1.678 ± 0.001
	1-*cis*-2-Dimethylcyclohexane	8.506	1.687 ± 0.005
	1-*trans*-2-Dimethylcyclohexane	8.642	1.714 ± 0.001
	Ethylbenzene	8.190	1.540 ± 0.005
	1,4-Dimethylbenzene (*p*-xylene)	5.283	1.014 ± 0.001

[a] Average of 2 experiments.

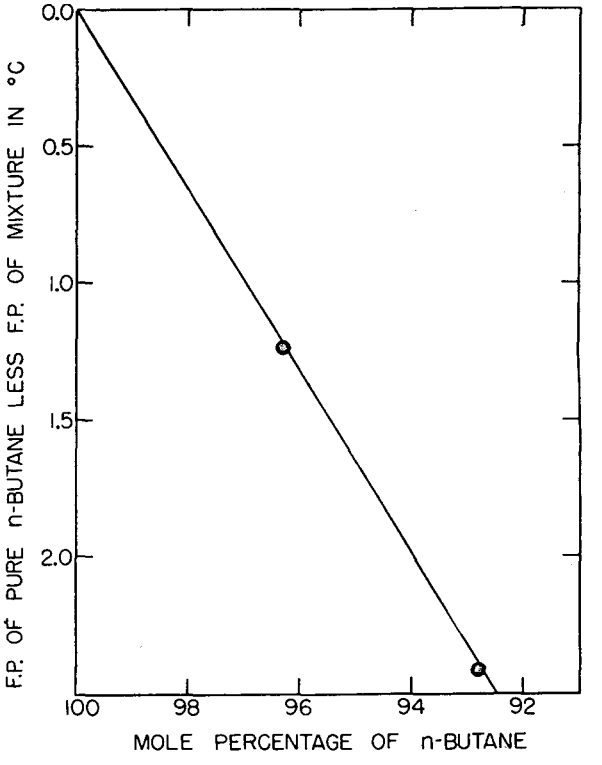

Figure 1. Lowering of Freezing Point of *n*-Butane on Addition of Known Amounts of Isobutane

The data obtained for the four substances are collected in Table I and are illustrated in Figures 1, 2, 3, and 4, where the experimental points are shown, together with the ideal line for the lowering of the freezing point. For the mixture of *n*-octane and *p*-xylene, only 5 mole % of *p*-xylene was used, in order to keep below the eutectic composition near 7 mole % of *p*-xylene. The ideal lines were calculated (*4*, *5*) from the cryoscopic constants for each compound, as taken from the tables of the API Research Project 44 (*1*), as follows, t_1^* being the freezing point for zero

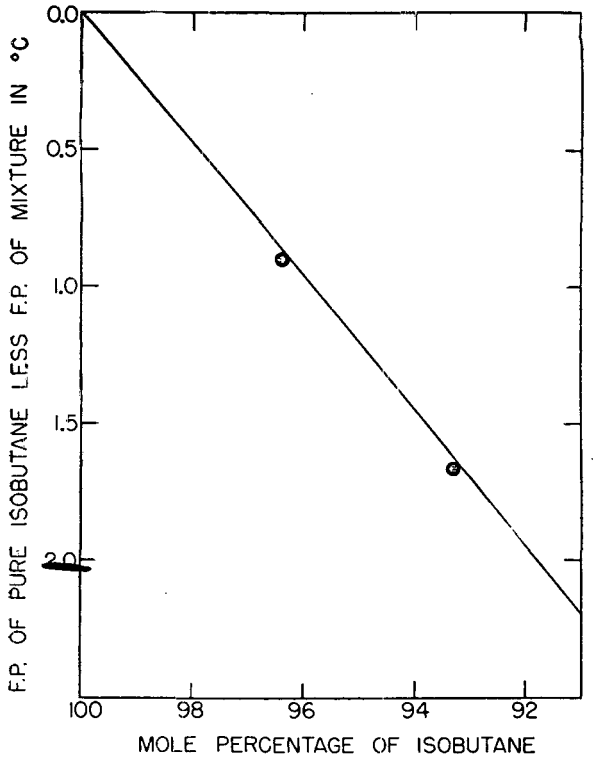

Figure 2. Lowering of Freezing Point of Isobutane on Addition of Known Amounts of *n*-Butane

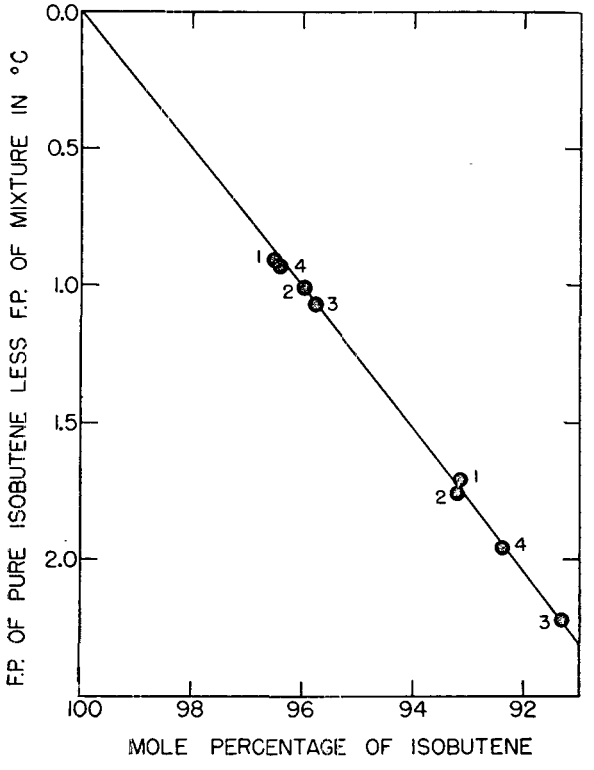

Figure 3. Lowering of Freezing Point of Isobutene on Addition of Known Amounts of Known Impurities

Impurities
1. Isobutane
2. 1-Butene
3. *cis*-2-butene
4. *trans*-2-butene

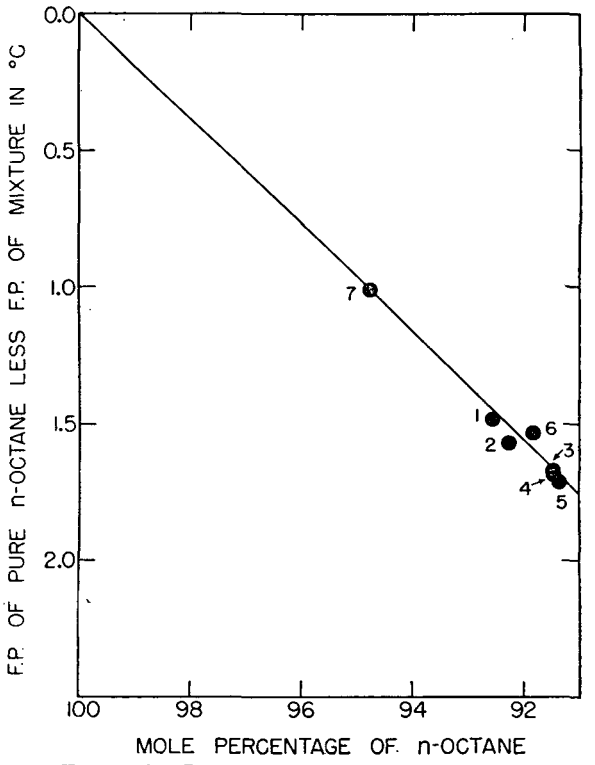

Figure 4. Lowering of Freezing Point of *n*-Octane on Addition of Known Amounts of Known Impurities

Impurities
1. 2,2,4-Trimethylpentane
2. 2,4-Dimethylhexane
3. 1-Methyl-3-ethyl-cyclopentane (*cis* + *trans*)
4. 1-*cis*-2-dimethylcyclohexane
5. 1-*trans*-2-dimethylcyclohexane
6. Ethylbenzene
7. 1, 4-Dimethylbenzene (*p*-xylene)

impurity in ° C., and A and B being the first and second cryoscopic constants (*5*) in mole fraction per ° C.; *n*-butane, $t_1^* = -138.350$, $A = 0.03085$, $B = 0.0048$; isobutane, $t_1^* = -159.600$, $A = 0.04234$, $B = 0.0057$; isobutene, $t_1^* = -140.350$; $A = 0.04044$, $B = 0.005$; *n*-octane, $t_1^* = -56.795$, $A = 0.05329$, $B = 0.0031$.

The deviations from the ideal lines are not significant and, therefore, these substances are ones for which the purity may be evaluated from measurements of freezing points. The results presented here justify the inclusion of *n*-butane, isobutane, isobutene, and *n*-octane in ASTM method of test (*2*).

LITERATURE CITED

(1) Am. Petroleum Inst. Research Project 44, "Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds," Petroleum Research Laboratory, Carnegie Institute of Technology, Pittsburgh, Pa.
(2) Am. Soc. Testing Materials, Philadelphia, Pa., ASTM Method of Test, D 1016.
(3) Glasgow, A. R., Jr., Krouskop, N. C., Beadle, J., Axilrod, G. W., and Rossini, F. D., ANAL. CHEM., 20, 410 (1948).
(4) Glasgow, A. R., Jr., Streiff, A. J., and Rossini, F. D., *J. Research Natl. Bur. Standards*, 35, 355 (1945).
(5) Rossini, F. D., "Chemical Thermodynamics," New York, John Wiley & Sons, 1950.
(6) Rossini, F. D., Mair, B. J., and Streiff, A. J., "Hydrocarbons from Petroleum," American Petroleum Institute Research Project 6, AMERICAN CHEMICAL SOCIETY Monograph 121, New York, Reinhold Publishing Corp., 1953.

RECEIVED for review March 25, 1954. Accepted July 27, 1954. Investigation performed as part of the work of the American Petroleum Institute Research Project 6 in the Petroleum Research Laboratory, Carnegie Institute of Technology. Part was completed at the National Bureau of Standards, Washington, D. C.

Direct Titration Method for the Determination of Barium

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The determination of barium by direct titration involves the titration of a barium solution with standard potassium sulfate solution, using tetrahydroxyquinone as an internal indicator. The end point of the titration is taken as the point of disappearance of the red barium salt of tetrahydroxyquinone. The method is particularly well suited for industrial control of the concentration of barium in solution.

A TITRATION method for the determination of barium was needed to check the strength of barium nitrate solutions used in the settling of phosphors of fluorescent screens. The inherently longer time required made a gravimetric method unsuitable for industrial control.

A considerable amount of experimental work on the direct titration of sulfate with standard barium chloride has been carried out (*1–12*). Schroeder (*6*) first proposed direct titration for the determination of sulfate using tetrahydroxyquinone as an internal indicator. His method involves the titration of sulfate in a sample with a standard barium chloride solution, the end point being indicated by the appearance of the red barium salt of tetrahydroxyquinone. Because solutions of tetrahydroxyquinone are unstable, Schroeder suggested addition of a few tenths of a gram of a dry dispersion of the indicator with a large amount of potassium chloride to each sample on which a sulfate determination is made.

Schroeder also studied the effect of acid concentration, temperature, and the presence of various other ions. The presence of hydroxyl, chloride, carbonate, silicate, calcium, magnesium, or aluminum was found to be inconsequential, except in high concentrations. The presence of iron caused interference.

Sheen and Kahler (*8*) extended the range of sulfate concentrations over which the titration method is applicable. They recommended the use of isopropyl alcohol in place of ethyl alcohol for lowering the solubility of barium sulfate and hastening precipitation. They also found that up to 60 p.p.m. of phosphate ion can be tolerated if the pH value of the sample is adjusted to approximately 4 before titration, using bromocresol green as an indicator, and that the use of a combination of sodium chloride and silver nitrate sharpened the end point.

In 1940, Kahler (*4*) found that the interfering effect of sulfite could be eliminated by adding a small amount of dilute hydrochloric acid to the solution, and then boiling, cooling, and neutralizing it with sodium hydroxide solution just to the acid side of phenolphthalein before titration.

Table I. Comparison of Volumetric and Gravimetric Methods[a]

Group	Barium Present, Mg.	Barium Found, Mg. Gravimetric	Volumetric
1	0.5	0.7	0.9
		0.7	1.0
		0.8	0.9
		0.8	1.0
		0.7	0.9
		0.7	1.0
2	2.5	2.5	3.9
		2.8	3.9
		2.8	4.1
		2.9	3.8
		2.8	4.1
		2.9	4.1
3	34.3	35.3	35.0
		34.8	35.0
		35.3	34.9
		35.1	35.0
		35.5	35.1
		35.0	35.1
4	105.0	105.9	108.1
		105.8	108.1
		105.9	107.9
		105.8	107.8
		105.8	108.0
		106.3	107.9

[a] Samples in groups 1, 2, and 3 were titrated with 0.025*N* K_2SO_4. A 0.1*N* K_2SO_4 solution was used for titration of group 4.

Table II. Determination of Barium in the Presence of Different Anions

Barium Solution	Ba Solution *N*	Ba Solution Ml.	0.025*N* K_2SO_4, Ml.	Ba ≅ Ba Soln., Mg.	Ba ≅ K_2SO_4, Mg.
$Ba(NO_3)_2$	0.0250	20.0	20.3	34.4	34.8
			20.4		35.0
			20.4		35.0
			20.4		35.0
			20.5		35.2
				Av.	35.0
$BaCl_2$	0.0250	20.0	20.9	34.4	35.9
			20.9		35.9
			20.9		35.9
			21.0		36.1
			20.9		35.9
				Av.	35.9
$Ba(C_2H_3O_2)_2$	0.0264	20.0	21.7	36.3	37.3
			21.6		37.1
			21.5		36.9
			21.7		37.3
			21.6		37.1
				Av.	37.1

The experimental process described in this paper is the reverse of the titration process described in the literature mentioned; it concerns the direct titration of barium with standard potassium sulfate solution, together with a study of the interfering effect of calcium and strontium.

COMPARISON WITH GRAVIMETRIC METHOD

Two series of determinations were carried out, one gravimetrically by the precipitation and weighing of barium as barium sulfate, and the second by the direct titration method. As shown in Table I, the results obtained through the use of the two methods agree closely with the theoretical results, and vary from the theoretical results to approximately the same degree. In the titration method, the results are slightly high, probably because the kinetics of the reaction necessitate the presence of a slight excess of sulfate ions to release the last traces of barium present as barium tetrahydroxyquinolate.

The gravimetric procedure consisted of the addition of 10 ml. of 1 to 1 hydrochloric acid to a 150-ml. sample solution. After the solution had been heated almost to the boiling point, 10 ml. of 1 to 1 sulfuric acid was added with constant stirring and the resulting precipitate digested on a hot plate for 20 minutes. The precipitate was then filtered, washed with hot water containing a small amount of sulfuric acid, and heated at 700° C. for 45 minutes before being cooled and weighed as barium sulfate.

EFFECT OF DIFFERENT ANIONS

Although the initial method for barium titration was used only for barium nitrate solutions, it was decided to determine the effect of several different anions on the titration. The results obtained with nitrate were compared with those obtained for chloride and acetate, as shown in Table II.

Table III. Effect of Calcium on Barium Titration[a]

0.0245N $BaCl_2$, Ml.	0.0245N $CaCl_2$, Ml.	0.0250N K_2SO_4, Ml.	Ca Present, Mg.	Barium, Mg. Present	Barium, Mg. Found
10.0	0	10.0	0	16.8	17.2
	0	9.9	0		17.0
	0	9.9	0		17.0
	10.0	10.0	4.9		17.2
	10.0	9.9	4.9		17.0
	20.0	10.0	9.8		17.2
	20.0	9.9	9.8		17.0
	30.0	10.0	14.7		17.2
	30.0	10.1	14.7		17.4
	40.0	10.0	19.6		17.2
	50.0	10.0	24.6		17.2

[a] 2 ml. of 1.5% silver nitrate and 2 ml. of saturated potassium chloride solution added to sharpen end point.

Interference by phosphate is prevented by adjustment of pH. Other anions that interfere with the titration, such as carbonate and sulfite, are eliminated by boiling at low pH.

EFFECT OF CALCIUM AND STRONTIUM

Relatively large concentrations of calcium can be tolerated during titration, as shown in Table III. The use of silver chloride end-point sharpener is necessary, however, in order to obtain a definite end point.

The presence of strontium in an appreciable amount leads to extremely high and inconsistent results. Strontium sulfate is soluble in water to the extent of 0.0114 grams per 100 ml. (*3*) at 30° C. but is much less soluble in an alcohol-water mixture. The use of a water solution for the titration of barium results in a much less intense color of the indicator and an indefinite end point.

Variation of pH had little effect. Results obtained in the titration of barium-strontium mixtures are presented in Table IV. Because only a small trace of strontium can be tolerated, a preliminary separation of barium and strontium is necessary when strontium is present to any appreciable extent.

EFFECT OF OTHER CATIONS

The following cations were found to have no effect when present in a concentration of 1500 p.p.m.: nickel, cadmium, magnesium, manganese, zinc, mercury(II), chromium(III), and cobalt(II). Interference was caused, however, by the presence of appreciable amounts of lead, aluminum, iron(III), tin(IV), and copper(II).

The majority of interfering cations can be removed by rendering the test solution basic and filtering off the hydrous oxide precipitates before titration.

METHOD OF ANALYSIS

The direct titration method for the determination of barium, as originally devised, was intended for use in controlling the concentration of barium in a barium nitrate solution.

The reagents used in this work include: standard 0.025*N* potassium sulfate solution, tetrahydroxyquinone indicator composed of an intimately ground mixture of disodium tetrahydroxyquinone and dried potassium chloride in a 1 to 300 ratio, and isopropyl alcohol.

A 20-ml. portion of aqueous solution containing 10 to 50 mg. of barium is transferred to a 250-ml. Erlenmeyer flask, 20 ml. of isopropyl alcohol are added, and the solution is mixed. Approximately 0.2 gram of the tetrahydroxyquinone indicator is introduced by means of a plastic measuring dipper; the solution is colored deep red when the flask is swirled. The solution is titrated rapidly with standard 0.025*N* potassium sulfate solution until the deep red changes to a rose color, indicating the approach of the end point. The titration is concluded by the addition of drops of potassium sulfate solution until an orange color appears throughout the body of the solution.

Although no difficulty was experienced in the titration of pure barium solution without the addition of an end-point sharpener, the presence of relatively large quantities of other cations resulted in a rather indefinite end point. The addition of silver nitrate necessitates the presence of an equivalent amount of chloride to prevent the formation of an excessive amount of silver tetrahydroxyquinolate and a resultant interference with the end point. Sharpening of the end point apparently is due to adsorptive forces at the surface of the silver chloride formed. The use of 2 ml. of a saturated solution of sodium chloride and 2 ml. of a 1.5% solution of silver nitrate effected a much sharper end point. The presence of the end point sharpener is especially desirable when large quantities of calcium are present.

Table IV. Effect of Strontium on Barium Titration[a]

0.0245N $BaCl_2$, Ml.	0.0250N $SrCl_2$, Ml.	0.0250N K_2SO_4, Ml.	Sr Present, Mg.	Barium, Mg. Present	Barium, Mg. Found
10.0	0	9.8	0	16.8	16.8
	0	9.8	0		16.8
	5.0	11.2	5.5		19.2
	5.0	11.1	5.5		19.1
	10.0	13.0	11.0		22.3
	10.0	12.6	11.0		21.6
	20.0	12.3	21.9		21.1
	20.0	16.6	21.9		28.5
	20.0	14.2	21.9		24.4
	20.0	17.5	21.9		30.0
	30.0	13.2	32.9		22.7
	40.0	17.6	43.8		30.2
	40.0	14.8	43.8		25.4
	50.0	15.0	54.8		25.8
	50.0	15.3	54.8		26.3

[a] 2 ml. of 1.5% silver nitrate and 2 ml. of saturated potassium chloride solution added to sharpen end point.

In solutions that are too basic, the barium salt of tetrahydroxyquinone is precipitated and yields little color. Barium tetrahydroxyquinone also exhibits little color in acid solutions. Adjustment of the pH is necessary, therefore, in solutions that vary markedly from the neutral point. A pH from 4.0 to 5.0 can be attained by the use of bromothymol blue indicator, and a pH from 8.0 to 9.0 by the use of phenolphthalein. An initial adjustment to a pH of 8.0 to 9.0 is necessary if iron or aluminum is present in the solution. Both iron and aluminum, as well as any other elements that form hydrous oxides in basic solution, are effectively removed at the higher pH. After the precipitated hydrous oxides have been separated by filtering, carbonate and sulfite can be efficiently eliminated in the filtered solution by adjustment to a pH of 3.0 with dilute hydrochloric acid and boiling for 2 or 3 minutes. A final adjustment to a pH of 4.0 to 5.0 and titration at this pH prevents interference by phosphate ion.

For the initial adjustment of pH 0.1*N* hydrochloric acid or 0.1*N* sodium hydroxide was added; 0.01*N* sodium hydroxide was added for the final close adjustment.

The titration range that can be covered by the use of 0.025*N* potassium sulfate solution is 25 to 3750 p.p.m. of barium. By using 0.1*N* potassium sulfate, the upper range can be extended from 3750 to 15,000 p.p.m. If 0.0025*N* potassium sulfate is used in the titration of very low barium concentrations, the end point is too indistinct.

Best results are obtained when the solution to be titrated contains 500 to 2500 p.p.m. of barium and a 0.025*N* potassium sulfate solution is used as titrant.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Betz, W. H., and Betz, L. D., "Water Analysis, Methods, Chemicals, Apparatus," 4th ed., Philadelphia, W. H. and L. D. Betz, 1944.
(2) Betz, W. H., and Betz, L. D., "THQ," Philadelphia, W. H. & L. D. Betz, 1939.

(3) Hodgman, C. D., "Handbook of Chemistry and Physics," 30th ed., p. 500, Cleveland, Ohio, Chemical Rubber Publishing Co., 1947.
(4) Kahler, H. L., IND. ENG. CHEM., ANAL. ED., **12**, 266 (1940).
(5) Peabody, W. A., and Fisher, R. S., *Ibid.*, **10**, 651 (1938).
(6) Schroeder, W. C., *Ibid.*, **5**, 403 (1933).
(7) Scott, W. W., "Standard Methods of Chemical Analysis," Vol. I, 5th ed., pp. 919–20, D. Van Nostrand Co., New York, 1939.
(8) Sheen, R. J., and Kahler, H. L., IND. ENG. CHEM., ANAL. ED., **8**, 127 (1936).
(9) *Ibid.*, **10**, 206 (1938).
(10) Sheen, R. J., Kahler, H. L., and Cline, D. C., *Ibid.*, **9**, 69 (1937).
(11) Smith–New York Co., Inc., Freeport, L. I., "Determination of Sulfate by the Tetrahydroxyquinone Method," 1948.
(12) Wilson, C. W., and Kemper, W. A., IND. ENG. CHEM., ANAL. ED., **10**, 418 (1938).

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Detection of Surface-Active Phenol Ethers with Sulfuric Acid and Formaldehyde

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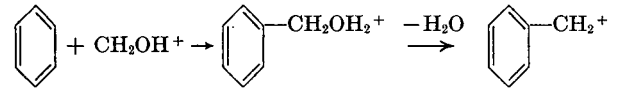
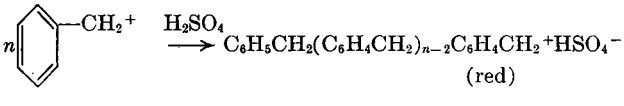
Surface-active agents containing an active benzenoid nucleus, such as *O*-substituted alkylphenols and monosulfonated diphenyls, may be detected by the dark red color which they produce when treated with concentrated sulfuric acid and formaldehyde. Other functional groups present in surfactants produce no interfering colors.

THE qualitative analysis of surface-active agents is a difficult and delicate task because of the multiplicity of types of compounds currently used as detergents, wetting agents, emulsifying agents, germicides, and similar products. Although a number of authors have described qualitative tests for various types of surfactants (*2–5*), the field still suffers from a lack of simple, definitive, qualitative tests for the various functional groups present in these materials.

The use of formaldehyde in the presence of concentrated sulfuric acid constitutes one of the most convenient tests for the presence of nucleophilic aromatic nucleus in organic compounds (*1*). The test consists of the reaction of formaldehyde with an aromatic nucleus in the presence of sulfuric acid to form a carbonium ion, which polymerizes to a colored complex.

$$CH_2O \xrightarrow{H_2SO_4} CH_2OH^+$$





$$n\,\text{—}CH_2^+ \xrightarrow{H_2SO_4} C_6H_5CH_2(C_6H_4CH_2)_{n-2}C_6H_4CH_2^+HSO_4^- \quad \text{(red)}$$

Since both the reaction of the formaldehyde with the aromatic nucleus and the subsequent polymerization are inhibited by electron-attracting substituents on the aromatic nucleus, a positive reaction is obtained only with compounds in which the aromatic nucleus is not deactivated by such substituents—e.g., —Cl, $-SO_3H$, $-NO_2$, —COOH, $-CH_2N^+(R)_3$. Compounds containing benzenoid nuclei give red complexes, while condensed polynuclear aromatics give green, blue, or purple complexes.

The aromatic nucleus appears in a number of types of surfactants, the most important being alkylbenzene sulfonates, alkylphenol ethers, and benzyltrialkylammonium salts. Of these types, however, only the alkylphenol ethers give positive results with this formaldehyde–sulfuric acid test, since, in the other cases, the aromatic nuclei present are deactivated by electron-attracting substituents. Table I lists the reactions to this test of the major classes of surfactants, with and without aromatic nuclei.

PROCEDURE

Fifty milligrams (or 1 drop) of anhydrous surfactant (obtained by methanol extraction or other suitable method from a surfactant-containing composition) is dissolved or dispersed in 0.5 ml. of carbon tetrachloride. One milliliter of 95% sulfuric acid is added, the mixture is agitated well, and the color is noted. One or 2 drops of formaldehyde (approximately 37%) are added, the mixture is agitated well, and the color is again noted.

DISCUSSION OF RESULTS

Of the various classes of surfactants commercially available only ethers of phenols (with the exception of the monosulfonated diphenyls mentioned below) give deep red colors upon addition of the formaldehyde. Other types of compounds give colors varying from very pale yellow to brown-black. However, in these cases the colors obtained are produced upon addition of the sulfuric acid alone, and the addition of the formaldehyde results only in a slight intensification of the existing color. In the case of esters of phenols, however, the deep red color is obtained only upon addition of the formaldehyde; the sulfuric acid by itself produces merely a yellow color.

The only other substances which give sharp color changes upon addition of the formaldehyde are sulfonated polynuclear hydrocarbons (diphenyl, naphthalene) in which at least one ring is unsulfonated. In these cases, the color formation is presumably due to the reactive (unsulfonated) aromatic nucleus present in these compounds. Diphenylmonosulfonates give the usual deep red color, while naphthalene monosulfonates produce a dark green color.

Although ethers of alkylphenols per se are classified as nonionic surface-active agents, the test is not confined to this class alone, but can be used to detect the aryl ether linkage in anionics or cationics as well, provided the aromatic nucleus has not been deactivated. Thus, although Triton X-400, Hyamine 2389, and BTC, whose aromatic nuclei are deactivated by attachment to $-CH_2N^+(R)_3$, all give negative results with this test; Triton X-770, concentrated, and Hyamines 10-X and 1622, which contain reactive alkylphenol linkages, give positive results. The positive results, obtained with both Igepal CO-850, which has a very long polyethoxyethanol side chain, and with octylphenoxyethanol indicate that the length of the chain attached to the aromatic nucleus by the ether linkage has no effect on this reaction.

Table I. Reaction of Commercial Surface-Active Agents to Treatment with Concentrated Sulfuric Acid and Formaldehyde

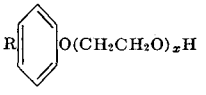
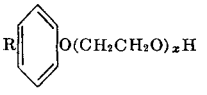
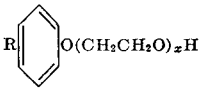
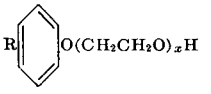
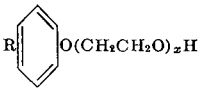
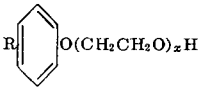
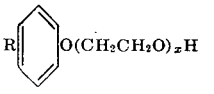
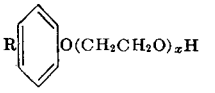
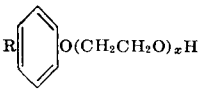
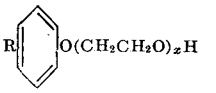
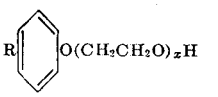
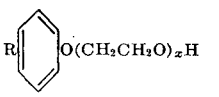
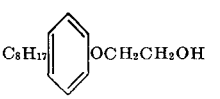
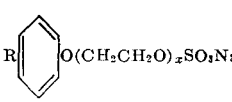
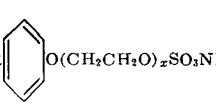
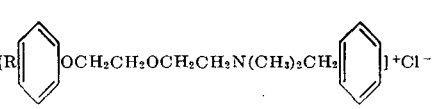
Product	Source	Chemical Structure[a]	Color With H_2SO_4	Color With $H_2SO_4 + CH_2O$	Result
Igepal CA-630	Antara		Yellow	Dark red	+
Igepal CA-710	Antara		Yellow	Dark red	+
Igepal CO-530	Antara		Orange-yellow	Dark red	+
Igepal CO-850	Antara		Orange-yellow	Dark red	+
Neutronyx 600	Onyx		Orange-yellow	Dark red	+
Triton X-100	Rohm & Haas		Orange-yellow	Dark red	+
Triton X-45	Rohm & Haas		Pale yellow	Dark red	+
Dispersant NI-O	Oronite		Orange-yellow	Dark red	+
Dispersant NI-W	Oronite		Orange-yellow	Dark red	+
Tergitol NP-14	Carbide & Carbon		Orange-yellow	Dark red	+
Tergitol NP-35	Carbide & Carbon		Orange-yellow	Dark red	+
Tergitol NPX	Carbide & Carbon		Yellow	Dark red	+
Octylphenoxyethano	Rohm & Haas		Yellow	Dark red	+
Triton X-770 (conc.)[b]	Rohm & Haas		Pale yellow	Dark red	+
Alipal CO-436	Antara		Yellow	Dark red	+
Hyamine 10-X	Rohm & Haas		Pale yellow	Dark red	+

Table I. Reaction of Commercial Surface-Active Agents to Treatment with Concentrated Sulfuric Acid and Formaldehyde (*Continued*)

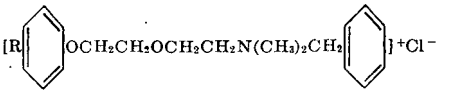
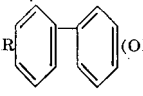
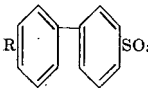
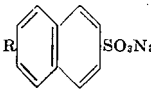
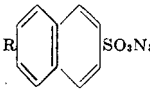
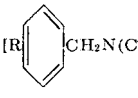
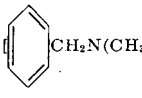
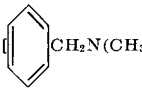
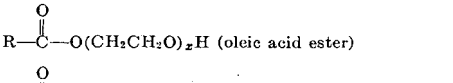
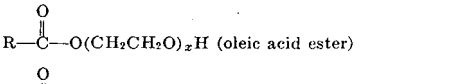
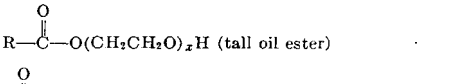
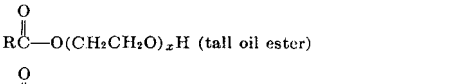
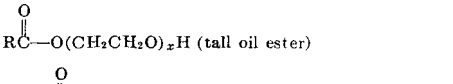
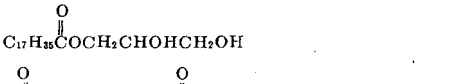
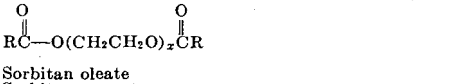
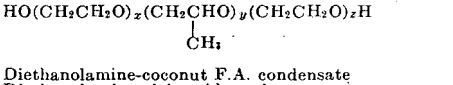
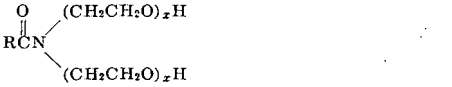
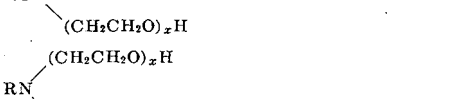
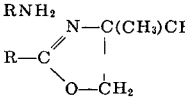
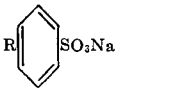
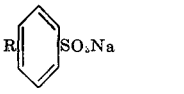
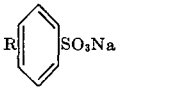
Product	Source	Chemical Structure[a]	Color With H_2SO_4	Color With $H_2SO_4 + CH_2O$	Result
Hyamine 1622	Rohm & Haas		Pale yellow	Dark red	+
Areskap 100	Monsanto		Yellow	Dark red	+
Aresket 300	Monsanto		Light amber	Dark red	+
Aerosol OS	Am. Cyanamid		Red-brown	Dark green	–
Alkanol B	Du Pont		Red-brown	Dark green	–
Hyamine 2389[b]	Rohm & Haas		Colorless	Yellow	–
Triton X-400[b]	Rohm & Haas		Colorless	Light amber	–
BTC[b]	Onyx		Colorless	Pale yellow	
Emulphor VN-430	Antara		Light orange	Light amber	–
PEG[c] 400 monooleate	Kessler		Light orange	Light amber	–
Sterox CD	Monsanto		Red-orange	Light amber	–
Ethofat 242/25	Armour		Red-orange	Red-orange	–
Renex	Atlas		Red-orange	Red-orange	–
Glyceryl monostearate	Glyco		Colorless	Pale yellow	–
PEG[c] 400 dioleate	Kessler		Light orange	Orange	–
Arlacel C	Atlas	Sorbitan oleate	Light orange	Orange	–
Span 60	Atlas	Sorbitan stearate	Pale yellow	Pale yellow	–
Emulphor ON-870	Antara	$RO(CH_2CH_2O)_xH$ (oleyl ether)	Light orange	Light orange	–
Tergitol TMN	Carbide & Carbon	$RO(CH_2CH_2O)_xH$ (alkyl ether)	Colorless	Yellow	–
Sterox SK	Monsanto	$RS(CH_2CH_2O)_xH$	Pale yellow	Pale yellow	–
Pluronic L62	Wyandotte		Pale yellow	Pale yellow	–
Ninol 2012A	Ninol	Diethanolamine-coconut F.A. condensate	Pale yellow	Pale yellow	–
Ninol 201	Ninol	Diethanolamine-oleic acid condensate	Light orange	Amber	
Ethomid C/15	Armour		Pale yellow	Light orange	–
Ethomeen C/15	Armour		Pale yellow	Pale yellow	–

Table I. Reaction of Commercial Surface-Active Agents to Treatment with Concentrated Sulfuric Acid and Formaldehyde (*Continued*)

Product	Source	Chemical Structure[a]	Color With H_2SO_4	Color With $H_2SO_4 + CH_2O$	Result
Armeen C	Armour	RNH_2	Colorless	Beige	–
Alkaterge C	Commercial Solvents		Yellow	Light orange	–
Ultrawet K	Atlantic Refg.		Pale yellow	Yellow	–
Detergent D-40	Oronite		Pale yellow	Pale yellow	–
Nacconol NR	Natl. Aniline		Pale yellow	Light orange	–
Hyponate L50[b]	Sonneborn	Petroleum sulfonate, oil-free	Dark brown	Brown-black	–
Igepon T-73	Antara	$RCN(CH_3)CH_2CH_2SO_3Na$	Yellow	Light orange	–
Aerosol OT	Am. Cyanamid	$ROOCCH_2CH(SO_3Na)COOR$	Pale yellow	Pale yellow	–
Duponol ME dry	Du Pont	$C_{12}H_{25}OSO_3Na$	Colorless	Colorless	–
Duponol LS paste[b]	Du Pont	$C_{18}H_{35}OSO_3Na$	Light orange	Orange	–
Aquasol 75AR	Am. Cyanamid	Sulfated castor oil	Orange	Red-orange	–

[a] R = alkyl.
[b] Dried at 110° C.
[c] PEG = polyethyleneglycol.

The color produced with sulfuric acid alone is indicative of the degree of unsaturation present in the nonaromatic molecules. Thus, a petroleum derivative (Hyponate L50) gives brown colors, tall oil derivatives (Renex, Sterox CD, Ethofat 242/25) give red-orange colors, oleic acid derivatives (Emulphors VN-430 and ON-870, Arlacel C, PEG 400 Dioleate, Ninol 201) yield light orange colors, and saturated compounds (Span 60, Pluronic L62, Ninol 2012A, Armeen C, etc.) produce pale yellow or no colors.

LITERATURE CITED

(1) Davidson, D., and Perlman, D., "A Guide to Qualitative Organic Analysis," p. 26, Brooklyn, N. Y., Brooklyn College Bookstore, 1952.
(2) Gilby, J. A., and Hodgson, H. W., *Mfg. Chemist*, **21**, 423 (1950).
(3) Goldstein, H., *Am. Dyestuff Reptr.*, **36**, 629 (1947).
(4) Linsenmeyer, K., *Melliand Textilber.*, **21**, 468 (1940).
(5) Wurzschmitt, B., *Z. anal. Chem.*, **130**, 105 (1950).

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Detection of Beta-Hydroxyethylamines by Pyrolysis with Sodium Chloroacetate

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β-Hydroxyethylamines, upon pyrolysis with sodium chloroacetate, decompose to yield acetaldehyde. The acetaldehyde, when led into a solution of sodium nitroprusside containing diethanolamine, produces a blue color which may be used as a qualitative test for these amines.

IN A continuation of the work on qualitative tests for the functional groups present in surface-active agents (*2*), it has been found that β-hydroxyethylamines may be detected by a modification of the Hofmann degradation of quaternary ammonium hydroxides (*1*). The β-hydroxyethylamine group is present in several classes of surfactants, such as diethanolamine–fatty acid condensates of the Ninol type, β-hydroxyethylimidazolines, and various hydroxyethylated long-chain amines. In addition, such β-hydroxyethylamines as triethanolamine, diethanolamine, and diethylaminoethanol are commonly used to form salts of fatty acids and other acidic compounds which are important emulsifying agents.

The test depends upon the thermal decomposition of a quaternized β-hydroxyethylamine to yield acetaldehyde, according to the following equation:

$$[R_3\overset{+}{N}CH_2CH_2OH] \longrightarrow CH_3CHO + [R_3NH]^+$$

The formation of acetaldehyde is detected by leading the volatile material produced during the pyrolysis into a solution of sodium nitroprusside containing a water-soluble secondary amine. If acetaldehyde is present, it reacts to form a blue color. This latter reaction is a reversal of the Simon test (*3*) in which water-soluble secondary amines are detected by the blue color (of unknown structure) which they produce with a solution of sodium nitroprusside containing acetaldehyde.

In order to ensure a uniform temperature for the thermal decomposition, an inert, high-boiling solvent was added to the reaction mixture, and constant temperature was maintained by conducting the reaction at its boiling point. After a number of trials using various solvents, the dimethyl ether of tetraethylene

glycol was selected as having physical and chemical characteristics most suitable for the purpose.

Sodium chloroacetate was found to be a more effective reagent than methyl iodide for quaternizing these amines, since its use yielded acetaldehyde with both secondary and tertiary β-hydroxyethylamines, whereas methyl iodide, under the same conditions, yielded acetaldehyde only from tertiary β-hydroxyethylamines. Ethanolamine, the only primary β-hydroxyethylamine, does not yield acetaldehyde when treated with either methyl iodide or sodium chloroacetate, and therefore does not respond to this test. Moreover, the use of sodium chloroacetate makes it unnecessary to convert the quaternary ammonium compound formed to its corresponding hydroxide in order for decomposition to occur. The action of sodium chloroacetate on a tertiary amine produces a betaine, $R_2\overset{+}{N}(CH_2CH_2OH)CH_2COO^-$, which under the conditions of the test, decomposes directly to acetaldehyde.

$$R_2NCH_2CH_2OH + ClCH_2COONa \longrightarrow R_2\overset{+}{N}(CH_2CH_2OH)CH_2COO^- + NaCl$$

Table I. Reactions of Various Amines and Nitrogen-Containing Surface-Active Agents to Pyrolysis with Sodium Chloroacetate

Product	Source	Structure	Color: After 5-min. pyrolysis	Color: + 10-min. standing	Result
Diethanolamine	Carbide & Carbon	$(HOCH_2CH_2)_2NH$	Light blue	"$CuSO_4$" blue	+
Diethanolamine·HCl	[a]	$(HOCH_2CH_2)_2NH_2^+Cl^-$	Royal blue	Deep blue	+
Triethanolamine	Carbide & Carbon	$(HOCH_2CH_2)_3N$	Royal blue	Deep blue	+
Triethanolamine phosphate	Beacon	$[(HOCH_2CH_2)_3NH^+]_3PO_4^{---}$	Royal blue	Deep blue	+
Duponol WAT[b]	Du Pont	$[(HOCH_2CH_2)_3NH]^+OSO_2OR$[c]	Royal blue	Deep blue	+
Diethylaminoethanol	Eastman Kodak	$HOCH_2CH_2N(CH_2CH_3)_2$	Royal blue	Deep blue	+
N-Ethyldiethanolamine	Eastman Kodak	$(HOCH_2CH_2)_2NCH_2CH_3$	Royal blue	Deep blue	+
N-Hydroxyethylpropylenediamine	Carbide & Carbon	$HOCH_2CH_2NHCH(CH_3)CH_2NH_2$	Royal blue	Deep blue	+
N,N'-Dihydroxyethylethylenediamine	Carbide & Carbon	$HOC_2H_4NHC_2H_4NHC_2H_4OH$	Royal blue	Deep blue	+
N-Hydroxyethylmorpholine	Carbide & Carbon	$HOCH_2CH_2N(C_2H_4OC_2H_4)$	Royal blue	Deep blue	+
N-α-Methylbenzyldiethanolamine	Carbide & Carbon	$(HOCH_2CH_2)_2NCH(CH_3)C_6H_5$	Royal blue	Deep blue	+
Amine 220	Carbide & Carbon	$HOCH_2CH_2N(C_2H_4N{=}C{-}R$[c]$)$	Royal blue	Deep blue	+
Amine O	Alrose	$HOCH_2CH_2N(C_2H_4N{=}C{-}R$[c]$)$	Royal blue	Deep blue	+
Versen-ol[b]	Bersworth	$(NaOOCCH_2)_2NC_2H_4N(CH_2COONa)C_2H_4OH$	Royal blue	Deep blue	+
Priminox 43	Rohm & Haas	$HOCH_2CH_2NH{-}R$[c]	Royal blue	Deep blue	+
Priminox 10	Rohm & Haas	$H(OCH_2CH_2)_xNH{-}R$[c] $(x = 5)$	Light blue	"$CuSO_4$" blue	+
Priminox 21	Rohm & Haas	$H(OCH_2CH_2)_xNH{-}R$[c] $(x = 15)$	Light blue	"$CuSO_4$" blue	+
Priminox 32	Rohm & Haas	$H(OCH_2CH_2)_xNH{-}R$[c] $(x = 25)$	Light blue	"$CuSO_4$" blue	+
Ethomeen T/15	Armour	$H(OCH_2CH_2)_x$ $H(OCH_2CH_2)_y$ $>N{-}R$[c] $(x + y = 5)$	Royal blue	Deep blue	+
Ethomeen S/20	Armour	$H(OCH_2CH_2)_x$ $H(OCH_2CH_2)_y$ $>N{-}R$[c] $(x + y = 10)$	Royal blue	Deep blue	+
Ethomeen C/25	Armour	$H(OCH_2CH_2)_x$ $H(OCH_2CH_2)_y$ $>N{-}R$[c] $(x + y = 15)$	Light blue	Royal blue	+
Ethomeen 18/60	Armour	$H(OCH_2CH_2)_y$ $>N{-}R$[c] $(x + y = 50)$	Light blue	"$CuSO_4$" blue	+
Di(β-hydroxyethyl)aniline	Eastman Kodak	$(HOCH_2CH_2)_2N{-}C_6H_5$	Light blue	"$CuSO_4$" blue	+
Diethanolamine-Coc. F.A. condensate	Alrose	Diethanolamine-Coc. F.A. condensate (1:1 molar ratio)	Royal blue	Deep blue	+
Ninol HA10	Ninol	Diethanolamine-Coc. F.A. condensate (1:1 molar ratio)	Royal blue	Deep blue	+
Ninol 2012A	Ninol	Diethanolamine-Coc. F.A. condensate (2:1 molar ratio)	Royal blue	Deep blue	+
Ninol AA62	Ninol	Diethanolamine-lauric acid (2:1 molar ratio)	Royal blue	Deep blue	+
Ninol 201	Ninol	Diethanolamine-oleic acid (2:1 molar ratio)	Royal blue	Deep blue	+
Alrosol O	Alrose	Diethanolamine-oleic acid (2:1 molar ratio)	Royal blue	Deep blue	+
Drisyn	Drew	Diethanolamine-fatty acid condensate	Royal blue	Deep blue	+
Monoethanolamine	Carbide & Carbon	$HOCH_2CH_2NH_2$	Pale yellow	Pale yellow	−
Isopropanolamine	Carbide & Carbon	$HOCH(CH_3)CH_2NH_2$	Pale yellow	Pale yellow	−
Diisopropanolamine	Carbide & Carbon	$[HOCH(CH_3)CH_2]_2NH$	Pale blue-purple	Gray-brown	−
Triisopropanolamine	Carbide & Carbon	$[HOCH(CH_3)CH_2]_3N$	Pale purple	Purple-gray	−
Tributylamine	Eastman Kodak	$(C_4H_9)_3N$	Pale yellow	Pale yellow	−
Tributylammonium chloride	[a]	$(C_4H_9)_3NH^+Cl^-$	Pale yellow	Pale yellow	−
Tribenzylamine	Eastman Kodak	$(C_6H_5CH_2)_3N$	Colorless	Colorless	−
Diethylenetriamine	Carbide & Carbon	$H_2NC_2H_4NHC_2H_4NH_2$	Pale yellow	Pale yellow	−
2-Amino-2-methyl-1-propanol	Commercial Solvents	$HOCH_2C(CH_3)_2NH_2$	Pale yellow	Pale yellow	−
2-Amino-2-methyl-1,3-propanediol	Commercial Solvents	$(HOCH_2)_2C(CH_3)NH_2$	Pale yellow	Pale yellow	−
Tris(hydroxymethyl)aminomethane	Commercial Solvents	$(HOCH_2)_3CNH_2$	Pale yellow	Pale yellow	−
N-Methylglucamine	Commercial Solvents	$CH_3NHCH_2(CHOH)_4CH_2OH$	Pale purple	Purplish gray	−
Ethomid C/15	Armour	$H(OC_2H_4)_x$ $H(OC_2H_4)_y$ $H(OC_2H_4)_x$ $>NCR$[c] $(x + y = 5)$	Purple-gray	Pale blue-gray	−
Ethomid RO/25	Armour	$H(OC_2H_4)_y$ $>NCR$[c] $(x + y = 15)$	Purple-gray	Pale blue-gray	−

[a] Prepared by adding dilute hydrochloric acid to the amine until acid to methyl orange, then evaporating the resulting solution to constant weight.
[b] Dried at 115° C.
[c] R = alkyl or alkenyl group of 12 to 24 carbon atoms.

$$R_2\overset{+}{N}(CH_2CH_2OH)CH_2COO^- \xrightarrow{\Delta} R_2\overset{+}{N}HCH_2COO^- + CH_3CHO$$

The water-soluble secondary amine chosen for use with sodium nitroprusside, in detecting the formation of acetaldehyde, was diethanolamine, since its stability, water-solubility, and low volatility made it appear eminently suitable for the purpose.

Table I lists the reactions to this test of various amines and nitrogen-containing surface-active agents.

PROCEDURE

Two hundred milligrams (or 4 drops) of a nitrogen-containing compound, 0.2 to 0.3 gram of sodium chloroacetate (Dow Chemical Co., technical grade, was used), and 1 to 1.5 ml. of tetraethyleneglycol dimethyl ether (Ansul Chemical Co.) are placed in a 5-inch test tube and agitated vigorously for a few seconds. The test tube is clamped at an angle of no more than 30° from the horizontal (to eliminate spattering during the pyrolysis) and a glass delivery tube with a 60°-angle bend is attached by means of a one-hole rubber stopper. The end of the delivery tube passes beneath the surface of the "detecting solution" contained in a 4-inch test tube supported by a wire gauze placed across an iron ring. In order to facilitate observation of color changes in the detecting solution, a piece of white paper is placed over the wire gauze supporting the test tube containing the solution. The detecting solution consists of 1 ml. of water to which have been added 2 drops of sodium nitroprusside solution (20 grams of $Na_2Fe(CN)_5NO.2H_2O$ dissolved in 50 ml. of water and diluted with 450 ml. of methanol) and 1 drop of diethanolamine.

The contents of the 5-inch test tube are now heated with a small flame at such a rate that bubbles of gas pass through the detecting solution at a rate not exceeding one per second. The contents should boil vigorously and the solvent should reflux from the upper portion of the test tube, but distillation of any appreciable amount of solvent must be avoided. The heating should be continued for no longer than 5 minutes.

The appearance of a definite blue color in the detecting solution during the pyrolysis period constitutes a positive result. As indicated by Table I, most β-hydroxyethylamines give a royal blue color (usually after heating the quaternized amine for about 3 minutes). In those cases where a light blue color is obtained, the detecting solution should be allowed to stand for up to 10 minutes and then re-examined. If the blue color has deepened to a brilliant royal or "copper sulfate" blue, the test is considered positive. If the light blue color persists or fades, the test is considered negative, since traces of blue may be due to β-hydroxyethylamine impurities present in commercial materials of related structure.

DISCUSSION OF RESULTS

All β-hydroxyethylamines tested give positive results with this test. In addition, amines containing one or two polyethoxyethanol groups attached to the amino nitrogen give positive results, although, as the size of the polyethoxyethanol group increases, with consequent decrease in the nitrogen content of the molecule, the results become less definite (Priminox 43 *vs.* 32; Ethomeen T/15 *vs.* 18/60). In di(β-hydroxyethyl)aniline, the presence of the aromatic nucleus, with its electron-attracting capacity, apparently inhibits the decomposition to acetaldehyde, and only a light blue color (which deepens on standing, however) is obtained. This tendency is intensified in the amides where the strongly electron-attracting C=O group inhibits the reaction to such an extent that ethoxylated amides give negative results (Ethomids C/15 and RO/25).

Isopropanolamines (mono-, di-, and tri-) all give negative results, since they cannot decompose to acetaldehyde. This makes the test valuable in distinguishing between emulsifying soaps made with triethanolamine or diethanolamine and those made with di- or triisopropanolamine.

The positive results obtained with diethanolamine hydrochloride, and triethanolamine phosphate indicate that in analyzing amine-containing compositions it should not be necessary to isolate the amine *per se*, but that a salt of the amine, often more conveniently obtained, may be tested instead. The triethanolamine salt of an anionic surfactant (Duponol WAT) can be detected with this method.

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LITERATURE CITED

(1) Hofmann, A. W., *Ber.*, **14**, 494, 659, 710 (1881).
(2) Rosen, M. J., ANAL. CHEM., **26**, 111 (1954).
(3) Simon, L. J., *Compt. rend.*, **125**, 536 (1897).

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Colorimetric Determination of Niobium in the Presence of Tantalum

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The major sources of error in the thiocyanate method for the colorimetric determination of niobium are loss of niobium due to hydrolysis of tantalum present and incomplete extraction of the niobium thiocyanate complex with ether. These effects have been minimized by adding tartaric acid to the reagents, changing the order of additions, and replenishing the thiocyanate and acid between extractions.

THE greatest drawback to the colorimetric determination of niobium with thiocyanate has probably been the interfering effect of tantalum at high ratios of tantalum to niobium. The authors have observed, in using a recently published procedure (*4*), that although satisfactory results are obtained at a 10 to 1 ratio of tantalum to niobium at low levels of niobium concentration, poor results are obtained at the same ratio with larger concentrations. They therefore extended their studies on the thiocyanate method with particular attention to the tantalum interference problem. The two main sources of error have been found to be: incomplete extraction of the niobium from the aqueous phase, and loss of niobium due to hydrolysis of tantalum present, the latter effect being the more important. It has been shown that addition of tartaric acid to the reagents and a change in the order of addition eliminate the erratic interferences of tantalum.

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EXPERIMENTAL

A majority of the reagents were prepared as in previous work (*4*). Niobium and tantalum stock solutions were made up from spectrographically analyzed high purity oxide as before. The oxides were fused in silica crucibles with potassium pyrosulfate, with special care to obtain clear melts, and the cooled masses were taken up in 10% tartaric acid. Close attention to detail was found necessary in high tantalum mixtures if clear solutions were to result. The pure niobium stock solutions were found to be stable, but tantalum stock solutions showed a tendency to hydrolyze on standing. The spectrographically pure oxide

samples were sometimes found to assay as low as 70% niobium pentoxide owing to the presence of moisture and volatile salts. The stock solutions were standardized gravimetrically by classical procedures.

Radioactive niobium-95 was prepared from a zirconium-95–niobium-95 mixture derived from uranium fission and obtained from the United States Atomic Energy Commission. Pure radioactive niobium tracer was prepared by carriage on manganese dioxide according to the method of Siegel, Bigler, and Hume (*5*). Gamma counting was done on liquid samples mounted in small glass cups and covered with lacquer films according to the technique of Freedman and Hume (*2*). A conventional scaling circuit and thin window, bell-shaped Geiger Müller tube was used for counting. Beta rays were removed by 435 mg. per sq. cm. of aluminum absorber.

The techniques of manipulation, extraction, and measurement with a Beckman DU spectrophotometer were essentially those of the previous publication.

ETHER EXTRACTION

In previous work, the presence of much tartrate appeared to affect the absorbance index of niobium in the thiocyanate complex. Since high tartrate concentrations offered the most promising path to avoidance of tantalum hydrolysis, the first efforts were directed to the elimination of direct interference by tartrate. It was observed that even traces of oxalate have a bleaching effect on the niobium thiocyanate color; however, careful analysis of the reagent grade tartaric acid used failed to show the presence of oxalate. Chemical analysis of the ether extracts revealed, however, that the initial portion of ether used to extract niobium also removed about 70% of the total thiocyanate and about 4% of the chloride. Since a high concentration of thiocyanate in the aqueous layer is necessary for efficient extraction, especially in the presence of tartaric acid, subsequent extractions were not removing much additional niobium. The effect of the volume of ether and removal of thiocyanic acid in a single extraction step is shown by the data in Table I.

Table I. Absorbance of 19.4γ of Niobium Extracted with Various Volumes of Ether and Diluted to 25 Ml. for Measurement

Extraction Volume, Ml.	Absorbance
2.0	0.302
5.0	0.302
10.0	0.292
15.0	0.282
25.0	0.208

The extensive removal of thiocyanic acid by a larger volume of ether results in a significant decrease in the efficiency of extraction. When the effect of dilution of the already-extracted color with more ether was determined, it was found that a 5-ml. extract which had been obtained in the usual way was significantly less intense in color when diluted to 25 ml. with pure ether than when diluted with ether which had been saturated with thiocyanic acid. When two extractions were made with 5-ml. portions of ether, with the addition of sufficient potassium thiocyanate and hydrochloric acid to bring back the original concentration, the addition of 15 ml. of fresh ether to the combined ether extract had no bleaching effect. Evidently, sufficient thiocyanic acid is extracted in two operations to prevent decomposition of the complex. In case of doubt, however, ether previously equilibrated with a potassium thiocyanate—hydrochloric acid mixture can be used profitably. It was concluded that extractions subsequent to the first should be made only if sufficient acid and thiocyanate is added to restore the optimum concentrations for niobium extraction, and that the final dilution of the ether extract should have a high enough concentration of thiocyanic acid to give the maximum color intensity of the niobium thiocyanate complex. Although a high thiocyanate concentration in the aqueous phase favors the formation of the complex and its extraction into ether, the thiocyanate concentration must not be raised too high or the blank correction becomes large. When 5 ml. of 20% (grams per 100 ml. of solution) potassium thiocyanate are used as in the standard procedure, the blank, against ether, amounts to only 0.01 or 0.02 absorbance unit. If 40% thiocyanate reagent is used, the blank increases fourfold; and with 80% thiocyanate, the blank increases 50- to 100-fold owing to the rapid formation of colored thiocyanate decomposition products.

The color intensity of the niobium in the aqueous phase and, under conditions of incomplete extraction, in the ether phase, is promoted by a high concentration of hydrochloric acid. It has been suggested that both the hydrogen and chloride ions are involved, inasmuch as color intensity is increased by the presence of magnesium chloride (*3*). In order to verify the importance of the chloride ion, the effects of hydrogen and chloride ions were studied separately.

On the assumption that the effect of hydrochloric acid is due to hydrogen ion alone, it was reasoned that other acids would be equally effective; and if the effect of magnesium chloride were due to magnesium ion, other magnesium salts would behave similarly. When perchloric acid was tried in place of hydrochloric acid, and sodium salts substituted for potassium, it was found that the color development was again increased by increasing the acid concentration in the same range. In another experiment, solutions of equivalent strength, of magnesium chloride and magnesium perchlorate, were added separately to solutions containing niobium, hydrochloric acid (1*M* rather than 4*M*, to allow the effect to be more readily observable), tartaric acid, and potassium thiocyanate. When the solutions were made 0.4*M* in magnesium chloride or magnesium perchlorate, the absorbances of the extracted samples were identical and some 35% greater than if the magnesium salts had not been used. It was therefore concluded that the effect of hydrogen ions and magnesium salts was due to their reaction with tartrate, thereby freeing the niobium for extraction as the thiocyanate complex, and that the chloride ion as such did not enhance the color. Too high a concentration of hydrochloric acid is undesirable, as it is then extracted into the ether. High concentrations of bromide bleach the thiocyanate color.

The efficiency of the ether extraction step was next determined using radioactive niobium-95 as a tracer. Amounts of niobium of the order of 25 γ were taken for extraction after the addition of sufficient niobium tracer to give a gamma counting rate of around 2000 counts per minute. The residual activity in the aqueous phase after two ether extractions was found to be very low, of the order of 20 to 30 counts per minute. This corresponds to a minimum extraction efficiency of 98 to 99%, if all the residual activity is actually niobium, and not traces of zirconium impurity. The estimate by Alimarin (*1*) of the efficiency as less than 50% for a two-step extraction is evidently not valid under the conditions of this procedure. For all intents and purposes, a two-step extraction is quantitative.

EFFECT OF TANTALUM

The interfering action of tantalum was studied in some detail. If niobium and tantalum solutions were extracted separately and the extracts combined, no interference due to tantalum was observable. It was found by the use of radioactive niobium, however, that although niobium could be extracted quantitatively when alone, the presence of equal or greater amounts of tantalum sometimes resulted in the stubborn retention of variable but significant amounts of niobium, even on repeated extraction. The tendency of niobium to remain in the aqueous phase increased with the proportion of tantalum and with the age of the niobium-tantalum solution. All evidence pointed to the co-separation of niobium with colloidal tantalum oxide as the source of the difficulty. Factors which tend to diminish the hydrolysis of tantalum likewise diminish the interference. The best results were obtained in the following manner.

The hydrochloric acid and stannous chloride reagents were

made up to be 1*M* in tartaric acid. The order of addition of reagents was changed so that thiocyanate was the first reagent to be added to the sample. Although the change in order of reagents made no difference when tantalum was absent, a great improvement was observed when tantalum was present. Under these conditions, the niobium was thus forced into the soluble and extractable thiocyanate complex before appreciable hydrolysis of tantalum could take place. For samples containing high percentages of tantalum, it was found necessary to carry through the analysis promptly after dissolution of the melt in tartaric acid: the longer the period of standing, the greater the chance for error, even though the solution appeared to be perfectly clear.

With these precautions, it was found that the presence of ten to twenty times as much tantalum as niobium had no effect on the niobium results, the average values of two series of determinations agreeing within 3%, an accuracy and precision comparable with the original method. Reliable standard samples containing high ratios of tantalum to niobium were not available, but the results of a few determinations run on oxide mixtures suggest that the method is applicable without modification at tantalum to niobium ratios as high as 100 to 1.

RECOMMENDED PROCEDURE

The sample is prepared for analysis by fusion with potassium pyrosulfate and dissolution of the melt in 10% tartaric acid. A volume of 1 to 2 ml. of the solution (containing 1 to 50 γ of niobium) is measured into a 60-ml. separatory funnel followed immediately by 5 ml. of 20% potassium thiocyanate, 2 ml. of 15% stannous chloride containing 1*M* tartaric acid, and 5 ml. of 9*M* hydrochloric acid, also containing 1*M* tartaric acid. The funnel is shaken thoroughly after the addition of each reagent. When all the reagents have been added, the mixture is allowed to stand for 5 minutes, and 5 ml. of ether are added. After 10 seconds of vigorous shaking, the mixture is allowed to stand for 5 minutes and the lower (water) portion is run off into a second separatory funnel. One milliliter of 9*M* hydrochloric acid and 0.7 ml. of 50% potassium thiocyanate, freshly prepared, are added to bring back the original concentration of reagents, and a second extraction is made with 5 ml. of ether. The combined ether extracts are diluted to 25 ml. with additional ether. After standing for 30 minutes to allow water droplets to settle out, the absorbance of the ether extract is read at 385 mμ against a blank treated in exactly the same way.

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LITERATURE CITED

(1) Alimarin, I. P., and Podvalnaja, R. L., *Zhur. Anal. Khim.*, 1, 30 (1946).
(2) Freedman, A. J., and Hume, D. N., *Science*, 112, 461 (1950).
(3) Freund, H., and Levitt, A. E., ANAL. CHEM., 23, 1813 (1951).
(4) Lauw-Zecha, A. B. H., Lord, S. S., and Hume, D. N., *Ibid.*, 24, 1169 (1952).
(5) Siegel, J. M., Bigler, W. P., and Hume, D. N., in "Radiochemical Studies, the Fission Products," ed. by C. D. Coryell and N. Sugarman, Book 3, p. 1532, New York, McGraw-Hill Book Co., 1951.

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Polarographic Determination of Cadmium and Zinc in Zinc Sulfide–Cadmium Sulfide Phosphors

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In a method for the quantitative determination of cadmium and zinc, in which lead is used as an internal standard, the diffusion currents of cadmium and zinc ions are measured in relation to the diffusion current of lead ion. The concentrations of cadmium and zinc are determined by comparison of the results obtained with a calibration curve. The materials used include a maximum suppressor containing methyl red and bromocresol green, a base solution of potassium chloride, and a modified electrolysis cell. The method is simple and rapid in application, and the necessity for constant temperature control is eliminated.

AN IMPROVED method was needed for the quantitative determination of cadmium and zinc in zinc sulfide–cadmium sulfide phosphors used in the manufacture of cathode ray tubes because the established chemical methods for the separation and determination of cadmium and zinc are difficult and time-consuming and not highly accurate.

Polarographic analysis of cadmium and zinc appeared to afford the best method for this determination because it eliminates the need for separating the cadmium from the zinc, which is the most difficult part of a chemical analysis because of the similarity in their chemical properties.

Experimental evidence presented by Lingane (*3*) indicated that the half-wave potential of cadmium ion in a 0.1*N* solution of potassium chloride referred to the saturated calomel electrode at 25° C. is −0.60 volt, and the half-wave value for zinc under the same conditions is −1.00 volt. Because a difference of only 0.1 volt between the half-wave potentials of two different ions is necessary for distinction of the polarographic "waves" obtained on a current-voltage curve, determination of the two ions in question is feasible.

Compensation for temperature variations was accomplished by the use of an internal standard as first suggested by Forche (*2*), who worked with cadmium and lead.

In 1948, Loofbourow and Frediani (*4*) discussed the internal standard method and provided experimental data for the system, lead, cadmium, zinc, in 0.1*N* potassium chloride.

Lead was selected as an internal standard because the half-wave potential of lead referred to the saturated calomel electrode is −0.40 volt. The solubility of lead chloride is sufficiently high for complete solution in the concentrations ordinarily employed in polarographic analysis.

Only four voltage settings and subsequent current readings were used in routine work in the manner described by Copeland and Griffith (*1*).

EXPERIMENTAL

Apparatus. The Fisher Elecdropode and a modified electrolysis cell requiring 5 to 10 ml. of test solution were used to obtain the current-voltage curves. A saturated calomel electrode was used as a reference electrode.

Reagents. The following reagents were used.

Potassium Chloride Solution. A 0.2*N* solution containing 5 ml. of a solution of methyl red and bromocresol green per liter.

Methyl Red–Bromocresol Green Solution. Three parts of a

0.2% alcoholic solution of methyl red added to 2 parts of a 0.2% alcoholic solution of bromocresol green.

Standard Lead Solution. One gram of spectrographically pure lead dissolved in the minimum amount of 1 to 1 nitric acid, and diluted with distilled water to 1 liter in a volumetric flask.

Standard Cadmium Solution. A solution containing 1 gram of cadmium in 1 liter prepared by dissolving 1.6309 grams of anhydrous cadmium chloride in a small amount of distilled water, and diluting to 1 liter.

Standard Zinc Solution. One gram of spectrographically pure zinc dissolved in the minimum amount of 1 to 10 hydrochloric acid, and diluted with distilled water to 1 liter in a volumetric flask.

Analytical Procedure. A phosphor sample of approximately 0.02 gram was weighed in a 1-ml. beaker, and the beaker and contents were lowered gently into a 50-ml. beaker to prevent loss of phosphor by brush transferral. The phosphor sample was dissolved in 10 ml. of 1 to 1 hydrochloric acid, and was heated slightly to hasten solution. After solution was complete, the 1-ml. beaker was raised above the surface of the liquid by means of a stirring rod, rinsed thoroughly with distilled water, and removed.

The solution was then evaporated to a small volume on a hot plate and to final dryness on a steam bath. Residual salts contained in the beaker after evaporation were dissolved in approximately 5 ml. of distilled water and were transferred with rinsing to a 100-ml. volumetric flask. Care was exercised in rinsing so that the total volume of solution did not amount to more than 35 ml. A 10-ml. portion of standard lead solution (1 mg. per ml.) was added as pilot ion or internal standard, and 50 ml. of potassium chloride solution containing a maximum suppressor of methyl red and bromocresol green were added as supporting electrolyte or base solution. The solution was then diluted to 100 ml. with distilled water, and mixed thoroughly, and a 5- to 10-ml. portion was transferred to the electrolysis cell of the polarograph.

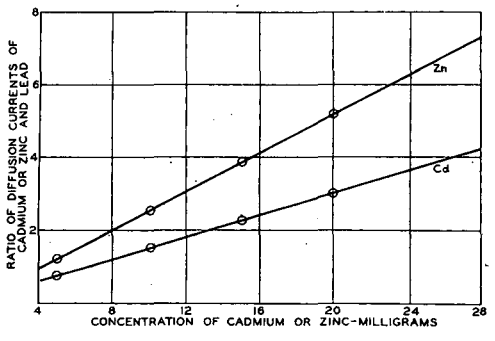

Figure 1. Calibration Curve

Nitrogen gas was bubbled through the solution in the electrolysis cell for a period of 5 minutes to ensure the complete removal of oxygen. At the end of the 5-minute oxygen-removal period, the flow of nitrogen through the solution was interrupted and directed over the surface of the solution during the course of the analysis preventing the entrance of oxygen from the air.

The rate of fall of mercury drops from the capillary tube was adjusted to 3 to 5 seconds per drop by adjustment of the content of the mercury reservoir above the capillary. After the proper rate was attained, the height of the mercury column in the filling tube of the reservoir was noted, as determined by a graduated scale attached to the upper end of the filling tube. A constant height of mercury was maintained in the filling tube by the addition of mercury to the noted level at the beginning of each series of readings.

The choice of voltage points for the current reading was based on a complete current-voltage curve for lead, cadmium, and zinc ions in solution.

Table I. Polarographic Determination of Cadmium and Zinc in Solutions of Known Concentration

Sample No.	Cadmium, Mg.		Zinc, Mg.	
	Added	Recovered	Added	Recovered
1	7.0	6.9	7.0	7.1
	7.0	6.9	7.0	7.1
2	9.0	8.9	9.0	9.1
	9.0	8.9	9.0	9.1
3	12.0	11.9	12.0	12.1
	12.0	11.9	12.0	12.1

PREPARATION OF CALIBRATION CURVE

A calibration curve was prepared by polarography of four solutions having known cadmium and zinc content. The solutions were made from the standard cadmium and zinc solutions. In each case, a 10-ml. portion of standard lead solution was added as internal standard.

The ratios of diffusion currents (ratios of i_d values) were calculated for both cadmium and zinc in relation to the lead internal standard.

The calibration curve obtained by plotting the ratios of i_d values with respect to concentration for both cadmium and zinc is shown in Figure 1.

ANALYSIS OF SYNTHETIC SAMPLES

The accuracy and precision of the polarographic analysis were ascertained by the analysis of solutions of cadmium and zinc having known concentrations. The results obtained are given in Table I.

ANALYSIS OF ZINC SULFIDE–CADMIUM SULFIDE PHOSPHORS

Phosphor samples having varying mole ratios of zinc sulfide to cadmium sulfide were treated in the manner given above. The diffusion current ratios for cadmium and zinc with respect to lead were determined, and the corresponding values for concentration of cadmium and zinc were determined from the calibration curve. Concentrations of cadmium and zinc were converted to the respective sulfides. A tabulation of the results obtained is shown in Table II.

Table II. Analysis Results for Zinc Sulfide–Cadmium Sulfide Phosphors

Blend	Cadmium Sulfide, %	Zinc Sulfide, %	Total, %
A	32.5	67.3	99.8
	33.0	66.8	99.8
	33.3	66.1	99.4
	32.9	67.6	100.5
B	50.2	50.8	101.0
	50.9	49.7	100.6
C	41.6	59.0	100.6
	40.4	59.9	100.3
D	70.2	30.5	100.7
	70.1	30.8	100.9
E	20.0	79.1	99.1
	20.2	80.1	100.3
F	9.6	90.8	100.4
	9.9	90.8	100.7
G	79.6	21.2	100.8
	79.3	21.1	100.4

The inherent error in the observation of galvanometer deflections was greatly reduced by the use of the maximum possible sensitivity of the Elecdropode rather than a constant sensitivity of 20 times. Sensitivities of 1, 2, 5, 10, and 20 times were used, and all galvanometer deflections were converted to a sensitivity of 1 before the diffusion-current ratios used in the calculation of the results given in Table II were obtained. A second calibration curve was prepared using maximum sensitivities instead of a constant sensitivity of 20 times.

CALCULATIONS

The percentage of cadmium or zinc in the test solution was calculated as follows:

Per cent cadmium sulfide

$$\frac{\frac{(CdS)}{(Cd)}(100)}{\text{Mg. of sample}} = \% \text{ CdS}$$

Per cent zinc sulfide

$$\frac{\frac{(ZnS)}{(Zn)}(100)}{\text{Mg. of sample}} = \% \text{ ZnS}$$

Galvanometer deflections were converted from one sensitivity to another as follows:

$$\text{(Galvanometer reading)} \frac{\text{(sensitivity used)}}{\text{(sensitivity desired)}} = \text{converted galvanometer reading}$$

The diffusion-current ratios were obtained as follows:

$$\frac{i_d \text{ of Cd or Zn}}{i_d \text{ Pb}} = \text{diffusion-current ratio}$$

CONCLUSION

The polarographic determination of cadmium and zinc in zinc sulfide–cadmium sulfide phosphors provides a simple and rapid means for the quantitative determination of these two elements. This method has the added advantage of providing a simultaneous determination of cadmium and zinc, thereby eliminating the necessity for time-consuming separations. In addition, the required sample size is reduced to a minimum.

By the use of the internal-standard procedure, the necessity for constant temperature control is eliminated. If the ratio of zinc to cadmium is the primary consideration, no internal standard need be used and no weighing of the sample is necessary. By this procedure different areas of a cathode ray tube fluorescent screen can be compared for zinc-cadmium ratio.

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LITERATURE CITED

(1) Copeland, L. C., and Griffith, F. S., ANAL. CHEM., 22, 1269 (1950).
(2) Forche, H. E., *Mikrochemie*, 25, 217 (1938).
(3) Lingane, J. J., IND. ENG. CHEM., ANAL. ED., 15, 583 (1943).
(4) Loofbourow, R., and Frediani, H. A., *Analyst*, 73, 384 (1948).

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Ultraviolet Spectrophotometry of Tellurium Sols

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Sols of elementary tellurium present two principal absorption bands. For the visible band, shifts in spectral positions are observed with variation of particle characteristics. The behavior and usefulness of the ultraviolet band are the subject of the present investigations. Good adherence to Beer's law and good reproducibility are obtained with the ultraviolet band. It does not shift spectral position with variation in particle size. Although its maximum lies at 280 to 290 mμ, any wave length between 240 and 290 mμ may be chosen for analytical purposes. This freedom of choice is a definite advantage.

THE trace determination of tellurium by spectrophotometry of the elementary tellurium hydrosol is complicated by the dependence of spectral properties upon size and geometric characteristics of sol particles. It has been shown that by varying the conditions appropriately, sols varying in color from blue to purple to red to amber are produced (*6*). The color depends upon the position in the visible range of a broad spectral band (Figure 1). Unfortunately, the band varies in character and position for a series varying only in tellurium concentration; hence, the wave length for quantitative visible spectrophotometry must be chosen with regard to certain unusual considerations described previously (*8*)—i.e., the wave length is intermediate in the range of wave length maxima found in a concentration series.

ULTRAVIOLET BAND

Tellurium sols also present an ultraviolet band which is specific for elementary tellurium. This band has an absorption maximum between 280 and 290 mμ in all sols so far investigated (Figure 1). It is suitable for quantitative analysis, yielding a satisfactory Beer's law relationship at any wave length in a rather wide spectral region, 240 to 290 mμ, for blue, purple, red, and amber sols. At longer wave lengths, there is danger that the two bands will overlap. The freedom of choice of wave length in the ultraviolet band gives it an advantage over the visible band, for which the choice of appropriate wave length for analytical purposes requires special attention.

The absorbance of the ultraviolet band increases considerably, with decrease in particle size being about one third greater in red sols than in blue sols. (The corresponding effect in the visible

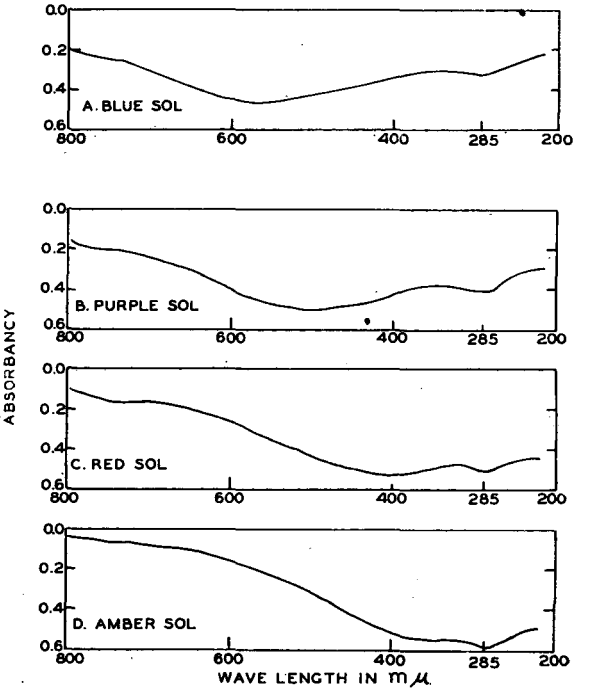

Figure 1. Absorption Spectra of Tellurium Sols

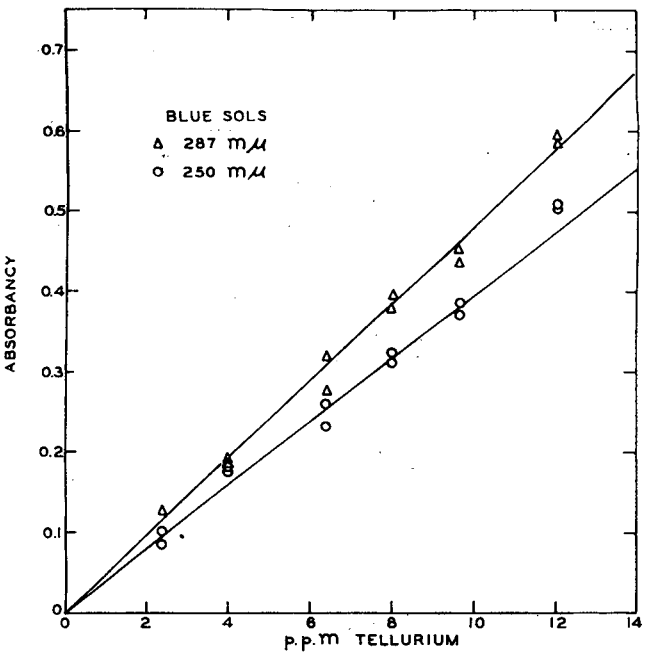

Figure 2. Beer's Law, Blue Sols

band is very small). Because this is so, the slope of the Beer's law curve may vary slightly with changes in reagents, techniques, and analysis. Frequent calibration checks are therefore indicated until the method has been well established in a given laboratory, and whenever changes are made which might affect particle formation.

REAGENTS AND SOL PREPARATION

Tellurium sols formed from the hypophosphorous acid—gum arabic system proved most satisfactory for analytical ultraviolet spectrophotometry. The conclusions reached in the previous investigation of this system generally hold for the present analysis, and the recommended procedure is based on them (*6*, *8*). A few points have received special or further attention in this investigation.

Both hypophosphorous acid and gum arabic absorb in the ultraviolet, and precision measurement of these reagents into the blank and each test solution is necessary.

All operations in the process of sol formation should be carried out as reproducibly as possible. The reaction medium should be vigorously swirled throughout addition of the reagent and for a few seconds afterward. Reagent solution may be blown from the pipet or allowed to drain by gravity. The former method yields slightly greater absorbance and linearity.

Adsorption of sol particles on glassware introduces an error, which is significant in higher sol concentrations and is more noticeable in blue than in red sols. This adsorption can be greatly decreased by applying a silicone film—e.g., Desicote—to the glass. The film is simply and readily applied, and should be frequently renewed. Satisfactory results may be obtained without the silicone treatment, but it is recommended when the best results are sought. Adsorbed tellurium is instantly removed with nitric acid. Silicone treatment of absorption cells is also recommended.

As a protective colloid, gelatin has both advantages and disadvantages in comparison with gum arabic. Gelatin is effective in lower concentrations than gum arabic is. Satisfactory sols are produced in gelatin concentrations as low as 0.01%. Hypophosphorous acid concentrations specified for various sols in 0.25% gum arabic must be approximately doubled to obtain the respective sol in 0.01% gelatin. Sols with gelatin do not foam as easily as those with gum arabic. Since gelatin absorbs very strongly at wave lengths shorter than 300 mμ, it must be used in minimal amounts and be very precisely measured out. Sols in minimal gelatin have disadvantages that outweigh the advantages of gelatin: The particles are very easily adsorbed on glass and even on silicone films, and sols show a more pronounced shift toward blue colors with increasing tellurium concentration than is observed with gum arabic sols. This shift is associated with increased deviation from Beer's law. In general, gum arabic is preferred to gelatin, for analytical use.

Stannous chloride, though effective in forming tellurium sols, is not useful in ultraviolet spectrophotometry because of its strong absorption in this region (*9*).

Instruments Used. Cary Model 11 recording spectrophotometer and Beckman Model DU spectrophotometer.

Reagents Used. Hypophosphorous acid, 3*M*, is made by diluting 32 ml. of Mallinckrodt purified 50% (9.5*M*) hypophosphorous acid [or 60 ml. of USP 30% (5*M*) hypophosphorous acid] with water to 100 ml.

Gum arabic powder (Schaar and Co.) in 100 ml. of hot water, centrifuged to remove large particulate matter. (For best results, gum arabic solution is prepared fresh daily.)

Tellurium standard, 50 p.p.m. of tellurium in 0.2*N* hydrochloric acid; 0.537 gram of potassium tellurium hexabromide (*5*) is dissolved in 100 ml. of 4*N* hydrochloric acid and diluted to 2 liters.

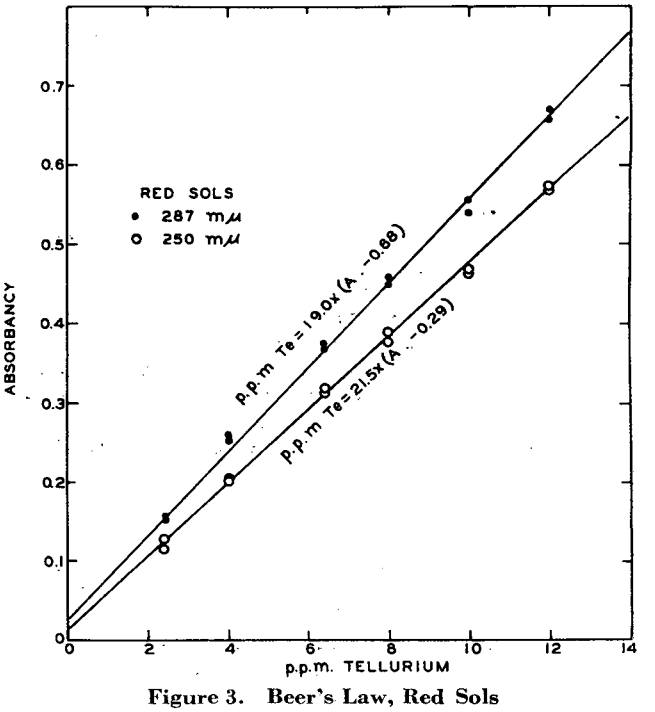

Figure 3. Beer's Law, Red Sols

Recommended Procedure (Red Sols). To a solution containing 0.1 to 0.7 mg. of quadrivalent tellurium and 1 to 8 meq. of hydrochloric acid in a 125-ml. Erlenmeyer flask, add 3 ml. of 4% gum arabic and sufficient water to make the volume 35 ml. Heat to boiling. While rapidly swirling the mixture, add rapidly from a pipet 5 ml. of 3*M* hypophosphorous acid. Continue swirling for a few seconds after addition is complete. Allow to digest near the boiling point for 15 minutes and cool in a bath of tap water for 15 minutes. Transfer to a 50-ml. volumetric flask and dilute to the mark. Read the absorbance at a wave length in the region 240 to 290 mμ.

BEER'S LAW AND REPRODUCIBILITY

Prototype blue sols and red sols were prepared, development taking place in 0.06*M* and 0.4*M* hypophosphorous acid, respectively. Absorbances were measured at 287 and 250 mμ as

Table I. Precision of Method in an Analysis of Variance

Error	Sum of Squares	Degrees of Freedom	Mean Square, A	F	Standard Error, P.P.M. Te
Blue sols at 250 mμ					
About regression	0.00110	4	0.000274	2.1	
Within replications	0.00078	6	0.00013		
Total	0.00188	10	0.000188		0.32
Blue sols at 287 mμ					
About regression	0.00102	4	0.00026	1.0	
Within replications	0.00159	6	0.00027		
Total	0.00261	10	0.00026		0.33
Red sols at 250 mμ					
About regression	0.000229	4	0.000057	1.8	
Within replications	0.000194	6	0.000032		
Total	0.000423	10	0.000042		0.14
Red sols at 287 mμ					
About regression	0.000314	4	0.000078	1.4	
Within replications	0.000335	6	0.000057		
Total	0.000549	10	0.000065		0.15

The error "about regression" represents deviations from Beer's law (linearity).
The error "within replications" represents errors not attributable to changes in tellurium concentration—e.g., errors in pipetting or reading absorbances.
The "total" error includes both of the aforementioned errors and best represents the error in a determination in which a linear relationship (Beer's law) is assumed for the data.
The mean squares are variance estimates, s^2 for absorbances.
The F values compare deviations from linearity with deviations within replications, a high F value indicating nonlinearity.

shown in Figures 2 and 3. The wave length 287 mμ is at the maximum; 250 mμ is an arbitrarily chosen wave length not on an absorption plateau. There is no significant effect on reproducibility (standard deviation in parts per million of tellurium) from choice of wave length between 290 and 240 mμ (Table I). Measurements made at 260 and 240 mμ further support this statement. In this greater freedom of choice of wave length lies a distinct advantage of the ultraviolet band over the visible band.

The superior reproducibility of red sols recommends them for analytical purposes. All curves are linear according to analyses of variance—i.e., no significant F-values are found (Table I). Red sols give a negative intercept on the concentration axis, which becomes more negative with increase in wave length. In these sols, tellurium concentration of 2 p.p.m. or less tend to give low absorbances relative to the linear calibration curve. Reproducibility and linearity characteristics for the ultraviolet band are very similar to those for the visible band; the sensitivity is slightly less for the ultraviolet band.

APPLICATIONS AND INTERFERENCES

Elementary tellurium sols have been used in visible colorimetry and spectrophotometry for analysis of ores (*12*, *13*), steels (*2*), industrial atmospheres (*4*, *11*) and biological material (*4*). Preliminary separations are usually indicated.

Notable among methods for separating trace amounts of tellurium is coprecipitation of tellurium in selenium by hypophosphite followed by volatilization of selenium as the bromide (*10*). Reduction with stannous chloride is also useful (*1*, *2*, *4*, *7*). If stannous chloride is used, measurements should be made at 280 to 290 mμ, because of strong absorption by chloro tin complexes at shorter wave lengths. Other ions whose chloro complexes absorb in the ultraviolet are ferric, bismuth(III), vanadium(V), molybdenum(VI), titanic, cuprous, plumbous, mercuric, and thallous. Absorption spectra of these ions are given by Rogers *et al.* (*3*, *9*). In most cases, interferences can be eliminated by proper selection of wave length for measurement. Alkaline earth ions and chloro complexes of chromic, nickelous, zinc, and aluminum ions do not absorb in the ultraviolet. Other general classes of interferences are strong oxidizing agents, agents which strongly complex tellurium, substances forming sols under similar conditions, and indifferent electrolytes in concentrations greater than 0.5M (*8*).

LITERATURE CITED

(1) Brown, E. G., *Analyst*, **79**, 50 (1954).
(2) Crossley, P. B., *Ibid.*, **69**, 206 (1944).
(3) DeSesa, M. A., and Rogers, L. B., *Anal. Chim. Acta*, **6**, 534 (1952).
(4) DeMeio, R. H., ANAL. CHEM., **20**, 488 (1948).
(5) "Inorganic Syntheses," Vol. 2, p. 189, New York, McGraw-Hill Book Co., 1946.
(6) Johnson, R. A., ANAL. CHEM., **25**, 1013 (1953).
(7) Johnson, R. A., and Kwan, F. P., *Ibid.*, **23**, 651 (1951).
(8) Johnson, R. A., Kwan, F. P., and Westlake, D., *Ibid.*, **25**, 1017 (1953).
(9) Merritt, C., Jr., Hershenson, H. M., and Rogers, L. B., *Ibid.*, **25**, 572 (1953).
(10) Southern, H. K., *Rept.* **Br-606**, May 31, 1945, cited in Natl. Nuclear Energy Ser., Div. VIII, **1**, Anal. Chem. Manhattan Project, p. 318, New York, McGraw-Hill Book Co., 1950.
(11) Steinberg, H. H., Massari, S. C., Miner, A. C., and Rink, R., *J. Ind. Hyg. Toxicol.* **24**, 183 (1942).
(12) Volkov, S. T., *Zavodskaya Lab.*, **5**, 1429 (1936).
(13) Zemel, V. S., *Ibid.*, **5**, 1433 (1936).

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Coulometric Determination of Orthophosphate

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Orthophosphate is determined by passing the sample through a cation exchange column in hydrogen form and determining orthophosphoric acid by differential titration with electrolytically generated base.

A SHORT investigation of the use of the method of Helrich and Rieman (*2*) for the determination of orthophosphate in microsamples has been made. The sample is converted to a mixture of acids by passing it through a cation exchange column in the hydrogen form; the orthophosphoric acid is then determined by differential titration with standard base. For microsamples, the base is added coulometrically in order to avoid the interference of carbonate in the procedure. Excess strong acid does not interfere; weak acids such as acetic must be removed prior to titration.

To determine the first end point of the differential titration, Helrich and Rieman used methyl red, which the authors also found to be useful. The second end point is in the range of phenolphthalein or thymol blue; for microsamples, thymol blue is preferred because of the sharper color change.

EQUIPMENT

The resin is used on a 3-ml. coarse fritted funnel (Corning 36290). About 1 gram of resin is placed on the filter and covered with carbon dioxide–free water. The flow through the filter should be slow, so that the sample can be held for 5 to 7 minutes.

The coulometric apparatus for titration with base has been described (*1*). A bromide medium was used with a silver anode and a platinum cathode. Sample vials of 8-ml. capacity were the titration vessels. Stirring was done with a magnetic stirrer.

Table I. Titration of Phosphate in Standards

Sample Compn., M				No. of	Found, Av.	Precision,
UO_2^{++}	PO_4^{---}	SO_4^{--}	NO_3^-	Detns.	M of PO_4^{---}	M
0.24	0.277	...	3.2	13	0.282	±0.013
0.24	0.139	0.6	3.2	7	0.145	0.004
0.24	0.208	0.5	3.2	8	0.202	0.005
0.28	0.416	0.5	3.3	9	0.408	0.016

PROCEDURE

To exchange metallic cations for hydrogen ion: Pipette sample onto a filter holding 1 ml. of resin covered with carbon dioxide–free water. Hold in the filter 5 to 7 minutes. The sample must be 1 to 2N mineral acid. It should contain 0.005 to 0.05 millimole of phosphate.

Slowly pass treated sample into titration vessel. Rinse with 5 ml. of carbon dioxide–free water. Rinse with 1% hydrochloric acid if much iron or aluminum is present to prevent precipitation of basic phosphates.

To provide electrolyte for the base generation: Add 500 μl. of 500 grams per liter of potassium bromide solution to the vessel. Add 100 μl. 0.1% methyl red indicator.

To preneutralize the strong acids: Add 0.1N sodium hydroxide dropwise until the methyl red end point is passed. Add 0.01N nitric or hydrochloric acid until the end point is reached, then about 10 to 25 μl. more. The sodium hydroxide must be carbon dioxide–free. Prepare from 50% sodium hydroxide and carbon dioxide–free water daily.

To achieve precise adjustment of strong acid neutralization: insert electrodes and titrate to the methyl red end point. A buffer solution with indicator can be used to match the end point. See (*2*).

Titration step: Reset the clock. Add 100 μl. of 0.1% thymol blue indicator. Titrate to the blue end point. The end point can be matched as indicated above.

At the completion of the titration: Record the time. Clean the assembly. Clean the electrodes. Replace the resin. The electrodes must be freed of silver bromide. Use hydrogen iodide (specific gravity, 1.7), followed by acetone, then rinse with water.

CALCULATIONS

$$\text{Grams per liter of orthophosphate} = \frac{i \times t \times 60}{96{,}500} \times \frac{10^3}{S} \times PO_4 = 59.054 \frac{i \times t}{S}$$

i = current, ma.
t = time, minutes
S = sample size, μl.

RESULTS

Table I gives the results of a series of runs made on samples containing uranium, orthophosphate, sulfate, and nitrates. The precision is the standard deviation of a single value. Sample size was 25 μl. The samples were made up from aliquots of standardized solutions to give the indicated solution composition. The phosphate solution was standardized by precipitation as ammonium phosphomolybdate and titration with a base.

These results show that the method is practical on the micro scale and that orthophosphate determination in the presence of uranium is feasible.

LITERATURE CITED

(1) Carson, W. N., Jr., and Ko, Roy, ANAL. CHEM., **23**, 1019 (1951).
(2) Helrich, K., and Rieman, W., IND. ENG. CHEM., ANAL. ED., **19**, 651 (1947).

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Automatic Titrating and Recording Apparatus

For Microbiological Assays

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An automatic titrating and recording apparatus for microbiological assays has been developed to facilitate the handling of the vast numbers of titrations that must be performed in running routine assays. This instrument can automatically titrate and record results on 225 samples consecutively with a high degree of precision (less than 1% standard error) and an accuracy of better than 97%. The precision and accuracy of the instrument are much better than the limits of error normally experienced in the microbiological procedures themselves. By suitable modification, the principles involved can be adapted to assays of vitamins and amino acids, acid base titrations, oxidation and reduction titrations; and similar procedures.

IN ROUTINE analytical procedures the trend toward time-saving, automatic instruments with recording attachments has gained momentum in recent years. One of the simple operations which has been notably improved with respect to the time factor is volumetric titrations. Great strides have been taken to remove the buret from the hands of the chemist and technician and place it in the steel clasp of an automatic titrator. Various techniques are used for adding titrant and measuring its volume (*1*, *2*, *5–8*, *10*, *11*). Lingane (*8*) pointed out that the motor-driven, screw-pushed syringe could be timed to measure the volume delivered. The Coleman and Beckman titrators use burets; the Precision titrator uses a syringe; whereas Cannon in his dispenser-titrator (International Instrument Co., Los Angles, Calif.) uses a timed rate of flow of liquid through a calibrated orifice, the liquid being under a controlled constant head of pressure. However, none of the present devices incorporates a mechanism for automatically recording the titration value, nor provides for mechanically and automatically changing the sample.

APPARATUS

The instrument (Figure 1) is an automatic titrating and recording apparatus designed primarily for use in microbiological assays but offering, through suitable modifications, several other applications.

The apparatus consists of: a pH-sensitive electronic controller, a glass-calomel electrode assembly, a sample carriage and automatic sample changer, a solenoid-operated polystyrene valve (*9*), an automatic recording interval timer, and electronic manometers for maintenance of constant pressure head (of nitrogen) on the alkali supply for titration, and the nitrogen gas supply for stirring samples during titration.

The pH-sensitive electronic controller was designed and built to include the following features:

Sensitivity to inputs of 2 to 3 mv. (less than 0.1 pH unit) with input electrode currents of the order of 10^{-14} ampere at normal

pH equivalent potentials—i.e., 58 mv. per pH unit from a glass-calomel electrode system.

Operation with power from 117 volts alternating current.

Extended time stability within 0.1 pH unit (5.8 mv.).

Adjustability to provide output control action when input pH increases from a lower pH to a higher preset value such as 7; ("off" to "on" action being achieved with a change of as little as 5.8 mv.).

Provision for output control action of: a solenoid-operated polystyrene titration valve to open when the sample pH is below that set in the controller and to close when the desired pH is reached; an automatic sample changer which controls the sample change action following completion of a titration; and the starting of an automatic recording interval timer when the titration valve is open and the stopping of the timer when the valve is closed.

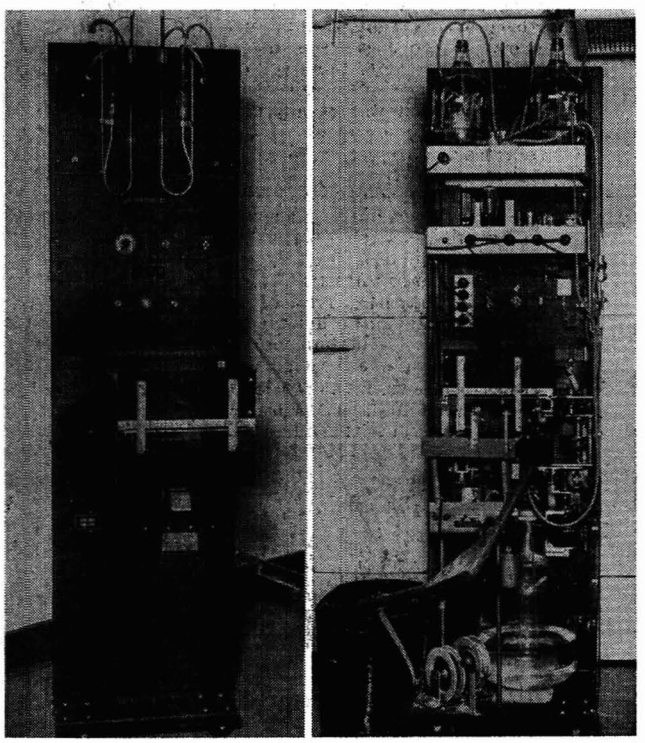

Figure 1. The Apparatus

Left. **Front view**
Right. **Back view**

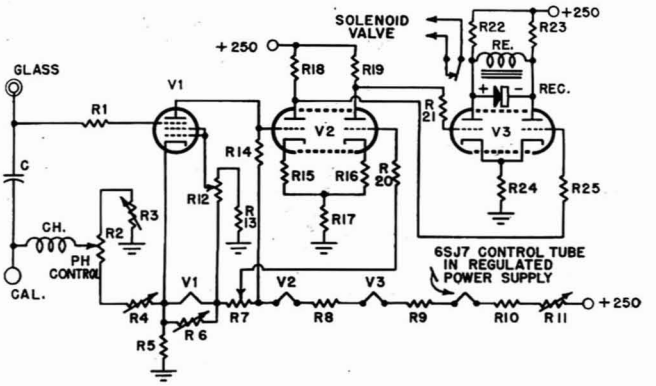

Figure 2. Schematic Diagram of pH-Sensitive Electrical Controller

R1. 6 megohms
R2. 25 ohms
R3, *R4*, *R5*. 50 ohms
R6. 200 ohms
R7, *R9*. 100 ohms
R8. 300 ohms
R10, *R11*, *R13*. 500 ohms
R12, *R15*, *R16*. 1 kilo-ohm
R14, *R20*. 3.9 megohms
R17. 6 kilo-ohms
R18, *R19*. 470 kilo-ohms
R21, *R25*. 1 megohm
R22, *R23*. 33 kilo-ohms
R24. 10 kilo-ohms
C. 275 mmfd.
CH. 2.5 mh.
RE. Advance, Type 850, 16 kilo-ohms, 5 ma. pull-in
REC. 1N34 diode
V1. 954 (selected)
V2. 12AX7
V3. 12 AU7

Resistors *R2* to *R12* and *R24* are 5-watt wire-wound.
Resistors *R1*, *R13*, *R14*, *R17*, *R18*, *R19*, *R22*, *R23* are Continental Carbon Co., 5% Nobeloy, Type X-1

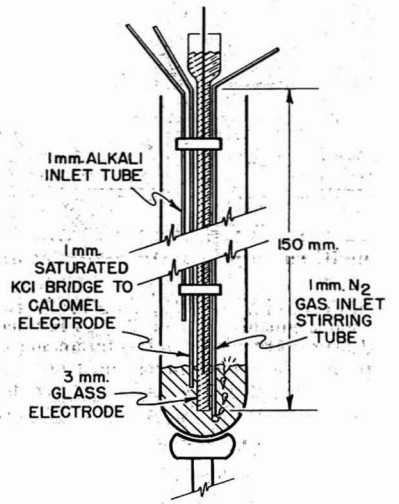

Figure 3. Glass-Calomel Electrode Assembly

A schematic diagram of this controller is shown in Figure 2.

Gapus and Pool (*4*) and Wu and Rainwater (*12*) have shown that it is not difficult to select standard Type 954 tubes for use in electrometer service. The authors' results confirm their findings with regard to the use of these tubes in measuring currents as low as 10^{-14} ampere. Therefore, the 954 was chosen for the input tube and wired for electrometer service, using the suppressor grid as the control element, and tying the control and screen grids together to a positive potential. The 954 tube is followed by a balanced direct current amplifier utilizing a 12AX7; a 12AU7 operates a plate current relay for output control. Electronically regulated direct current is used to provide filament current as well as high voltage and bias potentials. The Victoreen 5800 tube can serve as well as the 954 in a similar circuit.

The Glass-Calomel Electrode Assembly is shown in Figure 3.

This assembly includes a glass electrode (prepared by Milton T. Bush, Department of Pharmacology, Vanderbilt University, Nashville, Tenn.) and three plastic tubes. The glass electrode makes junction with the calomel electrode by means of a saturated potassium chloride bridge carried in one of the 1-mm. diameter plastic tubes. Another of the tubes conveys the alkali from the polystyrene valve to the sample being titrated; the third tube admits bubbles of nitrogen gas in a continuous stream to provide stirring of the sample during the titration. This assembly, of necessity, is small in its cross-sectional area in order that the volume it will displace within the sample tube may be at a minimum. Actually, the volume of titrant that can be added is of the order of 10 to 15 ml. in 18 × 150 mm. culture tubes containing 2 to 5 ml. of sample. Obviously, the concentration of the titrant must be adjusted to accommodate the sample and the volume of the sample tube. The authors' experience has involved the use of 0.04*N* sodium hydroxide with a 5-ml. culture or 0.02*N* sodium hydroxide with a 2-ml. culture.

The automatic sample changer system and sample tube carriage are shown in Figures 4 and 5, respectively.

A supply of 225 test tubes to be titrated may be loaded in the test tube carriage. This consists of a rectangular cart divided by polystyrene separators into 15 rows capable of holding 15 tubes each.

Test tubes from the carriage are presented to a rotating turnstile at the top of the changer mechanism (Figure 6), by weight-actuated sliding push plates in each row of the carriage (Figure

7). As the pH-sensitive controller calls for a change, one tube at a time is removed from the carriage for each quarter turn of the turnstile. A tube moved through 180° of the turnstile is presented to a rack and pinion-lifting mechanism, and raised into the electrode assembly.

The lower limit of the lifting rack's travel actuates a switch for reversing the motor drive. As the lifting rack rises, a Geneva motion is engaged to give the turnstile another quarter turn.

When the last test tube from one row of the carriage is delivered, the push plate of that row engages a trip lever, releasing the test tube carriage. This allows the carriage to move by weight actuation to a new row of tubes. The action is repeated until all tubes have been delivered and titrated. The mechanical change system is automatically turned off, and a signal buzzer informs the operator of completion when the carriage moves from the last row into a stop position.

The polystyrene valve has been described (*9*). This valve, when open, permits alkali to flow from the constant pressure-head reservoir to the sample. The flow is at a constant rate and is timed by the interval timer. Whenever the valve is closed the timer is also stopped. The use of this type of valve, rather than the pinching of a rubber tube, eliminates lag in the beginning of flow and "squirt" of alkali when flow is cut off. The plastic tubing which conveys the alkali from the valve to the sample is not elastic enough to permit "ballooning." The use of this valve

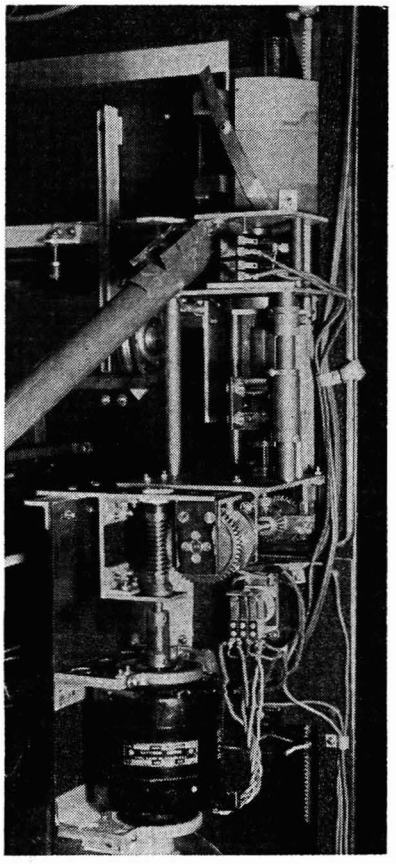

Figure 4. Automatic Sample Changer System

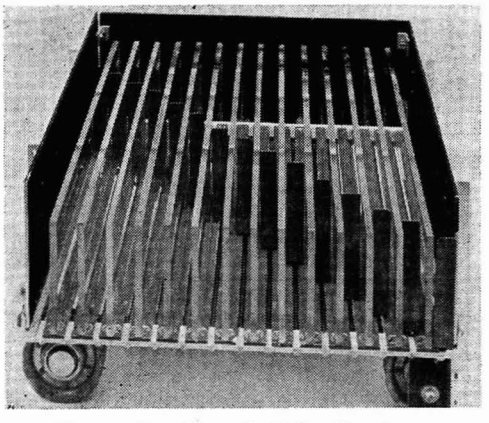

Figure 5. Sample Tube Carriage

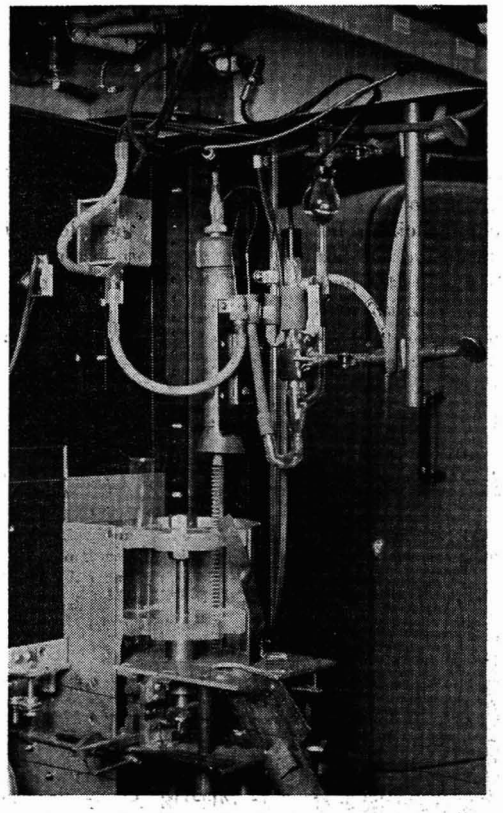

Figure 6. Sample Changer Turnstile Mechanism

affords the system a constant volume, and as a result, the smal error due to the lag in flow is eliminated. The solenoid whicl opens and closes the valve is operated by the pH-sensitive con troller in response to the pH sensed by the electrode assembly.

The automatic recording interval timer (Simplex Time Re corder Co., Gardner, Mass.) is a commercially available instru ment modified by installing auxiliary relays to make the time start and stop with the opening and closing of the polystyren valve and to print on the recording tape the total time the valv was open. Also, the reset to zero time is automatically per formed after the titration time.

The electric manometers used in controlling the pressure head o nitrogen on the alkali and in regulating the flow of nitrogen to th sample for stirring are similar to those used by Cannon.

The principle of the electronic manometer is well known, in volving simply a mercury manometer, one side of which is con nected to the gas pressure system, the other side making contac with a carbon electrode. The electrical contact makes or break an electronic circuit that operates a release valve in the gas pres sure system. In operation, the pressure is maintained at a con stant value within a few tenths of a millimeter of mercury.

This constant pressure on the alkali reservoir permits the ex pression of the flow of alkali in terms of time, the volume bein directly proportional to time under constant rate of flow. Thus only time of flow must be recorded. Actual volumes of alka

delivered can be obtained by knowing the rate of flow in milliliters per minute, but this is unnecessary in microbiological assays. Calibration of the instrument is accomplished daily by timing the flow of a definite quantity of titrant in order that the desired volume to be delivered per unit of time may be obtained.

OPERATION

The principles of operation and sequence of events are given in Figure 8 in block diagram.

The sample carriage is loaded with samples to be titrated in the order in which the titration is to proceed. The carriage is shifted until the first tube is directly in contact with the Geneva-controlled turnstile. The tubes are moved to the electrode assembly by successive 90° turns of the turnstile. The first tube is raised by the lifting mechanism, so that the sample comes in contact with the glass electrode assembly. If the pH is below 7 (assuming that 7 is the value present on the electronic controller), the electronic controller causes the polystyrene valve to open (permitting alkali to flow) and the recording interval timer to start. When the pH reaches 7, the controller stops the flow of alkali and the interval timer. If the pH remains at 7 for a predetermined delay time, the sample changer is activated, and the titrated sample is lowered from around the electrode assembly back into the turnstile. The delay period may be varied, depending on time required for the sample to be mixed to a uniform pH throughout. Five to 10 seconds have been found satisfactory for this work. After the sample is lowered, the time of titrant flow in units of seconds and tenths of seconds is printed on a tape, and the interval timer is automatically reset to zero in preparation for the next sample. After the titrated sample is lowered into the turnstile, the changer shifts the next sample in sequence under the electrode assembly and lifts it up around the electrodes. Again, if the pH is below 7, the titration proceeds automatically and is repeated until all samples in the rack are titrated.

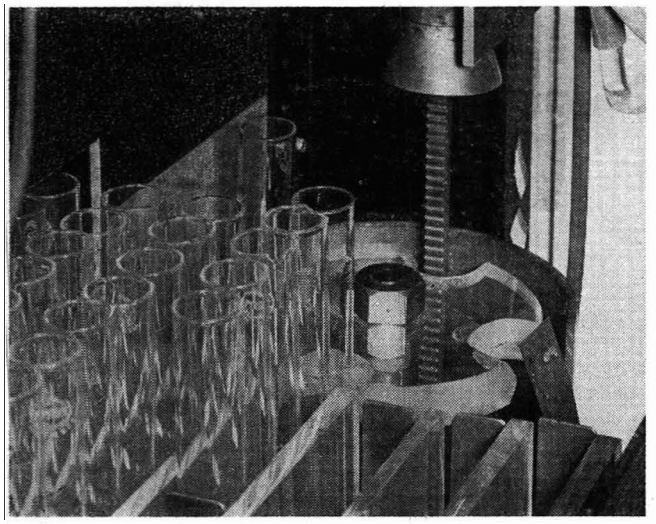

Figure 7. Weight-Actuated Push Plates Moving Tubes into Turnstile

The titrated sample is disposed of down a chute into a container of washing solution. If the pH of any sample is above that set on the pH controller as the desired end point, the sample will remain around the electrodes only the preset time (5 to 10 seconds) and be changed. A recording of zero will be made for such a sample, since no titrant was added. A signal indicates completion of titration for a batch of samples and the mechanical tube changer is inactivated. The technician in charge may then place more samples in the machine and start the process over. The time consumed in titrating a sample is governed by the rate of the flow of the alkali through the Leur-Lok needle on the outlet side of the polystyrene valve, which is controlled by the pressure head on the alkali and the size opening of the needle. The volume of titrant to be added is governed by the amount of acid to be titrated and the concentration of alkali used as the titrant. The alkali flow under the authors' conditions of operation gave approximately 10 ml. per minute. One minute, or 10 ml., is registered as 600 on the printing timer which records 600 time units per minute—i.e., a resolution of 0.1 second. For more precision, the time could be lengthened by slowing the rate of alkali flow.

Table I. Precision of Titrations

Sample	Average of 12 Seconds	Standard Error, Seconds
1	12.6	0.1
2	24.0	0.1
3	30.4	0.1
4	35.5	0.0
5	41.7	0.1
6	46.7	0.2
7	53.2	0.2
8	59.1	0.2
9	66.4	0.3
10	72.0	0.2
11	78.1	0.1
12	85.5	0.3

Table II. Accuracy of Titrations

Count Average of 4, Seconds	Milliequivalents of Acid	
	Found	Theoretical
27.6	0.206	0.200
39.6	0.296	0.300
46.1	0.344	0.350
53.8	0.402	0.400
65.3	0.489	0.500
80.1	0.598	0.600

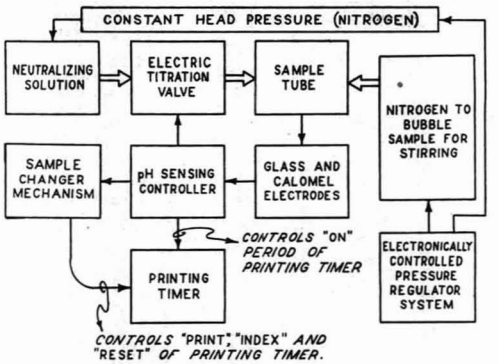

Figure 8. Operation Sequence Diagram

PRECISION AND ACCURACY

In operation, the instrument yields reproducible results well within the limits of desired accuracy obtainable in routine assays. Table I summarizes the results of 144 titrations. The standard error of precision does not exceed 1% and in most cases is less than 0.5%. Table II shows the data from a recovery experiment to determine the accuracy that could be expected with the use of the instrument. The accuracy is well within the limits of error inherent in the microbiological assay techniques. Differences observed in this recovery experiment may actually be reflecting, in part, a certain amount of error involved in pipetting the samples.

The instrument can be modified for other routine assays by relatively simple changes. Any acid-base titration technique can be used, whether it be for microbiological assay of vitamins, amino acids, or other essential nutrients or for the acidity from some other source. By using a different set of electrodes, oxidation-reduction titrations may be run and recorded.

By replacing the electrode assembly with a dipping type Geiger counter, and using a modifier scaler instead of the pH-sensitive controller, liquid samples containing radioactive materials could be handled. Collection of fractions from chromatographic columns on either a time or volume basis could be accomplished by using suitable controlling systems in place of the pH controller,

and receiving the samples into a collecting rack instead of disposing of the tubes. There are many possibilities in which the sample changer and principles involved in this instrument could be incorporated to reduce the time which is used in routine analytical procedures.

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LITERATURE CITED

(1) Austin, R. R., *Am. Gas Assoc. Proc.*, **31**, 505 (1949).
(2) Dunn, E. B., Melpolder, F. W., Taylor, R. C., and Young, W. S., *Proc. Mid-Year Meeting Am. Petroleum Inst.*, 30MIII, 45 (1950).
(3) Eades, C. H., Jr., McKay, B. P., and Romans, W. E., *Federation Proc.*, **II**, 205 (1952).
(4) Gapus, G. H., and Pool, M. I., *Review of Scientific Instruments*, **8**, 197 (1937).
(5) Hawes, R. C., Strickler, A., and Petterson, T. H., *Elec. Mfg.*, **47**, 76, 212 (1951).
(6) Jacobsen, C. F., and Léonis, José, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **27**, 333 (1951).
(7) Juliard, A., and Cakenberghe, J. van, *Anal. Chim. Acta*, **2**, 542 (1948).
(8) Lingane, J. J., ANAL. CHEM., **20**, 285 (1948).
(9) McKay, B. P., and Eades, C. H., Jr., *Ibid.*, **27**, 123 (1950).
(10) Shaffer, P. A., Jr., Briglio, A., Jr., and Brockman, J. A., Jr., *Ibid.*, **20**, 1008 (1948).
(11) Wise, E. N., *Ibid.*, **23**, 1479 (1951).
(12) Wu, C. S., and Rainwater, James, U. S. Atomic Energy Commission, *AEC Document*, **MDDC-1671** (1944).

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Paper Chromatography of Cobalt(III), Copper(II), and Nickel(II) Acetylacetonates

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Acetylacetone, one of the simplest β-diketones, was selected for study because its availability and low cost make it a desirable reagent for chromatographic separations. Cobalt(III), copper(II), and nickel(II) acetylacetonates were separated using mixtures of cyclohexane, dioxane, and methanol as the developing solvent. A mixture of 84% cyclohexane, 10% dioxane, and 6% methanol gave good separations. The mean R_f values were reproducible to ±0.02. Solubilities of the metal chelates were measured in the developing solvents. Relative adsorption affinities were obtained from dielectric constant measurements. A qualitative relation was found to exist between the relative sequence of R_f values and the relative solubility and adsorption affinity (polarization) of the metal chelates.

ALTHOUGH a large number of metal β-diketone complexes are well known (*10, 16*), these complexes have not been extensively used for chromatographic separations. Increasing interest in the use of chelating agents for inorganic chromatographic separations has been shown in the appearance of a number of recent articles (*1–7, 9, 11–15*). Acetylacetone, one of the simplest β-diketones, was selected for this study because its availability and low cost make it a desirable reagent.

Burstall *et al.* (*5*) and Pollard *et al.* (*11*) have used solvent systems containing acetylacetone in the chromatographic separation of some inorganic ions. A number of factors may influence this type of separation—namely, the rate of chelate formation, the presence of excess chelating agent, and the possibility of chelate hydrolysis in the presence of strong acids.

In order to avoid these factors the authors have preferred to spot the paper with the preformed metal acetylacetonates and to develop the chromatogram with a solvent mixture in which the chelates are stable.

REAGENTS

Acetylacetone (Matheson Co.), redistilled.
Methanol, C.P.
Cyclohexane (practical grade), redistilled.
Dioxane (technical grade), redistilled.
Aqueous solutions, 1%, of the metal ions prepared from: C.P. cobalt(II) nitrate, C.P. copper(II) nitrate, and C.P. nickel(II) nitrate.
Solution of dimethylglyoxime in ethyl alcohol, 1%.
Solution of dithio-oxamide in ethyl alcohol, 0.3%.

PROCEDURE

Acetylacetonates of cobalt(III), copper(II), and nickel(II) were prepared by shaking 1% solutions of the ions, adjusted approximately to a pH of 7 with sodium acetate, with acetylacetone. The nickel acetylacetonate was extracted with *n*-butyl alcohol and shaken with distilled water to remove nickel ions. The copper and cobalt acetylacetonates were extracted with methyl isopropyl ketone and shaken with water to remove any ions. In the extraction of the cobalt acetylacetonate, the methyl isopropyl ketone was kept in contact with the original solution until the ketone layer developed a dark green color. This chelate corresponded to the cobalt(III) acetylacetonate described by Gach (*8*). The oxidation of the cobalt(II) to cobalt(III) was probably due to impurities in the methyl isopropyl ketone. Final colors of the extracted and washed solutions of cobalt, copper, and nickel acetylacetonates were dark green, blue-green and yellow-green, respectively.

Hydrometer cylinders, 43 cm. tall and 7 cm. in diameter, served as chromatographic chambers. A part of the cylinder was lined with filter paper soaked with the solvent in order to saturate the chamber more efficiently. Twelve hours were then allowed for complete saturation of the chamber.

Whatman No. 1 filter paper strips 2.5 inches wide were spotted with the extracted solutions of the metal acetylacetonates and dried in air for 1 hour. The strips were then placed in the chamber saturated with vapor and equilibrated for 1 hour before immersion in the solvent. The chromatograms were developed completely in 3 hours, the solvent front having ascended approximately 25 cm.

Preliminary work indicated that methanol and cyclohexane would be desirable solvents for this study. The cobalt(III), copper(II), and nickel(II) acetylacetonates all moved with rather large R_f values in methanol, whereas only the cobalt acetylacetonate moved in cyclohexane. An appreciable amount of methanol was not soluble in cyclohexane; therefore, a third component, dioxane, was used to form a completely miscible solvent.

Table I. R_f Values of Metal Acetylacetonates in Mixed Solvent System (Cyclohexane, Dioxane, Methanol)

	Cobalt(III) Acetylacetonate			Copper(II) Acetylacetonate			Nickel(II) Acetylacetonate		
	Methanol, %			Methanol, %			Methanol, %		
Dioxane, %	3	6	9	3	6	9	3	6	9
5	0.58	...	...	0.18	...	...	0.00	...	...
7	...	0.57	...	...	0.22	...	...	0.00	...
10	0.69	0.64	0.56	0.25	0.27	0.27	0.00	0.00	0.00
15	0.76	0.72	0.65	0.29	0.34	0.37	0.00	0.00	0.00
20	0.79	0.77	0.73	0.34	0.39	0.44	0.00	0.00	0.02
25	0.83	0.81	0.75	0.43	0.47	0.48	0.00	0.00	0.02
35	0.90	0.88	0.84	0.56	0.57	0.60	0.00	0.01	0.03

reproducible with an average deviation of ±0.02.

Table I shows the average R_f values of the metal acetylacetonates related to an increasing concentration of dioxane in three fixed concentrations of methanol with cyclohexane complementing the dioxane concentration.

It is noted that the R_f value of the copper(II) acetylacetonate increases with increasing concentration of dioxane and/or methanol. The solubility data for copper(II) acetylacetonate in Table II show a similar relationship between solubility and concentrations of dioxane or methanol. Both the R_f and the solubility values of the copper acetylacetonate give a straight line relationship when plotted against increasing concentration of dioxane.

The position of the cobalt acetylacetonate was easily detected by its green color and by the orange coloration when sprayed with dithiooxamide. The nickel acetylacetonate was detected by spraying the paper with an alcoholic solution of dimethylglyoxime and then exposing it to ammonia fumes. The copper acetylacetonate was detected last by spraying with dithiooxamide and exposing it to ammonia fumes.

To verify that the metal acetylacetonates were being chromatographed and not the ions, a paper was spotted with the metal nitrates and chromatographed under conditions identical to the procedure used with the metal acetylacetonates. When the chromatogram was sprayed with an alcoholic solution of dithiooxamide, the metals were detected only in the position of the original spot. No migration of ions was observed.

The applicability of this technique to the separation of a mixture of cobalt(II), copper(II), and nickel(II) ions was determined in the following manner.

Table II. Solubility of Metal Acetylacetonates in Mixed Solvent System (Cyclohexane, Dioxane, Methanol)

	Cobalt(III), Grams/Liter		Copper(II), Grams/Liter		Nickel(II), Grams/Liter	
	Methanol, %		Methanol, %		Methanol, %	
Dioxane, %	3	9	3	9	3	9
5	5.3	...	0.64	...	0.63	...
10	...	17.4	0.82	2.02	...	1.35
15	10.1	...	1.24	2.60	1.07	...
20	...	27.3	1.64	3.05	...	...
25	17.5	...	2.13	3.76	3.13	8.6
35	...	...	3.08	5.08	...	...

An equimolar mixture of the three ions was converted to their metal chelates using the following procedure. Sixty milliliters of a 2% solution of cobalt, copper, and nickel nitrates were treated with 20 ml. of 3% hydrogen peroxide, and heated on a steam bath for 15 minutes. The solution was then adjusted to a pH of 7 with sodium acetate, and 20 ml. of a 1 to 1 mixture of acetylacetone–ethyl alcohol was added to form the metal chelates. The solution was heated for a few minutes and then treated with 50 ml. of a 1 to 1 mixture of methyl isopropyl ketone and *n*-butyl alcohol, heated on a steam bath for 30 minutes, and allowed to stand for several hours or until the organic layer was dark green. The organic layer was then removed and used to spot the chromatographic paper. The chromatographs of the mixture were identical with those of the individual chelates spotted together.

Sufficient amounts of solid metal chelates for solubility determinations were obtained by the following procedures.

Copper(II) acetylacetonate was precipitated by shaking acetylacetone with a 1% solution of copper(II) nitrate, adjusted approximately to a pH of 7 with sodium acetate. The pale blue precipitate was washed with hot water, dissolved in hot methanol and recrystallized by addition of water.

The cobalt(III) and nickel(II) acetylacetonates were obtained by evaporating the solutions of extracted chelates to dryness in a vacuum desiccator. The solid cobalt(III) and nickel(II) acetylacetonates were, respectively, dark green and pale green.

The solubility of each metal acetylacetonate in various compositions of the developing solvent was determined by saturating the solution with the solid chelate, evaporating an aliquot of the saturated solution to dryness in a vacuum desiccator, and weighing.

A Sargent Model V chemical oscillometer with cell holder-Type A and a fixed frequency of 5×10^6 cycles per second was used for the relative dielectric constant measurements of 0.01M solutions of the metal chelates in methanol.

RESULTS

The most effective separations occurred with the lower concentrations of methanol and dioxane. Mixtures of cyclohexane and dioxane did not give chromatograms suitable for measurement. Solvent mixtures near 6% methanol, 10% dioxane, and 84% cyclohexane gave good separations. The R_f values were

The R_f values of the cobalt acetylacetonate increased with increasing concentration of dioxane but decreased slightly with increasing concentration of methanol over the concentration range studied. The R_f values of the nickel acetylacetonate are very small, but seem to increase slightly with increasing concentration of dioxane and/or methanol.

If solubility of the metal acetylacetonates in the developing solvent were the governing factor in this system, then the solubility of the metal acetylacetonates would be expected to be cobalt > copper > nickel. Table III shows that cobalt acetylacetonate is the most soluble chelate as one might anticipate, but also shows that the solubilities of the copper and nickel acetylacetonates are too similar for these compounds to be separated on the basis of solubility alone.

Table III. Solubility of Cobalt(III), Copper(II), and Nickel(II) Acetylacetonates in Pure Solvents

(Grams per liter)

Chelate	Cyclohexane	Dioxane	Methanol
Co	1.0	89.8	58.0
Cu	0.2	16.3	3.5
Ni	0.2	26.0	78.2

Table IV. Capacitance Measurements of 0.01M Solutions of Metal Acetylacetonates in Methanol

Chelate	Capacitance
Cu	29,800
Co	30,140
Ni	>32,000

Capacitance measurements for the oscillometer cell filled with 0.01M solutions of the various metal acetylacetonates in methanol are given in Table IV. The relative dielectric constants of the solutions are directly proportional to the measured capacitance. It is also recognized that the relative dielectric constants of the

equal molar solutions are a measure of the relative total polarization of the solute molecules. Thus the relative total polarization of the various metal chelates should be in the order, nickel>> cobalt> copper. The greater the polarization the greater the strength of adsorption.

Comparison of the solubility and polarization data indicates that the relative R_f values fall in a logical sequence:

Solubility	Co >> Cu, Ni
Polarization	Ni >> Co > Cu
R_f values	Co > Cu > Ni

The small difference in solubility of the copper and nickel chelates coupled with the large polarization of the nickel chelate gives a R_f sequence copper > nickel, whereas the much larger solubility of the cobalt chelate coupled with only a slightly larger polarization than the copper chelate gives a R_f sequence cobalt > copper. Thus the relative R_f values can be explained qualitatively by these two factors. Another β-diketone chelate system has been studied by the authors (not yet published) in which five chelates have been measured with a similar correlation between solubility, polarization, and R_f values.

LITERATURE CITED

(1) Al-Mahdi, A. K., and Wilson, C. L., *Mikrochemie ver. Mikrochim. Acta*, 36/37, 218 (1951); 40, 138 (1952).
(2) Anderson, J. R. A., and Lederer, M., *Anal. Chim. Acta*, 5, 396 (1951).
(3) Ashizawa, T., *Repts. Balneol. Lab. Okayama Univ.*, No. 5, 1–42 (1951).
(4) Berg, E. W., and McIntyre, R. T., ANAL. CHEM., 26, 813(1954).
(5) Burstall, F. H., Davies, G. R., Linstead, R. P., and Wells, R. A., *J. Chem. Soc.*, 1950, 516.
(6) Erlenmeyer, H., and Dahn, H., *Helv. Chim. Acta*, 22, 1369 (1939).
(7) Fernando, Q., and Phillips, J. P., ANAL. CHEM., 25, 819 (1953).
(8) Gach, F., *Monatsh.*, 21, 98 (1900).
(9) Laskowski, D. E., and McCrone, W. C., ANAL. CHEM., 23, 1579 (1951).
(10) Martell, A. E., and Calvin, M., "Chemistry of the Metal Chelate Compounds." New York, Prentice-Hall, Inc., 1952.
(11) Pollard, F. H., McOmie, J. F. W., and Elbeih, I. I. M., *J. Chem. Soc.*, 1951, 466, 470.
(12) Pollard, F. H., McOmie, J. F. W., and Elbeih, I. I. M., *Discussions Faraday Soc.*, No. 7, 183 (1949).
(13) Pollard, F. H., McOmie, J. F. W., and Stevens, H. M., *J. Chem. Soc.*, 1951, 771; 1952, 4730.
(14) Reeves, W. A., and Crumpler, T. B., ANAL. CHEM., 23, 1576 (1951).
(15) Venturello, G., and Ghe, A. M., *Ann. chim. (Rome)*, 43, 267 (1953).
(16) Welcher, F. J., "Organic Analytical Reagents," New York, D. Van Nostrand Co., 1947.

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Determination of Tetraethyllead in Gasoline by X-Ray Fluorescence

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This work was undertaken to determine the possible advantages in speed and accuracy offered by x-ray fluorescence for the determination of tetraethyllead in gasoline. A study of the matrix effect has shown that: the same analytical curve may be used to determine tetraethyllead in gasoline whether present alone or as motor mix or aviation mix antiknock compound; the error due to sulfur is very small, the error due to possible gasoline additives such as phosphorus is negligible; and the effect of the carbon-hydrogen ratio is directly related to density. The method is rapid (5 to 10 minutes per sample) and has an average error of ±0.026 ml. of tetraethyllead per gallon.

IN 1950, Birks *et al.* (*4*) reported on the application of x-ray fluorescence to the determination of lead and bromine in aviation gasoline. From their work, it was readily apparent that the x-ray fluorescence method had certain advantages in principle, at least, over the direct x-ray absorption method for the determination of tetraethyllead (TEL) in gasoline because of its specificity. Absorption methods using polychromatic radiation lack selectivity of the measurement, x-ray absorption being an additive function of all the elements present in the sample. Factors in the determination of tetraethyllead in gasoline by this method, according to a number of investigators (*7, 12, 20*) are: the effect of absorption by sulfur, and the effect of absorption of the type of antiknock mixture present. In practice, data on the sulfur content and the type of antiknock mixture present are usually available, and, therefore, these factors are not serious drawbacks to the x-ray absorption method, which has proved to be a very useful tool and a great time saver in the routine analysis of gasoline samples (*14*). A rapid, instrumental method which is based on a unique property of lead and is free from the above factors would be desirable, however. The x-ray fluorescence method offers such a possibility and an investigation has been made to determine its advantages and disadvantages for this particular application.

The principles of x-ray fluorescence and its earlier applications to elemental analysis are fully discussed by von Hevesy (*11*). Recent advances in instrumentation, particularly the replacement of the photographic film with a Geiger counter for accurately recording the intensity of radiation, have resulted in more widespread use of the method. Several applications have been reported recently in the metallurgical field (*6, 9, 15, 17*) and for the analysis of elements difficult to separate and determine by chemical analysis (*2, 3, 5, 16*). For the most part, these applications represent the analysis of elements present in binary or ternary systems in concentrations from 1 to 50% or more. Interest in the petroleum field has turned to the analysis of much smaller concentrations of elements in a matrix of low atomic number—for example, the analysis of tetraethyllead in gasoline, wherein the range of lead concentration is from 0.02 to 0.24 weight %. With certain variations in instrumentation, the intensity of the fluorescent radiation may be greatly increased. In reporting on recent advances in instrumentation, Behr (*1*) has predicted that it will be possible to increase the intensity of fluorescent radiation and the sensitivity of detectors to the point that it will be possible to determine 10 to 100 p.p.m. of sulfur in lubricating oils.

MATRIX EFFECT

In x-ray fluorescence the sample is irradiated with a primary x-ray beam of sufficient energy to excite the elements at the surface and within the sample to emit their characteristic fluorescent x-rays. These characteristic x-rays excited within the sample radiate in all directions, a small portion emerging from the sample surface in a direction suitable for the analysis and the recording of their intensities. The fluorescent x-rays are subject to a decrease in intensity depending on the absorbing properties of the matrix elements for the particular wave length in question. Also, the fluorescent x-rays are subject to an increase in intensity

if the characteristic radiation from any of the matrix elements is close to but slightly lower than (0.1 to 0.2 A.) the wave length of the absorption edge of the element being determined (*11*).

Quantitative knowledge of the matrix absorbing and exciting effects may be obtained from published data. In the present system the possibility of an enhancement of the lead L_α line at 1.17 A., by the elements present in the matrix, may be virtually discarded. The elements which by theory should prove most effective for this purpose are rubidium, strontium, yttrium, zirconium, and elements of higher atomic number up to and possibly including rhodium. The relative matrix absorbing effects of the various compounds concerned in the present problem may be obtained from a consideration of their linear absorption coefficients at 1.17 A., the wave length of the lead L_α line. The data as calculated from the published mass absorption coefficients of the elements are presented in Table I.

Table I. Mass and Linear Absorption Coefficients of Compounds Occurring in Gasoline at λ = 1.17 A.

Compound	μ_m	μ_l
Benzene, C_6H_6	1.954	1.717
Iso-octane, C_8H_{18}	1.817	1.267
1,2-Dibromoethane, $C_2H_4Br_2$	36.51	79.61
1,2-Dichloroethane, $C_2H_4Cl_2$	34.56	43.30
Thiophene, C_4H_4S	16.84	18.02
Tetraethyllead, $(C_2H_5)_4Pb$	74 63	123.32
Aviation mix (equivalent to neat TEL)	98.62	162.91
Motor mix (equivalent to neat TEL)	97.64	160.80

Table II. Linear Absorption Coefficients of TEL in Benzene and Iso-octane at λ = 1.17 A.

Ml. TEL per Gallon of Hydrocarbon	TEL Added as TEL	Motor mix	Aviation mix
C_8H_{18} + 0.0	1 267	1.267	1.267
+ 1.0	1.2992	1.3090	1.3095
+ 2.0	1.3314	1.3510	1.3520
+ 3.0	1.3637	1.3931	1.3946
+ 5.0	1.4281	1.4771	1.4796
C_6H_6 + 0.0	1.717	1.717	1.717
+ 1.0	1.7491	1.7588	1.7593
+ 2.0	1.7812	1.8006	1.8017
+ 3.0	1.8133	1.8424	1.8440
+ 5.0	1.8775	1.9260	1.9287
+ 5.0 + 0.1% sulfur		1.9504	
+ 5.0 + 1% sulfur		2.2704	

Calculations were also made of the linear absorption coefficients of 1, 2, 3, and 5 ml. per gallon of neat tetraethyllead, and tetraethyllead as motor mix and as aviation mix antiknock compound in iso-octane (2,2,4-trimethylpentane) and in benzene. (In these calculations, "milliliters of tetraethyllead per gallon" is used as defined by the petroleum industry to be 1.65 grams of tetraethyllead per gallon of base stock at 15.5° C. The term "neat" tetraethyllead refers to commercial tetraethyllead to which no halogen compounds have been added. The term "tetraethyllead as motor mix antiknock compound" refers to tetraethyllead in combination with 1,2-dichloroethane and 1,2-dibromoethane in the amounts required to convert all the lead to lead chloride and one half the lead to lead bromide, respectively. The term "tetraethyllead as aviation mix antiknock compound" refers to tetraethyllead in combination with the amount of 1,2-dibromoethane to convert all the lead to lead bromide). These data are given in Table II.

From the data of Table II it might be predicted that the matrix absorbing effects of tetraethyllead, motor mix, and aviation mix in a specific hydrocarbon would be so nearly the same for any one concentration level of tetraethyllead that a single analytical curve would serve for their analysis. Further, one would expect that the same analytical curve would be suitable for variations in the lead-to-halogen ratios of tetraethyllead antiknock mixtures. A comparison of the absorption coefficients for the benzene and iso-octane standards shows an appreciable increase in absorption with increase in carbon to hydrogen ratio, indicating a need for a correction factor based on density. It would appear that the presence of 0.1% sulfur should not increase the absorbing power of the matrix sufficiently to decrease the measured tetraethyllead content at the 5-ml. level by more than 0.05 ml. An experimental investigation of the method has shown it to be even more favorable than these predictions indicate.

EXPERIMENTAL

The instrument used for the x-ray fluorescence measurements was a General Electric XRD-3 spectrometer with fluorescent attachment. A Machlett AEG-50 T type tungsten target tube operated at 47.5 ma. and 50 kv. was used to produce a continuous spectrum of sufficient energy to excite the L_α line of lead at a wave length of 1.17 A. to a suitable intensity. The sample was placed in a specially designed aluminum cell having a 1.5 × 1.75 inch window of 0.25-mil thickness of Mylar. The volume of the cell is about 40 ml. Two cells were used in this study, one having the window fixed at an angle of 45° to the primary radiation and the other at 60°. The cell holder was water-cooled to a temperature of 20° ± 1° C.

The fluorescent radiation, consisting of the characteristic radiation of lead and bromine plus a certain amount of scattered or background radiation, passes through a focusing soller slit on to a bent mica crystal oriented so as to produce reflections from the 331 planes. The mica crystal used was 4.4 mils thick and gave a relatively high intensity of the lead L_α radiation with relatively poor but satisfactory resolution. To increase the intensity further, the 0.3° detector slit supplied with the instrument was not used, since the 0.4° slit of the Geiger tube housing proved to give sufficient resolution.

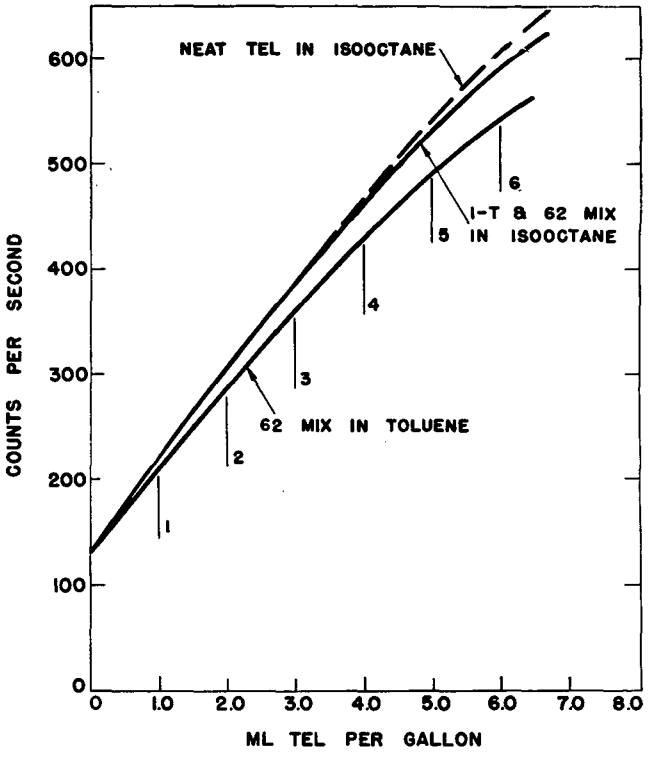

Figure 1. Calibration Curves for Neat Tetraethyllead, 1-T Aviation, and 62 Motor Mix Antiknock Compounds in Iso-octane, and for 62 Motor Mix Antiknock Compound in Toluene

The instrument performance was standardized daily by means of a Lucite block containing a uniform admixture of lead bromide. The angular setting of the goniometer for a maximum intensity of the lead L_α line was determined and ten intensity measurements were obtained in terms of the time required for 16,384 counts. The standard period of time for the conditions selected was 32.0 ± 0.3 seconds. The probable counting error for 16,384 counts is ±0.5%. For the mica crystal used, the goniometer setting

for the maximum intensity of the lead $L\alpha$ line was at 46.25°, the bromine $K\alpha$ at 40.60°, and the lead $L\beta$ at 38.35°.

CALIBRATION DATA AND METHOD

Using the standardized conditions as described, intensity measurements were obtained for a series of standards in iso-octane and in toluene containing neat tetraethyllead, Motor mix, and aviation mix antiknock compound at levels of 1, 2, 3, 4, and 5 ml. of tetraethyllead per gallon. All intensity measurements, including the background measurements, were made at the goniometer setting for the lead $L\alpha$ line. The data obtained are shown graphically in Figure 1. The intensity measurements were the average values for three to five time-readings of 16,384 counts each. The intensity for 3 ml. of tetraethyllead per gallon of iso-octane was 390 c.p.s. (counts per second) and that of unleaded iso-octane was 131 c.p.s. The limiting accuracy, when the instrument is operating properly, is entirely dependent on the statistical accuracy of the Geiger counter. The equation for calculating the relative probable error of a line above background as given by Birks and Brooks (*2*) is:

$$\%\ \text{relative P.E.}\ (N_S - N_B) = \frac{2/3\ (N_S + N_B)^{1/2}}{N_S - N_B} \times 100$$

where N_S is the total number of counts for the line plus background and N_B the total number of counts for the background. Applying this equation to the data for the 3-ml. level, the per cent relative probable error of the line above background measurement for a 1-minute count is 0.76%.

The calibration data of Figure 1 show that the same analytical curve is obtained for tetraethyllead in a specific hydrocarbon whether it is present as neat tetraethyllead, motor mix, or as aviation mix for concentrations up to 3 ml. of tetraethyllead per gallon. At higher tetraethyllead concentrations, the intensity readings of the neat tetraethyllead standards are slightly higher than are those for the motor mix and aviation mix antiknock compound. This presents no difficulty, however, because a rapid manual scanning through the position of the bromine $K\alpha$ line at 1.04 A. (2θ of 40.60°) will serve to determine whether the neat tetraethyllead curve is required for the analysis of a specific sample.

A comparison of the calibration data for neat tetraethyllead, motor mix, and aviation mix antiknock compound in Figure 1 with the calculated linear absorption coefficients of Table II shows good agreement between the experimental and theoretical results. The fact that the experimental results are more favorable than could be predicted from a direct comparison of the absorption coefficients is because of the variations in the effective absorbing paths. The data reported by Birks (*4*, Figure 5) show a much greater divergence between the calibration data for neat tetraethyllead and aviation mix than was observed in the present study. This difference in observed data may be due to some difference in instrumentation although, in view of the calculated absorption data of Table II, it is not readily understandable.

The calibration data of Figure 1 also show that the higher the carbon to hydrogen ratio of the base stock the greater is its absorptivity and the lower the measured intensity of the lead $L\alpha$ radiation. The two curves are related to each other by a linear function of the ratio of the densities of the two hydrocarbons. A comparison of the data for the two different hydrocarbon curves is given in Table III.

The data of Table III show that for a specified intensity level the average ratio of the milliliters of tetraethyllead concentration in toluene to that in iso-octane is 1.133. This value will vary depending on the standardized conditions selected, geometrical differences of the optical system, etc. The density ratio of these two hydrocarbons is 1.241. For the standardized conditions selected, the procedure, therefore, is to refer the determined average intensity measurement of a sample to the iso-octane curve to obtain milliliters of tetraethyllead per gallon and then multiply by the calculated density factor, D_F, to correct to milliliters of tetraethyllead per gallon of sample. The calculated density factor for a particular sample is obtained from the following equation:

$$\left(\frac{d_s}{d_{iso}} - 1\right) \times \left(\frac{1.133 - 1}{1.241 - 1}\right) + 1 = D_F$$

in which d_s and d_{iso} are the densities at 15.5° C. of the sample and iso-octane, respectively. If greater accuracy is desired, the experimental ratio corresponding to the measured intensity level (Table III) should be used instead of the average experimental ratio of 1.133. If a quick manual scanning at the position of the bromine $K\alpha$ line shows absence of bromine, the neat tetraethyllead curve must be used for tetraethyllead levels above 3 ml.

Table III. Comparison of Milliliters of Tetraethyllead per Gallon in Iso-octane and Toluene at Same Intensity Level

Intensity Level, C.P.S.	Ml. of TEL per Gallon in Iso-octane	Toluene	Ratio
200	0.75	0.86	1.146
300	1.90	2.15	1.131
400	3.15	3.57	1.133
500	4.52	5.01	1.121
		Average	1.133

During the course of this investigation, the possibility of observing Compton scattering was called to the authors' attention by Van Nordstrand (*19*). Data were obtained which showed modified scattering of the characteristic tungsten lines from the primary source, as described by Compton and Allison (*8*). In accordance with theory (briefly, the intensity of modified scattering varies inversely with the atomic number of the scattering element), the intensity of the modified radiation was greater from unleaded iso-octane than from unleaded benzene. Moreover, these respective intensities were observed to decrease with the addition of tetraethyllead. However, the intensity of the Compton scattering was small, and also, the difference in intensity from the two extreme scatterers, iso-octane and benzene, was not sufficient to make this a satisfactory method for differentiating base stocks on the basis of their carbon to hydrogen ratio.

TEMPERATURE EFFECT

On first thought, one might expect that the measured intensity of the lead $L\alpha$ line for a specified tetraethyllead concentration would vary inversely with temperature. Several tests were made which showed, however, that there was no appreciable change in the measured intensity of the lead $L\alpha$ radiation at the 5-ml.-per-gallon level for a temperature range of 12° to 30° C. This temperature range represents about a 2% change in sample volume.

It is realized from a more careful consideration that the measured intensity of the lead radiation would not be altered because the ratio of the concentration of lead to the other elements present (carbon, hydrogen, chlorine, bromine, sulfur, etc.) would remain constant regardless of the amount of contraction or expansion of the sample. As the sample expands the absorbing effect of the matrix is correspondingly decreased and the effective depth, from which the fluorescent radiation generated within the sample might emerge from the surface, is increased. Within certain limits, this compensating effect results in a constant intensity regardless of temperature. Considering the necessary geometry of the sample cell, area of the soller slits, etc., there should, however, be a small decrease in intensity with increased temperature, because as the sample expands, the effective depth of the sample increases and as this takes place the effective cross-sectional area of the sample decreases. For this reason, it is advisable to hold the temperature of the sample at some selected temperature ±1° C.

The temperature selected is unimportant as long as all tetraethyllead values of standards are expressed as milliliters of tetraethyllead per gallon at 15.5° C. and all measured densities of samples are corrected to 15.5° C. A temperature of 20° ± 1° C. was used for the present study because of the convenience in handling samples at a temperature close to room temperature.

EFFECT OF SULFUR AND OTHER IMPURITIES

To determine the effect of sulfur, 1% sulfur as thiophene was added to a standard sample of 5 ml. of tetraethyllead per gallon as motor mix in toluene. Measurements before and after the addition of sulfur showed that 1% sulfur decreased the intensity of the lead L_α line by 3.3%. The presence of 0.1% sulfur in a gasoline sample containing 3.0 ml. of tetraethyllead per gallon would therefore decrease the determined value by only 0.01 ml. The error at the 5-ml. level would be −0.0165 ml. This error is small compared with the direct x-ray absorption method in which 0.1% sulfur increases the determined tetraethyllead value by 0.185 ml. of tetraethyllead per gallon at all tetraethyllead levels (*7*). The most recent motor gasoline survey (*18*) shows that, with the exception of the Pacific coast states, the range of sulfur concentration in commercial gasolines is 0.014 to 0.210%, the average increasing from 0.043 to 0.095% as one travels west across the United States. For these average sulfur values, the resulting error in tetraethyllead determination would be negligible by x-ray fluorescence. At the maximum sulfur content of 0.21%, the measured tetraethyllead values will be 0.02 ml. low at the 3.0-ml. level as compared with 0.39 ml. high by the x-ray absorption method, if no correction is made for sulfur. The range of sulfur concentration for the Pacific coast states is 0.072 to 0.432% the average varying from 0.132% in premium grade gasoline to 0.262% in regular grade gasoline. Even with these high sulfur values, the error due to sulfur by the fluorescence method would not be excessive for routine control analysis.

Tests with fuels containing phosphorus additives in concentrations ten times those normally added to gasoline have shown no measurable absorption effect on the intensity of the lead line at the level of 3 ml. of tetraethyllead per gallon.

The possible error introduced by other impurity elements has not been completely investigated, but would depend on the amount present and its absorptivity at 1.17 A. For example, measurements made on nickel in a manner similar to those on sulfur showed that 1% nickel decreased the measured intensity of the lead L_α line by 8.5%.

COMPARISON OF 45° AND 60° SAMPLE CELLS

The GE fluorescent instrument was supplied with a sample holder which fixed the sample at an angle of 60° to the primary radiation. The first gasoline sample cell fabricated for this study also had the window at an angle of 60°. In an effort to decrease the matrix absorbing effects, as discussed by Friedman (*10*) and Noakes (*17*), a second sample cell was made with the window at an angle of 45° to the primary beam. If the effective sample area were the same in both cells, the matrix absorbing effects should vary with the tangent of the angle of the sample to the beam or be 1.732 times greater in the 60° cell than in the 45° cell. A comparison of the measured fluorescence in the two cells tends to confirm this.

The matrix absorbing effect in the two cells was compared for a standard containing 5 ml. of tetraethyllead per gallon as motor mix in toluene before and after the addition of 1% sulfur as thiophene. The original intensity of the fluorescent radiation was decreased to 96.7% in the 45° cell and to 93.1% in the 60° cell. Using the standard Lambert absorption equation and the calculated linear absorption coefficients in Table II, the effective sample depth or absorbing path was found to be 0.11 cm. in the 45° cell as against 0.20 cm. in the 60° cell. These two effective absorbing paths are roughly proportional to the ratio of the tangents of the two angles.

A similar experiment was performed by adding 1% nickel as a nickel organometallic compound to the standard containing 5 ml. of tetraethyllead per gallon as motor mix in toluene. Fluorescent measurements made before and after the addition of nickel showed a decrease in the original fluorescent intensity of the lead line to 91.5% in the 45° cell and to 87.7% in the 60° cell. The calculated effective absorbing paths for the two cells were 0.055 and 0.09 cm., respectively. These results clearly show a smaller matrix absorption effect with the 45° sample cell, which was therefore selected as the preferable cell for the present study.

RESULTS AND DISCUSSION

The fluorescent method has been applied to the analysis of 46 samples giving an average difference of 0.026 ml. of tetraethyllead per gallon and a standard deviation of 0.028 ml. of tetraethyllead per gallon based on a comparison with the chemical method of analysis, ASTM D526-42 (molybdate titration). These samples were selected to cover gasolines containing 0.1 to 6.0 ml. of tetraethyllead per gallon as neat tetraethyllead, as motor mix, and as aviation mix. The sulfur content varied from 0.003 to 0.125 weight %.

The time required for a complete tetraethyllead analysis is from 5 to 10 minutes, depending on the tetraethyllead concentration. Sample preparation consists simply of rinsing and filling the fluorescent cell. The method compares with the direct x-ray absorption method with respect to time required per determination and to average error. It is less effected by sulfur and other impurities in the order of 10 to 15 times, which enhances its value for the analysis of gasolines. Further experience with the method and subsequent development in instrumentation should result in an improvement in its accuracy and its application. The present accuracy, however, approaches the limit imposed on the method from statistical counting errors, which for the present selection of conditions is 0.76%, equivalent to 0.023 ml. at the 3.0-ml. level. It has come to the authors' attention that similar results have been obtained in other laboratories (*13*, *19*).

LITERATURE CITED

(1) Behr, F. A., "Some New Aspects Regarding High Intensity X-Ray Spectrography," Pittsburgh Conference on X-Ray and Electron Diffraction, Nov. 7, 1952.
(2) Birks, L. S., and Brooks, E. J., ANAL. CHEM., **22**, 1017 (1950).
(3) *Ibid.*, **23**, 707 (1951).
(4) Birks, L. S., Brooks, E. J., Friedman, H., and Roe, R. M., *Ibid.*, **22**, 1258 (1950).
(5) Brissey, R. M., *Ibid.*, **24**, 1034 (1952).
(6) *Ibid.*, **25**, 190 (1953).
(7) Calingaert, G., Lamb, F. W., Miller, H. L., and Noakes, G. E., *Ibid.*, **22**, 1238 (1950).
(8) Compton, A. H., and Allison, S. K., "X-Rays in Theory and Experiment," 2nd ed., New York, D. Van Nostrand Co., 1949.
(9) Cordovi, M. A., *Steel*, **123**, 88 (1948).
(10) Friedman, H., and Birks, L. S., *Rev. Sci. Instr.*, **19**, 323 (1948).
(11) Hevesy, G. von, "Chemical Analysis by X-Rays and Its Applications," New York, McGraw-Hill Book Co., 1932.
(12) Hughes, H. K., and Hochgesang, F. P., ANAL. CHEM., **22**, 1248 (1950).
(13) Humphries, J. T., Wright-Patterson Air Force Base, Ohio, *Air Force Tech. Rept.* 6520 (1951).
(14) Kellogg, H. W., Mahlke, E. F., and Jones, J. T., *Petroleum Processing*, **7**, 1430 (1952).
(15) Koh, P. K., and Caugherty, B., *J. Appl. Phys.*, **23**, 427 (1952).
(16) Mortimore, D. M., and Romans, P. A., *J. Opt. Soc. Amer.*, **42**, 673 (1952).
(17) Noakes, G. E., "Application of Fluorescent X-Ray Spectroscopy in Metallurgy," Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 4, 1953.
(18) U. S. Dept. Interior, Bur. Mines National Motor Gasoline Survey, for Winter, 1953–54, *Rept. Invest.* 5066.
(19) Van Nordstrand, R. A., Sinclair Research Laboratories, Inc., Harvey, Ill., private communication.
(20) Vollmar, R. C., Petterson, E. E., and Petruzzelli, P. A., ANAL. CHEM., **21**, 1491 (1949).

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Acetic Anhydride in Nonaqueous Titrimetry

Determination of Sulfuric Acid Catalyst in Ethyl Alcohol Esterification Mixtures

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Acetic anhydride has been used to determine sulfuric acid catalyst in industrial ethyl alcohol esterification mixtures, and other solvents in the mixture have been studied. Acetic anhydride has been used as a solvent for the titration of a number of weak organic bases. Pyridine, some amines, and ureas have been titrated with considerable sharpness of end point, and in some cases large potential changes at the end point. A technique for use of this solvent in titrating weak primary and secondary amine bases is applicable to the determination of some bases whose K_b values in water are 10^{-15} or larger.

THE concentration of sulfuric acid catalyst in continuous industrial ethyl alcohol esterification mixtures is important as regards yield of ethyl acetate and the corrosion of some types of plant equipment. These esterification mixtures are composed in the main of varying amounts of ethyl alcohol, acetic acid, ethyl acetate, and water. A simple method of analysis for the sulfuric acid catalyst consists of the potentiometric titration of the sulfuric acid in a sample of esterification mixture with a standard acetic acid solution of sodium acetate after an equal volume of acetic anhydride has been added to the sample.

The successful use of acetic anhydride in this instance has prompted its use in the titration of a number of weak organic bases including some with primary and secondary amine function In addition, these results have been viewed as a part of the whol position of acetic anhydride in nonaqueous titrimetry (*2–4, 6–8*)

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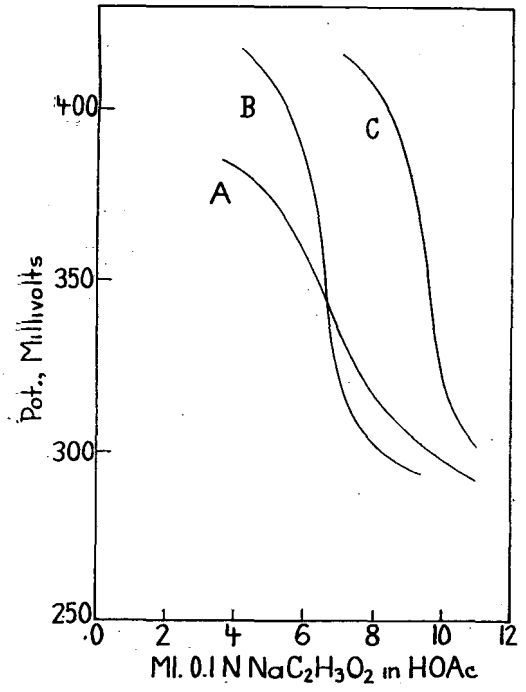

Figure 1. Titration Curves of Sulfuric Acid in a Low Water Content Industrial Ethyl Alcohol Esterification Mixture

A. Without acetic anhydride
B. With acetic anhydride
C. With acetic anhydride plus 0.0285 gram of sulfuric acid

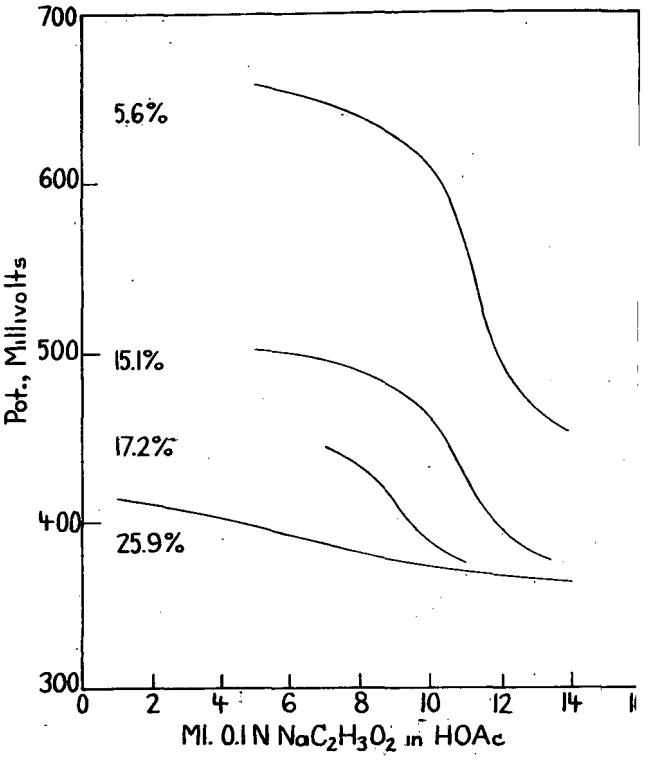

Figure 2. Titration Curves of Sulfuric Acid in Some Industrial Ethyl Alcohol Esterification Mixtures with Weight Per Cent of Water Shown

Performed in presence of acetic anhydride

REAGENTS

Acetic acid, A.C.S. reagent grade.
Acetic anhydride, A.C.S. reagent grade.
Ethyl alcohol, Carbide and Carbon Chemicals Co.
Ethyl acetate, Carbide and Carbon Chemicals Co.
Sulfuric acid, Baker and Adamson, reagent grade.
Sodium acetate, 0.1*N*, A.C.S. reagent grade in acetic acid.
Allylthiourea, 0.02*N*, A.C.S. reagent grade in acetic acid.
p-Aminodimethylaniline, 0.019*N*, technical grade in aceti acid.
Dimethylaniline, 0.022*N*, technical grade in acetic acid.
Diphenylamine, 0.02*N*, technical grade in acetic acid.
Glycine, 0.02*N*, A.C.S. reagent grade in acetic acid.
Pyridine, 0.018*N*, A.C.S. reagent grade in acetic acid.
Urea, 0.02*N*, A.C.S. reagent grade in acetic acid.
Perchloric acid, 0.1*N*, A.C.S. reagent grade in acetic acid.

PROCEDURE

Sulfuric Acid Determination. A 100-ml. sample of th esterification mixture is pipetted into a beaker containing magnetic stirring bar. An equal volume of acetic anhydride i added. The sulfuric acid contained is titrated using a pH meter (Beckman, Model H2 glass electrode) fitted with a fiber tip calomel and a glass electrode. A standard acetic acid solu tion of sodium acetate is used as titrant.

In adding acetic anhydride to those esterification mixtures containing appreciable quantities of water or ethyl alcohol, a large amount of heat may be given off, so that it may be necessary to carry out the operation in a flask fitted with a reflux condenser.

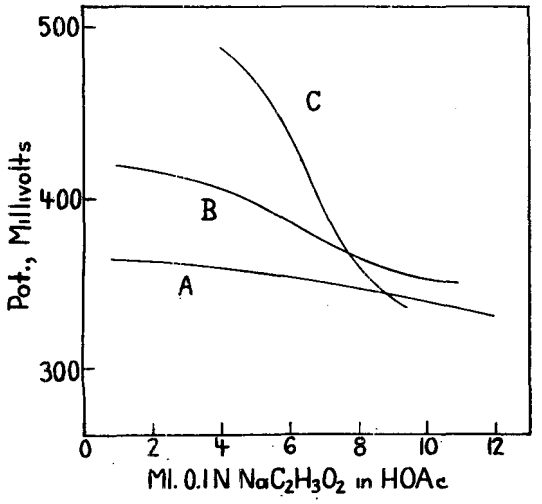

Figure 3. Titration Curves of Sulfuric Acid in a High Water Content Industrial Ethyl Alcohol Esterification Mixture

Under various conditions of exposure in acetic anhydride

In some cases the reaction between the acetic anhydride and the water and/or ethyl alcohol caused the generation of heat so that the mixutres came to a boil before the titrations were completed. However, in many cases duplicates of the titration mixtures remained as much as 15 to 30 minutes longer than would be necessary for titration before they came to a spontaneous boil. This boiling was preceded by a rapid change in temperature.

Titration of Amine Bases. In the titration of a base containing primary or secondary amine function, an aliquot portion of an acetic acid solution of the base is added to 100 ml. of acetic anhydride at 0° C. The base is then rapidly titrated with a standard solution of perchloric acid in acetic acid or acetic anhydride.

For bases that do not readily react with acetic anhydride, the titration may be carried out at room temperature.

ROLE OF ANHYDRIDE IN SULFURIC ACID TITRATION

In all cases the presence of acetic anhydride in the titration mixture is of value. Without the acetic anhydride the titration proceeds sufficiently well in some cases (Figure 1), but in other cases (Figures 2, 3) acetic anhydride must be added if the presence of the sulfuric acid is to be known. This has prompted an investigation of the influence of the constituents of the esterification mixture.

Sulfuric acid titrates as a monobasic acid in the solvent systems used. The advantage of using acetic anhydride as a solvent in the titration of sulfuric acid is well illustrated in Figure 4. The influence of ethyl acetate is exemplified by the data of Figure 5. These data were more readily obtained than the data in the ethyl acetate curve of Figure 4.

An adverse effect is attendant with the presence of water as illustrated by the data of Figure 2 collected for several industrial esterification mixtures. The weight per cent of water for each case was determined by the Karl Fischer method. For the cases of the larger water concentrations this effect can be prevented by partial removal of the water through reaction with acetic anhydride. Three titrations performed on one esterification mixture containing 19.7% water are shown in Figure 3. Curve *A* represents the titration performed in the absence of acetic anhydride. Curve *B* represents the titration of the same material in the presence of 100 ml. *of acetic anhydride* and curve *C* is for the titration after some of the acetic anhydride had reacted with only 47.7% of the water present and undoubtedly some or all of the ethyl alcohol. Ethyl alcohol effects the titration in the same manner as does water. After the titration, 52.3% of the water was still present, as determined by the Karl Fischer method.

The removal of some water from all of these systems tends to decrease the "leveling" power of the solvent. Total removal of water is not essential, but very large concentrations of water must be decreased somewhat before its adverse influence is sufficiently

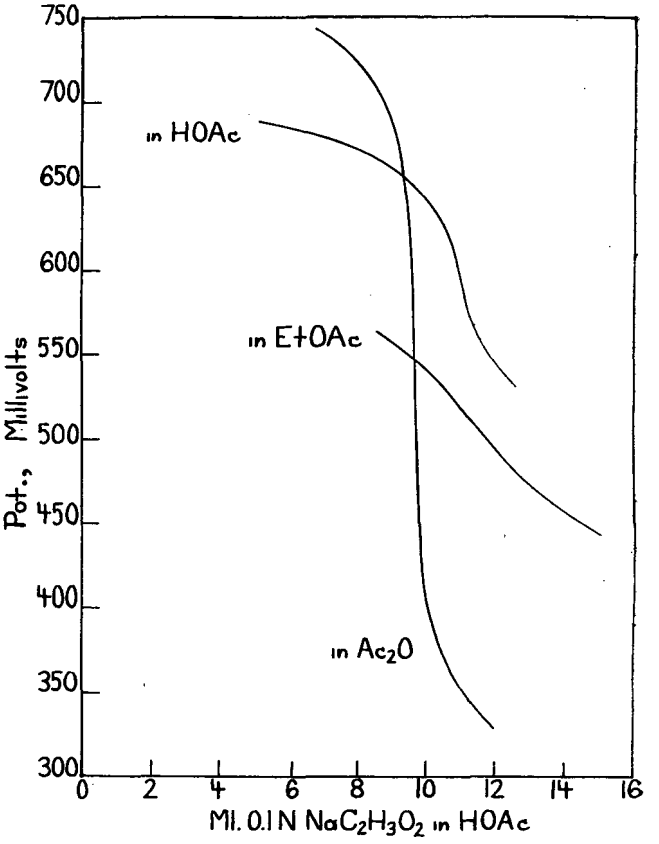

Figure 4. Titration Curves of Sulfuric Acid in Several Solvents

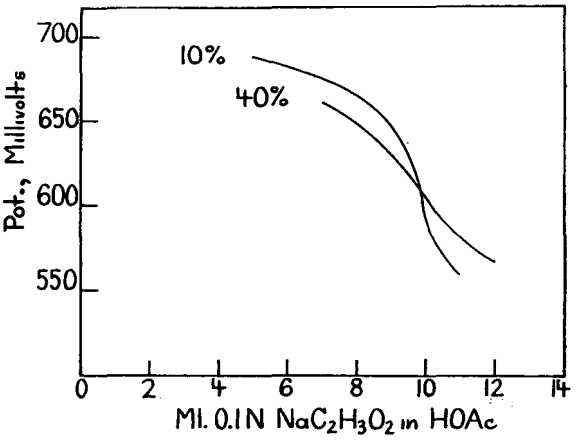

Figure 5. Titration Curves of Sulfuric Acid in Acetic Acid–Ethyl Acetate Mixtures

With per cent ethyl acetate shown

removed. In some cases not all of the water originally present had been removed. Therefore, it may be that the results are not attributable to complete removal of water only. Russell and Cameron (7) have concluded that "sulfuric acid, perchloric acid, and certain sulfonic acids show increased acidities in the presence of acetic anhydride which cannot be accounted for by dehydration of the solution."

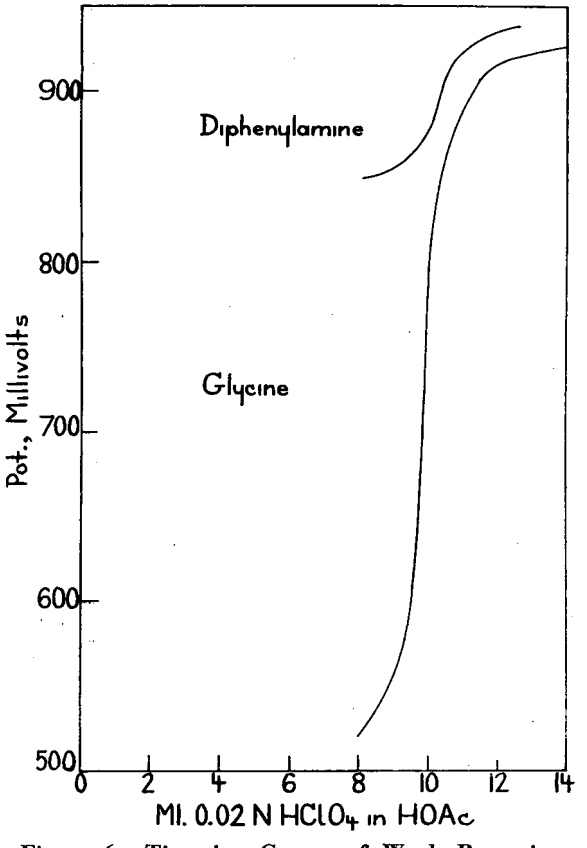

Figure 6. Titration Curves of Weak Bases in Acetic Anhydride at 0° C.

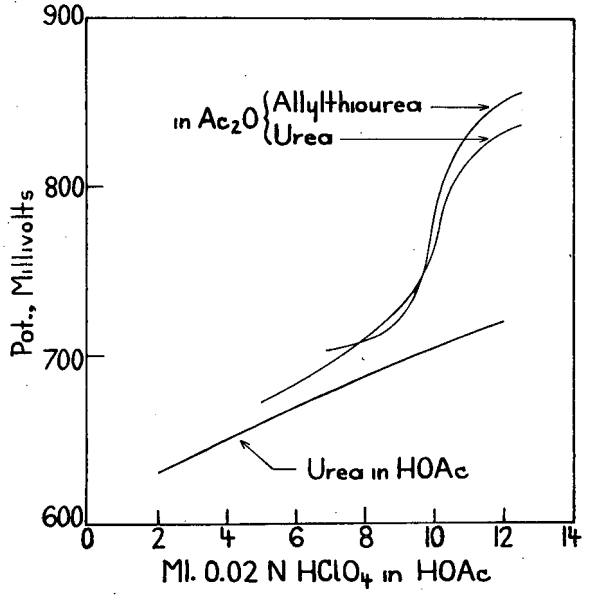

Figure 7. Titration Curves of Weak Bases at 0° C.

Table I. Base Dissociation Constants in Water

Base	K_b	Reference
Diphenylamine	6.3×10^{-14}	(1)
Glycine	2.26×10^{-12}	(6)
Pyridine	1.4×10^{-9}	(6)
Thiourea	1.1×10^{-15}	(6)
Urea	1.5×10^{-14}	(6)

TITRATION OF BASES IN ACETIC ANHYDRIDE

The use of acetic anhydride as a solvent has permitted very sharp end points to be obtained in the titrations of some weak bases (Figures 6, 7, and 8). In the titration of a tertiary amine there has been no difficulty due to reaction with acetic anhydride. However in the case of a primary or secondary amine the reaction with acetic anhydride has been prevented only by titrating in systems at 0° C. This has been accomplished in four cases (Figures 6 and 7).

ACETIC ANHYDRIDE IN TITRIMETRY

Acetic anhydride has been used as a dehydrating agent for either titrant (2, 3, 6) or some organic solvent (4) or as a reactant for the removal of some interfering substance. Wagner, Brown, and Peters (8) have used it in this latter way in the determination of tertiary nitrogen. In titrating some weak organic bases, Fritz and Fulda (4) used acetic anhydride–nitromethane mixtures as the solvent. They concluded that completely anhydrous con-

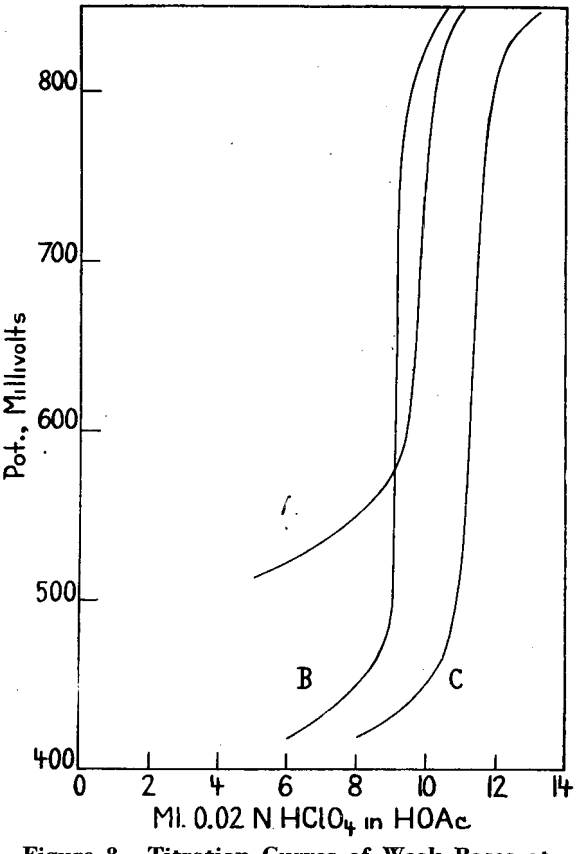

Figure 8. Titration Curves of Weak Bases at Room Temperature in Acetic Anhydride

A. 10 ml. of 0.019*N* *p*-aminodimethylaniline in acetic acid added to acetic anhydride
B. 10 ml. of 0.018*N* pyridine in acetic acid added to acetic anhydride
C. 10 ml. of 0.022*N* dimethylaniline in acetic acid added to acetic anhydride

Table II. Titrations of Sulfuric Acid in Several Solvent Mixtures[a]

Solvent Mixture, 100 Ml.	Sulfuric Acid Content, Used, gram	Found, gram
Acetic acid	0.057	0.053
Acetic acid, 50% / Acetic anhydride, 50%	0.057	0.057
Ethyl alcohol, 30% / Acetic anhydride, 70%[b]	0.057 0.171	0.058 0.172
Ethyl acetate, 50% / Acetic anhydride, 50%	0.057 0.171	0.056 0.172
Water, 30% / Acetic anhydride, 70%[b]	0.057 0.171	0.057 0.170

[a] Mixtures were brought to a boil, then cooled to room temperature before titrating.
[b] Because of spontaneous reaction of water and ethyl alcohol with acetic anhydride, concentration of each component of solvent was uncertain at moment of titration.

ditions so obtained give rise to the large potential changes observed at the end point.

Although the conditions under which the sulfuric acid has been titrated seem favorable for dehydration, the titrations have been carried out in the presence of water. Acetic anhydride makes possible the simple and rapid determination of some weak organic bases (Table I) whose base ionization constants in water are less than 10^{-12}. In the absence of K_b for allylthiourea the value for thiourea has been included in Table I. It is suspected that the K_b values for these two substances are about the same. Markunas and Riddick (*6*) have recommended the use of acetic acid in titrating weak organic bases whose K_b values in water are 10^{-12} or larger. Urea, which does not give an insoluble perchlorate in acetic acid, has been listed by Fritz (*2*) as giving an unsatisfactory end point in acetic acid. The use of acetic anhydride in this instance removes this difficulty (Figure 7).

The data of Table II have been collected on a number of mixtures into which a known amount of sulfuric acid had been added. Table III gives the quantitative data on the titration of several weak bases in acetic anhydride.

Table III. Titrations of Weak Organic Bases in Acetic Anhydride[a]

Base	Purity Found, %
Allylthiourea	100[b]
Dimethylaniline	99[c]
p-Aminodimethylaniline	98.5[c]
Diphenylamine	100[c]
Glycine	100[b]
Pyridine	100[b]
Urea	100.5[b]

[a] All bases were used as received.
[b] Reagent grade materials.
[c] Technical or practical grade.

LITERATURE CITED

(1) Bell, R. P., "Acids and Bases. Their Quantitative Behavior," p. 66, London, Methuen and Co., Ltd., 1952.
(2) Fritz, J. S., ANAL. CHEM., **22**, 1028–9 (1950).
(3) *Ibid.*, **25**, 407–11 (1953).
(4) Fritz, J. S., and Fulda, M. O., *Ibid.*, **25**, 1837–9 (1953).
(5) Lange, N. A., "Handbook of Chemistry," 8th ed., p. 1234, Sandusky, Ohio, Handbook Publishers, Inc., 1952.
(6) Markunas, P. C., and Riddick, J. A., ANAL. CHEM., **23**, 337–9 (1951).
(7) Russell, J., and Cameron, A. E., *J. Am. Chem. Soc.*, **60**, 1345–8 (1938).
(8) Wagner, C. D., Brown, R. H., and Peters, E. D., *Ibid.*, **69**, 2609–10 (1947).

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Semimicromethod for Determination of Cyanate Ion in Presence of Interfering Substances

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The method described employs ion exchange removal of interfering cations, conversion of cyanate ion to ammonium ion by dilute acid, separation of ammonium ion by ion exchange, and photoelectric colorimetric analysis of ammonium ion with Nessler's reagent.

A COLORIMETRIC method for the determination of cyanate ion in the presence of interfering substances has been developed. The mixture containing cyanate ion is passed through a cation exchanger; the effluent is collected; the column is eluted with a sodium hydroxide solution and rinsed, thus freeing it from all cations except the sodium ion. The original effluent containing only anions, sodium ions, and neutral molecules is acidified, and cyanate ion is rapidly converted to ammonium ion. The resultant solution is again passed through the cation exchanger. Ammonium ion is quantitatively retained, and the anions, exchanged sodium ions, and neutral molecules are rinsed from the column and discarded. Ammonium ion is eluted, the elutriate is treated with Nessler's reagent, and the absorbance is determined. A calibration curve relates absorbance to the cyanate ion concentration. Interference may be expected from a rather limited number of substances that react with dilute acid to produce cations which, in turn, interfere with the Nessler's reaction.

At elevated temperatures in aqueous solutions, urea decomposes according to the following reaction

$$CO(NH_2)_2 - NH_4^+ + CNO^- \qquad (1)$$

In the course of a kinetic study of this reaction (*13*), an extremely rapid conversion of cyanate ion to ammonium ion by dilute sulfuric acid was observed.

$$CNO^- + 2H^+ + 2H_2O - NH_4^+ + H_2CO_3 \qquad (2)$$

This same reaction had been employed by Hertig (*4, 5, 14*), and others at elevated temperatures for the quantitative determination of cyanate.

Previous work (*6–8, 12*) had established a convenient method for the determination of the ammonium ion based on an ion exchange separation and subsequent colorimetric analysis with Nessler's reagent. Consequently, if Reaction 2 were quantitative at room temperature, cyanate ion could be easily determined by a technique similar to that employed for ammonium ion. Since this method had proved useful in the presence of interfering substances, and treatment of complex mixtures containing

cyanate at a lower temperature than that employed by Hertig seemed desirable, the proposed technique was subjected to further investigation.

APPARATUS AND REAGENTS

Colorimeter. A Lumetron photoelectric colorimeter Model 402E equipped with a blue glass filter M 440 and 10-mm. rectangular cuvets was employed.

Ion Exchange Columns. A convenient column may be prepared by joining a 38 × 200 mm. test tube to 32 cm. of 15-mm. borosilicate glass tubing. A 2-mm. stopcock is attached to the tubing to regulate the drop rate. Twelve columns were used in the work.

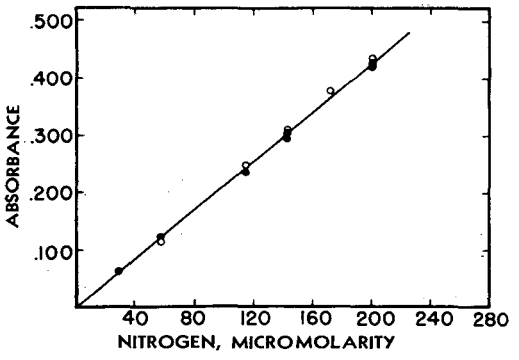

Figure 1. Absorbance as a Function of Nitrogen Content

○ Standard ammonium chloride
● Standard potassium cyanate

Nessler's Reagent. The preparation of this reagent has been described (*1*).

Ion Exchange Resin. For each column 11 ml. of low color Dowex 50 (20 to 50 mesh) was employed after it had been purified by recycling three times from the hydrogen to the sodium form.

Ammonia-Free Water. Distilled water passed through a large capacity Dowex 50 exchanger was used throughout the work.

All other reagents were of analytical reagent grade and conformed to A.C.S. specifications.

PROCEDURE

The separation and determination of cyanate, when interfering substances are present, are accomplished by the following steps:

1. To an appropriate aliquot of the solution containing cyanate add several drops of citrate buffer, to effect easy attainment of a sharp end point, and enough bromothymol blue to ensure a good color change.
2. Titrate the solution to a light green end point with dilute sulfuric acid or sodium hydroxide. Proper pH is essential for quantitative adsorption on the column.
3. Quantitatively transfer the solution to the ion exchange column (sodium form).
4. Adsorb at the rate of 1 drop every 3 seconds, retaining the effluent in a 100-ml. volumetric flask. Interfering cations are retained on the column; anions, including the cyanate ion, exchanged sodium ions, and neutral molecules are in the effluent.
5. Wash, retaining washes until total volume is about 90 ml.
6. Fill the columns with about 90 ml. of water and add 10 drops of 4*M* sodium hydroxide. Allow to flow at full rate and rinse thoroughly with water. This removes the interfering cations trapped in step 4.
7. To the retained effluent (steps 4 and 5) add 10 ml. of 1.0*N* sulfuric acid.
8. Dilute to volume, mix, and let stand 10 minutes or more. Cyanate ion is converted to ammonium ion.
9. Pipet appropriate aliquot into a beaker.
10. Repeat steps 1, 2, 3, and 4, but do not retain the effluent. Interfering anions and neutral molecules are discarded in this step.
11. Wash until effluent is free of indicator color.
12. Add approximately 90 ml. of water and 10 drops of 4*M* sodium hydroxide.
13. Elute into a 100-ml. flask at a rate of 1 drop every 2 seconds.
14. Add 4 ml. of Nessler's reagent and dilute to mark.
15. Allow to stand 15 minutes, and determine absorbance.

When interfering substances are known to be absent, only steps 6 through 15 need be followed.

RESULTS

Figure 1 shows a representative calibration curve obtained with both standard potassium cyanate and standard ammonium chloride. Figure 2 demonstrates the effect of increasing acid concentration with a fixed cyanate concentration. Complete conversion requires a mole ratio of acid to cyanate of at least 2.5×10^3. At high acid concentration a slight decrease in the apparent concentration of cyanate is observed. This presumably occurs because of the very high sodium content of these solutions after neutralization (step 10 in procedure). A competition of the sodium ion and ammonium ion for the resin takes place, producing the observed effect. No interference from urea, ammonium ion, or common cations and anions at moderate concentrations was observed (*6*). A series of experiments showed that the conversion of cyanate ion to ammonium ion in 0.1*N* sulfuric is complete in 10 minutes or less (step 8). The range of the method as described is approximately 40 to 200 micromoles of cyanate per liter of nesslerized solution (step 14). Appropriate dilution before adsorption (step 3) can readily be made to cover a wide range of concentrations. The standard deviation of a set of ten duplicate analyses of a solution containing 170 micromoles per liter was 1.5%.

DISCUSSION

A search of the literature has revealed several methods for cyanate determination; a gravimetric method (*3*) based on the comparative insolubility of silver cyanate, argentometric methods (*10, 11*), and two colorimetric methods (*9, 2*).

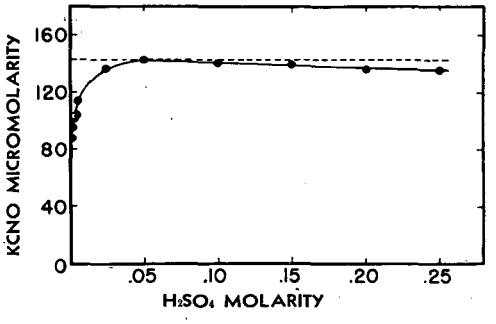

Figure 2. Effect of Acid Concentration on Cyanate-Ammonium Conversion

Dashed line represents complete conversion

The gravimetric and argentometric methods were not well suited to semimicrodeterminations, and, like the first colorimetric method, had not been extensively evaluated in the presence of interfering substances. Dodge and Zabban (*2*), however, employed a method similar in some respects to the one reported here. In their determination cyanate ion was converted to ammonium ion by digestion with sulfuric acid (*4, 5, 14*), for 0.5 hour, keeping the solution near the boiling point. The ammonium ion formed was analyzed with Nessler's reagent. If interfering substances were present, the method was somewhat difficult to apply. In the presence of ammonium salts or other nitrogenous materials cyanate was precipitated as silver cyanate,

which was subsequently digested with acid. Before analysis, the silver ion had to be removed to prevent reaction with the Nessler's reagent. If interfering cations were present a Rochelle salt solution was used to complex them. This procedure, however, could be applied to only a limited number of cations present in low concentration. In some instances, a modified Kjeldahl method was employed to separate the ammonia.

The present method is well suited to the analysis of mixtures. The preliminary pass through the column removes interfering cations, which are subsequently eluted and discarded. The comparatively gentle treatment of the elutriate containing the anions, including cyanate, exchanged sodium ions, and neutral molecules with 0.1*N* acid at room temperature for 10 minutes should convert only the most easily hydrolyzable substances to ammonium ion. The second pass of the solution through the column retains only sodium ion and anions or molecules that are converted by mild acid treatment to cations. Of these cations only those that on elution interfere with the Nessler's reaction will prove troublesome. It seems unlikely that many substances other than the cyanate ion will exhibit this sequence of chemical behavior.

Ammonium ion and cyanate ion can be readily determined in solutions containing both, without resorting to a method of differences.

The procedure is easy to perform and fairly rapid. In the current study, 24 analyses per day are made routinely by a single analyst.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) American Public Health Association, New York, "Standard Methods for the Examination of Water and Sewage," 1933.
(2) Dodge, B. F., and Zabban, W., *Plating*, **39**, 381 (1952).
(3) Duval, C., *Anal. Chim. Acta.*, **5**, 506 (1951).
(4) Hertig, O., *J. Soc. Chem. Ind. (London)*, **20**, 838 (1901).
(5) Hertig, O., *Z. angew. Chem.*, **14**, 585 (1901).
(6) Kistiakowsky, G. B., Manglesdorf, P. C., Rosenberg, A. J., and Shaw, W. H. R., *J. Am. Chem. Soc.*, **74**, 5015 (1952).
(7) Kistiakowsky, G. B., and Shaw, W. H. R., *Ibid.*, **75**, 866 (1953).
(8) *Ibid.*, p. 2751.
(9) Martin, E. L., and McClelland, ANAL. CHEM., **23**, 1519 (1951).
(10) Ripan-Tilici, R., *Z. anal. Chem.*, **99**, 415 (1934).
(11) *Ibid.*, **102**, 32 (1935).
(12) Rosenberg, A. J., thesis, Harvard University, 1952.
(13) Shaw, W. H. R., and Bordeaux, J. J., work in progress.
(14) Williams, H. E., "Cyanogen Compounds," pp. 400–2, London, Edward Arnold and Co., 1948.

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Determination of High Molecular Weight Ketones

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A simple rapid method for the determination of high molecular weight ketones uses hydroxylamine hydrochloride and a high molecular weight amine in nonaqueous solvents. The method has been used to determine varying amounts of carbonyl compounds in mixtures, and has been used successfully as a control procedure.

HYDROXYLAMINE hydrochloride has been used as a reagent for the determination of aldehydes and ketones in analytical procedures for a number of years.

The reactions involved are

$$RCHO + NH_2OH \cdot HCl \rightarrow RCH{=}NOH + H_2O + HCl \quad (1)$$

$$RCOR' + NH_2OH \cdot HCl \rightarrow RR'C{=}NOH + H_2O + HCl \quad (2)$$

The use of the reagent was reported by Brochet and Cambier (*2*) as early as 1895 for the quantitative determination of formaldehyde. Bennett and Donavan (*1*) and Marasco (*5*) later used it to determine acetone.

The procedures employing hydroxylamine reagent to determine carbonyl compounds are of four general types: titration of the hydrochloric acid produced as shown in the above equations (*3*, *9*); neutralization of the hydroxylamine hydrochloride liberating free hydroxylamine to react with the carbonyl groups, followed by titration of unreacted hydroxylamine (*10*, *11*); determination of the water produced in the reaction using Karl Fischer reagent (*7*); and measurement of the change in pH caused by the liberation of hydrochloric acid as indicated in Equations 1 and 2 (*4*, *8*). An excellent summary of hydroxylamine procedures has been prepared by Mitchell (*6*).

A procedure has been developed to fill the need for a rapid and simple determination of high molecular weight aliphatic ketones in the presence of varying amounts of free fatty acid. No previously mentioned method could be applied to the determination of such ketones as stearone and palmitone, because of their limited solubility in all but a few suitable solvents. Using as the reagent 0.5*N* hydroxylamine hydrochloride in a mixed solvent of 65% isopropyl alcohol and 35% methanol, the analytical procedure has been used effectively to determine these ketones. A measured excess of an organic base (octadecenylamine) in isopropyl alcohol is added to facilitate complete reaction between the ketone and hydroxylamine hydrochloride by combining with the hydrochloric acid liberated. At the end of the reaction period, unreacted amine is titrated with standard hydrochloric acid solution in isopropyl alcohol to a bromophenol blue end point. A titration is run on a blank containing the exact quantities of hydroxylamine hydrochloride and octadecenylamine used with the sample. The difference between blank and sample titrations gives a direct measure of the carbonyl groups present in the sample. Since hydrochloric acid formed in the reaction is never liberated and is constant, it does not affect the titration of either the sample or the blank. Under these conditions, any free fatty acid present will not affect the titration. If amine salts of the fatty acid are formed, they will titrate as free amine.

A procedure using free hydroxylamine in a proper solvent mixture, omitting the organic base, may be used. However, since the stability of a solution of free hydroxylamine is poor, this procedure is impractical where a great number of control analyses must be made constantly.

Although the results of this paper deal entirely with ketones, the procedure has been applied successfully to some high molecular weight aldehydes.

REAGENTS

Hydroxylamine Hydrochloride Reagent. Dissolve 35 grams of hydroxylamine hydrochloride (reagent grade) in 350 ml. of

methanol, heating if necessary. Dilute to 1 liter with isopropyl alcohol.

Amine Reagent. Dissolve 140 grams of octadecenylamine (Armeen SD, Armour and Co., Chicago, Ill.) in isopropyl alcohol to make 1 liter of solution. Theoretically, any amine that gives a sharp bromophenol blue end point when titrated with standard hydrochloric acid in isopropyl alcohol and which forms an isopropyl alcohol–soluble hydrochloride may be used as a substitute for octadecenylamine. Armeen SD satisfies these requirements.

Standard Hydrochloric Acid in Isopropyl Alcohol. Prepare a 0.5*N* solution by dissolving 43.5 ml. of concentrated hydrochloric acid in 300 ml. of isopropyl alcohol. Dilute this solution to 1 liter with additional isopropyl alcohol. Standardize according to one of the usual procedures. This solution should be standardized weekly, although it will keep without changing strength for several weeks if stored in a well-stoppered bottle.

Bromophenol Blue Indicator. Prepare a 0.1% solution in Formula 3A alcohol.

ANALYTICAL PROCEDURE

Melt the sample, and if turbid, filter in an oven at 100° C. to obtain a sparkling clear sample. Weigh into a 250-ml. glass-stoppered volumetric flask. Choose the sample weight so that about half the reagent will remain unreacted after the reaction is complete (see Calculations for equation by which size of sample may be estimated). Pipet 25 ml. of the amine reagent into the flask, and then pipet 25 ml. of the hydroxylamine hydrochloride reagent into the flask. It is important to add the reagents in this sequence if this method is applied to aldehydes, to prevent the formation of acetals with the isopropyl alcohol. For difficultly soluble samples, heat the flask moderately on a hot plate, swirling the contents until the sample is dissolved.

Place the flask in a water bath at 70° C., loosen the stopper momentarily to expel air, then stopper firmly. After heating for 30 minutes, remove the flask from the water bath. Add 0.5 ml. of bromophenol blue indicator and titrate with the standard alcoholic hydrochloric acid to a green color; then continue adding the acid in increments of 0.1 ml., shaking after each addition until a yellow end point is reached. Make a blank determination, following the same procedure without the sample. Care should be taken to titrate the blank and the sample at about the same temperature. Titration of some samples will not give a sharp end point; hence, it is helpful to run the blank first and then titrate the sample to the same color.

When an electrically heated water bath is not available, the procedure is modified as follows. The samples are weighed into 500-ml. glass-stoppered Erlenmeyer flasks, the reagents are then added as before, and the flasks are stoppered. A sheet of heavy asbestos paper is placed on top of an ordinary steam bath, and the flasks are placed on the asbestos paper. The asbestos keeps the alcohol from boiling. The procedure is followed exactly as before. With a light background the end points of the samples are very easily compared with the blank determination using the 500-ml. Erlenmeyer flasks.

CALCULATIONS

$$\% \text{ ketone} = \frac{(\text{ml. blank} - \text{ml. titration}) \times N \text{ HCl} \times \text{mol. wt. of ketone}}{\text{wt. of sample (grams)} \times 10 \times \text{number of carbonyl groups in molecule}}$$

$$\text{Apparent molecular weight of ketone} = \frac{\text{wt. of sample (grams)} \times 1000 \times \text{no. of carbonyl groups}}{(\text{ml. blank} - \text{ml. titration}) \times N \text{ HCl}}$$

An equation for estimating the sample weight of an unknown having one carbonyl group and consisting mostly of ketone is as follows: weight of sample in grams = 0.007 × molecular weight of ketone.

DISCUSSION AND RESULTS

Strong acids or bases in the sample will interfere with the titration. They may be removed effectively by washing the samples with boiling water. Weak organic bases will interfere also; they may be removed by washing the sample with hot dilute aqueous mineral acid, after which the acid must be removed by washing the sample with boiling water. Some metals and their salts may oxidize the hydroxylamine. Such contaminants can be removed by filtering the sample in an oven at 100° C.

Some results obtained on high molecular weight ketones using the described method are summarized in Tables I, II, III, and IV. The ketones were prepared and purified in the Research Division Laboratories of Armour and Co.

Tables III and IV show the results of analyses of samples subjected to various reaction periods at 70° C. The period commenced from the time solution of the sample was attained by heating the flask gently on a hot plate.

Table I. Determination of Carbonyl in Purified Ketones

Compound[a]	No. of Detns.	% of Theoretical[b] Carbonyl Found
Laurone	5	99.5 ± 1.0
Myristone	5	99.2 ± 1.0
Palmitone	5	99.5 ± 1.0
Stearone	5	99.0 ± 1.0
Diheptyl ketone	4	99.0 ± 1.0
Oleone	5	99.1 ± 1.0
Nonyl heptadecyl ketone	3	99.0 ± 1.0
Dionyl ketone	2	98.2 ± 1.0

[a] All compounds were of 95% purity or higher.
[b] All samples were run for 30 minutes at 70° C.

Table II. Determination of Carbonyl in Ketone–Fatty Acid Mixtures

Mixture[a]	No. of Detns.	Ketone Found, %
Laurone, 80%–lauric acid, 20%	3	78.5
Laurone, 50%–lauric acid, 50%	3	49.0
Laurone, 20%–lauric acid, 80%	3	20.7
Palmitone, 80%–palmitic acid, 20%	3	78.2
Palmitone, 50%–palmitic acid, 50%	3	51.4
Palmitone, 20%–palmitic acid, 80%	3	20.4
Stearone, 80%–stearic acid, 20%	3	78.9
Stearone, 50%–stearic acid, 50%	3	52.5
Stearone, 20%–stearic acid, 80%	3	21.6

[a] Prepared gravimetrically using materials of known purity.

Table III. Per Cent Ketone Determined in Crude Laurone at Various Reaction Periods

Reaction Time (at 70° C.), Min.	Ketone Found, %
0	88.5
5	90.5
15	91.6
30	90.8
120	91.6

Table IV. Per Cent Ketone Determined in Purified Stearone at Various Reaction Periods

Reaction Time (at 70° C.), Min.	Ketone Found, %
0	91.5
15	96.2
30	98.8
60	98.8

LITERATURE CITED

(1) Bennett, A. H., and Donavan, F. R., *Analyst*, **47**, 146–52 (1922).
(2) Brochet, A., and Cambier, R., *Compt. rend.*, **120**, 449–54 (1895).
(3) Bryant, W. M. D., and Smith, D. M., *J. Am. Chem. Soc.*, **57**, 57–61 (1935).
(4) Byrne, R. E., Jr., ANAL. CHEM., **20**, 1245 (1948).
(5) Marasco, M., *Ind. Eng. Chem.*, **18**, 701–2 (1926).
(6) Mitchell, J., Jr., "Organic Analysis," Vol. I, p. 243, New York, Interscience Publishers, 1953.
(7) Mitchell, J., Jr., and Bryant, W. M. D., *J. Am. Chem. Soc.*, **63**, 573–4 (1941).
(8) Roe, H. R., and Mitchell, J., Jr., ANAL. CHEM., **23**, 1758–60 (1951).
(9) Smith, D. M., and Mitchell, J., Jr., *Ibid.*, **22**, 750–5 (1950).
(10) Stillman, R. C., and Reed, R. M., *Perfumery Essent. Oil Record*, **23**, 228 (1932).
(11) Trozzolo, A. M., and Lieber, E., ANAL. CHEM., **22**, 764–8 (1950).

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Permanent Color Standards for Determination of Phosphate by Molybdenum Blue Method

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This note describes the characteristics of solutions which have been found to be very suitable as permanent color standards for the routine estimation of phosphate in the concentration range from 10 to 100 parts per billion of phosphorus. Application of the standards to the field determination of phosphate in natural waters is described elsewhere (7).

IN THE course of the development of a field method for the determination of phosphate, it became evident that there is a definite need for stable, well-matching, permanent color standards for use with the molybdenum blue method of phosphate determination.

The most widely used method for the determination of traces of phosphorus involves the reduction of a heteropoly molybdophosphate to give a blue color, commonly referred to as molybdenum blue (*8*). The need for standards is emphasized by the fact that the molybdenum blue solutions fade after about 15 minutes. This method can be adapted for field use in the concentration range from 10 to 100 parts per billion phosphorus by using tall-form Nessler tubes of 50-ml. capacity as the viewing tubes and permanent solution color standards for comparison of the unknowns.

Several permanent solution color standards have been proposed for visual color comparison of the molybdenum blue solutions in the phosphate determination (*1, 2, 6*).

However, Woods and Mellon (*8*) have found that most of the color standards suggested do not give a good visual match with the reduced molybdophosphate; one mixture (*6*) is no more stable than the reduced molybdophosphate itself. Permanent color standards are needed which are not only stable upon exposure to air and sunlight but also closely match the color of the reduced molybdate.

Color standards composed of mixtures of copper sulfate and bromophenol blue in an acetate buffer of pH 4.53 make very satisfactory and useful color standards for phosphate determination in the range from 10 to 100 parts per billion of phosphorus. The color developed by this system is stable, and matches the color well enough so that unknown phosphate solutions can be determined to ±5 parts per billion. Table I gives the amounts of the components needed to prepare the standards. Although these values will prepare solutions which show a reasonably close color match, in every instance the final adjustment should be made empirically against a known phosphate solution under conditions identical with those to be used in the actual unknown determination. Changes in hue of the standard can easily be made by changing the pH of the solution, as this changes the hue of the bromophenol blue.

Figure 1 compares the spectral transmittancy curves of a molybdenum blue solution containing 500 parts per billion of phosphate phosphorus and its visually matched color standard. A Model B Beckman spectrophotometer with 1-cm. cells was used to obtain the curves. The fact that the spectrophotometric curves do not exactly match does not necessarily mean that the colors do not match by visual comparison. To obtain a measure

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Table I. Volumes of Copper Sulfate (0.00883 Gram[1] of Copper per Ml.) and Bromophenol Blue (0.010%) Needed to Prepare Color Standards

(Diluted to 30 ml. with buffer of pH 4.53)

Concn. Phosphorus, P.P.B.	$CuSO_4$, Ml.	Bromophenol Blue, Ml.
10	0.40	0.085
20	0.70	0.150
30	0.96	0.210
40	1.17	0.330
50	1.40	0.400
60	1.60	0.500
70	1.79	0.620
80	1.95	0.750
90	2.17	0.900
100	2.40	1.000
120	2.82	1.145
150	3.40	1.540

Table II. Variation of Spectral Transmittance with Time for Copper Sulfate–Bromophenol Blue Color Standard Solution

A mixture of 4.88 mg. of $CuSO_4$ and 0.920 mg. of bromophenol blue in 10 ml. of a sodium acetate–acetic acid buffer solution of pH 4.53, in solutions continually exposed in east window for time indicated

Wave Length, Mμ	Transmittance, %				
	0 days	3.5 days	9 days	18 days	24 days
500	...	...	...	97.2	96.5
525	...	...	...	96.5	96.2
550	...	...	...	94.7	94.4
575	...	...	...	91.6	91.3
600	...	...	...	88.8	88.9
625	89.5	89.5	89.3	90.3	91.0
650	88.2	88.4	87.5	88.0	88.6
675	85.0	84.0	84.3	84.7	85.0
700	81.6	81.6	81.1	81.3	81.6
725	79.2	79.3	78.7	79.0	79.3
750	78.0	78.0	77.5	77.6	78.3
800	78.8	78.7	78.3	78.4	79.0
850	81.6	81.3	81.1	81.2	81.5

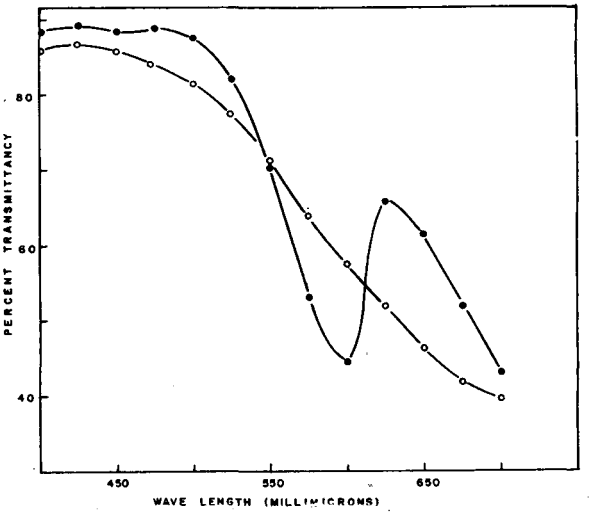

Figure 1. Spectral Transmittancy Curves for Molybdenum Blue Test on 500 Parts per Billion of Phosphorus and Its Visibly Matched Color Standard

1-cm. cells used in Model B Beckman spectrophotometer
● Color standard
○ Standard phosphate

of visual matching the chromaticity coordinates, x and y, based on the International Commission on Illumination standard observer and coordinate system (*3*), were calculated. The method of ten selected coordinates (*5*) and Standard Illuminant C were used. For the reduced molybdophosphate, values of x and y were 0.153 and 0.200, respectively; for the visually matched color standard, 0.149 and 0.190. These figures show that the solutions match well in color hue. With the aid of the Maxwell triangle (*4*) to indicate hue deficiency, an even closer match probably could be obtained by a slight empirical adjustment of solution pH.

The stability of the color standards is demonstrated by the data of Table II. Spectral transmittancy curves were determined periodically on a mixture of 4.98 mg. of copper sulfate and 0.920 mg. of bromophenol blue in 10 ml. of acetate buffer of pH 4.53 during the time it remained in an east window exposed to considerable direct morning sunlight. There was no change in the absorption spectrum of the mixture even after 24 days, indicating that the color standards have good stability characteristics.

The color standards should prove valuable for routine use in the visual estimation of traces of phosphate by the molybdenum blue method.

LITERATURE CITED

(1) Denigès, *Bull. soc. pharm. Bordeaux*, **65**, 107 (1927).
(2) Florentin, *Ann. chim. anal. et chim. appl.*, **3**, 295 (1921).
(3) Mellon, M. G., "Analytical Absorption Spectroscopy," p. 522, New York, John Wiley & Sons, 1950.
(4) *Ibid.*, p. 525.
(5) *Ibid.*, p. 535.
(6) Meyer, *Science*, **72**, 174 (1930).
(7) Parry, E. P., and McClelland, A. L., *Progressive Fish Culturist*, **16**, No. 4, 158 (1954).
(8) Woods, J. T., and Mellon, M. G., IND. ENG. CHEM., ANAL. ED., **13**, 760 (1941).

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Method for Sparking Thin Sheet Samples for Spectrographic Analysis

Application to Manganese and Niobium Determination in Stainless Steel

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A technique has been developed for sparking thin sheets of steel and applied to the spectrographic determination of manganese and niobium. The sample is cooled by helium during sparking.

A TECHNIQUE has been developed for sparking thin sheets of steel for the spectrographic determination of manganese and niobium. Normally, reproducibility and accuracy are achieved by using massive samples which do not become appreciably warmer than room temperature when sparked. However, when thin sheets of stainless steel are sparked, the heat produced by the spark is sufficient to cause oxidation of the sheet on the unsparked side. Whenever this evidence of overheating occurs, manganese and niobium values are very erratic and unusually high, manganese being affected much more than niobium. It is believed that, because of the high local temperatures of the steel, excessive volatilization of manganese and niobium occurs (with respect to the amount of iron volatilized).

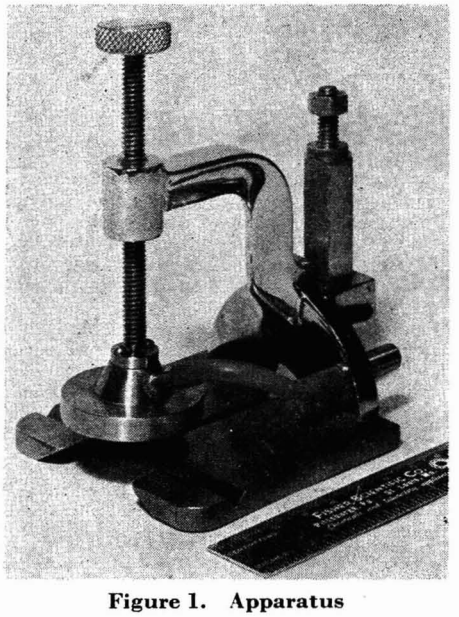

Figure 1. Apparatus

An attempt to cool the sample while sparking by attaching it to a solid block of steel or copper was unsuccessful, probably because of the poor thermal contact of the thin sample with the cooling block. More successful was the technique of using a flow of cooled helium on the upper or unsparked side of the sample during the analysis. Helium was chosen because of its high thermal conductivity.

To accomplish helium cooling, the clamp on the Petrey stand sample holder was replaced with a hollow fitting into which the cooled gas could flow and impinge on the upper surface of the thin sample (Figure 1). The gas was passed through a flowmeter and then through a copper coil immersed in an ice bath and from there into the Petrey stand clamp. Ice was used as a cooling

Table I. Effect of Variation in Coolant Flow

He Flow, Liters/Min.	Apparent % Mn	Apparent % Nb
0	0.76	0.61
	1.09	0.95
	1.98	1.73
	2.80	2.75
4	1.38	0.95
	1.00	0.92
	1.15	0.93
	1.17	0.99
9	0.79	0.71
	0.74	0.70
	0.74	0.71
	0.70	0.61
15	0.68	0.67
	0.61	0.60
	0.63	0.60
	0.53	0.55
28	0.57	0.54
	0.62	0.53
	0.58	0.51
	0.60	0.49

agent rather than dry ice or liquid nitrogen because of the possibility of condensing atmospheric water on the sample and thereby causing erratic sparking conditions.

With the cooling system described, a flow of helium of from 5 to 25 liters per minute, depending upon sample thickness, produced the desired results. In all cases, the results were read from working curves that had been prepared using massive standard samples. Given in Table I are manganese and niobium values determined from a sheet of 0.010-inch steel. For this thickness of sample, optimum flow is approximately 28 liters per minute. The apparent values for manganese and niobium are not reduced to the true values of 0.40 and 0.38, but they do approach minimum, reproducible values. These values must be corrected to the true value by a chemical analysis of one piece of each proposed sample thickness from a given heat of steel. Heat identification of large quantities of sheet stock can then be rapidly performed. For a general application of this technique to thin sheet stock of varied manganese and niobuim concentrations, complete working curves based on chemical analyses would have to be drawn for each sample thickness.

The precision of this method is now as good as that obtained with massive samples.

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Reduced-Scale Reid Vapor Pressure Apparatus

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An apparatus to measure Reid vapor pressure of small samples consists of a sample cup to fix the liquid-vapor ratio and a pressure transducer to convert the pressure into an electrical equivalent, which is measured by an auxiliary detecting circuit. The range is 0 to 20 pounds. A sample of only 3 ml. is required. The time required to handle a sample properly and make a measurement is about one third less by this method than by standard methods, and less bench space is required. The precision is as good as that of standard methods.

THE Reid vapor pressure test is widely used in the petroleum industry as a measure of the vapor pressure of volatile, nonviscous petroleum products. The standard ASTM method (*1*) uses a bomb-type apparatus and requires at least a 5-ounce sample. Although quarter-sized bombs are available, the amount of sample required is still too much to be taken directly at the carburetor or other parts of the fuel system where only small quantities of fuel are available. Such information is often desirable in studying vapor lock and weathering characteristics of fuels. Therefore, an apparatus to measure Reid vapor pressures on small samples was developed and tested. It differs from apparatus previously reported for this type of measurement (*2*).

DESCRIPTION OF APPARATUS

Figure 1 is a photograph of the apparatus with a cell in the constant temperature oil bath. The cell, a drawing of which is shown in Figure 2, consists of a sample cup tightly clamped to a pressure transducer. A polyethylene gasket, lightly greased, is used to ensure a vapor-tight seal. The transducer element is a full-bridge transducer operating on the unbonded strain wire principle (*3*). This transducer converts pressure applied to a bellows element into an exact electrical equivalent, which is then measured by a detector circuit. The opposite side of the bellows is open to the atmosphere; the pressure measured by the circuit is a differential pressure. The detector is essentially a null potentiometer operating so that the measured reading is independent of the voltage applied to the transducer. A simplified diagram is shown in Figure 3.

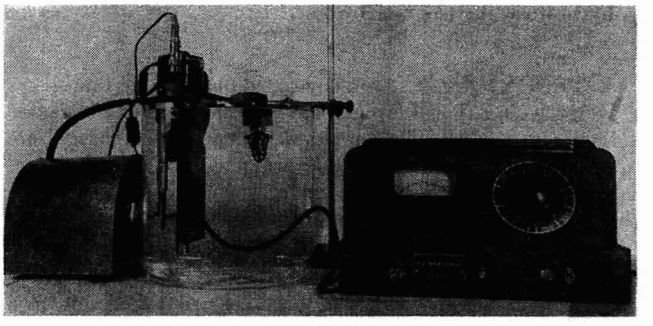

Figure 1. Apparatus

Hot air required to dry the vapor space is obtained from a 2-foot coil of 0.25-inch stainless steel tubing (not shown) heated by connecting each end of the coil to the low voltage side of a 115- to 5-volt transformer. The 115-volt winding is across a Variac. Air is blown directly through the steel tubing. On the outlet side, the steel coil is connected through a piece of rubber tubing to a short length of $^1/_{16}$-inch outer diameter steel tubing which can be inserted inside the pressure transducer. The heat generated and the temperature of the air can be controlled by adjusting the Variac.

The scale on the calibrated balance control, Helipot, in the bridge circuit has 1000 divisions, and the bridge can be balanced to ±1 division or ±0.02 pound. The transducers were calibrated against a mercury manometer and are linear over the 0 to 20-pound range. A typical calibration is shown in Figure 4.

Electrical stability was determined by measuring the vapor pressure of pure acetone. The results are listed in Tables I and III. These results show that the standard deviation due to factors which include bridge stability is 0.044 over a period of 2 months, and that stability is not the limiting factor in the repeatability of the method.

Table I. Stability of Reduced Scale Reid Vapor Pressure Apparatus

(Measurements on pure acetone)

Date	Operator	Scale	Pressure, Lb./Sq. Inch
7-3-53	E	365	7.30
7-6-53	E	370	7.40
7-7-53	E	370	7.40
7-22-53	B	367	7.34
7-22-53	C	365	7.30
7-22-53	D	368	7.36
7-22-53	B	365	7.30
7-22-53	A	367	7.34
7-23-53	B	364	7.28
7-23-53	D	362	7.24
7-23-53	A	362	7.24
8-3-53	C	366	7.32
8-4-53	D	369	7.38
8-5-53	D	363	7.26
9-1-53	B	371	7.42

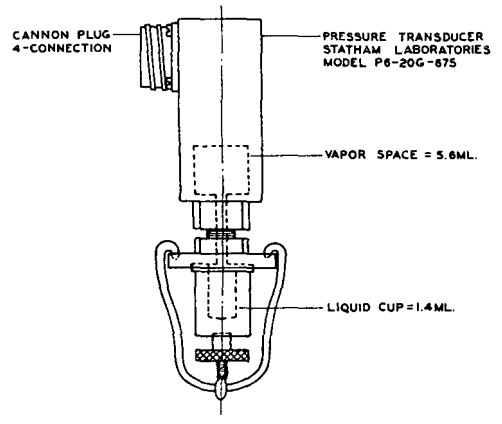

Figure 2. Pressure Transducer and Cell Assembly

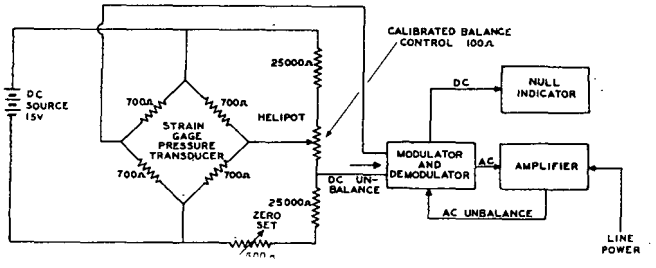

Figure 3. Simplified Diagram of Reid Vapor Pressure Indicator

Operation. The method of operation is as nearly the same as that of ASTM D 323-52 as is practical with the reduced scale apparatus.

Samples are drawn into chilled containers through a cooling coil if possible. The size of the sample container should be such that it is approximately 80% filled, and stored cold until used. Dewar flasks filled with ice water make convenient baths if the samples are to be stored for only a few hours; otherwise, cold room storage is more convenient.

Before the first determination in a series is made, the assembled cell is preheated in the 100° F. constant temperature oil bath for 10 to 15 minutes. The cell is removed from the bath and carefully cleaned. The vapor space in the transducer is flushed with isopentane, the isopentane is removed by suction, and the vapors are blown out by use of the steel tubing connected to the hot air source. Hot air is blown into the transducer for at least 10 minutes. The air temperature is such that the temperature in the vapor space of the transducer is as near 100° F. as possible when the sample cell is connected to the transducer. Experiments have shown that a temperature of 102° F. from the air preheater will compensate for the drop that takes place while the cell is being connected.

The sample cup is wiped clean and immersed almost to the top in an ice water bath. A piece of absorbent paper or a cork is inserted in the liquid space to prevent moisture from condensing in the cup. To prevent cooling of the air in the vapor space of the transducer and heating of the cup and sample while the apparatus is being assembled, the following sequential steps are carried out as quickly as possible. When the cup has cooled sufficiently and the sample is ready to charge, the gasket is coated lightly with vacuum stopcock grease. The sample bottle is inverted and shaken vigorously to mix the liquid and the air. The cup is removed from the ice water bath, the water is wiped off, and the gasket is placed on the top of the cup. The hot air tube is removed from the vapor chamber, the Helipot dial is set at zero, the potentiometer circuit is adjusted to the null position by means of the 500-ohm zero adjustment in series with the Helipot, and the air jet is replaced to keep the vapor chamber at the proper temperature. The sample is charged to the chilled cup, the hot air jet is removed, and the cup and transducer are clamped together. After the cell is assembled, it is shaken, upside down, for 30 seconds. The cell is placed in the 100° F. bath in an upright position. After a minimum of 15 minutes, the galvanometer is again brought to null position by use of the Helipot and the dial is read. The Helipot dial reading times a constant factor gives the Reid vapor pressure in pounds.

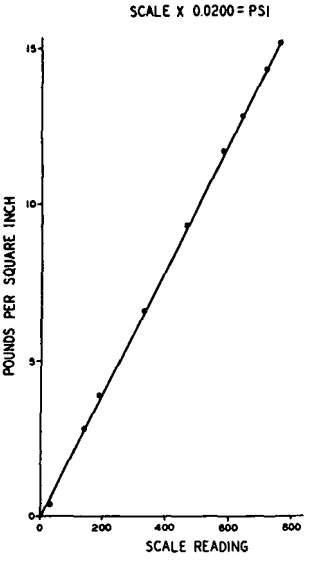

Figure 4. Calibration of Reid Vapor Pressure Apparatus

Table II. Typical Replicate Measurements of Reid Vapor Pressure of Gasolines on Reduced Scale Apparatus

Sample	Date Run	Operator	Reid Vapor Pressure, Lb./Sq. Inch
10X	7-28	B	7.24
	7-28	B	7.46
	7-28	B	7.30
	7-29	B	7.34
	7-30	A	7.66
	7-31	B	7.08
	7-31	A	7.18
	7-31	A	7.66
	8-4	B	7.44
	8-4	D	7.20
	8-5	D	7.26
	8-5	B	7.16
	8-5	B	7.50
13X	7-28	B	13.62
	7-28	A	13.90
	7-28	B	13.70
	7-29	B	14.28
	7-30	A	14.12
	7-31	A	13.86
	8-3	C	13.70
	8-3	A	13.98
	8-4	D	13.64
	8-4	A	14.08
	8-5	A	13.96

Although the apparatus is not checked against a mercury manometer each time, as in the standard method, the original calibration of the dial is done in this way. A standard sample is run frequently to check the accuracy. This procedure also detects leaks or other difficulties in operation. The new apparatus has one scale to cover the 0 to 20-pound pressure range, eliminating the necessity for three separate gages. The sample storage container can be of any size larger than 2 ml., and an elaborate transfer procedure is replaced by simply pipetting the sample from a chilled pipet.

It is important that the sample be kept cold while it is being transferred from the sample container to the cell. This can be accomplished by using chilled pipets. The ends of the pipet are sealed with rubber policemen while they are being chilled, to prevent internal condensation of moisture; the outside should be wiped dry to keep extraneous moisture out of the sample. If sufficient sample is available nearly to fill a 4-ounce

Table III. Standard Deviation of Replicate Samples

Sample	No. of Observations	Av. Reid Vapor Pressure	Standard Deviation
Pure acetone	15	7.33	0.044
10X	13	7.34	0.18
6X	12	9.04	0.15
2X	12	12.55	0.19
35	5	13.17	0.14
30	5	13.66	0.23
13X	11	13.89	0.22
13	3	14.00	0.12
	101[a]		0.17

[a] Based on all gasoline samples measured. 76 degrees of freedom.

sample bottle, the sample can be transferred to the cup through a wash bottle top in place of the cork. If 10 to 12 ml. of sample are forced through the delivery tube before charging sample to the cup, the system will be cold enough for safe transfer of the sample. Heating the vapor space to 100° F. eliminates the need for correcting for change in air pressure on heating and for change in the vapor pressure of water. This technique also eliminates the necessity for measuring the temperature of the air chamber when the apparatus is assembled.

Performance. The repeatability of the method by different operators within one laboratory was evaluated by determining the Reid vapor pressure approximately 100 times. The standard deviations and replicate results are summarized in Table III. The vapor pressures of the samples listed in these tables were determined in a random order by various operators over a period of about 2 weeks. The individual samples were identified only by numbers, and the relationship of the samples was unknown to the operators. The smaller samples permitted easier handling and storage than macro samples so that the small scale measurements require about one third less time than the standard measurements.

ACKNOWLEDGMENT

The authors are indebted to Robert Matteson and V. B. Waithman for the original design of this instrument. They wish to thank V. C. Davis for designing and constructing the bridge and R. K. Stone for his participation in many helpful discussions of this problem.

LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa., "ASTM Standards on Petroleum Products and Lubricants," Method D 323–53, November 1953.
(2) Levin, H., Morrison, A. B., and Reed, C. R., ANAL. CHEM., **22**, 188 (1950).
(3) Statham Laboratories, 12401 West Olympic Blvd., Los Angeles 64, Calif., *Instrument Notes*, No. 4, (November 1949).

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Determination of Boron in Silicates after Ion Exchange Separation

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Existing methods for the determination of boron in silicates are not entirely satisfactory. Separation as the methyl ester is lengthy and frequently erratic. An accurate and rapid method applicable to glass, mineral, ore, and water samples uses ion exchange to remove interfering cations, and boron is determined titrimetrically in the presence of mannitol, using a pH meter to indicate the end point.

RECENTLY Martin and Hayes (*3*) have shown that boron can be separated from interfering cations by ion exchange. Their experimental data are restricted to the analysis of steel samples, although their basic data imply wider applications. This investigation confirms the earlier work and tests the method for the determination of boron in silicates.

The conventional procedure for the isolation of boron is by distillation as the methyl ester. This method was investigated and, as others have found (*3*, *4*), incomplete volatilization was obtained from large amounts of aluminum, and frequently a double distillation was necessary in the presence of both iron and silicon. As such, the method is unwieldy and time-consuming.

In this investigation only minor modifications were made in the procedure described by Martin and Hayes. Boron is brought into solution either by an acid extraction or by fusion, eluted through an ion exchange bed, and determined titrimetrically in the presence of mannitol, using a pH meter to indicate the end point.

METHOD

Reagents. Methyl red indicator solution.
Concentrated hydrochloric acid.
Sodium carbonate, C.P., anhydrous.
Sodium hydroxide, standardized 0.05*N*.
Sodium hydroxide, 20% weight per volume (carbonate free, prepared from 50% sodium hydroxide).
Mannitol, neutral, boron-free.
Amberlite IR 120 (H), analytical grade (exchange capacity of the dry resin is approximately 5 meq. per gram). This material may be regenerated after use by transferring the accumulated resin from a number of determinations to a large glass tube and washing with (1 + 9) hydrochloric acid until the issuing liquid gives a negative test for adsorbed ions. The hydrochloric acid is removed by washing with distilled water.

Apparatus. Beckman, Model 2H, line-operated pH meter with saturated calomel and glass electrodes.

Preparations of Ion Exchange Column. A borosilicate glass chromatograph tube, 20 × 400 mm., with sealed-in, coarse porosity, fritted disk provided with a small rubber tube extension and screw clamp is used. Fill the tube with water and add the resin slowly as a slurry until a column 10 inches in length is formed. The column should be free of air spaces.

Before using, wash the column with 100 ml. of (1 + 9) hydrochloric acid and follow with 50-ml. portions of water until the effluent gives a negative test for chlorides.

Solution of Borates. ACID-SOLUBLE BORATES. Weigh a sample containing 10 to 20 mg. of boron oxide into a 125-ml. Erlenmeyer flask. Add 30 ml. of (5 + 25) hydrochloric acid and connect the flask to a reflux condenser. Heat the mixture to boiling and reflux slowly for 20 to 25 minutes. After allowing the mixture to cool slightly, pour 5 ml. of water through the top of the reflux condenser; disconnect the condenser and wash the tip of the condenser carefully with water. Filter the mixture while hot through a 9-cm. Whatman 41 H filter paper, and wash the residue with hot water to a volume of about 50 ml.

ACID-INSOLUBLE BORATES. Weigh a sample containing 10 to 20 mg. of boron oxide (not more than a 1-gram sample) into a platinum crucible, add six times the sample weight of anhydrous sodium carbonate, and, with a platinum stirring rod, mix the sample and flux intimately. Cover the crucible and heat gently for 5 to 10 minutes to expel moisture. Now gradually increase the heat so that after 5 to 10 minutes more, a liquid melt is formed. When fusion is complete, grasp the crucible with tongs and give it a rotary motion so as to spread the contents over the sides of the lower half of the crucible, thus expediting subsequent solution. Cool, and place the crucible in a 150-ml. beaker containing 20 ml. of water. Cover the beaker with a watch glass and add concentrated hydrochloric acid down the sides of the beaker until there is an excess of 1 ml. over the theoretical amount

calculated to decompose the carbonate. After decomposition of the melt dissolve any carbonate adhering to the crucible or cover with hot dilute hydrochloric acid; remove any adhering material with a rubber policeman, and rinse into the beaker with a stream of hot water. Filter the mixture through a 9-cm. Whatman 41 H paper, and wash with hot water to a volume of about 50 ml.

PROCEDURE

Neutralize the solution obtained from the above treatment with 20% sodium hydroxide until a precipitate just starts to form; add concentrated hydrochloric acid dropwise until the precipitate just dissolves or until the solution is just acid to litmus paper. Pass the solution through the ion exchange column into a 400-ml. beaker and follow with four 50-ml. portions of water, adding the wash water when there is 0.5 inch of supernatant solution above the resin. Adjust the rate of flow so that the total elapsed time for the solution and the four washings is 15 minutes (flow rate of 16.7 ml. per minute). At the conclusion of the last washing the effluent should be only slightly acid to pH test paper. (If not, the amount of resin in the column should be increased to ensure the complete removal of cations. No additional amount was ever necessary in this investigation.) Add 2 or 3 drops of methyl red indicator to the solution and make alkaline with 20% sodium hydroxide, and then barely acid with concentrated hydrochloric acid. Cover the beaker with a watch glass and boil gently for 3 to 5 minutes to remove carbon dioxide. Cool the solution to room temperature, preferably in a water bath. Introduce the pH meter electrodes and stirrer into the beaker, and adjust the pH of the solution to 7.0 with 0.05*N* sodium hydroxide. The indicator needle of the pH meter should be steady and not drift from the reading of 7.0. Add 40 grams of mannitol, and, using a microburet (calibrated 0.05 ml.), titrate rapidly with standardized 0.05*N* sodium hydroxide until the pH of the solution approaches a value of 7 and then slowly near the end point to allow for any slight lag in response of the pH meter. When the indicator needle remains steady on 7.0 for at least 10 seconds, record the volume of standard base used.

A blank correction (usually less than 0.10 ml. of 0.05*N* sodium hydroxide) for the reagents is subtracted from the sample titration and the boron oxide is calculated:

$$1 \text{ ml. of } 0.05N \text{ NaOH} = 1.741 \text{ mg. of } B_2O_3$$

If the titer is small, repeat the determination, collecting the eluate in a 250-ml. volumetric flask, and determine the boron colorimetrically.

Tin, rarely encountered in boron analysis, should be removed before the ion exchange separation is made. This is most easily done by plating out the tin with granular zinc after the borate is in acid solution.

EXPERIMENTAL DATA

A master solution was prepared containing a number of cations to test the effectiveness of the outlined procedure. It contained: sodium chloride 12.71 grams, potassium chloride 1.91 grams, calcium chloride 13.84 grams, magnesium chloride 20.90 grams, barium chloride 0.79 gram, ferric chloride 24.20 grams, aluminum chloride 23.49 grams, zinc chloride (metal dissolved in hydrochloric acid) 0.50 gram, water to make 500 ml.

The master solution was analyzed for boron, and none was found. Various amounts of boric acid were then added to 10 ml. of the master solution. Results by the proposed procedure are given in Table I.

National Bureau of Standards glass samples were fused and analyzed by the proposed procedure. The results are given in Table II.

Hollander and Riemann (*2*) report boron oxide values for NBS 92 as 0.65% and for NBS 93 as 12.51%, and conclude that their values are closer to the actual content.

Synthetic mixtures of colemanite ($Ca_2B_6O_{11}.5H_2O$) and bentonite [(Mg, Ca) $O.Al_2O_3.5SiO_2.nH_2O$], and howlite [$Ca_2SiB_5O_9(OH)_5$] and bentonite were also analyzed. The results are presented in Table III.

Table I. Recoveries of Boric Acid

Boric Acid Taken, Mg.	Recoveries	
	Mg.	%
4.96	4.97	100.2
4.96	4.97	100.2
19.83	19.83	100.0
19.83	19.83	100.0
39.66	39.76	100.3

Table II. Analysis of National Bureau of Standards Glasses

Glass	B_2O_3 Reported, %	B_2O_3 Found, %
NBS 92	0.70	0.63
	0.70	0.62
NBS 93	12.7	12.43[a]
	12.7	12.44
	12.7	12.45

[a] Sample dried at 500° C.

Table III. Analysis of Synthetic Mixtures

B_2O_3 Taken, Mg.	Recoveries		Method of Solution
	Mg.	%	
16.07 (colemanite)	16.05	99.9	Acid leach
	16.07	100.0	Acid leach
20.32 (colemanite)	20.36	100.2	Fusion
	20.37	100.2	Fusion
17.95 (howlite)	18.02	100.4	Acid leach
	18.02	100.4	Acid leach
17.95 (howlite)	17.98	100.2	Fusion
	17.85	99.4	Fusion

Several "refractory borosilicates" were analyzed using the methanol distillation as described by Hillebrand *et al.* (*1*) and the proposed procedure. The results are presented in Table IV.

Table IV. Analysis of Refractory Borosilicates

Mineral	Methanol Separation, % B_2O_3	Ion Exchange Separation, % B_2O_3
Black tourmaline (Nuevo, Calif.)	9.79	10.03
Green tourmaline (Calif.)	10.51	10.70
Dumortierite (impure) (Carge Mucharcho Mts., Imperial Co., Calif.)	1.93	2.02
Axinite (North Hills Quarry, Riverside, Calif.)	5.86	5.98

The high iron, alumina, and silica contents of these minerals preclude complete volatilization of the boron in the first distillation. Only one distillation (collected in two fractions) per sample was made.

CONCLUSIONS

The proposed method has decided advantages over the methods currently used in that the accuracy and reproducibility are good; less manipulative skill is involved; blank corrections are very low; and time of analysis is greatly reduced.

ACKNOWLEDGMENT

The author gratefully acknowledges the mineral specimens supplied by W. C. Oke, Minerals Curator, California Institute of Technology, Pasadena, Calif., for use in this investigation.

LITERATURE CITED

(1) Hillebrand, W. F., Lundell, G. E. F., Bright, M. S., and Hoffman, J. I., "Applied Inorganic Analysis," p. 756, New York, John Wiley & Sons, 1953.
(2) Hollander, M., and Riemann, W., 3d, ANAL. CHEM., **18**, 789 (1946).
(3) Martin, J. R., and Hayes, J. R., *Ibid.*, **24**, 182 (1952).
(4) Rader, L. F., Jr., and Hill, W. L., *J. Agr. Research*, **57**, 901 (1938).

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Determination of Carbon and Fluorine in Highly Fluorinated Substances

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In a general method for the determination of carbon and fluorine in organic compounds containing a high percentage of fluorine, a combustion procedure is used in which the sample is burned with moist oxygen in a quartz tube. Gases, liquids, and solids can be handled. The fluorine is determined by an acid-base titration of the hydrofluoric acid formed and the carbon, which is simultaneously converted to carbon dioxide, is absorbed by Ascarite. Carbon and fluorine can be determined in a large variety of compounds with a relative error of less than 1%.

FLUORINE-containing organic compounds have become of general interest and importance. A method more universally applicable than those available was needed for the simultaneous determination of carbon and fluorine in these materials. The first problem encountered is that of decomposing the material so as to convert the fluorine into the ionic form. Belcher and Goulden (*1*) in their review article have conveniently arranged these methods into five main groups—combustion in oxygen, combustion in other gases, alkali metal fusion, fusion with alkalies, and decomposition in solution. Decomposition by combustion in oxygen appeared to be the most suitable and the least objectionable in regard to handling of reagents. Of the various combustion methods available for the determination of fluorine (*1–10*) the only methods which appeared applicable were those of Teston and McKenna (*10*), Milner (*7*), and Clark (*3*).

Teston and McKenna (*10*) reported a semimicromethod for the simultaneous determination of carbon, fluorine, and chlorine. Their method of determining fluorine does not, however, give correct fluorine values in hydrogen containing compounds.

Milner (*7*) described a procedure in which the sample is burned in a stream of moist oxygen, utilizing a platinum tube. This method is reported to be useful for compounds boiling at about 60° C. or higher. Milner's procedure with various modifications was used successfully in this laboratory. Several disadvantages in addition to the high cost of the platinum tube were soon realized. Controlled vaporization of the sample is difficult when the sample cannot be observed. Maintaining an even flow of steam without increasing the flow rate of the sample through the tube is bothersome. The over-all size of the apparatus is relatively large and the length of time per analysis is somewhat long for routine analyses.

The quartz tube micromethod of Clark (*3*) for the determination of fluorine only was then investigated. It was faster and less costly than Milner's platinum tube method, but yielded low results with certain types of compounds. A study of this method was made in an attempt to apply it to the types of compounds which gave erroneous results. The proposed procedure differs from Clark's in several important respects. First, a simultaneous carbon determination has been incorporated in the procedure. Second, owing to difficulties encountered in decomposing many compounds, the temperature has been raised from 900° C. to about 1150° C., and a small amount of moisture has been introduced into the combustion tube. Third, the Grote absorber has been modified slightly, and modifications have been made in the apparatus to facilitate handling of gas samples. Fourth, the acid-base titration has been modified. With these modifications of the apparatus and procedure, carbon and fluorine have been determined successfully in a large variety of fluorinated materials including the compounds which gave erratic results originally.

APPARATUS

The equipment listed is advised where a permanent setup capable of handling a variety of samples is desired (see Figure 1).

Standard pressure regulator, *A*, giving sufficient pressure to allow an oxygen flow rate of approximately 25 ml. per minute.

Gas sample bulb, *B*, of 10-ml. capacity, 7/10 standard-taper joints, is used for all gas samples and low boiling liquids.

Two gas bubble traps, *C*, filled with water which serve as flow rate meters as well as a source of water vapor.

A movable electrically heated furnace, *D*, 3 to 5 cm. in length, and operating at 800° to 900° C., is used to vaporize the liquid and solid samples.

Fused quartz combustion tube, *E*, with body length of approximately 650 mm. and outside diameter of 11 mm. The tube is equipped with a side arm at the entry end and a 10/30 male joint at the exit end (available from The Arthur H. Thomas Co., Philadelphia, Pa.). The joint is sparingly greased with Celvacene heavy vacuum grease. The section of the combustion tube within the hot part of the main furnace, *F*, is packed alternately with quartz chips and rolls of platinum gauze freshly etched with aqua regia. The packing used consists of four sections of platinum gauze and three sections of quartz chips. The section of platinum at the entrance end protrudes out of the furnace for 1 to 2 cm.

An 8-cm. silver gauze roll is placed in the section of the combustion tube within furnace *N*. This serves to remove interfering sulfur compounds and halogens other than fluorine from the gas stream.

Lindberg high temperature micro furnace, *F*, operating at 1100° to 1200° C. (available from The Arthur H. Thomas Co.).

A 10-cm. electrically heated furnace, *N*, which is kept at 350° C.

Spray trap and modified Grote absorber with a medium frit, *G*. Ideally, this should be made of quartz, but borosilicate glass has been found to be satisfactory. This has been modified by using a standard-taper joint which facilitates removal of the acidic material from the absorber (Figure 2). The entrance end of the absorber is so constructed that the condensed water vapor will drain into the absorber and not form a pool of water near the standard-taper joint.

Concentrated sulfuric acid trap, *H*.

Anhydrone absorber, *I*.

Standard-taper microabsorption tubes, *J*, *K*, filled with Ascarite. Tube *J* is the carbon dioxide absorption tube and *K* is used as a tare.

Guard tube containing Anhydrone, *L*.

Standard Mariotte bottle assembly, *M*.

REAGENTS

Sodium hydroxide, 0.01*N*, carbonate-free, stored in polyethylene bottle equipped with an automatic buret. Alkali remaining in glass buret after a titration is discarded and not allowed to return to the bottle.

Phenolphthalein solution, 1% in ethyl alcohol.

Saturated mannitol solution, reagent grade.

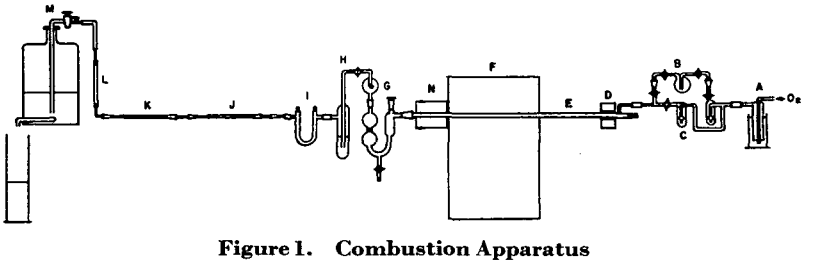

Figure 1. Combustion Apparatus

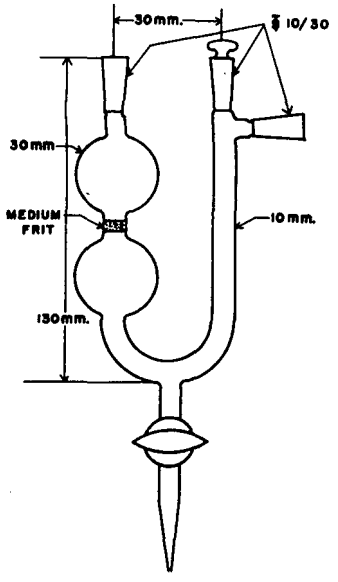

Figure 2. Standard-Taper Joint

PROCEDURE

Samples of 8 to 10 mg. are used. Solids are weighed in platinum boats, liquids in glass capillaries, and gases either directly in the gas bulb or the weight is calculated by the method of taking gas aliquots. The usual oxygen purification train is used, and the oxygen flow rate is adjusted to approximately 25 ml. per minute. The joint connecting the combustion tube with the modified Grote absorber is cooled with a stream of air. Glass capillaries for liquid samples are sealed on both ends before weighing. The liquid is frozen by immersing one end of the capillary in liquid air. The other end is snapped off and both pieces are quickly introduced into the combustion tube. The solid or liquid sample is slowly vaporized into the hot portion of the tube, taking about 15 to 20 minutes for combustion with a 20-minute sweeping time. In the case of a gas sample, the apparatus is so arranged that the main flow of oxygen bypasses the sample bulb and only one bubble every 2 seconds goes through the bulb. After about 10 minutes, the ratio of oxygen going through to that bypassing the bulb is increased until finally the entire flow of oxygen is directed through the bulb.

After about 40 minutes the Ascarite tubes are removed and weighed. Cooling of the joint between the combustion tube and modified Grote absorber is discontinued. This allows the joint to warm up so as to drive any condensed moisture into the absorber. When the joint is dry and free of acid, the oxygen flow is turned off, the absorber is removed, and its contents are transferred to a 125-ml. flask. To remove all acid from the absorber, the titration is started by adding a few drops of phenolphthalein and small amounts of standard 0.01*N* sodium hydroxide directly to the absorber. After the latter is scrubbed with a swab made of cotton on a platinum wire, the washing is added to the 125-ml. flask. This process is repeated until 1 drop of alkali in the absorber remains pink to phenolphthalein. The titration is continued by titrating the combined solutions in the flask with standard 0.01*N* sodium hydroxide. Before the end point is reached, the solution is boiled a few minutes on the acid side to remove carbon dioxide absorbed from the atmosphere, is quickly cooled, and the titration is completed to the permanent pink color of phenolphthalein.

The titer of the alkali is determined by running a series of determinations for fluorine on fluorine-containing organic compounds whose purity is well established. Purified samples of perfluorobutyric acid; polytetrafluoroethylene; 1,1-dihydroperfluorobutyl acrylate; or perfluoropropane have been used. In order to check the "mannitol increment" occasionally, as discussed below, 10 ml. of neutralized saturated mannitol solution are added after the end point has been reached. The titration is then continued to a reappearance of the pink phenolphthalein color.

The carbon is weighed as carbon dioxide, which is absorbed by the Ascarite. A carbon dioxide blank is determined, using the same volume of oxygen over the same period of time. The blank is usually about 50 γ or less.

CALCULATION FOR PER CENT FLUORINE

[Titration volume (ml. of standard alkali)] × [titer of alkali (mg. of F/ml. of NaOH)]/sample weight in mg. × 100 = % F

DISCUSSION

The decomposition of a fluorocarbon sample in a quartz tube, in the presence of oxygen and at elevated temperatures, can be represented as in Equation 1 for perfluoropropane.

$$C_3F_8 + 2SiO_2 + O_2 \rightarrow 2SiF_4 + 3CO_2 \quad (1)$$

$$SiF_4 + 2H_2O \rightarrow 4HF + SiO_2 \quad (2)$$

In Clark's (*3*) procedure, as in the present one, the silicon tetrafluoride is absorbed by hydrolysis in a Grote absorber containing water according to Equation 2. The hydrogen fluoride can then be titrated with sodium hydroxide, using phenolphthalein as the indicator. Clark postulated that some of the fluorine was tied up as the weak acid, monohydroxyfluoroboric acid which was formed from the reaction of silicon tetrafluoride or hydrogen fluoride with boron in a Grote absorber.

$$BF_3 + H_2O \rightarrow HBF_3OH \quad (3)$$

$$BF_3 + HF \rightarrow HBF_4 \quad (4)$$

$$HBF_4 + H_2O \rightleftharpoons HBF_3OH + HF \quad (5)$$

Accordingly, he added mannitol after reaching the first end point and then titrated to a second end point. The difference between the two end points he assumed represented the monohydroxyfluoroboric acid present. Hence, he added three times this value to the first titration volume to obtain the volume of base equivalent to the fluorine present.

The experience in this laboratory is in agreement with that of Clark's, in that there is definitely a mannitol increment using the procedure described. Furthermore, if the base is standardized acidimetrically, fluorine values are low if no increment is added and near-theoretical values can be obtained by adding three times the mannitol increment to the titration volume.

Nevertheless, there is some question as to the validity of this use of the mannitol increment. In attempting to reduce the increment to a minimum, the following was observed. The 0.01*N* alkali on standing slowly picks up boron from the glass. Very little boron appears to result from combustion in the quartz tube—most of it comes from the absorber. As was shown by Clark (*4*) and also found in this laboratory, the pH of the colorimetric equivalence points before and after adding mannitol are not the same. Borate ion at concentrations which might be found in the Grote absorber does not affect the titration of hydrofluoric acid. The mannitol increments from a large number of individual determinations were small (about 0.10 ml. of 0.01*N* sodium hydroxide); in the range of 1 to 10 mg. of fluorine, the variation was no greater than the titration error.

The use of the mannitol increment decreases the precision of the titration, since three times the error in its determination is added to the error in ascertaining the titration volume. Hence an empirical standardization of the alkali, eliminating the mannitol increment, was adopted. This simplifies the titration and at the same time increases the accuracy. It is advisable to run reference samples periodically to ensure that the conditions have remained constant.

In the course of deciding on the best conditions for the quantitative decomposition of highly fluorinated materials, it became apparent that both temperature and moisture are important factors.

Table I. Combustion at 900° C. without Water Vapor

Sample	Theoretical Values	Values Found: Sample A	Values Found: Sample B	Low in Ratio of C/F: Sample A	Low in Ratio of C/F: Sample B
C_8F_{18}	21.9% C 78.1% F	20.0% C 64.5% F	19.5% C 63.5% F	1/4.4	1/3.8
C_3F_8	19.1% C 80.9% F	17.9% C 73.3% F	16.8% C 68.2% F	1/3.9	1/3.4

Table II. Decomposition of Tetrafluoromethane at Various Temperatures in Presence of Water Vapor

Temperature ° C.	Observed Values: % C	Observed Values: % F	Theoretical Values: % C	Theoretical Values: % F
1000	0.4	2.1	13.6	86.4
1200	0.6	3.1		

Table III. Effect of Moisture in Decomposing Some Compounds at 1100° C.

Compound	Without Moisture	With Moisture	Theoretical
C_8F_{18}	19.5% C 63.5% F	22.0% C 77.7% F	21.9% C 78.1% F
$C_8F_{16}O$	21.1% C 62.8% F	23.1% C 72.9% F	23.1% C 73.1% F

Table IV. Effect of Temperature in Presence of Moisture

Compound	Found at 1000° C.	Found at 1100° C.	Found at 1200° C.	Theoretical
$C_8F_{16}O_2$	21.8% C 64.2% F	22.4% C 70.0% F	... ...	22.2% C 70.4% F
C_4F_8O	... ...	18.1% C 54.5% F	22.2% C 69.8% F	22.2% C 70.4% F

Following are some results obtained on a few compounds analyzed under various conditions which will serve to demonstrate these effects.

It was observed in the analysis of perfluoro-octane and perfluoropropane at 900° C. with no water vapor present that both carbon and fluorine results were low in a ratio of approximately 1 carbon to 4 fluorines (Table I). This would lead one to believe that the results were probably low owing to the formation of tetrafluoromethane. If this were formed during the combustion, one would expect to obtain low carbon and fluorine values since tetrafluoromethane does not, under the conditions of the analysis, decompose quantitatively at even higher temperatures and in the presence of water vapor (Table II). An effort was then made to isolate and identify the breakdown products formed in the combustion to see whether tetrafluoromethane could actually be found in the gas stream from compounds which did not yield quantitative results under the conditions used. The exhaust gases from a typical run of perfluoro-octane and a cyclic perfluoro ether (c-$C_6F_{12}O$) were collected and identified by infrared spectroscopy. In both cases with decomposition at 900° C. and no water vapor present, tetrafluoromethane was identified in the exhaust gases. It must be realized, however, that other breakdown materials might be found in the exhaust gases if the sample is introduced into the hot part of the tube too rapidly. For example, when the cyclic perfluoro ether (c-$C_6F_{12}O$) was volatilized in about one fourth the normal time, carbon monoxide and hexafluoroethane were produced in addition to tetrafluoromethane.

From these observations it appears that if one could prevent tetrafluoromethane from forming during the combustion, quantitative results might be obtained. Several investigators have reported that the addition of moisture aids in the decomposition (*1, 8*). Since tetrafluoromethane was realized with compounds containing no hydrogen, the introduction of hydrogen-containing material, such as water, should alleviate this difficulty.

Table III shows the results obtained on two compounds which demonstrates the effect of the addition of moisture into the combustion tube. Of course, repeated runs without water vapor gave variable results. The percentages listed are averages of several individual determinations.

The addition of moisture alone is not always sufficient to decompose the compound quantitatively, and an increase in temperature as well is required. In the case of c-$C_8F_{16}O_2$, 1100° C. was sufficient to yield quantitative results whereas cyclic perfluorobutyl ether gave low carbon and fluorine results at 1100° C. but yielded quantitative results at 1200° C. (Table IV).

No one set of conditions is necessarily essential to decompose the various fluorine-containing compounds quantitatively. Polytetrafluoroethylene gives correct carbon and fluorine values at 900° to 1000° C. with no water vapor present. On the other hand, several of the cyclic ethers were quantitatively decomposed only after temperatures of 1200° C. were used in the presence of water vapor. By using the apparatus and procedure as described, a large variety of compounds has been analyzed for carbon and fluorine with a relative error of 1% or less (Table V). The reproducibility of this method is demonstrated by the results from ten analyses of 1,1-dihydroperfluorobutyl acrylate (Table VI). Most of these and many other research samples were supplied by the Central Research Department, Minnesota Mining and Manufacturing Co.

Table V. General Table of Results

Compound	Carbon, % Calcd.	Carbon, % Found	Fluorine, % Calcd.	Fluorine, % Found
$C_{14}F_{30}$	22.8	22.7	77.2	77.4
$(C_2F_4)_x$	24.0	24.0	76.0	75.8
$C_5F_{11}H$	22.2	22.4	77.4	77.4
$C_7F_{16}H$	22.7	22.6	77.0	76.7
$C_4F_9OC_2F_4OC_4F_9$	21.1	21.1	73.3	73.4
$C_6F_{13}OC_6F_{13}$	22.0	21.8	75.5	75.0
CF_3CH_2OH	24.0	24.1	57.0	56.9
$C_9F_{19}CH_2OH$	24.0	23.9	72.2	71.8
C_2F_5COOH	22.0	22.0	57.9	57.8
$C_2F_5COOC_2H_5$	31.3	31.2	49.5	49.8
$C_4F_7COOCH_2C_5F_{11}$	24.2	24.2	69.0	68.6
$CH_2{=}CHCO_2CH_2C_3F_7$	33.1	33.1	52.4	52.3
$C_3F_7CH(OH)_2$	22.2	22.2	61.6	61.3
$(C_5F_{11}CO)_2O$	23.6	23.4	68.5	68.0
$C_5F_{11}CCl_3$	18.6	18.5	53.9	54.0
C_3F_7I	12.2	12.2	44.9	44.8
$CF_2BrCFBrCO_2CH_2C_3F_7$	18.0	18.1	40.6	40.7
$C_2F_5CH_2NCO$	27.4	27.5		
$C_6H_5NHCH_2C_3F_7$	43.6	43.3		

Table VI. Reproducibility of Results from Ten Analyses of 1,1-Dihydroperfluorobutyl Acrylate[a]

	By Analysis % C	By Analysis % F	Theoretical % C	Theoretical % F
	32.9	52.2		
	33.1	52.4		
	33.1	52.3		
	33.2	52.5		
	33.1	52.6		
	33.0	52.5		
	33.0	52.2		
	33.1	52.0		
	33.1	52.2		
	33.1	52.3		
Average	33.1 $\pm 0.0_5$	52.3 $\pm 0.1_6$	33.07	52.36

[a] Obtained by three analysts using two combustion trains.

SULFUR-CONTAINING COMPOUNDS

Most sulfur compounds present no particular problem with the usual procedure. However, in the case of certain sulfur compounds in which the sulfur is in a low state of oxidation, both the carbon and fluorine results were high. To overcome the high fluorine values it has been found advantageous to have one of the sections of quartz chips (in the hot zone of the furnace) impregnated with vanadium pentoxide. Vanadium pentoxide has long been used as a catalyst for the oxidation of sulfur to sulfur trioxide. At these temperatures, fluoride ion is not held by vanadium. In addition, the carbon values on this type of sulfur-containing compound were high, probably because some acidic material other than hydrofluoric acid was swept out of the

Table VII. Summary of Results and Conditions Used for Analyzing Certain Sulfur Compounds

Compound	Calcd.	Per Cent Fluorine Found: With general packing	Per Cent Fluorine Found: With V_2O_5 in packing
$(C_3F_7)_2S_2$	66.2	69.5	66.0
$(C_3F_7)_2S_3$	61.4	65.6	61.2

Compound	Calcd.	Per Cent Carbon Found: With general packing	With V_2O_5 in packing	With V_2O_5 in packing with $KMnO_4$ trap
$(C_3F_7)_2S_2$	17.9	18.9	18.8	18.0
$(C_3F_7)_2S_3$	16.6	19.8	21.7	16.7

absorber. To correct this difficulty, a small bubble trap filled with acidic potassium permanganate was inserted in the train between the modified Grote absorber and sulfuric acid trap. Table VII gives a summary of the results for two sulfur-containing compounds analyzed under various conditions.

INTERFERENCES

Metal ions which retain fluorine at the operating temperature of the furnace will give low fluorine results. In the case of nitrogen compounds, acidic oxides of nitrogen are formed, so that the fluorine cannot be determined by an acid-base titration. Quantitative carbon values are, however, obtained. Several phosphorus-containing compounds have been successfully analyzed for both carbon and fluorine.

LITERATURE CITED

(1) Belcher, R., and Goulden, R., *Ind. Chemist*, **27**, 322, 323 (1951).
(2) Calfee, J. D., *et al.*, *J. Am. Chem. Soc.*, **62**, 267 (1940).
(3) Clark, H. S., ANAL. CHEM., **23**, 659 (1951).
(4) Clark, H. S., and Rees, O. W., Illinois State Geol. Survey, *Rept.* **169** (1954).
(5) Grosse, A. V., *et al.*, *J. Phys. Chem.*, **44**, 275 (1940).
(6) Hubbard, D. M., and Henne, A. L., *J. Am. Chem. Soc.*, **56**, 1078 (1934).
(7) Milner, O. I., ANAL. CHEM., **22**, 315 (1950).
(8) Rickard, R. R., Ball, F. L., and Harris, W. W., *Ibid.*, **23**, 919 (1951).
(9) Schumb, W. C., and Radimer, K. J., *Ibid.*, **20**, 871 (1948).
(10) Teston, R. O'D., and McKenna, F. E., *Ibid.*, **19**, 193 (1947).

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Interference of Sulfhydryl Groups in Analysis of Urinary Mercury and Its Elimination

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The mercury in the Salyrgan-cysteine complex, which contains a mercury-sulfur bond, was found to be more difficult to determine quantitatively than either the mercury in Salyrgan or inorganic mercury. The same difficulty was encountered in the analysis of mercury in the urine of dogs treated with Salyrgan. The urinary mercury is believed to exist in a form analogous to the Salyrgan-cysteine complex. Minor changes in Kozelka's method for mercury analysis made this procedure satisfactory for the quantitative determination of mercury in these sulfur containing compounds.

THE usual procedure for studying the applicability of an analytical method to biological material is to analyze for known amounts of a particular substance added to tissues or body fluids. However, in the intact animal such a substance may be converted to a form which cannot be duplicated in dead tissue, and which might complicate the analysis. This was found to be true in the case of mercury excreted in urine, in contrast to mercury added to urine.

In earlier experiments performed in this laboratory with a variety of analytical procedures, recoveries of mercury were poor. The authors suspected the deficiency was due to the loss of mercury through volatilization and, therefore, turned to an ingenious method by Kozelka (*2*), which actually takes advantage of this volatility. The authors found it to be adequate for standard solutions of either inorganic mercury or Salyrgan (mersalyl), an organic mercurial. In the course of studies on the excretion of mercury after the administration of Salyrgan to dogs, simultaneous determinations of urinary mercury were made with Kozelka's method and the polarographic method. Results obtained by these two methods showed considerable discrepancies which had not been present in the control experiments. The reason for this was suspected to be the formation, in vivo, of a compound considerably more resistant to digestion than Salyrgan itself. The nature of this compund was suggested by further polarographic study (*5*) to be RHgSR′, where R represents the organic part of the Salyrgan molecule and —SR′ represents a small, unidentified sulfhydryl compound. To test this hypothesis an analogous compound was prepared from Salyrgan and cysteine, and analyzed by Kozelka's method. This compound was found to be more difficult to analyze, but this difficulty can be surmounted by relatively minor changes in the original method. The interference by sulfhydryl moieties is not noticeable when solutions of mercurials are added to urine, because urine does not usually contain enough neutral sulfur to form an appreciable amount of the mercury complex. In this respect, Simonsen (*3*) implied that mercury determinations in urine were unsatisfactory if the urine contained much protein.

METHODS

In Kozelka's original method, 100 ml. of urine is concentrated and then digested with 50 ml. of concentrated sulfuric acid, 20 grams of ammonium sulfate, and 1 gram of copper sulfate in a Kjeldahl flask fitted to a condenser which ends in a water trap. Any mercury that is not distilled over during the digestion procedure is carried over as a complex chloride by a stream of chlorine gas and heat. The mercury in the distillate is determined colorimetrically with dithizone. This method was used by the authors with the following modifications.

Kozelka did not specify the time necessary for digestion nor the rate of chlorine flow. The time of digestion and the time of chlorine flow were therefore varied while the rate of chlorine flow was controlled at approximately 150 ml. per minute with a differential water manometer as an indicator.

Urine from animals treated with Salyrgan contains enough mercury to allow analyses to be made on 0.1 to 3 ml. of urine. Therefore, the procedure for concentrating solutions was eliminated. In addition to the previously mentioned chemicals, 2 grams of sodium chloride were added to the digestion mixture to simulate the chloride content of concentrated urine.

Instead of an Evelyn colorimeter at 470 mμ, a Beckman DU spectrophotometer was used at 480 mμ, which is closer to the absorption maximum of mercury dithizonate (*2*).

The grade of dithizone (Eastman, white label) used did not require purification (*4*), but some difficulty was experienced with the carbon tetrachloride in which it was dissolved. The dithizone solution was tested by subjecting it to the same procedure as in the analysis—that is, 25 ml. of dithizone solution was washed twice with 50-ml. portions of 9*N* ammonium hydroxide and the absorption of the solution at 480 mμ determined. Different brands and even different lots of the same brand of carbon tetrachloride (all marked "suitable for dithizone test") gave different blank readings. Moreover, a given solution increased in absorbance from day to day. Redistillation of the carbon tetrachloride did not remedy this. As a consequence, daily dithizone blanks were run in addition to the usual reagent blanks, which were obtained at the beginning of each series of analyses.

In the authors' experience the extraction of mercury is best made from less than 75 ml. of total fluid, distillate plus wash water.

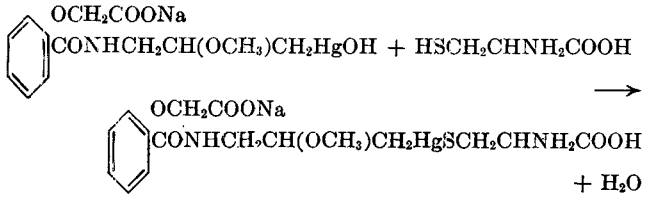
Because the most critical step in the procedure is the extraction of all the mercury containing distillate and wash water with exactly 25 ml. of dithizone solution, even minute losses through

a stopcock (which obviously cannot be greased) must be avoided. The distillate was therefore transferred directly to a 250-ml. glass-stoppered Erlenmeyer flask where the initial extraction was then performed. The aqueous layer was drawn off by suction. In the subsequent operations where the preservation of total volume is not critical, separatory funnels with water lubricated stopcocks were used.

S & S No. 589 filter paper was found to be suitable for use in this analysis without previous extraction with dithizone.

The standard solution of inorganic mercury was prepared from metallic mercury (*2*). The standard solution of Salyrgan was prepared from salyrganic acid (powder, Sterling Winthrop, Control No. N-360HH). The concentration of mercury calculated from the weight of Salyrgan in solution was 37.9 γ per 2 ml., by the modified procedure, 37.8 and 38.2 γ.

The standard solution of the RHgSR′ was prepared from the standard Salyrgan solution and cysteine. An equivalent amount of cysteine hydrochloride was placed in a volumetric flask and made up to the mark with standard Salyrgan solution, after clearing the latter of oxygen to prevent the oxidation of the cysteine. Removal of oxygen was accomplished by bubbling nitrogen saturated with water vapor at room temperature through the solution for 3 hours. The completeness of the reaction was verified polarographically (*1*).



In experiments performed to ascertain the effect of urine on the analysis, 5 ml. of urine from an untreated dog were added to the mercury solution in the digestion flask.

RESULTS

The data presented in Table I indicate that for small quantities of mercury the procedures employed were satisfactory whether the mercury was present as inorganic mercury or as Salyrgan in the presence or absence of urine. However, for the 50- to 60-γ range the results were low. In these experiments it was not necessary to concentrate the urine; consequently the final concentration of salts in the digestion flask was lower than that in Kozelka's experiments. Since the resulting lower boiling temperature might possibly be responsible for the incomplete distillation of the mercury, the amount of ammonium sulfate was doubled in the digestion flask. The last two analyses of Table I show that this procedure gave satisfactory recoveries.

However, in Table II, the recovery was poor whenever the mercury was present as the Salyrgan-cysteine complex, even when relatively small amounts of mercury were analyzed. Doubling the concentration of ammonium sulfate and lengthening the digestion period to 2 hours did not improve the recoveries.

Table I. Analysis of Sulfur-Free Mercury Compounds

Type of Mercury	Urine Added, Ml.	Digest Time, Min.	Cl_2 Time, Min.	$(NH_4)_2SO_4$, G.	Hg, γ Expected	Hg, γ Found
Hg^{++}	..	20	55	20	30	30
	..	20	55	20	50	50
	..	20	50	20	40	39.5
	..	20	50	20	20	19.5
Salyrgan	5	30	60	20	9.5	9.5
Hg^{++}	..	20	50	20	50	47.5
Salyrgan	5	30	60	20	56.8	51.5
	5	30	60	20	56.8	51.5
	5	30	60	40	56.8	57.8
	5	30	60	40	56.8	57.8

Table II. Interference of Sulfur

Type of Mercury	Digest Time, Min.	Cl_2 Time, Min.	$(NH_4)_2SO_4$, G.	Hg, γ Expected	Hg, γ Found
Salyrgan-cysteine	60	60	20	37.7	34
	60	60	20	37.9	33
	120	60	40	37.9	34.5
	120	60	40	37.9	31.0

Table III. Effects of Strong Heat

Type of Mercury	Digest Time, Min.	Cl_2 Time, Min.	$(NH_4)_2SO_4$, G.	Hg, γ Expected	Hg, γ Found
Salyrgan-cysteine	60	60	40	37.9	38
	60	60	40	37.9	36.5
	60	60	40	37.9	37.5

Table IV. Results with Modified Procedure

Type of Mercury	Digest Time, Min.	Cl_2 Time, Min.	$(NH_4)_2SO_4$, G.	Hg, γ Expected	Hg, γ Found
Salyrgan-cysteine	60	120	40	37.9	39
	60	120	40	37.9	38
	60	120	40	37.9	37
	60	120	40	37.9	37
	60	120	40	37.9	38.5
	60	120	40	56.8	57.5
	60	120	40	56.8	57.8
	60	120	40	9.5	9.5
	60	120	40	9.5	9.5

The digestion can be somewhat improved (Table III) if, during the passage of chlorine gas, the solution is very strongly heated. Unfortunately this causes so much sulfuric acid to be distilled over that the neutralization of the distillate becomes hazardous.

By doubling the time of chlorine flow and using moderate heat (enough to keep the solution boiling) all the organic mercury was digested and distilled without the hazards of excess acid distillation (Table IV). The total time involved in the last two unsuccessful analyses in Table II is 3 hours, the same as that for the successful analyses in Table IV. The only difference is the time of exposure to chlorine gas. The superior oxidizing power of the digestion mixture in the presence of chlorine gas probably accounts for the completeness of the digestion.

CONCLUSION

The results show that it is possible to make a complex which cannot be accurately analyzed by Kozelka's method unless the procedure is modified. The essential modifications are: a higher boiling temperature produced by adding 40 grams of ammonium sulfate to the digestion mixture, and a 2-hour period of chlorine flow at 150 ml. per minute. With these modifications the method gives results accurate to 1 γ in the range of 9.5 to 57 γ.

In general, it may be concluded that any method based solely on the analysis of inorganic mercury added to urine or other biological material is open to question. An additional criterion for the adequacy of a method should be simultaneous analysis of aliquots of the same biological material by an independent and widely different technique, if possible. In the authors' case the polarographic technique did not require digestion of the urine. In a series of simultaneous analyses of 18 samples of dog urine containing excreted mercury the modified Kozelka method was found to yield results in good agreement with the polarographic method.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Benesch, R., and Benesch, R. E., *Arch. Biochem. and Biophys.*, **38**, 425 (1952).
(2) Kozelka, F. L., ANAL. CHEM., **19**, 494 (1947).
(3) Simonsen, D. G., *Am. J. Clin. Pathol.*, **23**, 789 (1953).
(4) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Vol. II, p. 3, Van Nostrand, New York, 1949.
(5) Weiner, I. M., and Müller, O. H., unpublished paper.

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Hydrolysis Constants of *n*-Aliphatic Amine Salts

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The hydrolysis constants of *n*-primary amines in approximately 22% ethyl alcohol solutions were determined from potentiometric titration data. The pK_h values determined by extrapolation to zero ionic strength were in good agreement with published values of pK_h in water solutions determined by conductance and e.m.f. methods.

IN AN ordinary titration of an amine solution with a strong acid, using a glass electrode pH meter, it was noted that the data obtained at the end point provided a very simple method of calculating the hydrolysis constant of the amine ion. In view of the agreement of this method with more rigorous and conventional methods, it was considered of interest to apply this titration method to a group of amines in order to assess its value.

An excellent summary of references and data is made by Hoerr and coworkers (*4*) who used the conductance ratio method and by Hall and Sprinkle (*2*) who measured electromotive force values of cells without liquid junctions. Both groups of workers overcame the problem of solubility of the higher amines in water by making measurements on alcohol solutions of various concentrations and subsequently extrapolating the results to zero alcohol concentration. These workers have shown that the change in conductance and electromotive force, both attributed to the ionization of the systems involved, is relatively small in dilute alcohol solutions. Consequently, the present study was based on amine solutions which contained approximately 22% ethyl alcohol at the end point of the titration.

In titrating a weak base with a strong acid, a salt is formed which undergoes partial hydrolysis according to the equation

$$B^+ + H_2O \leftrightarrows BOH + H^+$$

According to typical derivations (*3*) of the hydrolysis constant

$$pK_h = 2pH - pC \qquad (1)$$

the expression was used to determine the values for the hydrolysis constant at various concentrations, C, of the amine salt.

By applying the principle used by Everett and Wynne-Jones (*1*) the values for pK_h at zero ionic strength were determined. This method involved three determinations at approximately 0.05, 0.025, and 0.012 ionic strength.

EXPERIMENTAL METHODS AND MATERIALS

In order to use Equation 1, it was necessary to measure accurately the pH of the amine hydrochloride solutions. This was accomplished by titrating a solution of the amine in a 44% ethyl alcohol–water solvent with standard hydrochloric acid in water having a normality of the same order of magnitude as the amine solution. The end point of a potentiometric titration can be satisfactorily determined by means of plotting the change in pH (ΔpH) against the milliliters of acid added. However, there is considerable difficulty in determining the exact pH at which the pure amine hydrochloride exists. To find the pH of the end point, a graphical analysis, as in Figure 1, was made by bisecting the distance between the linear portions of the curve prior to and following the stoichiometric end point.

A Beckman, Model H2, glass electrode pH meter was calibrated at pH 4, 7, and 10 with Clark and Lubs' buffer mixtures, and rechecked at pH 7 with a buffer supplied by the manufacturer of

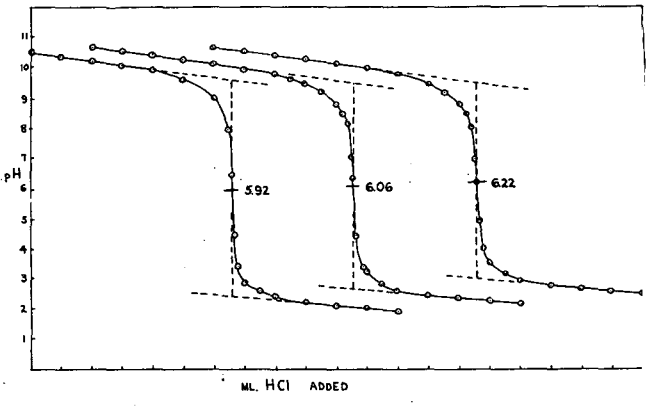

Figure 1. Potentiometric Titration Curves

For 0.1, 0.05, and 0.025*N* $C_3H_7NH_2$ with standardized hydrochloric acid at similar concentrations

Table I. Hydrolysis Values Based on Titration Data and Compared with Published Values

Amine	End Point Data: pH	Ethyl alcohol by weight	Salt concn.	pK_h	$\sqrt{\mu}$	pK_h (alc.) Extrapolated to $\sqrt{\mu}=0$	pK_h (H_2O): ICT (*5*)	Hoerr (*4*)	Hall (*2*)
NH_3	5.26	23.3	0.0459	9.20	0.214				
	5.40	23.1	0.0242	9.18	0.156				
	5.56	22.6	0.0122	9.20	0.110	9.22	9.25	...	...
CH_3NH_2	5.83	21.4	0.0495	10.36	0.222				
	6.00	21.3	0.0246	10.39	0.157				
	6.19	21.4	0.0124	10.43	0.111	10.56	10.59	...	10.64
$C_2H_5NH_2$	5.90	22.0	0.0482	10.48	0.220	(10.60)	10.65	...	10.67
$C_3H_7NH_2$	5.92	23.6	0.0504	10.54	0.224				
	6.06	23.6	0.0273	10.56	0.165				
	6.22	23.6	0.0136	10.57	0.117	10.58	10.58	...	10.58
$C_4H_9NH_2$	5.96	20.0	0.0458	10.57	0.214				
	6.10	20.1	0.0228	10.60	0.151				
	6.28	20.1	0.0114	10.62	0.107	10.64	10.55	10.60	10.71
$C_5H_{11}NH_2$	5.94	21.4	0.0495	10.57	0.222				
	6.16	21.4	0.0248	10.60	0.157				
	6.28	21.4	0.0124	10.65	0.111	10.68	...	10.62	10.70
$C_6H_{13}NH_2$	5.96	22.3	0.0515	10.63	0.227				
	6.12	22.3	0.0255	10.65	0.160				
	6.25	22.2	0.0128	10.61	0.113	10.62	...	10.63	...
$C_7H_{15}NH_2$	5.94	22.6	0.0523	10.60	0.229				
	6.10	22.6	0.0261	10.62	0.162				
	6.24	23.1	0.0130	10.59	0.114	10.60	...	10.65	...
$C_8H_{17}NH_2$	5.82	22.1	0.0510	10.35	0.226				
	5.98	22.1	0.0255	10.37	0.160				
	6.18	22.1	0.0127	10.46	0.113	10.55	...	10.64	...
$C_{10}H_{21}NH_2$	5.34	22.0	0.0487	9.37	0.220	9.40	...	10.63	...
$C_{12}H_{25}NH_2$	5.22	21.7	0.0501	9.06	0.224				
	5.35	21.8	0.0251	9.10	0.158				
	5.52	21.8	0.0126	9.12	0.112	9.14	...	10.62	...
$C_{14}H_{29}NH_2$	4.98	22.7	0.0472	8.64	0.217	(8.70)	...	10.61	...
$C_{16}H_{33}NH_2$	4.80	21.8	0.0502	8.32	0.224				
	4.98	21.4	0.0248	8.36	0.158				
	5.16	21.5	0.0124	8.39	0.111	8.42	...	10.60	...

the instrument. Temperature was controlled to ±0.1° C. for calibration and to ±0.5° C. for the titrations. Ammonium hydroxide and hydrochloric acid (C.P.), 95% ethyl alcohol, and freshly distilled water were used. The lower amines were purchased directly from Eastman Kodak and Matheson & Coleman Bell Co. according to their current catalog listings. The higher amines, beginning with octyl amine were kindly supplied by Armour & Co. as distilled grade Armeens. All amines were used from freshly opened packages in order to minimize the presence of carbon dioxide which is absorbed by the amines to form carbamates. All titrations were performed as rapidly as possible in order to eliminate the effect of atmospheric carbon dioxide.

RESULTS

The results of this study are summarized in Table I. The end-point data were derived from a series of graphical analyses similar to that in Figure 1. An error of ±0.04 pK_h unit was attributed to errors in reading and interpreting these graphs. Values for pK_h, calculated by using Equation 1, were plotted against the square root of the ionic strength for each amine, and the extrapolated values of pK_h were obtained. The experimental error, attributed to all factors concerned, was ±0.85% of the pK_h values or ±0.09 pK_h units. Reliable values from three published sources are listed for comparison. The $pK_{(alc.)}$ values refer to those determined in alcohol solutions, while the $pK_{(H_2O)}$ refer to values determined or calculated for water solutions of the amine salt.

For the higher amines, a considerable deviation from Hoerr's values was noted. These deviations, which follow a definite trend, may be attributed to the formation of micelles even in the alcoholic solutions. The micelle reduces the concentration of the amine ion and at the same time introduces a different ionic species. These effects would undoubtedly affect the activities of the ions concerned and the resulting values of the hydrolysis constants.

LITERATURE CITED

(1) Everett, D. H., and Wynne-Jones, W. F. K., *Trans. Faraday Soc.*, 48, 531 (1952).
(2) Hall, N. F., and Sprinkle, M. R., *J. Am. Chem. Soc.*, 54, 3469 (1932).
(3) Hamilton, L. F., and Simpson, S. G., "Calculations of Analytical Chemistry," 4th ed., p. 194, McGraw-Hill Book Co., New York, 1947.
(4) Hoerr, C. W., McCorkle, M. R., and Ralston, A. W., *J. Am. Chem. Soc.*, 65, 328 (1943).
(5) International Critical Tables, Vol. VI, McGraw-Hill Book Co., New York (1929).

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Amperometric Method for Mercaptan Sulfur in Hydrocarbons

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The amperometric titration of total mercaptan sulfur with silver nitrate has been applied to synthetic blends of mercaptans in hydrocarbons. The average deviation from the true value was 1 p.p.m. for liquid hydrocarbons containing mercaptan sulfur in the range of 0 to 100 p.p.m., and 3 p.p.m. for gaseous hydrocarbons containing mercaptan sulfur in the range of 0 to 400 p.p.m. Organic sulfides, disulfides, thiophene, and tetraethyllead do not interfere. Free sulfur in amounts of less than 0.001% does not interfere. The apparatus is simple, compact, and portable. The amperometric titration can be completed in less than 10 minutes, and is especially suitable for use in routine determinations of total mercaptan sulfur in hydrocarbons.

THE increasing use of high-sulfur crudes has made it desirable to have rapid, accurate methods for the determination of mercaptan sulfur (thiols) in liquid and gaseous petroleum products. Previously reported methods include those based on the reaction of the mercaptan with iodine (*7, 13, 15*), with a copper salt (*2, 6, 14, 17, 19*), and with silver nitrate (*1, 3–5, 8–12, 16*). Kolthoff and Harris (*10, 11*) described the amperometric titration of mercaptan sulfur with silver nitrate using a rotating platinum electrode.

The method presented here is based on the work of Kolthoff and Harris (*11*), and has been used successfully in this laboratory for several years. It was chosen over other reported methods because of its speed, accuracy, and adaptability to routine determinations. Liquid samples are dissolved in ammoniacal acetone and titrated with silver nitrate, according to Kolthoff and Harris (*11*). The mercaptan sulfur from a gaseous sample is absorbed in an excess of silver nitrate, and the excess silver is titrated with standard dodecyl mercaptan according to the procedure used by Warner (*18*).

The apparatus is simple, inexpensive, compact, and portable (Figure 3). For use in routine analysis it was found more convenient to substitute a commercial pencil-type calomel electrode for the reference electrode used by Kolthoff and Harris (*11*). This modification requires the application of a negative potential to the electrode system.

APPARATUS

Microammeter, direct current, 0- to 30-μa. range, with a sensitivity of 0.2 μa. per division, and an internal resistance of approximately 490 ohms. A Weston Model 931 is suitable.

Voltmeter, direct current, 0- to 0.25-volt range, with a sensitivity of 0.01 volt per division, and an internal resistance of 40,000 ohms per volt.

Stirrer. A stirrer is required that will maintain a constant speed of approximately 1000 r.p.m., and in which electrical connection can be made between the rotating electrode and the potential divider. A Cenco No. 18802A stirrer with electrode holder attachment No. 18809 is suitable.

Rotating electrode. This electrode is similar to the one described by Kolthoff and Harris (*11*).

Reference electrode. A sleeve-type calomel pencil electrode, such as Beckman No. 1170–71 is used. (Warning. Select an electrode that has a low internal resistance. Some of these electrodes have a relatively high resistance, and would cause difficulty in this titration.)

Electrical circuit. The electrical wiring diagram is shown in Figure 1.

Gaseous sample container. A 100- to 200-ml. cylinder of stainless steel or aluminum, and fitted with a stainless steel needle valve, is used.

REAGENTS

Supporting electrolyte. Dissolve 100 grams of reagent grade ammonium nitrate in 500 ml. of concentrated ammonium hydroxide.

Cadmium sulfate solution, 12%. Dissolve 150 grams of reagent grade cadmium sulfate ($3CdSO_4.8H_2O$) in distilled water, add 10 ml. of 6*N* sulfuric acid, and dilute to 1 liter.

n-Dodecyl mercaptan solution, standard 0.01*N*. Dissolve 2.1 grams of the purified *n*-dodecylmercaptan in isopropyl alcohol and dilute to 1 liter. Standardize this solution daily against 0.01*N* silver nitrate amperometrically.

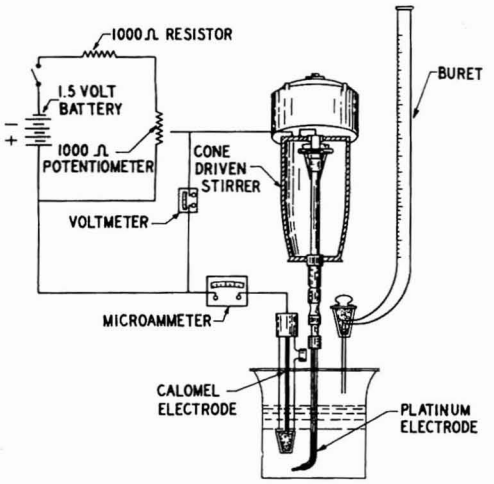

Figure 1. Diagram of Apparatus

n-Dodecyl mercaptan may be purchased from Bios Laboratories, Inc., 17 West 60th St., New York 23, N. Y. The commercial product may be purified as follows. Distill a large portion in an efficient column and collect the center cuts boiling from 150° to 158° C. at 24 mm. Shake a portion of this product with small portions of metallic mercury until the mercury remains bright and shows no evidence of reaction after 2 hours of shaking in a mechanical shaker.

PROCEDURE

Preparation of Apparatus. Assemble the titration apparatus as shown in Figure 1, using soldered connections wherever practical in the electrical circuit.

Place 100 ml. of acetone and 5 ml. of the supporting electrolyte solution in a beaker, and immerse the tips of the electrodes at least 1 inch below the surface of the liquid. Adjust the speed of the rotating electrode to approximately 1000 r.p.m.; maintain this same speed throughout a titration. Close the electrical circuit and adjust the potential divider so that -0.23 ± 0.02 volt is applied to the electrode system. The current may increase to 15 or 20 μa. at first, but it will decrease to near zero in a few minutes.

Analysis of Liquid Samples. If hydrogen sulfide is present, it can be removed by shaking the sample with acid cadmium sulfate solution.

Measure with a pipet the quantity of sample as shown in Table I into a beaker, and add 100 ml. of acetone and 5 ml. of the supporting electrolyte. Titrate with 0.01*N* silver nitrate to the amperometric end point, as described by Kolthoff and Harris (*11*).

Analysis of Gaseous Samples. REMOVAL OF HYDROGEN SULFIDE AND ABSORPTION OF MERCAPTAN. Introduce 50 ml. of the acid cadmium sulfate solution into a gas-washing bottle. In another gas-washing bottle place 50 ml. of acetone and an accurately measured quantity of standard 0.01*N* silver nitrate solution as indicated in Table II. Weigh the sample container to the nearest 0.01 gram.

If the sample container is too bulky to be weighed conveniently, attach a wet-test meter to the exit side of the second gas washing bottle. Liquefied petroleum gas samples should be taken from the liquid phase. Regulate the flow of sample through the gas-washing bottles at a rate of about 1 gram per minute. After sufficient sample has passed through the system, as indi-

Table I. Size of Sample

Estimated Weight % Mercaptan Sulfur	Ml. of Sample
0.0001–0.001	50
0.001 –0.01	20
0.01 –0.1	5

Table II. Size of Sample

Estimated Weight % Mercaptan Sulfur	Grams of Sample	Ml. of 0.01*N* Silver Nitrate
0.0001–0.001	100	10.00
0.001 –0.01	20	10.00
0.01 –0.1	5	25.00

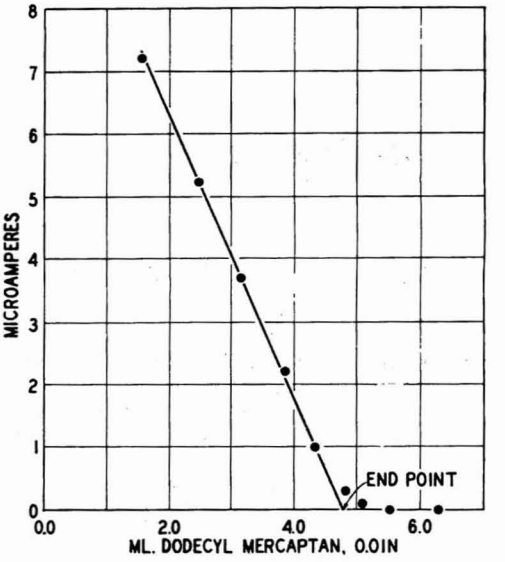

Figure 2. Typical Titration Curve for Gaseous Hydrocarbon Samples

Table III. Analyses of Liquid Hydrocarbons of Known Mercaptan Sulfur Conten

Sample No.	Description	Weight % Mercaptan Sulfur Added	Silver Nitrate Method Used: Colorimetric Found	Colorimetric Deviation	Potentiometric Found	Potentiometric Deviation	Amperometric Found	Amperometric Deviation
A	Iso-octane[a]	0.0005	....	....	....	....	0.0005	0.0000
							0.0006	+0.0001
							0.0005	0.0000
							0.0005	0.0000
B	Jet fuel[b]	0.0010	0.0013	+0.0003	0.0011	+0.0001	0.0011	+0.0001
			0.0013	+0.0003	0.0012	+0.0002	0.0010	0.0000
			0.0012	+0.0002			0.0010	0.0000
			0.0014	+0.0004			0.0010	0.0000
C	Iso-octane[a]	0.0035	....	....	....	....	0.0035	0.0000
							0.0035	0.0000
							0.0036	+0.0001
D	Jet fuel[b]	0.0050	0.0054	+0.0004	0.0054	+0.0004	0.0052	+0.0002
			0.0052	+0.0002	0.0054	+0.0004	0.0051	+0.0001
			0.0052	+0.0002			0.0053	+0.0003
			0.0053	+0.0003			0.0052	+0.0002
E	Jet fuel[b]	0.0082	0.0083	+0.0001	0.0083	+0.0001	0.0082	0.0000
			0.0083	+0.0001	0.0085	+0.0003	0.0083	+0.0001
				Av. 0.0003		0.0003		0.0001

[a] Mercaptan sulfur added as *tert*-butyl mercaptan.
[b] Mercaptan sulfur added as *n*-dodecyl mercaptan.

cated in Table II, close the sample cylinder valve and sweep out the system for about 5 minutes with nitrogen. Reweigh the sample cylinder, or record the wet-test meter reading.

Titration. Quantitatively transfer the contents of the gas-washing bottle containing the silver mercaptides to a beaker, rinsing with acetone. Add 5 ml. of supporting electrolyte to the silver nitrate solution and titrate amperometrically with the standard 0.01*N* dodecyl mercaptan solution. A typical titration is plotted in Figure 2.

ACCURACY

The data in Table III indicate that for liquid hydrocarbons containing mercaptan sulfur in the range of 0 to 100 p.p.m. the average deviation from the true value was 1 p.p.m. Similarly, the data in Table IV indicate that the average deviation from the true value was 3 p.p.m. for gaseous hydrocarbons containing mercaptan sulfur in the range of 0 to 400 p.p.m.

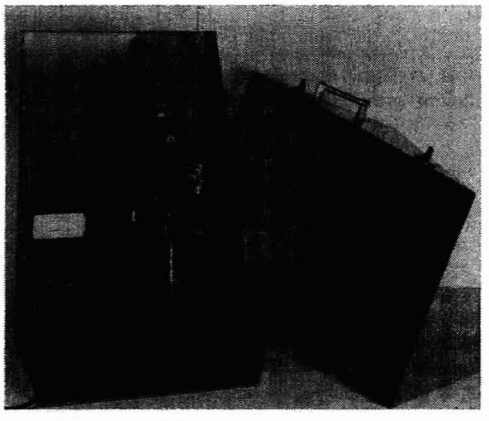

Figure 3. Photograph of Apparatus

Table IV. Analyses of Propane of Known Mercaptan Sulfur Content

		Weight % Mercaptan Sulfur			
		Iodometric Method		Amperometric Method	
Sample	Added	Found	Deviation	Found	Deviation
A	0.0000	0.0000	0.0000	0.0000	0.0000
		0.0000	0.0000	0.0000	0.0000
B[a]	0.0004	0.0005	+0.0001	0.0005	+0.0001
		0.0005	+0.0001	0.0005	+0.0001
				0.0005	+0.0001
C[b]	0.0018	....	....	0.0020	+0.0002
				0.0019	+0.0001
				0.0018	0.0000
D[a]	0.0038	0.0043	+0.0005	0.0040	+0.0002
		0.0041	+0.0003	0.0041	+0.0003
				0.0038	0.0000
E[a]	0.0397	0.0444	+0.0047	0.0410	+0.0013
		0.0451	+0.0054	0.0402	+0.0005
		0.0437	+0.0040	0.0407	+0.0010
				0.0406	+0.0009
		Av.	0.0017		0.0003

[a] Mercaptan sulfur added as ethyl mercaptan.
[b] Mercaptan sulfur added as *tert*-butyl mercaptan.

DISCUSSION

The data in Table III for the colorimetric and potentiometric methods were obtained by methods essentially the same as those of Borgstrom and Reid (*3*) and Tamele and Ryland (*16*), respectively. In preparing the iso-octane–mercaptan blends A and listed in Table III, a weighed amount of 2-methyl-2-propanethiol (*tert*-butyl mercaptan, National Bureau of Standards sample 905–5S) was added to a measured quantity of Phillips Petroleum Co. pure grade iso-octane. The theoretical mercaptan-sulfur contents of the jet fuel samples B, D, and E represent the average values obtained by 13 laboratories during a cooperative testing program of instrumental methods for the determination of mercaptan sulfur (conducted by the Research Division III, Section A, American Society for Testing Materials).

Table V. Effects of Interfering Substances on Amperometric Determination of Mercaptan Sulfur in Iso-octane

	Weight %	Weight % Mercaptan Sulfur		
Substance	Added	Present	Found	Deviation
Elemental sulfur	0.0001	0.0036	0.0034	−0.0002
	0.0005	0.0016	0.0015	−0.0001
	0.0058	0.0139	0.0110	−0.0029
	0.0066	0.0036	0.0030	−0.0006
	0.0289	0.0615	0.0569	−0.0046
Organic sulfide[a]	0.017	0.0045	0.0046	+0.0001
	0.238	0.0045	0.0045	0.0000
Disulfide[b]	0.010	0.0029	0.0029	0.0000
	0.110	0.0029	0.0029	0.0000
Thiophene	0.020	0.0029	0.0029	0.0000
	0.250	0.0029	0.0029	0.0000
Tetraethyllead	3.00 ml./gal.	0.0029	0.0028	−0.0001

[a] Added as isobutyl sulfide.
[b] Added as isobutyl disulfide.

The data in Table IV by the iodometric method were obtained by a modification of the method of Shaw (*15*). The propane-mercaptan blends were prepared by adding a weighed amount of previously analyzed Eastman Kodak, white label, ethylmercaptan, or National Bureau of Standards *tert*-butylmercaptan (905–5S) in the case of sample C, to a weighed amount of Phillips Petroleum Co. pure grade propane.

Kolthoff and Harris (*11*) reported that chlorides and small amounts of bromide do not interfere but that cyanides and other ions, such as iodide and sulfide, which form insoluble silver salts in ammoniacal medium do interfere with the amperometric silver nitrate titration for mercaptan sulfur. The results tabulated in Table V indicate that organic sulfides, disulfides, thiophene, and tetraethyllead do not interfere. If the free sulfur content is less than about 0.001%, there is practically no interference with the mercaptan analysis.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) *Am. Gas J.*, **162**, No. 6, 47 (1945).
(2) Bond, G. R., Jr., IND. ENG. CHEM., ANAL. ED., **5**, 257 (1933).
(3) Borgstrom, P., and Reid, E. E., *Ibid.*, **1**, 186 (1929).
(4) Davies, E. R. H., and Armstrong, J. W., *J. Inst. Petroleum*, **29**, 323 (1943).
(5) Eberle, J. F., Phillips Petroleum Co., unpublished communication.
(6) Ellis, E. W., and Barker, T., ANAL. CHEM., **23**, 1777 (1951).
(7) Hakewill, H., and Rueck, E. M., *Proc. Am. Gas Assoc.*, **28**, 529 (1946).
(8) Hallikainen, K. E., and Pompeo, D. J., *Instruments*, **25**, 335 (1952).
(9) Institute of Petroleum, 26 Portland Place, London, unpublished communication.
(10) Kolthoff, I. M., and Harris, W. E., ANAL. CHEM., **21**, 963 (1949).
(11) Kolthoff, I. M., and Harris, W. E., IND. ENG. CHEM., ANAL. ED., **18**, 161 (1946).
(12) Mapstone, G. E., *Australian Chem. Inst. J. & Proc.*, **15**, 236 (1948).
(13) Sampey, J. R., and Reid, E. E., *J. Am. Chem. Soc.*, **54**, 3404 (1932).
(14) Schulze, W. A., and Chaney, L. V., *Natl. Petroleum News*, **25**, No. 34, 37 (1933).
(15) Shaw, J. A., IND. ENG. CHEM., ANAL. ED., **12**, 668 (1940).
(16) Tamele, M. W., and Ryland, L. B., *Ibid.*, **8**, 16 (1936).
(17) Turk, E., and Reid, E. E., *Ibid.*, **17**, 713 (1945).
(18) Warner, P. F., Phillips Petroleum Co., unpublished communication.
(19) White, D. L., and Reichardt, F. E., *Gas*, **25**, No. 6, 38 (1949).

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Analytical Procedures Using a Combined Combustion-Diffusion Vessel

An Improved Method for the Degradation of Carbon-14–Labeled Lactate and Acetate

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Simple combustion-diffusion vessels may be used for the complete degradation of carbon-14–labeled lactate and acetate. The results obtained compare favorably with those of other standard methods. This flask is useful for general degradation procedures of carbon-14–labeled compounds.

THE use of combustion-diffusion vessels of very simple construction in analytical procedures has been described (*2, 6*). The application of this technique to the degradation of carbon-14–labeled lactate and short-chain fatty acids is reported here. Although several methods for the degradation of these compounds are available (*1, 3, 8, 10, 12*), they do not lend themselves readily to simultaneous determinations, and often require specialized and expensive equipment.

The procedure used here involves the oxidation of lactate to acetate and the degradation of the latter by means of a modification of the Phares method (*8*). The degradation scheme is outlined below.

Degradation Scheme

$$\underset{(3)\ (2)\ (1)}{CH_3\text{-}CHOH\text{-}COOH} \xrightarrow{KMnO_4,\ H^+} \underset{(3)\ (2)}{CH_3\text{-}COOH} + \underset{(1)}{CO_2} \quad (1)$$

$$\underset{(3)\ (2)}{CH_3\text{-}COOH} \xrightarrow{NaN_3,\ H_2SO_4} \underset{(3)\ (2)}{CH_3NH_2} + \underset{(1)}{CO_2} \quad (2)$$

$$\underset{(3)}{CH_3NH_2} \xrightarrow{KMnO_4,\ OH^-} \underset{(1)}{CO_2} \quad (3)$$

EXPERIMENTAL

The combustion-diffusion vessel has been described (*6*). It consists of a 50-ml. Erlenmeyer flask provided with a center well for carbon dioxide absorption, and closed with a rubber serum cap. However, a variety of vessels or screw-top vials can also be employed (*2*).

Oxidation of Lactate (Equation 1). Dichromate has been used for decarboxylation of lactate to acetate (*12*). In the authors' hands, however, about 10% of the α-carbon was also oxidized, a finding in agreement with that of Daus *et al.* (*5*). Use of permanganate as oxidant reduces contamination to 2 to 3%. In this reaction the yields of both acetate and carbon dioxide were nearly quantitative (Table I).

Table I. Oxidation of Lactate–Carbon-14 with Permanganate

Compound[a]	Yield, %		Specific Activity, Counts/Min./Mg. $BaCO_3$		% Activity in Carboxyl Carbon of Lactate
	CO_2	Acetate	CO_2	Av.[b] lactate carbon	
Lactic acid-1-carbon-14	97	98	24.6	8.5	96.5
Lactic acid-2-carbon-14	99	96	2.4	35.8	2.3
Lactic acid-3-carbon-14	95	96	1.3	18.1	2.4

[a] Obtained from Richard Lemmon, Donner Laboratory, University of California.
[b] Obtained by persulfate combustion (*6*) of lactate.

Procedure. Two hundred micromoles of lactate were introduced into the main compartment of the vessel. One milliliter of standardized, carbonate-free alkali was placed in the center well, and immediately thereafter 2 ml. of 5% potassium manganate in 2*N* sulfuric acid were added. The flasks were quickly capped and evacuated through a hypodermic needle (*6*). The vessels were heated for 30 minutes in an oven set at 80° C., and allowed to cool. The barium carbonate was precipitated and assayed in the usual manner (*6*). The acetate was recovered by steam distillation of the permanganate solution in a Markham still (*7*); 20 volumes of distillate were collected, and the acetic acid was titrated with alkali. Results of typical experiments with variously labeled lactates are presented in Table I.

Degradation of Acetate (Equation 2). The sodium acetate solution was evaporated to dryness on a steam bath, and the residue was taken up in a small volume of water (exactly 1 or 2 ml.). Aliquots were taken for persulfate combustion (*6*) and for decarboxylation by the Schmidt reaction. The carbon dioxide obtained from the persulfate combustion is derived from the α and β carbons of lactate.

The aliquot used for the Schmidt reaction was transferred to a shell vial and evaporated to dryness. About 0.2 ml. of 100% sulfuric acid (*8*) was added, followed by the addition of about 30 mg. of recrystallized sodium azide. The contents were mixed with a short stirring rod which was left in the vial.

The vial was put in the center well of the reaction flask, and the flask was capped and evacuated. In most cases the Schmidt reaction does not begin until the flask is heated, but with some samples of 100% sulfuric acid, considerable heat was evolved immediately after the addition of the acid to the acetate-azide mixture. For this reason, an alternate procedure was employed. The vial containing acetate and azide was placed in the center well of the flask, and the flask was capped and evacuated. The acid was cautiously injected into the vial with 0.5-ml. syringe to which was attached a 2-inch, 23-gage needle.

The flask was placed in an oven, at about 80° for an hour. After cooling, about 2 ml. of carbon dioxide–free alkali were injected into the main compartment of the flask with a hypodermic syringe. One hour was allowed for carbon dioxide absorption and the vacuum was then released by inserting a hypodermic needle through the cap. The vial was removed for subsequent methylamine distillation.

In the Schmidt reaction, the sulfur dioxide formed is also absorbed by the alkali. To eliminate this contamination it was necessary to regenerate the carbon dioxide. Base was delivered into the center well, and the flask was capped and evacuated. The carbon dioxide was liberated by injecting several milliliters of 2*N* sulfuric acid containing 1 to 2% of hydrogen peroxide into the main compartment. The hydrogen peroxide served to oxidize the sulfite, and eliminated the variable blanks obtained otherwise. The carbon dioxide was collected and assayed as described previously.

The amount of sample used for degradation by the Schmidt reaction is limited by the flask size, as two equivalents of gas are formed during the reaction (carbon dioxide and nitrogen). With the 50-ml. reaction flasks used here, as much as 0.5 millimole of acetate can be degraded.

Oxidation of Methylamine (Equation 3). The vial containing the methylamine was put into a small distillation flask containing a few milliliters of water, and the solution was made alkaline. The contents of the flask were concentrated to a small volume, and the methylamine distillate (about 5 ml.) was trapped in an excess of sulfuric acid contained in another combustion flask. One milliliter of 2*N* sodium hydroxide and 3 ml. of 5% potassium permanganate were next added, and the flask was evacuated and heated for about 30 minutes in an oven set at 80° to 90° C. After cooling, alkali was put into the center well and the flask was re-evacuated. The carbon dioxide liberated by injecting excess sulfuric acid into the main compartment was collected in the manner described above.

An alternative procedure for the combustion of methylamine with potassium persulfate can be used. In a previous paper (*6*) it was reported that the carbon dioxide yields from this oxidation were low. However, amines of this type can be completely oxidized, provided a large excess of persulfate is utilized (*9*). When 200 micromoles of methylamine were being burned, 1 gram of potassium persulfate was found to yield satisfactory results.

Table II. Degradation of Acetate-Carbon-14

Compound	Reaction	Products of Reaction: Per Cent Recovery CO_2	Per Cent Recovery CH_3NH_2[b]	Specific Activity[a] CO_2	Specific Activity[a] CH_3NH_2[c]
Acetate-1-carbon-14	$K_2S_2O_8$ oxidation	96	..	22.7	...
	Azide reaction	92	90	46.0	0
Acetate-2-carbon-14	$K_2S_2O_8$ oxidation	96	..	18.2	...
	Azide reaction	90	91	0	35.9
Acetate-carbon-14[d]	$K_2S_2O_8$ oxidation	90	..	54.7	...
	Azide reaction	95	93	34.7	79.9

[a] Counts/min./mg. $BaCO_3$ assayed with an end-window Geiger-Müller tube.
[b] Determined by titration of volatile base.
[c] Oxidized to CO_2 with $KMnO_4$.
[d] Biologically prepared (4).

Methylamine can also be combusted directly after the Schmidt reaction, without distillation. The contents of the vial are transferred, with water, to the main compartment of a reaction vessel, and the combustion is conducted as previously described (6). This direct combustion greatly simplifies the procedure. However, if any unreacted acetate is present, it also will be oxidized.

Table II shows that the recoveries of both carbon dioxide and methylamine were about 90%. The specific activities of the labeled carbons of either acetate-1-carbon-14 or acetate-2-carbon-14 were twice the average values obtained by persulfate combustion. It appears that there is no significant cross-contamination by this method.

The advantages of this procedure are especially apparent when many samples are degraded simultaneously. The method was also found suitable for the degradation of propionate and butyrate. It is likely that a variety of degradations can be carried out by means of the simple apparatus described here, with a considerable saving of time. In this laboratory the complete degradation of serine by the periodate method of Sakami (11) was performed in the combustion-diffusion vessel.

LITERATURE CITED

(1) Aronoff, S., Barker, H. A., and Calvin, M., *J. Biol. Chem.*, **169**, 459 (1947).
(2) Baker, N., Feinberg, H., and Hill, R., ANAL. CHEM., **26**, 1504 (1954).
(3) Barker, H. A., and Kamen, M. D., *Proc. Natl. Acad. Sci., U. S.*, **31**, 219 (1945).
(4) Dauben, W. G., Abraham, S., Hotta, S., Chaikoff, I. L., Bradlow, H. L., and Soloway, A. H., *J. Am. Chem. Soc.*, **75**, 3038 (1953).
(5) Daus, L., Meinke, M., and Calvin, M., *J. Biol. Chem.*, **196**, 77 (1952).
(6) Katz, J., Abraham, S., and Baker, N., ANAL. CHEM., **26**, 1503 (1954).
(7) Markham, R., *Biochem. J.*, **36**, 790 (1942).
(8) Phares, E. F., *Arch. Biochem.*, **33**, 173 (1951).
(9) Phares, E. F., personal communication.
(10) Roseman, S., *J. Am. Chem. Soc.*, **75**, 3854 (1953).
(11) Sakami, W., *J. Biol. Chem.*, **187**, 369 (1950).
(12) Wood, H. G., Lifson, N., and Lorber, V., *Ibid.*, **159**, 475 (1945).

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Argentimetric Procedure for Borohydride Determination

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In order to avoid errors arising from loss of borohydride under acidic conditions, an analytical procedure was developed which permits the determination of borohydride under alkaline conditions. The analysis is based upon the reaction: $8Ag^+ + BH_4^- + 8OH^- \xrightarrow{\text{ethylene-diamine}} 8Ag \downarrow + H_2BO_3^- + 5H_2O$. The precipitated silver is removed by filtration and the excess silver ion in the solution is determined by standard volumetric procedures. The procedure allows the determination of borohydride in the presence of iodate and shows that the reaction of iodate with borohydride is very slow. No interference occurs with potassium chlorate, sodium formate, ethyl alcohol, acetone, and cyclohexanone. Benzaldehyde interferes, resulting in high borohydride values. An ammoniacal solution of silver nitrate provides a convenient sensitive spot test for borohydride solutions.

IN THE original investigations of the chemistry of the borohydrides, the determination of active hydrogen utilized hydrolytic decomposition of the borohydride ion under acidic conditions, followed by measurement of the hydrogen evolved (4, 5, 9). Volumetric methods based upon the oxidation of borohydride by iodine (7), hypochlorite (3, 8), and iodate (6) have been proposed. The iodate method is a simple procedure which appears to have many advantages for the rapid analysis of borohydride solutions. An excess of standard potassium iodate solution is added to the aqueous borohydride sample, stabilized by alkali. A large excess of solid potassium iodide is added, followed by 4*N* sulfuric acid. After 2 to 3 minutes in the dark, the liberated iodine is titrated with standard sodium thiosulfate solution.

An attempt was made to simplify the iodate procedure by eliminating the need for two standard solutions. Lyttle *et al.* had stated that the borohydride reduction of iodate appears to be an instantaneous reaction (6). It therefore appeared that the analysis could be carried out by the addition of a large unknown excess of iodate to the borohydride solution, conversion of the iodide (presumably formed in the rapid reduction) to free iodine by acidification, followed by titration of the free iodine by standard arsenious oxide under controlled pH.

The results obtained were erratic. In the course of investigating the cause of the difficulties, it became evident that the reaction of iodate with borohydride is not so fast as it had been postulated to be. The iodate procedure must depend upon the formation of iodine or other intermediate oxidation products, upon the acidification of the iodate-iodide solution, followed by the reaction of these products with the borohydride.

In the course of this study an argentimetric procedure for borohydride determination was developed which permits the analysis of borohydride solutions under strongly alkaline conditions and the determination of borohydride in the presence of iodate. Application of the method definitely established the slowness with which iodate and borohydride react under alkaline conditions.

The new analytical procedure depends upon the reduction of silver ion by borohydride ion under alkaline conditions. In order

$$8Ag^+ + BH_4^- + 8OH^- \rightarrow 8Ag \downarrow + H_2BO_3^- + 5H_2O$$

to maintain the silver ion in solution under alkaline conditions,

a complexing agent must be used. Both ammonia and ethylenediamine were investigated. The latter proved advantageous in a number of respects. Consequently, all analytical data reported in the present paper are derived from the procedure utilizing this complexing agent.

PROCEDURE

Aqueous sodium borohydride solutions, 2*M* in sodium hydroxide, were employed for the analyses. In the presence of the strong base such solutions were observed to be relatively stable for long periods of time. The ethylenediamine solution contained 40 grams of ethylenediamine per liter of solution (approximately 0.7*M*). Standard solutions of silver nitrate (0.2000*N*) and ammonium thiocyanate (0.1000*N*) were prepared and standardized.

The reagent is prepared by mixing 25.00 ml. of the standard silver nitrate solution with 25 ml. of the ethylenediamine solution. As this mixture is swirled in an Erlenmeyer flask, a 2.00-ml. sample of the borohydride solution is added. The solution immediately darkens as metallic silver precipitates. The mixture is immediately poured into a sintered-glass funnel of fine porosity. The filtrate is collected under slight vacuum. The flask in which the reduction had occurred is washed with 10 to 20 ml. of water, and this wash water is used to rinse the precipitated metal in the funnel. The wash water is drawn into the flask containing the original filtrate. At least three washings are made similarly to ensure quantitative removal of the silver ion. The precipitate is not permitted to become dry until the final washing has been made.

The filtrate is now made acid with 5 ml. of concentrated nitric acid, 2 ml. of standard ferric ammonium sulfate indicator are added, and the solution is titrated in the usual manner with standard ammonium thiosulfate solution (*10*). Addition of 20 ml. of nitrobenzene shortly before the stoichiometric point sharpens the end point considerably (*2*).

RESULTS AND DISCUSSION

Typical results obtained by the argentimetric procedure as compared with the iodate procedure are summarized in Table I.

In order to ascertain whether the analysis was sensitive to interference by substances that might be present in solutions being analyzed for borohydride, a number of tests were made by adding several millimoles of the substances examined to the silver nitrate–ethylenediamine solution immediately before addition of the borohydride sample and carrying out the analysis as described. The results are summarized in Table II.

The precision of these results is somewhat lower than those reported in Table I, presumably because of the necessity for rapid addition and mixing of the reagents. The results, however, clearly establish that the reaction of borohydride with the silver ion reagent is much faster than the reaction of the reducing agent with the aldehyde or ketones used, or with the other added materials investigated.

Table I. Determination of Borohydride

Dilution[a]	Borohydride Found, Meq./Ml. Iodate method	Silver method
None	1.574	1.576
	1.581	1.576
	1.581	1.592
	1.597	1.588
	1.605	1.590
	Av. 1.588 ± 0.011	1.584 ± 0.007
3:1	1.196	1.186
	1.204	1.195
	1.196	1.197
	1.194	1.187
	1.206	1.194
	Av. 1.199 ± 0.005	1.192 ± 0.004
1:1	0.806	0.792
	0.815	0.799
	0.817	0.800
	0.805	0.795
	0.803	0.801
	Av. 0.809 ± 0.005	0.798 ± 0.003

[a] A standard solution of sodium borohydride in 2*M* sodium hydroxide was diluted with 2*M* sodium hydroxide in ratio indicated.

Table II. Borohydride Determination in the Presence of Added Substances

Substance Added	Borohydride Found, Meq./Ml. Detn. 1	Detn. 2	Av.
None	1.611	1.593	1.60
2.5 mmole cyclohexanone	1.630	1.593	1.61
2.5 mmole benzaldehyde	1.859	1.717	1.79
2.5 mmole acetone	1.622	1.609	1.62
None	1.592	1.566	1.58
2.5 mmole ethyl alcohol	1.599	1.618	1.61
2.5 mmole benzaldehyde	1.755	1.748	1.75
2.5 mmole sodium formate	1.590	1.557	1.57
5.0 meq. potassium chlorate	1.622	1.633	1.63

Of the materials tested, only benzaldehyde interfered. In the absence of borohydride, the addition of freshly distilled benzaldehyde to the silver nitrate–ethylenediamine reagent in the presence of alkali results in the reduction of silver and the consequent loss of silver ion from solution. The high figures for borohydride in the presence of benzaldehyde (Table II) presumably are due to this side reaction.

The reaction was applied to an examination of the postulated rapid rate of reaction of borohydride with iodate. Two solutions were prepared which contained identical concentrations of sodium borohydride in the presence of 0.1*M* sodium hydroxide. One solution was made 0.1*N* with respect to potassium iodate. The solutions were maintained at room temperature. At appropriate times, aliquots were removed from each solution and analyzed for borohydride content (Table III). It is apparent that in 3 hours the decrease in borohydride concentration is approximately 1% and after 102 hours more than half of the original borohydride is still present.

Table III. Decrease of Borohydride Concentration with Time in Presence and Absence of Potassium Iodate[a]

Time, Hr.	Borohydride Found, Meq./Ml. Blank[b]	Iodate soln.[b]
0.3	2.58	2.63
3.5	2.57	2.61
8.0	2.51	2.44
24	2.48	2.23
102	2.36	1.59

[a] Room temperature.
[b] Solutions 0.1*N* in sodium hydroxide.

Reduction of iodate would presumably form iodide ion. This should remove silver ion from solution. Consequently, the analyses in Table III should be considered to represent only approximately the amount of borohydride remaining. It is probable that the amount left is somewhat less than that given by the analytical figures. The data nevertheless establish that iodate does not interfere with the proposed method of borohydride analysis and that the reaction of potassium iodate with sodium borohydride is very slow.

The analytical procedure could be considerably simplified if the excess silver ion could be titrated in the presence of the precipitated silver. However, the black color of the precipitate makes a colorimetric determination of the end point impractical. Thus an attempt to apply the titration method of Bloom and McNabb (*1*) failed for this reason. No attempt was made to apply conductometric or potentiometric methods to such a titration.

An ammoniacal silver nitrate solution provides a sensitive qualitative test for borohydride ion. The test is run by placing 1 drop of a 1*M* silver nitrate solution in concentrated aqueous ammonium into a depression of a white, glazed porcelain spot test plate and allowing a drop of the borohydride test solution to fall into the silver nitrate drop. A black precipitate forms immediately. The limit of detection by this method was determined to be $1 \times 10^{-4}M$ with respect to hydride ion, or $2.5 \times 10^{-5}M$ with

respect to borohydride ion. (The usual precautions with ammoniacal silver nitrate solutions should be observed.)

The iodate method has consistently yielded excellent results in this laboratory. This method has yielded low results in other laboratories. In view of the present study, it is probable that these low results arise from failure to acidify the borohydride-iodate solution promptly and carefully, thus resulting in hydrolytic loss of active hydrogen. Where acidification of the reaction solution can be tolerated, the iodate method appears the most satisfactory of those now available. The argentimetric procedure of the present paper provides a satisfactory alternative procedure for borohydride determination, which should be especially useful in cases where acidification is undesirable.

LITERATURE CITED

(1) Bloom, A., and McNabb, W. M., IND. ENG. CHEM., ANAL. ED., 8, 167 (1936).
(2) Caldwell, J. R., and Moyer, H. V., *Ibid.*, 7, 38 (1935).
(3) Chaikin, S. W., ANAL. CHEM., 25, 831 (1953).
(4) Chaikin, S. W., and Brown, W. G., *J. Am. Chem. Soc.*, 71, 122 (1949).
(5) Davis, W. D., Mason, L. S., and Stegeman, G., *Ibid.*, 71, 2775 (1949).
(6) Lyttle, D. A., Jensen, E. H., and Struck, W. A., ANAL. CHEM., 24, 1843 (1952).
(7) Mathews, M. B., *J. Biol. Chem.*, 176, 229 (1948).
(8) Pecsok, R. L., *J. Am. Chem. Soc.*, 75, 2862 (1953).
(9) Schlesinger, H. I., *et al.*, *Ibid.*, 75, 215 (1953).
(10) Volhard, J., *J. prakt. Chem.*, 117, 217 (1874).

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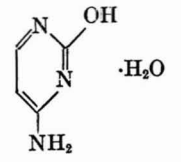
CRYSTALLOGRAPHIC DATA

90. Cytosine Hydrate (4-Amino-2-hydroxypyrimidine Hydrate)

Contributed by HARRY A. ROSE, Lilly Research Laboratories, Indianapolis, Ind.



Structural Formula for Cytosine Hydrate

CYTOSINE may be easily recrystallized from water, giving flat blades. The hydrate may lose water upon standing at room conditions for some time. The dehydration process can be speeded by heating the hydrate to 110° C. for 15 minutes.

The optical properties of the hydrate have been briefly described (*2*, *3*). X-ray powder diffraction data have also been given for the anhydrous crystals (*1*), but the fact that the data were for the anhydrous material was not made clear in the paper.

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Blades lying on {100} elongated parallel to *b*, showing the prism {110}, clinodome {011}, and basal pinacoid {001}.

Figure 1. Crystals of Cytosine Hydrate from Hot Water on Microscope Slide

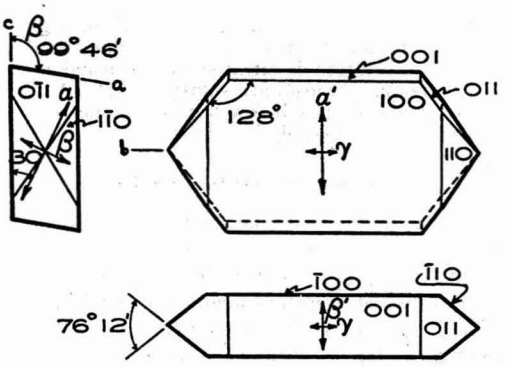


Figure 2. Orthographic Projection of Typical Crystal of Cytosine Hydrate

Cytosine Hydrate Powder Data

d	*I/I*1	*hkl*	*d*(Calcd. from *a*, *b*, and *c*)
7.70	1.00	100	7.70
6.05	0.33	110	6.06
5.06	0.20	11$\bar{1}$	5.07
4.92	0.20	020	4.91
4.12	0.33	120	4.14
3.85	0.07	200	3.85
3.78	0.53	002	3.78
3.53	0.66	121, 012	3.50, 3.53
3.19	1.00	102, 201	3.19, 3.19
3.03	0.07	220, 112, 211	3.03, 3.03, 3.03
3.01	0.07	130	3.01
2.939	0.07	22$\bar{1}$, 20$\bar{2}$	2.931, 2.939
2.865	0.07	13$\bar{1}$	2.865
2.828	0.13	21$\bar{2}$	2.816
2.740	0.07	131	2.737
2.566	0.07	300, 30$\bar{1}$	2.567, 2.567
2.451	0.27	040	2.455
2.435	0.20	13$\bar{2}$	2.436
2.237	0.13	123	2.245

Anhydrous Cytosine Powder Data

d	*I/I*1	*d*	*I/I*1
6.46	1.00	2.45	0.03
5.33	0.66	2.39	0.27
4.43	0.66	2.27	0.07
3.65	0.27	2.22	0.07
3.53	0.66	1.849	0.07
3.40	0.66	1.820	0.03
3.28	1.00	1.716	0.13
3.09	0.20	1.640	0.07
3.07	0.20		
2.97	0.13		
2.89	0.13		
2.85	0.53		
2.73	0.13		
2.70	0.07		
2.55	0.27		

Axial Ratio. 0.7951:1:0.7812 (x-ray).
Beta Angle. 99° 46′.
Interfacial Angles (polar). $110 \wedge 1\bar{1}0 = 76°12'$, $011 \wedge 0\bar{1}1 = 75° 10'$.

X-Ray Diffraction Data

Cell Dimensions. $a = 7.81$ A.; $b = 9.82$ A.; $c = 7.67$ A.
Formula Weights per Cell. 4 (3.99 calculated from x-ray data).
Formula Weight. 129.12.
Density. 1.476 grams per cc. (flotation), 1.481 grams per cc. (x-ray).

Optical Properties

Refractive Indices. $\alpha = 1.445$, $\beta = 1.747$, $\gamma = 1.782$ (*2*).
Optic Axial Angle. $2V = (-)36°$ (calcd.) (*3*); $2E = 65°$ (calcd.).
Dispersion. Not observed.
Optic Axial Plane. Perpendicular to 010.
Acute Bisectrix. α.
Extinction. $\alpha \wedge c = 30°$ (in obtuse β).
Molecular Refraction (R) (5893A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.685$. R(calcd.) = 33.3; R(obsd.) = 33.2.

Fusion Data. On heating, cytosine hydrate loses water in the range from 65° to 75° C. and melts with decomposition in the range 320° to 325° C.

X-Ray Powder Diffraction Data. All x-ray powder diffraction data were obtained using a camera 114.6 mm. in diameter and chromium radiation with vanadium pentoxide filter. A wave-length value of 2.2896 A. was used in the calculations.

LITERATURE CITED

(1) Clark, C. C., *Arch. Biochem. Biophys.*, **31**, 18–28 (1951).
(2) Hendricks, S. B., *J. Am. Chem. Soc.*, **57**, 552 (1935).
(3) Winchell, A. N., "Optical Properties of Organic Compounds," p. 239, Academic Press, New York, 1954.

Contributions of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

MEETING REPORTS

Society for Analytical Chemistry

An ordinary meeting of the Society for Analytical Chemistry was held October 6 in London, at which the following papers were presented.

Theoretical Basis of Sensitivity Tests and Their Application to Potential Organic Reagents for Metals. H. M. N. H. Irving and Mrs. H. S. Rossotti, Inorganic Chemistry Laboratory, South Parks Road, Oxford.

The concentration of a given metal—ligand complex in a solution containing fixed total amounts of a metal ion and a reagent is expressed in terms of the pH of the solution and of the stability constants of the metal—ligand and ligand—proton systems. Equations describing the absorbancy of such a solution and the solvent extraction and precipitation of a given complex are derived, and the factors governing precipitation in a solution containing two metal ions were discussed. If the stability constants and relevant physical properties of each complex were known, the value of a potential analytical reagent could be predicted. In practice, as insufficient data are available and the equations are often complicated by competitive complex formation, a potential reagent is best investigated more empirically.

The results of "sensitivity tests" on 8-hydroxycinnoline, 8-hydroxyquinazoline (and its 2,4-dimethyl derivative) and 5,8-dihydroxy-2,3-dimethylquinoxaline with 33 cations were reported and discussed.

The effect of substituents in the ligand on the stability of a complex, and hence on the sensitivity of an analytical reaction, was discussed. The influence of solvent on stability was considered, and it was shown that, in favorable circumstances, approximate stability constants in water can be calculated from values found in other solvents. Reference was also made to the influence of the central metal ion on the intrinsic solubility of the complex in water and in organic solvents.

Investigation of 5-Nitroso-oxine as an Analytical Reagent. H. M. N. H. Irving and R. G. W. Hollingshead, Inorganic Chemistry Laboratory, South Parks Road, Oxford.

The results of sensitivity tests suggested that 5-nitroso-oxine should be a more selective reagent than oxine, for it resembled 2-methyloxine (8-hydroxoquinaldine) in giving no precipitate with aluminum and failed also to give insoluble complexes with gallium, indium, or magnesium. Unfortunately, this reagent proved to be of no practical value, for it did not precipitate quantitatively any of the commoner metals—even copper and zinc. Reasons for this behavior were suggested from a study of its absorption spectrum, dissociation constants, and lack of reactivity toward bromine.

The 47th ordinary meeting of the Physical Methods Group was held in Oxford on October 22, jointly with the London Section of the Royal Institute of Chemistry. The following papers on radiochemistry were presented and discussed.

Assay Equipment for a Radiochemical Laboratory. J. E. Johnston, Isotope School, A. E. R. E., Harwell, Nr. Didcot, Berks.

The measurement of radioactive isotopes by ionization chambers, proportional counters, Geiger counters, and scintillation counters was briefly outlined. The features of each of these instruments of possible interest to the analytical chemist were described, such as sensitivity, stability, and specialized applications, and a list (including cost) was given of the electronic equipment required for each of the measuring methods. Three years' experience in the Isotope School with equipment supplied by the various manufacturers was described.

Determination of Gamma Isomer in Crude Benzene Hexachloride by a Carbon-14 Isotope Dilution Method. D. E. Palin, General Chemicals Division, I.C.I. Ltd., Widnes, Lancs.

An isotope dilution method for the determination of gamma isomer in crude benzene hexachloride has been developed for use as a check on other analytical procedures. A weighed amount, a, of radioactive pure gamma isomer of known specific activity, x, was mixed with a suitable weight, b, of the crude benzene hexachloride and a sample of pure gamma isomer of diluted activity was separated by a combination of solvent extraction and partition chromatography. The specific activity, y, of this sample was determined by beta-particle counts, made in a methane flow proportional counter, on thick specimens of barium carbonate prepared by standard techniques of microcombustion, absorption, precipitation, and filtration. The percentage of gamma isomer, g, in the original crude sample was then given by the expression:

$$g = \frac{100a(x - y)}{by}$$

Physical and Analytical Control of the Radioactive Effluent from A. E. R. E. Harwell. R. H. Burns, Industrial Chemical Group, A. E. R. E., Harwell, Nr. Didcot, Berks.

The paper gave details of the permissible levels of activity in the effluent from the Atomic Energy Research Establishment. These levels were based on the internationally accepted drinking water tolerances for radioactive isotopes. In order to ensure that the limits laid down were not exceeded, it was necessary to estimate the radium, other alpha emitters, calcium, and strontium and the other beta-active isotopes in the liquid wastes. Details of the methods developed for this analytical control were given.

The main source of activity in the effluent was derived from the isotopes formed during the fission of uranium. These fission products were numerous and very varied in chemical character. Brief details were given of the treatment processes used to decontaminate the effluent prior to discharge to the river Thames.

Physical control of the waste material was accomplished by the design of special containers, a separate active drainage system, and treatment in a plant erected for this purpose. A description was given of the controls exercised at each stage of the disposal system from the laboratories to the Thames.

A meeting of the Scottish Section of the Society for Analytical Chemistry was held October 27 in Glasgow.

Determination of Sodium Carboxymethylcellulose. K. Sporek and A. F. Williams, Research Department, Nobel Division, I.C.I., Ltd., Stevenston, Ayshire.

Sodium carboxymethylcellulose is a product derived from cellulose materials by treatment with alkali and monochloroacetic acid. In addition to sodium carboxymethylcellulose, the reaction mass contains sodium glycollate and inorganic salts, much of which may be removed by washing the product with aqueous alcohol. Depending upon the degree of washing and type of cellulose, different grades of sodium carboxymethylcellulose are obtained and these have many industrial applications.

A method of analysis based on precipitation of the free acid of sodium carboxymethylcellulose in water–alcohol solutions of mineral acid has been compared with methods based on precipitation of the insoluble uranyl and copper salts. The behavior of relatively pure and impure samples has been examined by a procedure involving dialysis.

Determination of sodium carboxymethyl by precipitation in acid solution, in material derived from wood pulp, gave values which were considerably lower than those obtained by dialysis whereas similar values were obtained for material prepared from pure α-cellulose. A colorimetric method was described for the determination of the glycollic acid which may be present in the manufactured product.

Properties of Ling (Heather) Honey. T. J. MITCHELL, Department of Technical Chemistry, Royal Technical College, Glasgow.

Ling honey from the nectar of *Calluna vulgaris* may contain relatively large amounts of colloidal matter, a considerable proportion of which is nitrogenous, consisting of proteins, amino acids, and melanoidins. Ling honey has been shown to possess properties of thixotropy and elastic recoil which prevent its extraction from the combs by centrifugal force in a honey extractor. This colloidal property of thixotropy is shown by setting or gelatinizing of the liquid when at rest, with reconversion to liquid on shaking. It has an important part in many biological processes. It is believed that these unusual properties are due to the presence of a protein which is readily precipitated by heat or by the addition of various reagents.

By the cooperation of leading beekeepers, samples were obtained from widely scattered districts in Scotland and Northumberland. These honeys were examined for water content, pH value (protein content), ash or mineral matter, colloid content, color, and taste.

Correlation was found between the pH and the ash content of the honeys. There was some evidence of relationship between the colloid content and the total nitrogen and thixotropy. The relation between these properties was not exact, probably because of the variation in floral source of the honeys.

An ordinary meeting organized by the Biological Methods Group was held November 3, in London. The following papers on the biological evaluation of the purity of water and effluents were presented.

Introduction. B. A. SOUTHGATE, Water Pollution Research, D. S. I. R., Watford, Herts.

The extent to which the quality of water is evaluated by biological methods depends on the use to which the water is put. A source of domestic supply is usually examined bacteriologically, but chemical or physical methods are used to evaluate most of its other important qualities, including the concentration of poisonous or physiologically undesirable substances, such as toxic metals or fluoride, and substances that cause corrosion or deposition of scale. The ability of a stream to support fish can be examined either by conducting tests with fish in the laboratory or by maintaining captive fish in special boxes in the river itself; usually such tests are supplemented by chemical examination of the river water. In a fishing stream the concentration of dissolved oxygen is very important; it is usually governed by the rate of oxidation of organic matter in the river by bacterial activity. Hence it is important to be able to determine the rate of oxidation, in respirometers or by other methods, of effluents discharged to the stream. The direct determination of the toxicity to fish of effluents—particularly industrial effluents—is also very desirable. In the examination of streams, which usually fluctuate rapidly in composition, it is a great advantage to use automatic sampling gear or, better, automatic devices to record continuously the concentration of constituents of particular importance.

Measurement of Toxicity to Fish. D. W. M. HERBERT, Water Pollution Research, D. S. I. R., Watford, Herts.

One method of attempting to determine whether an effluent, when discharged to a river, will injure fish, is to make laboratory tests of the toxicity of dilutions of the effluent. A toxicity test can conveniently last for only a few days, while waste waters are often discharged continuously; consequently, long-term effects must be predicted from short tests. The logarithm of period of survival is linearly related to the logarithm of the concentration of many poisons; with some this relation holds approximately from a few hours to 3 months. Use of this relation to extrapolate from tests of short duration might predict the mortality expected from prolonged exposure to high dilution, although precision would be low. Studies on a river and in the laboratory show that survival is affected by the interaction of many factors. Toxicity of some poisons is altered by changes of temperature, dissolved oxygen concentration, and pH value within the ranges tolerable to fish. Although such variables can be controlled in a laboratory test, they vary in streams, and allowance must be made for this in the interpretation of results. One poison in a river may alter the toxicity of others—for example, in a sewage effluent, carbon dioxide seems to reduce the toxicity of ammonia but to increase sensitivity to lack of oxygen.

Some Aspects of the Biology of Polluted Rivers. J. E. FORREST, Queen Mary College, Mile End Road, London E. 1.

To the biologist, effluents discharged into rivers fall roughly into three classes: heated effluents, which, at best, may consist solely of cooling water, effluents containing harmful inorganic chemicals, such as cyanides, and effluents containing organic matter. Combinations of these may occur, and frequently cooling water is discharged mixed with an effluent of another kind. The effects of heated and inorganic effluents were not considered in detail. The discharge of a significant amount of organic matter usually has a far-reaching effect on the plant and animal life of a river. Depending upon the degree of organic pollution, an abnormal and easily recognizable type of fauna is found, leading, in extreme examples, to a dead river. Below the point of discharge, a sequence of changes in the fauna occurs, and this is passed through in the reverse order farther downstream during the recovery of the river. Changes in the river depend to a great extent on its nature. There are indications that traces of certain metals in an effluent may have a damaging effect on water plants. The establishment and maintenance of a normal growth of weed in a river are of extreme importance. An excessive growth of *Cladophora* may cause widespread damage, and it usually thrives in an organically polluted river. Many present-day effluents contain traces of detergents, and little is known of the biological effects of these.

Determination of the Safety of Water. E. WINDLE TAYLOR, Metropolitan Water Board, New River Head, Rosebery Ave., London E. C. 1.

The chief object of the bacteriological analysis of water is to ensure that the water is free from pathogenic bacteria by the time it passes into supply to consumers. The criterion of purity from the bacteriological standpoint has been based, since the inception of this form of examination, on the isolation of normal intestinal microorganisms from water. If it can be shown that the water is free from bacteria normally present in human and animal intestines, it can be reasonably assumed that the water is free from any disease-producing bacteria.

For larger parasites, such as leptospira, protozoa, helminth ova, etc., it is necessary to examine a deposit under the microscope obtained from a large quantity of water by means of filtration or a centrifuge.

The examination of water for virus infection is a difficult procedure, but such infection is usually associated with other evidence of gross pollution which can be detected by the usual bacteriological methods.

At a joint meeting of the Scottish Section with the Glasgow and West of Scotland Section of the Royal Institute of Chemistry, held November 26 in Glasgow, a talk on "Sea Water" was given by H. Barnes, Scottish Marine Biological Association, Millport, Scotland.

Oceanography—pure and applied—is a complete science. Its study calls on many disciplines, among which that of the chemist is by no means the least important. Sea water is a complex medium containing dissolved gases, inorganic ions, and soluble, colloidal, and particulate organic matter in varying quantities. Some of these constituents are present in relatively constant proportions, while others, in particular those which take part in cyclical nutritive processes, vary considerably in time and place. A knowledge of the chemistry of these nutritive cycles is fundamental to our comprehension of the whole economy of the sea. The chemistry of the bottom sediments is a necessary background to our knowledge concerning past and present geological processes. Apart from being considered a source of vast quantities of food, the mineral wealth of the sea is of tremendous importance; in this field applied chemistry plays a dominant part.

The tenth annual general meeting of the Biological Methods Group was held in London, December 10. The following papers were presented and discussed.

Evaluation of Vegetable Purgatives. J. W. FAIRBAIRN, Department of Pharmacology, School of Pharmacy, 17 Bloomsbury Square, London, W.C. 1.

Various methods have been suggested for the evaluation of vegetable purgatives. For the past 5 years the author has used white mice as test animals for the assay of the anthraquinone group of purgatives, and he compared results with chemical methods and mentioned certain clinical trials that are being carried out.

Disk Plate Method of Assay with Neurospora Mutants for Aneurin, Pyridoxine, Choline, Inositol, and *p*-Aminobenzoic Acid.

E. C. BARTON-WRIGHT AND N. J. BUTLER, 33 Hyde Park Gardens Mews, London W. 8.

To overcome the tedious and cumbersome assay procedure usually followed with *Neurospora* mutants, a modified method has been adopted. In place of growing the organisms in a liquid medium, they are grown on a solid agar medium in Petri dishes.

To operate the method, the different levels of standard and test solutions are pipetted into Petri dishes and the total volume in each case is made up to 5 ml. with water. 15 ml. of melted basal medium are added to each plate, and the contents are well mixed and allowed to set.

The "inoculum" for the assay is also grown on solid medium containing the vitamin which is to be assayed. Disks, 8 mm. in diameter, are cut out with a sterile cork borer, and a single disk is transferred to the center of each plate. The plates are incubated at either 22° or 25° C. and the diameters of the zones of growth formed at the end of the incubation period are measured.

The results are plotted as log dose against diameter of growth. In a valid assay parallel straight lines are obtained for standard and test solutions. The medium used is a modification of the synthetic one usually employed for these assays. This modified method of assay has a number of advantages over the older method, not least of which is the fact that it is approximately ten times more sensitive.

Biological Estimation of Vitamin E. T. MOORE, Dunn Nutritional Laboratory, University of Cambridge, Cambridge.

The classical method of testing for vitamin E depends on its ability to prevent resorption of the fetuses in rats which have been made pregnant after prolonged restriction to a diet deficient in the vitamin. This method is very laborious, requires large numbers of animals, and usually takes 5 to 6 months to complete. Alternative methods might be based on the long established effects of the vitamin in preventing brown discoloration of the uterus, depigmentation of the incisor teeth, or degeneration of the testes. Such methods might be less laborious than the classical procedure, but would allow no saving of time. Recently hopes of much more rapid assays have been raised by Gyorgy's observation that the red blood corpuscles of rats deficient in vitamin E are hemolyzed in vitro on treatment with dialuric acid, a substance related to alloxan. Sensitivity of the corpuscles to dialuric acid may be produced by restricting rats to a deficient diet for only a few days, and may be rectified within a few hours by the administration of a single adequate dose of vitamin E. Only a drop of blood, obtainable from the tip of the tail, is required for each examination. Cures are temporary, and the same rat may therefore be used many times. Repeated small doses of vitamin E tend to be less effective than a single large dose, which makes the method more suitable for assaying strong sources of the vitamin than weak sources. Promising results have been obtained, however, with cereal products. Methylene blue, and certain other redox dyes, present a problem to the bioanalyst in being able to prevent some of the injuries in vitamin E deficiency but not others.

Conference on Molecular Spectroscopy

THE Conference on Molecular Spectroscopy was held by the Hydrocarbon Research Group, Spectroscopic Panel, Institute of Petroleum, in London on October 28 and 29. The papers presented included the following:

Use of Fluorescence for Industrial Analysis and Examination. E. J. BOWEN, Physical Chemistry Laboratory, Oxford University, Oxford, England.

The value and limitations of fluorescence observations for detecting and estimating substances were examined. General principles of measurement with special reference to fluorescence spectrometry were given and attention was drawn to matters leading to error. The proper way of plotting fluorescence spectra was also discussed.

Spectroscopic Studies of the Phosphorescent States of Aromatic Hydrocarbons. GEORGE PORTER AND MAURICE W. WINDSOR, Department of Physical Chemistry, Cambridge University, Cambridge, England.

Many aromatic molecules on illumination pass to an excited metastable form which is identified with the phosphorescent state. By means of the technique of flash photolysis and spectroscopy, the absorption spectra of these labile molecules in degassed fluid solvents have been recorded for a large number of polycyclic aromatic hydrocarbons and also for many benzene derivatives. Lifetimes investigated lie between 10^{-5} and 10^{-2} second, and are longer the more viscous the solvent medium. Decay of the phosphorescent molecule occurs, in solution, by a predominantly nonradiative process. The triplet state and other theories of the phosphorescent state were discussed, none being found to give a completely satisfactory account of the observed phenomena.

Raman Spectrometry. Instrumentation and Application. B. F. DUDENBOSTEL, JR., Standard Oil Development Co., Linden, N. J.

The development of photoelectric detection and high speed pen recording of Raman spectra has tremendously extended the applications of the Raman effect to the analysis of liquid samples. The widest application of this technique has been in the petroleum field. Since photoelectric recording Raman spectrometers have not been commercially available until recently, these instruments have been restricted to laboratories which have constructed their own. Consequently, much more rapid advance in analytical application has been achieved in the fields of infrared and mass spectrometry, where excellent commercial instruments have been available for many years.

Determination of Aromatic Hydrocarbons in Lubricating Oil Fractions by Far Ultraviolet Absorption Spectroscopy. R. A. BURDETT, L. W. TAYLOR, AND L. C. JONES, JR., Wood River Research Laboratory, Shell Oil Co., Wood River, Ill.

The most intense absorption band system of the benzenes occurs in the region just beyond the lower wave-length limit of conventional photoelectric spectrometers. The intensity of this absorption band is independent of the number and location of alkyl substituents. This band system has been used for the determination of homologs of benzene in lubricating oil fractions and found to give results in good agreement with chromatography. Near ultraviolet absorption bands have been used for the estimation of the naphthalenes and phenanthrenes in the same oils.

Infrared Instrumentation, Present and Future. VAN ZANDT WILLIAMS, The Perkin-Elmer Corp., Norwalk, Conn.

Specific infrared instrumentation is available today for laboratory, pilot plant, and process control applications. The laboratory instrument meets present problem requirements as set by a centralized group servicing its own research facilities for "unknown" identification, molecular structure analysis, or quantitative multicomponent analysis. Future requirements include a simple, inexpensive infrared spectrophotometer which the individual chemist can afford on a "standby" basis, an attachment for automatic measurement of integrated absorption coefficients, and a basic study of quantitative analysis to permit transfer of usable data from one site to another. In general, the present status of pilot plant and process control infrared equipment is well ahead of the ability of the industry to make use of such instruments.

Preparation and Use of Additively Colored Alkali Halide Crystals as Infrared Transmission Filters. W. G. BURNS, Hilger and Watts, Ltd., London, AND J. GAUNT, Chemistry Division, Atomic Energy Research Establishment, Harwell, England.

Crystals containing high concentrations of *F*-centers, made by heating alkali halides with potassium metal and cooling them quickly, have already been shown to have suitable characteristics for use as near infrared transmission filters. The effect of controlling the cooling of such crystals has been studied, and it has been shown possible to vary the infrared transmission from a sharp cut-in at about 1 to 2 microns to a slow increase in transmission starting at 10 to 15 microns, caused by very slow cooling. The extension of the visible absorption into the infrared is accompanied by a decrease in the sharpness of the transmission edge. The outer layer of the crystal has also been shown to have a great effect in extending the infrared absorption. Work on very thin sections has shown that in the center of the crystal the effect of slow cooling is to cause the formation of *R*, *M*, and *N* centers, but at the edge the colloidal band predominates. The use of such additively colored alkali halide crystals as infrared transmission filters was demonstrated and discussed.

Further Infrared Measurements with a Cesium Iodide Prism. E. K. PLYLER, National Bureau of Standards, Washington, D. C.

Infrared measurements with a cesium iodide prism have been made to 54 microns. The single-pass and the Walsh double-pass systems were compared and their advantages noted. The transmittances of crystals of CsBr, TiBr-I, and CsI, and of films of polystyrene and polyethylene have been measured. In addition, the absorption spectra of three halomethanes and seven halobenzenes have been observed. Prism spectrometry from 24 to 54 microns with a CsI prism is handled by the same methods as those used with a CsBr prism.

Pressed Disk Technique in Spectroscopy. M. A. FORD, G. R. WILKINSON, AND W. C. PRICE, King's College, London University, London, England.

Equipment for the production of transparent disks from alkali halide powders was described. The technique of using these disks for obtaining the spectra of solid materials finely dispersed within them was discussed. The nature of the process has been investigated with special reference to the production of good quality disks.

Intensities of Vibrational Absorption Bands. H. W. THOMPSON, St. John's College, Oxford University, Oxford, England.

The significance of vibrational band intensities was explained, and methods were outlined for determining the true intensities from the "apparent" integrated band areas. The relative merits of molecular extinction coefficients at peak maxima, or of integrated band areas, were considered. Applications of measurements on band intensities were surveyed with particular reference to analysis and structural diagnosis, and typical values were collected for some key groups. The derivation of bond polar properties from band intensities was outlined and results of recent work were reviewed critically. Interesting conclusions have been drawn with regard to the properties of C—H bonds. The limitations of this method were indicated and the importance of studying the electrical anharmonicity of bonds was emphasized.

Characteristic Vibration Frequencies of Substituted Benzenes. R. R. RANDLE AND D. H. WHIFFEN, Department of Chemistry, Birmingham University, Birmingham, England.

Tables of the vibration frequencies, which appear to be essentially independent of substituent but depend only on the substituent positions, were given for all types of substituted benzenes. The frequencies were assigned as far as possible to the type of mode involved, C—H deformation, C—C stretch, etc.

Infrared Spectra of Some Monodeutero Aromatics and Their Analytical Application. E. D. KUNST, Koninklijke/Shell-Laboratorium, Amsterdam, Holland.

The infrared spectra between 3 and 15 microns of several monodeutero aromatics were studied in order to develop a method for the determination of these compounds in the presence of their parent compounds. It appeared that some of the empirical rules correlating the spectrum with the type of substitution around the aromatic nucleus in alkyl aromatics remain approximately valid if one of the alkyl groups is replaced by a deuterium atom. The bands to which these rules apply and which lie in the region between 11.5 and 15.0 microns are fairly intense and are very useful in quantitative analysis.

Low Temperature Infrared Spectroscopy. N. SHEPPARD, Department of Colloid Science, Cambridge University, Cambridge, England.

An account was given of the applications of low temperature techniques to the study of infrared spectra, with examples taken from recent work. Changes occurring in such spectra on passing from the gaseous to the liquid, or the liquid to the solid states, and on lowering the temperature in any one of these states, were described for rigid molecules. Additional effects which occur on change of state for flexible molecules exhibiting rotational isomerism were discussed. The applications of low temperature techniques to the spectroscopic study of molecular complexes and problems of qualitative and quantitative analysis were outlined.

Chemical Applications of Nuclear Magnetic Resonance Spectroscopy. R. E. RICHARDS, Lincoln College, Oxford University, Oxford, England.

Many atomic nuclei behave as though they possess a nuclear spin, and when placed in a magnetic field a number of energy levels become available to them. Nuclear resonance spectra occur when transitions among these energy levels are induced by radio-frequency radiation. Interactions of different kinds occur between neighboring nuclei which broaden or split the energy levels, and from a study of these effects much information of interest to the chemist can be derived. The spectra yield information on molecular structure and internuclear distances in crystals, on the nature of chemical bonds and electron distribution in them, and on the potential barriers which hinder molecular motion in solids. The spectra can be used for the analysis of samples for different elements, for specific groupings in molecules, and for the analysis of mixtures. Rates and equilibria in chemical reactions can be studied and estimates of the rates of very rapid chemical exchange processes can be obtained. Many other important chemical properties can be studied, and the possible applications have not been by any means fully explored. Only very small amounts (1 to 0.001 cc.) of material are used, which can be recovered unchanged after measurement.

An attempt was made to present a simple explanation of the processes which occur, with particular emphasis on the chemical applications. Examples were given and the applications were summarized. A brief description of the experimental method indicated the sort of equipment used.

Spectroscopic Studies on Reactions at High Temperatures. D. F. HORNIG, Metcalf Chemical Laboratories, Brown University, Providence, R. I.

Strong shock waves may be used to heat substances several thousand degrees in times short compared to those required for chemical reactions. Such shock waves are often intensely luminous. Emission spectra from BrCH and ClCN show lines from CN and C_2; spectra of CH_3Br and CH_3I show a continuum, CH and C_2. Absorption spectra have also been obtained. Methods of following spectral changes through intervals of a few microseconds were described and results presented.

The Emission Spectra of Molecules and Radicals in the Infrared. G. R. WILKINSON, M. A. FORD, AND W. C. PRICE, King's College, London University, London, England.

The infrared emission from gases excited by an R. F. discharge has been studied in the region 1 to 5.5 microns. The conditions for maximum emission were discussed. Preliminary spectra were presented for several molecules, the essential features being the occurrence of high vibrational and rotational transitions, which should lead to more accurate values of the rotational constants. It was shown that the emission from HCl and CO forms a convenient method of calibration of high resolution spectrometers. The favorable frequency factor occurring in the transition probability for emission enables overtone bands to be observed with a far smaller quantity of gas than that required in absorption.

Infrared Absorption Bands of Hydrocarbons under High Resolving Power. H. W. THOMPSON, St. John's College, Oxford University, Oxford, England.

The objects of an analysis of vibrational-rotational absorption bands of polyatomic molecules, measured under high resolving power, were outlined, and the method was compared with others leading to similar information. Some results obtained recently for simple hydrocarbons were considered in relation to each other.

Interpretation and Use of Infrared Reflection Spectra. T. S. ROBINSON AND W. C. PRICE, King's College, London University, London, England.

A description was given of the technique for obtaining infrared reflection spectra from small samples of material, such as small crystals. The theory of the process was developed and it was shown how the absorption spectrum and the dispersion can be calculated from the reflection data. The method can be applied where the absorption is so strong that direct measurements of extinction coefficient are difficult owing to the small thickness involved. The use of polarized radiation gives information concerning the direction of certain bonds with respect to the crystal axes. Applications to polythene, polytetrafluoroethylene, urea, and glycine served to illustrate certain features of the method.

Diffraction Gratings and Their Use in Infrared Spectroscopy. L. A. SAYCE AND A. JACKSON, National Physical Laboratory, Teddington, Middlesex.

The high cost of equipment has limited the use of infrared spectroscopy in identification and estimation of molecular species and in process control. Diffraction gratings can be made at low cost and an indefinite number of identical gratings can be generated from one master helix. Thus, if suitably inexpensive detecting systems can be made available, routine molecular spectroscopy ceases to be a luxury and the way is open for infrared monochromators to be used for the general control of chemical processes.

Spectroscopic Studies on Oxidation of Hydrocarbon Mineral Oils. H. LUTHER.

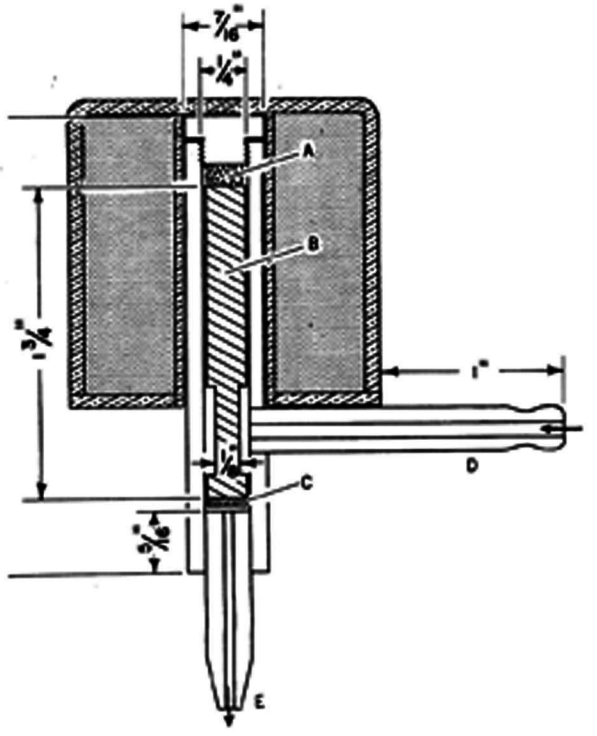
This complex subject has received a great deal of study by others, but an examination of the literature shows that results and conclusions vary according to conditions. The author's study of the reaction mechanism involves the continuous analysis of the circulating gases containing carbon monoxide and carbon dioxide after the reaction is started—first through a magnetic-oxygen analyzer and then into a carbon monoxide–infrared gas analyzer. An apparatus for carrying out the oxidation studies is described which permits the following: operation as a closed system, control of temperature and pressure, continuous measurement of oxygen consumption, proper mixing of inserted material with oxygen to permit study of reaction in the liquid or gas phase, control of gaseous reaction products and their separation from the oxygen, provision for separation of gaseous products in a cold trap at −180° C., means of estimation of amount of material consumed, and finally the means for continuous or discontinuous characterization of the liquid reaction products.

Chemical, chromatographic, and infrared methods were used in identification. While the author adds little to clear up the complexities of this subject, it is stated that under the conditions used, in contradiction to published data, no peroxides were found as intermediate products.

Electromagnetic Laboratory Valve

B. P. McKay and Charles H. Eades, Jr., Department of Biochemistry, University of Tennessee, Memphis, Tenn.

The control of fluid flow is an important factor in many laboratory operations. An electromagnetic valve has been developed in these laboratories in connection with an automatic recording titrator [Eades, C. H., Jr., McKay, B. P., Romans, W. E., and Ruffin, G. P., ANAL. CHEM., 26, 123 (1954)] for the control of alkali flow to the titrating mechanism. The valve is automatically opened and closed by remote control.



The valve chamber consists of a polystyrene barrel fitted into the plunger well of a Guardian Type No. 4-AC 115-volt alternating current solenoid. A polystyrene side-arm inlet tube, 1/4 inch in outside diameter, 3/32 inch in inside diameter, is cemented to the plunger barrel. The outlet tube, *E*, 1/4 inch in outside diameter has a bore of 3/64 inch. The valve seat of the outlet tube is smoothly turned on a lathe to ensure a flat seat.

The plunger of the valve, *B*, consists of a chrome-plated steel rod with a reduced diameter near the valve seat end. This permits inlet head-pressure to give increased speed and positive closure to the valve. The part of the plunger that backs pad *C* is also reduced slightly in size to permit proper flow of fluid to outlet *E*.

A small piece of foam rubber, *a*, acts as a closing spring. Fast positive closure of the valve is achieved by the combined action of this foam rubber, the weight of the steel plunger, and inlet head-pressure acting against the back of the valve seat. A small piece of smooth-surfaced (chemical-resistant) rubber, *C*, serves as a valve seat. For easy cleaning and decontamination, the top of the plunger barrel is fitted with a threaded cap and neoprene gasket.

The valve has fast, positive action with 40 volts alternating current supplied to the solenoid coil. This is obtained with a 175-ohm 20-watt resistance in series with the coil and 115 volts alternating current applied to the circuit. The valve may be used in continuous duty applications with no significant rise in temperature. For intermittent duty cycles where the on period does not exceed 5 minutes and the off period is at least 10 minutes, 115 volts alternating current may be used on the coil.

In operation the valve is actuated by a line-operated pH-sensitive controller. When the pH of a sample of acid in contact with a glass–calomel electrode system is below the preset end point, the valve is opened and alkali flows. When the end point is reached, the circuit is inactivated and the valve closes, stopping the fluid flow. The amount of liquid that passes through the valve is measured by timing the calibrated rate of flow under a constant pressure head. If this valve is used in conjunction with a buret, the volume delivered may be read directly.

The possible applications of this valve are many. It is easily constructed, greaseless, easily cleaned, and fast acting, and can be adapted to automatic or remote control.

Simple Apparatus for Comparing Emulsions and Suspensions

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A NUMBER of methods of evaluating emulsion stability of emulsible concentrates have appeared in recent literature. Griffin and Behrens (*1*) described a device by means of which a large number of emulsions may be compared under uniform lighting conditions. Kelly (*2*) described a method of evaluation of the emulsion stability of herbicide formulations. Selz (*3*) listed and described a number of the methods in current use. Suggitt (*4*) stated (with regard to emulsion characteristics of emulsifiable concentrates): "For routine evaluation the simpler procedure of comparing the results obtained against a standard formulation is more rapid and meaningful than any absolute method." The apparatus here described was designed with the objects in view of preparing several emulsions (or suspensions) simultaneously for direct comparison and comparing the emulsions or suspensions in the same apparatus in which they were prepared. This, in the author's experience, has resulted in a definite saving in time and manipulation; in addition, the test method is truly comparative.

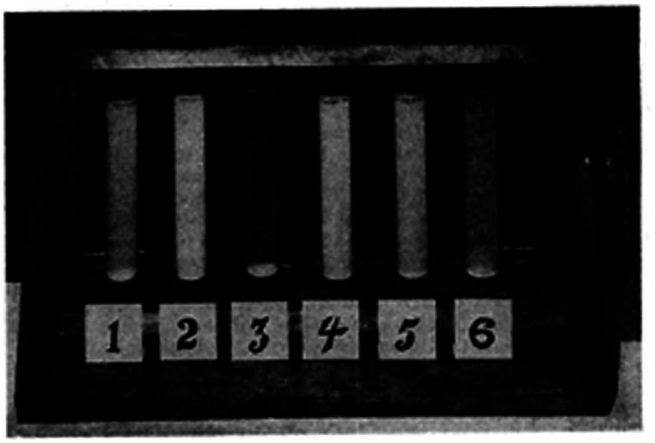

Figure 1. Typical Comparison Using Apparatus Described

All emulsions contained 1% by volume of one emulsifiable concentrate. Concentrate contained (by weight) 25% technical grade DDT, 71% Esso heavy aromatic naphtha, and 4% Emcol H-77 emulsifier. Emulsions were allowed to stand 24 hours for maximum photographic effect; however, differences between emulsions were readily apparent by inspection as little as 5 minutes after agitation.

APPARATUS

As shown in Figure 1, the emulsion comparator is simply a wooden frame supported on a bearing at each end, in which up to six glass cylinders may be fastened. The emulsions are prepared in the glass cylinders. The cylinders used were those described under Fisher Scientific Co. Catalog No. 8-535. These are plain, ungraduated glass cylinders, approximately 10 inches in height, with flared base and approximately 1.5 inches in internal diameter. They were calibrated by marking each cylinder at an arbitrary height of 20 cm. from the bottom of the inside of the cylinder, and then measuring the amount of water which the cylinder will hold up to the mark. This value, in milliliters, is

marked on the cylinder along side the 20-cm. mark. In use, it is desirable that all the cylinders in any one test have substantially the same dimensions—e.g., hold the same volume up to the 20-cm. mark. When calibrated, the cylinders generally held from 185 to 195 ml.; the greatest number held 185 ml. A pulley wheel is attached to the end of one of the axles, and also a handle for manual turning. Manual turning has been found to be quite sufficient for all ordinary comparisons; however in some cases it might be desirable to use a constant-speed motor attached to the pulley wheel.

TEST METHODS

Concentrated Emulsions, containing 10% or more of oil phase.

EMULSIFIABLE CONCENTRATES HEAVIER THAN WATER. The emulsifiable concentrate is measured directly into the bottom of the glass cylinders, then the water used is added slowly down the side of each cylinder so as to leave the concentrate as a discrete layer beneath. A small amount of emulsion almost invariably forms at the interface, but the two layers must be clear. Keeping the frame slightly tilted aids in this operation. Afterward, the tubes are stoppered (No. 7 rubber stoppers or corks may be used) and the frame is closed and tightened. Rotation of the frame 10 to 20 times then forms all emulsions simultaneously, except for the traces of emulsion which formed during the previous operation. Comparisons are made at convenient time intervals thereafter—for example, 1, 5, and 10 minutes, 1 hour, and 24 hours. If it is desired to take numerical readings of creaming or sedimentation rates, a thin strip of millimeter paper may be cemented to the side of each cylinder and readings taken from this.

EMULSIFIABLE CONCENTRATES LIGHTER THAN WATER. The procedure is exactly as described above, except that the water is measured into the tubes first, then the emulsifiable concentrate(s) are measured carefully onto the surface of the water in each tube.

DENSITY OF EMULSIFIABLE CONCENTRATE IS EXACTLY 1.000. It is desirable to run a preliminary test at room temperature to see whether sediment rises or falls. Either may occur through slight composition changes in the two phases. Thereafter by raising or lowering the temperature at which the test is run, it should be possible to carry out the test as described for the two types above.

Dilute Emulsions, containing 2% or less of oil phase. The emulsifiable concentrate(s) are measured into 5-ml. Griffin beakers, which in turn are floated on the surface of the water used in the respective cylinders. The cylinders are stoppered, the rack is closed and tightened, and the frame turned through 10 to 20 rotations, as before. If the volume of the concentrate is less than about 1 ml., it is necessary to weight the bottom of each beaker to prevent tipping. This may be done by cementing a small disk or metal washer to the outside.

In use, the beaker almost invariably comes to rest in an upright position on the bottom of the emulsion tube. In the rare instance when one does not, it can nearly always be righted by allowing all the beakers to fall through one more complete revolution. Turning the rack to an angle about 30° with the vertical, then allowing it to come to rest in vertical position, causes all the beakers to move to one side, after which sedimentation rates are observed from the other side. Since the vertical edge of each beaker occupies a relatively small, nearly constant area, it does not interfere seriously with reading of sedimentation rates, even though some of the sediment falls inside the beaker.

Wettable Spray Powders, suspension and foaming. The powders are weighed directly onto the surface of the water. It is desirable to weigh the powders first, then drop them all into the cylinders containing water, at as nearly the same time as possible. The cylinders are stoppered and frame is closed, tightened, and rotated 20 to 30 times (more if necessary to break up agglomerates). If foaming is to be compared, it is desirable to fill the tubes only about half full and use vigorous agitation (rotate the frame quickly for about 30 seconds).

Re-emulsification and Resuspension. These qualities may be determined after a suitable time interval by rotating the frame again and noting the number of complete revolutions necessary to resuspend or re-emulsify the sediment, in each case.

DISCUSSION

Where dilute emulsions containing 1% or less of oil phase are evaluated, it has been the experience of the author that variable and erratic results are obtained when there is a delay between the introduction of the emulsifiable concentrate into the water and agitation of the mixture. While believed to be due to extraction of part of the emulsifying agent by the water before agitation, this effect has not been investigated beyond establishing that it exists. A typical example is shown in Figure 1; the emulsifiable concentrate in tube 1 was added slowly down the side of the tube into the water 3 minutes before agitation, while the same concentrate in tube 2 was added to the floating beaker; thus, introduction of concentrate into the water was simultaneous with agitation. Otherwise the two emulsions received identical treatment (experimental details appear in Table I). This does not appear to be a problem with emulsions containing 10% or more of oil phase, when evaluated as described above, and may not be a problem with all emulsifier-solvent systems. The range between 1% oil phase and 10% oil phase is doubtful.

Table I. Details of a Typical Comparison[a]

Tube No.	Water Used	Hardness (as $CaCO_3$), P.P.M.	Method of Adding Concentrate
1	Tap	40 (approx.)	Added down side of tube 3 minutes before agitation
2	Tap	40 (approx.)	Added to floating beaker
3	Distilled	0	Added to floating beaker
4	Artificial hard[b]	250	Added to floating beaker
5	Artificial hard[b]	500	Added to floating beaker
6	Artificial hard[b]	1000	Added to floating beaker

[a] Results shown in Figure 1.
[b] Equal moles of calcium chloride and magnesium chloride.

LITERATURE CITED

(1) Griffin, W. C., and Behrens, R. W., ANAL. CHEM., 24, 1076–8 (1952).
(2) Kelly, J. A., *J. Agr. Food Chem.*, 1, 254–7 (1953).
(3) Selz, E., *Ibid.*, 1, 381–6 (1953).
(4) Suggitt, J. W., "Procedures for Evaluating Herbicides," presented before Agricultural Pesticide Technical Society, Macdonald College, Quebec, Canada, June 23, 1954.

Trap for Attenuating Mercury Vapors in the Mass Spectrometer

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THE presence of mercury parent peaks at m/e 198 to 204 and of their half-peaks at m/e 99 to 102 has caused difficulty in the interpretation of some mass spectra.

Mercury in the ionization chamber of the Consolidated mass spectrometer Model 21-103 arises from three different sources: the manometer in the gas inlet system, the diffusion pumps in the exhaust vacuum unit, and the mercury orifice (liquid inlet). Mercury vapors from the manometer of the gas-inlet system are

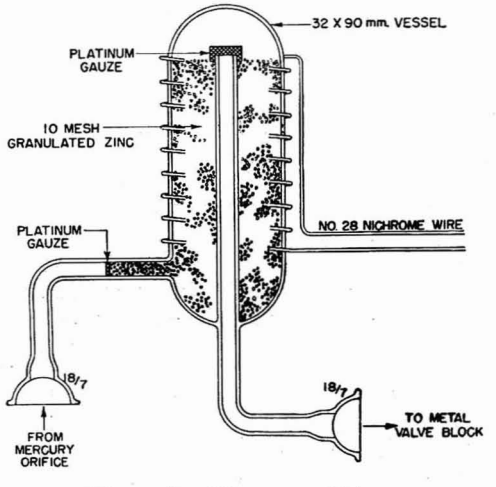


Figure 1. Diagram of Trap

of little importance because this portion of the spectrometer is usually closed to the metal valve block; a trap of solid carbon dioxide and another of oxygen (or nitrogen) prevents mercury vapors in the diffusion pumps from entering the ionization chamber to any great extent. Mercury vapors most often arise from the sample inlet region. The entire inlet system becomes saturated with mercury; passage of the vapors into the ionization chamber is, therefore, unavoidable, particularly with the introduction of higher boiling liquids.

Many of the metals readily form amalgams with mercury; however, the material selected to attenuate the vapors must be sufficiently inert to organic vapors. Although gold foil is commonly used to check mercury in vacuum systems, the expense of the metal makes the use of such a trap less attractive, especially if the trap must be changed periodically. While zinc is known to be active toward the more polar materials, it was selected in this investigation because of ready availability. In order to test the efficiency of zinc as an attenuator, mercury vapors at a pressure of a few microns were pulled through a trap filled with 10-mesh (Baker's analyzed) zinc, followed by a liquid oxygen trap. There was no evidence of mercury in the cold trap even after 48 hours.

The installed trap is illustrated in Figure 1. The trap is between the mercury orifice and the metal valve block. The gas inlet line has not been modified, and therefore, mercury may still enter the chamber from this region. To facilitate removal of the trap for cleaning and refilling, ball joints were used. Platinum gauze was placed over the two openings, thus preventing migration of zinc to other parts of the spectrometer. Because of the large increase in surface area of the inlet system, considerable background effects from absorbed materials will be noticed. This can be avoided by heating the trap with resistance wire. The temperature should be held below 100° C. to prevent damage to the wax joints. Numerous runs at 50° C. using air, water, or methanol as flushing agent indicated that background effects were not increased, if the pump-out time was at least 5 minutes.

The height of the mercury half-peak (m/e 101) is shown in Table I for different compounds expanded in the inlet system before and after installation of the trap.

Table I. Effect of Zinc Trap

Compound Examined	Height of m/e 101 Arbitrary Units	
	Before installation	After installation
Methanol	4.0	2.5[a]
Ethyl alcohol	2.1	2.1[a]
2,4-Hexadienal	4.6	0.2
	15.1	0.0
Acetic acid	6.3	0.0
	5.0	0.0
Isopropyl alcohol	4.1	0.0
Dodecene	18.3	0.1
	76.1	0.3
Methanol	0.3	0.1
Ethyl alcohol	1.1	0.0

[a] Examined shortly after installing trap.

Samples admitted through the gas inlet are obviously not affected by the trap, whereas those admitted through the mercury orifice show a marked decrease in peak height at m/e 101. These data were taken several months after installation of the trap; mercury peaks were detectable at normal ionizing current (10 μa.) for about 2 weeks after installation because of adsorbed mercury in the metal valve block; after this period, however, the device functioned satisfactorily for several months without service.

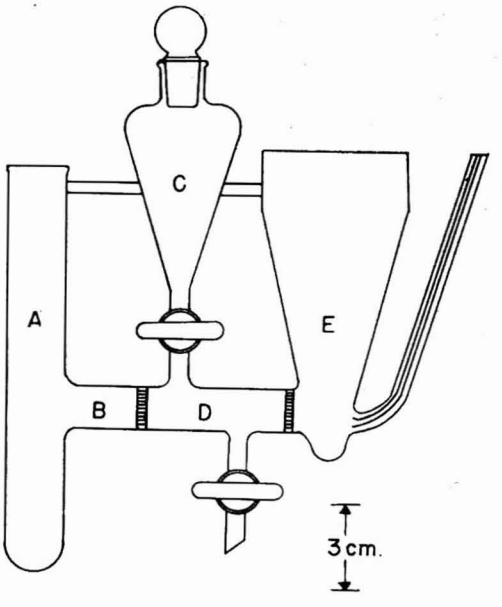
In order to determine the effect of zinc on organic samples, the following compounds were examined mass spectrometrically before and after fabrication of the trap: ethylene dichloride, methanol, n-butyl alcohol, 2,4-hexadienal, acetic acid, water, propylenediamine, acrolein, ethyl acetate, and benzene. Mixtures containing ethylene chlorohydrin, ethylene dichloride, and ethylene oxide—20 hydrocarbons, saturated, unsaturated, and aromatic—acetic anhydride, and acetic acid, were also examined. Comparison of the spectra obtained with and without the trap revealed no apparent differences in peak ratios or intensities. This indicates that there is no discrimination of the more polar molecules while passing through the trap. Sensitivity values were not affected in any way; indeed, there should be no change in base peak height per micron because the trap does not alter the calibrated volume. The micromanometer measures the pressure within the 3-liter preleak bulb, and the trap is in the line prior to the metal valve block. The authors do not use calibrated volumes for sensitivity measurements, but instead use a micromanometer for measuring the pressure in the expansion volume. If calibrated dippers are used, there will obviously be a volume change, and, consequently, a pressure change; therefore the volume of the system must be determined after installation of the trap.

The zinc in the trap was changed after 6 months (1500 complex nonroutine samples) of continuous use. Mercury peaks are obtained only after prolonged pumping of the orifice system and operating the instrument with five times the normal sensitivity (50 μa.).

Versatile Polarographic Cell

Robert L. Pecsok and Richard S. Juvet, Jr., University of California, Los Angeles 24, Calif.

A POLAROGRAPHIC "dilution" cell of conventional design (Kolthoff, I. M., and Lingane, J. J., "Polarography," Vol. I, p. 364, New York, Interscience Publishers, 1952) fails when strongly acidic or basic test solutions are to be analyzed. In these cases, the test solution slowly dissolves the agar plug, resulting in contamination of both the test solution and the reference electrode. Carritt (Carritt, D. E., Ph.D. thesis, Harvard University, 1947) recommended a modified H-cell which prevented the diffusion of chloride ion from the reference electrode into the test solution. However, his cell was somewhat fragile, and did not prevent the eventual contamination of the reference electrode.



A cell of rugged construction has been designed to eliminate these problems. Volumes of solution from 2 to 55 ml. may be analyzed, and the opening is large enough so that a glass electrode may be permanently mounted in the stopper, if desirable.

Compartment A contains the saturated calomel electrode. B is an agar plug saturated with potassium chloride. A sintered-

glass disk, coarse porosity, separates B from D. Compartment C contains saturated potassium chloride solution which is used to flush D from time to time. Compartment E, which contains the test solution, is separated from D by a Corning fine-porosity sintered-glass disk.

Resistance. The cell resistance was measured with an Industrial Instruments Model RC-16 conductivity bridge with a decade capacitor in parallel using 1000 cycles per second. The resistance was ca. 195 ohms when compartment E contained $0.1M$ potassium chloride and a platinum foil electrode of 1 sq. cm. A second measurement, made through a dropping mercury electrode at the instant of the drop fall, gave a value of ca. 265 ohms. The first value was checked by a less sensitive oscillographic method with agreement of ca. 5%.

Diffusion Experiment. Fifty milliliters of distilled water was allowed to stand in E for 21 hours with a slow stream of nitrogen passing through. At the end of this period, the chloride content of the water had increased to only $0.0064M$. Therefore, over normal periods of time, the diffusion of ions from D to E, or vice versa, is negligible for most purposes. Moreover, C and D may be filled with an indifferent electrolyte in special cases where even traces of chloride ion must be excluded from the test solution.

Electric Heater for Van Slyke-Folch Carbon Combustion Apparatus

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IN THE original Van Slyke–Folch manometric carbon combustion [Van Slyke, D. D., and Folch, J., *J. Biol. Chem.*, 136, 509–41 (1940)] a micro gas burner is used as a source of heat. Air drafts sometimes make the control of the heat difficult. An easily constructed electric heater, which provides more readily regulated heat and a temperature range adequate for the various steps of the process, simplifies the technique of the combustion.

Figure 1 shows how the heater unit and power control appear when assembled on the Van Slyke machine for a carbon combustion. The control illustrated is a variable auto-transformer (Variac Type No. 200-B, General Radio Co., Cambridge 39, Mass.) of power rating suitable for the heater used. This control is mounted in a standard 4 × 5 × 6 inch aluminum box which has been reworked to receive it. At places on the inside of the container where electrical contacts might possibly touch, pieces of insulating plastic were cemented to the walls. The cost of the two basic items, the small heater and a control (rated at 1 ampere and 115 volts) is from $10 to $17. For direct current operation, a

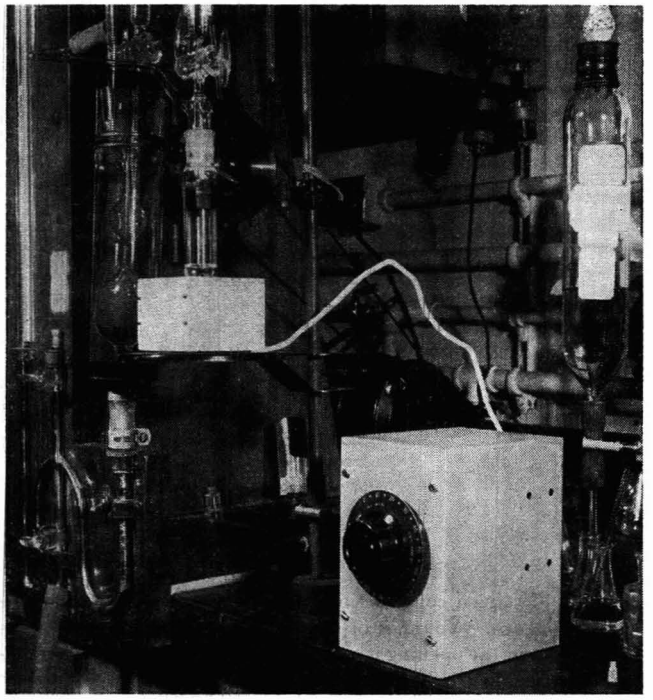

Figure 1. Heater Assembly with Resistance Assembled with Van Slyke Combustion Equipment

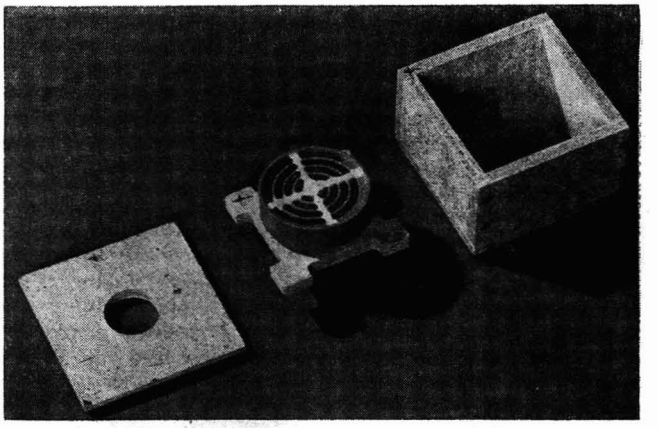

Figure 2. Separated Parts of Heater Assembly

Box, cover, and plate to which heating unit is attached are of asbestos board

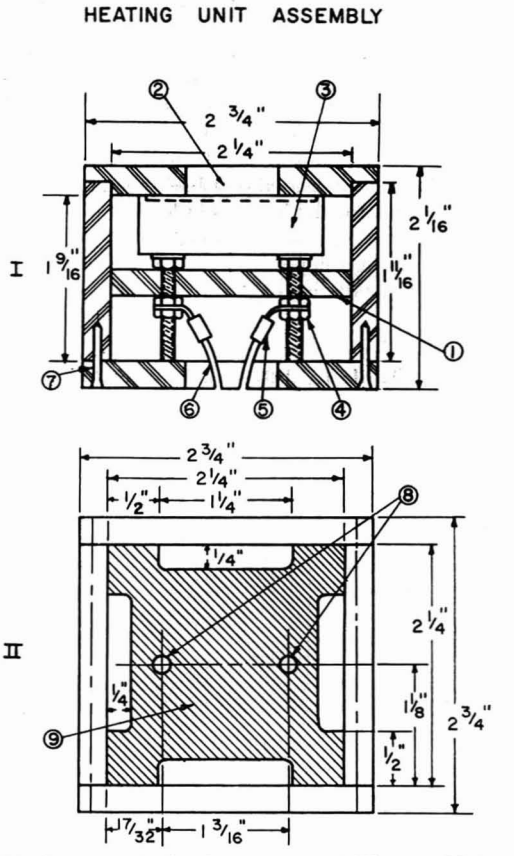

Figure 3. Scale Drawings of Heater Unit Assembly

I. Vertical section through middle of assembly
   1. Heater spacing plate (details in II)
   2. 7/8-inch circular holes in top and bottom
   3. Heating unit, *A*
   4. No. 4-40 hexagonal nut (brass), four required
   5. Pinch lugs, two required
   6. Insulated wires from rheostat
   7. No. 16 gage brad (steel), four required

II. Horizontal section across assembly at level of heater support plate
   8. 5/32-inch diameter holes
   9. Darker area is heater spacing plate, which slip-fits into box

rheostat must be used in place of the autotransformer. The use of a 1-ampere rheostat of proper resistance for either alternating or direct current operation would reduce the cost of construction to possibly $5.00 to $7.00. An autotransformer was preferred for the heater, as a transformer does not heat up as readily as a rheostat and, thus, provides a more constant volt-amperage supply to the heater.

An exploded view of the heater (rated at 110 watts and 115 volts, in Figure 2 shows how it may be disassembled for replacement of burned out heating units (available from American Instrument Co., Silver Spring, Md., as replacement parts of an electric micro-Kjeldahl digestion apparatus). When assembled, the small plate (containing side vents for air cooling) which has the heater attached to it, fits into the box and the lid fits tightly over the top.

The details of the heater construction are shown in scale drawings in Figure 3. This box and support plate are made of Transite. The heater unit, *A*, is fastened to the support plate beneath it with four small spacing nuts. A similar set of spacing nuts fastens the electrical contacts to the unit; the nuts are easily removed to replace a heater unit. As the heating unit never becomes extremely hot during an analysis, ordinary coated wire was found to be satisfactory for lead wire from the heater to the power control. Complete heater units will be available from Arthur H. Thomas Co., Philadelphia, Pa.

ACKNOWLEDGMENT

This research was supported by the Atomic Energy Commission.

Self-Balancing System for Continuous Control of Current or Voltage

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THE thermal conductivity method has long been used for the analysis of binary gas mixtures, including mixtures of isotopes (*2–6*). The accuracy usually claimed for this method is approximately 0.05 to 0.1%, and the gas analyzed may be recovered unaltered and undiluted. These facts led to the adoption of this method of analysis for hydrogen-deuterium and hydrogen-tritium mixtures during the course of an extended series of separations of these isotopes, employing Hertz-type diffusion pumps (*1*). Because these separations proceeded almost continuously, it was advantageous to achieve as nearly automatic operation of the analysis apparatus as was feasible. Reproducibility to 0.02% has been achieved with the aid of the servo-system described below.

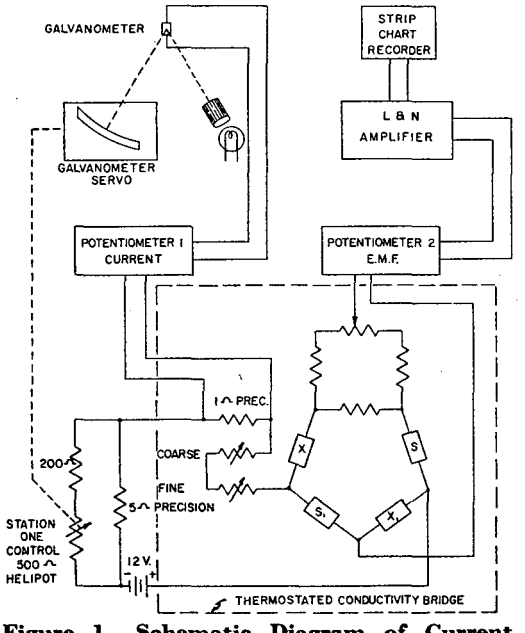

Figure 1. Schematic Diagram of Current Control for Gas Analysis Apparatus

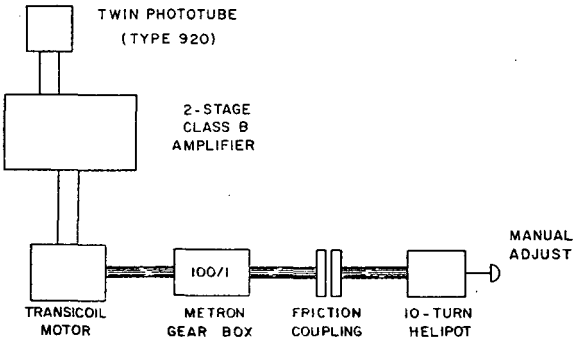

Figure 2. Block Diagram of Galvanometer Servo

In general, the thermal conductivity method consists of passing a constant current through a wire surrounded by the gas to be analyzed, and measuring the wire's resistance. The experience of the authors has been that the greatest single difficulty with the method is in the precise control of this heating current. Since gases vary in their abilities to conduct heat, the temperature of the filament and hence its resistance will be a function of the gas and of its pressure. Empirical calibration curves may be constructed by using known gases or known mixtures of gases.

CURRENT CONTROL

The bridge circuit employed is outlined in Figure 1. Upon operation of the thermal conductivity cells as received from the manufacturer (Leeds and Northrup), it became apparent that the limiting factor in the sensitivity was instability of the heating current, even though three thermostated 120 amp.-hr., 12-volt storage cells were employed in parallel to supply this current. Therefore, a 5-ohm resistor in series with the bridge and a 500-ohm, 10-turn Helipot and a 200-ohm fixed resistor in parallel with this resistor were added. The galvanometer employed had a sensitivity of 0.02 μv. per mm. This combination served to allow more precise current control, and with the servo-driven Helipot, allowed this control to be accomplished automatically. The servo is of the closed-loop type (Figure 2); its operation depends on the differential signal received by a twin phototube (Type 920), masked except for a narrow vertical slit at the front to prevent interference from room lighting, and also to increase the slope of the signal *vs.* beam deflection function. It is now possible to control the 0.5-ampere current to 1 part in 100,000, which allows reproduction of analyses to $\pm 0.02\%$.

For continuous monitoring of the e.m.f. developed across the bridge, a Rubicon Type B potentiometer, a Leeds and Northrup microvolt direct current amplifier, and a Brown 2.5-mv. Electronik potentiometer were employed. The potentiometer is used to oppose all but a few microvolts of the e.m.f. to be measured, and the remainder is fed to the direct current amplifier, where its magnitude is increased to a value suitable for recording on the Brown instrument. Thus, the output e.m.f. is recorded, making possible continuous and automatic observation of such phenomena as the self-equilibration of $H_2 + T_2 = 2HT$.

VOLTAGE CONTROL

Recently the servo system has been adapted to assist in the continuous and accurate control of the temperature of an electric furnace, as shown in Figure 3. The Brown recorder is fed

a boosted signal corresponding to the difference between the setting of the Rubicon Type B potentiometer and the e.m.f. of a Chromel-Alumel furnace thermocouple. The recorder is equipped with a cam, relay, and resistors so that it controls the temperature that it measures. By this arrangement—essentially zero suppression—it is possible to record temperature over a wide range without loss of sensitivity. It was found that room temperature changes overnight were sufficient to change the potentiometer working battery voltage by more than 1 mv., or the opposition voltage by more than 12 μv. (equivalent to a temperature change in the furnace of about 0.3° C.). The servo system monitors and adjusts the 6-volt supply by comparing it continuously with an Eppley standard cell (unsaturated type). The experience of the authors is that after 16 hours of unattended operation the working supply voltage seldom differs by more than 50 μv. from the desired value, and is usually within 10 μv. of this value in spite of the fact that room temperature changes of 20° F. are commonplace. All the components of this system, including the components of the servo, are commercially available.

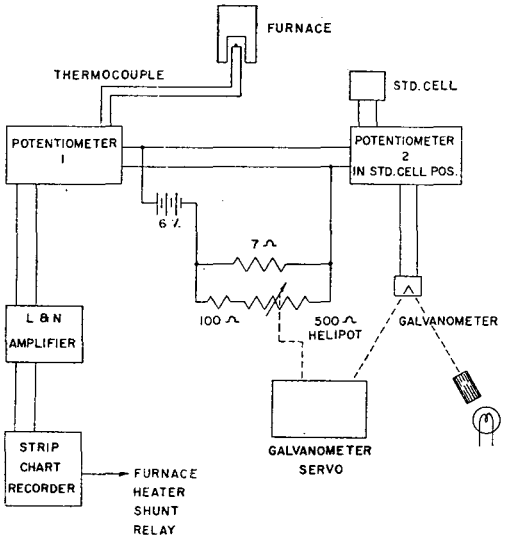

Figure 3. Schematic Diagram of Voltage-Controlling System

ACKNOWLEDGMENT

The authors are indebted to William L. Briscoe of this laboratory for the design of the servo amplifier employed. This work was performed under the auspices of the AEC.

LITERATURE CITED

(1) Dunn, F. J., Mosley, J. R., and Potter, R. M., ANAL. CHEM., **27**, 63 (1955).
(2) Farkas, A., and Farkas, L., *Nature*, **132**, 894 (1933).
(3) Farkas, A., Farkas, L., and Rideal, E. K., *Ibid.*, **137**, 315 (1936).
(4) Gregory, H. S., and Archer, C. T., *Phil. Mag.* (7), **15**, 301 (1933).
(5) Newell, W. C., Purcell, R. H., Gregory, H. S., and Ellingham, H. J. T., *Nature*, **137**, 69 (1936).
(6) Reilly, J., and Rae, W. N., "Physico-Chemical Methods," Vol. I, p. 449, New York, D. Van Nostrand Co., 1943.

Water-Flow Safety Switch for Gas or Electric Heaters

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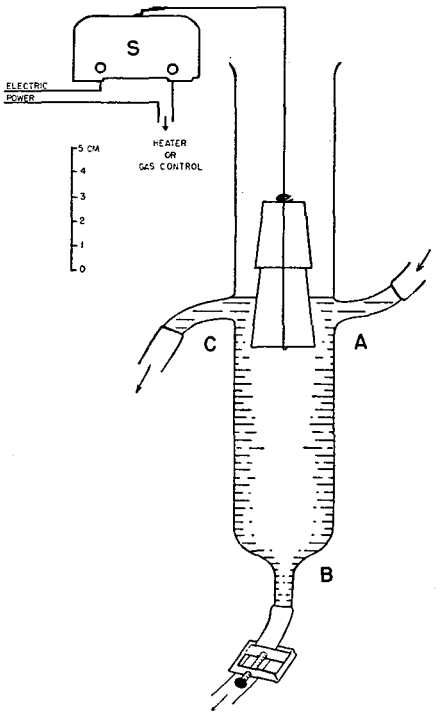
With the dependence of many laboratory operations upon water cooling of condensers, there exists the hazard of water pressure failure. If the condenser is part of a mercury diffusion pump, insufficient cooling may permit the mercury to distill into the remainder of the system. Safety devices described include a thermoregulator sealed into the system for protection of electrically heated water stills (*3*), a pressure-sensitive relay (*1*), and more recently, a bellows-type pressure switch (*2*) for use in conjunction with a mercury diffusion pump or other electrically heated equipment. The device described here is of simple construction and has been in use for some time with a mercury diffusion pump.



A Type W lever arm microswitch, *S* (normally closed and requiring 35 grams for opening), is mounted on the wall above a large glass test tube (3.5 cm.), which serves as a float chamber, as shown in the figure. A length of No. 16 copper wire is soldered to the switch lever and after bending is run through two corks (No. 8 and No. 10). A piece of solder wire is placed around the copper wire above the corks so that its weight is just sufficient to operate the switch to the down or open position.

The return water from the condenser enters the float chamber by the inlet tube, *A*, and leaves by the drain tube, *B*, at a rate determined by the setting of the pinchcock. The pinchcock is adjusted to pass water at a rate below that desired for cooling the system. When the rate of water flow increases beyond this amount, the water level rises to the overflow tube, *C*, and thereby raises the float to switch on the heater. Conversely, a decrease in water flow will switch off the heater, and if a double throw type of switch were used an alarm could be operated. There is an automatic check every time the still is switched on, as power is not supplied to the heater until the water has been turned on. Furthermore, should the cork float become waterlogged after long continuous use, power failure rather than failure to switch off would result.

If a separate pilot flame is used, gas burners (using butane in this laboratory) may be controlled electrically by the use of a relatively inexpensive and commercially available magnetic gas valve (Minneapolis-Honeywell V495A). Thus, an electric safety switch may be used for gas or electrical heating.

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LITERATURE CITED

(1) Poole, J. H. J., and Gilmour, J. C., *J. Sci. Instr.*, **20**, 49 (1943).
(2) Roth, W., ANAL. CHEM., **25**, 1940 (1953).
(3) White, L. M., and Secor, G. E., IND. ENG. CHEM., ANAL. ED., **18**, 332 (1946).