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## This Month's Cover. Ana-

lysts may look with pride at their record of achievement in aiding the progress and accomplishments of medical research. Although their work has not been so loudly proclaimed as the efforts of others, it has been a significant factor in pushing outward the frontiers of medical science. A new tracer technique, an improved instrument design, or a better analytical method often provides the key to open up a new or hitherto blocked line of medical research. The cover fea-tures a newly developed instrument for scanning body areas in which radioactive isotopes may have localized. This was one of many have analytical instruments and methods discussed at the recent Symposium on Analytical Methods sponsored by the National Institutes of Health (see page 21 A) and at the Fourth Annual Research Equipment Ex-hibit held in conjunction. This hibit held in conjunction. This meeting and others give strong indi-cation that the role of the analyst in medical research is an expanding one.

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WE COMMENTED editorially in the February issue on the streamlined name of the "Society of Public Analysts and Other Analytical Chemists" to "The Society of Analytical Chemistry." Analysis in Britain started with the demand for control of food and drug products, so that the public could be protected against fraud and poisoning. The same conditions in this country brought into being the food and drug laws which necessitated development of adequate analytical procedures for the guidance of enforcement agencies.

For those whose libraries subscribe to Food, Drug, Cosmetic Law Journal, we recommend as interesting reading an article by George Taylor in the March issue, Vol. 9, page 133, dealing with the history of analytical chemistry in Britain beginning with the formation of the Society of Public Analysts in 1874. For those who do not have the article available, we quote:

Despite its name, the membership of the society was not confined to public analysts, although it was restricted to "analysts in practice" until 1899, when it was broadened to include persons having a bona-fide interest in analytical chemistry. But the appointment as a public analyst was the handiest of instruments for the endeavor to establish the science of analytical chemistry with particular reference to the composition of foods and their methods of analysis. Even so, the work was not omitted nor circumscribed; the laboratory of the public analyst was often the center of activities dealing with matters of the health and amenities of the public within the purview of the particular local authority concerned. .

Following the historical outbreak of arsenical poisoning in 1901—consequent on the use of arsenical glucose in a brewery which resulted in beer containing poisonous amounts of arsenic—which caused a number of deaths and many cases of serious illness, the society was associated with the Society of Chemical Industry in devising methods of analysis. Members gave evidence before a royal commission whose final report recommended limits for arsenical contamination in foods which have been generally accepted and have been officially acted on for more than 50 years. The recent recommendations of the Ministry of Food in respect of statutory limits can scarcely be said to differ significantly from those earlier recommendations....

In 1929 the society collaborated with the Association of British Chemical Manufacturers in recommending permissible limits for the arsenical contamination of coloring matters for foodstuffs, in 1930 with the Food Manufacturers Federation in rec-



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The society was now rapidly extending its interest to all branches of analytical chemistry. Men and women joined because it provided the readiest opportunity for discussion and publication of purely analytical papers. It had become largely concerned with industrial analysis and research. Even during the war years of 1939 to 1945, while under the authority of the Defence Research Department of the government it accepted responsibility for examination of foodstuffs for contamination with war gases, many members were engaged in war work in other fields. The rapid advance in the knowledge and application of scientific methods had resulted in specialization, and our society grasped the opportunity afforded by this development by setting up the three subject groupsmicrochemistry, an analytical advance without which much of the progress of biochemistry with its requirements for intimate knowledge of the composition of minute vital bodies, such as chromosomes and genes, could not have been made; physical methods, whereby, among other issues, those of instrumentation have gone far to do away with the difficulties arising from the limitations of the hand and eye; and biological methods, wherein even the vital activities of unicellular microorganisms are employed as analytical reagents. It thereby, in fact, acknowledged the change that had been slowly taking place in the fundamental objects of the society— namely, the change from a society largely concerned with food, and its composition and analysis, to a society engaged in the promulgation of the science of analytical chemistry.

In regard to the development of the *Analyst*, journal of the society, first published in 1876, Taylor writes that:

Very early, too, abstracts of analytical methods published in other scientific journals began to appear. In due course, these abstracts developed a characteristic personal to the *Analyst*—they were all "work-ing abstracts"—that is, the method of analysis given in the abstract could, if necessary, be carried out by any skilled analyst without reference to the original publication. They became a recognized feature of the journal, often occupying more than half the space of an issue. Year by year the *Analyst* grew in size and reputation until now it may quite fairly be said that it is the premier journal devoted to analytical chemistry in the United King-dom and is recognized as such throughout the world. It is on the same level with us as is ANALYTICAL CHEMISTRY in the United States. The year 1949 marked one new departure, however. At the end of that year abstracts were divorced from the Analyst, and issued by the bureau of abstracts as a separate publication entitled British Abstracts C. But this diversion is proving to be brief; the bureau of abstracts is being closed down, and once again the society will be responsible for the publication of these abstracts, now under the name Analytical Abstracts, but still as a separate monthly issue.



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The distilling flask proper, i.e., inner jacket of principal glass part, is 175 mm long, with body 35 mm diameter, enclosed in a steam jacket 225 mm long  $\times$  60 mm outside diameter. The flask

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## ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

## What's Ahead in the Next 25 Years?

LAST YEAR ANALYTICAL CHEMISTRY completed 25 years of service to the profession of analytical chemistry. It is both interesting and instructive to note the growth of the journal in that period. What is of much greater significance, however, is the change of attitude concerning the importance of analytical chemistry that has occurred in the lifetime of the publication. Mostly it is a change of attitude on the part of analysts themselves.

The January 1929 issue of *Industrial and Engineering Chemistry*, in announcing the establishment of an ANALYTICAL EDITION, had this to say about the role of analytical chemistry:

Without regard to the way in which the work of chemistry may be classified or subdivided, analytical chemistry is vital to all. Its accomplishments may not be spectacular, much of it seems routine, and usually the part it contributes to the success of important work is unnoticed. The contributions of analytical chemistry may not be appreciated by the plant manager, nor by the director of the laboratory, nor the chief of the bureau, but every chemist knows to what extent we depend upon accuracy in this field of work....

These words were penned by the late Harrison E. Howe who, as editor of *Industrial and Engineering Chemistry*, was mostly responsible for the idea of establishing the ANALYTICAL EDITION. His description of the status of the analytical chemist of 25 years ago was accurate for that period. It provides us with an opportunity for true evaluation of the progress that subsequently has been made.

Today in most companies, certainly the progressive ones, the worth of the analyst and the analytical department is fully appreciated by top management, by the director of research, and by the plant manager. In most organizations the head of the analytical department is a full-fledged member of the team and participates in major decisions concerning research and production.

The history of the growth of ANALYTICAL CHEMISTRY in many respects reflects the progress of the profession. This is as it should be. A scientific and technical journal can and should provide professional leadership, as well as being a medium for mass dissemination of data.

In its first year of existence, the ANALYTICAL EDITION contained 245 editorial pages. In 1953, a total of 2078 editorial pages were printed, a gain of nearly 800% in the 25-year period. In 1933, it became necessary to issue the ANALYTICAL EDITION on a bimonthly rather than a quarterly basis. Four years later the decision was made to make the journal a monthly. In 1948 the big gamble was taken and the ANALYTICAL EDITION was completely divorced from *Industrial and Engineering Chemistry* and given the title ANALYTICAL CHEMISTRY.

These successive steps were taken because the broad field of analysis was expanding phenomenally. With this expansion have come greater recognition and responsibility for members of the profession of analytical chemistry.

It is but seven years ago that ANALYTICAL CHEMISTRY in an editorial offered a four-point program designed to obtain proper recognition and appreciation of the analyst. Anyone reading that program now will agree that a great deal of real progress has been made in seven short years. However, there still remains much to be done; indeed, there will always be much to be done.

It always is interesting to review the past, but what we are really concerned with is the future. What elements of the original four-point program need to be reemphasized: what new objectives should be added? We are members of a learned profession and, therefore. have a feeling of responsibility to society. At the same time, all of us have a perfectly legitimate desire for both professional and financial success. If these are the general goals, what are the best means of achieving these objectives? Much of the past progress has been brought about because the analysts discarded a defeatist attitude. They replaced it with a realistic approach based on a sound evaluation of the contribution they are making to the advancement of chemistry and science and industry generally. This was a necessary first step in the attempt to improve the professional and economic status of the analyst. It is a firm foundation upon which to build in the future.

We lack a crystal ball with which to prophesy the future. We honestly believe, however, that when 1978 arrives, the editor of ANALYTICAL CHEMISTRY will be able to speak in glowing terms of the contributions of analytical chemistry to the health and welfare of society; also about further gains registered in the professional standing of the analyst. With greater professional recognition will come better monetary rewards.

## Separability of Hydrocarbons by Elution Chromatography

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A quantitative method has been developed for measuring the relative adsorbabilities of hydrocarbons. Adsorbability data are presented for compounds representative of various hydrocarbon classes, on silica gel, alumina, active carbon, and combinations of silica with magnesia and alumina. Adsorbability is expressed in terms of the volume of developing solvent per volume of free space in the adsorbent bed required to carry the adsorptive front through this bed and also to effect its removal. The geometry of the adsorbent bed is not critical if the bed depth is about 50 cm. or greater. Neither rate of eluent flow nor particle size of the adsorbent is a critical factor. In the order of increasing affinity for silica gel the hydrocarbons form the series: paraffins, olefins, diolefins, alkylbenzenes, alkenylbenzenes, acetylenes, naphthalenes. The effect of ring closure is much less than that of unsaturation. Neither ring closure nor conjugation affects the adsorbability of diolefins. The quantitative data presented here predict rather well the separability of hydrocarbons on a practical analytical scale. Thus, using silica gel, mono-olefins appear to be separable from diolefins and diolefins from acetylenes. In some cases partial separation of acyclic from cyclic olefins can be effected. Poor separability is indicated for diolefins from alkylbenzenes. Alumina is superior to silica gel for the separation of alkylbenzenes from naphthalenes. Normal paraffins may be partially separated from other saturated types over activated carbon, using oxygenated compounds of low molecular weight as developing solvents.

**F** OR some years solid adsorbents have been extensively utilized for the separation of hydrocarbon mixtures according to molecular type (2-5, 9, 10). Three classes of techniques have been employed: continuous introduction method, sometimes referred to as frontal analysis, in which the mixture is continuously added to the column and one or more properties of the effluent are measured (3, 5, 9); displacement development, in which the mixture is forced through the adsorbent bed by a liquid which is more strongly adsorbed than all components of the mixture (2); and elution development, in which a small amount of the mixture is washed through the adsorbent with a liquid which is usually less strongly adsorbed than some or all the components of the mixture (4).

Generally, it is considered (8) that the last of these techniques is most effective for complete separation of a complex mixture. Such separation problems could be approached more intelligently if quantitative data were available on the relative adsorbability of the various classes of hydrocarbons. These data should make it possible to predict rather well for a given system to what extent the several classes may be separated.

While some information is available, nothing approaching a complete picture for the hydrocarbons has been presented by a uniform method of study. Included among the efforts in this direction is the work of Smit (7), who measured the "transport factors" for several hydrocarbons of higher molecular weight, representing paraffins, olefins, alkyl benzenes, and polynuclear aromatics. He defined the transport factor as: T = g/V where g is the weight of adsorbent in a given volume and V is the volume of developer necessary to carry the adsorptive through this amount (g) of adsorbent.

Transport factors were determined for six hydrocarbons using silica gel and in some cases for as many as four different developing solvents. Fink *et al.* (1) report data on the relative adsorbabilities of several olefins and aromatics as a result of determining the capacities of silica gel for these compounds.

In the study reported here the objective was to obtain quantitative data on the relative adsorbabilities of different hydrocarbon types using various adsorbents, to do this in terms of the volume of developer necessary to carry the adsorptive front through a given volume of solid adsorbent, and also to determine the volume of developer necessary for essentially complete removal. In this study, these volumes are expressed in terms of the liquid free space in the adsorbent bed. These two quantities are here referred to as appearance factor and disappearance factor, respectively. Their physical significance is more clearly elucidated under the discussion of experimental technique. These quantities turn out to be numerically equal to the reciprocals of the quantities  $R_1$  and  $R_t$  as defined by LeRosen *et al.* (6). These latter values are defined as ratio of movement of adsorptive to movement of developing solvent. It is believed that the concept proposed herein offers the advantage of a more obvious physical significance in considering the separability of different molecular types.

#### TECHNIQUE

Figure 1 shows a schematic diagram of the simple apparatus used.



Figure 1. Schematic Diagram of Apparatus Used in Adsorption Studies

A small column of adsorbent was mounted in close proximity to a Perkin-Elmer infrared spectrometer. For most of the measurements the diameter of the adsorbent bed was 5 mm. The volume of adsorbent was about 12 ml. The effluent from the bottom of the column was carried to the sample cell mounted on the spectrometer. In practice the column was first evacuated. The vacuum was broken by introduction of a known volume of developer solvent at the top of the column. The solvent was allowed to flood the column and to flow through the bed until just level with its top surface. Noting the volume of effluent at this point permitted the determination of the free liquid volume

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of the adsorbent bed. At this time a small amount (usually  $0.1 \pm 0.01$  ml.) of the substance under study was introduced at the top of the bed and washing was continued with the effluent passing through the absorption cell. The spectrometer was set on an absorption band sensitive to low concentrations of the adsorptive in the developer solvent. The recorder was operated and the appearance of the first trace of the adsorptive in the sample cell gave rise to a sharp break in the recorded trace. The volume expressed in terms of the liquid free space in the adsorbent bed-i.e., ml. of effluent per ml. of liquid free spaceis called the appearance factor.



Variation of Relative Concentra-Figure 2. tration of 1-Hexene in Eluent (Absorbance) with Extent of Removal from Silica Gel

Absorbent. No. 923 silica gel, 12.0 ml. Column. 5-mm. diameter, 48-cm. height Adsorptive. 1-Hexene, 0.1 ml. Column. 5-mm. diameter Adsorptive. 1-Hexene, 0.1 Eluent. n-Heptane Liquid free space. 8.2 ml.

It became apparent that it was equally important to know the number of volumes of developer required to wash the gel free of the adsorptive. The volume point at which the concentration of the adsorptive in the effluent approaches zero is rather elusive, since this approach is asymptotic. Figure 2 shows the shape of the concentration vs. volume curve for 1-bexene. This is typical of the curves obtained for other compounds. In these curves the absorbance—i.e., relative concentration—has been measured and plotted against the volume collected after the appearance of the adsorptive in the effluent. The purpose of replotting these curves was to find what fraction of the total amount of adsorptive had been removed at some finite relative concentration. It was observed by making planimeter measurements on the areas that when the relative concentration reaches 5% that of its maximum value, 98% or more of the adsorptive has been washed out. Such a degree of removal is adequate for present considerations. The total volume of eluent (in terms of liquid free space) required for 98+% removal is called the disappearance factor. In some cases, particularly with the more strongly adsorbed

materials, an excessively long time is required to reach the 5%



Figure 3. Correlation for Short-Cut Method for **Estimation of Disappearance Factor for Silica Gel** 

Disappearance factor =  $\frac{\text{volume of eluent}}{C}$ at 5% maximum concentration



Figure 4. Effect of Ratio of Gel to Charge on Appearance Factor Free space proportional to amount of

gel No. 923 silica gel. *n*-Heptane eluent

relative concentration. short-cut method utilized in these cases is based on an empirical relationship between the volumes at 50 and 5% relative concentrations. This relationship for silica gel is shown in Fig-ure 3. A similar relationship was found for other adsorbents.

#### EFFECT OF EXPERI-MENTAL CONDITIONS

Effect of Ratio of Gel to Charge. It might be assumed that if a large ratio of gel

to adsorptive were used, the appearance factor and disappearance factor would be independent of sample size. At infinite dilution the appearance factor probably would approach the value of the disappearance factor. Figure 4 shows a plot of appearance factor for several compounds vs. the ratio of free space to charge size (proportional to ratio of gel to charge). These data are for Davison No. 923 silica gel. As smaller charges of adsorptive are used, the appearance factor becomes larger. This is consistent with the known fact that adsorption isotherms are not linear with concentration but curve toward the concentration axis. One would expect the disappearance factor to be independent of sample size. Figure 5 shows that this is not quite true, but the reason becomes apparent when it is remembered that the disappearance factor represents not the point of zero absolute concentration but only the point at which 98+% of the material has been washed from the gel.

Effect of Col-

Geometry. umn A study has been made of the effect of diameter and depth of the adsorbent bed on the relative adsorbabilities of hydrocarbons. Appearance and disappearance factors were measured for 1,5-hexadiene on Davison No. 923 silica gel using nheptane as eluent. In this study two bed diameters, 5 and 10 mm., were used, and bed depth was varied from 170 to 920



Figure 5. Effect of Ratio of Gel to Charge on Disappearance Factor

Free space proportional to amount of gel No. 923 silica gel. *n*-Heptane eluent

mm. The data tabulated below would indicate that the appearance factor is independent of the diameter but increases somewhat with increasing depth approaching a limit as the bed depth is increased to a value of approximately 500 mm. This is shown further in Figure 6.

Column	Free	Appear-	Disap-
Dimensions,	Space,	ance	pearance
Mm.	Ml.	Factor	Factor
$5 \times 460$	$8.3 \\ 16.4 \\ 8.9 \\ 25.0$	2.77	7.5
$5 \times 920$		2.91	7.2
$10 \times 170$		2.22	7.0
$10 \times 460$		2.71	7.1

For the major portion of the work which has been done on hydrocarbons of various types, a sample size of 0.1 ml. has been used with an adsorbent bed 5 mm. in diameter by 460 mm. in height, containing about 12 ml. of adsorbent and having a liquid free space of about 8.2 ml.

Effect of Particle Size. A study has been made of the effect of particle size of gel on the appearance and disappearance factors. The factors were determined for 1,5-hexadiene on screened No. 923 silica gel using *n*-heptane as eluent. The data show that particle size has no important effect on the appearance or disappearance factors.

	No. 923 Screened Silica Gel, Mesh Size	Appearance Factor	Dis- appearance Factor
1,5-Hexadiene	80–100 100–150 150–200	$2.60 \\ 2.55 \\ 2.64$	$\begin{array}{c} 6.60 \\ 6.07 \\ 6.14 \end{array}$

Effect of Flow Rate. The effect of rate of flow of developer through the adsorbent bed has been determined. Again the system, 1,5-hexadiene, No. 923 silica gel (100 to 150 mesh), and *n*-heptane eluent was employed. These data, shown below, prove that rate of flow as measured by milliliters effluent per minute per milliliter of free space in the column has no pronounced effect on the adsorption of the material being measured.





Figure 6. Effect of Adsorbent Bed Depth on Appearance Factor No. 923 silica gel. n-Heptane eluent

Hydrocarbons Used. When available, the hydrocarbons used were those provided by the American Petroleum Institute as calibration standards. Several of the diolefins were purchased from the Farchan Research Laboratories and used without further purification.

#### RESULTS

Silica Gel Studies. Measurements on individual compounds are shown in Table I and presented graphically in Figure 7. The



Figure 7. Relative Adsorbabilities of Hydrocarbons on Silica Gel

Shown by presence of adsorptive in effluent No. 923 silica gel. *n*-Heptane developer. Ratio of liquid free space to charge volume, 80

Table I. Appearance and Disappearance Factors for Various Compounds

(Davison No. 923 silica gel, 24° C.)

Free Annear annear Eree Annear	annoar-
Space/ spice spice Spice/ spice	appear-
Compound Developer Charge Factor Factor Compound Developer Charge Factor	Factor
Acyclic mono-olefins Aromatics	
1-Hexene n-Heptane 82 1.42 2.48 Benzene n-Heptane 82 3.76	9.00
1-Hexene n-Heptane 33 1.22 2.43 Benzene n-Pentane 82 3.34	6.92
I-Hexadecene n-Heptane 82 1.06 1.93 Benzene Chloroform 82 0.95	
2,4,4-Trimethyl-1- Benzene Carbon tetra- 82 1.78	4.39
pentene n-Heptane 82 1.37 2.76 chloride	
2,4,4-Trimethyl-2- Toluene n-Heptane 82 4,56	11.83
pentene n-Heptane 75 1.68 2.88 Toluene n-Heptane 33 3.23	10.80
2-Ethyl-1-hexene n-Heptane 75 1.79 2.79 Ethylbenzene n-Heptane 75 4.45	8.34
trans-4-Octene n-Heptane 75 1.24 2.11 n-Butylbenzene n-Heptane 38 3.13	
2,3-Dimethyl-2-butene n-Heptane 75 1.68 2.59 tert-Butylbenzene n-Heptane 82 4.23	11.8
2-Methyl-1-pentene n-Heptane 82 1.90 3.70 n-Decylbenzene n-Heptane 82 3.35	8.73
Styrene n-Heptane 82 5.27	13.1
Cristian Indene n-Heptane 82 6.88	24.0
Cyclonexene $n$ -Heptane $82$ 1.71 3.41 Indane $n$ -Heptane $83$ 4.85	12.9
Cyclonezene $n$ -Heptane 33 1.49 3.07 Indane $n$ -Heptane 33 2.73	
Cyclonexene $n$ -Pentane $82$ 1.55 2.88 Tetralin $n$ -Heptane $83$ 5.42	13.7
Cyclonexene Chloroform 82 0.77 1.18 Naphthalene n-Heptane 82 8.26	24.5
Cyclonexene Carbon tetra- 82 1.12 2.05 Naphthalene Chloroform 82 0.84	1 43
chloride Naphthalene Carbon tetra- 82 2.83	5.55
4-Ethyl-1-cyclohexene n-Heptane 75 1.73 2.85 chloride	0.00
Diolefins $1$ -Methylnaphthalene $n$ -Heptane $82$ 6.43	17.1
1.5-Hexadiene n-Heptane 82 2 93 6 77 2-Methylnaphthalene n-Heptane 82 8.58	26.0
1.5-Hexadiene n-Heptane 33 1.90 6.60 Dimethylnaphthalene n-Heptane 82 8.95	22.1
15-Heradiane n-Pantana 82 2 32 5 31	
1.5-Hexadiene Chloroform 82 0.70 1.10 Miscellaneous	
1.5-Hexadiene Carbon tetra, $82 = 1$ , $83 = 4$ , $40 = 1$ -Hexyne n-Heptane $82 = 6.75$	18.4
chloride 82 2.27	4.39
2-Methyl-1.3-pentadiene n-Heptane 75 2 72 5 34 Methylene chloride n-Heptane 82 3.50	9.54
1.3-Cyclobexadiene n-Heptane 75 2 75 5 63 Carbon tetrachloride n-Heptane 70 1.13	
1.4-Cyclohexadiene n-Hentane 82 3 10 7 20	
I-VinvI-3-cyclobexene n-Hentane 82 2 94 7 06	

data in Figure 7 were obtained using 0.1-ml. samples on a 5  $\times$ 460 mm. gel bed with a liquid free space of 8.2 ml, and with n-heptane developer. It can be seen from Figures 4 and 5 that as the ratio of gel to charge (ratio of free space to charge) increases, the appearance factors approach the disappearance factors; thus, the ultimate separability of any two compounds is dependent on the differences in disappearance factors. However, there is an upper usable limit to the ratio of free space to charge. The ratio used in obtaining the data in Figure 7, approximately 80, is large enough to resolve differences in adsorbabilities of compounds, yet it is a practical value which allows the separations to be carried out. The column length has approached the limit where appearance factor is independent of bed depth.

From Figure 7 it is possible to predict the feasibility of separating different materials by considering whether one material will be washed from the gel prior to the appearance of the second material. If present in different concentrations, the ratios of gel to charge will differ for the two components, and this should be considered in order to make precise predictions.

Table	II.	Appearance	and	Disappear	ance	Factors	fo
		Component	ts of ]	Binary Mix	tures		

	Free Space/ Charge	Appearance Factor	Dis- appearance Factor
Cyclohexene (alone)	82	1.71	3.41
with 1,5-hexadiene)	82 <sup>a</sup>	1.59	2.86
1,5-Hexadiene (alone) 1,5-Hexadiene (50–50 mix. with cyclohexene)	82	2.93	6.77
	824	2.86	•••
Benzene (alone)	32.8	2.45	
Benzene (50-50 mix. with tert-butylbenzene) tert-Butylbenzene (alone)	32.8ª	2.00	
	32.8	3.11	•••
mix. with benzene)	32.84	1.95	
<sup>a</sup> Ratio with respect to compone.	nt under con	sideration.	



Adsorbent. No. 923 silica gel, 250 ml., 15 mm.  $\times$  150 cm. Charge. 5 ml. of 50-50 mixture of cyclohexene and 1,5hexadiene Eluent. n-Heptane

Effect of One Component on Another. It would also be expected that one component will have an effect on the appearance and disappearance factors for other components in a mixture. Data presented in Table II simply show that with the ratio of gel to charge constant for a given hydrocarbon, the addition of a second hydrocarbon tends to lower both the appearance and disappearance factors.

Tests on Separability of Components. Several tests have been carried out to determine how well the data presented in Table I or Figure 7 may be used to predict the feasibility of separation

in a larger column. For the first of these tests 5 ml. of a 50-50 blend of cyclohexene and 1,5-hexadiene were used with a column containing 250 ml. of silica gel. These values correspond to a ratio of free space to individual component of about 82. In Figure 7 it is observed that these two components will yield some overlap singly under these conditions-that is, cyclohexene disappears at 3.41 volumes, whereas 1,5-hexadiene appears at 2.93 volumes. Cuts were taken of the effluent and analyzed for both components by infrared methods, and the data are plotted in Figure 8. Area measurements indicate that two fractions could be obtained with about 98% recovery and 98% purity for each component.

Tests have been made to check the predictions of the data of Table I and Figure 7 relative to the separability of cyclic and acyclic olefins. Referring to Table I it is observed that 2-methyl-1-pentene is present in the effluent from 1.90 to 3.70 volumes of the wash (in terms of the free liquid space in the adsorbent bed). Cyclohexene is present from 1.71 to 3.41 volumes of the wash. One would conclude that a separation of these compounds is not feasible. A test was made in a column containing 250 ml. of gel. In this test the ratio of the free space to the volume of each component would be very nearly 82 or comparable to that used in obtaining the data listed above. The results of this test are shown in Figure 9 and check exactly with what would be predicted from the appearance and disappearance factors.



Charge, 5 ml. of 50-50 blend of cyclohexene and 2-methyl-1-pentene Eluent. n-Heptane

Considering the possible separation of cyclohexene and 1hexene, it is observed that the factors for the latter are 1.42 and 2.48 and for cyclohexene 1.71 and 3.41. This would indicate that a partial separation would be feasible. Tests were made on this separation both at room temperature (33° C.) and at a reduced temperature ( $-6^{\circ}$  C. at the bottom inlet of the jacket and 4° C. at the top outlet). Figure 10 presents the results obtained at the higher temperature. The results at the lower temperature were similar. If two fractions were made by cutting at the intersection of the two curves, the results (obtained by area measurements) would be as follows:



These results indicate that a fair separation is achieved, as

Comparison of Developing Solvents. While most of the data have been obtained using n-heptane as the developer, a few data have been obtained using other solvents.

	Appearance Factor				Disapp Fa	earance ctor		
Compound	n-C7	n-C5	CHCl <sub>3</sub>	CCl <sub>4</sub>	n-C7	n-Č₅	CHCla	CC14
Cyclohexene 1,5-Hexadiene Benzene Naphthalene	$1.71 \\ 2.93 \\ 3.76 \\ 8.26$	$1.55 \\ 2.32 \\ 3.34 \\$	$\begin{array}{c} 0.77 \\ 0.79 \\ 0.95 \\ 0.84 \end{array}$	$1.12 \\ 1.83 \\ 1.78 \\ 2.83$	$3.41 \\ 6.77 \\ 9.00 \\ 24.5$	$2.88 \\ 5.31 \\ 6.92 $	1.18 1.19 1.43	$2.05 \\ 4.40 \\ 4.39 \\ 5.55$

These data indicate that *n*-pentane is a more effective washing solvent than *n*-heptane, as in all cases both the appearance and disappearance factors are lower. It is concluded that essentially the same degree of separation is possible using either paraffin, however. It would appear that switching from a paraffinic developer to a more strongly adsorbed material at some stage in a separation procedure might have practical advantages in time requirement and in reducing the volume of the effluent but no advantage in feasibility of separations.



It was, at first, surprising to observe appearance factors of less than unity using chloroform as the developer (this was also observed for various other systems discussed in this paper). A possible explanation is that if the developer is as strongly adsorbed as the material being studied, the latter does not necessarily have access to all of the space within the adsorbent. Thus, the available free space actually is something less than that value which was determined in flooding the column with developer initially. Any tendency toward "channeling" likewise could yield low values for the appearance factor.

General Conclusions for Studies on Silica Gel. It is interesting to note the effect of substituents on the adsorption characteristics of alkyl benzenes (Figure 7). Both the appearance and disappearance factors are higher for toluene, ethylbenzene, and *tert*-butylbenzene than for benzene—that is, substitution enhances the degree of adsorption. This is also shown in Figure 4. This is not true, however, when a mixture of alkyl benzenes is displaced through a column with a highly adsorbing material such as ethyl alcohol. A long paraffin chain such as *n*-decyl does, however, result in a reduction of both factors. In considering the data of Table I and Figure 7 it is concluded that the following separations are feasible:

- . Mono-olefins from diolefins.
- 2. Mono-olefins from aromatics.

3. Partial separation in some cases of acyclic from cyclic mono-olefins.

4. Partial separation of bicyclic aromatics from monocyclic aromatics.

Definitely not feasible is the separation of aromatics from diolefins as general classes.

Studies on Activated Alumina. PRETREATMENT. Before making measurements on individual hydrocarbons with activated alumina, the optimum pretreatment was studied. For this work Alorco Grade F-20 activated alumina was used. Dimethylnaphthalene (mixed isomers) was used as a test material and both appearance and disappearance factors were measured. The results are shown below:

Treatment of Grade F-20 Activated Alumina			Арреагансе	Dis-	
Hours	° C.	Pressure	Factor	Factor	
Un	treated, as re	eceived	2.90	10.9	
2	145	Atm.	3.07	9.0	
2	210	Atm.	2.94	10.2	
2	425	Atm.	3.26	14.6	
96	425	Atm.	1.94	6.32	
2	165	Vac. oven	3.58	15.6	
36	235	Vac over	3 40	12 3	

It is concluded that heating for 2 hours in a vacuum oven at 165° C. gives the best activity, and material so treated was used in subsequent work.

ADSORBABILITY DATA. Table III presents the appearance and disappearance factors that have been measured for various

Table III. Appearance and Disappearance Factors for Various Compounds

(Grade F-20 activated alumina, 24° C.)

n:-

		Free	Appear-	appear-
Compound	Developer	Space/ Charge	ance Factor	ance Factor
Acyclic mono-olefins				
1-Hexene	<i>n</i> -Heptane	83	0.96	1.35
4-Methyl-2-pentene	<i>n</i> -Heptane	83	0.91	1.32
2-Ethvl-1-hexene	n-Heptane	83	0.97	1.39
2,3-Dimethyl-2-butene 2,4,4-Trimethyl-2-	n-Heptane	83	0.91	1.29
pentene	<i>n</i> -Heptane	83	0.87	1.25
1-Hexadecene	n-Heptane	83	1.02	1.54
Cyclic mono-olefins				
Cyclohexene	n-Heptane	41	1.02	1.46
Cyclohexene	<i>n</i> -Heptane	83	1.06	1.45
Cyclohexene	n-Heptane	277	1.07	1.54
Diolefins				
1,5-Hexadiene	n-Heptane	41	0.96	1.57
1,5-Hexadiene	<i>n</i> -Heptane	83	1.11	1.84
1,5-Hexadiene	<i>n</i> -Heptane	166	1.24	1.83
1,5-Hexadiene	<i>n</i> -Heptane	277	1.29	1.87
1,5-Hexadiene	Chloroform	83	0.78	
1,5-Hexadiene	Carbon tetra- chloride	83	0.92	1.30
1,4-Cyclohexadiene	<i>n</i> -Heptane	83	1.24	2.24
1.3-Cyclohexadiene	n-Heptane	83	1.23	2.05
1-Vinyl-3-cyclohexene	n-Heptane	83	1.23	2.06
Aromatics				
Benzene	n-Heptane	41	1.15	2.51
Benzene	n-Heptane	83	1.37	2.90
Benzene	<i>n</i> -Heptane	277	1.52	3.04
Benzene	Chloroform	83	0.84	1.15
Benzene	Carbon tetra-	83	0.95	1.50
mala and		41	1 16	0 40
Loluene	<i>n</i> -neptane	41	1.10	2.40
Totuene	<i>n</i> -neptane	000	1.04	2.8/
loiuene	n-neptane	211	1.09	2.90
tert-Butyibenzene	<i>n</i> -neptane	80	1.37	2.49
n-Decylbenzene	n-neptane	80	1.00	3.08
Naphthalene	n-Heptane	83	3.00	14.5
I-Methylnaphthalene	n-Heptane	83	2.73	14.3
2-Methylnaphthalene	n-Heptane	83	4.14	16.5
Dimethylnaphthalene	n-Heptane	83	3.58	15.6
Dimethylnaphthalene	Chlorotorm	83	0.77	1.21
Dimethylnaphthalene	Carbon tetra- chloride	83	1.16	2.88
Phenanthrene	Carbon tetra- chloride	83	2.15	4.47
Diphenyl	Carbon tetra- chloride	83	1.41	2.70
Styrene	<i>n</i> -Heptane	83	1.88	5.27
Indene	n-Heptane	83	2.30	10.28



Figure 11. Relative Adsorbabilities of Hydrocarbons on Alumina

Shown by presence of adsorptive in effluent Grade F-20 activated alumina. *n*-Heptane developer. Ratio of liquid free space to charge volume, 80

hydrocarbons over activated alumina. Several developers have been tried. Data showing the effect of ratio of adsorbent to charge on the appearance and disappearance factors for several different compounds indicated that this effect was similar to that observed for silica gel. Figure 11 presents the appearance and disappearance factors for various hydrocarbons in bar graph form. In this figure all data are on a comparable basis to facilitate predictions

of separability.

On the basis of these data, alumina afford a better separation between alkyl benzenes and naphthalenes than does silica gel. Solubility limitations made it difficult to obtain data on more highly condensed compounds using n-heptane as developer. Poor separations are predicted for any combination of mono olefins and diolefins. As with silica gel, poor separation is predicted between diolefins and alkyl benzenes.



Figure 12. Relative Adsorbabilities of Hydrocarbons on Carbon

Shown by presence of adsorptive in effluent Columbia activated carbon 80- to 200mesh. *n*-Heptane developer Ratio of liquid free space to charge volume, 80

Studies on Activated Carbons. Preliminary studies on activated carbons were directed toward selection of carbons that might show promise for separating hydrocarbon types which were not found separable on either silica gel or activated alumina —viz., separation of diolefins from alkyl benzenes and separation of the saturated hydrocarbon types. Table IV and Figure 12 present the appearance and disappearance factors of several cyclic and acyclic diolefins and of several alkyl benzenes on an activated carbon using n-heptane developer. The data reveal that while

Table IV.	Appearance	and	Disappe	arance	Factors	for
Various	Hydrocarbons	on C	olumbia	Activat	ed Carbo	n

(80 - 200)	mesh,	n-Heptane	developer,	24° C	;.)
		E			

	Free Space/ Charge	Appearance Factor	Dis- appearance Factor
1-Hexene Cyclohexene 1,5-Hexadiene 2-Methylpentadiene <sup>a</sup> 1.Vinyl-3-cyclohexene 1,3-Cyclohexadiene 1,4-Cyclohexadiene Benzene <i>tert</i> -Butylbenzene	80 80 86 86 86 86 86 80 80	$\begin{array}{c} 0.73 \\ 0.83 \\ 0.93 \\ 1.43 \\ 0.99 \\ 1.14 \\ 1.33 \\ 1.98 \\ 0.97 \end{array}$	1.52 1.79 3.70 3.12 3.24 3.0 6.3 3.93
<sup>a</sup> Mixed isomers.			

activated carbon may be useful for the separation of certain individual compounds, it does not allow type separation of diolefins and alkyl benzenes.

A study was made to determine just what carbon and solvent wash system would show greatest promise for separation of the saturated hydrocarbon types. To this end *n*-heptane was used as the adsorptive, on the basis that the highest appearance factor possibly would point to the system having greatest resolving power. Because, in general, saturated hydrocarbons are more strongly adsorbed on activated carbon than are oxygenated compounds, it was reasoned that the latter might prove effective as developers in resolving the saturated hydrocarbon types. Alcohols of low molecular weight and acetone were the most promising of the compounds tested. Results indicated the Columbia Grade L activated carbon offered greater promise than Columbia Grades G, S, and 4SXA carbons, and chars derived from petroleum sources. The data in Table V show results obtained using different solvents with Grade L carbon varying in size and in

Table V.	Арреа	rance	and	Disappearan	ce Fac	ctors	for
n-Heptane	with	Various	s Ca	rbon-Solvent	Wash	Syste	ems

Grade L Activat	Columbia ed Carbon	Free		Appear-	Dis- appear-
Mesh	Heat treatment	Space/ Charge	Solvent	ance Factor	ance Factor
60-160	Unheated	85	2,2,4-Tri- methyl- pentane	1.14	2.57
60~160	Unheated	82	CCl₄	0.65	
60 - 160	Unheated	74	CHCl <sub>3</sub>	0.81	
60-160	Unheated	84	Acetone	1.26	2.88
Unsized	Unheated	87	Acetone	1.39	2.52
100-160	Unheated	85	Acetone	1.53	2.03
120 - 160	Unheated	85	Acetone	1.47	2.31
60-160	(Vac. heated	85	Acetone	1.15	2.87
120-160	175° C.,	85	Acetone	1.35	2.52
00 100	4 hours)	07	O IL O IL	1 64	
60-160	Unneated	80	CILOU	1.04	7 0
60-160	Unheated	28	CH3OH	3.61	7.0



(Grade L, 100-160-mesh, unheated)

Dis-

Acetone Acetone Acetone	85. 86	1.53	2.03
Acetone Acetone	86		
Acetone		0.97	
	86	1.09	2.15
Acetone	86	0.92	
Methanol	27	1.2	10.0
Methanol	27	1.5	8.0
Methanol	28	19	8.3
Methanol	28	3.6	7.0
Acetone	86	1.51	4.54
Acetone	86	2.10	5.34
Acetone	86	2.14	7.94
Acetone	86	3 75	9.75
Acetone	86	2 75	12.0
Acotone	86	3 42	12.3
Acotone	86	1 34	3 58
Agetone	86	1 61	3 36
	Methanol Methanol Methanol Methanol Acetone Acetone Acetone Acetone Acetone Acetone Acetone Acetone Acetone	Methanol 27 Methanol 27 Methanol 28 Methanol 28 Acetone 86 Acetone 86 Acetone 86 Acetone 86 Acetone 86 Acetone 86 Acetone 86 Acetone 86 Acetone 86 Acetone 86	Methanol         27         1.2           Methanol         27         1.5           Methanol         28         1.9           Methanol         28         3.6           Acetone         86         2.10           Acetone         86         2.14           Acetone         86         2.75           Acetone         86         2.75           Acetone         86         3.42           Acetone         86         1.34           Acetone         86         1.61



Figure 13. Relative Adsorbabilities of Hydrocarbons on Carbon

Shown by presence of adsorptive in effluent Columbia Grade L activated carbon, 100- to 150-mesh. Acetone and methanol developer

heat treatment. It was concluded that 100- to 160-mesh material with no heat treatment is slightly superior.

Table VI and Figure 13 show the appearance and disappearance factors for the four classes of C7 saturated hydrocarbons using acetone and methanol as developers. It does not appear feasible to effect good separation of any one class, but the possibility does exist of partially separating the normal paraffins from the other three classes.



Column. Column. 15 mm. inside diameter, 150-cm. length Adsorbent. Columbia Grade L activated carbon, 60- to 150mech

Developer. Methanol Mixture. 5 ml. of 50-50 methylcyclohexane and *n*-heptane

In order to demonstrate this prediction, a 5-ml. sample of a 50-50 blend of methylcyclohexane and n-heptane was percolated through a bed of activated carbon, 15 mm. in diameter and 150 cm. in depth, using methanol as a developer. Fractions of 50 ml. each were taken from the column and analyzed by mass spectrometer. The data are shown graphically in Figure 14. If two fractions were made by cutting at the intersection of the two curves, the results would be as follows:

	Purity, %	Recovery %
Methylcyclohexane	98	93
n-Heptane	93	98

#### Table VII. Appearance and Disappearance Factors for Various Hydrocarbons on Silica-Alumina and Silica-Magnesia

(100-160-mesh; n-heptane developer; 24° C.)

Adsorptive	Adsorbent	Free Space/ Charge	Appear- ance Factor	Dis- appear- ance Factor
1-Hexene Cyclohexene 1,5-Hexadiene 2-Methyl-1,3-penta- diene	Silica-alumina <sup>a</sup> Silica-alumina <sup>a</sup> Silica-alumina <sup>a</sup> Silica-alumina <sup>a</sup>	83 81 81 86	1.10 1.29 1.45 1.87	1.63 1.58 2.64 Reacted on column
1,3-Cyclohexadiene Benzene <i>tert</i> -Butylbenzene	Silica-alumina <sup>a</sup> Silica-alumina <sup>a</sup> Silica-alumina <sup>a</sup>	86 86 83	$1.58 \\ 2.05 \\ 2.29$	$2.92 \\ 5.05 \\ 7.0$
1-Hexene 1-Hexadecene Cyclohexene 1,5-Hexadiene 1,4-Cyclohexadiene 1,3-Cyclohexadiene	Silica-magnesia <sup>b</sup> Silica-magnesia <sup>b</sup> Silica-magnesia <sup>b</sup> Silica-magnesia <sup>b</sup> Silica-magnesia <sup>b</sup>	78 78 78 76 76 76	$1.05 \\ 1.00 \\ 1.28 \\ 1.59 \\ 1.79 \\ 1.61$	2.05 2.39 2.35 3.53 5.00 Reacted on column
Benzene 2-Methyl-1,3-penta- diene	Silica-magnesia <sup>b</sup> Silica-magnesia <sup>b</sup>	78 76	2.18 Reacted	5.89 on column
<sup>a</sup> 87% silica, 13% alumin <sup>b</sup> 70% silica, 30% magne	a. sia.			

Relative adsorbabilities of several alkyl benzenes and a representative diolefin on Grade L carbon with acetone developer are also shown in Figure 13. This acetone-carbon system indicates no separation between diolefins and alkyl benzenes as classes.

Silica-Alumina and Silica-Magnesia Adsorbents. Two commercial cracking catalyst materials were tested for possible selectivity in hydrocarbon separations: alumina-silica (13% alumina) and magnesia-silica (30% magnesia). Both adsorbents were screened to give a 100- to 160-mesh fraction and heated in a vacuum oven at 175° C. for 2 hours for activation. Table VII and Figure 15 show data obtained for representative hydrocarbons of several classes on these two adsorbents. These data indicate both adsorbents, in general, to be inferior to silica gel for effecting separations.

While there is some indication of an advantage over silica gel



Figure 15. Relative Adsorbabilities of Hydrocarbons on Mixed Solids

Shown by presence of adsorptive in effluent Silica-alumina and silica-magnesia, 100- to 150-mesh. *n*-Heptane developer. Ratio of liquid free space to charge volume, 80

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in the separation of alkyl benzenes from diolefins, the reactivity of the latter may preclude their recovery. For analytical purposes, it may prove useful in some cases to make separations on silica gel to obtain a fraction containing alkyl benzenes and conjugated and nonconjugated diolefins. A subsequent percolation over silica-alumina or silica-magnesia might effectively remove the conjugated diolefins.

### CONCLUSIONS

A method has been applied for establishing quantitatively the relative adsorbabilities of hydrocarbons according to molecular type on various solid adsorbents. Reliable predictions have been shown to be possible for the feasibility of analytical separations on a practical scale.

Thus, on silica gel the following separations are possible: (1) mono-olefins from diolefins, (2) mono-olefins from aromatics, and (3) partial separation in some cases of acyclic- from cyclic mono-olefins.

Alumina affords a better separation of aromatics based on the

### number of aromatic nuclei in the molecule. Activated carbon affords a partial separation of straight-chain paraffins from other saturated hydrocarbons.

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## **Circular Filter Paper Chromatography**

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Most of the work on paper chromatography done to date has been of an empirical nature. This paper attempts to elucidate factors of somewhat more fundamental nature. Data obtained using filter paper squares show the effect of various factors on the rate of solvent flow and the degree of resolution of a known cation mixture. The circular technique offers many advantages over the strip method. The solvent flow can be adjusted to the desired rate, thereby shortening considerably the time necessary for a chromatographic separation. This technique appears to produce clearer, better-defined zones. The reflectance densitometer is a convenient instrument for measuring zone widths, points of maximum color density, and  $R_f$  factors.

THE use of circular filter paper chromatography has not been widespread. Early workers who used this technique included Goppelsroeder (7), Skraup (17), Holmgren (8), and Brown (2). Recently, Rutter has called attention to its advantages (15, 16). Rosebeek separated many of the amino acids using this technique (14). Giri et al. have developed quantitative chromatographic procedures for separating amino acids in circular sheets (5, 6). Lüderitz and Westphal extended Rutter's technique by applying discrete spots of material in a circle about the center of the paper rather than as a single spot at the origin, so that many different substances could be compared (9). Bersin and Muller separated many sugars on filter paper disks and compared their results with those using paper strips (1).

As work in paper chromatography is still largely empirical, an attempt has been made to reveal information of a more basic nature which will help workers in this field.

This paper is reports on a continuation of the study of the flow of liquids through paper (12); the use of a reflectance densitometer in measuring zone widths and  $R_f$  values on completed paper

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chromatograms (12); and the efficiencies of separations using filter paper squares as compared to those using strips.

#### EXPERIMENTAL DETAILS

Most of the present work has been performed using filter paper squares.

Two parallel cuts are made from the same edge of a square piece of filter paper to the center of the paper. The "tab" is bent at 90° with the plane of the paper and cut to suitable length. The mixture to be separated is placed on the tab at the point where it joins the center of the square. The paper is then supported on a crystallizing dish (8 cm. in diameter  $\times$  4 cm.) containing the solvent. Another crystallizing dish is used as a cover in order to prevent solvent evaporation. When the solvent flows through the paper, the components of the mixture are separated into circular zones.

Filter paper strips which were attached to a cork by a thumbtack were suspended in a 1000-ml. graduated cylinder containing the solvent. Also in the cork were holes for two glass tubes. One tube, which reached almost to the bottom of the cylinder, carried nitrogen saturated with the solvent being used. The other tube carried away excess gas. The mixture to be analyzed was placed on the strip 1 inch from the end that was immersed in the wash liquid.

A mixture of bismuth trichloride, cupric chloride, and mercuric chloride was separated using butanol saturated with 3N hydrochloric acid (3). One hundred and thirteen micrograms of each of these salts dissolved in excess hydrochloric acid was applied to the paper. The solvent was the top layer obtained after shaking equal quantities of 1-butanol and 3N (2.95N) hydrochloric acid. A large quantity of solvent was prepared initially, so that in subsequent experiments there was no question as to the uniformity of solvent used. After formation of the chromatogram, the positions of the bands were revealed by using hydrogen sulfide. glass rack was made to fit inside a desiccator and support 10 to 20 The air was evacuated from the desiccator and hydrosquares. gen sulfide gas was passed into it. Complete development of colors occurred in a few hours. These colored zones faded imperceptibly when exposed to the atmosphere for many hours

Whatman No. 1 paper was purchased in  $18^{1}/_{4}$  by  $22^{1}/_{2}$  inch sheets. For comparative experiments squares or strips were cut from the same sheet in the machine direction.

Throughout these experiments the temperature was kept at  $24^{\circ} \pm 1^{\circ}$  C.

In experiments with squares, the front of the mercury band was

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indistinguishable from the solvent front, so that the position of the former was used to calculate solvent radii and areas.

### FACTORS AFFECTING RATE OF SOLVENT FLOW IN SQUARES

Width of Tab. Nine squares of paper, 4 by 4 inches, were cut from one sheet of paper. Tabs, 3 to 8 mm., were cut, and the length of each was adjusted to 34 mm. Twenty-five milliliters of butanol saturated with 3N hydrochloric acid was placed in each of nine crystallizing dishes. The squares were placed on the dishes, and similar dishes were used as covers. The flow of liquid through the paper was allowed to proceed for 180 minutes after the solvent had reached the top of the tab.

The area wet by the solvent was calculated from the following relationship:

$$A = \pi r_a r_b - r_b w$$

where A = area,  $r_a = \text{radius}$  of major axis,  $r_b = \text{radius}$  of minor axis, and w = width of tab. The width of each tab was determined by measuring the widths at different points with a traveling microscope and averaging the results.

The values for the tab widths and the wet areas are shown in Table I. As zero width must correspond to zero area, the average slope was calculated by averaging all values of A/w. The resultant equation was:

$$A = 26.2w$$

The average deviation of A (observed) from A (calculated) was 2.43%.

Table I. Effect of Tab Width on Rate of Solvent Flow

Wet Area, Sq. Mm.	Width, Mm.
2065	8.0
2090	8.0
1793	6.7
1721	6.7
1504	6.0
1402	5.1
1363	5.0
1039	4.0
770	3.0

Table II. Effect of Time on Area of Paper Covered by Solvent

Wet Area, Sq. Mm.	Time, Min.	
1170	112	
1070	112	
1452	142	
1820	142	
1784	180	
2446	242	
2955	298	

The proportionality existing between area of the paper covered by solvent and the width of the tab can be shown from known facts. On strips of filter paper (12), the relationship between the height, h, to which liquid rises in paper in a given time, t, is:

$$h^2 = at + b \tag{1}$$

where a and b are constants. At any height, h, A = hw. Substituting A/w for h in Equation 1 gives:

 $A^2 = w^2(at + b)$  $4 = w \sqrt{at \pm b}$ and Therefore, dt

$$\frac{dA}{dt} = \frac{wa}{2\sqrt{at+b}}$$
(2)

Thus the increase in area developed in unit time is proportional to the tab width.

In order to cut tabs of uniform width, two single-edged parallel razor blades were clamped to adjustable brass spacers. A cutter like this will cut tabs in paper squares with a variation of less than 0.05 mm.

Time of Solvent Flow. In order to study the rate of solvent flow, eight squares of paper were cut from one large sheet of filter paper. The widths of the tabs (6.68 mm.) were kept constant by using the parallel razor blade cutter. The same amount of solvent (25 ml.) was placed in each crystallizing dish. Solvent was permitted to flow for varying times and the resultant wet areas were measured. Table II shows the results.

Equation 3 was computed from the results in Table II.

2

$$4 = 10.0t$$
 (3)

The average deviation of A (observed) from A (calculated) was 2.44%

The proportionality of area and time is a direct consequence of  $h^2 = at + b$ . The rate of solvent flow in a strip is considered to be made up of two factors, the capillarity factor and the accessibility factor. At any given height, the capillarity factor is constant and if the width of tab is constant, the accessibility factor does not change. Therefore, the increase in area of the square wet by solvent should be proportional to the time.

$$A = k_1 t$$

This relationship confirms earlier measurements made on paper (11).

If the area developed is plotted against time for squares having tabs of various width, a family of straight lines is formed. The validity of the relationship between area and width can also be shown from Table III. If the slope of each line is divided by the width of the tab. a constant value of  $1.491 \pm 0.020$  is obtained. This corresponds to an average deviation of 1.34%. The precision is better than obtained previously, as the average slopes are taken in each case and the errors involved in individual measurements tend to cancel one another.

Distance between Paper and Solvent. The effect of the distance between the plane of the paper and the solvent on the rate of flow was studied. In order to keep this distance constant

Table III. Relationship of Tab Width and Time to Area of Paper Wet by Solvent

		-	•		
Width, Mm.	Area, Sq. Mm.	Time, Min.	A/t	$\frac{A/t}{w}$	Dev.
4.0	1039	180	5.77	1 510	0 091
4.0	2158	340	6.34}	1,512	0.021
5.0	1363	180	7.57	1 404	0 002
5.0	2137	290	7.37	1,494	0.003
6.7	Slope taken	from Table 1	I 10.00	1.493	0.002
6.0	1575	180	8.75	1 440	0.040
6.0	2070	242	8.55	1.442	0.049
8.0	1645	130	12.66)	1 - 1 - 1 - 2	0.005
8.0	2090	180	11.60	1.516	0.025
			Av.	1,491	0.020

#### Table IV. Effect of Distance between Paper and Solvent on Rate of Flow

Height, Mm.	Time, Min.
6.9	65
15.7	173
$16.0 \\ 19.0$	180 234
26.0 32.0	340 407
32.0	415

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during an experiment it was necessary to prevent the slight sagging at the center of the square when the paper is wet.

Glass vials, 1 cm. in diameter, were ground with emery dust until, when placed inside the crystallizing dishes, the glass vials were of the same heights as the dishes. The bottoms of the crystallizing dishes were covered with solvent and varying amounts of solvent were placed in each vial. Eight squares of paper having tabs of similar width (3.60 mm.) were placed on the dishes. The tabs were immersed in the solvent contained in the vials, while the vials themselves supported the paper at the center of the squares. Solvent was allowed to flow so that equal areas of paper were wetted, and the times were noted. The results of this experiment are shown in Table IV.

By the method of least squares, the equation for the straight line produced was:

$$t = 13.8h - 31$$

The average deviation of t (observed) from t (calculated) was 3.06%.

This line does not intercept the Y axis at zero. It is probable that for very small h's, t would approach zero, although probably not in a linear manner. That the above should be true can be shown from known facts.

Take the case of two squares with tabs of the same width. Let the distance between solvent and paper in one case be  $h_1$ , and in the second case  $h_2$ . Therefore,

$$\begin{array}{rcl} h_1{}^2 &=& Dt_1\\ h_2{}^2 &=& Dt_2 \end{array}$$

The velocity is defined as dh/dt.

$$2hdh = Ddt$$
  

$$dh/dt = D/2h = V$$
  

$$V_1 = D/2h_1 \text{ and } V_2 = D/2h_2$$

Therefore,

and

$$V_2/V_1 = h_1/h_2$$

-----

For equal areas developed, the time, t, is proportional to 1/V. Therefore,  $t_1/t_2 = h_1/h_2$ 

Machine Direction in Filter Paper. The orientation of fibers in a particular paper can be measured by admitting a drop of dilute dye solution to the paper from a fine capillary (12). The ratio of the major to the minor axis is constant in each case, independent of the size of the spot. Using a dilute alcoholic solution of methyl orange, this factor was found to be  $1.15 \pm 0.02$  for Whatman No. 1 paper. Even for a paper with no machine direction, an ellipse will be formed during a chromatographic experiment, because of the effect of the width of the tab.

At any time, t, let  $r_a$  be the radius of paper wet by solvent in a direction parallel to the tab, and  $r_b$  be the radius in the direction perpendicular to the tab. Then, as  $r^2 = kt$ ,

$$r_a = \sqrt{kt}$$
  
 $r_b = \sqrt{kt} + w/2$ 

where w is the width of the tab. At all times, ellipticity defined as  $r_b/r_a = E$ .

Therefore

$$E = \frac{\sqrt{kt} + w/2}{\sqrt{kt}} = 1 + \frac{w}{2\sqrt{kt}}$$

This equation checks in the limits, for when t is 0—that is, when the solvent has just started to enter the square—E is infinite. When t is infinite, E is 1, since the paper has no machine direction.

For a paper with a machine direction, the same derivation is applicable. Thus, if the ellipticity of the wet area produced in such a paper is 1.15, and this is made to coincide with direction b, then

$$r_{a} = \sqrt{kt}$$

$$r_{b} = 1.15 \sqrt{kt} + w/2$$

$$E = \frac{1.15 \sqrt{kt} + w/2}{\sqrt{kt}} = 1.15 + \frac{w}{2\sqrt{kt}}$$

and

In the limits, when t is 0, E is infinite, and when t is infinite, E =1.15.

#### Table V. Effect of Time of Solvent Flow on Ellipticity of **Resultant Zones**

Time, Min.	$1\sqrt{t}$	E (Obsd.)	E (Calcd.)	Dev.
112	0.0953	1.220	1.216	0.004
112	0.0953	1.210	1.216	0.006
142	0.0840	1.200	1.205	0.005
142	0.0840	1.205	1.205	0.000
180	0.0746	1.196	1.195	0.001
180	0.0746	1.188	1.195	0.007
242	0.0643	1.181	1.184	0.003
298	0.0579	1 169	1.177	0.008
			Av.	0.004

The validity of this equation can be checked by comparing the ellipticity of the wet areas of the squares in Table II against E(calculated) at various times, since the width of the tab (6.68 mm.) and k, the diffusion constant (10.0), are known. This comparison is made in Table V.

The extrapolated value of E when  $1/\sqrt{t}$  is 0—that is, when t is infinite-is the same value as would be obtained by directly add-



Figure 1. Recorder Tracing of Completed Strip Chromatogram

ing a drop of dilute dye solution to a piece of filter paper. If this value is used, the equation is:

$$E = 1.116 + \frac{w}{2\sqrt{kt}} \tag{4}$$

The value 1.116 checks fairly well with the value 1.15  $\pm$  0.02 obtained using the dye solution.

#### CHROMATOGRAPHIC MEASUREMENTS ON FILTER PAPER

Use of a Reflectance Densitometer.  $R_f$  values, the thickness of zones, and the distance between zones on completed paper chromatograms were determined with a reflectance densitometer. The output of the cell is connected directly to a 100-ohm poten-

tiometer, and the center tap and one end of the potentiometer are connected through shielded and grounded wires to the Brown Electronik recording potentiometer. Thus, the output of the photocell can be varied from zero to a maximum.

As the sample drum rotates uniformly, the photocurrent varies in proportion to the reflectivity and this is faithfully reproduced on the recorder chart. As the chart is also synchronously driven, the chart distance corresponding to 1 mm. on the drum can be calculated from the known speeds of drum and recorder paper.

Although the use of this instrument for concentration measurements is inferior to a transmittance technique, it is very useful for other factors such as width of zones, separation between zones, and  $R_{f}$  values.

Interpretation of Reflectometer Scanning. The mechanism of the scanning process may be represented as follows:

Imagine the scanning patch of light to be rectangular and moving across the opaque zone from left to right. As long as the light is moving along white areas in the paper, the reflectance will be a maximum. As soon as the edge of the light patch reaches a colored zone, the pen starts to move in a direction corresponding to decreased reflectance. When the light patch is within the colored zone, the recorder indicates minimum reflectance. When the light patch has passed the colored zone, maximum reflectance occurs again.

Figures 1 and 2 show typical patterns produced in scanning paper chromatograms. In order to relate distances on the chromatogram to distances on the recorder paper, the width at the base of the recorder curve, indicating maximum reflectance, is divided by a known factor (12.7 in one case). This distance, W, is related to w, the width of the zone, and x, the width of the light beam as shown below.

It can be seen that while the recorder paper has moved a distance W, the scanning drum has moved a distance w + x. Since w, the width of the zone, is what we are interested in: w = W - x. The same line of reasoning may be applied to the measurement of distances between zones. While the scanning drum has moved a distance w' - x, the recorder paper has moved W'. Thus w', the distance between two zones on paper, is given as: w' = W' + W'x.

The analysis given above was confirmed by scanning a strip of paper on which were drawn several India-ink rectangles of various known widths and distances apart.

In analyzing a chromatogram the situation is not as ideal as pictured above. A light beam is not of uniform intensity. Furthermore, a zone is not sharply defined at its edges. The result is that the recorder curve will show a pronounced "toe." In practice, the straight-line portion of the curve has been extended to coincide with the "base" line. This value for the "width" of a zone is repeatable. Furthermore, in comparing the widths of zones under various conditions, any "error" will be canceled, owing to the consistency of the recorder.

In analyzing paper strips the procedure has been to scan the chromatograms from beginning to end at a point of maximum zone width. In analyzing squares, the procedure was first to scan the squares at right angles to the direction of the tab, starting at the solvent front and ending at the solvent front. This gave two

Separations in Squares after Development Figure 3.

96 minutes

151 minutes 200 minutes

R

D

4 cm.-

D. 252 minutes E. 332 minutes



Table VI.	Effect of Time of Development on Band Widths, Band	d
	Separations, and $R_r$ Factors	

Time.	Solvent	Band	Widths,	Mm.	Ba Separatio	nd ons, Mm.	Rr Factors		
Min.	Radius	Cu	Bi	Hg	Cu-Bi	Bi-Hg	Cu	Bi	Hg
9696151200252252332332	$19.2 \\ 19.4 \\ 25.4 \\ 28.3 \\ 27.9 \\ 30.8 \\ 31.5 \\ 34.1 \\ 35.7 \\$	$\begin{array}{r} 4.58\\ 4.39\\ 4.00\\ 4.26\\ 4.14\\ 4.19\\ 4.32\\ 4.26\\ 4.56\\ 4.60\end{array}$	$\begin{array}{r} 4.68\\ 4.83\\ 4.36\\ 4.36\\ 4.60\\ 4.80\\ 4.52\\ 4.94\\ 5.35\\ 5.47\end{array}$	$1.66 \\ 1.85 \\ 1.31 \\ 1.39 \\ 1.40 \\ 1.35 \\ 1.28 \\ 1.25 \\ 1.33 \\ 1.43$	$\begin{array}{c} 2.73\\ 2.80\\ 5.34\\ 4.92\\ 6.32\\ 5.83\\ 7.15\\ 7.41\\ 7.78\\ 8.17\end{array}$	$\begin{array}{c} 0.43\\ 0.58\\ 1.70\\ 1.48\\ 2.19\\ 2.23\\ 2.40\\ 2.33\\ 2.73\\ 2.78\end{array}$	$\begin{array}{c} 0.452\\ 0.452\\ 0.456\\ 0.448\\ 0.447\\ 0.450\\ 0.452\\ 0.449\\ 0.459\\ 0.443\\ \end{array}$	$\begin{array}{c} 0.818\\ 0.828\\ 0.818\\ 0.823\\ 0.819\\ 0.822\\ 0.826\\ 0.826\\ 0.827\\ 0.828\\ 0.825 \end{array}$	$\begin{array}{c} 0.982 \\ 0.975 \\ 0.987 \\ 0.988 \\ 0.989 \\ 0.991 \\ 0.989 \\ 0.995 \\ 0.988 \\ 0.988 \\ 0.989 \end{array}$
Av.		4.33	4.79	1.43			0.451	0.823	0.987

Table VII. Effect of Tab Width on Band Widths, Separation between Bands, and  $R_r$  Factors

Tab Width.	Tab Vidth, Band Widths, Mn		Mm.	Ba Separatio	ond ons, Mm.		Time		
Mm.	Cu	Bi	Hg	Cu-Bi	Bi-Hg	Cu	Bi	Hg	Min.
$4.0 \\ 4.1 \\ 5.1 \\ 5.0 \\ 6.0 \\ 0 \\ 1$	$\begin{array}{r} 4.13 \\ 4.20 \\ 4.40 \\ 4.20 \\ 4.21 \\ 4.21 \end{array}$	$\begin{array}{r} 4.38 \\ 4.38 \\ 4.40 \\ 4.56 \\ 4.60 \end{array}$	1.20 1.12 1.12 1.20 1.20 1.20		$1.85 \\ 1.72 \\ 1.88 \\ 1.83 \\ 1.89 \\ $	$\begin{array}{c} 0.440 \\ 0.434 \\ 0.438 \\ 0.446 \\ 0.437 \end{array}$	$0.829 \\ 0.840 \\ 0.834 \\ 0.834 \\ 0.828 \\ 0.828 \\ 0.822 \\ 0.828 \\ 0.82$	$\begin{array}{c} 0.985 \\ 0.982 \\ 0.981 \\ 0.984 \\ 0.978 \\ \end{array}$	$350 \\ 340 \\ 300 \\ 290 \\ 242 \\ 242 \\ 300 \\ 300 \\ 242 \\ 300 \\ 300 \\ 300 \\ 242 \\ 300 $
$6.1 \\ 6.7 \\ 6.7 \\ 8.0 \\ 8.0 \\ 8.0$	$\begin{array}{r} 4.24 \\ 4.27 \\ 4.40 \\ 4.56 \\ 4.50 \end{array}$	$\begin{array}{r} 4.58 \\ 4.94 \\ 4.70 \\ 5.32 \\ 5.58 \end{array}$	$     \begin{array}{r}       1.30 \\       1.28 \\       1.24 \\       1.32 \\       1.38 \\       1.38 \\       \end{array} $	$\begin{array}{c} 6.50 \\ 6.24 \\ 6.35 \\ 5.79 \\ 6.08 \end{array}$	$     \begin{array}{r}       1.92 \\       1.89 \\       1.95 \\       1.97 \\       1.88 \\     \end{array} $	$\begin{array}{c} 0.440 \\ 0.443 \\ 0.437 \\ 0.456 \\ 0.450 \end{array}$	$\begin{array}{c} 0.830 \\ 0.828 \\ 0.824 \\ 0.828 \\ 0.832 \end{array}$	$\begin{array}{c} 0.982 \\ 0.980 \\ 0.983 \\ 0.980 \\ 0.976 \end{array}$	$248 \\ 217 \\ 210 \\ 180 \\ 180$

values for the width of each zone and the distance between any two zones. Next, the square was scanned from the center of the square to the solvent front in a direction parallel to that of the tab. This gave another value for each zone. The three values obtained for each distance were then averaged to obtain the mean value. Typical recorder tracings are shown in Figures 1 and 2.

#### SEPARATIONS IN FILTER PAPER SQUARES

Filter paper squares were employed to study various factors which affect the separation of a mixture of cupric chloride, bismuth trichloride, and mercuric chloride.

Separations as a Function of Time of Development. Ten filter paper squares with tabs of the same width (6.68 mm.) were immersed to the same height of solvent. After being developed for different times, the squares were sprayed with hydrogen sulfide and the various distances measured. The resultant data are shown in Table VI. Figure 3 shows five of these squares. The  $R_r$ factor is defined as the radius to the point of maximum density in a band divided by the radius to the solvent front.

From Table VI it is seen that the metal zones get thinner at first and then begin to widen gradually. The widening of zones, or "tailing," is always noted in the case of filter strips. However, this effect appears to be less in the squares, as the increased diameter of each zone partly compensates for the tailing effect. In fact, over the times measured, the greatest deviation in the thickness of any band is less than 1 mm.

The  $R_r$  factors, measured at right angles to the direction of the tab, are shown to be constant and independent of time of develop-

ment. The copper  $R_r$  was  $0.451 \pm 0.0034$ , for an average deviation of 0.75%. The bismuth  $R_r$  was  $0.823 \pm 0.0034$ , for a deviation of 0.41%. The mercury  $R_r$ was  $0.987 \pm 0.0032$ , or 0.35%.

An experiment was performed which was the same as that just described, except that the tabs were cut at right angles to the machine direction of the paper. The results confirmed the data in Table VI.

Separations as a Function of Tab Width. Since increasing the tab width increases the rate of solvent flow, its effect on the separation of copper, bismuth, and mercury was studied. The widths of the tabs were varied from 4 to 8 mm. Each square was developed to the same total area (approximately). The results are shown in Table VII.

From Table VII it is seen that the copper zone is only slightly widened by increasing the tab width. This is due in part, no doubt, to the low  $R_r$  factor that is, under all the conditions studied, this zone moves slowly enough to make approach to equilibrium between paper and solvent phase possible. The mercury band behaves almost ideally. This zone is virtually at the solvent front and in each case remains concentrated in a very narrow zone. The bismuth zone shows significant tailing. This spreading is almost entirely at the rear of the zone, having the effect of decreasing the copper-bismuth separation but having little effect on the bismuth-mercury separation.

The rate of development of the squares with 4-mm. tabs is about half that of the squares with 8-mm. tabs; yet there

is no perceptible change in  $R_r$  factors. This would indicate that the ratio of the metal zone distance to the solvent distance is a true constant, independent of rate of flow of solvent.

Separations as a Function of Distance between Solvent and Paper. Using the parallel razor-blade cutter, tabs 3.6 mm. wide were cut in five similar squares. The distances between plane of paper and solvent level were varied and all squares were developed with solvent to the same total areas. The results are shown in Table VIII.

The first two columns show the times taken for development and the distances between plane of paper and solvent level. Columns 3, 4, and 5 show the effect of increasing the rate of solvent flow on the width of the metal zones. The copper and mercury zones again are fairly constant regardless of speed (except for the mercury zone in the first case). However, the change in width of the bismuth zone is marked. For a 400% increase in rate, the bismuth zone increases in width about 50%.

Table VIII. Effect of Distance between Solvent and Paper on Band Widths, Separation between Bands, and  $R_r$ Factors

Time	Dis-	Bai	Band Widths, Mm.			nd itions, n.	Rr Factors		
Min.	Mm.	Cu	Bi	Hg	Cu-Bi	Bi-Hg	Cu	Bi	Hg
97	9.0	4.07	6.07	1.80	5.34	0.00	0.445	0.829	0.962
180	16.0	3.99	4.57	1.20	6.20	1.32	0.439	0.832	0.968
234	19.0	3.92	4.60	1.12	6.20	0.40	0.462	0.850	0.973
340	26.0	4.15	4.42	1.35	5.94	1.39	0.466	0.842	0.970
407	32.0	4.16	4.18	1.26	5.68	2.17	0.458	0.827	0.971

#### Table IX. Effect of Time of Development on Separations Obtained Using Filter Paper Strips

Time.	Solvent	Band Widths, Mm.			Ba Separatio	and ons, Mm.	R <sub>f</sub> Factors		
Min.	Ht., Mm.	Cu	Bi	Hg	Cu-Bi	Bi-Hg	Cu	Bi	Hg
90	82	16.6	27.5	11.2	9.55		0.220	0.708	0.840
90	80	16.4	26.0	12.0	8.90		0.210	0.680	0.836
160	110	18.2	32.4	15.2	17.7	1.1	0.209	0.650	0.857
190	118	20.6	34.7	15.2	20.2	6.8	0.196	0.641	0.867
230	126	19.4	35.8	14.3	28.0	3.2	0.211	0.684	0.870
300	143	19.0	37.3	13.3	34.2	14.7	0.216	0.655	0.892
360	158	25.0	37.0	14.8	41.5	12.2	0.212	0.690	0.880
420	171	27.2	38.6	14.7	47.9	17.4	0.214	0.708	0.918
						Av.	0.211	0.677	0.873

The last five columns show the separations between the zones, and the  $R_r$  factors. Although it is apparent that separations become better with increasing time of development, the values for the copper-bismuth and bismuth-mercury distances do not seem to vary in an orderly manner. The reason for this lies in the  $R_r$ factors. The  $R_r$  factor for copper is 0.454  $\pm$  0.0096; that for bismuth is  $0.836 \pm 0.0088$ , but individual values vary beyond these limits. A small change in R, greatly changes the distances between zones.



Speed of Separation in Strip and Square Figure 4.

Comparison of Paper Square and Strip in Separations. EFFI-CIENCY OF SEPARATIONS. In filter paper squares, the constancy of the  $R_r$  factor (the ratio of the radii of solute and solvent) measured at right angles to the direction of the tab has been shown. By taking 31 squares which were developed for varying times and had tabs of different widths, the following values were obtained:

Copper	0.442	±	0.0086	=	1.94%
Bismuth	0.828	±	0.006	=	0.73%
Mercury	0.974	±	0.003	=	0.36%

To compare separations in strips and squares, eight strips of paper were cut. 1 inch wide, and developed with solvent for varving times. The results are shown in Table IX.

A linear relationship exists between the square of the distance traveled by the solvent and time. By the method of least squares, the equation is:

$$h^2 = 66.8t + 958 \tag{5}$$

The average deviation of  $h^2$  (observed) from  $h^2$  (calculated) is 3%

From columns 3 and 4 it is seen that the widths of the copper and bismuth zones vary from 1.5 to 7 hours. The mercury zone remains narrow throughout this time interval. Columns 6 and 7 show the separations between zones. It is apparent that after sufficient time these separations are greater than in the squares.

#### ANALYTICAL CHEMISTRY

However, it is of interest to find out which technique gives the fastest ones

In a strip, the relationship between height of solvent and time is given as:

$$h_s^2 = at + b \tag{6}$$

(7)

The  $R_f$  factor for a solute,  $m_1$ , is  $h_{m_1}/h_s$ .  $h_s = h_{m_1}/R_{f_{m_1}}$ Therefore,

Substituting in Equation 6:

 $h_{m_1} = R_{f_{m_1}} \sqrt{at+b}$ 

And for another solute, 
$$m_2$$
:  
 $h_{m_2} = R_{\ell_{m_2}} \sqrt{a\ell + b}$ 

Subtracting,  $h_{m_2} - h_{m_1} = (R_{l_{m_2}} - R_{l_{m_1}}) \sqrt{at + b}$ 

The actual separation between zones is somewhat less than  $(h_{m_2}$  $h_{m_1}$ ), which is the distance between the centers of the bands. If  $(S_{m_2}-S_{m_1})$  is the actual separation and  $W_{m_1}$  and  $W_{m_2}$  are the average widths of the bands, the following is approximately true:

$$h_{m_2} - h_{m_1} = (S_{m_2} - S_{m_1}) + \frac{1}{2} (W_{m_1} + W_{m_2})$$

Substituting in Equation 7:

$$(S_{m_2} - S_{m_1}) = (R_{I_{m_2}} - R_{I_{m_1}}) \sqrt{al + b} - \frac{1}{2} (W_{m_1} + W_{m_2}) (8)$$

From this equation it is seen that if the difference in  $R_f$  factors is constant and the widths of the solute zones do not change too much, a plot of the separation between zones against  $\sqrt{at+b}$ should be linear. Furthermore, the value of t, when the separation is zero, gives the time necessary before separation begins. If we define  $R_r$  in the square as the

ratio of the radii of solute and solvent, Equation 9 may be derived in the same way.

$$\frac{(S_{m_2} - S_{m_1}) = (R_{r_{m_2}} - R_{r_{m_1}})}{\sqrt{kt + c} - \frac{1}{2} (W_{m_1} + W_{m_2})}$$
(9)

The only difference between Equations 8 and 9 is in the values of the constants.



Separations in Strips after Development Figure 5. 90 minutes 300 minutes S. T. 181 minutes 420 minutes
	(Calculated values	)
Time, Min.	Cu-Bi, Mm.	Bi-Hg, Mm.
90	7.4	0.0
90	8.5	0.0
160	20.5	00
190	22.7	3.7
230	27.1	5.6
300	37.9	9.8
360	39.5	13.2
420	43.1	15.5

## Table X. Effect of Development Time of Band Separa-

Equations 8 and 9 make it possible to compare speed of separation of square and strip. However, these equations assume unchanging R factors. In the case of the squares these factors are reasonably constant, but, as seen in Table IX, the values for the strips vary. In order to test Equations 8 and 9, the values for the separations between zones were recalculated, assuming that the  $R_f$  factors for each filter paper strip were identical with the average values given. These "adjusted" values are given in Table X. In Figure 4, the separation between zones is plotted against  $\sqrt{66.8t + 958}$  (from Equation 5). From the values of  $\sqrt{66.8t + 958}$  when the separations are zero, the times necessary for separations to occur are 47 minutes for the copper and bismuth zones (plot A), and 141 minutes for the bismuth and mercurv zones (plot B).

Figure 4 also shows the separations for squares plotted against the square root of the time. From the values of  $\sqrt{t}$  when separations are zero, it is seen that 23 minutes are required for the copper and bismuth zones to separate (plot C) and 45 minutes for the bismuth and mercury (plot D).

Although the actual figures given for initial separations are of little accuracy, they show that separations occur faster in the squares in this case. This may be shown pictorially by referring to square A in Figure 3 and strip S in Figure 5. In the case of the square, after 90 minutes, separation is already complete, while in the strip it is not. (Actually, it appears that separation of the bismuth and mercury zones is complete in the strip, but, as shown by the photometric record, it is not. The light space between the zones is yellow, indicating incomplete separation.)

The speed of separations in the square is at least as fast as in the strip. For efficiency and clear definition of zones the square method is often superior. The volume of solvent necessary for separation is much less in the square. The height of solvent in strip, or radius of solvent in square, necessary before separation occurs depends on the widths of the zones. Since these zones are much thinner in the square, less development with solvent is required for the desired separation.

Measurements of R factors, zone widths, or areas are much more accurate with the square method. There is less tailing of zones and the "center of gravity" of each zone is much more clearly defined. This will be apparent from Figures 1 and 2.

This discussion is not meant to minimize the importance of paper strip chromatography, which for many purposes, especially two-dimensional chromatography, is very valuable. Rather it is desired to point out the advantages of using squares, so that this technique will be used more widely.

COMPARISON OF  $R_f$  and  $R_r$  FACTORS. The factor R was originally introduced by Martin and Synge in their theoretical treatment of partition chromatography (10). The term  $R_f$  was introduced by Consden in extending this theory to paper chromatography (4), and was defined as follows:

$$R_f = \frac{\text{movement of zone}}{\text{movement of advancing front of liquid}}$$

Mathematically, the following relationship was shown to exist:

$$R_f = \frac{A_1}{A_1 - \alpha A_s}$$

where  $A_1$  = area of cross section of the mobile phase,  $A_s$  = area of cross section of the nonmobile phase, and  $\alpha$  = partition coefficient.

Thus, the  $R_f$  factor is a ratio of areas. In the case of the filter paper strip, the area is directly proportional to the height of solute and solvent, and to the width of the strip. As the width of the strip is constant, the ratio of areas reduces to the ratio of the distances traveled by solute and solvent, respectively.

In dealing with squares, the areas of paper traversed by solute and solvent are proportional to the square of their respective radii. Thus, the  $R_f$  factor obtained from an experiment with squares would be the square of the ratio of the radii of solute and solvent.

Table XI shows the  $R_r$  values (ratio of radii of solute and solvent) obtained in this work. The squares of these values are then compared with  $R_f$  values obtained from experiments with paper strips. The agreement for copper and bismuth is seen to be good. The mercury values do not check closely, but a large factor in this discrepancy can be attributed to the use of an average  $R_f$  value for mercury. As Table IX shows, this value increases steadily as the length of the experiment increases.

Table XI.	Comparison of R	Factors in S	Square and Strip
	Rr (Square)	$R_{\tau^2}$ (Square)	Rf (Strip)
Copper Bismuth Mercury	$\begin{array}{c} 0.442 \\ 0.828 \\ 0.974 \end{array}$	$\begin{array}{c} 0.195 \\ 0.685 \\ 0.948 \end{array}$	0.211 0.677 0.873

In experiments with squares, the ratio of the radii of solute and solvent is the criterion for possible separations. Therefore, the use of a new constant would seem convenient. Of course, this constant could be easily deduced from the known  $R_f$  value.

Confirmation of this relationship between strip and square can be found in the work of Bersin and Müller (1). Using the method of Partridge and Westall (13), they separated many sugars using filter paper disks and compared the " $R_f$ " values obtained with those reported by Partridge for paper strips. Although the relationship was not shown in the article, the squares of their " $R_f$ " values compare very favorably with the  $R_f$  values obtained with strips.

Many of these considerations would not be applicable to systems having variable  $R_f$  values.

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# Characterization of Essential Oils by Chromatography

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A method for the rapid inspection of a large number of essential oils was needed. It was desirable that the method characterize various oils by the patterns they produced as well as give tentative identification of major constituents. Chromatoplates were prepared by coating glass plates with silicic acid using starch as a binder. After development, the plates were inspected under ultraviolet light for ultraviolet-active materials. The plates then were sprayed with an acidic solution of 2,4-dinitrophenylhydrazine and inspected under visible and ultraviolet light to detect ketones. After heating, the plate was again inspected under visible and ultraviolet light to detect heat- and acid-sensitive materials. As a result of this study the identity of many essential oils can be indicated. Variation in condition of the adsorbent is minimized by use of the plates. The five-stage inspection of the plates gives opportunity for characterization not often possible in a single chromatogram.

CENSITIVE tests for essential oil constituents were needed to in aid in evaluating the results of a mint-breeding program which has been under way for several years. The chromatographic method was indicated from the start. Classical methods of distillation and preparation of derivatives were not useful, since in most cases the amount of oil available was 0.1 to 1.0 ml., and many plants were to be studied. Infrared was not used in most of the work, since the large number of compounds present in the unfractionated oils would confuse the picture and make translation of the curve a most complex undertaking. After a survey of various chromatographic methods, the use of chromatoplates was adopted. These were glass plates coated with silicic acid, using starch as a binder (4). They are direct descendants of the chromatostrips developed by Kirchner, Miller, and Keller (3). These authors indicated the use of plates for a different purpose-namely, two-dimensional chromatography. Other methods of chromatography tried did not give good separations of the terpenes of interest. Another major problem was to locate the colorless constituents on the plates. Three new tests were applied to the plates in working with the mixtures of compounds in essential oils. These are of general usefulness with many types of organic materials. On a single chromatoplate many ketones and ultraviolet-active and heat-sensitive materials could be detected by a five-step inspection sequence.

Tests for ketones were emphasized, since it was apparent that ketones were often key compounds in the oils. This was particularly true in the mints. Terpenic ketone dinitrophenylhydrazone derivatives did not separate well and it was, therefore, of great value to run the whole oil on the chromatoplate and then subsequently prepare the derivative on the plate by spraying with a solution of dinitrophenylhydrazine. The derivatives formed on the plate had colors often characteristic of their degree of unsaturation. Thus, furfural gave a red spot, carvone, an orange-red spot, pulegone, an orange to yellow spot, and butyraldehyde, a yellow spot.

Ultraviolet-adsorbing materials could be detected on the same plate used for the ketone tests if an ultraviolet-active material were added to the coating mixture, and the active materials observed by masking effect. Kirchner, Miller, and Keller (3), adopting the suggestion of Sease (6), used zinc cadmium sulfide and zinc silicate. It was found that Rhodamine 6G also could be used for the same purpose. The latter dye was a useful additive for ketone tests, as suggested by White and Dryden (7), because after spraying with dinitrophenylhydrazine the dinitrophenylhydrazones were visible under ultraviolet light on a chromatoplate containing the dye. This was a convenient addition to the observation of the derivatives in the visible range and was more sensitive in some cases.

Heating the plate after spraying it with the hydrochloric acid solution of dinitrophenylhydrazine usually revealed new spots under visible and ultraviolet light. Heat- and acid-sensitive compounds gave red, brown, or violet colors in the visible under these conditions. Although the acid conditions were adequate to bring out colors due to some of the materials, the use of heat made additional materials visible and reduced the time for the test.

The chromatoplates rather than the strips were used to avoid the difficulties of variation of  $R_f$  values. Although the original description of chromatostrips indicated several precautions necessary for consistent results (3), the original workers still reported that the  $R_f$  of their control, limonene, varied from 0.41 to 0.55. It was easy to depart very slightly from the standard procedure and obtain great variation in  $R_f$  values as a result of differences in thickness of coating or moisture content. Often it was apparent that other constituents in the oils caused variation in the  $R_f$  values. Essential oils differ greatly in the type of constituents present, and these constituents essentially could affect the nature of the developer. Often the identification of a ketone in 5% concentration was attempted in the presence of 50% of an alcohol or ketone in the same oil. It was decided, therefore, to use the wide plates and run pure, suspected compounds on the same slide simultaneously with the unknown material and to mix the known compounds with the unknown material to see if the spots could be separated chromatographically with various developing solvents. In this way the uncertainty of many of the identifications was removed.

Figure 1 demonstrates the usefulness of the plates even though the  $R_f$  values varied. Various compounds including carvone were run on chromatoplates on which the  $R_f$  values of





carvone varied widely. For instance, if the  $R_f$  values for menthone, dihydrocarvone, and furfural were plotted against the  $R_f$ values of carvone on the same plate, a straight-line function was obtained. The relative positions of any two materials on a path were nearly constant, and a fairly consistent ratio of  $R_f$  values could be calculated.

Thus, by knowing the location of one compound, the general location of the others could be predicted. In the early work, furfural was used for a reference compound, since it gave a good test with dinitrophenylhydrazine and appeared in a region apart from most terpenic ketones. However, Figure 1 demonstrated that the slope of the furfural line was too slight to make that compound of much use. Carvone or butyraldehyde was more useful.

### EXPERIMENTAL

Preparation of the Chromatoplates. The preparation of the chromatoplates followed the directions for the chromatostrips  $(\mathcal{S})$ . Besides the usual technique of directly spreading the starch-silicic acid mixture on the glass, an alternative method of coating the plates was to add an additional volume of water to the mixture after heating, and spray it on the plate with a small paint sprayer. A mixture of 28.5 grams of silicic acid (Fisher, c. P., precipitated), 1.5 grams of starch (amylopectin, or cornstarch can be used), 0.0011 gram of Rhodamine 6G, and 54 ml. of water was stirred on a water bath at 85° C. until a short time after the mixture thickened. After the mixture was removed from the bath, 20 ml. of water was added. The mixture was then spread on plate glass 5 inches wide and 7 inches high. Metal separators were used to give about 0.02 inch of coating. Certain batches of silicic acid were inferior to others in adsorption power and were not used.

Development of the Chromatoplate. After the plates were dried 20 minutes in an oven at 105° C. followed by 30 minutes at 2 mm. over potassium hydroxide, 1 to 2  $\gamma$  of the materials were applied with ultramicropipets as rapidly as possible along a line 2 cm. above the bottom of the plate. For rough work syringes and hypodermic needles were used, with the materials in Skellysolve C solutions. The concentration used depended upon the relative amount of the particular test in question.

Development of the materials was accomplished in a covered battery jar, using 10 to 15% ethyl acetate in hexane (Skellysolve B purified through an alumina column) as developing agent. This gave results comparable to those obtained by Kirchner, Miller, and Keller (3) with 15% ethyl acetate in hexane. The use of hexane alone, also used by these earlier workers, was found to be very useful for the separation of terpene hydrocarbons in the present applications. Benzene was used for further tests of identity of compounds.

Location of Compounds. The detection of ultraviolet materials was made immediately after the developing solvent had traveled 12 to 14 cm. from the initial line. The chromatoplate was then sprayed with a solution of 0.4 gram of 2,4-dinitrophenylhydrazine (DNPH) in 100 ml. of 2N hydrochloric acid as used by Bland (2) for paper chromatography. After the position of the ketones and the color of the derivatives was marked, the plate was inspected under ultraviolet light to find trace ketones. The plate was then placed in an oven at 105° C. for 10 minutes and again inspected under visible light and ultraviolet light to detect heat- and acidsensitive materials. This consecutive series of observations, with the notation of the time of appearance of the spots in addition to their color and location, then became a characterization of the material under study.

**Recording of Data.** Tracings on onionskin paper were made of the chromatoplate and the areas colored appropriately with colored pencils. In many cases, slides were made directly by photographing the plate.

### APPLICATIONS OF THE TECHNIQUE

The tentative identification of specific compounds by this method is dependent upon a knowledge of the nature of the spots produced by known materials. Some of these have been reported by Kirchner, Miller, and Keller  $(\mathcal{J}, \mathcal{J})$ . Many others have been run during the present work. Examples of some materials which can be detected on a single chromatogram, using the described sequence of tests, are given in Table I. The  $R_f$  values given are to be regarded as relative and not as absolute ones.

Table I. Range of Compounds on a Chromatoplate

		-	-
Compound	$R_f \times 100$	Test	Color
Monocyclic terpenes	95-100	Ultraviolet and heat	
n-Valeraldehyde	75	DNPH spray	Yellow
Butyraldehyde	6 <b>8</b>	DNPH spray	Yellow
Menthone	58	DNPH sprav	Yellow
1,8-Cineol	54	Heat	Red-brown
Dihydrocarvone	50	DNPH spray	Yellow
Pulegone	47	Ultraviolet (faint) and	Yellow
-		DNPH sprav	
Carvotanacetone	44	DNPH spray	Red
Carvone	41	Ultraviolet and DNPH	Orange-red
Citral	39	Ultraviolet and DNPH	Yellow
Eugenol	36	Ultraviolet and DNPH	Brown-violet
Linalool	31	Heat	Red-brown
Furfural	13	DNPH	Red

The chromatoplate method has been used for characterization of several essential oils. A typical result is shown in Figure 2 where spearmint-type oils were used. Carvone is one of the main constituents of all the oils. However, the odor of each oil is distinctive, and comparison of the relative size of the spots showed significant differences. For instance, the commercial spearmints, such as the American, contained relatively little dihydrocarvone, and this spot did not appear when 1  $\gamma$  quantities were used. *Mentha crispa* on the other hand had nearly as large a dihydrocarvone spot as carvone, and *M. spicata*, Line 1 strain oil actually had a larger dihydrocarvone area. These results have been checked on oil from three different crop years and, in the case of the *M. spicata* and commercial oils, from plants grown both in Europe and the midwestern and western areas of the United States.



I, M. crispa; II, M. spicata, Line 1 strain; III, American spearmint; IV, Scotch spearmint \\\, spot visible under ultraviolet; ///, spot visible after DNPH spray; open spot, spot visible after spraying and heating

In Table II are given the patterns of some essential oils which have been examined in the above manner. The wide range of types is clearly shown. The developing solvents in all these cases was 10% ethyl acetate in Skellysolve B. One microgram of the oil was used in this survey work. The table indicates in the first column the location of the spots as the  $R_I \times 100$  value. The next three columns give the type of test obtained at each area. In the column headed "UV" are indicated those materials

	<b>n</b>	14	Tost	CHIOMA		Tus of Some Essentia	10115		Test		<b>.</b>
	$\frac{R_f \times 100}{100}$	ŪV	Spray	Heat	Intensity of Spot		$R_f > 100$	< <del>UV</del> -	Spray	Heat	of Spot
Anise oil	94			x	Low	Monarda punctata (hors	e-				
	$\frac{62}{28}$	x	Р	x	Low	mint oil)	93 65	x	Y	x	Low Low
	18		Or	-	Low		50	_	Ŷ	-	Low
Carvone	37			x	LOW		35 20	x	F 1	BrR	-
Bergamot oil	97			BrR	Low		11 5	x x	Ŷ Y	BrR	Low
	66 40		Or	BrR	Low		3		v	BrR BrB	
	$\frac{33}{25}$	x		BrR Br	Low	Carvone	32	*	1	DIR	
	18	x		Br	Low	Neroli oil	95	х		$\mathbf{BrR}$	Low
-	ő	x x	Or		Low		73 66		XO	$\mathbf{BrR}$	Low
Carvone	38						44 37		Y x <sup>b</sup>		Low <sup>a</sup>
Camphor oil	97 78	x	Or	BrR Vi	Heavy		33		-	BrR	
	45 33	x	Y		Low		20	x		BrR	
	28	x	v	BrR	Low	Carvone	0 47	x	Ŷ	BrR	
	13	x	x	Bror	Low	Orange oil, California, e	x-				
Carvone	38					pressed	92 54		v	$\mathbf{BrR}$	Low
Clove bud oil	97 71		Y	BrR	Low		49		v	$\mathbf{BrR}$	Low
	33 16	x	Vi OrB	BrR	Low		26		T	BrR	
0	2	x	ÖrR	Br	Low		18	x	Y	BrR Br	Low
Carvone	38				<b>.</b> .	Carvone	41				
Dill oli	67 44	x	Y OrR		Lowa	Pennyroyal oil, European	94 55		v	$\mathbf{BrR}$	Low
	33 18	x	OrR	OrR	Low		39	x	ÖrY	BrR	*
	11	x	Ör	BrR	101		18	x	Ŷ Y	BrR	Low
	3	x	Or OrR	BrR			3	×	v	BrR Br	Low
Carvone	$\begin{array}{c} 0\\ 32 \end{array}$	x	OrY	$\mathbf{BrR}$		Carvone	3ž	~	•	201	20.
Eucalyntus dives	89			BrR		Pettigrain oil, Paraguay	94			$\mathbf{BrR}$	Low
Elouis prus stres	52		Y	2.10	Lowa		67		Xo	BrR	
	31		Ŷ		Low		44 38	x	Y		
	$\frac{30}{17}$	x		Or BrR	Low		36		x <sup>b</sup>	BrR	Low
Carvone	$\frac{2}{42}$		Y	BrR	Low		22		X <sup>0</sup>	BrR	Fo.
Gerenium oil	94		v	BrB			3 0	x	OrY	BrR Br	
Geranium on	68		л 37	BrR	Low	Carvone	41				
	34 38		Y	BrVi	Low	Pycnanthemum pilosum	94 50		v	x	Low
	$\frac{32}{21}$		x	BrR BrR			35	х	ŌrR OrR	OrR	
	18		v	BrR	Lout		18	x	Ör	OrR	Low
~	ō		Ŷ	Br	10.0		11	x	Or Or	BrR	Low
Carvone	43						3	x	OrR	BrR BrB	
Lavender, spike	94 82		Y	BrR	Low	Carvone	32	•	011	DIR	
	56 50		v	$\mathbf{BrR}$	Low	Sage oil	94			BrR	Lout
	33		1 17	BrR			54		Y	Brn	LOW
	$\frac{25}{12}$		Y	BrR	Low		31 22			BrR BrR	
Carvone	0 43		Y	Br		Carvone	43				
Lemon oil. California. ex-						Tansyloil	92 63	x	v	$\mathbf{BrR}$	Lows
pressed	96 61			BrR	Low		40		x <sup>b</sup>	$\mathbf{BrR}$	2011
	41	x	OrY	A D_D	1		23	x	Ork	OrR	-
	$\frac{31}{27}$	x		BrR	Low		17 13		Ŷ Y	BrR	Low Low
	20 18	x x					3		Y	Br BrB	
	7	x	Y	Ð-		Carvone	32	•	011	DIR	
Carvone	43	x	I	Dr		Thyme_oil	95	x	77		T
Lemongrass oil	98			BrR	Low		46	x	P	_	Low
	$\frac{54}{38}$	x x	Or Y Or R	BrR			32 15		BrY	BrR BrR	Low
	28 15	x		BrR			7		B.V.	BrR	Low
<b>C</b>		x	Or	Br		~	Ō	x	OrR.	BrR	Low
	38			D. D		Carvone	47		701	<b>n D</b>	
Lime ou, distilled	95 43		Y	BrR	Low	wormwood oil	96 87	x	ы	BrK	
	25 7	x	Xp	BrR	Low		78 63			BrR BrY	
Carvone	0		BrY	$\mathbf{BrR}$	Low		58		Y	v	
Lime oil expressed	31 07			B*B			36		Or	1	
Lime on, capiesseu	64		Y o r	DIK	Low		28 18			BrR BrR	
	38 24	x x	Y	BrR	Low	Carvone	0 38	x	Br	Br.	
	18 8	x x	Y Y	BrR BrR	Low Low	<sup>4</sup> Long streak					
	5	w	Ŷ.	BrR	Low	<sup>b</sup> Ultraviolet active after s	praying	g.	0	л. р:	nk. R radi
Carvone	38	*	0r	DIR	LOW	Vi, violet; W, white; Y, ye	llow.	10wii;	JI, UIANG	ς, τ, ρι	ua, 10, 100,

Table II. Chromatoplate Patterns of Some Essential Oils

which showed up under ultraviolet light before spraying. In the column "Spray" are indicated those materials which gave a color upon spraying with a hydrochloric acid solution of dinitrophenylhydrazine. Some materials which are particularly unstable to acid appear here as pink or brown areas even though they do not contain a carbonyl group. For instance, the spot at 62 in anise oil gave a pink color on spraying, which is the same test given by anethol at that location. The "Heat" column is for those materials which appeared as visible spots after heating. Materials giving a positive test on spraying are not indicated on the heat test, unless a further significant color change occurred. The colors produced are also characteristic of the particular materials. The intensity of the spots is only a very rough approximation of the quantity, but this quantitative indication has been found useful with some oils. The corresponding value for carvone on the same chromatoplate is also given for the oils to serve as a marker to permit comparison between plates.

The next phase of the work is the identification of the spots by comparison with authentic materials such as in Table I. In some cases materials of sufficient purity were available and have been compared directly. The yellow spot appearing on spraying at or slightly below carvone in oils such as lemon, lemongrass, lime, or orange checks well for citral. Linaloöl is located as a red-brown spot after heating about 10  $R_f \times 100$  units below carvone, and its presence is indicated in geranium, spike lavender, expressed lemon oil, lemongrass oil, horsemint, neroli, orange, and thyme. Other types of compounds detected are phenols, such as eugenol, indicated at 33 in clove bud oil and at 38 in geranium oil, and thymol or carvacrol in horsemint and thyme oil at about the carvone area. Most monocyclic terpene hydrocarbons are indicated in oils as heat-sensitive spots above 90.

Certain limitations to the method have been found. The generalization of Kirchner, Miller, and Keller (3) that compounds whose  $R_I$  values differ by 0.05 could be distinguished has held true in this work also. Thus, valeraldehyde and butyraldehyde were separated readily, but pulegone and dihydrocarvone were not always separated. In some cases this difficulty was

not troublesome, since the nature of the tests identified materials that were nearly superimposed. The presence of cineol, which was heat sensitive, was detected despite partial superposition of dihydrocarvone, which gave a ketone test. Similarly, pulegone, which was ultraviolet active and gave a ketone test, could be distinguished from dihydrocarvone, which gave a ketone test only. Figure 2 also shows how overlapping of spots gave no particular difficulty in many cases. Very inactive ketones such as fenchone, which originally were reported to give no dinitrophenylhydrazone by the usual preparatory technique (1), did not appear on the plate. Other materials such as pulegone, which once was thought to give no dinitrophenylhydrazone by the usual method in solution because of instability (1), produced good spots on the plate.

The method is particularly convenient for detection of oxygenated constituents of essential oils. In those oils, which are of particular interest because of hydrocarbon constituents, use of another developer, such as hexane alone, would be indicated  $(\delta)$ . Other developers might be desirable for highly adsorbed materials.

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# Polarographic Behavior of Organic Compounds At the Mercury Pool Electrode

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The study of the mercury pool cathode for the polarography of organic compounds leads to significant differences, although the polarograms are qualitatively similar to those obtained for ion-metal reductions. In contrast to the behavior exhibited by metallic ions, the reduction potentials are often different from those obtained at the conventional dropping electrode and at times the number of waves is different. In general, the reproducibility of the mercury pool cathode is not as good as that of the dropping mercury electrode; half-peak potentials are precise to 10 mv., and current values, within 7% of their relative values. The groups which have been studied are: nitro, dinitro, keto, diketo, azo, hydrazo, nitroso, disulfide, and quinone. The new electrode extends the range of polarographic methods to more dilute solutions. It is sometimes possible, in the analysis of mixtures of organic compounds, to take advantage of the shift in the reduction potentials.

I N A recent paper, the application of a polarized mercury pool electrode to analytical polarography was demonstrated (10). This study was limited to ion-amalgam reductions, and it was shown that the polarographic waves obtained were theoretically predictable by equations previously derived for oscillographic reductions (8, 9). The mercury pool electrode has the advantage of greatly increased sensitivity over polarographic methods employing the conventional dropping mercury procedures. Because of this advantage, an attempt was made to use the mercury pool electrode for the reduction of organic compounds and to determine the limitations involved.

The compounds selected for study were chosen to represent typical examples of polarographically reducible organic functional groups. Because the equations of both Sevcik  $(\mathcal{P})$  and Randles  $(\mathcal{B})$  were developed for oscillographic polarography involving ionamalgam reductions, and because many organic compounds are irreversibly reduced, it was not expected that they would apply to organic work. It was found, however, that the polarograms were similar in shape to those previously reported for metal ion reductions.

### APPARATUS AND REAGENTS

A thermostated  $(25^{\circ} \text{ C.})$  cell used in the beginning of this work has been described (10). Later, the cell was modified as shown in Figure 1. This arrangement allowed much more rapid and efficient deaeration. The solution to be analyzed was placed in the cell and the oxygen removed by forcing nitrogen (Seaford grade) through a fritted glass disk at the bottom of the compartment. To facilitate oxygen removal, a small amount of absolute alcohol (about 1%) was added to the solution. The surface tension of the solution was thus changed so that the nitrogen entered in very fine bubbles. Under these conditions no oxygen was detectable after about 15 to 30 seconds. Three milliliters of mercury were then added through the small side arm with the desired potential already applied. After the current had reached a constant value, the voltage scanning was begun.



The cell shown used about 15 ml. of solution. A smaller cell was also designed and found to be satisfactory. It held 1 ml. of solution and had an electrode area of 0.8 sq. cm. Since the electrode area was smaller, the peak currents measured were lower. However, the absolute magnitude of the currents obtained is unimportant. Only the ratio of peak height to residual current and the slope of the residual current values, with increasing positive voltage, are important. With the smaller cell, the current values were still large enough to be recorded on a conventional recording polarograph. A large cell, with an electrode area of 8 sq. cm. con-taining about 100 ml. of solution, was also tried and yielded waves similar to those previously obtained. The current values were larger, but such a procedure seemed to offer no advantage. In fact, the smaller the cell, the more favorable is the ratio of volume to electrode area. A modified Leeds and Northrup Electro-Chemograph was used in recording the polarograms. To decrease electrical hash, this apparatus was modified by replacing the polarizing slide wire with one having six times as many convolutions. The area of the mercury pool was 3 sq. cm. and the dropping electrode, used in comparison work, had an  $m^{2/3}t^{1/6}$  value of 2.49, where m is the rate of mercury flow and t is the drop time.

All of the solutions studied, except those whose wave height varied with polarization rate, were scanned at a rate of 400 mv. per minute using an auxiliary polarizer (10). Duplicate determinations were made for every point reported, and the reported results are the average of the two determinations. Comparison



polarograms, carried out with the dropping mercury electrode utilized  $10^{-4}M$  solutions, and 0.01% gelatin was added if a maximum was encountered. The values of the peak heights were corrected for the contribution of residual current by extrapolation of the base line current.

### EXPERIMENTAL

Nitro Compounds. Because nitro compounds are frequently determined polarographically, and a large amount of electrochemical data is available for nitrobenzene  $(\gamma)$ , this compound was chosen for exploratory work.

A wave typical of those obtained for nitrobenzene with the quiet mercury pool is illustrated in Figure 2. It is similar in shape to those obtained for ion-amalgam reductions. A more abrupt frontal slope and sharper peak might have been expected for this wave, because a 4-electron change is postulated for the reduction at the dropping mercury electrode (4). Because of the irreversibility of most organic reductions, little can be predicted as to the theoretical shape calculated from the equations of Randles and Sevcik.

 
 Table I.
 Comparison of Reduction Potentials at Dropping Mercury and Mercury Pool Electrodes

•				
	Dropping Volt vs.	Electrode, S.C.E.	Pool El Volt vs.	ectrode, S.C.E.
Compound	lst	2nd	lst	2nd
Nitrobenzene	-0.22	-0.68	-0.40	None
Dinitrobenzene	-0.27	-0.38	-0.30	-0.51
Benzophenone	-1.21		-1.15	
Benzil	-0.93		-0.85	
Azobenzene	-0.83		-0.72	
1-Nitroso-2-naphthol	-0.27		-0.80	
Cystine	-0.46	-0.87	-0.56	-1.11
1.6-Anthraquinone disulfonate	-0.67	• • •	-0.66	
Hydrazobenzene	-0.83	• • •	-0.76	

Nitrobenzene, at a pH of 1, in 10% alcohol, yields two waves upon reduction at a dropping mercury electrode. The first wave, for reduction to phenylhydroxylamine, has a half-wave potential of -0.22 volt vs. S.C.E. ( $\theta$ ). The second wave, much smaller in magnitude, is caused by reduction of phenylhydroxylamine to aniline, and occurs at a half-wave potential of -0.68 volt. It is significant that only one wave was obtained at the mercury pool electrode and that it had a potential of -0.40 volt vs. S.C.E. It was further found that a pure solution of phenylhydroxylamine failed to give a reduction wave. This is in contrast to the results obtained for the reduction of metal ions where the half-peak potentials obtained at a mercury pool agreed closely with the half-wave potentials of the dropping mercury electrode. Table I



shows a comparison of the reduction potentials at the two electrodes for various compounds. It is possible that at the mercury pool the second wave of nitrobenzene was shifted to a value more negative than the potential at which hydrogen discharge becomes appreciable. When using the pool electrode, the hydrogen discharge wave becomes appreciable at a potential 0.2 to 0.3 volt more positive than when the dropping electrode was employed. This is possibly caused by the greater sensitivity of the electrode.

As would be expected, the half-peak potential for the reduction of nitrobenzene was dependent on the pH. In Table II the values are listed for solutions of pH 1 to 4 (1). A reg

average half-peak potential of 0.045 volt was noted for a unit change in pH. The peak heights increased with pH, but not in a regular manner. At the dropping mercury electrode, there is also an increase in diffusion current with higher pH values, but the effect is much less pronounced (6). The half-peak potential was found to be. constant with concentration as well as with the rate of potential scanning.

According to the equations of Sevcik and Randles, the peak heights obtained in oscillographic polarography vary with the square root of the rate of voltage polarization. This was found to be true for the reduction of nitrobenzene at a quiet mercury pool as shown in Figure 3. The average deviation from linearity was 2.4% and the maximum deviation, 4.8%.

The variation of peak height with concentration is given in Table III for various concentrations of nitrobenzene. The solutions were buffered at a pH of 3 and contained 5% ethyl alcohol. The concentration of ethyl alcohol was not critical, because solutions containing 10% gave essentially the same results. Although the calibration curve is linear at lower concentrations, it falls off in more concentrated solutions. Considerably greater sensitivity was obtained with the mercury pool than with the dropping electrode, making it possible to determine solutions containing 0.25  $\gamma$  of nitrobenzene per ml.

m-Dinitrobenzene was the second example of an organic nitro compound. The polarogram shown in Figure 4 is a typical example of the type of curve found for this compound. The solution was  $10^{-4}M$  with respect to *m*-dinitrobenzene, was buffered at a pH of 3, and contained 5% alcohol by volume. The two peaks are clearly resolvable, a phenomenon not possible at this pH with the dropping mercury electrode. For comparison purposes, a polarogram of the same solution, recorded at the dropping mercury electrode, is shown. With this compound two distinct waves are obtained for both electrodes. It is possible that the third, long-drawn-out wave is present at the dropping mercury electrode. With the dropping electrode, the half-wave potentials are -0.22 and -0.37 volt vs. S.C.E. for the first and second waves, respectively. The corresponding potentials at the mercury pool are -0.28 and -0.51 volt. Here again the reduction potentials obtained at the quiet pool are different from those obtained by conventional polarographic procedures.

Wave height dependency on concentration is shown in Table III. The first wave is proportional to concentration except for the most concentrated solution. The second wave does not vary linearly with concentration, as evidenced by the decrease in value of  $I_{\text{max}}/C$  as the concentration increases. This might be

Nitrobenzene with pH

(Polarization rate, 0.4 volt per minute; solution,  $10^{-5}M$ ; temperature  $25^{\circ}$  C.)

Half-Peak Potential

Variation of Half-Peak Potential for

Imax., µa

 $I_{\max}/C$ , Ampere/Mole

al fo Tal ulai	or the r ble II tl r increa	eduction he values use of the	1 2 3 4	$ \begin{array}{r} -0.400 \\ -0.45 \\ -0.490 \\ -0.54 \\ \end{array} $	$5   5.45 \pm 0   6.60 \pm 0   5   6.75 \pm 0   7.40 \pm 0$	25         0.54           20         0.66           .15         0.675           .20         0.74	$\begin{array}{c} \pm \ 0.02 \\ \pm \ 0.02 \\ \pm \ 0.015 \\ \pm \ 0.02 \end{array}$
I	00-						/
	50-		$\sim$				
	0		m – DIN	IITROBENZ	ZENE		
croamperes	6-		IO	<sup>4</sup> М рН	3		
Mid	4-	M				And Andrewski An	e e Service Se
	2 0	2	- <u></u> 4	6	8 -	-1.0 -	-1.2
		F:	Gure 4 Polo	olts vs. S	CE m-Dinitroha	nzona	
		F1	GUICTI IUID	rograms or	me-Dimitione	1120110	

Table II.

pH of Solution

caused by the growth of the first wave, which partially masks the second as concentration increases.

Carbonyl Compounds. Waves were obtained for the reduction of benzophenone in a 0.1M solution of ammonium acetate (Figure 5). Because the hydrogen current appeared so soon after the emergence of the carbonyl wave, the wave heights for benzophenone were measured by subtracting the background polarogram from the benzophenone polarograms, instead of extrapolating the base line as was done for the other compounds.

The dependence of wave height on concentration is given in Table III. As in the previous cases, the  $I_{\rm max.}/C$  value is not constant over the whole concentration range studied. The half-peak potential for these solutions was  $-1.15 \pm 0.01$  volts. In a buffer of pH 4, the half-peak potential was -1.02 volts and was unaffected by scanning rate.

A conventional polarogram of the  $10^{-4}M$  solution of benzophenone is also shown in Figure 5. The half-wave potential was -1.22 volts and the wave height was 0.80  $\mu$ a. The hydrogen discharge occurs at a much more negative potential than in the case of the pool polarogram. The  $i_d/Cm^{2/3}t^{1/6}$  value obtained was 3.21. Day (2) gives this value as 2.60 in his work on benzophenone.



Benzil was found to yield one well defined wave and two other waves much smaller in magnitude (Figure 6). The same material yielded only a single wave at the dropping mercury electrode with a half-wave potential of -0.93 volt vs. S.C.E. The value of  $I_{\rm max}$ ./C decreased somewhat with increasing concentration (Table III). A recent study by Marple and Rogers (5) has shown that by substituting a mercury-plated platinum electrode for the dropping mercury electrode, the number of reduction waves for dinitrobenzene can be increased from two to three.



Azo Compounds. Azobenzene in 0.1M lithium hydroxide and 1% ethyl alcohol gave a well defined wave with an unusually sharp peak (Figure 7). A polarogram of the same solution obtained at a dropping electrode is shown for comparison purposes. This compound is reduced at a more positive potential at the pool electrode, as shown in Table I. The value of the peak current is abnormally high when compared to the other compounds studied. The high value of  $I_{\text{max}}/C$  shown in Table III can be explained by postulating an adsorbed film of azobenzene on the mercury surface. An adsorbed film would increase the peak current values by increasing the concentration of the reducible compound at the electrode interface. When the surface film was depleted, there would be a sharp decline in the reduction current, which would result in a pronounced peak. Further evidence to substantiate this is that the peak values of this compound do not increase as the square root of the scanning rate, as would be expected for a diffusion controlled peak. The increase is almost linear. Increasing the amount of ethyl alcohol in the supporting electrolyte exerted a profound influence on the height and shape of the azobenzene wave. In raising the ethyl alcohol concentration from 1 to 50%, the value of the current was decreased to one tenth its original value. A further difference in behavior of this compound was the shift in half-peak potential with scanning rate. toward more positive values. The uniform behavior of the other

Table III. Variation of Peak Current with Concentration

		$I_{\max}./C$ , Ar	npere/Mole	
Concentration, Mole/Liter	Nitro- benzene	<i>m</i> -Dinitro- benzene, 1st wave	Benzo- phenone	Benzil
$\begin{array}{c} 2 \times 10^{-6} \\ 5 \times 10^{-5} \\ 10^{-5} \\ 2 \times 10^{-5} \\ 5 \times 10^{-5} \\ 7 \times 10^{-5} \\ 10^{-4} \end{array}$	0.67 0.67 0.66 0.67 0.61 0.58	0.70 0.73 0.72 0.71 0.69 0.61	0.67 0.67 0.54 0.52	0.72 0.67 0.68 0.65
	Azobenzene	Cystine	1,6-Anthra- quinone Disulfonate	Hydrazo- benzene
$2 \times 10^{-6} \\ 5 \times 10^{-6} \\ 10^{-5} \\ 2 \times 10^{-5} \\ 5 \times 10^{-5} \\ 7 \times 10^{-5} \\ 10^{-4} \\ $	1.00 1.04 1.04 0.98 0.98	0.32 0.32 0.32 0.35	0.29 0.31 0.31 0.32 0.30	1.05 0.93 0.97 0.96

organic compounds seems to indicate the lack of appreciable adsorption.

Figures 7 and 8 illustrate the sensitivity which is attainable with the mercury pool electrode. Figure 7 compares the polarograms for azobenzene obtained with both the pool electrode and a conventional dropping electrode. Further increase in sensitivity can be had by increasing the rate at which the electrode is polarized. Figure 8 shows a polarogram of azobenzene obtained at a voltage scanning rate of 6.0 volts per minute. At this concentration, a conventional polarographic wave was hardly noticeable ( $i_d = 0.03 \ \mu a$ .). The increased magnitude of the charging current at the higher scanning rate is seen in the initial sharp current rise before the plateau is reached. The narrower range of voltage, between the anodic dissolution of mercury and the evolution of hydrogen, decreases the value of the higher rates of polarization.



Figure 7. Polarograms of Azobenzene

Nitroso Compounds. 1-Nitroso-2-naphthol in a 0.1M ammonium hydroxide-0.1M ammonium chloride solution gave a wave in which the peak was less pronounced than in other polarograms (Figure 9). Reproducibility of wave height was distinctly poorer, and errors as large as 20% were recorded. The linearity of peak height versus concentration was thus difficult to evaluate, but the average value of all determinations was 0.40 ampere per mole. The reduction potential obtained at the mercury pool was more than 0.5 volt more negative than the half-wave potential at the dropping electrode.

Disulfides. At the mercury pool electrode, cystine was found to yield two waves as shown in Figure 10. The reduction process at the pool electrode is apparently similar to that which occurs at the dropping mercury electrode. Under the same conditions, the polarogram at the latter electrode also consisted of two waves. The first, at a half-wave potential of -0.46 volt, was considerably smaller than the second ( $E_{1/2} = -0.87$  volt). The second wave was used for analysis, and the peak heights of this wave were linear with concentration, within the precision of the method, as shown in Table III. Because of the discharge of hydrogen soon after the second peak, it was difficult to measure the current values at dilutions below  $2 \times 10^{-5}M$ . When a strong base was used as supporting electrolyte, no well defined reduction was found. This behavior is similar to that which occurs at the dropping mercury electrode.

Quinones. The ammonium salt of 1,6-anthraquinone disulfonate in 0.1*M* lithium hydroxide and 1% alcohol yielded a well defined wave (upper half of Figure 11). The peak currents were linear with concentration, and the half-peak potential was independent of concentration. The reduction potential, using the mercury pool electrode, was close to the value of -0.67 volt, which was obtained at the dropping mercury electrode under the same conditions as in Table I.



Unsaturated Compounds. Attempts were also made, using the pool electrode, to obtain polarograms of anthracene in solutions of 0.05M tetraethylammonium bromide and 5, 10, and 50% alcohol by volume. In no case was a wave obtained. However, a dropping mercury electrode polarogram of the solution containing 10% ethyl alcohol, yielded an excellent polarogram with a half-wave value of -1.63 volts vs. S.C.E. Apparently, the reduction potential of this compound at the mercury pool is shifted to





such a negative potential that it is completely masked by the hydrogen discharge.

Halogen Compounds. Organic compounds containing halogens were also found to yield reduction waves at the pool electrode. Carbon tetrachloride was found to be reducible, but because of the experimental difficulties involved in keeping the compound in solution during deaeration, no quantitative studies were made (3). The gamma isomer of hexachlorocyclohexane was also found to be reducible. Two waves were obtained in contrast to the single wave found at the dropping mercury electrode. Because of the current interest in this compound, this specific analysis was studied in detail (11).

### ANODIC OXIDATIONS

An attempt was made to perform anodic oxidations at the mercury pool electrode. The wave obtained for the oxidation of hydrazobenzene in 0.1M lithium hydroxide is shown in Figure 12. The general shape of the wave is what would be expected from the results of oscillographic polarography. The base lines obtained in these anodic polarograms were not as well defined as those of the cathodic waves.

### REVERSE POLARIZATIONS

In the previous work on ion-amalgam reductions, it was found that entirely different curves were obtained if the polarization was carried out from negative to positive potentials (10). Peaks are not obtained, because the diffusion layer is already set up before the half-peak potential is reached. Anodic currents, however, appear as valleys in these polarograms because of the dissolution of the deposited metal when the potentials become sufficiently positive. When reactions are irreversible, these valleys would not be expected, because no deposition occurs with organic compounds. In Figure 13 a polarogram is shown that was obtained by scanning the voltage from a negative to a positive potential. From the shape of the curve, it is evident that the product of the reduction of nitrobenzene is not reoxidized at the mercury pool electrode. The wave obtained, however, is still characteristic of nitrobenezene. The half-peak potential on the forward polarization was -0.50 volt vs. S.C.E., while the value of the half-wave potential on the reverse polarization was -0.49 volt. Similar results were obtained for m-dinitrobenzene, except that two waves were found. The half-wave potentials of both the waves obtained corresponded to half-peak potentials of the forward polar-

### ANALYTICAL CHEMISTRY

ization. In contrast to the nitrobenzenes, 1,6anthraquinone disulfonate yielded an anodic wave when the electrode was polarized from negative to positive potentials. The waves obtained in both directions are shown in Figure 11. The half-peak potentials of the two waves agree within experimental error. The existence of an anodic wave indicates the reduction product is reoxidizable. Because the half-peak potentials are the same, it might be inferred that the electrolytic process is reversible. This is the criterion used in oscillographic polarography for reversibility, although the time involved here is much longer.

Azobenzene also yielded a reduction product which was found to be reoxidizable at roughly the same potential. The reduction product can be shown to be hydrazobenzene, because the anodic wave obtained with the latter compound was identical to the wave found on the reverse polarization of azobenzene.

### DETERMINATION OF MIXTURES

Because the reduction potentials of various compounds at the mercury pool cathode are at times different from those obtained at the dropping electrode, it seemed possible that some mixtures, with similar half-wave potentials at the dropping mercury electrode, could be resolved at the quiet pool. An example is the determination of m-dinitrobenzene in nitrobenzene. At a pH of 3, the half-wave potentials of the two compounds, which are obtained by conventional polarography, are so close that resolution is difficult. At the mercury pool electrode, however, m-dinitrobenzene can be determined in twenty times as much nitrobenzene with an accuracy within 5%, as shown in Table IV. It is much more difficult, however, to determine nitrobenzene in the presence of dinitrobenzene. The second wave of the latter compound coalesces with the peak characteristic of nitrobenzene. Because the second wave of nitrobenzene is not linear with concentration, it is difficult to compensate for the contribution of this wave.





Table IV. Determination of *m*-Dinitrobenzene in Presence of Nitrobenzene Using Mercury Pool Electrode

Concentration,	Mole/Liter	in in in in in	nax.
m-Dinitrobenzene	Nitrobenzene	1st wave	2nd wave
$5 \times 10^{-6} \\ 5 \times 10^{-6} $	$5 \times 10^{-6}  5 \times 10^{-6}  10^{-5}  5 \times 10^{-5}  10^{-5}  10^{-4}$	$\begin{array}{r} 3.50 \pm 0.00 \\ 3.53 \pm 0.20 \\ 3.40 \pm 0.20 \\ 3.35 \pm 0.05 \\ 3.35 \pm 0.15 \end{array}$	$\begin{array}{c} 2.08 \pm 0.07\\ 3.40 \pm 0.05\\ 4.80 \pm 0.40\\ 8.90 \pm 0.30\\ 30.20 \pm 0.30\\ 60.5 \pm 0.50\end{array}$

### THE USE OF STIRRED POOLS

In order to increase further the sensitivity of the mercury pool electrodes, polarograms were carried out, in which the solution above the electrode was agitated by means of a stirrer. The current values were increased, and the ratio of current height to residual current was found to be more favorable. As would be expected, peaks are not obtained, and the shape of the curves are similar to conventional polarograms. Work is being done on the analytical application of such electrodes and will be reported in a subsequent publication.

### DISCUSSION

The mercury pool cathode has been applied to the polarographic determination of a small number of organic compounds and the results obtained are different in many respects from polarograms found by conventional methods. Obviously, further work is necessary before any over-all evaluation of the application of this electrode of polarography can be reached. However, certain outstanding differences between the two electrodes are evident.



The increased sensitivity of the new electrode makes possible the determination of much smaller quantities of reducible organic material. Besides this obvious advantage, it is possible to work in a lower range of concentrations and still use aqueous solutions. In some cases, where by conventional procedures it was necessary to use 80% ethyl alcohol because of solubility problems, the reduction could be carried out in 5% alcohol at the mercury pool. The precision of the various measurements using the pool electrode seemed to be poorer than that obtained by conventional procedures. The half-peak potentials, for example, were reproducible to only 10 mv., a value higher than the comparable halfwave potentials. Peak heights could be reproduced within about 5 to 7% of their relative values.

An outstanding difference between the two electrodes is that the reduction potentials are not the same in both cases. The potentials obtained at the quiet pool are sometimes positive and sometimes negative, when compared to the dropping electrode values. When resolution is poor by conventional procedures, the use of the mercury pool might well shift one of the potentials, allowing the two waves to be separated.



Some of the difference in reduction potentials might possibly be explained by kinetic effects. With the dropping electrode, a new diffusion layer is set up every few seconds. In the case of the quiet mercury surface, the same diffusion layer persists throughout the entire recording of the polarogram. Since many electroreductions occur slowly, this difference might explain some of the potential shifts and differences in the number of waves for organic compounds. The reduction of benzil at both electrodes is an example in which a different number of waves is obtained, At the dropping mercury electrode a single wave is obtained, which has been shown to correspond to a reduction to benzoin. No further reduction occurs, even though benzoin itself does yield a polarographic wave. The rearrangement to benzoin is apparently slow at the dropping mercury electrode (4). At the mercury pool electrode, however, if benzoin is the reduction product, it would remain in contact with the electrode long enough to be reduced further. Similar phenomena have been reported by Marple and Rogers (5). Their results are not comparable to the results for the reduction of dinitrobenzene reported here because of the great difference in pH in the two experiments.

In using the pool electrode, it was found that for most supporting electrolytes the hydrogen wave occurred at a potential of 0.2 to 0.3 volt more positive than it appeared at the dropping mercury electrode. Unfortunately, many important organic functional groups, such as conjugated double bonds and the aldehyde group, are reduced to a value quite close to hydrogen evolution, and cannot be determined by this procedure. The rate of polarization has a distinct effect on the potential at which hydrogen is discharged. At low rates of voltage scanning, the hydrogen wave becomes appreciable at more negative potentials (Figures 7 and 8). When polarizing at a rate of 0.4 volt per minute, the current becomes appreciable at -1.5 volts, while at 6.0 volts per minute a potential of only about -0.9 volt can be attained.

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# Polarographic Determination of the Gamma Isomer Of Hexachlorocyclohexane

## In the Presence of Other Isomers and Higher Chlorinated Material

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In the usual polarographic determination of the gamma isomer of hexachlorocyclohexane, difficulties are experienced from other compounds which are normally present. In particular, heptachlorinated compounds are reduced at a half-wave potential which overlaps that of the gamma isomer. If the dropping mercury electrode is replaced by a mercury pool electrode, the reduction potentials are shifted in such a way that interferences are eliminated. This method has a greater sensitivity than the conventional polarographic method and has been applied to pure lindane, natural isomeric mixtures, concentrates, dusts, and alpha-beta cakes.

THE widespread use of lindane as a pesticide has aroused recent interest in its analytical determination. Because of the difficulty of determination in the presence of the usual impurities, a solution to this problem has been attempted by a wide variety of analytical methods.

Simple colorimetric tests have been devised (7, 9), but these are not specific for the gamma isomer, and all other isomers interfere. Bioassay methods which employ the toxic effect of lindane on houseflies have been tried (5). These methods lack precision and have inherent uncertainties in any one determination. Polarographic methods have been proposed (2, 4, 6, 10)and are useful, because the alpha, beta, delta, and epsilon isomers yield no diffusion wave, but the gamma isomer is reducible. In the presence of hepta- and octachlorocyclohexane, the method is difficult to apply. These more highly chlorinated products give a wave which merges into the polarogram of the gamma isomer. Draght (3) has devised an empirical procedure for surmounting this difficulty if only small amounts of heptachlorocyclohexane are present. Infrared methods have been used for this analysis (2), but the other compounds absorb radiation at all wave lengths within the spectrum of the gamma isomer. Higher chlorinated material causes further uncertainties in the analysis. When the method is applicable, however, each individual isomer in a mixture can be determined. All components in the pesticide mixtures can be separated chromatographically (1, 8). In such procedures, the gamma isomer can be separated and weighed and, therefore, is not dependent on any interpretive procedures. The disadvantages of this method are the time and technique necessary and the fact that a large sample must be used.

A new polarographic procedure has been proposed, using a mercury pool cathode (11), in which a wave is obtained for the gamma isomer of hexachlorocyclohexane. None of the other isomers contributes to this wave nor do the hepta- and octachlorinated compounds. The method is rapid and has a sensitivity more than ten times the conventional polarographic procedure.

In applying the recently developed mercury pool cathode to organic polarography, distinctly different results from those found with the dropping mercury electrode were often obtained (12). Reduction potentials were shifted, sometimes in a positive direction and, at other times, toward negative potentials. In some instances, even the number of waves obtained was different. Because such results might be obtained at the mercury pool



Figure 1. Mercury Pool Polarograms of Hexachlorocyclohexanes



Figure 2. Polarograms of Hepta- and Octachlorocyclohexanes

electrode, it was hoped that a solution to the problem of determining the gamma isomer might be found.

A solution of 20% ethyl alcohol with 1% potassium chloride was chosen as the supporting electrolyte for the analyses. Polarograms were run in this medium, both at the dropping mercury electrode and the mercury pool cathode on the alpha, beta, and gamma isomers of hexachlorocyclohexane, as well as the heptaand octa- compounds.

The gamma isomer was found to give one long wave at the dropping mercury electrode at -0.94 volt vs. S.C.E. This potential is considerably more positive than the half-wave values usually recorded at higher alcohol concentrations. In contrast, two waves were found at the mercury pool electrode. The first had a half-peak potential of -0.83 volt, and the second occurred at -1.40 volts. The initial wave was different in shape from those usually obtained at the mercury pool in that the peak was flattened (12). A typical polarogram is shown in Figure 1.

No reduction wave was found for the alpha isomer at the dropping electrode. A wave was obtained at the quiet mercury pool, which occurred shortly after -1.0 volt. The wave was not fully developed in this medium, because of the appearance of the hydrogen discharge shortly after its initiation. The beta isomer did not give a wave with either electrode, and neither of the two other isomers was available for study. Polarograms are shown in Figure 1 for each of these samples at a concentration of 10  $\gamma$  per ml.

A mixture of the heptachlorocyclohexane isomers was also studied. A single wave was obtained at the dropping electrode with an approximate half-wave potential of -0.6volt. This polarogram was drawn out over the long range of -0.2 to -1.0 volt. At the mercury pool electrode, two reduction potentials were found (Figure 2). The first, a positive value, had already begun to develop at zero applied e.m.f.; the estimated half-peak potential was about -0.1 volt. The second wave occurred at negative values merging into the hydrogen wave. The proposed procedure was successful because of this difference between the two electrodes.

The octachlorocyclohexanes also were found to give two waves at the mercury pool. The first wave apparently occurred at a positive potential, because the polarogram begins at a high current value. The second wave is close to the hydrogen discharge and is not fully developed.

Because the difference between the reduction potentials of the various compounds indicated the feasibility of an analytical method, the linearity of the height of the gamma isomer wave was first checked against concentration. Lindane, which assayed 99.9% pure, was used in these analvses. Only the first reduction wave was considered, because the alpha isomer and the more highly chlorinated compounds interfere with the second peak. The wave height was measured as illustrated in Figure 4. The current was found to be linear within 5% over the range of 1 to 25  $\gamma$ per ml., as shown in Figure 3. In more concentrated solutions, the current values increase more rapidly than would be predicted. The peak heights also vary with the square root of the voltage scanning rate, a behavior noted with other organic molecules and inorganic ions (11, 12).

The greatest difficulty, in the application of the conventional dropping mercury electrode to the determination of lindane, is involved with the interference of the heptachlorinated compound. Synthetic mixtures of the gamma isomer of hexachlorocyclohexane with heptachloro- isomers were therefore analyzed for lindane content. The results are shown in Table I. It may be seen that the first wave of lindane shows no interfer-

### Table I. Effect of Heptachlorocyclohexanes on the Wave Heights of Gamma Hexachlorocyclohexane

$\gamma/\text{Ml}.$ $\gamma/\text{Ml}$	. Extrapolation, $\mu a$ .	μa.	Error,
$\begin{array}{cccc} 10 & 0 \\ 10 & 10 \\ 10 & 20 \\ 10 & 30 \end{array}$	23 32 39	16.5 16.2 17.0 16.8	-1.8 + 3.0 + 1.8



Figure 3. Polarogram of Lindane in a Natural Isomeric Mixture

 $\frac{7}{7}$ 

ence from the heptochlorinated compounds. There was a considerable contribution to the second wave, however. A minor difficulty was experienced with large amounts of the heptacompounds. The recorded currents from the first wave of heptachlorocyclohexane did not level off before the lindane wave appeared, and straight-line extrapolation was not possible. This difficulty could be easily avoided by applying a potential of -0.2 volt and not starting the voltage scanning until the current had reached a steady value. This usually occurred in 2 or 3 minutes. Under these conditions, the base line preceding the lindane wave could be easily extrapolated.

According to the preliminary work, the alpha isomer appeared to be the only one which might cause difficulty. This compound gave a reduction wave, which was initiated shortly after the wave for lindane, as shown in Figure 1. If the amount of the alpha isomer was very large, the initiation of its wave occurred at more positive voltages. When the amount of the alpha compound was greater than thirty times the gamma isomer, this wave encrouched upon the lindane peak, the length of the current plateau was considerably shortened, and an uncertainty was introduced in measuring its magnitude. This may be seen in the error introduced in the analysis of the alpha-beta cake as shown in Table II.

The method was tested on analyzed samples generously supplied by the Pennsylvania Salt Manufacturing Co. These

included natural isomers, concentrates, dusts, and alpha-beta cake, which had been analyzed by a chromatographic method tentatively approved by the Association of Official Agricultural Chemists. The polarograms were run at a quiet mercury pool electrode, and the amounts in each mixture were determined by reference to the calibration curve. Polarograms are shown in Figures 3 and 4 for a natural isomeric mixture and a sample of dust. The results of these analyses, as well as the mean deviation and the number of analyses, are shown in Table II.

### APPARATUS AND REAGENTS

The polarographic cell and circuit employed have been de-aribed (11). The area of the pool was calculated as  $2.86 \pm 0.04$ scribed (11). sq. cm. A saturated calomel electrode was used as the reference electrode, and the temperature control was maintained at  $25^{\circ} \pm 0.1^{\circ}$  C. The polarograph was a Leeds and Northrup Electro-0.1° C. chemograph with a voltage scanning rate of 200 mv. per minute. The cell was deaerated with Seaford nitrogen from Air Reduction Sales Co. Before entering the cell, it was passed through a scrub-ber containing a 20% by volume solution of ethyl alcohol. The mercury for the pool was obtained from the Bethlehem Apparatus Co. Its use eliminated the troublesome transients probably caused by traces of grease, sometimes observed in polarograms where mercury from other sources had been employed.

Reagent grade potassium chloride was used without further purification. Absolute ethyl alcohol was better than 95% alcohol in eliminating errors from impurities in the solvent. All water used was redistilled. The various isomers of hexachlorocyclohexane were supplied by Pennsylvania Salt Manufacturing Co. and were used without further purification. The heptachloro-and octachloro- compounds had been recrystallized from heptane and undoubtedly were mixtures of the various isomeric forms. They had a wide melting point range and analyzed slightly below the theoretical value for chlorine.

### PROCEDURE

A sample of appropriate size was dissolved in hot, absolute ethyl alcohol, cooled, and diluted to yield a solution of 20%

hepta % -1.0 - 1.2 -.4 -.6 -.8 Volts vs. SCE Figure 4. Polarogram of the Gamma Isomer in a Dust **Table II.** Analysis of Technical Samples Other Isomers (Chromatographic Analysis) Gamma Isomer, % By chromatog- Differ-By pool electrode Type of Material Alpha, % Beta, % Heptachloro-, % raphy ence Natural isomer  $11.2 \pm 0.5$  (6)  $12.9 \pm 0.5$  (10)  $11.1 \pm 0.3$  $12.3 \pm 0.3$  $^{+0.1}_{+0.6}$  $65 \\ 65$ 88  $\frac{2}{2}$  $9 \pm 0.8 (4)$   $6 \pm 1.2 (4)$   $1 \pm 1.1 (4)$  $\begin{array}{c} 35.6 \pm 0.5 \\ 36.6 \pm 0.6 \\ 42.7 \pm 0.5 \end{array}$ 25  $21 \\ 21 \\ 17$ 332 -1.0-0.6 $\frac{25}{20}$ 

13.0%

%

%

40

20

0

**Microamperes** 

 $\hat{2}$ 

3

Dust

 $\frac{2}{3}$ 

Concentrate

Alpha-beta

cake

gamma

alpha

beta

ethyl alcohol and 1% potassium chloride. About 15 ml. of the solution were placed in the polarographic cell and deareated for 10 minutes with an applied voltage of -0.2 volt. Five milliliters of mercury were then added and deareation was continued for an additional 5 minutes. In later models of the electrolysis cell, a small side arm with a ground-glass cap was supplied for introduction of the mercury. The nitrogen was then bypassed over the surface of the solution, and the polarogram was run from -0.2 to about -1.4 volts at a rate of 200 volts per minute. Wave heights were obtained by extrapolation of the base line, as shown heights were obtained by extrapolation of the base line, as shown in Figure 4, and the gamma isomer content was obtained by comparison with the calibration curve prepared from pure lindane.

 $\frac{10}{10}$ 

90

### ACKNOWLEDGMENT

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 $\pm 0.3 \\ \pm 0.3 \\ \pm 0.4$ 

 $2.3 \pm 0.2$ 

+0.6+1.6

+1.6

.5(4).5(4).8(2)

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 $3.9 \pm 0.25$  (2)

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# Determination of Barium, Calcium, and Zinc in Lubricating Oils

## **Use of Fluorescent X-Ray Spectroscopy**

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This method is capable of determining in 3 to 12 minutes for each element, concentrations of barium, calcium, and zinc normally found in lubricating oils. The accuracy is comparable to chemical methods. The use of a helium path extends the range of x-ray fluorescence to wave lengths beyond those otherwise available, making the determination of barium and calcium practicable. Interferences due to absorption by other elements in the sample are treated mathematically and experimentally.

UBRICATION oil blending, being in general a batch process, requires quick control analysis for additive components in order to use the blending facilities at their highest capacity. X-ray fluorescence was found to offer advantages of speed and accuracy seldom found in other analytical methods. The x-ray method, as applied to barium, calcium, and zinc in new lubricating oils, requires no ashing or sample preparation. The total concentration of each element sought is measured regardless of chemical form. A determination can be performed in 3 to 12 minutes for each element in the concentrations generally found in additive-type lubricating oils. This method is currently in use in a company refinery.

The use of x-ray fluorescence as an analytical tool has advanced rapidly since 1948, when equipment became available commercially. Instrumental improvements, which should make the method even more attractive, will soon be available. A good discussion of the method is found in papers by Birks, Friedman, et al. (2, 4).

#### DISCUSSION

The diagram shown in Figure 1 represents the optical system of the x-ray spectrograph. A beam of primary x-radiation from the tube is directed to the sample. The primary beam excites the elements in the sample to give off a secondary or fluorescent radiation, the wave lengths of which are characteristic of the elements emitting the radiation. This secondary radiation passes through a collimator, so that only a parallel beam of the secondary radiation impinges on the analyzing crystal. The crystal serves as a diffraction grating, separating the radiation into its various wavelength components. The Geiger tube scans an arc around the crystal, measuring the intensity of the x-radiation as a function of wave length.

When the determination of barium, calcium, and zinc is attempted by x-ray spectrography, some of the inherent limitations of the method are encountered. With the equipment now available, analysis is limited to the wave-length region between 0.8 and 2.4 A. Above or below this region, analyses are of low sensitivity. Table I shows that the useful wave lengths of both barium and calcium fall in this region of low sensitivity. A method of extending the useful range in the long wave-length region is to replace the air in the x-ray path by a light gas such as hydrogen or helium. This procedure uses helium, permitting the determination of both barium and calcium which would not be practical with an air path. Zinc, having wave lengths in the optimum region, does not require the helium path. However, for convenience, all determinations, even that of tetraethyllead in gasoline, are performed using the helium path.

The secondary x-rays emitted from the metals in the sample are partially absorbed not only in the air path, but also in the sample itself. The fluorescing metal atoms are located at varying distances below the sample surface, and the radiation from these atoms in reaching the surface is then subjected to absorption and scattering processes, which are related to the composition of the sample. Zinc radiation, for example, is absorbed by the carbon, hydrogen, sulfur, barium, and zinc in the sample. Each element has an absorption and scattering power which varies with the x-ray wave length. This introduces the possibility of interferences from the sample. For example, 0.1% zinc in an oil containing 1% barium gives a much lower intensity of zinc x-radiation than would the same concentration of zinc in the absence of barium. Interference problems such as this are corrected for by preparing calibration curves based on samples containing known concentrations of the interfering element or elements.



A. X-ray tube B. Sample holder C. Collimator D. Rock salt crystal E. Geiger tube

Absorption calculations were made to confirm the validity of the experimental results and as a method of predicting further interferences. The principal exciting radiation for any element is that of wave length just shorter than its absorption edge. This radiation is more penetrating in these dilute solutions than is the corresponding fluorescent radiation, whose wave length is appreciably longer than the absorption edge. Thus the exciting radiation penetrates to such depths in the sample that the longer fluorescent x-rays have little chance of returning to the surface. The absorption coefficient for the exciting radiation of deeper penetration is lower than that of the fluorescent radiation, so that the problem may be treated as one of uniform excitation independent of depth. Excitation of the zinc K-radiation, for example, will be proportional to the zinc concentration and to the milliamperage (m) of the x-ray tube.

The absorption of fluorescent radiation is handled with the usual absorption equation.

$$I = I_0 e^{-(\mu/\rho)\rho\chi} \tag{1}$$

where I is the intensity of radiation unaltered in direction and wave length after traversing a distance,  $\chi$ , in a medium whose

Table	I. Wave and L Lin	Lengths nes for Ba	and	Spectrometer Calcium, and	Angles d Zinc	of	K
	7.5		77.0		-	~	

	R	α	1	Кø		Lα	Lβ		
	λ,Α.	Angle	λ,Α.	Angle	λ,Α.	Angle	λ,Α.	Angle	
Zinc Barium Calcium	${1.435^a\ 0.389\ 3.353^a}$	$29.51^{a}$ 7.89 73.13 <sup>a</sup>	$1.292 \\ 0.340 \\ 3.083$	$26.55 \\ 6.93 \\ 66.44$	ר 2.770 ת	Coo long 58.83 Coo long	to be us 2.562ª to be us	eful 54.16ª eful	
<sup>a</sup> Values	used in	this wor	k.						

	Radi	ation	
Absorber	Zinc (1.43 A.)	Barium (2.78 A.)	Calcium (3.35 A.)
С	4.43	26	43
Р	59.4	(400)	(650)
S	75.0	454	780
Ca	142	(750)	(150)
Zn	49.3	283	<b>`480</b>
Ba	307	(300)	(470)
H	0	`0´	` O ´

mass absorption coefficient is  $\mu/\rho$ . Here  $\mu$  is the linear absorption coefficient,  $\rho$  is the density, and  $I_0$  is the initial intensity. The mass absorption coefficient of a mixture is expressed in terms of the coefficients of the elements in the following manner:

$$\mu/\rho = W_1 (\mu/\rho)_1 + W_2 (\mu/\rho)_2 + W_3 (\mu/\rho)_3 + \dots$$
 (2)

where  $W_1$ ,  $W_2$ ,  $W_3$ , etc., are weight fractions of the various elements in the mixture which have corresponding mass absorption coefficients  $(\mu/\rho)_1$ ,  $(\mu/\rho)_2$ ,  $(\mu/\rho)_3$ , etc. Pertinent coefficients, given in Table II, are taken from x-ray literature (1, 5, 6) or computed by accepted methods (3). The computed values are in parentheses.

Using as an example the zinc radiation (1.43 A.) in a sample containing a weight fraction of zinc,  $W_{Zn}$ , and other elements, barium, carbon, sulfur, phosphorus, and hydrogen, a mass absorption coefficient for the mixture is obtained:

$$\mu/\rho = 49.3 W_{\rm Zn} + 307 W_{\rm Ba} + 75 W_{\rm S} + 59.4 W_{\rm P} + 4.43 W_{\rm C}$$
(3)

Using the condition

$$W_{\rm C} = 1 - (W_{\rm Zn} + W_{\rm Ba} + W_{\rm S} + W_{\rm P} + W_{\rm H})$$
 (4)

gives the final expression for the mass absorption coefficient of the mixture

$$\mu/\rho = 44.9 W_{\rm Zn} + 303 W_{\rm Ba} + 70.6 W_{\rm S} + 55 W_{\rm P} + 4.43 (1 - W_{\rm H})$$
(5)

The change from Equation 3 to Equation 5 is for convenience only and takes into account the fact that changes in absorption due to increasing amounts of metal are partially compensated for by the reduced carbon content. In Equation 3 it is always necessary to allow for the changes in carbon concentration. Equation 5 is generally used without consideration for changes in hydrogen concentration.

The intensity of the zinc radiation before absorption is

$$I_{0\mathrm{Zn}} = K_{\mathrm{Zn}} m \rho W_{\mathrm{Zn}} \tag{6}$$

where  $K_{Zn}$  is a constant which includes such factors as voltage and target of x-ray tube, absorption and emission characteristics of the zinc, geometrical factors, and the detecting efficiency.

Allowing for absorption, the intensity of zinc radiation measured, coming from different depths,  $\chi$ , in the sample, is given by

$$I_{\rm Zn}\chi = I_{0\rm Zn}e^{-(\mu/\rho)\rho\chi} \tag{7}$$

This equation must be integrated from  $\chi = 0$  to  $\chi = \infty$  to give the total intensity of zinc radiation escaping from the sample and being measured. Experimental results show that the samples are effectively of infinite depth.

$$I_{Zn} = \int_0^\infty I_{Zn\chi} d\chi \tag{8}$$

$$= K_{\mathbb{Z}n} m \rho W_{\mathbb{Z}n} \int_0^\infty e^{-(\mu/\rho)\rho \chi} d\chi$$
(9)

$$=\frac{K_{\mathbf{Z}\mathbf{n}}\mathbf{m}W_{\mathbf{Z}\mathbf{n}}}{(\mu/\rho)}\tag{10}$$

$$= \frac{\Lambda Z_{\rm n} m W Z_{\rm n}}{[44.9 W_{\rm Z_{\rm n}} + 303 W_{\rm Ba} + 70.6 W_{\rm S} + 55 W_{\rm P} + 4.43 (1 - W_{\rm H})]}$$
(11)

This formula checks accurately the experimental data on the effect of barium on the zinc radiation. Comparison of observed values and calculated intensities are given in Table III. The value of 0.10% Zn and 0.0% Ba is used to establish the  $K_{Zn}$  value (Table IV). All other values are then valid comparisons.

Table IV.	Values of K Expressing Calibration Data Using
	Molybdenum Tube at 50 Kv.

Element	K Value
Ca	$1.10 \times 10^{3}$
Zn	19.5 × 10^{3}
Ba	2.05 × 10^{3}

### Table V. Effect of Base Oils on Background Count and Absorption Correction

				Counts p Zinc Wa	er Second at ave Lengths
	Viscosity, S.U. Sec.	°F.	C/H Ratio	Oil with- out zinc	Oil with 0.075% zinc
Pennsylvania bright stock Pennsylvania neutral South Texas pale oil South Texas pale oil Mid-Continent sol- vent refined neutral Mid-Continent sol- vent refined bright stock	$150 \\ 150 \\ 2000 \\ 100 \\ 160 \\ 120$	210 100 100 100 100 210	6.69 6.22 7.23 6.80 6.57 7.03	20.0 20.0 20.1 20.1 20.1	131.0 130.4 131.0 131.0 130.6 131.2

In the concentration range of interest, zinc showed no interference with the barium analysis. This has also been shown by calculations based on an equation analogous to Equation 11 using the  $K_{Ba}$  value in Table IV.

The variations in carbon-hydrogen ratio with stocks normally encountered have no significant influence on the analyses. This is shown in Table V for the determination of zinc.

> A more complete treatment of the absorption problem considering an average absorption coefficient  $(\mu/\rho)_{\ell}$  for the excited radiation as well as  $(\mu/\rho)_{\ell}$  for the fluorescent radiation gives Equation 12 replacing Equation 10

$$I_{\rm Zn} = \frac{K_{\rm Zn} m W_{\rm Zn}}{(\mu/\rho)_f + 0.5 \ (\mu/\rho)_e}$$
(12)

The factor 0.5 is a geometrical term required because the fluorescent radiation travels a greater distance, emerging at  $30^{\circ}$  to the surface, whereas the

Table III. Zinc Radiation from Oils in Counts per Second above Background (Comparison of calculated and experimental values)

				Counts	per Second			
	0.02	% Zn	0.07	% Zn	0.10	% Zn	0.1	2% Zn
% Ba	Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.
$\begin{array}{c} 0.00 \\ 0.30 \\ 0.60 \end{array}$	29 24 19	30 24 20.3	103 85 70	103 84 70	$147^{a}$ 120 99	$147^{a}$ 119.7 100.9	$175 \\ 143 \\ 118$	$176 \\ 143 \\ 119$

<sup>a</sup> This value used for calculating the  $K_{Zn}$  value for use in Equation 11. Other components of this oil were phosphorus 0.1%, sulfur 0.05%, and hydrogen 13%. The density of the oil is 0.865.



Figure 2. Polyethylene Bag as Installed on Instrument

excited radiation goes in nearly vertically. For fluorescent wave lengths less than about 1.4 A., the infinite-depth approximation is no longer correct. The integrals must each be evaluated for the true dish depth.

### INSTRUMENTATION

The x-ray equipment used in this work was a basic Norelco (North American Phillips Co.) diffraction unit with fluorescence accessories. (X-ray fluorescence equipment is also available from General Electric X-Ray Corp. and from Ohio X-Ray Co.) The equipment was used as supplied except for minor modification of the fluorescence assembly. A milliampere stabilizer, furnished by the manufacturer as an accessory, is a necessary addition to the equipment if accurate determinations are to be made. The analyzing crystal supplied and used with the instrument is rock salt with a *d* spacing of 2.819 A. The 7-inch nickel tubing collimator supplied was used in the position between the sample and crystal. No other collimator was used. The x-ray tube is a Machlett OEG50 with a molybdenum target.



Figure 3. Sample Dish with Indicator Attached

To establish a continuous helium path from sample to Geiger tube it is necessary to enclose the space between the exit end of the collimator and the Geiger tube. This enclosure must be flexible and capable of moving with the rotation of the crystal and Geiger tube. Such an enclosure (Figure 2) is constructed in the following manner: A Plexiglas disk, 4 inches in diameter and 0.5 inch thick, is affixed to the Geiger tube housing which has been shortened by cutting about 0.75 inch from the bottom of the housing. The space between the tube and the housing must be filled with a putty or mastic compound to prevent helium The space between the tube and the housing passing to the high voltage connection at the upper end of the tube. Helium present in this part of the tube will cause a discharge, giving erratic counting results. Another Plexiglas disk about 3 inches in diameter and 0.5 inch in thickness is fitted and cemented to the collimator and its supporting arm. A polvethylene film about 0.002 inch thick is made into a gas-tight bag fitting over and between the two disks when in their respective positions on the equipment. Rubber O rings of the proper diam975

abic	 Lincor	U1	nepiaeing		I utu		
			Counts	per	Second	above	

Table VI

	Backgr	ound
	Without He	With He
0.05% Zn 0.7% Ba	58.4 8.7	$78.8 \\ 28.2$
0.5% Ca	0.7	8.7

eter hold the polyethylene bag in place on the Plexiglas disks. A groove cut in the edge of the disk will aid in making a firm connection at this point. A hole 2 inches in diameter is cut in the side of the polyethylene bag to fit over the crystal holder. Another O ring holds the bag to the equipment at this point. Helium is admitted in both the x-ray tube housing and through the Plexiglas disk on the Geiger tube. The helium entrance to the x-ray tube housing is made by drilling the removable end plate and attaching a brass tubing connector. A right-angle bend should be made in the copper tubing attached to this fitting to prevent the passage of x-rays straight through the connector. The rate of flow of helium is adjusted by gradually increasing it until no further improvement in counting rate is observed. The apparatus used in this work required a helium rate of flow of about 600 ml. per minute. With some consideration to making a more efficient seal around the sample dish entrance, this rate of flow could be substantially reduced. Table VI indicates the importance of using the helium path.



Figure 4. X-Ray View of Alterations to Fluorescence Assembly

A critical point in obtaining reproducible determinations with this procedure is to arrive at and maintain a uniform positioning of the top surface of the sample in relation to the x-ray tube and the collimator. To achieve this, minor modifications were made in the fluorescence assembly and sample dish. So that the dish could be filled to a reproducible level, a reference pointer was attached in the manner shown in Figure 3. Filling is then done by placing the dish on a level surface and adding the sample until it just touches the pointer. The importance of using a level indicator of this type is shown in Table VII. This table also shows that the effect of infinite sample thickness is attained when the depth is 0.23 inch or more.

The primary x-ray beam has an appreciable heating effect on the sample, thus changing the volume and consequently the level in the sample dish. To minimize this volume change, a water-cooled aluminum block (Figure 4) replaced the sample dish slide track. The entire bottom of the aluminum sample dish is then in direct contact with the cooled block. Water is circulated through the block at a rate sufficient to keep the sample at approximately room temperature. The block is extended beyond the housing, to furnish a place to fill the dish and aid in getting it into the housing without spillage. To install this system, it was necessary to remove the safety shutter which blocks the x-ray beam when the sample dish is removed from the unit. This was replaced with a manually operated shutter, which is curved to match the contour of the tube and slides back and forth parallel to the axis of the tube. An interlock mechanism prevents the removal of the sample dish until the shutter is closed.

### 200 180 160 140 COUNTS PER SECOND 120 100 80 C 0.0% BARIUM ◬ 0.3% BARIUM 60 0.6% BARIUM 40 20 C .02 .04 .06 .08 -10 .12 % ZINC

Figure 5. Calibration Curve for Zinc Using a Molybdenum Tube at 50 Kv. and 30 Ma. with Helium

Table VII. Influence of Sample Position and Sample Thickness on Counting Rate

Distance above or below Normal Sample Level, Incl	Sample Thickness, Inch	Counts per Second
+0.015	0.280	120.0
0	0.266	113.3
0	$0.234^{a}$	113.3
0	$0.157^{a}$	105.0
-0.015	0.251	109.2
<sup>a</sup> Achieved by placing false bot	tom in sample dish.	

### CALIBRATION CURVES

Calibration curves are constructed for each of the metals through the concentration ranges which will be encountered. This is done by carefully preparing a series of standards for each metal, from known concentrations of metal sulfonates or naphthenates, and blending with a neutral oil of medium viscosity. Each of these is run as directed in the procedure. The number of counts is converted to counts per second and these values plotted against concentration of the metal in the standard. Typical calibration curves are shown in Figures 5, 6, and 7.

When zinc is determined in the presence of barium, a family of

Table VIII. X-Ray and Chemical Analyses for Barium, Calcium, and Zinc

-

ample 1 2 3 4 5 6 7 8 9	% ] X-ray 0.51 0.22 0.11 0.08 0.24	Barium Chemical 0.51 0.22 0.12 0.10 0.23	$\frac{\%}{\rm X\text{-}ray} \\ 0.068 \\ 0.101 \\ 0.089 \\ 0.086 \\ 0.103 \\ \end{array}$	Zinc Chemical 0.068 0.100 0.090 0.086 0.100	% C X-ray 0.021 0.099 0.196 0.586	Chemical 0.020 0.097 0.195 0.582
	40					
	36					+
	32-				$\square$	_
	28			- /*		-
anoc	24			$\bigwedge$		_
JER SE(	20					_
CL ND	16					_
C	12	/				
	a					
	4					
		0.2	0.4 % BA	0.6 0	5.8	

Figure 6. Calibration Curve for Barium Using a Molybdenum Tube at 50 Kv. and 40 Ma. with Helium

curves must be prepared by increasing the barium content of the zine standards in steps of 0.1%, through the concentration range of barium that will be encountered. The decrease in zine radiation with increasing barium in the sample is almost linear at barium concentrations of less than 1%, thus making interpolation between curves an easy matter.

To maintain the validity of the calibration curves with day-today fluctuation in instrument sensitivity, it is necessary to have a stable standard with which to make accurate checks. For this purpose a magnesium alloy containing a small amount of zinc is used. This is permanently mounted in a sample dish and a count taken on the zinc line every day. If this value changes from that obtained at the time the calibration curves were prepared, a correction factor is applied.

### PROCEDURE

The unit is allowed to warm up for 30 minutes before any quantitative measurements are made. Helium flow is adjusted



Figure 7. Calibration Curve for Calcium Using a Molybdenum Tube at 50 Kv. and 40 Ma. with Helium

to approximately 600 ml. per minute a few minutes before counting determinations are to be made. The sample dish is carefully filled to a fixed level and inserted in position under the x-ray tube. The scaler is set for fixed count operation, the number of counts in keeping with the accuracy desired. The statistics of counting are discussed by Birks *et al.* (2). Spectrometer angles, using a rock salt crystal for a d spacing of 2.819 A. for the elements to be determined, are shown in Table I. The spectrometer is adjusted to the angle of the element to be determined, and the times required for several successive counts are recorded.

The times required for the several counts taken are averaged and calculated as counts per second. The factor for instrument sensitivity is applied, if necessary, and the concentration of the element being determined is found from the calibration curve.

### RESULTS

The results for a series of calcium, zinc, and barium samples are shown in Table I. The accuracy of the x-ray data for these and other determinations was found to be at least equal to chemical methods. The method is capable, if sufficient counts are taken, of yielding accuracies within 2 or 3% of the barium and calcium, when present in concentrations greater than 0.05%, and within 1 to 2% of zinc in concentrations greater than 0.005%.

### ACKNOWLEDGMENT

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# Spectrochemical Determination of Impurities in Manganese Dioxide

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A spectrographic method has been developed for the semiquantitative determination of 23 elements that occur in manganese dioxide. Spectrochemical analyses for elements found in natural and synthetic manganese dioxide offer data that can be used to interpret the effects of impurities in the manganese dioxide used as the depolarizer in dry cells. The procedure is rapid, compared with conventional wet-chemical methods. The results are reproducible to within  $\pm 20\%$ . The sample is mixed with an equal weight of graphite and burned in a deep, thick-walled, graphite electrode in a direct current arc. When analytical data are obtained for the alkali elements, lithium carbonate is introduced as an internal standard. The analytical spectral lines and their usable ranges are given.

SOME of the elements that occur in the natural and synthetic manganese dioxide that is to be used as the depolarizer in dry cells act as poisoners to the battery system. Because other elements have undesirable effects it is desirable to evaluate chemically the number and concentration range of impurity

elements. These elements previously were determined by the wet-chemical method which often overlooked small quantities; therefore, this laboratory has developed a spectrochemical procedure that has proved to be both a rapid and satisfactory method for obtaining semiquantitative analyses of elements found in manganese dioxide.

In this procedure 19 elements are determined from one spectrogram and four elements (sodium, potassium, strontium, and barium) are determined from a second spectrogram. The procedure covers the wide range in quantity of the elements found in manganese dioxide, but no attempt is made to obtain the maximum accuracy possible for any one given element.

### METHOD AND EXPERIMENTAL DETAILS

The sample is mixed with an equal weight of graphite, placed in a deep, thick-walled, graphite electrode, and burned in a direct current arc for 5 seconds after the arc begins to sputter. A manganese spectrum line is used as the internal standard for all elements. To obtain analyses of the alkali elements, lithium carbonate is introduced as an internal standard and the sample is burned in a direct current arc for 10 seconds.

Apparatus. A 1.5-meter grating spectrograph (Applied Re-search Laboratories) is used in all of the work. It is supplied

with two optical benches that are arranged to permit simultaneous photographing of the ultraviolet and visible regions. A two-step filter is placed in the ultraviolet optical path. A 60micron slit opening is used in all exposures.

The excitation source is an Applied Research Laboratories Multisource unit, which is operated on settings of 60 mfd., 400 microhenries, and 18 ohms. These settings give a direct current, short-circuit current of 13 amperes and a current of 10 amperes with a 1-cm. arc.

The film, Eastman Spectrum Analysis No. 2, is developed in D-19 at 69° C. for 3 minutes in an Applied Research Laboratories developing machine.

The films are measured with an Applied Research Laboratories comparator-densitometer.

Standards. This laboratory uses as standards nine manganese dioxide samples, which contain the majority of elements encountered and the usual range of impurities. These samples have been analyzed chemically by several laboratories and have proved to be satisfactory standards for analysis.

The standards include samples of the following manganese dioxides: ores from the African Gold Coast, the Caucasus, Chile, and Phillipsburg, Mont.; synthetic manganese dioxides are from Burgess, Bright Star, National Carbon, and Baker (c.r. reagent grade).

Electrodes. The anode is a rod of ultra-high-purity graphite (United Carbon Products Co.), and has a diameter of 0.25 inch, and length of 1.25 inches. A hole, which is 0.25 inch deep and 0.125 inch in diameter, is drilled in the top of the anode. When the analyses of the alkali metals are obtained, a second, similar anode is used in which the hole in the top is 0.166 inch deep. The cathode is a graphite rod (not pointed at the end) with dimensions of 0.125 inch diameter and 1.5 inches in length.

### PROCEDURE

The original sample is mixed thoroughly and quartered, in order to obtain a representative batch. The selected batch is ground so that it will pass through a 100-mesh screen. A 100-mg. portion of the sample and an equal amount of high-purity, 100-mesh graphite are weighed on a Roller-Smith Precision Balance and mixed for 20 seconds in a modified, dental Wig-L-Bug amalgamator. Twenty milligrams of the mixture is placed in the bottom electrode. The sample occupies about half the depth. The electrodes are placed in the excitation source unit. The intensity of exposure is controlled by a combination of quartz filters which are adjusted so that the internal standard line for manganese yields transmittance values between 20 and 40% in the lighter of two steps. A two-step filter is held in the same mount as the The top electrode is touched to the bottom elecquartz filters. trode and the arc is drawn to a gap of 1 cm. and burned at 10 amperes. The initial burn is a very smooth glow, which persists for about 80 seconds before the arc begins to sputter. The length of the glow period is dependent on the quantity of im-purities present in the manganese dioxide. The glow or smooth period is longer in purer samples and shorter in the less pure The total length of exposure is the smooth period plus samples. 5 seconds of the sputtering period. Five two-step spectra are recorded on one film length. One spectrum is a standard

Table I.	Analytical Lines and Concentration Range of	əf
	Impurities in Manganese Dioxide	

(Internal standard line manganese 2695.4 A.)

Element	Analysis Line A	Concn. Range,
Diement	Line, A.	10
Berium	2335 3	1 -5
Boron	2497 7	Õ 02-Õ 2
Lord	2922 1	0.02-0.2
Leau	2000.1	0.02-0.2
Manager	2010.0	0.2 -2
Magnesium	2892.1	0.01-0.2
<b>a</b>	2781.4	0.2 - 2
Silicon	2881.6	0.01 - 0.4
	2435.2	0.4 - 5
Iron	3020.6	0.01-0.2
	2983.6	0.1 - 2
Aluminum	3092.7	0.01-0.3
	2660.4	0.3 - 2
Molvbdenum	3170.3	0.01 - 0.1
Vanadium	3185.4	0.01 - 0.1
Conner	3274 0	0 01-0 1
ooppor	2961 2	0 2 -1
Zine	3282 3	0.2 -2
Titonium	2279 8	0.01 0.2
Silaa	2200.0	0.01-0.2
Silver	0002.9	0.01-0.03
Nickel	3414.8	0.01-0.2
Cobait	3453.5	0.01 - 0.2
Chromium	4254.3	0.01-0.1
Calcium	4302.5	0.01-0.2

sample with approximately the same composition as the unknown sample, whereas the other spectra are of the unknown samples. The film is processed and placed in the comparator-densitometer for measurement. The percentage transmittance of the internal standard line for manganese and of the lines of impurity elements are measured and listed in Table I. The intensity ratio of the internal standard line and the impurity element line is established by means of the emulsion calibration curve. Churchill's (2) preliminary curve method is used for the film calibration. The amount of each impurity element present in a sample, expressed as percentage by weight, is obtained from previously established working curves, based on concentration values of the internal standard line and the impurity element line. Because each film contains its own appropriate standard, that standard may be used to detect trends (deviations) in the analytical working curves.



Figure 1. Effect of Buffer on Selective Volatilization

The procedure for determining sodium, potassium, barium (for values below 0.1%), and strontium is a modification of a method suggested by Harvey (3). One per cent of high-purity lithium carbonate is mixed with the sample and an equal weight of powdered graphite is added to this mixture. A 30-mg. charge, which completely fills the cup, is weighed into the 0.166inch-deep bottom electrode. The electrodes are burned for ten seconds in a 10-ampere direct current arc. A lithium spectrum line is used as the internal standard. The analytical spectrum lines used are shown in Table II.

Table II. Analytical Lines and Concentration Range for Other Elements in Manganese Dioxide

(Internal	standard	line	lithium	2741.3	A.)	
-----------	----------	------	---------	--------	-----	--

Element	Analysis Line, A.	Concn. Range, %
Sodium	3302.3	0.1 - 2
Potassium	4044.1	0.1 -3
Strontium	4077.7	0.01-0.1
Barium	4554.0	0.01-0.1

### DISCUSSION

The procedure described is not a burn-to-completion method. Further analyses of the residual powdered graphite reveal the presence of manganese and of impurity elements, in about the same relative concentrations as the original material. A minimum amount of graphite is consumed in the time necessary to volatilize most of the manganese. Selective volatilization is eliminated very largely by the buffering effect of the manganese dioxide and the powdered graphite. Ahrens and Liebenberg (1) note that the presence of powdered carbon or graphite, when mixed with a sample other than manganese dioxide, tends to eliminate selective volatilization during the first period of arcing. Figure 1 shows that manganese dioxide and graphite buffer the

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burning conditions for a longer period and more effectively than do graphite and a material other than manganese dioxide.

When the correct exposure is obtained (transmittance values between 20 and 40% for the internal standard line) the background is negligible and no background correction is necessary.

No thorough study has been made of the interelement effect, or of the mineralogical effect of the various forms of manganese dioxide ores encountered. Preliminary study of these effects showed that a procedure to eliminate them would complicate the method unnecessarily. Selection of the proper, closely related, chemically analyzed standard makes possible partial corrections for these effects.

Elements of low-ionization potential, such as sodium and potassium, in concentrations greater than 0.5%, have a markedly suppressive effect on most other impurity elements. The internal standard line for manganese also is suppressed and, by increasing the over-all exposure, the ratio between the internal standard line and the impurity lines remains approximately the same as the corresponding ratio for a sample that contains no elements of low-ionization potential.

The manganese spectrum is relatively simple and there is little interference between manganese lines and analytical lines. A faint manganese line interferes with the lead line at 2833.1 A., at values of the impurity below 0.02%. Manganese interference with the most persistent zinc line limits the detection of that element to 0.2%. There is zinc interference with the sodium line at 3302.5 A.

Thus far no interpretive data have been obtained from impurity values below 0.01%, a value that has been set by this laboratory as the lowest quantity to be reported. Because the majority of the analytical spectrum lines show sensitivities below this value and the synthetic manganese dioxides have few impurities above 0.01%, their analyses are greatly facilitated by estimations rather than measurements, provided a standard with impurities approximating this value is used for comparison. As a rule, natural manganese ores contain most of the elements sought in a measurable quantity.

The nine chemically analyzed standards are supplemented by special standards for high values of lead and barium. No working curves have been established for the analysis of arsenic, antimony, or tin, as most samples do not contain them. When these elements are present, an estimation is made from special chemically analyzed standards.

When the element barium appears in quantities greater than 1% (the mineral hollandite is one form of naturally occurring manganese dioxide that contains high barium), it is determined from the film, using manganese as an internal standard line. When the quantity is below 0.1%, barium is determined along with the alkalies.

Lead and iron are chemically analyzed and spectrochemical analyses are used only as a check on the polarographic determination of lead and the wet-chemical determination of iron. Table

Table III. Spectrographic and Chemical Determinations of Other Elements in Manganese Dioxide

Lea	d, %	Iro	n, %	Silicon, %		
Spec.	Chem.	Spec.	Chem.	Spec.	Chem.	
0.15	0.14	1.3	1.0	6.0	6.1	
0.18	0.16	1.1	0.96	5.0	6.3	
< 0.02	< 0.02	1.2	1.1	0.80	1.0	
< 0.02	<0.02	1.4	1.2	0.75	0.95	
0.06	0.04	2.0	1.5	1.0	1.4	
0.27	0.25	2.0	1.8	5.1	4.8	
0.29	0.20	1.2	1.1	5.7	4.9	
0.30	0.41	0.13	0.16	0.06	0.06	
0.03	0.02	0.01	0.01	0.01	0.005	
<0.02	< 0.02	0.08	0.09	0.19	0.14	

Table IV. Reproducibility of Results from Ten Analyses of Same Sample of Manganese Dioxide

		Lead		Iron	ŝ	Silicon		
	%	Deviation from mean	%	Deviation from mean	%	Deviation from mean		
	$\begin{array}{c} 0.036\\ 0.037\\ 0.038\\ 0.038\\ 0.039\\ 0.038\\ 0.037\\ 0.037\\ 0.037\\ 0.037\\ 0.037\\ 0.033\end{array}$	$\begin{array}{c} 0.001 \\ 0.000 \\ 0.001 \\ 0.001 \\ 0.002 \\ 0.001 \\ 0.000 \\ 0.000 \\ 0.000 \\ 0.000 \\ 0.000 \\ 0.004 \end{array}$	$\begin{array}{c} 0.31 \\ 0.37 \\ 0.39 \\ 0.41 \\ 0.34 \\ 0.39 \\ 0.31 \\ 0.41 \\ 0.41 \\ 0.34 \end{array}$	$\begin{array}{c} 0.06\\ 0.00\\ 0.02\\ 0.04\\ 0.03\\ 0.02\\ 0.06\\ 0.04\\ 0.04\\ 0.03\\ \end{array}$	$\begin{array}{c} 0.077\\ 0.071\\ 0.082\\ 0.097\\ 0.066\\ 0.077\\ 0.066\\ 0.082\\ 0.087\\ 0.077\end{array}$	$\begin{array}{c} 0.001 \\ 0.007 \\ 0.004 \\ 0.019 \\ 0.012 \\ 0.001 \\ 0.012 \\ 0.004 \\ 0.009 \\ 0.001 \end{array}$		
Mean	0.037	0.001	0.37	0.034	0.078	0.007		
% Av. d from 1	ev. nean	2.7		9.2		9.0		

III gives comparative values between spectrographic and chemical values for silicon, lead, and iron for ten samples.

### REPRODUCIBILITY

A statistical study, conducted over a period of one year, indicates that the procedure is reproducible to well within  $\pm 20\%$ for the majority of the elements, and better than  $\pm 10\%$  for some of the elements. Table IV shows the results of the same sample analyzed ten times for three elements.

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# **Calibration Method for X-Ray Fluorescence Spectrometry**

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A first-order linear correction for the absorption effects of the various elements in an alloy on fluorescent x-ray intensities is obtained by essentially assuming a monochromatic incident beam. The result is a set of simultaneous equations; the unknowns represent composition, the diagonal coefficients are determined by intensity measurements on the unknown alloy and pure elements, and the nondiagonal coefficients are calculated from intensity measurements on analyzed binary alloys. The simultaneous equations are overdetermined, and the calculating procedure, which minimizes the error introduced by the assumption, has been determined empirically. Analyses based on this method, where only pure elements are needed as standards, have been performed on quaternary alloys containing molybdenum, nickel, iron, and chromium. The calculated values agreed in nearly all cases to within  $\pm 5\%$  of the values obtained by accepted chemical procedures.

S HERMAN has treated the problem of fluorescent x-ray analysis statistically (3). The following development considers the physical mechanism of x-ray absorption and indicates an empirical elemental calibration.

If it were not for absorption effects, the fluorescent x-ray intensity due to an element in an alloy would be proportional to the atomic density of that element, and a quantitative analysis could be made from the ratio of intensity of the element in the alloy to that of the pure element. The absorption effects arise because the incident x-rays penetrate a significant distance below the specimen surface, although Koh and Caugherty (2) have shown that this distance is not more than 0.004 inch. An expression for the absorption effects can be derived, but its precise form is not usable owing to the continuous spectrum of the incident beam. The excitation probability, however, is very high only for a limited region on the short-wave-length side of the absorption edge. By assuming the incident intensity and the absorption coefficients of all the elements to be constant in this region of high excitation probability, the first-order absorption effects of all elements can be easily determined empirically from binary alloys. The assumption of constant incident intensity and absorption coefficients introduces an appreciable error when the alloy contains elements whose absorption edges or characteristic spectra lie in the region of high excitation probability. These conditions are abundant in the chromium-iron-nickel alloys; therefore, this ternary system would be a severe test for the assumption. In the investigation of the chromium-iron-nickelmolybdenum quaternary system, molybdenum was added to test for large differences in atomic number.

Gillam and Heal dealt with the mathematical problem of fluorescent x-ray intensities (1). Expressions of similar form are derived here.

The fluorescent x-ray intensity due to a slab of thickness, dx, at a depth, x, below the surface is determined (Figure 1).

Let  $I_0(\lambda)d \lambda$  be the intensity of the incident radiation in the range of wave lengths between  $\lambda$  and  $\lambda + d\lambda$ . At the slab, the incident intensity is reduced by a factor of

$$\exp\left[-\mu_{e}(\lambda)x\alpha\right]$$

where  $\mu_{\epsilon}(\lambda)$  is the linear absorption coefficient of the specimen for the incident wave length,  $\lambda$ , and  $\alpha$  is a geometric constant of the

apparatus. Roughly,  $\alpha = \csc \theta$ ; more precisely, it is complicated owing to the incident beam divergence.

If A is the area of the mask in front of the specimen, and if  $N_a$  is the atomic density of the element, a, in the specimen, there are  $N_a A dx$  atoms of a in the slab. If  $Q_a(\lambda)$  is the excitation probability of element a at wave length  $\lambda$ , the intensity of fluorescent x-radiation emitted by the slab in all directions is

$$I_0(\lambda)d\lambda \exp\left[-\mu_e(\lambda)\alpha x
ight]Q_a(\lambda)N_aAdx$$

A fraction of this radiation within the infinitesimally small solid angle,  $d\omega$ , which makes an angle  $\phi$  with the specimen surface in the plane of incidence, will be diffracted by the spectrometer crystal to the Geiger counter. On emerging from the surface, this radiation from the slab has been further reduced in intensity by a factor of

$$\exp\left(-\mu_f x \csc \varphi\right)$$

where  $\mu_f$  is the linear absorption coefficient of the specimen for the monochromatic fluorescent x-radiation of element a. The fluorescent intensity due to element a in the slab, excited by the range of wave lengths between  $\lambda$  and  $\lambda + d\lambda$  is

$$d^{2}I_{a} = I_{0}(\lambda)Q_{a}(\lambda)N_{a}Ad\omega \exp\left[-(\mu_{e}(\lambda)\alpha + \mu_{f}\csc\varphi)x\right]dxd\lambda \quad (1)$$

The intensity due to the entire volume of the specimen is obtained by integrating Equation 1 from a depth of x = 0 to  $x = \infty$ , giving

$$dI_{a} = \frac{I_{0}(\lambda)Q_{a}(\lambda)N_{a}Ad\omega d\lambda}{\mu_{e}(\lambda)\alpha + \mu_{f}\csc\varphi}$$
(2)

To obtain an exact expression for fluorescent x-ray intensity, Equation 2 must be integrated with respect to  $\lambda$ ; however, three quantities in Equation 2 are unknown functions of  $\lambda$ . At wave lengths longer than the absorption edge of a,  $Q_a(\lambda)$  is zero; it is maximum at the absorption edge, and decreases rapidly as  $\lambda$ decreases from the edge. If we assume that  $I_0(\lambda)$  and  $\mu_a(\lambda)$  are essentially constant over the range of high  $Q_a(\lambda)$ , the approximate expression for fluorescent x-ray intensity due to a is

$$I_{a} = \frac{I_{0}Q_{a}N_{a}Ad\omega}{\mu_{e}\alpha + \mu_{f}\csc\varphi}$$

$$= \frac{K_{a}N_{a}}{\mu_{e}\alpha + \mu_{f}\csc\varphi}$$
(3)

where  $K_a = I_0 Q_a A d\omega$  is a constant of the apparatus and the element, a, and is independent of the absorption in the specimen. The intensity is seen to be proportional to the atomic density,  $N_a$ , of a, modified by the absorption effect of the alloy in the denominator of Equation 3. We shall denote this absorption effect by  $\rho_s M_{as}$ , where  $\rho_s$  is the density of the alloy, subscript a refers to the radiating element, and subscript s refers to the alloy matrix of all the elements. The mass absorption coefficients,  $\mu/\rho$ , of a mixture of elements is the sum of the mass absorption coefficients of all elements in proportion to their composition by weight. If  $W_b$  is the fractional weight of element b in the alloy, then

$$M_{as} = \alpha \sum_{b} W_{b} \left(\frac{\mu_{e}}{\rho_{b}}\right)_{ab} + \csc \varphi \sum_{b} W_{b} \left(\frac{\mu_{f}}{\rho_{b}}\right)_{ab}$$
(4)

the double subscript, ab, indicating the mass absorption coefficient of element b for the excitation (first term of Equation 4) or characteristic (second term of Equation 4) radiation of element a. From Equation 4, it follows that

$$M_{as} = \sum_{b} W_{b} M_{ab} \tag{5}$$

If the fluorescent x-ray intensity  $I_{aa}$ , from a pure element specimen of a is divided by that from the alloy,  $I_{aa}$ , the ratio is (using Equations 3 to 5)

$$R_{a} = \frac{I_{aa}}{I_{as}} = \frac{K_{a}N_{aa}}{\rho_{a}M_{aa}} \times \frac{\frac{\rho_{s}\sum_{b}W_{b}M_{ab}}{K_{a}N_{as}}}{K_{a}N_{as}}$$
$$= \frac{N_{aa}}{\rho_{a}} \times \frac{\rho_{s}}{N_{as}}\sum_{b}W_{b}\frac{M_{ab}}{M_{aa}}$$
(6)

 $N_{as}/\rho_s$  is the number of atoms of *a* per gram of alloy, and  $N_{aa}/\rho_a$  is the number of atoms of *a* per gram of *a*. Therefore,

$$\frac{N_{as}}{\rho_s} \times \frac{\rho_a}{N_{aa}} = W_a \tag{7}$$

the number of grams of a per gram of alloy, or the fractional weight of a. Multiplying Equation 6 by  $W_a$  and setting  $A_{ab} \equiv M_{ab} = \overline{M_{ab}}$ .

 $R_a W_a = \sum A_{ab} W_b = W_a + \sum A_{ab} W_b$ 

or

$$b \qquad b \neq a$$

$$(1 - R_a)W_a + \sum_{b \neq a} A_{ab}W_b = 0 \tag{8}$$

The absorption parameters,  $A_{ab}$ , can be easily obtained empirically by reading intensity ratios on chemically analyzed binary alloys. Thus, from Equation 8,

$$A_{ab} = \frac{W_a}{W_b} (R_a - 1)$$

$$A_{ba} = \frac{W_b}{W_c} (R_b - 1)$$
(9)



Figure 1. X-Ray Fluorescence

Once these absorption parameters are determined over an alloy system, any alloy in the system can be analyzed by reading fluorescent x-ray intensity ratios,  $R_a$ ,  $R_b$ , . . ., for each element in the alloy and substituting them into simultaneous Equations 8:

$$-(R_a - 1)W_a + A_{ab}W_b + A_{ac}W_c + \dots = 0 A_{ba}W_a - (R_b - 1)W_b + A_{bc}W_c + \dots = 0 A_{ca}W_a + A_{cb}W_b - (R_c - 1)W_c + \dots = 0$$
 (10)

The unknowns are further governed by the constraint:

$$W_a + W_b + W_c + \dots = 1$$
 (11)

### EXPERIMENTAL PROCEDURE

Fifteen specimen blocks were prepared: Four were the highpurity elements, chromium, iron, nickel, and molybdenum, with respect to which the intensity ratios were taken; six were all of the binary combinations of these elements for determining the empirical absorption parameters of the system; the remaining five were test alloys, three being chromium-iron-nickel ternaries, and two being chromium-iron-nickel-molybdenum quaternaries.

The binaries, ternaries, and quaternaries were chemically analyzed. These analyses were conducted with exceptional care and the values from duplicate samples averaged. The procedures were checked by analyzing National Bureau of Standards' samples at the same time.

All elemental, binary, and ternary samples were checked qualitatively on the x-ray spectrometer for impurities, and none was detected.

Fluorescent x-ray intensities were read using the General Electric XRD-3S unit. The line intensities were read in all samples for a given element without changing the goniometer setting or the stabilized x-ray tube current. The latter was adjusted so that the intensity from the pure element did not exceed 3500 counts per second. Appropriate background intensities. Table IV lists intensity ratios for the alloys and the actual intensities of the elements in counts per second. The total count for each reading was 65,536, giving a statistical probable error of 0.26%.

Table I. Chemical Analyses of the Binary Alloys

Analysia	Binary Partner								
for	Cr	Fe	Ni	Mo					
Cr Fe Ni Mo	50.83 48.19 23.53	$\begin{array}{r} {\bf 48.24} \\ {\bf 46.65} \\ {\bf 34.44} \end{array}$	48.07 51.53 46.27	74.28 65.33 53.70					

### Table II. Intensity Ratio for Binary Alloys

sting		Binary Partner							
Element	Cr	Fe	Ni	Mo					
Cr	1.000	1.760	1.815	1.841					
Fe	3.360	1.000	1.613	1.835					
Ni	2.860	3.670	1.000	1.954					
Mo	3.770	2.787	2.461	1.000					

Table III. Absorption Parameters

Radi- ating	Absorbing Element								
Element	Cr	Fe	Ni	Mo					
Cr	1.000	0.721	0.813	2.660					
Fe	2.482	1.000	0.676	1.582					
Ni	1.863	2.420	1.000	1.108					
Mo	0.877	0.9435	1.260	1.000					

The compositions of the binary alloys are given in Table I, and the x-ray intensity ratios of the binaries are listed in Table II. The data in these two tables are substituted into Equations 9 to determine the absorption parameters (Table III). With these evaluated, the simultaneous equations for the chromiumiron-nickel-molybdenum quaternary system are:

 $-(R_{\rm Cr} - 1)$ Cr + 0.721Fe + 0.813Ni + 2.660Mo = 0 (A)

2.482Cr - ( $R_{Fe}$  - 1)Fe + 0.676Ni + 1.582Mo = 0 (B)

1.863Cr + 2.420Fe -  $(R_{Ni} - 1)$ Ni + 1.108Mo = 0 (C)

$$0.877$$
Cr +  $0.9435$ Fe +  $1.260$ Ni -  $(R_{Mo} - 1)$ Mo = 0 (D)

An alloy of this system is analyzed by reading the four x-ray intensity ratios from the alloy and the standard elements, substituting them into the above equations, and solving the equations.

Since there are no constant terms in these equations, we can merely obtain ratios among the unknowns. The equations are,

therefore, overdetermined, since there are only n-1 independent ratios among n quantities. A set of ratios can be obtained by dividing through by any of the four unknowns. We can then omit any one of the four equations to solve for the three unknown ratios with the remaining three equations. Thus there are at least 16 independent ways to solve the above equations.

These 16 independent procedures yield 16 different sets of solutions, because the equations are based on a rough assumption and the coefficients are empirical. Thus the equations are mutually inconsistent to some extent. It is desirable to choose that calculating procedure which tends to minimize the errors introduced by the assumption. Such a calculating procedure was developed empirically; however, the result indicates that it can also be developed from the symmetry of the equations.

### CALCULATING PROCEDURE

Initially, one element must be distinguished from the others. Empirically it develops that the best solutions are obtained by distinguishing the most abundant element in the alloy. By dividing throughout by the unknown representing the relative weight of the abundant element, the effective number of unknowns is reduced by one.

The result is four equations in three unknowns, each equation containing a constant term. The triple ratio among the three unknowns, which is the triple ratio among the three elements other than the abundant element, is best determined from the three equations which contain the intensity ratios of these elements. Therefore, the equation which contains the intensity ratio of the abundant element is omitted, and the triple ratio is obtained by evaluating the three numerator determinants of the remaining three equations. These determinants are then multiplied by an adjustment factor, whose value depends on the ratios of each of the elements to the abundant element, and, therefore, whose value is best obtained by substituting the three determinants into the fourth equation, which contains the intensity ratio of the abundant element. The final four individual solutions are determined by using Equation 11.

The use of the equations in combinations other than that just described consistently yields solutions further from the accepted values obtained by chemical analysis. The range of values calculated among various combinations of the equations is particularly large for chromium-iron-nickel alloys, because the abundant anomalies in the ranges of high excitation probability introduce large errors to the assumption. Therefore, this calculating procedure apparently uses all equations in the best possible manner to minimize these errors. The relation of this procedure to the symmetry of the problem fortunately provides convenient rulesof-thumb.

### SAMPLE CALCULATION

If Alloy 4 from Table IV, which gives the intensity ratios of the ternary and quaternary alloys, is chosen iron is seen to

Table IV.	Intensity I	Ratios for T Alloys	ernary and	Quaternary
		$Ra = I_{aa}^{a}/I_{a}$	18	
Alloy No.	Cr	Fe	Ni	Mo
1 2 3 4 5	$\begin{array}{r} 3.610 \\ 3.535 \\ 2.477 \\ 5.04 \\ 4.01 \end{array}$	$\begin{array}{c} 1.918 \\ 6.620 \\ 3.665 \\ 2.346 \\ 1.942 \end{array}$	$14.34\\2.152\\5.373\\6.640\\14.72$	15.34 41.35
Iaa <sup>b</sup>	597	2795	3270¢	1076
<sup>a</sup> Intensity	in counts per	second on put	re element correc	sted for back-

ground. <sup>6</sup> Intensity from a 0.75  $\times$  0.75 inch sample area. Bent mica crystal was 0.0016 inch thick. <sup>6</sup> Nickel intensities were determined with a W-target x-ray tube operat-ing at 24.1 ma. and 48.5 kvp. All other elements were determined under 49.6 ma. and 48.5 kvp.

have the lowest intensity ratio; therefore, it is taken as the most abundant element. Equation B is omitted, the equations are divided through by Fe, the constant terms are transposed, and the intensity ratios from Table IV are substituted.

-4.04Cr/Fe + 0.813Ni/Fe + 2.660Mo/Fe = -0.721  $(\mathbf{A})$ 

$$1.863$$
Cr/Fe -  $5.640$ Ni/Fe +  $1.108$ Mo/Fe =  $-2.420$  (C)

$$0.877 \text{Cr/Fe} + 1.260 \text{Ni/Fe} - 14.34 \text{Mo/Fe} = -0.9435$$
 (D)

If the denominator determinant of the coefficients times the adjustment factor is denoted by k, then the numerator determinants are

$$k \operatorname{Cr/Fe} = \begin{vmatrix} -0.721 & 0.813 & 2.660 \\ -2.420 & -5.640 & 1.108 \\ -0.9435 & 1.260 & -14.34 \end{vmatrix} = -108.61$$
  
$$k \operatorname{Ni/Fe} = \begin{vmatrix} -4.04 & -0.721 & 2.660 \\ 1.863 & -2.420 & 1.108 \\ 0.877 & -0.9435 & -14.34 \end{vmatrix} = -163.42$$
  
$$k \operatorname{Mo/Fe} = \begin{vmatrix} -4.04 & 0.813 & -0.721 \\ 1.863 & -5.640 & -2.420 \\ 0.877 & 1.260 & -0.9435 \end{vmatrix} = -39.37$$

Multiplying Equation B by k/Fe and transposing the resulting constant term (with  $R_{\rm Fe}$  substituted in from Table IV) yields

2.482 k Cr/Fe + 0.676 k Ni/Fe + 1.582 k Mo/Fe = 
$$1.346 k$$
 (B)

The above three numerator determinants are substituted into this equation, and the result is solved for k, giving

$$k = -328.62$$

Equation 11 can now be used to evaluate the four unknowns as follows (disregarding the negative signs):

$$k \operatorname{Cr}/\operatorname{Fe} = 108.61$$
  

$$k \operatorname{Ni}/\operatorname{Fe} = 163.42$$
  

$$k \operatorname{Mo}/\operatorname{Fe} = 39.37$$
  

$$k = k \operatorname{Fe}/\operatorname{Fe} = 328.62$$
  

$$k/\operatorname{Fe}(\operatorname{Cr} + \operatorname{Ni} + \operatorname{Mo} + \operatorname{Fe}) = 640.02 = k/\operatorname{Fe}$$

giving

$$Cr = \frac{108.61}{640.02} = 16.97\%$$

$$Fe = \frac{328.62}{640.02} = 51.35\%$$

$$Ni = \frac{163.42}{640.02} = 25.53\%$$

$$Mo = \frac{39.37}{640.02} = 6.15\%$$

for the analysis.

### DISCUSSION

The results on all five test alloys, using the calculating procedure outlined above, are given in Table V, together with the chemical analyses. For Alloy 3, whose three elements are equally abundant, the best analysis is obtained by taking the mean of the three sets of solutions, using each of the elements as the abundant one. This is not true for the remaining alloys, which, like most practical alloys, have only one element in high abundance. The single calculating procedure, therefore, should be adequate in most cases.

In the derivation of Equation 2, all of the excitation radiation was assumed to come from the direction of the x-ray source. When a certain element in the alloy emits  $K \alpha$  radiation in the region of high K excitation probability for the element being read, there will be a strong monochromatic component of the excitation spectrum coming from all directions. This secondary excitation radiation, however, will still be strongest from the

Ele- ment	Composi- tion by Chemical Analysis, %	Composi- tion by X-Ray Using Param- eters, %	% Error	Composi- tion by X-Ray by Propor- tional Intensities, %	% Error
Cr Fe Ni	$19.85 \\ 65.94 \\ 13.98$	$20.29 \\ 65.95 \\ 13.76$	+2.22 + Nil -1.57	$27.7 \\ 52.1 \\ 6.98$	+39.5 -21.0 -50.0
Cr Fe Ni	$21.23 \\ 13.85 \\ 64.84$	$20.39 \\ 15.04 \\ 64.57$	$   \begin{array}{r}     -3.95 \\     +8.60 \\     -0.42   \end{array} $	$28.7 \\ 15.1 \\ 46.5$	$^{+35.2}_{+9.0}_{-28.3}$
Cr Fe Ni	$\begin{array}{r} {32.13} \\ {35.43} \\ {31.86} \end{array}$	$32.44 \\ 35.86 \\ 31.66$	+0.97 + 1.21 - 0.63	$40.4 \\ 27.3 \\ 18.6$	$^{+25.8}_{-22.9}_{-41.5}$
Cr Fe Ni Mo	$\begin{array}{r} 16.45 \\ 50.20 \\ 26.80 \\ 6.55 \end{array}$	$16.96 \\ 51.35 \\ 25.54 \\ 6.15$	+3.10 +2.29 -4.70 -6.10	19.881.015.16.52	+20.4 +61.4 -43.6 - 0.5
Cr Fe Ni Mo	18.4366.2612.992.32	19.6364.9313.232.22	$+6.51 \\ -2.01 \\ +1.85 \\ -4.31$	$24.9 \\ 51.5 \\ 6.79 \\ 2.42$	+35.1 -22.3 -48.4 + 4.3
	Ele- ment Cr Fe Ni Cr Fe Ni Cr Fe Ni Cr Fe Ni Cr Fe Ni Cr Fe Ni Cr Fe Ni	Composi- tion by Chemical Analysis, Cr 19.85 Fe 65.94 Ni 13.98 Cr 21.23 Fe 13.85 Ni 64.84 Cr 32.13 Fe 35.43 Ni 31.86 Cr 16.45 Fe 50.20 Ni 26.80 Mo 6.55 Cr 18.43 Fe 66.26 Ni 12.99 Mo 2.32	Composition by tion by Chemical         Composition by X-Ray Using           Ele- ment         Malysis, %         Param- eters, %           Cr         19.85         20.29           Fe         65.94         65.95           Ni         13.98         13.76           Cr         21.23         20.39           Fe         13.85         15.04           Ni         64.84         64.57           Cr         32.13         32.44           Fe         35.43         35.86           Ni         31.86         31.66           Cr         16.455         6.95           Ni         26.80         25.54           Mo         6.55         64.93           Ni         12.99         13.23           Mo         2.32         2.22	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table V. Final Results

direction of the surface. Gillam and Heal (1) gave, without derivation, an expression for the effect, which they called mutual fluorescence, and which was to be added to a term analogous to Equation 2. The inclusion of this expression would prohibit the use of linear equations with empirical coefficients.

The use of empirically determined absorption parameters compensates for this omission. This can be seen from the values of the absorption parameters (Table III). Thus  $A_{\rm CrFe}$ ,  $A_{\rm crNi}$ , and  $A_{\rm FeNi}$  have values less than unity—i.e., less than the selfabsorption value. Since the absorbing element in each case is a little heavier than the radiating element, the low absorption parameters can be due only to mutual fluorescence. Hence, the empirically determined parameters give an automatic correction for mutual fluorescence. The three corresponding parameters with the subscripts reversed, on the other hand, are appreciably greater than unity. Close examination shows that there is a correspondence in the departure from unity between the two sets of three parameters. This stems from the reciprocal relation: high excitation of A from B's  $K \alpha$  radiation means high absorption of B's radiation by A.

That the absorption parameters with their automatic correction for mutual fluorescence are not strictly constant can be seen in Table V. In Alloy 2, having a high nickel-low iron content, the x-ray reading of iron is 8.6% too high; whereas, for Alloy 5, in which the nickel-iron composition is reversed, the x-ray reading of iron is 2% too low. Thus it is well to have the binaries, from which the parameters are determined, near the 50-50 composition.

The last two columns in Table V give the compositions and relative errors, assuming the relative compositions to be inversely proportional to the intensity ratios of Table IV. The effects of absorption and mutual fluorescence are readily observed, and the effectiveness of the absorption parameters in correcting for these effects is in evidence.

Analyses based on this procedure offer the significant advantages of a physical method of analysis that is not restricted by the need for reference or standard samples with a composition similar to the sample being analyzed.

### CONCLUSIONS

Absorption-excitation effects can be expressed in terms of empirical parameters relating pairs of elements.

These parameters and flourescent intensity data can be combined in theoretically simultaneous, linear equations.

A solution of these equations ultimately gives the percentage by weight of each element in an alloy.

This definite system for solving these overdetermined equations greatly simplifies the mathematical manipulation.

The accuracy of results obtained on ternary and quaternary alloys compares favorably to that obtained by routine chemical procedures.

This method is not restricted by need for reference samples.

### ACKNOWLEDGMENT

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# Spectrophotometric Determination of Humulone Complex and Lupulone in Hops

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A rapid method for determination of lupulone and of humulone complex (humulone plus cohumulone) in petroleum ether extracts of hops is based on absorbance measurements at wave lengths of 275, 325, and 355 m $\mu$ . The influence of solvent and of alkali in methanol on the spectrophotometric stability of lupulone and humulone complex has been studied. The background absorption of hop extracts due to components other than these two has been investigated by adsorption on ion exchange resin and silica gel, by molecular distillation, and by alkaline extraction of hop extracts. Conditions to which a background absorption must conform in a ternary spectrophotometric analysis, so that it makes no contribution to values for each of two real components, are pointed out. These conditions are shown to be approximated well by the variable background absorption of hop extracts due to components other than lupulone and humulone complex.

THERE is need for a rapid method for determination of the resinous constituents of hops, which are important in brewing. These include humulone, lupulone, and cohumulone. Cohumulone was discovered recently by Rigby and Bethune (11), who isolated it by countercurrent distribution. It is precipitated with humulone by lead and o-phenylenediamine, and its absorption spectrum and optical rotation are nearly identical with those of humulone  $(\mathcal{P})$ . Since humulone, cohumulone, and possibly a third component (11) are not distinguished by the ultraviolet spectrophotometric method described or by any other method, with the exception of countercurrent distribution (10), the term "humulone complex" is used for the mixture and "humulone" for the pure compound.

A tentative proposal for spectrophotometric determination made by the authors in 1949 (6) was based on their observation that lupulone and humulone complex give rise to the principal absorption of hop extracts in the spectral region approximately from 300 to 400 m $\mu$ . Although the tentative method proved very useful in following the isolation of lupulone and humulone complex, subsequent work by Rigby and Bethune (10) and work in this laboratory has shown that the content of humulone complex is overestimated systematically because of interference by nonspecific absorption, which increases with decreasing wave length.

The modified spectrophotometric method of Rigby and Bethune (10) was based on a preliminary purification of the hop extract by adsorption on silica gel, following the procedure of Govaert and Verzele (4). Investigations of various methods of fractionation of hop extracts have led to the wholly spectrophotometric method reported here. The influence of the illdefined absorbing impurities is corrected by measuring the absorption at 275 m $\mu$ , a wave length of minimum absorption for both lupulone and humulone complex. The method is rapid; results on prepared extracts can be obtained within 5 minutes.

The nature of the interfering background absorption was investigated by fractionating the impurities, that absorb at low wave lengths, away from lupulone and humulone complex by molecular distillation, by adsorption on ion exchange resin and silica gel, and by alkaline extraction. Although the background spectra thus obtained did not conform to varying concentrations of a single hypothetical compound, they did follow the relationship  $A_{355}:A_{225}:A_{275} = 1:(0.93 + 0.18X):X$ , which allows a solution for lupulone and for humulone complex by ternary simultaneous equations. It is possible that such a property of ternary equations, in allowing a certain variation in the spectrum of background absorption, has not been reported previously and may be useful in other applications. For example, backgrounds of constant spectra will rarely, if ever, be found for plant extracts.

Lupulone and humulone complex, although relatively stable in hop extracts, were found to be much less stable in more highly purified form in various solvents, so that special precautions are necessary in fractionation and standardization.

### SPECTROPHOTOMETRIC PROPERTIES OF LUPULONE AND HUMULONE COMPLEX

Spectrophotometric observations usually were made with a Cary Model 11 recording spectrophotometer. Photometric accuracy was verified with a yellow and a blue reference filter (NBS-G-5145) and alkaline potassium chromate solutions prepared according to Haupt ( $\delta$ ). Mercury and cadmium emission lines were used as standards for wave-length calibration. In general, absorption spectra were scanned between 400 and 220 mµ. Although the Beckman Model DU spectrophotometer was used only occasionally, the instrument may be used to advantage in the analytical procedure where absorbance is to be measured at only a few wave lengths. Concentrations should then be somewhat lower than shown here, in order to fall within the best precision of this instrument.

In the following discussion the absorbance of a solution at a given wave length,  $\lambda$ , is given by the expression:

$$A_{\lambda} = \log_{10} \frac{I_0}{I} = \alpha \ cd$$

where  $I_0$  is the energy transmitted by solvent-filled reference

cell, I, the energy transmitted by sample cell, c, the sample concentration in grams per liter, d, the cell depth (path length) in centimeters, and  $\alpha$ , the specific absorption coefficient. Thus, where specific absorption coefficients are given, they are in units of liters per gram centimeter.

Another symbol,  $A'_{\lambda}$ , is useful in describing the attempts to separate the ultraviolet-absorbing impurities from the lupulone and humulone complex in hop extracts by liquid-liquid extraction, by adsorption analysis, and by vacuum distillation. In such cases the fractional absorbance  $A'_{\lambda} = A_{\lambda}D$ , where  $A_{\lambda}$  is the observed absorbance for an aliquot of any fraction, and D the dilution factor from the starting hop extract.

It was found early in this investigation that both lupulone and humulone complex in methanol solution separately obey Beer's law, and that the absorbance is additive in mixtures. These conditions are, of course, essential to the development of convenient equations for the analytical procedure.



Figure 1. Absorption Spectra of Lupulone and Humulone Complex in Acidic (0.002N) and Alkaline (0.002N) Methanol

Absorption spectra of lupulone and humulone complex in methanol are shown in Figure 1; details of the purification procedures are given in the next section. Spectra usually were scanned immediately after mixing 0.03 ml. of 0.2N sodium hydroxide or 0.2N hydrochloric acid with the 3.0-ml. contents of the 1.00-cm. fused-silica absorption cells. The solutions, 0.002N in acid or alkali, were found to give reproducible spectra over reasonable periods of time, as shown in the section on stability. The alkaline spectra proved most useful, and undefined readings refer to this condition. Reagent-grade absolute methanol was used as a reference solvent; the small changes in solvent absorption caused by the acid were very reproducible. The alkali pro-

rable I.	Stabilit	y of Hu Met	mulone hanol	Comple	ex in N	leutral
Concn. of	Sto	rage Conditio	ns	Increase	in Absort	ance, %
Humulone Complex, Mg./Ml.	Approx. temp., °C.	Time	Illumi- nation	355 mµ	325 mµ	275 mµ
$ \begin{array}{c} 10 \\ 5 \\ 1 \\ 0.054 \\ 0.054 \\ 0.054 \\ 0.040 \\ 0.040 \end{array} $	25 5 25 25 25 25 25 25 25	7 days 28 days 90 days 24 hours 4 hours 2 hours 1 hour 4 hours 4 hours	Room Dark Room Room Room Room Room	-25 1 -47 0.5 -2 -1 0.5 -5 0.5	-24 1 -44 0.5 -2 -1 0.5 -6 0.5	67 27 83 1 •• 19 2

	5	Sodium Hy	droxide					Amm	ionia		
NaOH concn	H <sub>2</sub> O concn.,	Storage	Absorbance Change, %		NH <sub>3</sub> concn.,	H <sub>2</sub> O ·	) Storage	1	Absorbance Change, %		
N	%	min.	355 mµ	325 mµ	275 mµ	N	%	min.	$355 m\mu$	$325 m\mu$	$275 \text{ m}\mu$
				H	lumulone	Complex					
$\begin{array}{c} 0.02 \\ 0.01 \\ 0.02 \\ 0.002 \\ 0.02 \\ 0.02 \\ 0.012 \end{array}$	1 0.5 1 1 10 0.3	$10 \\ 15 \\ 15 \\ 15 \\ 15 \\ 15 \\ 180$	-0.7 <0.5 <0.5 <1 <1 <1 <1	<0.5 <0.5 <0.5 <1 <1 <1 <1	1 <2 <2 <2 <3 <3	$\begin{array}{c} 0.03 \\ 0.01 \\ 0.02 \\ 0.002 \\ 0.02 \\ 0.02 \\ 0.01 \end{array}$	$1 \\ 0.3 \\ 1 \\ 10 \\ 1 \\ 0.5$	$960 \\ 15 \\ 15 \\ 15 \\ 15 \\ 15 \\ 180 \\ 20$	-9 <0.5 <1 <1 <1 -3 <0.5	$     \begin{array}{r}       -9 \\       <0.5 \\       <1 \\       <1 \\       <1 \\       -2 \\       <0.5     \end{array} $	$     \begin{array}{r}       49 \\       <2 \\       <3 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\       <2 \\$
					Lupu	lone					
0.02 0.02 0.001	1 1 0.5	20 10 32	0.5 0.5 0.5	$0.5 \\ 0.5 \\ 0.5 \\ 0.5$	3 3 3	$\begin{array}{c} 0.02 \\ 0.02 \\ 0.02 \\ 0.002 \\ 0.002 \\ 0.0005 \\ 0.006 \end{array}$	< 0.1 1 0.7 1 0.3 3	$10 \\ 10 \\ 120 \\ 20 \\ 20 \\ 10 $	$   \begin{array}{r}     -2 \\     -4 \\     -17 \\     -1 \\     -0.5 \\     -2   \end{array} $	-1.5 -3 -11 -0.5 -0.5 -1	

Table II. Stability of Lupulone and Humulone Complex in Alkaline Methanol

duced precise results if the reagent was kept free of carbonate, which may precipitate in anhydrous methanol and cause light scattering.

Stability. Methanol was chosen as the solvent for spectrophotometry because humulone complex and especially lupulone are relatively stable in it. Solutions of lupulone in methanol (250  $\gamma$  per ml.) showed no change in 3 months at room temperature in diffuse daylight. Humulone complex, although much less stable, is adequately stable for analytical purposes at scanning dilutions if dark storage is used or if the final dilution into methanol is delayed until a few minutes before reading (Table I).

The stability of humulone complex and especially of lupulone is least in nonpolar solvents. The spectrum of lupulone in 2,2,4trimethylpentane, although similar to that in acidified methanol. changed within 20 minutes (10% decrease in  $A_{320}$ ). On further storage (up to 3 months at room temperature) the spectrum approached that of a simple carbonyl compound. Similar, but

somewhat slower, changes occur in petroleum ether and carbon tetrachloride. However, lupulone appears to be fairly stable in chloroform. Humulone complex changes in ethylene dichloride to give a spectrum similar to that of isohumulone (3)when scanned in alkaline methanol. The change occurs somewhat more rapidly in diffuse light than in the dark, but is complete within 24 hours at room temperature for a 0.03% solution.

The concentration and kind of alkaline give small but analytically important effects upon both the spectra and the stabilities of purified lupulone and humulone complex in methanol. A scanning solu-

tion composed of 99% methanol and 1% water, and 0.002N in sodium hydroxide was chosen for analytical purposes because lupulone is unstable in ammoniacal methanol (Table II) and because the spectrum of humulone complex is influenced by concentration of sodium hydroxide (Table III).

The concentration of water in the range 0.1 to 3% had no effect on the spectra of lupulone or humulone complex in weakly alkaline methanol. Higher concentrations affected the spectrum of humulone complex, particularly with the higher concentrations of sodium hydroxide. Variation of water concentration in the range 0.3 to 10% had little effect upon stability, except for lupulone in the presence of 0.02N ammonium hydroxide.

Precautions regarding stability of lupulove and humulone complex are impor-

cause it causes unexplained losses of both lupulone and humulone complex. Samples of this preparation were recrystallized repeatedly by four different procedures.

1. By solution in petroleum ether and chilling to  $-20^{\circ}$  C. (seven times)

2. By solution in methanol and addition of 10% of water (three times).

3. By partial neutralization with hydrochloric acid of a solu-tion of sodium lupulonate in 60% methanol (three times).

4. By cooling a 4.5% solution in petroleum ether successively to  $30^\circ$ ,  $20^\circ$ ,  $0^\circ$ , and  $-20^\circ$  C., crystal crops being removed at each temperature.

Table IV shows melting points and specific absorbances at a few selected wave lengths for several stages of each treatment. Inasmuch as the melting point of lupulone varies with rate of heating, these melting points were determined by inserting the capillary in a bath at 85° C. with the temperature rising 6° per minute.

### Table III. Variation of Spectrum of Humulone Complex in Methanol with Sodium Hydroxide Concentration

Nº0H				A	bsorbance	$e^a$			
Concn., N	$380 \text{ m}\mu$	370 mµ	360 mµ	$355 \text{ m}\mu$	$345 \text{ m}\mu$	335 mµ	325 mµ	$275 \text{ m}\mu$	220 mµ
$\begin{array}{c} 0.02 \\ 0.01 \\ 0.002 \\ 0.001 \end{array}$	$1.070 \\ 1.090 \\ 1.095 \\ 1.093$	$1.344 \\ 1.357 \\ 1.369 \\ 1.371$	$1.414 \\ 1.426 \\ 1.441 \\ 1.441 \\ 1.441$	$\begin{array}{c} 1.405\\ 1.412\\ 1.426\\ 1.428\end{array}$	$1.436 \\ 1.447 \\ 1.463 \\ 1.462$	$1.615 \\ 1.627 \\ 1.636 \\ 1.634$	$\begin{array}{c} 1.707 \\ 1.706 \\ 1.712 \\ 1.711 \\ 1.711 \end{array}$	$\begin{array}{c} 0.419 \\ 0.416 \\ 0.408 \\ 0.402 \end{array}$	$2.18 \\ 2.13 \\ 2.13 \\ 2.13 \\ 2.12$

<sup>a</sup> Both lupulone and humulone complex have spectra in methanol which are independent of the concentration of ammonia (from 0.0001 to 0.02*N*, or from 0.7 to 200 times the stoi-chiometric equivalent) and which agree with those observed with low concentrations of so-dium hydroxide. The spectrum of lupulone is independent of the concentration of sodium hydroxide in the range 0.001 to 0.02*N*. Secondary maxima (227 mµ for humulone complex and 222 mµ for lupulone) become evident in the more dilute alkali, because absorption by the sodium hydroxide, not compensated in the scanning procedure used, obscures them at all but the lowest alkali concentrations.

### Table IV. Purification of Lupulone

			Specific Absorption Coefficients <sup>a</sup>									
		Reiting		In A	lkali			In Acid				
Procedure	Stage	°C.	355 mµ	331.5 mµ	$325 \text{ m}\mu$	275 mµ	355 mµ	325 mµ	275 mµ			
Starting material	0	93-93.5	45.7	36.4	32.5	3.6	18.4	21.0	15.5			
1	$1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \\ 7$	93.5-94.5 94-95 94-95 94-94.5 93.5-94.5 89-92 85-90	$\begin{array}{r} 45.6 \\ 46.3 \\ 45.0 \\ 46.1 \\ 46.0 \\ 45.0 \\ 44.1 \end{array}$	36.6 37.0 36.7 37.0 36.8 36.1 35.5	$\begin{array}{r} 32.5\\ 32.9\\ 32.7\\ 32.8\\ 32.5\\ 32.0\\ 31.5\\ \end{array}$	3.5 3.7 3.6 3.9 3.9 3.9 3.6	$18.3 \\ 18.5 \\ 18.5 \\ 18.4 \\ 18.2 \\ 17.9 \\ 17.6 \\$	$21.1 \\ 21.5 \\ 21.3 \\ 21.3 \\ 21.1 \\ 20.8 \\ 20.5$	$\begin{array}{c} 15.8\\ 16.2\\\\ 16.0\\ 15.8\\ 15.6\\ 15.5\end{array}$			
2	$\frac{2}{3}$	•••	$\substack{\textbf{46.1}\\\textbf{46.3}}$	$\begin{array}{c} 36.8\\ 37.4 \end{array}$	$\begin{array}{c} 32.7\\ 33.0 \end{array}$	3.5 3.5	$\begin{array}{c} 18.4 \\ 18.6 \end{array}$	$\begin{array}{c} 21.2\\ 21.7\end{array}$	$\begin{array}{c} 15.8\\ 16.1 \end{array}$			
3	$\frac{2}{3}$	• • •	$\begin{array}{c} 45.2 \\ 45.7 \end{array}$	$   \begin{array}{c}     36.0 \\     36.3   \end{array} $	$\begin{array}{c} 32.0\\ 32.1 \end{array}$	$\begin{array}{c} 3  .  6 \\ 3  .  7 \end{array}$	$\begin{array}{c} 17.9 \\ 18.9 \end{array}$	$\begin{array}{c} 20.8\\ 22.0 \end{array}$	$\begin{array}{c}15.7\\17.2\end{array}$			
4	$     \begin{array}{c}       1 \\       2 \\       3 \\       4     \end{array} $	95–96 95–96 95–95 . 5 92–93	$46.0 \\ 45.9 \\ 45.8 \\ 45.7$	$37.2 \\ 37.1 \\ 36.9 \\ 37.0$	33.3 33.0 32.8 33.2	$3.7 \\ 3.6 \\ 3.6 \\ 4.4$	$18.1 \\ 18.4 \\ 18.2 \\ 18.0$	$21.2 \\ 21.2 \\ 21.2 \\ 21.2 \\ 21.1$	$15.7 \\ 15.8 \\ 15.6 \\ 15.6$			

tant primarily in fractionation,

purification, and standardization. Unlike similar solutions of the purified components, petroleum ether extracts of hops are unexpectedly stable. Absorption Coefficients of Lupulone and Humulone. Lupulone was prepared by direct crystallization from the petroleum ether-soluble solids of commercially dried hops as previously described (7), with three crystallizations from petroleum ether and two from 90% methanol. The use of dry ice as an aid in grinding hops (7) is to be avoided, be-

		Tabl	eV. I	Purifica	tion of	Humu	lone Co	mplex	. 1			
	Temn	Ontical		In A	81	Decine AD	sorption	In Acid				
Fraction	° C.	Rotation, °4	355 mµ	$331.5 \text{ m}\mu$	325 mµ	275 mµ	355 mµ	$345 \text{ m}\mu$	331.5 mµ	$325 \text{ m}\mu$	275 mµ	
Original <sup>c</sup>			31.8	37.4	38.0	8.3	16.6	17.8	20.6	21.4	20.7	
AI	25		31.8	37.4	38.0	8.3	16.6	17.8	20.6	21.5	20.7	
A2	12	• • •	31.6	37.6	38.0	8.2	16.5	17.7	20.6	21.3	20.4	
A3	5		31.6	37.6	38.0	8.2	16.5	17.7	20.6	21.3	20.6	
Ala			31.6	37.4	38.0	8.7	16.5	17.6	20.4	21.3	20.5	
Blad	37	- 208	31.7	37.1	37.8	9.0	16.7	17.8	20.5	21.4	20.2	
Bib			31.7	37.1	37.8	8.9	16.6	17.9	20.4	21.4	20.2	
B3a	iś	-207	31.8	37.3	38.0	8.9	16.8	18.0	20.6	21.5	20.5	
<b>B</b> 3h			32.0	37 7	38.5	9.0	16.8	18.0	20.7	21.5	20.5	
<b>B</b> 49	13	-208	31.7	37.0	37.6	8.9	16.8	17.9	20.5	21.5	20.5	
B4b			31.6	37.5	38.1	9.1	16.7	17.9	20.5	21.4	20.4	

<sup>a</sup> In methanol (c2,1L). <sup>b</sup> Solvent is methanol, 0.002.N in sodium hydroxide or hydrochloric acid. <sup>c</sup> Except for A1a, the measurements for Series A were made on the o-phenylenediamine salt and corrected for absorption due to the o-phenylenediamine content. <sup>d</sup> For Series B, duplicate samples of each humulone-complex fraction were weighed for scanning to obtain an estimate of the precision of the absorption curves. The difference between duplicates is generally less than 0.5%. The averages of set B3a and b have been chosen as the best values for the absorption coefficients of humulone complex in methanol under the above conditions.

Repeated recrystallization by chilling in petroleum ether (Procedure 1) caused the melting point and absorbance at high wave lengths to pass through a maximum at the second and third stages while  $A_{275}$  showed a minimum in the same region. Procedure 3 slightly degraded the starting material. Inasmuch as the over-all changes in spectrum on repeated recrystallization were very small (0.5% at higher wave lengths), the standard lupulone sample is assumed to have high purity. The first and second stages of Procedure 4 gave products with a 1° higher melting point than any others. Because lupulone melts with decomposition making melting points highly sensitive to small changes in technique, this treatment may not have produced lupulone of significantly higher purity than that from the other treatments. The specific absorption coefficients for lupulone chosen for analytical calculations are:  $355 \text{ m}\mu$ , 46.0;  $325 \text{ m}\mu$ , 33.1; 275 mµ, 3.7.

Humulone complex was isolated, from the petroleum ethersoluble solids of hops remaining after lupulone crystallization, as the o-phenylenediamine salt by the method described previously (7). The absorption coefficients of humulone complex in acidic and alkaline methanol were studied with both free humulone complex and with the o-phenylenediamine salt (with corrections for the absorption of the contained o-phenylenediamine). Spectra were scanned for various samples crystallized by chilling benzene solutions of the o-phenylenediamine salt of humulone

complex to successively lower temperatures. For Series A of Table V, 10 grams of o-phenylenediamine salt (crystallized three times from benzene) in 125 ml. of benzene at 40° C. was cooled to 25° C., a crop of crystals was removed, washed with petroleum ether, and dried in vacuum. Two additional crops of crystals were removed at 12° and 5° C., the latter with the addition of 10% petroleum ether. Humulone complex (458 mg., 99% of theory) isolated by shaking with aqueous hydrochloric acid and ethyl ether from 600 mg. of fraction A1 gave the results shown for entry A1a of Table V, in good agreement with the coefficients measured on the o-phenylenediamine salt.

A similar procedure on a larger scale was used for Series B of Table V, except that free humulone complex was made for the absorption and optical rotation measurements reported for each fraction. The specific absorption coefficients for humulone complex chosen for analytical calculations are: 355 mµ, 31.8; 325 mµ, 38.1; 275 mµ, 9.0.

After the discovery by Rigby and Bethune (11)of cohumulone in hop extracts two countercurrent distributions were conducted with humulone complex isolated by ether extraction (after acidification with hydrochloric acid) from the parent batch of ophenylenediamine salt on which the purification studies of Table V were carried out. The solvent system was 0.43Mpotassium phosphate buffer (pH 8.25) and 2,2,4-trimethylpentane (>99 mole %), approximating that of Rigby and Bethune, who used pH 8.5 phosphate buffer. The concentration of humulone complex influenced the distribution markedly; 7.5 mg. per ml. gave a distribution ratio, K, about twice as high as that at 1 mg. per ml.

The first 59-tube run gave

two peaks with approximate areas under the curves corresponding to 14.5% of the slower component (K = 0.51) and 85.5% of the faster component (K = 1.27). The second run gave 15% of the slower component (K = 0.53) and 85% of the faster component (K = 1.34) as indicated by  $A_{325}$ . It appears that the humulone complex on which the absorption coefficient studies were made contained less than 15% cohumulone, because the parent material was recrystallized from benzene and from a benzene-petroleum ether mixture, a treatment which, Rigby and Bethune (11) state, enriches the precipitate in humulone.

### INVESTIGATIONS OF HOPS

Extraction Procedure. In contrast to petroleum ether solutions of lupulone and humulone complex, petroleum ether extracts of hops are remarkably stable. After 24 hours at room temperature the spectra of hop extracts (equivalent to 2.5 mg. hops per ml.) in petroleum ether (boiling point 30° to 60° C.) show essentially no change. After 72 hours, the adsorption in the 275 m $\mu$  region rises moderately (approximately 30%) but little change occurs at the higher wave lengths characteristic of lupulone and humulone complex. The reason for this marked difference in stability is not known. Direct extraction of dried hops with petroleum ether has obvious advantages, with respect

Table VI. Effect of Extraction Procedure on Extract Purity

		Hop 1		Hop 2				
Extraction Method	Lupulone, %ª	Humulone complex, % <sup>a</sup>	Excess A 275, %	Lupulone, %ª	Humulone complex, % <sup>a</sup>	Excess A275, %		
Direct methanol	5.2	8.4	150	4.8	7.8	167		
tract of methanol	4.2	7.0	58	4.3	6.7	85		
Direct petroleum ether <sup>b</sup>	4.7	7.6	49	4.1	6.5	51		
<sup>a</sup> Calculated using F	oustions 1a	and th						

b Direct extraction with petroleum ether by rocking at room temperature was found to be complete in about 30 minutes in confirmation of earlier reports (2).

Table VII. Fractionation of Hop Extract by Aqueous Alkali Extraction Assay Values, Dry Wt. of Hop, %

						Lupulone		Humulone Complex		
Fraction	A' 275	A'325	A (355	Amount	<u>4′275</u> %	Ternary analysis	Binary analysis	Ternary analysis	Binary analysis	
Original	52.2	196.0	204.1	15.0	40	4.56	4.41	5.89	6. <b>46</b>	
1st extract 2nd extract 3rd extract 4th extract Residue Sum of fractions	$     \begin{array}{r}       37.9 \\       4.0 \\       1.8 \\       1.0 \\       9.3 \\       54.0     \end{array} $	$   \begin{array}{r}     158.0 \\     20.3 \\     6.3 \\     1.9 \\     5.8 \\     192.3   \end{array} $	$ \begin{array}{r} 160.3 \\ 26.3 \\ .8.2 \\ 2.3 \\ 5.1 \\ 202.2 \end{array} $	7.0 1.3 1.0 0.7  10.0	23 48 125 250	3.161.030.330.080.114.71	$   \begin{array}{r}     3.09 \\     1.02 \\     0.32 \\     0.08 \\     0.03 \\     \hline     4.54   \end{array} $	5.340.140.02-0.04-0.02 $5.44$	5.61 0.18 0.05 0.03 0.28 6.15	

to simplicity and speed, over methanol extraction followed by successive liquid-liquid extractions with petroleum ether, as commonly employed.

The low absorption of both lupulone and humulone complex near 275 m $\mu$  provides a region of relatively high sensitivity to absorbing impurities. An index of absorbing constituents, other than lupulone and humulone complex, in extracts is calculated by subtracting from the observed  $A_{275}$ , the theoretical  $A_{275}$  for the lupulone and humulone complex, which a binary analysis of  $A_{325}$  and  $A_{555}$  indicates should be present: this excess absorbance may be expressed as a percentage of the calculated A 975.



Figure 2. Absorption Spectra of Hop Extract and Background (Separated by Molecular Distilla-tion) in Alkaline (0.002N) Methanol

Methanol extraction of dried hops gave extracts with approximately 150% excess  $A_{275}$  while petroleum ether extracts showed about 50 to 60% excess  $A_{275}$  (Table VI). Liquid-liquid petroleum ether extracts of methanol extracts give intermediate values for excess  $A_{275}$ . Moreover, the difference curve between the spectrum for a direct petroleum ether extract and that for a methanol extract of the same hop sample has a different shape than a typical

hop-extract spectrum (Figure 2), being high at low wave lengths and low at high wave lengths.

Fractionation of Hop Extracts. Although lupulone and humulone complex obviously account for most of the ultraviolet absorption of petroleum ether extracts of hops, the presence of excess absorption in the 275 m $\mu$  region shows other absorbers to be present. The larger part of this work has been devoted to attempts to determine the nature of this interfering absorption. The problem has proved to be difficult, and no procedure has been found for separating completely the lupulone and humulone complex from the other absorbers. Four approaches to the fractionation of hop extracts have been tried: Alkaline extraction, ion exchange absorption analysis, silica gel adsorption analysis, and molecular distillation.

A major difficulty in any fractionation of hop extracts is the instability of the separated constituents. In petroleum ether and ethylene dichloride, whole hop extract is stable in comparison with isolated lupulone and humulone complex, which are markedly unstable. Thus, in adsorption analyses, conservation of absorption of the various fractions is not achieved, absorption being augmented at low wave lengths (below 300 m $\mu$ ) and lost at higher wave lengths during fractionation. However, these changes, during chromatography on both ion exchangers and silica gel, can be minimized sufficiently by control of solvent choice, time, and illumination to yield useful qualitative and semiquantitative information on the interfering absorbers. Molecular distillation and alkaline extraction were the only operations in which good absorbance conservation at all wave lengths was obtained.

Alkaline Extraction. A petroleum ether extract (10 ml. of a 5% extract) of a good quality hop was extracted rapidly four times with 2 volumes of aqueous 5% potassium carbonate solution. The over-all conservation of absorbance was good (line 1 versus line 7, Table VII). Because of its ascending shape toward lower wave lengths, the residue from alkaline extraction calculates almost exclusively as humulone complex in the binary analysis based on 325 and  $355 \text{ m}\mu$ . Its contribution to the humulone-complex value (0.28) is approximately 5% of the apparent

Humulone

								Excess A	/ 275	Lupul	oneb	Com	plex <sup>b</sup>
Sample <sup>a</sup>	Quality	Fraction	Cut No.	A 275	$A_{325}'$	A355	%	Amount	% removed	Ternary analysis	Binary analysis	Ternary analysis	Binary analysis
Hop A <sup>c</sup>	Poor	Original extract		51.2	68.8	62.0	241	36.2		0.86	0.50	1.81	3.18
		Luphum. cuts Interference	$1-14 \\ 15-24$	13.4 40.4	$38.0 \\ 27.0$	$38.1 \\ 19.0$	53 570	$\begin{array}{c} 4.8 \\ 33.4 \end{array}$	87	0.75	0.69	1.17	1.39
		Sum of cuts	1-24	53.8	65.0	57.1	273	38.4		0.71	0.31	1.65	3.14
Hop A	Poor	Original extract		38.5	55.6	52.6	235	27.4		0.95	0.67	1.31	2.33
		Luphum. cuts Interference	16 7-9	$14.6 \\ 23.8$	$\substack{\textbf{42.6}\\\textbf{13.1}}$	$43.7 \\ 8.7$	78 580	$\begin{array}{c} 6.5\\ 20.3 \end{array}$	76	0.95	0.88	1.22	1.47
		Sum of cuts	1–9	38.4	55.7	52.4	231	26.8		0.91	0.65	1.35	2.37
Hop B	Fair	Original extract		49.0	127.1	129.0	100	24.4		2.74	2.50	3.59	4.51
		Luphum. cuts Interference	$1-14 \\ 15-17$	$\begin{array}{c} 27.0\\ 24.3 \end{array}$	$\substack{111.2\\16.0}$	$\begin{array}{r} 118.6\\10.9\end{array}$	34 500	$\begin{array}{r} 6.7 \\ 20.0 \end{array}$	72	2.87	2.81	3.15	3.40
		Sum of cuts	1-17	51.3	127.2	129.5	110	26.8		2.81	2.55	3.46	4.47
Humulone complex		<b>Original solution</b>		264	1128	941	1.5	4		-0.15	-0.1	100	100
		Humulone complex Interference	1-7 8-11	247 55	1027 43	$\substack{848\\29}$	3.3 380	8 43		-1.6	-1.7	92.2	92.4
		Sum of cuts	1-11	302	1070	877	20	51		-2.2	-2.9	94.3	97.2
Distillate of hop B	Fair	Distillate		25.8	93.4	94.8	42	7.6		1.91	1.83	3.02	3.31
		Luphum. cuts Interference	$^{1-6}_{7-9}$	17.9 10.2	$\begin{array}{c} 79.8 \\ 10.2 \end{array}$	$\begin{array}{c} 85.0 \\ 7.1 \end{array}$	$\begin{array}{c} 22 \\ 280 \end{array}$	$3.2 \\ 7.5$	58	2.03	2.00	2.33	2.45
		Sum of cuts	1-9	28.1	90.0	92.1	60	10.6		1.96	1.85	2.71	3.12
Distillate of hop C	Good	Distillate		42.3	191.3	200.6	19	6.7		4.52	4.46	5.94	6.18
		Luphum. cuts Interference	16 79	$35.5 \\ 12.5$	$\substack{172.0\\18.0}$	$\substack{187.3\\13.3}$	$\begin{array}{c} 15 \\ 66 \end{array}$	4.7 8.3	30	4.81	4.77	4.71	4.90
		Sum of cuts	1-9	48.0	190.0	200.6	35	13.0		4.71	4.58	5. <b>52</b>	6.00

Table VIII. Fractionation of Hop Extracts by Adsorption Analysis on Silica Gel

<sup>a</sup> The sample (petroleum ether extractables) in ethylene dichloride (or benzene extract in Run A) was added to a 0.6 × 7 cm. column (0.6 × 16 cm. for Run B) of ethylene dichloride-washed silica gel (Davison). Eluate was collected in 10-ml. cuts with a rate of flow of 0.6 ml. per minute. Methanol was used to elute the interference after complete elution of lupulone and humulone complex. The light intensity was less than 2 foot-candles, the time of elution was approximately 3 hours, and the cuts were scanned immediately after collection. <sup>b</sup> Percentage of original weight. <sup>c</sup> Benzene extract and developer. <sup>d</sup> Decomposition exceeded purification.

content (6.15%). The residue contained about 25% of the petroleum ether-soluble solids, or about 4% of the weight of this hop, and it accounts for about 50% of the excess  $A_{275}$  of the original extract.

Silica Gel Adsorption Analysis. Adsorption analysis on silica gel with benzene as a developer was introduced by Beyaert and Cornand (1) for preparation of lupulone and humulone complex and by Govaert and Verzele (4) for preliminary purification of hop extracts in the analytical method for these substances. Rigby and Bethune (10) adapted the procedure to the spectrophotometric determination. Ethylene dichloride has been substituted in the present investigation because benzene extracts of hops are unstable to light. Thus, with ethylene dichloride, no change in  $A_{355}$  was noted in 66 hours, whereas a loss of approximately 5% was noted in 30 hours with benzene with a light intensity less than 2 foot-candles. Separated humulone complex is unstable in ethylene dichloride also, so that losses occurred even with the duration of experiments limited to 3 hours.

Summarized in Table VIII are six fractionations on silica gel of hop extracts, molecular distillates of hop extracts, and humulone complex. It may be noted that ethylene dichloride gave better recovery of adsorbance than benzene, but that approximately 8% decomposition of humulone complex occurred. The excess  $A_{275}$  formed during adsorption analysis of humulone complex appeared principally in the methanol eluate. With all

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distillation seemed most likely to offer easy control of decomposition. The expectation was borne out by the excellent conservation of absorption at all wave lengths.

Extracts were prepared as follows, to eliminate a methanolinsoluble waxlike material of very low specific absorption: Handground hops (usually 5.0 grams) were rocked at room temperature with 100 ml. of petroleum ether for about 40 minutes. The solids were allowed to settle, and an aliquot of supernatant liquid was evaporated to dryness under mechanical pump vacuum at a low temperature. The solids were chilled in a dry ice bath before release of vacuum and then dissolved in a volume of methanol equal to that of the petroleum ether aliquot. A small amount of insoluble wax was filtered off and the filtrate used for molecular distillation. The wax represented less than 0.1% of the ultraviolet absorption of the petroleum ether extract.

For distillation, 2 or 3 ml of such a 5% extract of hops plus an equal volume of methanol were evaporated slowly to dryness under mechanical pump vacuum in the molecular still to obtain a film of uniform thickness. This is not difficult if heat is supplied only by air conduction. Unless the films are protected from contact with air (by vacuum or by chilling), excess absorbance develops at low wave lengths and loss of absorbance at high wave lengths. The still containing the solids was then chilled to dry ice temperature, the vacuum released, and the still transferred to a vacuum system consisting of a mercury-diffusion pump backed by a two-stage mechanical pump. When the vacuum reached <1 micron, the Dewar-type still pot was heated to 60° C. for 2 hours (sufficient for complete distillation of lupulone and humulone complex, as judged from the shape of the residue

plex appeared principally in hop extracts the sum of  $A'_{275}$ for all cuts exceeded the original  $A_{275}$  whereas the reverse was true for  $A'_{325}$  and  $A'_{355}$ . Most of the composition change appears to be at the expense of humulone complex, the lupulone values remaining fairly constant.

It is evident that silica gel adsorption analysis does not completely fractionate lupulone and humulone complex from interfering absorbers. The percentage of excess  $A_{275}$ of the original extract removed by adsorption analysis gives a rough measure of the efficiency of the procedure. Approximately 72 to 87% of the interference was removed from hops A and B, whereas with hop distillates, the decomposition exceeded the purification.

Specially prepared silica gel has been recommended (1, 10), but was not tested in the investigation. Rigby (9) has stated that conservation of the absorbance of humulone complex was obtained by its use.

The spectra of the methanol eluates are similar to those for the residues of molecular distillation. The relative absorbances of this material from the hop extracts are approximately 1:1.5:2.1 to 2.7 for  $A_{355}$ :  $A_{325}$ :  $A_{275}$ .

Molecular Distillation. Since adsorption analysis had shown hop extracts to contain very labile systems, short-path

Table IX.	Fractionation	of Hon	Extracts by	Molecular	Distillation
I aDIC LZ.	riacuonación	OT TTOD	D'ALTACLS DY	TOICCUIAL	Distination

					Е	xcess	A 275	Lupu	lone <sup>a</sup>	Humulone Complex <sup>a</sup>	
Hop umber	Fraction	, A 275	, A 325	, A 355	Amt.	%	% re- moved	Ternary analysis	Binary analysis	Ternary analysis	Binary analysis
1	Extract	25.2	44.7	48.7	17.4	222		1.42	1.24	0.61	1.27
	Distillate Residue	$15.4 \\ 9.6$	38.4 4.4	$\begin{array}{c} 44.4\\ 3.0 \end{array}$	9.2	150	47	1.44	1.34	0.50	0.85
	Dist. $+$ res.	25.0	42.8	47.4				1.45	1.27	0.48	1.14
$^{2}$	Extract	23.9	30.3	32.0	18.3	325		0.92	0.73	0.26	0.96
	Distillate Residue	$\begin{smallmatrix}14.5\\9.3\end{smallmatrix}$	$\tfrac{26.4}{3.7}$	$\tfrac{29.7}{2.0}$	10.1	230	44	0.94	0.83	0.28	0.66
	Dist. $+$ res.	23.8	30.1	31.7				0.90	0.72	0.27	0.96
3	Extract	48.4	72.0	70.0	33.9	<b>234</b>		1.42	1.08	1.56	2.84
	Distillate Residue	26.0 22.6	$\begin{array}{c} 61.7 \\ 10.2 \end{array}$	$63.8 \\ 6.0$	14.4	125	58	1.48	1.34	1.53	2.08
	Dist. + res.	48.6	71.9	69.8				1.41	1.07	1,56	2.85
4	Extract	38.5	105.3	109.9	19	97		2.58	2.40	2.74	3.45
	Distillate Residue	$\tfrac{25.8}{13.9}$	$\begin{array}{r} 98.4 \\ 6.0 \end{array}$	$\frac{104.5}{3.9}$	8	45	58	2.51	2.44	2.76	3.05
	Dist. $+$ res.	39.7	104.4	108.4				2.52	2.31	2.71	3.47
5	Extract	64. <b>3</b>	140.7	133.7	35.4	122		2.12	1.77	4.52	5.85
	Distillate Residue	$\begin{array}{c} 37.7 \\ 26.1 \end{array}$	$\frac{122.1}{13.3}$	118.3 9.2	13.1	53	63	1.91	1.70	4.38	4.86
	Dist. $+$ res.	63.8	135.4	127.5				1.93	1.58	4.40	5.74
6	Extract	51.7	145.0	146.6	23.8	85	50	3.02	2.78	4.31	5.19
	Distillate Residue	$\frac{35.6}{16.2}$	$\frac{136.3}{7.8}$	$\frac{140.4}{5.5}$	9.9	38	98	2.98	2.90	4.28	4.04
	Dist. $+$ res.	51.8	144.1	145.9				3.03	2.79	4 25	5,14
7	Extract	52.5	139.6	137.6	24.9	90	60	2.54	2.30	4.41	5.33
	Distillate Residue	$\frac{35.0}{17.8}$	$\frac{129.3}{8.7}$	$\frac{130.1}{6.0}$	10.0	40	60	2.51	2.41	4.30	4.09 5.27
_	Dist. + res.	32.8	138.0	130.1				2,00	2.20	+.32	0.21
8	Extract	54.6	193.5	200.0	18.3	50	79	4.37	4.18	5.85 5.85	6.00
	Distillate Residue	$\frac{38.7}{15.9}$	$\frac{182.9}{9.3}$	$\frac{190.7}{6.8}$	5.0	15	73	4.19	4.14	5.92	6.58
0	Dist. + res.	51.0	134.4	017 0	15 5	20		4 51	4.95	6.92	7 20
9	Distillato	55.9 44.6	212.8	217.0	15.5		57	4.31	4.30	6 65	6.86
	Residue Dist $\pm res$	13.5	$\frac{9.2}{211.3}$	$\frac{7.3}{215.6}$	0.1	10	0.	4.44	4.27	6.74	7.38
10	Extract	60 4	220 8	225 7	18 4	44		4 69	4 51	7 01	7.68
10	Distillate	45.4	209 1	216 6	6.3	16	65	4.64	4.58	6.79	7.00
	Residue $Dist. + res.$	$\frac{16.0}{61.4}$	$\frac{10.2}{219.3}$	$\frac{7.7}{224.3}$	0.0	10	00	4.69	4.49	6.89	7.61
11	Extract	45 1	213 0	224 4	6.1	16		5.13	5.08	6.57	6.77
11	Distillate	39.7 7.1	206.6 5.2	221.1 4.1	2.6	7	57	5.32	5.30	6.18	6.34
	Dist. + res.	46.8	211.8	$\overline{225.2}$				5.35	5. <b>27</b>	6.25	6.54
<sup>a</sup> Percer	ntage of original	weight.									

curves), while the cold-finger condenser was cooled with a mixture of alcohol and dry ice. After distillation the still pot was cooled in dry ice, the vacuum was released, and the distillate and residue were dissolved quickly in absolute methanol.

Table IX gives results on extract, distillate, and residue for 11 hops of varying quality. The absorbance balances are excellent and superior to those for fractionation on silica gel. The results have been selected (from results with 24 hops) to show the widest ranges of lupulone and humulone-complex content and ratio. Hop 2, with a brown color and strong valeric acid odor, had about one twentieth as much lupulone or humulone complex as the best hop (No. 10). The ratio of lupulone to humulone complex varied from about 0.9 to 0.3.



Separated Hop Backgrounds

 ☐ Silica gel chromatography
 △ Alkaline extraction
 ○ Molecular distillation
 ● Ion exchange chromatography
 Regression line Azis/Azis = 0.933 + 0.1824 Azis/Azis. estimate = 0.068 Standard error of

Molecular distillation removed about two thirds of the excess  $A_{275}$  of the extracts and showed the binary analysis for humulone complex to be high by at least 7 to over 100%, depending on hop quality, or by about 10 to 12% for the samples most frequently encountered. The relative coefficients of nonvolatile residue varied with hop quality as shown in Figure 3 where  $A_{355}$  has been set at 1.00. The residues of hops with more than 5 to 7% humulone complex are fairly well represented by the relative coefficients 1:1.35:2.4 for  $A_{355}:A_{325}:A_{275}$ , whereas those with 3 to 5% humulone complex have residue coefficients of about 1:1.4:3.0. Table IX shows that lupulone values by the binary analysis are relatively unaffected by the presence of the interfering materials removed by molecular distillation. As will be shown below, humulone-complex values by the ternary equations are much less sensitive to variations in the relative coefficients of the interference than those by the binary equations.

Ion Exchange Resin Adsorption Analysis. Lupulone and humulone complex are adsorbed quantitatively from methanol (although not from petroleum ether) by the strongly basic ion exchange resins such as IRA 400 and Permutit S, and are eluted quantitatively by 0.1N hydrochloric acid in methanol (Table X). Inasmuch as lupulone and humulone complex have maximum stability in methanol, these adsorbents should be preferable to silica gel or other nonexchange materials from which these acids are eluted by neutral methanol (and therefore require the use of other solvents).

In preparation for fractionation of hop extracts on IRA 400, elution constants were determined by the method described by Tompkins (12) from batch equilibrations in 0.2N solutions of various acids in methanol. The distribution values, Kd, for lupulone and humulone complex, respectively, were as follows: acetic acid, 100 and 942; formic acid, 24 and 67; chloroacetic acid, 21 and 30; and malonic acid, 20 and 22. Experience with columns has confirmed the prediction that formic acid is best suited for the separation of lupulone and humulone complex, whereas acetic acid is best suited for the resolution of those com-

Table X. Sorption of Lupulone and Humulone Complex by Ion Exchange on IRA 400

Resin <sup>a</sup> Mg.	Fraction	, A 355	4276	Excess Amt.	4 275 %	Lupulone, <sup>b</sup> Mg.	Humulone Complex <sup>b</sup> , Mg.
	Original extract	2544	663	175	36	21.7	32.7
10	Filtrate Eluate	$2473 \\ 117$	${}^{626}_{51}$	$\begin{array}{c} 172 \\ 16 \end{array}$	$\begin{array}{c} 38 \\ 44 \end{array}$	$\begin{array}{c} 21.6 \\ 0.0 \end{array}$	30.5 2.9
75	Filtrate Eluate	$1667 \\ 888$	$\begin{array}{c} 410 \\ 275 \end{array}$	$\substack{180\\35}$	78 14	20.1 1.4	$\begin{smallmatrix}12.5\\20.2\end{smallmatrix}$
150	Filtrate Eluate	$\begin{array}{r} 858 \\ 1724 \end{array}$	$\begin{array}{c} 260 \\ 475 \end{array}$	160 88	$\begin{smallmatrix}160\\23\end{smallmatrix}$	$\begin{array}{c} 11.8\\9.4 \end{array}$	$\begin{array}{c} 4.3\\ 29.5 \end{array}$
300	Filtrate Eluate	$\begin{array}{r} 236 \\ 2334 \end{array}$	$\begin{array}{c} 160 \\ 512 \end{array}$	$^{116}_{75}$	$\substack{138\\17}$	2.0° 19.3	3.0° 30.3

<sup>a</sup> The resin was rocked with 25 ml. of hop extract (equivalent to 625 mg. of hops) in methanol for 1 hour, after which it was washed twice by decanta-tion and then rocked for 1 hour with 0.11N hydrochloric acid in 99% meth-anol to effect elution. <sup>b</sup> By binary analysis. <sup>c</sup> The shape of the spectrum showed that less than half of the indicated where a shape of the spectrum showed that less than half of the indicated

values actually could be lupulone or humulone complex.

ponents that are adsorbed less strongly than lupulone. Table X shows that nearly all of the material contributing to excess  $A_{275}$  is less firmly bound than humulone complex, and the greater part is less firmly bound than lupulone. With 300 mg. of resin, an amount sufficient to adsorb humulone complex and lupulone quantitatively, 66% of the excess  $A_{275}$  remained in the filtrate.

A control experiment gave quantitative recovery of humulone complex, when developed on a column of IRA 400 formate with 0.2N formic acid in methanol, if the column was kept in the dark. With a column exposed to diffuse light, substantial losses of both lupulone and humulone complex were incurred (Table XI). The period of contact on the column appeared to have less effect than exposure to light. Excellent resolutions of lupulone and humulone complex were obtained, because these substances were well separated from each other and from most of the strongly adsorbed impurities of hop extracts that contribute markedly to excess  $A_{275}$ , as well as from the impurities that are adsorbed less strongly than lupulone. The latter were investigated by developing with 0.05N acetic acid in methanol. At least five distinct components may be recognized (Table XII).

Fractionation of molecular distillates of hop extracts showed that the absorbing materials coming off the IRA 400 column ahead of lupulone were removed almost quantitatively by the distillation procedure, because less than 0.2% of  $A_{325}$  or  $A_{355}$  was recovered in cuts prior to lupulone. In these experiments on molecular distillates, 0.5N hydrochloric acid in methanol eluted material with  $A_{355}$ :  $A_{325}$ :  $A_{275}$  equal to approximately 1:2:5, in amounts which accounted fairly well for the excess  $A_{275}$  of the distillates. Interpretation of the origin of this material was complicated, as with whole hop extracts, by breakdown of some lupulone and humulone complex which was evidenced by failure to recover quantitatively the absorbance at high wave lengths.

It is thought that better recoveries could be obtained by development of columns in the dark at low temperature; and that ion exchange sorption analysis might yield the absolute values for lupulone and humulone complex required for confirming the validity of the spectrophotometric method proposed. The evidence obtained from the fractionation experiments is discussed in connection with the ternary analysis.

Analysis of Hop Extracts. Several possibilities exist with respect to a spectrophotometric estimation of hop quality and particularly of the humulone complex which is of prime importance to the bittering power of hops. Binary analyses based on two absorbance readings in the neighborhood of the absorption maxima, 355 and 325 m $\mu$ , for lupulone and humulone complex, and ternary analyses of greater accuracy gained through the use of an additional absorbance reading are discussed. Combinations of spectrophotometric measurements and some method of separation of lupulone and humulone complex from other ultra-

				Recove Ab	ery of C sorbance,	original %
$Expt.^{a}$	Test Load	Conditions	Fraction	A 355	A 325	A' 75
A	Lupulone, 16 mg.	Darkness, 4 hours; eluted in diffuse light	Lupulone Decomposition	98.5	100 <1	101
В	Humulone complex, 30 mg.	$Darkness^b$	Humulone complex Decomposition	99 1.2	$100 \\ 1.2$	103 1.8
С	Lupulone, 16 mg., plus humulone complex, 30 mg.	Diffuse light, 9 hours	Lupulone <sup>¢</sup> Humulone complex <sup>¢</sup> Decomposition	38 47 0.7	27 57 1	19 77 21
			Total	86	85	118
D	Extract of 500 mg. of hops <sup>d</sup>	Dark column; elu- ates collected over- night without light shielding	Impurity before lu- pulone	3.4	3.8	11.3
			Lupulone Humulone complex Impurity after humu- lone complex	$51 \\ 38 \\ 0.25$	39 49 0.87	$\begin{array}{c} 24\\61\\3.4\end{array}$
			Total	93	92	100
Е	Extract of 400 mg. of hops <sup>e</sup>	Dark column; elu- ates collected over- night without light shielding	Impurity before lu- pulone Lupulone Humulone complex Impurity after humu- lone complex	0.6 55 38 0.7	$1.1 \\ 42 \\ 49 \\ 1.4$	9 33 37 9
			Total	94	93	88
<sup>a</sup> The until lup	columns, approximatel	y $0.9 \times 22$ cm., were mplex were removed co	eluted first with $0.2N$ for $0.2N$ is the second	formic a $0.5N$ h	cid in me ydrochloi	ethanol ric acid

Table XI. Recovery of Lupulone and Humulone Complex from IRA Formate

in methanol until the decomposition products or impurities were recovered. The flow rate was approximately 0.5 ml. per minute. The resin was 80 to 100 mesh. <sup>b</sup> The collection was interrupted, and the column with adsorbed humulone complex was allowed

to stand overnight.
It was calculated that 86% of the lupulone and 83% of the humulone complex were recovered.
Lyophilized hops; 5.1% lupulone and 6.7% humulone complex by binary analysis.
Poor quality hops; 2.4% lupulone and 3.4% humulone complex by binary analysis.

violet-absorbing constituents are not further considered here, although alkaline extraction and batch adsorption with ion exchange resins appear promising.

Binary Analyses. A spectrophotometric method, based on the assumption that the ultraviolet absorption of hop extracts is due solely to the humulone-complex and lupulone content, must be limited in its accuracy and precision in view of the variable contribution made by minor components as reported. Both the tentative method proposed earlier (6) and the modification of Rigby and Bethune (10) are in this category. Both employ absorbancies measured at two wave lengths in alkaline methanol solutions, the former using 332 and 355 m $\mu$  and the latter 320 and 355 mµ. Considerations of wave-length error, sensitivity, effect of minor components, and errors in solution preparation indicate that these choices are near the optimum. In this work 325 and 355 m $\mu$  and the specific absorbancies listed above for lupulone and humulone complex are used, yielding

> $C_H = -47.28 A_{355} + 65.71 A_{325}$ (1a)

$$C_L = 54.43 A_{355} - 45.43 A_{325} \tag{1b}$$

where  $C_H$  and  $C_L$  are given in milligrams per liter of the solution for which A is the absorbancy for 1-cm. path length.

The results labeled "binary analysis" in Tables VII, VIII, and IX were calculated using Equations 1a and 1b. By comparison of these results in Table IX with those calculated by the more accurate ternary analysis method, it is seen that Equation 1a gives

Table XII. Fractionation on IRA 400 of Hop Extract **Components Less Acid than Lupulone** 

		Rela	% of Total		
Component	Cut No.	$355 \text{ m}\mu$	325 mµ	275 mµ	325 mµ
1 2 3 4 5	1-2 3-8 9-13 14-25 26-30	1 1 1 1	1.5 1.3 1.7 1.4 1.1	$\begin{array}{c} 4.1 \\ 1.7 \\ 3.5 \\ 2.2 \\ 1.5 \end{array}$	0.25 2.6 0.8 1.3 0.5
Sum	1-30	1	1.3	1.8	5.4

values for humulone complex approximately 10% too high for good quality hops and as much as 100% too high for stored hops of poor quality. In the latter case the larger error is due to both the greater contribution of components other than lupulone and humulone complex and the larger ratio of  $A_{325}$ :  $A_{355}$ because of such components. The errors in lupulone content arising from the use of Equation 1b are usually much smaller than for humulone complex with the corresponding Equation 1a.

Equations 1a and 1b should give accurate results for a procedure in which the components other than lupulone and humulone complex are satisfactorily removed prior to spectrophotometric measurements.

Ternary Analysis. Background components, other than lupulone and humulone complex, with reproducible spectrophotometric properties may be eliminated readily by considering the mixture as a three-component system, making readings at three wave lengths and solving three simultaneous equations. However, in the estimation of only one component, the background absorption need not be that of a single pure component  $(A_1: A_2: A_3 \text{ are ratios of con-}$ 

stants) but rather may vary widely in spectral shape if the following relationship holds:

$$A_1: A_2: A_3 = 1: (k + aX): X$$

where  $A_{1, 2, 3}$  are relative absorbancies of the background at the three wave lengths in question, k and a are constants, and X is variable. This relationship amounts to the restriction that the background be made up of algebraically additive combinations--that is, including negative values-of the second real component and a hypothetical third component. This restriction on the properties of noninterfering backgrounds is broad enough to accommodate widely varying spectral shapes-e.g., in the equation presented below for humulone complex, backgrounds with relative absorbancies at  $355:325:275 \text{ m}\mu$  of 1:0.7:0.0 (descending); of 1:0.96:1 (flat); of 1:3.3:10; of 1:26.5:100 (steep); etc., are corrected. The separated backgrounds for the hop extracts tested, by conforming approximately to the relationship  $A_{355}: A_{325}: A_{275} = 1: (0.933 + 0.183X): X$ , are eliminated reasonably well in a ternary analysis for humulone complex and lupulone such as is described below.

The choice of wave lengths for multicomponent analyses may be made by plotting the ratio of the absorbance of each component to that of the probable mixture against wave length (8). When this is done for the ratios of absorbancies in alkaline methanol of lupulone, humulone complex, and residue from a molecular distillation of hop extract, respectively, to the absorbance of a low-quality hop, several choices of three wave lengths are offered from the maxima that occur at 400, 390, 355, 325, and 275 m $\mu$ . The last three were chosen as combining maximum sensitivity with minimum influence of wave-length error.

The absorbancies (relative to  $A_{355}$ ) of the backgrounds separated from hop extracts (by molecular distillation, alkaline extraction, or silica gel or ion exchange resin adsorption analysis) at 275 m $\mu$  plotted against those at 325 m $\mu$  yield a good approximation of a straight line (Figure 3). On the assumption of a background component B, with specific absorbancies of 1.0 at  $355 \text{ m}\mu$ , 1.5 at  $325 \text{ m}\mu$ , and 3.1 at 275 m $\mu$  (a set of values picked from this regression line and typical of average quality hops), the following equations hold for the absorbancies of a hop extract:

$$A_{275} = 3.7 C_L + 9.0 C_H + 3.1 C_B$$
  

$$A_{325} = 33.1 C_L + 38.1 C_H + 1.5 C_B$$
  

$$A_{355} = 46.0 C_L + 31.8 C_H + 1.0 C_B$$

where  $C_L$ ,  $C_H$ , and  $C_B$  represent the concentrations, respectively, of lupulone, humulone complex, and the assumed background in milligrams per liter of solution measured. The equations yield

$$C_H = -51.56 A_{355} + 73.79 A_{325} - 19.07 A_{275}$$
(2a)

(2b)

and

$$C_L = 55.57 A_{355} - 47.59 A_{325} + 5.10 A_{275}$$

Setting  $C_H$  and  $C_L$  equal to zero, one obtains

$$\frac{A_{325}}{A_{355}} = 0.699 + 0.258 \frac{A_{27}}{A_{35}}$$

$$\frac{A_{325}}{A_{355}} = 1.168 + 0.107 \frac{A_{27}}{A_{35}}$$

and

$$\frac{A_{325}}{A_{355}} = 1.168 + 0.107 \frac{A_{275}}{A_{355}}$$

Setting  $A_{355} = 1$  and  $A_{275} = X$ ,

$$A_{355}: A_{325}: A_{275} = 1: (0.699 + 0.258X): X$$
(3a)

is obtained for a background that would not interfere in the calculation of humulone complex, and

$$A_{255}:A_{325}:A_{275} = 1:(1.168 + 0.107X):X$$
(3b)

for a background that would not interfere in the calculation of lupulone. Plots of Equations 3a and 3b will be called "correction lines." The relationships between  $\frac{A_{325}}{A_{355}}$  and  $\frac{A_{275}}{A_{355}}$  by Equations 3a and 3b and those found experimentally with actual separated background fractions are plotted in Figure 3.

Figure 3 shows that the two correction lines cross the regression line of the naturally occurring backgrounds at the point representing the assumed background used in setting up the three simultaneous equations. The slopes of the correction lines, and thus the point of their intersection with the regression line, may be varied by choice of relative absorbancies of the assumed background. Other choices could make the correction lines intersect the regression line in the region of  $A_{325}/A_{355}$  characteristic for low-quality stored hops, or any other point. The intercept of the correction lines on the  $A_{325}/A_{355}$  axis is substantially independent of the choice of background absorbancies, if the latter are picked from the regression line above  $A_{275}/A_{355} = 1$ .

The approximations to absorbance ratios for the actual backgrounds of hop extracts, shown in Figure 3, were obtained in various ways. A regression line based on the residues of molecular distillations, which are subject to the least errors of decomposition, would not deviate markedly from that based on the other estimates. A more serious problem arises from the possibility that the observed backgrounds may not be completely representative of the actual backgrounds. That this is not likely to be so is indicated by finding that the spectrum of residual background remaining in molecular distillates, as estimated by subtracting the contributions of lupulone and humulone complex (calculated by ternary analysis) from the spectrum of the distillate, conformed to the general shape of the distillation residues.

A rigorous proof of the reliability of the ternary analysis might be obtained by completely separating the background from lupulone and humulone complex without spectrophotometric change. It is believed that this could be accomplished best by ion exchange sorption analysis in the dark at low temperatures.

If the fractions of the backgrounds shown in Figure 3 for various hops are assumed to be representative of the actual backgrounds, some estimates of error may be made. In Table IX, lupulone and humulone-complex concentrations calculated by Equations 2a and 2b have been listed along with binary calculations by Equations 1a and 1b. Comparison of the humulonecomplex and lupulone contents of the distillate with those of the sum of distillate plus separated background shows that the backgrounds caused errors of the order of 1 to 4% when calculated by Equations 2a and 2b, whereas in binary analysis the same backgrounds caused errors of about 10 to 50% in humulone complex. Lupulone values by the binary method are much less sensitive to background. The poorer agreement between distillates and original extracts than that between distillate and sum of distillate and background is due to slight decomposition during the distillation procedure.

In the case of humulone complex separated by silica gel adsorption (Table VIII), the same comparisons are much poorer owing to greater departure of the relative absorbancies of the backgrounds from the correction line.

In Figure 3, departure of a point from the correction line by 0.05 causes an error of about 12% in humulone complex, if the given background and humulone complex are present in amounts such that their absorbancies at 355 m<sub>µ</sub> are equal. For lupulone, a departure of 0.05 produces about 11% error, if its absorption is equal to that of the background. However, the average background absorption in the extracts examined is about 10% of the absorption of the humulone complex or lupulone at 355 m<sub>µ</sub>—i.e., approximately 5% of the total absorption. The range of the ratio, of background absorption to humulone-complex absorption at 355 m<sub>µ</sub> is from about 4 to 40%, the median value being about 10%. Only three samples of badly degraded hops gave the high ratios. The range of the same ratio for lupulone was 3 to 20% with most samples around 10%.

The random error (standard deviation), contributed by the variation of background absorbance ratios from the regression line of Figure 3, may be calculated as follows:  $(\pm 0.07)$  (73.8)/ (0.1) (31.4) =  $\pm 1.6\%$  for humulone complex and  $(\pm 0.07)$  (47.6)/(0.1)(21.5) =  $\pm 1.5\%$  for lupulone in good quality hops. In explanation, the first calculation represents the ratio [(error of estimate of the regression line) × (coefficient of  $A_{325}$  in Equation 2a)] to [(average  $A_{355}$  of the background relative to  $A_{355}$  for humulone complex) × (value for  $C_H$  obtained by substituting the coefficients for humulone complex, relative to  $A_{355}$ , in Equation 2a)].

Lack of coincidence of the regression line and the correction lines contributes additional bias errors, which vary for humulone complex from zero, at  $\frac{A_{275}}{A_{355}} = 3.1$ , to (0.15) (73.8)/(0.1) (31.4) = 3%, at  $\frac{A_{275}}{A_{375}} = 1$  or 5 and for lupulone from zero to (0.2) (47.6)/ (0.1) (21.5) = 4%, at 1 or 5, for good quality hops. A positive deviation of a background from the correction line causes a positive error in humulone complex and a negative error in lupulone. The backgrounds of the majority of hops tested fall within 0.1 of both correction lines for Equations 2a and 2b.

If the points in Figure 3 representing extremely poor hops and decomposition products of humulone complex and lupulone were excluded, new equations could be set up to fit the correction lines more closely to the data for the samples most frequently encountered. However, before setting up different equations for extracts of hops of different quality classes, it would be desirable to get many more data on background absorption of such hops by such refinements of techniques as have been described. The procedure described below is based on Equations 2a and 2b, which best fit all of the data regardless of hop quality.

### SUMMARY OF PROCEDURE FOR ANALYSIS OF HOPS

To 5 grams of hand-ground hops in a glass-stoppered graduate, sufficient low-boiling petroleum ether is added to bring the volume to 100 ml. The mixture is agitated by gentle rocking at room temperature for 30 minutes and then allowed to stand until the solids are settled, leaving a clear supernatant liquid. An aliquot of this clear extract is diluted with reagent-grade absolute methanol to a volume convenient for spectrophotometric reading. The required dilution for the Beckman Model DU spectrophotometer is usually 100- to 200-fold. The diluted solution is then mixed with 1% of its volume of 0.2N sodium hydroxide (aqueous) in the absorption cell and immediately read in the spectrophotometer at 355, 325, and 275 mu. Reagent blanks should be run. For ternary analysis the resulting absorbance readings are substituted in Equations 2a and 2b to give the humulone-complex and lupulone content of the solution read in milligrams per liter. These values are then multiplied by the dilution factor for the hop sample to give percentage of humulone complex or lupulone.

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## **Coprecipitation from Homogeneous Solution**

Manganese(II) on Basic Stannic Sulfate

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The extent and nature of coprecipitation occurring during precipitation from homogeneous solution were studied. Among the many factors influencing the extent of coprecipitation of manganese(II) with basic stannic sulfate, precipitated by the urea method, are the initial supersaturation effect, the initial concentration of manganese, the rate of precipitation, and adsorption on the surface of the completely precipitated carrier. Contamination by occlusion during the formation of the major portion of the precipitate is not a significant factor. However, considerable contamination may occur in the early stages of the precipitation process owing to the initial supersaturation effect. During the precipitation of the last portions of the carrier and especially in the presence of excess precipitant, coprecipitation by adsorption becomes appreciable. When micro quantities of manganese are present, essentially the same coprecipitation is obtained with both the heterogeneous and the homogeneous methods of precipitation. However, with macro quantities, owing to the elimination of initial supersaturation and to the formation of the precipitate under conditions in which the Paneth-Fajans-Hahn rule is effective, contamination during the formation of a carrier can be minimized by the technique of precipitation from homogeneous solution.

THE problem of the contamination of precipitates is a major L aspect of investigations in gravimetric separations. The separation of normally soluble substances from solution during the formation of a host precipitate, or upon the addition of a preformed carrier, constitutes the phenomenon of coprecipitation. Other terms such as occlusion, adsorption, and inclusion are used to describe the specific processes by which coprecipitation occurs, although there often is disagreement about the precise meaning of the particular term. These processes have been con-

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sidered in detail in the generalized theory of coprecipitation which Kolthoff (7) presented in 1932.

Investigations with preformed carriers led to the formulation of the Paneth-Fajans-Hahn rule (15). Although the rule is subject to some exception, it furnishes useful criteria for the prediction of adsorption phenomena. Studies by other investigators (8-10) have also included determinations of the total coprecipitation resulting from the formation of a precipitate.

In general, as has been demonstrated many times, experimental conditions strongly influence coprecipitation, especially in the case of surface adsorption. If, however, the formation of a mixed crystal is possible, there is less dependence on experimental conditions. Instances of anomalous mixed-crystal formation have also been observed by Hahn (4) who has referred to these as isodimorphism. Kolthoff (7) has indicated that coprecipitation due to mixed-crystal formation cannot be readily eliminated since the contamination is inherent in the nature of the substances involved. Kolthoff has maintained that, in the absence of mixedcrystal formation, coprecipitation due to occlusion is real coprecipitation. He defines occlusion as the process which occurs when foreign ions are adsorbed at the surfaces of a growing precipitate and are then covered over by the subsequent formation of new layers; this definition is used in this study. The occlusion process is markedly affected by such experimental conditions as concentrations of initial reagents, stirring, temperature, and aging.

In order to study properly the occlusion process, Kolthoff suggested that studies be made of the change in concentration of the impurity during crystal growth. A major obstacle in such a study with an insoluble precipitate would be the high local supersaturation effect produced by the direct addition of a precipitant to a solution. Because of this, Henderson and Kracek (5) obtained erratic results in their studies of the barium-radium chromate system. The technique of precipitation from homogeneous solution (3, 16) minimizes these local supersaturation effects.

The objective of this study was to determine the extent and nature of coprecipitation during the process of precipitation from homogeneous solution. Basic stannic sulfate (18), which is precipitated by the urea method, was chosen as the carrier and manganese(II) as the contaminant. For comparative data, tin was also precipitated as the hydrous oxide with ammonium hydroxide.

### EXPERIMENTAL

**Reagents.** All reagents used, except where otherwise noted, were either C.P. or reagent grade quality. **Tin(IV) Solution.** Metallic tin (Kahlbaum or J. T. Baker Chemical Co.) was dissolved in hydrochloric acid and the tin Chemical 60.7 was dissolved in hydrothic actual in the unit subsequently oxidized with chlorine gas. The solution was gravimetrically standardized by precipitation with ammonia ( $\mathcal{C}$ ); impurities in the stannic oxide were accounted for by the procedure of Caley and Burford ( $\mathcal{Z}$ ). The solution contained 150 m cf tip( $\mathcal{U}$ ) are 25 ml 150 mg. of tin(IV) per 25 ml. Manganese(II) Solution. Manganese(II)

chloride tetrahydrate was dissolved in water acidified with sulfuric acid. The solution was gravimetrically standardized by precipitation with

diammonium hydrogen phosphate (17). Manganese-54. Cyclotron-produced manganese-54 was ob-tained carrier-free from the Oak Ridge National Laboratory. The material was supplied as manganese(II) chloride in 0.1Nhydrochloric acid. Two working solutions containing this nuclide were prepared. Ten milliliters of one solution contained about  $5 \times 10^{-3}$  mc. of manganese-54, 0.05 mg. of carrier man-ganese, and 4 ml. of hydrochloric acid. The second solution was prepared carrier-free and contained only the radioactive nuclide







Sulfate Initial concentration of manganese, approximately 10<sup>-3</sup>M

and hydrochloric acid. The  $5 \times 10^{-3}$  mc, were known to about  $\pm 10$  to 20%; this corresponds to about 6.5  $\times$  10<sup>-10</sup> gram. Gamma rays emitted by the radioisotope were measured with a scintillation counter.

Urea. The urea used was obtained from the Matheson Co., Inc., East Rutherford, N. J. The required quantity, 20 grams, was dissolved in about 40 ml. of water, and the solution filtered prior to each precipitation. Reaction Vessel. The reaction vessel and sampling device

Reaction Vessel. Ine reaction vessel, used in the investigations are illustrated in Figure 1. In use, a condenser was placed at A to maintain constant volume. reaction vessel, C, was calibrated at 10-ml. intervals from 150 to 220 ml. by the addition of known volumes of water. Intermediate volumes could be read to  $\pm 1$  ml. A fine frit of B permitted the withdrawal of clear liquid samples. The 10.00-ml. sampling tube, D, was calibrated in the same manner as C. The apparatus was checked experimentally as described in the general procedure.

Constant Temperature Bath. The oil bath used had a temperature control of  $\pm 0.3^{\circ}$  C.

### **GENERAL PROCEDURE**

Solutions were prepared to contain varying quantities of man-ganese(II), about 150 mg. of tin(IV), 20 grams of urea, 14 ml. of 12N hydrochloric acid, 5 ml. of 36N sulfuric acid, and 1 gram of ammonium sulfate. The volumes were adjusted to about 210 ml., and the pH was adjusted to 0.50 with ammonia. The pH meter was standardized at pH 1.00. The solutions were heated for varying times, and samples of supernatant liquid were re-moved and analyzed. Because of refluxing, the temperature of the reaction mixture was 4° below that of the bath. The residual tin was determined by the hematoxylin method of Teicher and Gordon (13). Manganese was determined by the standard procedure of oxidation to permanganate (12) and subsequent photo-metric determination. This photometric method was modified by elimination of the recommended phosphoric acid, the presence of which produced a turbidity by reacting with tin(IV)

The general procedure was checked with known quantities of manganese and tin. These were added in separate experiments to vessel C of Figure 1, the entire apparatus was thermostated, and samples were withdrawn into D and analyzed. The results of two determinations, one with 10.13 mg. of manganese initially added to C, and one with 59.2 mg. of tin, showed 10.11 and 591. mg. of the two elements, respectively, to be initially present in C. This technique-i.e., indirect analysis-is subject to error. Be-This technique i.e., inductor analysis is subject to the inclusion of this, the percentages of tin and manganese reported in this paper are estimated to be in error by an absolute  $\pm 1\%$  in the range from 0 to 100%. Indirect analysis of the solution was chosen in order to avoid the uncertainties encountered being the percentage. in the direct analysis of a precipitate-for example, the washing of a precipitate to separate it from mother liquor can lead to spurious results (18).

Radiochemical Procedure. Radiochemical techniques were utilized to determine the manganese when its initial concentra-tion was  $10^{-6}M$  and below. Activity determina-tions were made using 3-ml. liquid samples in 5-ml. glass vials.

### RESULTS

Precipitation at 97° C. Precipitations were performed at 97° C. with solutions containing 10.13 mg. of manganese (9  $\times$  10<sup>-4</sup>M). Stirring of these solutions was provided by the carbon dioxide evolved by the hydrolysis of urea. The results are shown in Figure 2. The data are somewhat erratic; the curve merely shows the general trend. The impure precipitate, which was formed, appeared somewhat colloidal and exhibited a very slight, off-white color. These observations indicated that precipitation might have been too rapid. The effect of precipitation at a lower temperature was then investigated.

Precipitation at 86° C. A similar series of precipitations was then performed at 86° C. The results are shown by the second curve in Figure 2. These precipitates also exhibited the off-white color. If the reaction was allowed to proceed beyond pH 3.0, it was difficult to stop it at any given pH, because the pH would rapidly rise to 6.0, and manganese dioxide would begin to precipitate. At pH 6.2, approximately 40% of the manganese would be precipitated; at pH 7.1, the corresponding value was about 75%.

The greatest amount of coprecipitation seemed to occur during the early stages of precipitation, when less than approximately 25% of the tin had precipitated. It was of interest then to determine the effect of adding the manganese to the solution after a pure 25% fraction of tin had precipitated. Identical solutions were prepared with the exception that the manganese was omitted. After about 25% of the tin had been precipitated, manganese was added. The reaction was then allowed to continue for varying times, and samples were removed and analyzed for residual tin and manganese. The results are also shown in Figure 2. The precipitates did not exhibit the slight, off-white color previously obtained.



Precipitation at 76° C. The observations at 76° C. are also shown in Figure 2. Figure 3 summarizes the pH versus time relationships at the various temperatures studied; at 76° C. the rate of precipitation was reduced considerably. The precipitates seemed to be pure white until more than 0.05 mg. of manganese was coprecipitated; beyond this point they appeared to be just off-white.

Willard and Gordon (18) reported that basic stannic sulfate produced from boiling solutions had a molar ratio of tin to sulfate of 44. The precipitates obtained in the present study at 76° and 86° C. had a molar ratio of about 32. The analyses for the molar ratios were made by washing the precipitates with hot 2% ammonium nitrate and then with distilled water. After air-drying, samples were fused with sodium peroxide in a nickel crucible. The melts were taken up in water, treated with an excess of hydrochloric acid, and transferred to a 500-ml. volumetric flask. The tin was determined by the hematoxylin

procedure. The sulfate was determined as barium sulfate. The composition of the precipitate appeared to be the following: stannic oxide, 93.7%; stannic sulfate, 3.4%; and water, 4.3%.

Although the precipitates formed at  $86^{\circ}$  and  $97^{\circ}$  C. showed no definite pattern under the microscope, the precipitate formed at  $76^{\circ}$  C. was composed of clusters of spheres.

It.was found that complete precipitation of tin occurred at pH 1.0 at 76° C., rather than at pH 1.2 as observed at 86° C. The initial coprecipitation effect was eliminated at the lower temperature, but as quantitative precipitation was approached, the amount of coprecipitation increased rapidly.

Since the least amount of contamination of basic stannic sulfate occurred at 76° C., another series of experiments, in which the tin was precipitated as the hydrous oxide by the direct addition of ammonia, was performed under the same experimental conditions. The reaction vessel was placed in the bath and about 30 minutes were allowed for the system to come to constant temperature. The solution was then mechanically stirred. Varying quantities of ammonia were added dropwise at a rate of approximately 2 ml. per minute, and the solutions were stirred for an additional 15 to 20 minutes. During the time the reaction vessel was in the bath, there was no observable change in pH due to the hydrolysis of the urea present in the solution. Samples were removed and analyzed; the results are shown in Figure 2.

The precipitates were voluminous and gelatinous; the data indicated that they were considerably more contaminated than the basic stannic sulfate. Several of these gelatinous precipitates were allowed to digest in the mother liquor at varying temperatures and for varying lengths of time. In one case, after 92% of the total tin had been precipitated, the precipitate was digested at 76° C. for 4 hours, during which time some additional tin came down, so that 98% was precipitated. However, there was no detectable change in the concentration of manganese in the mother liquor. In another case, after the tin was quantitatively precipitated at 76° C., the solid phase was then digested at 15° C. for 16.5 hours, during which time the precipitate released about one fifth of its coprecipitated manganese.

Since these studies had indicated that a very small amount of contamination of basic stannic sulfate occurred at 76° C. at an initial manganese concentration of about  $10^{-3}M$ , it became desirable to study the effect of even lower initial concentrations of manganese, using radiochemical techniques as described.

Preliminary experiments indicated that the nuclide, as received, was in the bivalent state. The coprecipitation data observed, with both stannic sulfate and hydrous stannic oxide from solutions initially  $10^{-6}M$  in manganese (0.05 mg. per 210 ml.), are illustrated in Figure 4.

Limited observations made with carrier-free solutions, initially  $10^{-11}M$  in manganese, are shown in Figure 5.

Initial experiments indicated that no loss of radioactive material due to adsorption on the glass surfaces occurred at both initial manganese concentrations of  $10^{-6}$  and  $10^{-11}M$ .

### DISCUSSION OF THE RESULTS

The extent of coprecipitation of manganese(II) with basic stannic sulfate appears to be directly related to the rate of precipitation of the basic sulfate. Thus, at 97° C. with solutions  $10^{-3}M$  in manganese, quantitative precipitation of the tin required about 3 hours and was accompanied by a large amount of coprecipitation. The relatively large amount of coprecipitation obtained with the initial 25% fraction of the tin precipitate suggested that a supersaturated condition may have been present. A slight flocculation effect was observed after 25% of the tin had



Initial concentration of manganese, 10<sup>-4</sup>M
precipitated; this effect would decrease the total surface area and may be related to the decrease in coprecipitation observed in Figure 2. Finally, as the rate of hydrolysis of urea was decreased by reducing the temperature from 97° to 76° C.—about 30 hours being required for complete precipitation of the tin the initial amount of coprecipitated manganese was reduced from 30% to a barely detectable amount. The flocculation effect by the precipitate was also no longer apparent. The interpretation of these results is in accord with the general observations reported by von Weimarn (14). La Mer and Dinegar (11) have also demonstrated a limiting degree of supersaturation in the precipitation of barium sulfate when the latter was precipitated from homogeneous solution.

Thus, it would appear that the initial coprecipitation with basic stannic sulfate was due to supersaturation, the magnitude of which was reduced as the rate of hydrolysis of urea was decreased. When the manganese was added to the reaction mixture after the precipitation of an initially pure 25% fraction of the basic sulfate at 86° C., the extent of coprecipitation was negligible (Figure 2). As shown in Figure 2, the plateau obtained in this case very closely approximates the other plateau obtained the initial supersaturated effect, and appears merely to be displaced by the quantity of manganese coprecipitated by the initial 25% fraction of basic stannic sulfate. This observation also supports the view that a supersaturation effect is responsible for the initial coprecipitation. If the rate of precipitation of tin is reduced by lowering the reaction temperature, the supersaturation effect becomes negligible (Figure 2).



Initial concentration of manganese, 10<sup>-11</sup>M

The results of 86° C. also illustrate the Paneth-Fajans-Hahn rule. The plateau observed has not been previously reported and is associated with the greater affinity of the precipitate for the tetravalent tin.

Willard and Gordon (18) obtained typical Freundlich adsorption isotherms with basic stannic sulfate, when precipitation took place from boiling solutions, and the final pH was from 1.3 to 1.9. The present study did not investigate this pH range at  $97^{\circ}$  C. It is possible that any given fraction of the precipitate might follow the Freundlich relationship, even before quantitative precipitation.

At 76° C. and at the same initial concentration of manganese  $(10^{-3}M)$  as shown in Figure 2, coprecipitation was not observed until about 50% of the tin had precipitated. From this point on, the amount of coprecipitation slowly increased. After the tin was quantitatively precipitated, a large increase in the amount of coprecipitation was again observed, followed by an apparent leveling-off at about 15% of the manganese initially present. Normally, one would expect a greater amount of adsorption at the lower temperature. This effect was not too apparent here, although there is somewhat more coprecipitation at 76° C. in the case of the larger fractions of tin precipitated. It may well

From these observations it appears that adsorption following complete precipitation of the carrier must be considered an important factor in precipitation reactions. Willard and Sheldon (19) also found a considerable amount of adsorption by cations following complete precipitation of iron as the basic formate. As a result of their work, they suggested a two-stage process which produced excellent separations. The quantity of contaminant found by this method with a given amount of precipitate is smaller than is obtained if the precipitate is separated in a single-stage continuous manner. Willard and Sheldon ascribed the success of their two-stage process to diminished adsorption at low pH, but their results are better interpreted by a more generalized description which emphasizes the role of the Paneth-Fajans-Hahn rule.

It is also of interest to examine these results in the light of Kolthoff's emphasis that occlusion is the significant factor in real coprecipitation (?), as has been discussed. By following the change in concentration of the tracer in the mother liquor during the precipitation, as suggested by Kolthoff, this study has demonstrated that adsorption on the completely precipitated carrier contributes more to the final coprecipitation than does occlusion.

In this study there was a possibility that some of the manganese was coprecipitated as the dioxide. However, the large quantity of chloride ion acts as an effective reducing agent at the working acidity. This was confirmed by the rapid dissolution of a small quantity of freshly precipitated manganese dioxide added to the solution. Furthermore, work by Anderson (1) and by Willard and Sheldon (19) on the coprecipitation of manganese(II) with hydrous ferric oxide and basic ferric formate, respectively, indicates a lack of oxidation to manganese(IV), even at the higher pH used to precipitate iron(III).

There is little doubt that some manganese(II) is oxidized to manganese(IV), as is indicated by the very slight, off-white color exhibited by the basic stannic sulfate. However, the extent of oxidation is probably small; dark brown manganese dioxide did not begin to precipitate until pH 6.2 was reached. The tin precipitates obtained at lower initial concentrations of manganese were pure white.

It was surprising, in the case of solutions initially  $10^{-6}$  and  $10^{-11}M$  at 76° C., to find that there was essentially no difference, despite the obvious difference in surface area, in the amount of coprecipitation by either the heterogeneous or homogeneous methods of precipitation. This has been illustrated in Figures 4 and 5. This could be indicative of anomalous mixed-crystal formation—i.e., Hahn's isodimorphism (4).

The extent of coprecipitation from less than  $10^{-3}M$  manganese solutions was appreciably affected by the rate of addition of ammonia. Thus, when a 50% fraction of tin was precipitated by the addition of the reagent in 1-ml. portions rather than dropwise, the amount of coprecipitation increased from 2% to more than 5% of the manganese initially present. The subsequent results indicated that the precipitations were performed under conditions approximating precipitation from homogeneous solution. By varying these conditions, such as dropwise addition of ammonia and vigorous stirring, it is possible that any desired coprecipitation values could be obtained.

Although the results obtained at low initial concentrations of manganese  $(10^{-6} \text{ and } 10^{-11}M)$  are essentially the same with either method of precipitation, the gelatinous nature of the precipitate, produced by the addition of ammonia and the retention of mother liquor, are undesirable characteristics. The results at  $10^{-3}M$  manganese are heavily in favor of precipitation from homogeneous solution.

This investigation suggests means for the separation of relatively pure carriers. These include such factors as elimination of the initial supersaturation effect and removal of the carrier

before it is quantitatively precipitated. Although the technique of precipitation from homogeneous solution can be used to attain these factors and is favorable in other ways-i.e., slow precipitation rate and concomitant reduction in surface adsorption due to larger particle size-a certain amount of contamination seems inevitable.

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## **Determination of Silica and Fluoride in Fluorsilicates**

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In the separation and determination of silica and fluoride, silica is determined by combined colorimetric and gravimetric methods and fluoride, by distillation as fluosilicic acid and titration with thorium nitrate at a constant pH of  $3 \pm 0.10$ . A new method for separating fluoride and silicate in a one-filtration operation uses zinc oxide in the fusion, and ammoniacal zinc oxide as precipitant. Residual silica, left in solution after the single filtration, is estimated colorimetrically. The interference of ions in the distillation has been investigated extensively, including a special study of silicate and borate. By evaporating the distillate to dryness with sodium carbonate and fusing, all fluoride is recovered in the presence of borate. This makes possible the accurate estimation of fluoride by distillation when borates are present in the sample. Incomplete fusion of sample is a larger factor in fluoride loss than is volatilization. Direct distillation of a small fused sample is an excellent means of determining fluoride without making any separations.

THE work reported in this article was done so that more L accurate analyses of synthetic fluorphlogopite mica and its isomorphs, and of synthetic fluoramphiboles, might be obtained. Many of these compounds contain boron in addition to fluorine. Other types of natural minerals were included to make the results generally applicable. A more detailed presentation will be made in a subsequent report.

If the fluoride present is appreciable, silica cannot be determined accurately, unless the fluoride is removed prior to the silica determination. Many combinations of sodium carbonate, sodium borate, and boric acid, singly or together were tried, but none gave accurate data for silica. This work substantiates that of Lundell and Hoffman (5), who found that acidic recovery of silica in presence of fluoride, even with borates or arsenites, was unsatisfactory. An alkaline medium for separating fluoride from silicate was therefore necessary. The method of Berzelius (2) was evolved for precisely this situation. However, it is quite lengthy, requiring three precipitations and filtrations, as well as prolonged boiling and adjustment of pH. Further, recovery of all the silica is not easy, and the Berzelius determination of fluoride is notorious for the difficulties involved in obtaining accurate results.

In experiments designed to find shorter and more accurate ways of estimating silica and fluoride, a modification of the Berzelius method was tried, reducing it to two filtrations with a colorimetric determination of residual silica in solution. This modification was adequate for many samples, and was actually used for over a year, but the residual silica was not always low enough for accurate estimation. The presence of 1% or less of silica, on the basis of the original sample, was preferred for colorimetric estimation, since experience showed that too much error might be obtained with 5 to 15% residual silica. In the quest for a means of getting low residual silica, the discovery was made that zinc oxide in the original sodium carbonate fusion, plus ammoniacal zinc oxide in the solution of the fusion, gave reliably low values for residual silica. The zinc oxide actually assisted in giving more complete fusion of fluoride-containing samples with sodium carbonate, instead of hindering it as did aluminum hydroxide, calcium oxide, or magnesium oxide. Moreover, the low residual silica was obtained with only one filtration, because the ammoniacal zinc oxide was added to the unfiltered solution of the fusion.

Previous difficulties with the lead chlorofluoride method (4, 13) plus the intriguing versatility and accuracy obtainable with the fluosilicic acid distillation method (14), led to study of the latter in detail. Application of the distillation procedure was easy; it was only necessary to evaporate the filtrate obtained as above to 100 ml. or less, and distill with perchloric acid. The small residual silica obtained with the zinc oxide-fusion method was no hindrance to distillation, because sufficient silica to form fluosilicic acid must either be present or added.

Borates caused no difficulty in accurate fluoride distillation and estimation, provided the distillate with excess sodium carbonate was taken to dryness in platinum, then fused to break up borofluoride salts. Details are given in Section C. An extensive bibliography on determination of silica and fluoride is given by Hillebrand et al. (4). McKenna has published a literature review of methods of fluorine and fluoride analysis covering 516 réferences (7).

## ANALYTICAL CHEMISTRY

Table	I.	Effect	of	рН Tho	on riun	Titration Nitrate	of	Fluoride	with

$(20.0 \text{ mg. of } \text{F}^- \text{ taken})$								
$\mathbf{p}\mathbf{H}$	F-Found, Mg.	$\mathbf{pH}$	F - Found, Mg.					
1.7 2.4 2.6 2.9 3.05	>23 20.62 20.14 19.96 19.99	$3.2 \\ 3.4 \\ 4.0 \\ 4.5$	19.95 19.50 18.54 <17					

#### A. GENERAL CONDITIONS FOR DISTILLATION AND TITRATION OF FLUORIDE

Distilling Apparatus and Procedure. The apparatus used was modified from a design by the U. S. Department of Com-merce (Ace Glass, Inc., Vineland, N. J., No. 6431). The capacity of the Claissen flask was 250 ml. Because sulfate from sulfuric acid volatilization caused high results and an indistinct end point in the titration with thorium nitrate, 25 ml. of 72% perchloric acid was always used as the distilling acid. The total distillate collected was 500 ml., 75 to 125 ml. of which was distilled before 140° C. was reached. Under ideal conditions, fluoride was completely recovered by distillation at 120° and 130° C.; how-ever, 140° C. is recommended. As a source of silicon(IV), 150 mg. of very fine sand, 300 mesh or finer, was added. With-out this addition, silicon(IV) and boron(III) would be dissolved from the glass flask by the fluoride of the sample. In the ordinary procedure this boron(III) contamination could cause slightly low results for fluoride. An asbestos pad, with a hole cut to fit the distilling flask, was used to prevent hot spots. After a temperature of  $120^{\circ}$  to  $125^{\circ}$  C. was reached, steam from an external source was admitted through the hole used for water additions, and the temperature was raised to 140° C. Glass beads or silicon carbide chips were used to prevent bumping. If the amount of fluoride was small, a slight excess of sodium hydroxide was added, and the entire 500 ml. of distillate was evaporated in platinum to 100 ml. Porcelain and glass vessels should not be used for this evaporation (6). The distilling flasks were thoroughly cleaned with hot 10% sodium hydroxide between distillations (9

The rate of distillation was at least 4 ml. per minute, but not over 6 ml. per minute. Under ideal conditions, with 100 mg. of fluoride present and an original volume of 75 ml., 98 mg. of fluoride was distilled in the first 100 ml. of distillate, 1.9 mg. in the second 100 ml., and 0.1 mg. in the last 300 ml. These data are based on 500 ml. of distillate, and it is recommended that 500 ml. be collected, unless actual experimentation with the samples involved indicates that less is satisfactory.

volved indicates that less is satisfactory. In a normal distillation, very little of the liquid being distilled is carried over as spray or otherwise. This is proved by the fact that the distillate from 100 mg. of silver ion in the Claissen flask gave no test for silver. Also, with 4339 mg. of sodium ion present in the liquid being distilled, only 1 mg. of sodium was found in the distillate.

Titration of Fluoride with Thorium Nitrate. A close control of the pH of the solution during titration is necessary (Table I) if accurate results are to be obtained. The limits of pH allowable are 2.8 to 3.2; however, a closer control of pH at 3.0 to 3.05 is recommended. This was achieved by use of a glass-electrode pH meter and mechanical stirrer during the entire titration. Dilute sodium hydroxide or dilute perchloric acid was added with a dropper to give the correct initial pH of 3.05, and dilute sodium hydroxide was added continuously during the titration with thorium nitrate to maintain a constant pH of 3.0 to 3.05. By this technique buffer was neither needed nor used. This procedure gave the most well-defined end point of any tried, and was particularly useful for titration of larger amounts of fluoride. Adjustment of pH by using sodium alizarin sulfonate as an indicator for the pH, as has been recommended, proved inadequate for accurate work. Also 1 ml. of buffer (94.5 grams of monochloroacetic acid and 10.0 grams of sodium hydroxide per liter) was insufficient for more than 3 to 4 mg. of fluoride. Even 4 ml. of this buffer was insufficient to maintain the correct pH while titrating 20 mg. of fluoride.

The reason for control of pH with dilute sodium hydroxide is that much hydrogen ion is produced from the reaction:  $Th^{++++}$  + 4HF (weak acid)  $\rightarrow$  ThF<sub>4</sub> + 4H<sup>+</sup>. A quantitative estimation

showed that 1.000 mg. of hydroxide was needed for each 2.035 mg. of fluoride titrated. On the ionic basis this was 1.000 ion of hydroxide to each 1.820 ions of fluoride. Dilute sodium hydroxide for pH control permitted the use of the minimum amount of reagent necessary for each titration, regardless of the quantity of fluoride present.

Titration to the maximum pink color of sodium alizarin sulfonate, in which traces of yellow were absent, was the end point used. With this technique a titration blank of 0.10 ml. or less was obtained. The end point is fairly easy to judge, but a little experience gained by titrating standard solutions is recommended.

For most accurate results, the thorium nitrate solution should be standardized by an actual distillation of lithium or sodium fluoride, using the same distilling conditions as for the samples. However, the difference between direct and distillation standardization was only about 1 part in 150. In amounts of fluoride from about 0.5 to 50 mg., a 0.0800N thorium nitrate solution was a satisfactory concentration.

Because the fluoride largely distills as fluosilicic acid, no interference from silicates was expected in the titration. Amounts of sodium metasilicate nonahydrate up to 1 gram did not interfere when added directly to the solution being titrated. Also, boric acid in amounts up to 1 gram did not interfere.

Table II. Effect of Certain Ions on Recovery of 100 Mg. of Fluoride by Distillation of 500 Ml.

on and Amount Added, Mg.	Compound Added	F-Recovered, Mg.
Al <sup>+++</sup> . 100	AlCl <sub>3</sub>	96.5ª
BO <sub>3</sub> , 100 (as B <sub>2</sub> O <sub>3</sub> )	H <sub>3</sub> BO <sub>3</sub>	93.0
$BO_3 =, 40$ (as $B_2O_3$ )		97.7
$BO_3, 20$ (as $B_2O_3$ )		98.1
BO <sub>3</sub> , 10 (as B <sub>2</sub> O <sub>3</sub> )		99.1
Ce + + + +, 100	(NH <sub>4</sub> ) <sub>2</sub> Ce(NO <sub>3</sub> ) <sub>6</sub>	98.9
Sc + + +, 100	Sc <sub>2</sub> O <sub>3</sub>	98.0
SiO <sub>3</sub> , 127	$Na_2SiO_3 \cdot 9H_2O$	99.8
SiO <sub>3</sub> , 253		95.9
Th + + + + +, 100	$Th(NO_3)_4$	98.0
U+++++, 100	UO2(NO3)2-6H2O	98.0
Zr + + + +, 100	$ZrO(NO_3)_2$	76.4
a Ensure the second 500 ml	of the distillate 2 mm of	

<sup>a</sup> From the second 500 ml. of the distillate, 3 mg. of fluoride was recovered.

Additional Titration Tests. Since the perchlorate ion has the least complexing tendency for thorium(IV) of the common anions, it was thought that substitution of thorium perchlorate for thorium nitrate might improve the sharpness of the end point. The difference, however, was not great enough to warrant the use of one salt over the other.

Room temperature was best for the titration, since  $8^{\circ}$  C. gave slightly high results, and  $38^{\circ}$  C., slightly low.

High concentration of sodium ion, and presumably other alkalies, should be avoided since they cause a lowering of results. Up to 0.5 gram of sodium carbonate per titration could be present without causing excessive lowering of fluoride recovered.

Ethyl alcohol solutions, 50%, were less satisfactory for titration of macro amounts of fluoride than water alone (1).

Direct titration of the fluoride in the filtrate from the zinc ion separation of fluoride and silicate proved unsatisfactory. Recoveries of fluoride were excessively low. The interference of sodium, zinc, and other ions possibly present in the filtrate excluded the use of this short cut. The titration is so sensitive to the effects of cations which form complexes with fluoride, and of anions which form complexes with thorium(IV), that data obtained from any new titration, where other ions might be present, should be rigorously checked.

### B. INTERFERENCE OF IONS IN THE DISTILLATION OF FLUORIDE AS FLUOSILICIC ACID

Determination of the extent of interference of certain ions was made by distilling 100 mg. of lithium or sodium fluoride directly in the presence of the ion. The distillation procedure was as described in Section A. Data for interfering ions are in Table II. Surprisingly, very few ions seriously interfered in the complete distillation of fluoride. These ions were aluminum(III), borate, metasilicate, and zirconium(IV). Ions that interfered slightly were cerium(IV), scandium(III), thorium(IV), and uranium(VI). Ions that did not interfere under conditions of the test were: antimony(III), arsenic(III), barium, beryllium, bismuth(III), cadmium(II), calcium, chromium(III), cobalt(II), copper(II), gadolinium(III), gallium(III), germanium(IV), indium(III), iron(III), lanthanum(III), lead(II), lithium, magnesium, manganese(II), mercury(II), molybdenum(VI), neodymium(III), nickel(II), niobium(IV), platinum(IV), potassium, rhenium(VII), rhodium(III), samarium(III), selenium(IV), silver, sodium, strontium, tantalum(IV), tellurium(IV), thallium(I), tin(II), titanium(IV), tungsten(VI), yttrium(III), and zinc.

Distillation and quantitative recovery of fluoride in presence of borate is described in Section C. A special technique was devised to eliminate borate interference. Aluminum(III) will not interfere in fluoride recovery by the zinc oxide-fusion procedure, because it is largely separated with the silica. However, in direct distillations of fused samples, aluminum(III), if present in large quantities, could become a factor in recovery (Table III).

Table III. Effect of Aluminum on Fluoride Recovery by Distillation

	Al Taken, as	F <sup>-</sup> Recovered, Mg.				
F <sup>-</sup> Taken, Mg.	Al(ClO <sub>4</sub> ) <sub>3</sub> , Mg.	1st 500 ml.	2nd 500 ml.			
50.0	20	49.4	0.09			
50.0	40	49.5	0.40			
50.0	60	48.8	0.96			

Table IV. Effect of Sodium Metasilicate, Nonahydrate on Recovery of Fluoride by Distillation (100 mg. of F<sup>-</sup> taken)

SiO2 Taken (as Na2SiO3·9H2O), Mg.	F <sup>-</sup> in Distillate, Mg.	SiO2 in 500 Ml. Distillaté <sup>a</sup> , Mg.
0	98,95	63
20	98.50	61
40	99.8	65
60	100,2	65
80	99.7	67
100	99.8	67
120	97.4	65
160	97.4	64
200	95.9	61
200 <sup>a</sup> Quantity distilled wit	95.9 th fluoride.	61

<sup>b</sup> Slightly low value caused by borate from distilling flask.

Silica, in some form, is essential to the distillation of fluoride as fluosilicic acid. If not provided, silica will be removed from the glass distilling apparatus. With borosilicate glass, a slight lowering of fluoride recovered would be expected, unless a special technique is used (see Section C). Although silica or some silicate is necessary, too much silicate will interfere by causing a loss of fluoride (Table IV). This loss is not caused by an excess of fine sand, but appears to be connected with the precipitation of gelatinous silica during the distillation. Whether the loss is caused by mechanical entrapment or by chemical combination is not known. Fluoride and oxygen ions are of similar size. and differ in charge by only one unit, so it is not unlikely that some of the fluoride may substitute for oxygen in the polymerized silicic acid. A peculiarity of the silica interference is that small amounts of fluoride (0 to 6 mg.) can be largely recovered in the presence of a large excess of silicate, while large amounts of fluoride (12 to 40 mg.) are not quantitatively recovered under the same conditions (Table V). The recovery of microgram quantities of fluoride in presence of excess silicate has been given detailed study in two recent articles (8, 11).

Stoichiometric amounts of silicate and fluoride gave quantitative recovery, but silicate in excess of the theoretical ratio caused

(60 mg.	SiO2, as Na2SiO3.9H2O, ta	ken)
F <sup>-</sup> Taken, Mg.	F-Recovered, Mg.	SiO <sub>2</sub> in 500 Ml. Distillate, Mg.
$\begin{array}{c} 0.1 \\ 4.0 \end{array}$	$\begin{array}{c} 0.16 \\ 3.95 \end{array}$	0.3
$\begin{array}{c} 6.0\\ 12.0 \end{array}$	$\begin{array}{c} 6.0\\ 11.2\\ \end{array}$	4
20.0 50.0 100.0	18.9 48.9	<b>8</b>  65
200.0	198.6	
able VI. Recover by Fusion of I	y of Fluoride in the Distillate with Sodi	Presence of Bora ium Carbonate
able VI. Recover by Fusion of I F <sup>-</sup> Taken, Mg.	y of Fluoride in the Distillate with Sodi B2O3 (as H2BO3), Mg.	Presence of Bora ium Carbonate F <sup>-</sup> Found, Mg.
able VI. Recover by Fusion of I F <sup>-</sup> Taken, Mg. 100.0	y of Fluoride in the Distillate with Sodi B2O3 (as H2BO3), Mg. 100	Presence of Bora ium Carbonate F <sup>-</sup> Found, Mg. 99.7

progressively lower recovery of fluoride. The theoretical quantity of silica needed for distillation of 100 mg. of fluoride as fluosilicic acid is 53 mg. Since 60 to 67 mg. of silica actually came over (Table IV), it is likely that other compounds of fluorine which are enriched in silicon are also being distilled. The best source of silica was 150 mg. of a very fine sand, because tests showed that an excess of sand did not cause any loss of fluoride.

The simultaneous presence of calcium ion and phosphate, such as in phosphate rock, prevents the complete separation of fluoride from silicate by either the Berzelius method (10) or the zinc oxide-fusion method. Two fusions (sodium carbonate) and leachings recovered only 2.5% fluoride when 3.3% was actually present. With the zinc oxide-fusion, a phosphate rock containing 3.67% fluoride gave 2.43% in the filtrate and 1.24% with the silica precipitate. In the absence of phosphate, calcium fluoride is converted to calcium carbonate by reaction with hot sodium carbonate solution. While interfering in separations, phosphate does not interfere in the distillation of fluoride. Hence the best method for samples such as phosphate rock is a direct distillation, preferably after a sodium carbonate fusion.

#### C. DETERMINATION OF FLUORIDE BY DISTILLATION IN PRESENCE OF BORATES

Table II shows that 100 mg. of fluoride could not be recovered quantitatively in the presence of 100, 40, or 20 mg. of borate as boric acid. Only 93 out of 100 mg. of fluoride was recovered in the presence of 100 mg. of boric oxide. After several unsuccessful experiments to determine the location of the unrecovered fluoride (including adding sodium silicate to the distilling flask, prolonged distillation, distillation with sulfuric acid at several temperatures, trapping of any possible volatile gas, and evaporation of distillate to dryness with sodium hydroxide), it was decided that the missing fluoride must be in the distillate in the form of a nonionized fluoboric acid or its salt. Accordingly the distillate from a solution containing 100 mg. of fluoride and 100 mg. of boric oxide was evaporated to dryness in platinum with 2 grams of sodium carbonate. The salts were carefully dried, then fused for 2 minutes with a Fisher burner at medium heat. The resulting fusion was dissolved, diluted to 500 ml., and 100 ml. was titrated. Recovery of fluoride, even with 300 mg. of boric oxide present, was complete (Table VI). The fusion had presumably converted the nonionized fluoborate into the simple ions fluoride and borate. Borate in this form does not interfere in the titration with thorium nitrate.

A peculiarity of these fluoride-borate-metasilicate distillations is that even with excess borate present, most of the fluoride distills as fluosilicic acid, while most of the borate that goes into the distillate does so because of its volatility rather than its combination with fluoride. In fact, it appears that the only borate distilling in combination with fluoride is the portion which forms nonionized fluoboric acid. Recovery of fluoride in the presence of the interfering ions aluminum(III), zirconyl, and metasilicate was not improved by additions of borate to the distilling flask.

Apparently the volume-concentration ratio has a pronounced effect on the fluoride-borate distillation. For example, complete recovery of 2 to 20 mg. of fluoride in the presence of 100 mg. of boric oxide was obtained even without the evaporation to dryness and fusion. This distillation was made using the same liquid volumes as was used for the 100 mg. fluoride-100 mg. boric oxide mixtures. High volume and low concentration gave best recovery. This is of interest, because these amounts of fluoride and boric oxide are in a range found in many materials, particularly if small samples (100 to 200 mg.) are taken for analysis.

#### D. ZINC OXIDE-SODIUM CARBONATE FUSION PROCEDURE FOR DETERMINATION OF FLUORIDE AND SILICA

This procedure requires only one filtration to separate fluoride from most of the silica. The residual silica accompanying the fluoride is estimated colorimetrically. Fluoride is determined by distillation with perchloric acid and titration of the distillate with thorium nitrate. The main portion of the silica is determined by standard gravimetric methods.

**Decomposition of Sample.** If complete decomposition of the sample is to be attained in one fusion, the sample should be very finely ground; minus 200 mesh is necessary for some samples. Incomplete fusion can be a serious source of error, and should be carefully guarded against, even though the zinc oxide fusion gives better decomposition of the sample than sodium carbonate alone. Silica should be added to the fusion mixture for high fluoride-low silica samples. Possible reasons for the effectiveness of zinc are: (1) It is a smaller ion, with higher field strength than sodium. (2) It forms no ternary compounds with aluminum and silicon.

Loss of Fluoride during Sodium Carbonate Fusion. Fifteen minutes of fusion at medium heat, plus 15 minutes of fusion at the highest heat of a Fisher burner  $(1100^{\circ} \text{ C.})$ , did not cause loss of fluoride by volatilization. With a flame, the temperature of the top of the crucible was at least 200° less than that of the bottom. Samples of topaz, opal glass, and granite plus sodium fluoride were fused in an electric furnace, giving the following results: 950° C. for 30 minutes showed no fluoride loss; 1050° C. for 20 minutes showed a loss of less than 0.05% fluoride; and 1200° C. for 10 minutes showed a loss of about 0.3% fluoride (original sample basis). Each of these series was started in a cold furnace. The upper limit of fusion temperature would therefore appear to be 1050° C. in the electric furnace.

Detailed Procedure. 1. FUSION OF SAMPLE. Using a gas burner, fuse a 0.500-gram sample with 1.0 gram of zinc oxide and 5 grams of sodium carbonate, just above the melting temperature of the sodium carbonate, for 20 minutes. Then 1050° to 1100° C. for an additional 20 minutes. Then fuse at about If an electric furnace is used, start with a cold furnace, and fuse for 30 minutes between 950° and 1050° C. Platinum crucibles are satisfactory for use under oxidizing conditions, but many gas burner flames appear to be oxidizing but are really reducing (12). A reducing flame leads to reduction of zinc and subsequent alloying with platinum. Nickel crucibles appear to be satisfactory for use with flames that are slightly reducing. (To check completeness of fusion for new types of samples, transfer the ammoniacal zinc oxide precipitate of Section D-2 to a platinum dish, add 5 grams of sodium carbonate, and carefully dry. Either burn the filter paper separately, or in the platinum dish, then mix well. Fuse, and distill the entire fusion without separation of any kind. Evaporate the 500-ml. distillate as described in Section A, and titrate. Silica will of course have to be determined on a new sample.)

2. PRECIPITATION WITH AMMONIACAL ZINC OXIDE. Transfer the fused sample to a 500-ml. stainless steel beaker containing a stainless steel or nickel spatula. Add 200 ml. of cold water. If the fusion sticks to the crucible, the entire crucible and lid may be placed in the beaker. Allow to stand until soluble salts are dissolved, overnight if convenient. Break up the cake completely to make sure no undissolved salts remain. Remove and police crucible and lid if they were placed in beaker. Add slowly, with much stirring, 25 ml. of ammoniacal zinc oxide (containing 1.0 gram of zinc oxide, 1.3 grams of ammonium carbonate, and 2 ml. of concentrated ammonium hydroxide dissolved in 10 ml. of water and diluted to 25 ml.).

Heat to boiling with constant swirling over a flame. Cover and set the beaker in an aluminum cup or similar device on a hot plate. This is necessary to prevent bumping and loss of sample. Boil for 8 to 10 minutes.

Remove from plate and let set 5 minutes. Filter through 12.5cm. No. 40 Whatman filter paper or equivalent, catching the filtrate in a 500-ml. volumetric flask. Transfer the precipitate to the paper and wash twice with boiling water. Transfer the bulk of the precipitate back to the stainless beaker with the spatula, then wash the remainder from the paper into the beaker with hot water. Adjust the volume to 60 or 75 ml., and stir thoroughly. Filter through the same paper, transfer the precipitate to the paper, and police the beaker. Wash the paper and precipitate 5 times with hot water. Washing must be carefully done to avoid loss of fluoride to the voluminous precipitate.

3. COLORIMETRIC ESTIMATION OF SILICA IN FILTRATE. Adjust the volume of the filtrate to 500 ml. and mix well. Transfer with a pipet a 25-ml. sample of the filtrate to a borosilicate glass beaker containing 70 ml. of water. This gives a 25-mg. sample; another size of sample may be used if more convenient. Adjust pH at 1.3 to 1.4 with 1 to 1 hydrochloric acid, using a glass-electrode pH meter. Immediately add 5 ml. of a 10% aqueous solution of ammonium molybdate. Stir well and let set 5 minutes. Add 10 ml. of 10% tartaric acid in water, stir, and let set 1 minute. Add 10 ml. of reducing reagent, stir, and transfer to a 250-ml. volumetric flask. [The reducing reagent is made according to Bunting (3). Solution A contains 90 grams of sodium bisulfite in 800 ml. of distilled water; solution B contains 7 grams sodium sulfite in 100 ml. of water plus 1.5 grams 1-amino-2-naphthol-4-sulfonic acid, which is stirred until dissolved. Add B to A and dilute to 1 liter.] After mixing, let solution set 30 minutes. Measure per cent transmittance at 820  $m\mu$ , or with suitable filters, in a spectrophotometer or photometer.

The reducing reagent will keep better if stored in a refrigerator in small, completely filled bottles until needed. It is recommended that the molybdate, tartaric acid, and reducing reagent be made fresh each month.

4. DETERMINATION OF MAIN PORTION OF SILICA. Transfer the paper and residue to a platinum dish, char carefully, and finally burn the paper at  $650^{\circ}$  C., preferably in a muffle furnace. Dissolve the small residue left in the stainless steel beaker with 25 ml. of hot water plus 4 ml. of concentrated hydrochloric acid. Police into the platinum dish. Wipe the inside of the beaker with a small piece of filter paper, and place this in the platinum crucible to be used for silica determination. The crucible used for fusion should also be used for silica (unless a nickel crucible was used).

Add 20 ml. of concentrated hydrochloric acid to the platinum dish and set the dish on a steam bath. Break up lumps thoroughly with a blunt borosilicate glass rod. Evaporate to dryness on a steam bath. Add 50 ml. of hot water plus 5 ml. of concentrated hydrochloric acid, stir well, and let set on a steam bath 10 minutes to dissolve salts that might otherwise cause excessive nonvolatile matter. Filter through 11-cm. No. 40 Whatman filter paper, and wash well with hot 5% hydrochloric acid in the manner usual for silica determinations.

Add 3 to 5 ml. of nitric acid plus 20 ml. of perchloric acid to the filtrate. Evaporate on a hot plate just to fumes of perchloric acid, using a raised cover on the beaker. Place a closely fitting watch glass on the beaker, and reflux for 20 minutes. Use precautions generally advised for perchloric acid (4). Reflux at such a rate that perchloric acid is not lost from the beaker. Remove from the hot plate, cool, and dilute with 125 ml. of hot water. Add a small amount of macerated filter paper, filter through 11 cm. No. 40 Whatman paper, and wash well with hot water particularly around the top of the paper. Place both papers containing silica in the same crucible originally used for the fusion (if platinum), and burn the paper at a low temperature in an oxidizing atmosphere. Heat at 1200° C.

Place both papers containing silica in the same crucible originally used for the fusion (if platinum), and burn the paper at a low temperature in an oxidizing atmosphere. Heat at  $1200^{\circ}$  C. for 1 hour, remove to a desiccator, cover the crucible with a 50ml. beaker previously set in the desiccator, and weigh when cool. Add 4 drops of 1 to 1 sulfuric acid, 15 ml. of hydrofluoric acid, and take to dryness on a hot plate in a hood. Heat carefully over a flame to decompose sulfates; then heat for 5 minutes at  $1000^{\circ}$  C. in oxidizing atmosphere, preferably in an electric muffle furnace. Cool and weigh as before. The difference in weight is silica.

Add the gravimetric and colorimetric values (both reduced to per cent) to obtain the total per cent of silica of the sample. For more accurate data, further recovery, or a correction, must be made for the silica which is nearly always lost, even after two

	Fluoride, %						
Sample	In <sup>a</sup> residue	In filtrate	Total found	Present			
Opal glass 91 Synthetic mice 3692	0.12	5.70	5.82	5.750			
Phosphate rock 56a	1.24	2.43	3.67	3.560			
Diabase W-1° Granite G-1°	0.19 0.18 0.16	10.90	15.07 11.08 11.27	15.08 11.04 11.34			

Table VII. Results Obtained for Fluoride by the Use of Zinc Oxide-Fusion Procedure

<sup>a</sup> The silica-zinc oxide precipitate, from part 2, was distilled instead of being used for determining silica. <sup>b</sup> National Bureau of Standards certificate value. <sup>c</sup> Fluoride was added.

Table VIII. Res	sults Obtained	for	Silica by	Zinc	<b>Oxide-Fusion</b> Procedure	
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	Sample Size.	ZnO.	Na <sub>2</sub> CO <sub>3</sub> .	SiO <sub>2</sub> , %						
Sample	Grams	Grams	Grams	Color.	Grav.	Total found	Present			
Opal glass 91	$\begin{array}{c} 0.5000 \\ 0.5000 \\ 1.0000 \\ 1.0000 \\ 0.0000 \end{array}$	$1.0 \\ 1.0 \\ 1.0 \\ 1.5 $	5 5 5 7.5	$\begin{array}{c} 0.76 \\ 0.74 \\ 0.54 \\ 0.40 \end{array}$	$\begin{array}{c} 66.52 \\ 66.48 \\ 66.78 \\ 66.85 \end{array}$	$67.28 \\ 67.22 \\ 67.32 \\ 67.25 \\ 07.25 \\ 07.25 \\ 07.25 \\ 07.25 \\ 0.25 \\$	67.53ª			
Granite G-1 <sup>b</sup>	2.0000 0.5000 0.5000 1.0000	$3.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0$	15 5 5 5	0.43 0.83 0.60 0.85	$     \begin{array}{r}       66.84 \\       71.64 \\       71.82 \\       71.61 \\     \end{array} $	67.27 72.47 72.42 72.46	72.64¢			
Diabase W-10	0.5000	1.0	5	0.77	51.64	52.41	52.66°			
<sup>e</sup> National Bureau of Standards reported value.										

<sup>6</sup> About 10% fluoride, as sodium fluoride, added. <sup>c</sup> United States Geological Survey reported value.

acidic dehydrations. This correction was about 0.20% (see Table VIII), and varied slightly with conditions.

5. DETERMINATION OF FLUORIDE. Transfer the remaining portion of the 500-ml. filtrate (from step 2) to a stainless steel beaker. Evaporate on a steam bath to 75 ml. or less, and transfer to the 250-ml. Claissen flask used for distillation. **Evaporation** may be done in the Claissen flask. The small amount of residue in the beaker is best transferred to the flask by policing with 2 ml. of perchloric acid in 20 ml. of cold water. Add to the flask 150 mg of very finely ground quartz sand, which is free of fluo-ride, and a few silicon carbide chips or glass beads to prevent bumping. Assemble the entire distillation apparatus including a 500-ml, volumetric flask for receiver. Add 23 ml. of perchloric acid for a total of 25 ml. Distill, using a flame, until the temperature is  $123^{\circ} \pm 3^{\circ}$  C. Then add steam from an auxiliary flask at such a rate that the condenser is not overloaded. Continue heating briskly until the temperature reaches 140° C. At this point, adjust the flame so that this temperature is held for the remaining portion of the distillation. The rate of distillation At this point, adjust the hand so that the remaining portion of the distillation. The rate of distillation the remaining  $5 \text{ m}^3$  per minute. Collect 500 ml. of total distillate

Adjust the volume to the mark, mix well, and if fluoride is high, take a 100-ml. portion for direct titration with thorium nitrate. take a 100-mi. portion for direct titation with thorinin intrace. If fluoride is low, transfer the entire distillate to a platinum dish, which contains 3 pellets of sodium hydroxide, and evaporate to 100 ml. or less. The pH for this evaporation must be higher than the phenolphthalein end point. In either method adjust the volume to  $100 \pm 10$  ml. Add 2 ml. of sodium alizarin sulfonate solution, prepared by dissolving 0.50 gram in 1 liter of water, and titute with the deviced therium pitters equation. and titrate with standardized thorium nitrate solution. The use of a glass-electrode pH meter, mechanical stirring, and dilute sodium hydroxide to maintain a pH of 3.05, is recommended for the titration. In the calculations, remember that 25 ml. or more was used for the colorimetric silica determination; hence the sample titrated above is not exactly 100 or 500 mg.

RESULTS OBTAINED FOR FLUORIDE BY USE OF ZINC OXIDE-FUSION PROCEDURE. Results for six different materials analyzed for fluoride by the described method, with the single exception that the silica precipitate was distilled directly instead of being used for silica determination, are in Table VII.

The results found for fluoride were very accurate, provided that the fluoride distilled from the residue was added to the total. Excluding phosphate rock, which is best analyzed by a direct distillation, the average amount of fluoride in the silica residue was 0.15%. It is believed that most of this is derived from incomplete washing of the rather large precipitate which results from the addition of the ammoniacal zinc oxide. This slight loss of fluoride is not peculiar to the zinc oxide fusion. The same samples, as in Table VII, were analyzed by the Berzelius method,

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as modified by Hillebrand and Lundell, except that the silica precipitate was distilled to determine fluoride. With the modified Berzelius method, the silica precipitate was found to contain an average of 0.19% fluoride. Also, the sodium carbonate fusion of the Berzelius method did not give complete decomposition of topaz and synthetic mica, as was done by the sodium carbonate-zinc oxide fusion of the zinc oxide-fusion method. About the only conclusion arising from these facts is that for most accurate fluoride results with either method, the silica precipitate should be distilled, and silica determined on a separate

sample. For most work a correction would be satisfactory.

7. RESULTS OBTAINED FOR SILICA BY ZINC OXIDE-FUSION PROCEDURE. The silica found by the above procedure on three different materials is reported in Table VIII. The residual silica left was less than 1%, an amount well suited for colorimetric estimation. Total silica, found by the summation of colorimetric plus gravimetric determinations, averaged 0.20% low. Previous work with 0.5-gram samples of mullite refractories and clavs indicated that this low value is caused by incomplete recovery of silica in two dehydrations. It

is also generally known that some silica is not recovered in two acid dehydrations, and that for highly accurate work, this silica must be recovered and added to the main portion.

The small amount of fluoride, which averages 0.15%, left with the silica could account for some of the unrecovered silica. The theoretical loss of silica for 0.15% of fluoride is about 0.10%, but it is not believed that the theoretical loss is obtained during an actual analysis. The only remedies for the slightly low values are a correction, long dehydrations, or estimation of the unrecovered silica by standard methods. Until experience is gained, it is recommended that standard samples containing fluoride, or known samples plus added fluoride, be analyzed concurrently with the unknown material.

The ratios of sample to zinc oxide to sodium carbonate were apparently not critical, for as seen in Table VIII, the lowest residual silica was obtained with 1 gram of sample, 1.5 grams of zinc oxide, and 7.5 grams of sodium carbonate.

#### E. DIRECT DISTILLATION OF FLUORIDE AFTER SODIUM CARBONATE FUSION OF SAMPLE

The remarkable freedom from interference by most elements in the fluosilicic acid distillation of fluorides prompted an interest in the possibility of recovering fluoride without any separation at all. Accordingly, certain samples were analyzed for fluoride by distillation after a preliminary fusion with 5 grams of sodium carbonate. The entire fusion was transferred to the distilling flask, and the distillation and titration were made as usual.

Table IX.RecoveryDistillation of Sample	of Fluoride Fused with	by a Sing Sodium C	le Direct arbonate
	Sample	Fluori	de, %
Sample	Size, Mg.	Found	Present
Muscovite mica	1000	0.27	0.35
Opal glass 91	1000 200 100	$   \begin{array}{r}     0.13 \\     5.44 \\     5.60 \\   \end{array} $	$0.15 \\ 5.75 \\ 5.75 \\ 5.75 \\ 100 \\ $
Na2SiF6 Fluorspar 79	200 200	60.50 46.25	$\begin{array}{c} 60.62\\ 46.14\end{array}$
Granite G-1 + F <sup>-</sup> Diabase W-1 + F <sup>-</sup>	100 100	9.75 9.75	$10.12 \\ 10.07 \\ 15.08$
Lepidolite mica	100 200	14.00 14.48 0.78	$15.08 \\ 15.08 \\ 0.79$
	200 200	2.08 7.10	2.25 7.79
Synthetic phiogopite mica	200	8.75	9.30

Table X. Recovery of Fluoride by Two Direct Distillations with Intervening Fusion of Residue

			Fluoride, %		
Sample	1st Dis- tillation, %	2nd Dis- tillation, %	Total found	Present	
Synthetic phlogopite mica	$10.92 \\ 8.75 \\ 8.67$	$\begin{array}{c} 0.38 \\ 0.45 \\ 0.50 \end{array}$	11.30 9.20 9.17	$11.34 \\ 9.30 \\ 9.24$	

Results from several types of materials are in Table IX. The data indicate that sample size should be kept as low as possible, nearly complete recovery may be expected if fluoride is low, and interference is about what would be expected from the amounts of metasilicate, aluminum(III), and fluoride present.

Nearly complete recovery of fluoride was obtained by filtering the residue in the distilling flask, washing well to remove perchloric acid, and again fusing in platinum with 5 grams of sodium carbonate. This second fusion was distilled as before and the fluoride added to that obtained in the first distillation. Preferably the same perchloric acid should be used in the second distillation. Results are in Table X. If borates are present, the method presented in Section C must be used.

A single direct distillation of a sample after fusion with 5 grams of sodium carbonate may therefore be expected to indicate the approximate quantity of fluoride present in the sample. This

procedure makes an excellent qualitative test for fluoride. Quantitative results may be obtained by again fusing the residue from the distilling flask, redistilling, and combining the two results.

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## **Determination of Low Concentrations of Oxygen in Hydrogen**

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Oxygen in hydrogen can be determined by the formation of water vapor on a platinum catalyst followed by separation by condensation at the liquid nitrogen temperature, and measurement of the volume at 10-mm. pressure. The method is standardized to take into account the adsorption of water vapor on the glass apparatus. The lower limit in the apparatus is 0.00005% oxygen on a 1.3-liter sample with an error of The error decreases as the concentration in-±10%. creases, becoming  $\pm 2\%$  above 0.002% oxygen. The sensitivity and accuracy of the method are illustrated in the determination of current efficiency in the electrolytic formation of oxygen between platinum electrodes in sulfuric acid. The current efficiency rises from 53 to 93% between 10 and 68 μa.

THE determination of small amounts of oxygen in gases has been attempted in many ways. Measured as molecular oxygen, magnetic methods (10, 25) have been applicable to concentrations above about 0.01% and biochemical methods (22, 26) to concentrations below about 0.0001% (1 p.p.m.). Although the mass spectrometer does not appear to have been used for this purpose, it would probably not be useful below about 0.005%. Thus, Inghram et al. (14, 18) have reported that the minimum change in the oxygen content of tank hydrogen detectable with the mass spectrometer is 0.0006%. If the oxygen is converted to water vapor, the determination may be made with the infrared gas analyzer (39), the frost-point hygrometer (4, 16, 35), or the inorganic film hygrometer (9, 12, 37). These methods are applicable to concentrations above about 0.001%, but, owing to problems arising from the adsorption of water vapor on the walls of the apparatus and the fact that in a hygrometer measurement a small amount of water is either condensed from or released to the

gas, they serve better for continuous analysis in flow systems. Also suitable only for continuous analysis is a calorimetric method (7, 20) in which oxygen is catalytically converted to water vapor and the temperature rise measured. If the gas is passed over carbon at 1120° C., oxygen and its compounds are converted to carbon monoxide (13, 36), which may be determined with the infrared gas analyzer or by conventional means. A sensitivity of 0.003% oxygen in hydrogen-nitrogen mixtures has been reported (34) for this method, but other work (13) suggests that a much higher sensitivity is possible.

To support a study of oxygen poisoning on iron-synthetic ammonia catalyst, it was necessary to have an analytical method for oxygen applicable to the range 1 to 100 p.p.m. Because there appeared to be no approach to a sufficiently sensitive continuous method for insertion in a flow system which would leave oxygen in molecular form, it was necessary to develop a method for individual samples. To avoid the inconvenience of wet chemical procedures, such as those based on the oxidation of manganous hydroxide (40), ferrous hydroxide (32), copper (5), sodium anthraquinone- $\beta$ -sulfonate (3), or pyrogallol (38), the limitations of a direct method were examined, in which oxygen is catalytically converted to water vapor on a platinum filament, the water condensed, and its volume measured under reduced pressure.

The determination of a small quantity of water by measuring its volume at low pressures was first investigated by Langmuir (19) in 1912. In his classic research on low-pressure gas reactions, he developed a microanalytical technique for as little as 0.001 ml. of gas, based on simple reactions such as the combination of oxygen and hydrogen, or oxygen and carbon monoxide. on a platinum filament, the condensation of carbon dioxide and water vapor, and the use of the McLeod gage to measure pressure changes so produced. Finding, however, that a direct pressure measurement of water vapor was inaccurate, owing to its adsorption on glass walls, such measurements were excluded from his analytical procedure. In low-pressure micromethods of gas analysis described since the adsorption of water vapor on glass has been recognized. Oxygen and hydrogen have usually been determined indirectly by contraction methods (17, 27, 28), although in some cases they are determined by a pressure measurement on water vapor in calibrated apparatus (6, 30, 31). The latter approach has also been taken for the microdetermination of hydrogen in organic compounds (23).

At higher pressures and with larger amounts of water, adsorption may be neglected. Thus, water has been directly determined by pressure-volume measurements in the gases evolved on heating glass (8, 33), in the hydrogen reduction method for oxygen in metals such as copper, lead, tin, and their alloys (1, 15, 24). and in a method of gas analysis described by Bamford and Baldwin (2), in which oxygen is determined through its conversion to water vapor.

The method described is essentially a refinement of the technique of Bamford and Baldwin, which was not extended below 0.01% oxygen. In the present apparatus the lower limit is 0.00005% oxygen (0.5 p.p.m.) on a 1.3-liter sample, with an error of  $\pm 10\%$ . At concentrations approaching 0.002% and higher the error becomes  $\pm 2\%$ . A single measurement requires about 1 hour.

## EXPERIMENTAL

In this apparatus a mercury piston pump circulates the gas sample over a platinum catalyst and through a liquid nitrogen cold trap in which the water vapor formed is condensed. This water is then determined by measuring its volume at 10-mm. pressure in a McLeod-type gage.

Since an amount of gas is usually measured in terms of volume in milliliters and pressure in millimeters of mercury, it is con-In mininters and pressure in mininters of mercury, it is convenient to denote it in liter-micron units, where a micron is  $10^{-6}$  meter of mercury. Thus, at a given temperature, 1.3 liters of hydrogen at 760-mm. pressure is 1,000,000 liter-microns, and at 0.0001% oxygen (1 p.p.m.), the amount of water vapor to be formed is 2 liter-microns, 0.2 ml, measured at 10-mm. pressure.

Apparatus. A diagram of the apparatus is shown in Figures 1 and 2. Figure 1 gives the general arrangement, showing the location of a McLeodtype water vapor gage of capacity 5500 liter-microns. Figure 2 shows how a second gage, of capacity 150 liter-microns, has been inserted between cold trap T (Figure 1) and the vacuum manifold. Construction details for the platinum catalyst tube are shown in Figure 3 and the mer-cury piston pump is pictured in Figure 4.

The direction of gas flow in a determination for oxygen (Figure 1) is from the pump through stopcock 1, cold trap T, stopcock 2, over platinum catalyst P, returning to the pump through stopcocks 3 and 5. Stopcocks 4 through 8, and 10 remain closed. Water vapor condensed in T is then isolated by closing 1 and 2, and the hydrogen evacuated through 10 and 11. Finally, depending on the amount to be measured, the water is evapo rated into the 5500-liter-micron gage or distilled into the 150-liter-micron gage, and its volume determined at 10-mm. pressure.

If it is possible to sample a gas stream directly, or if a sample is to be drawn from a cylinder, the sample may be introduced via I (Figure 1). Tf direct sampling is not possible it is convenient to bring the sample to the apparatus in a 5-liter bulb and introduce it as shown in Figure 5. T paratus is inserted ahead of stopcock 1. This ap-Thus, the sample bulb is set in place, air evacuated via 17 and 18, then 17 and 18 turned to admit the sample to the circulating system. The gas is circulated via 17 and 18 to 1, bypassing P via 7 (Figure 1), until the water remaining in the gas and adsorbed until the water remaining in the gas and the point on the glass walls has been reduced to the point for ovvern is not affected. Stopthat an analysis for oxygen is not affected. Stop-cock 17 (Figure 5) is then closed, and by manipcock 17 (Figure 5) is then closed, and by manp-ulating the Toepler pump, a suitable portion of gas is left in the circulating system. Stopcock 18 is then closed, and the gas circulated via 19 to 1 for an oxygen determination. When this is completed, a second portion is taken for analysis, and so on.

## ANALYTICAL CHEMISTRY

Cold trap T (Figure 1) is a length of 8-mm. tubing folded as shown to allow snow carried forward from a to melt and condense in b. It is always evacuated from the outlet side (via stopcock 10) to avoid the loss of water vapor condensed on warmer upper parts of a. If a sample of condensate is to be taken for mass spectrometer analysis, a sample tube may be sealed on at C.

A convenient arrangement for automatically supplying liquid nitrogen to the cold trap Dewar, when circulating gas overnight, is shown in Figure 6. It consists of a methane-filled gas thermometer which acts as a switch for a solenoid air valve, and so controls the flow of compressed air to a 50-liter liquid nitrogen container from which the Dewar is fed. The cup around the methane-filled finger is a simple device for delaying the on-off action and so eliminating chatter. Liquid nitrogen does not contact the finger until it enters the cup at level a, and so the liquid nitrogen level fluctuates between a and b. Normal evaporation from the liquid nitrogen container, plus the air flow needed to force liquid nitrogen into the Dewar, pass through the normally open solenoid value and escape at c.

The catalyst is an 8-inch length of No. 28 gage 90/10 platinumrhodium wire on No. 18 gage platinum leads. Its temperature is derived from current-voltage measurements, taking the coef-ficient of resistivity as 0.0013. Since the current required to maintain a given temperature is about one tenth as much in a vacuum as in one atmosphere of hydrogen, it should be off when not in use, or when a large pressure change is to be made. The spiral is supported by a thin-walled quartz tube so that it will not touch and crack the glass wall. The water-jacketed construction cools the glass, which otherwise would become heated and desorb considerable amounts of gas. The cold trap is placed ahead of the catalyst for its protection from mercury vapor. It is wise to shield the catalyst tube from possible damage; it could be broken with hydrogen in the apparatus and lead to an explosion.

The water vapor gage, in which the pressure-volume measure-ment is made, is a series of graduated cylindrical volumes, cali-brated by mercury fillings. The larger has a volume of 550 ml. above the cutoff point, the smaller, a volume of 15 ml.

The mercury piston pump consists of two 250-ml. bulbs con-



Figure 1. General Arrangement of Apparatus



Figure 4. Mercury Piston Pump

nected by a 20-mm. tube through which mercury flows back and forth as the platform rocks. Each bulb is therefore a cylinder, and the mercury a piston; each has an inlet and outlet mercury valve, and the two pumps operate in parallel and  $180^{\circ}$  out of phase. The glass coils serve to connect the moving parts to the fixed inlet and outlet tubes. The pump was run at 4 cycles per minute through an angle of about 25°, displacing approximately 600 ml. per minute.

The apparatus in Figure 1, marked off by dotted line X, is for the introduction of known amounts of oxygen for calibration purposes. Volume A is filled with oxygen to the desired pressure and then turned into the main flow. If very small amounts are to be introduced, the amount in A can be further reduced by expansion into B.

By introducing known amounts of oxygen the recovery of water vapor can be measured. To obtain an analysis the volume of the circulating system must also be known; in this case, the volume was 1840 ml., measured by gas fillings.

**Procedure.** The amount of water vapor recovered from the gas is found to be the net result of condensation in the cold trap and the adsorption or desorption of water vapor from glass walls. The recovery depends, therefore, on the procedure which is followed. For example, it is important that the times allowed for circulation, evacuation, and distillation of water vapor be standardized. The room temperature should be held to within 1° or 2°. The glassware should not be heated nor the high

frequency vacuum tester applied. In particular it is essential that the catalyst tube have a cooling water jacket. The following procedure was adopted:

A. In the initial preparation of the apparatus for analysis it is necessary, after evacuation and filling with hydrogen, to circulate gas through the cold trap and over the heated platinum filament for several hours. This will remove adsorbed oxygen from the catalyst and clear the system of water vapor. It is

GLASS



Figure 5. Apparatus for Introducing Samples



Figure 6. Liquid Nitrogen Supply and Level Control

necessary that adsorbed water vapor in the catalyst chamber between stopcocks 2 and 3 (Figure 1) be reduced sufficiently that the determination is not affected. Pure hydrogen should remain in the bores of stopcocks 1 through 7 and a vacuum in 8 and 13.

and 13. B. The system is evacuated, a sample introduced as described, and the entire sample circulated, bypassing P via 7, to remove water vapor and other condensable gases. Because adsorbed water vapor in the system must be reduced to the point that an analysis is not affected, the time required will depend on the oxygen concentration to be measured. If the oxygen concentra-

		,	Table I.	Calibrat	ion Data,	5500	-Liter-	Micro	on Gage		
No.	Hydro- gen Batch	Day	Liter-Micr Added, as oxygen	ons of H <sub>2</sub> O Measured, as water	Resid- ual, Liter- Microns	No.	Hydro- gen Batch	Day	Liter-Micr Added, as oxygen	ons of H <sub>2</sub> O Measured, as water	Resid- ual, Liter- Microns
1	1	1	295 310 304 292	244 269 263 247	$\begin{array}{c} 0.53 \\ 0.35 \\ 0.28 \\ 0.31 \end{array}$	6		4	$10.8 \\ 11.0 \\ 11.0 \\ 11.0 \\ 11.0 \\ 10.8 $	2.9 0.98 0.79 0.89 0.83	$\begin{array}{c} 0.17 \\ 0.20 \\ 0.20 \\ 0.12 \\ 0.10 \end{array}$
2		2	59.5 59.3 59.7 59.7	$32.1 \\ 32.7 \\ 32.7 \\ 32.4$	$\begin{array}{c} 0.16 \\ 0.15 \\ 0.15 \\ 0.14 \end{array}$	7	4	5	10.8 187.8 189.6	0.52 134 148	0.09 0.41 0.34
3	2		30.4 30.7 30.4 30.6	$10.0 \\ 11.7 \\ 11.7 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.6 \\ 11.7 \\ 11.6 \\ 11.7 \\ 11.6 \\ 11.7 \\ 11.6 \\ 11.7 \\ 11.7 \\ 11.6 \\ 11.7 \\ $	$   \begin{array}{c}     0.25 \\     0.20 \\     0.22 \\     0.25   \end{array} $	8			189.9 190.1 99.9 100.2	$152 \\ 150 \\ 64.5 \\ 67.5 \\ 150 \\ 67.5 \\ 150 \\ 1$	0.26 0.33 0.19 0.18
4		3	1022 1010 1011	943 967 978	0.25 0.20 0.18	9	5	6	99.6. 99.1 13.2	67.6 67.9 3.5	0.16 0.14 0.60
5	3		1013 600 599 603	974 555 556 557	0.16 0.47 0.34 0.41				$13.5 \\ 13.6 \\ 13.9 \\ 13.6 \\ $	$2.7 \\ 2.4 \\ 2.3 \\ 2.3 \\ 2.3$	$0.25 \\ 0.21 \\ 0.28 \\ 0.25$
			602	564	0.32		6	7	13.8 13.7 13.7 13.7 13.7 13.7 13.7	2.7 2.5 3.0 2.8 2.5 2.6	$\begin{array}{c} 0.22 \\ 0.25 \\ 0.22 \\ 0.22 \\ 0.22 \\ 0.26 \\ 0.23 \end{array}$

tion exceeds about 25 p.p.m. (50 liter-microns of water vapor in a 1.3-liter sample), a drying time of 1 hour is usually sufficient. Below about 25 p.p.m. of oxygen, a longer drying time is needed, and as drying proceeds, the amount of water vapor recovered should be measured to determine when an analysis can be made. For oxygen concentrations of 1 to 2 p.p.m., this may require 50 to 75 hours. It is evident, therefore, that glassware must be clean, so that only the desorption of water is involved and not the slow removal of water from a trace of impurity such as alkali.

If, however, a gas stream is sampled directly, it is simple and convenient to draw the sample through a drying train and admit it to the previously dried circulating system. When this is done, an analysis for any oxygen concentration can be made immediately.

C. A suitable portion of gas is circulated over the catalyst, and the water vapor is condensed at liquid nitrogen temperature. In this work, the method was calibrated using liquid nitrogen only. If dry ice is used, the vapor pressure of water at  $-78^{\circ}$  C. gives the method a lower limit of perhaps 1 p.p.m. of oxygen. For amounts of water vapor in

For amounts of water vapor in excess of about 50 liter-microns, measured in the 5500-litermicron gage, the sample is circulated for 15 minutes. For smaller amounts of water vapor, measured in the 150liter-micron gage, circulation time is 30 minutes. D. Hydrogen is evacuated from the region bewaded by

D. Hydrogen is evacuated from the region bounded by stopcocks 1, 2, 7, 8, 10 (Figure 1), and 13 (Figure 2) for 2 minutes through stopcock 10.

E. In preparation for a measurement, the water vapor gages are evacuated as follows: Water from the previous measurement is evacuated from the 5500-liter-micron gage for 5 minutes, through stopcock 9 (Figure 1). The 150-litermicron gage is evacuated for 1 minute through stopcock 14 (Figure 2), then by means of stopcock 15, a measured amount of water vapor is admitted to the gage. This amount is about 75 litermicrons, obtained by allowing water in g to evaporate into the 3-ml. volume, h, at room temperature. After a moment the gage is evacuated again, for 5 minutes. F. A water bath at  $25^{\circ}$  C. is placed around trap T (Figure 1), and depending on the amount to be measured, the water is either evaporated through stopcock 8 (Figure 1) into the 5500-liter-micron gage, for 3 minutes, or distilled through stopcock 13 (Figure 2) into the 150-liter-micron gage, for 10 minutes, putting liquid nitrogen in the glass cup built into the gage for this purpose. The mercury is then raised above the cutoff and the gage allowed to warm to room temperature.

If the amount of water to be measured exceeds 5500 litermicrons, it can be measured in portions. A water bath on T(Figure 1) at 11° C. (pressure of the water is 10 mm.) will allow successive portions to be evaporated either into the gage or into bulbs of known volume at f (Figure 2). G. The mercury in the gage

G. The mercury in the gage is raised, and the amount of water vapor determined by several pressure-volume meas-

urements between 9- and 11-mm. pressure. The pressure is then increased further to condense water and allow a measurement of other gases which may be present.

Here the contribution of the previously dried sample is admitted and analyzed immediately. A delay of several hours will alter the contribution which the adsorption or desorption of water vapor on glass makes to the analysis, particularly when measuring small amounts. It is desirable to run one portion after another and obtain a result which represents steady-state conditions throughout.

### EXPERIMENTAL RESULTS

Calibration. The method was calibrated by adding known amounts of oxygen to a batch of dry oxygen-free hydrogen, making several measurements at each level to obtain a steady-state result. With each evacuation of the cold trap, the amount of hydrogen remaining is reduced. A series of calibration measure-

## Table II. Calibration Data, 150-Liter-Micron Gage

NT -	Hydro-	D	Liter-Micr Added, as	ons of H <sub>2</sub> O Measured,	Resid- ual, Liter-	Ν.	Hydro- gen	D	Liter-Mic Added, as	rons of H <sub>2</sub> O Measured,	Resid- ual, Liter-
No.	Batch	Day	oxygen	as water	Microns	No.	Batch	Day	oxygen	as water	witerons
1	1	1	$\mathbf{Blank}$	0.11	0.20				Blank	0.63	0.14
			$1.00 \\ 1.00 \\ 1.00$	$\begin{array}{c} 0.25 \\ 0.31 \\ 0.29 \end{array}$	$0.19 \\ 0.18 \\ 0.17$	6			$58.8 \\ 60.2 \\ 60.2$	$rac{46.8}{50.2}$	0.14 0.14 0.13
			1.00	0.35	0.17				60.2	50.5	0.15
			Blank	0.13	0.17				Blank	0.66	0.10
2			3.00 3.00 3.00 3.00	0.66 1.01 1.14 1.07	$\begin{array}{c} 0.18 \\ 0.18 \\ 0.17 \\ 0.15 \end{array}$	7			101.0 101.0 101.0 101.0	86.2 90.2 90.6 90.7	$\begin{array}{c} 0.12 \\ 0.13 \\ 0.11 \\ 0.14 \end{array}$
			Blank	0.30	0.13	9	3	3	Blank	0.80	0.09
3			$5.00 \\ 5.00 \\ 5.00 \\ 5.00 \\ 5.00 $	${}^{1.63}_{2.11}\\{}^{2.15}_{2.18}$	0.17 0.17 0.14 0.14	0	0	5	18.5 18.9 18.6 18.8	$     11.9 \\     12.1 \\     11.8 \\     11.2   $	$0.34 \\ 0.25 \\ 0.22 \\ 0.20$
	2	2	Blank Blank	$     \begin{array}{c}       0.34 \\       0.28     \end{array} $	$\begin{array}{c} 0.13 \\ 0.31 \end{array}$				Blank	0.36	0.17
4			10.12 9.92 9.81 9.95	Lost 4.89 5.21 4.98	$0.25 \\ 0.26 \\ 0.20$				18.9 18.7 18.9 18.9	$10.6 \\ 10.4 \\ 11.5 \\ 12.3$	$\begin{array}{c} 0.20 \\ 0.16 \\ 0.16 \\ 0.16 \\ 0.16 \end{array}$
			Blank	0.37	0.16				Blank	0.42	0.12
5			30.0 30.1 29.9 29.8	19.5     21.7     21.6     21.6     21.6	$\begin{array}{c} 0.23 \\ 0.16 \\ 0.15 \\ 0.15 \end{array}$						

ments would begin, therefore, in hydrogen at about 700-mm. pressure and end when the pressure had fallen to about 200 mm. A second batch of hydrogen would then be introduced and measurements continued.

Calibration data are assembled in Tables I and II, for the 5500- and 150-liter-micron gages, respectively, and summarized in Table III. The measurements were made over several days, and the water vapor recovered at various hydrogen pressures.

In Tables I and II the initial measurement at each level of calibration, for reasons discussed below, is less accurate. The column headed "Residual, Liter-Microns" gives the amount of condensable gas other than water vapor which deposits in the cold trap during a determination, and is measured as in Paragraph G of the procedure. In Table II, "Blank" denotes a measurement in which oxygen has not been added, but which is otherwise identical in all respects with a regular measurement.

	Та	ble II	I. Calil	bration	Summa	ry	
5500-Li Liter-	ter-Micros Microns o	n Gage, f H₂O	Re-	150-Lit Liter-	er-Micron Microns o	Gage, f H <sub>2</sub> O	Re-
Added	Meas- ured, p	Lost, v	covery, %	Added	Meas- ured, p	Lost, v	covery, %
$   \begin{array}{c}     10.9 \\     13.7 \\     30.6   \end{array} $	$0.8 \\ 2.6 \\ 11 7$	10.1 11.1 18.9	38	$1.00 \\ 3.00 \\ 5.00$	$0.32 \\ 1.07 \\ 2.15$	$0.68 \\ 1.93 \\ 2.85$	32 36
59.6 99.6	32.6 67.7	27.0 31.9	55	9.89 18.8	5.03 11.6 21.6	4.86	79
302 601	260 559 973	42 42 38		$\begin{array}{c} 23.3\\ 60.2\\ 101.0\end{array}$	50.5 90.5	$9.7 \\ 10.5$	84
	0.0	50					

The data summarized in Table III are plotted in Figures 7 and 8, as  $\log p vs. \log v$ , where p is the measured and v, the lost water vapor—i.e., the difference between the amount of water vapor introduced (as oxygen) and the amount measured. Since each set of data represents steady-state conditions, this loss is incurred in the water vapor gage, by absorption. The absorption loss associated with the amount of water vapor measured in an analysis is then taken from these curves.



Figure 7. Calibration Curve, 5500-Liter-Micron Gage

**Discussion.** From Tables I and II the precision of the measurements is seen to be very satisfactory. The condensation of water vapor, its handling, and its adsorption in the gage are evidently reproducible, even when the total amount is only 1 liter-micron, and the adsorption loss is 70%. It is conservative to give the error of the method as  $\pm 10\%$ , approaching  $\pm 2\%$  as the amount of water vapor involved rises to 40 liter-microns and beyond.

Because the amount of water vapor recovered and measured depends on the condition of the glassware and the establishment of steady-state conditions, a single measurement is not sufficient for an accurate analysis. Nevertheless the error is small, unless a sample low in oxygen follows one that is high. This mem-



Figure 8. Calibration Curve, 150-Liter-Micron Gage

ory effect is shown in Table IV, in which the first measurement of each set of data of Table I is treated as an unknown, using the calibration curve in Figure 7. To obtain greater accuracy at least three measurements should be made.

When measuring very small amounts of water vapor, it is also necessary that the blank be reduced to the levels indicated in the data of Table II. Thus, if the sample contains 3 liter-microns of water vapor (as oxygen) the blank must be reduced to 0.30; otherwise the results will be high, because at this water vapor level, a steady state will not be reached in two or three measurements. The lower the water vapor level, the lower the rate of drying, and the slower the approach to the steady state from a higher level. However, the steady state is rapidly reached from a lower water vapor level, so that the apparatus should always be dried beyond the requirements of the sample.

## Table V. Recovery vs. Evacuation Time, 5500-Liter-Micron Gage

A	dded, as	Measured, after Evacuating for		
-	oxygen	5 minutes	10 minutes	
	99.7	66.1		
	99.8	67.8		
	99.9	68.8		
	99.7	67.8		
	99.8		61.7	
	99.8		64.3	
	99.7		66.3	
	99.9		65.6	
	99.8		62.2	
	99.8	67.0		
verage	99.8	$67.5 \pm 0.8$	$64.0 \pm 1.7$	

In the measurement of a given amount of water vapor, the portion lost by adsorption depends on the amount of preadsorbed water in the gage; it increases as preadsorption is reduced. In the procedure using the 5500-liter-micron gage, this is determined simply by a 5-minute evacuation of the previous sample. The effect of reducing preadsorption is shown in Table V, which presents a series of measurements comparing 5- and 10-minute evacuation periods. To measure very small amounts of water, the adsorption loss must be reduced, not only by reducing the adsorptive surface, but also by preadsorbing a definite amount of water. Thus, the 150-liter-micron gage is prepared for measurements by filling with 75 liter-microns of water vapor and evacuating for 5 minutes. In this way preadsorption is held at a high level. Therefore, in a single measurement using the 5500-liter-micron gage there is memory in both the circulating system and the gage; with the 150-liter-micron gage, there is memory in the circulating system only.

The degree to which the adsorption loss has been reduced in the smaller gage is indicated in the recovery figures of Table III. The 30- and 60-liter-micron levels are compared, and the recovery at 3 liter-microns is seen to equal that at 30 liter-microns in the larger gage.

A factor affecting precision is the pressure at which the volume of water vapor is measured. Ten millimeters of mercury is large enough to be read by the unaided eye, yet not too near the saturation pressure of approximately 25 mm. The higher the relative pressure the greater the adsorption of water vapor. Thus, the pressure-volume measurement at 11-mm. pressure is about 3% lower than the measurement made at 9 mm.

The rapid attainment of the steady state in each calibration indicates that the conversion of oxygen to water vapor and its removal are substantially complete in the time allowed.

Table VI. Catalytic Formation of Water from Oxygenand Carbon Monoxide

Oxy	ygen,	Carbon Monoxide,		
100 to 1000	Liter-Microns	500 to 900 Liter-Micron		
Temp.,	Recovery,	Temp.,	Recovery	
°C.	%	°C.	%	
$\begin{array}{c} 40\\110\end{array}$	2	230	<1	
	9	270	<1	
$   \begin{array}{r}     160 \\     200   \end{array} $	40 73	330 400	$\frac{1.5}{2}$	
230	100	470	7	
270	100	530	9	

#### OXYGEN DETERMINATION IN THE PRESENCE OF IMPURITIES

Because the oxygen determination depends on the measurement of water vapor, it is necessary to remove any substances which might poison the catalytic formation of water, or which will condense with water and interfere in its measurement. This is not as severe a limitation as it might appear at first sight. In the preliminary treatment of the sample to remove water vapor, by condensation in a liquid nitrogen cold trap, there is also a complete removal of all potentially interfering substances except nitric oxide, carbon monoxide, and nitrogen. Thus, ammonia, carbon dioxide, nitrogen peroxide, hydrocarbons and compounds of nitrogen, sulfur, and halogens are eliminated. Ethylene, which would be hydrogenated to ethane and so condense with water vapor, has a vapor pressure of  $0.54 \times 10^{-3}$  mm. at 77.5° K. (21), or the equivalent of 0.4 p.p.m. of oxygen in a 1.3-liter sample. The only way in which these substances might interfere is through the loss of oxygen in the condensate. Although not investigated, this loss is believed to be very small, if measurable. If a gas stream is to be sampled directly, suitable purification can be provided for the continuous removal of water vapor and other impurities. The purified sample will contain, therefore, hydrogen and oxygen, and perhaps nitric oxide, carbon monoxide, and nitrogen.

The effect of carbon monoxide in the oxygen determination was investigated extensively. The conversion of carbon monoxide to water and methane, as a function of catalyst temperature, is given in Table VI, together with comparable data for oxygen. The conversion is seen to be negligible at 270° C., at which

#### Table VII. Determination of Oxygen in the Presence of Carbon Monoxide

		(Catalys	t temperat	ure, 260°	' to 270° C	.)	
Day	Hydrogen Pressure, Mm.	Liter-M of J Added as O <sub>2</sub>	dicrons H <sub>2</sub> O Present <sup>a</sup> as CO	$c \overset{\%}{\mathrm{O}^a}$	Measure H2O	ed, Liter- CO2	Microns Total
1	738 695 656 619 581	99.8 99.7 99.9 99.8 99.7	964 893 825 761 700	0.071 0.070 0.069 0.067 0.066	76.9 81.6 80.3 80.3 80.1	17.9 19.3 19.2 19.2 19.2 19.2	$\begin{array}{r} 94.8 \\ 100.9 \\ 99.5 \\ 99.5 \\ 99.3 \end{array}$
2	$546 \\ 517 \\ 488 \\ 459 \\ 434 \\ 410 \\ 386 \\ 365 \\ 344 \\ 324$	$\begin{array}{c} 317\\ 319\\ 326\\ 323\\ 326\\ 328\\ 328\\ 319\\ 322\\ 326\\ 326\\ \end{array}$	$\begin{array}{c} 643\\ 559\\ 477\\ 398\\ 325\\ 257\\ 198\\ 146\\ 103\\ 67\\ \end{array}$	$\begin{array}{c} 0.064\\ 0.059\\ 0.053\\ 0.047\\ 0.041\\ 0.034\\ 0.028\\ 0.022\\ 0.016\\ 0.011\\ \end{array}$	212 235 244 260 275 266 287 289 311 310	50.7 53.5 55.4 54.4 53.0 47.1 43.3 37.0 31.8 24.6	263 289 299 314 328 313 330 326 343 335
3	304 289 273 258 243 228	325 326 326 328 330 328	40 26 6	0.007 0.005 0.001 	268 336 321 348 336 323	$12.9 \\ 19.4 \\ 8.9 \\ 5.0 \\ 2.1 \\ 0.7$	281 355 330 353 338 324

 $^a$  In this series of measurements on a batch of purified hydrogen containing initially 964 liter-microns of carbon monoxide, the falling carbon monoxide partial pressure is calculated from the amount removed as carbon dioxide and the amount removed with each evacuation of the cold trap, 5.6%. Since hydrogen removal is mainly by evacuation the carbon monoxide concentration is falling.

temperature oxygen is completely recovered, suggesting that a selective catalysis is possible.

When both oxygen and carbon monoxide were present, although a temperature of 270° C. suppressed methane-water formation, carbon monoxide competed with hydrogen for the oxygen and the products were carbon dioxide and water, respectively. Table VII gives data for the recovery of oxygen in the presence of approximately a 15-fold concentration of carbon monoxide. The recovery, as water plus carbon dioxide, is quantitative. However, when these measurements were continued the following day, after overnight exposure of the catalyst to the carbon monoxidecontaining gas, recoveries were low and erratic, indicative of catalyst poisoning. The catalyst had also become poisoned for water-methane formation; a second temperature study showed a recovery of less than 1% (down from 7%) at 470° C.

It may be concluded, therefore, that it is possible to make an accurate determination of oxygen in the presence of carbon monoxide, except, perhaps, at very low oxygen concentrations. The carbon dioxide product is determined by increasing the pressure beyond the saturation pressure of water vapor and making a second pressure-volume measurement; the error arising from its solubility in water at room temperature is negligible. The slight loss in catalytic activity is quickly restored by heating to 670° C. in pure hydrogen.

Despite the low carbon monoxide concentration in the hydrogen, about 0.06%, the amount of carbon dioxide formed is relatively high. It is proportional to the first power of the oxygen partial pressure and independent of the falling partial pressure and decreasing concentration of carbon monoxide, except at low levels. It indicates not only strong and preferential adsorption of carbon monoxide over hydrogen but also that carbon monoxide is monopolizing only a certain area of the surface, amounting to about 10% (assigning two elementary sites to hydrogen and one to carbon monoxide), and suggesting an interesting catalytic investigation of surface heterogeneity.

The effect of nitric oxide in the oxygen determination was not investigated. However, it would presumably be readily converted to nitrogen and water vapor. If the sample received has attained equilibrium at room temperature in the reactions

$$2NO + O_2 \rightleftharpoons 2NO_2 \rightleftharpoons N_2O_4$$

then, after passage through the liquid nitrogen cold trap, it will not contain both nitric oxide and oxygen in measurable amounts. Therefore, a qualitative analysis for nitric oxide should supplement the oxygen determination in such cases.

Although nitrogen is potentially a source of ammonia in the oxygen determination, such formation was never observed. None was found after 15 minutes at  $670^{\circ}$  C. nor after 30 minutes, and longer, at  $350^{\circ}$  C.

#### MEASUREMENT OF CURRENT EFFICIENCY

An interesting application was the measurement of current efficiency in the electrolytic formation of oxygen at very low currents.

In a study of the poisoning of iron-synthetic ammonia catalyst by oxygen, small amounts of oxygen were added to the gas stream by electrolyzing a sulfuric acid solution between platinum wire electrodes, 1 cm. long, 0.7 cm. apart. It was necessary to measure the amount actually introduced, especially at very low currents where the current efficiency might not be 100%. Since the gas stream,  $N_2 + 3H_2$ , was purified before the addition of oxygen, and subsequently purified further over potassium hydroxide pellets, Ascarite, activated alumina, and phosphorus pentoxide, the stream could be sampled into the dry apparatus and analyzed immediately. This was confirmed by an examination of the purified gas itself, which showed that the equivalent of 0.1 p.p.m. of oxygen could be taken as an upper limit on the impurity content. The data are shown in Table VIII.

The accuracy of the method is demonstrated by the results for 30  $\mu$ a.; the same current efficiency is obtained at two concentration levels, 0.44 and 1.22 p.p.m. Further evidence of the reliability is indicated in the approximately constant recombination current of about 6  $\mu$ a., the assumption being that the difference from 100% current efficiency represents recombination of dissolved hydrogen and oxygen in the cell.

Table	VIII.	Current	Efficiency	in	Oxygen	Electrolysis
	Calculat	ted Oxygen A	Addition		Analysis.	Current.
Cur #	rent, a.	Gas flow, liters/hr.	P.p.m. O2		P.P.M. O <sub>2</sub>	Efficiency, %
10	0.1	3.8	0.56		$0.26 \\ 0.32$	59
10	).4	3.75	0.58		$0.41 \\ 0.28 \\ 0.30 \\ 0.35$	48
29	9.7	10.0	0.62		$0.23 \\ 0.43 \\ 0.44 \\ 0.44$	71
30	).5	3.9	1.64		$     \begin{array}{r}       0.46 \\       1.21 \\       1.27 \\       1.27 \\       \end{array} $	74
68	3	3.75	3.8		3.5	93
			Summary			
	Current, µa.		Current Efficiency, %		Reco	ombination Current, μa.
	10 30 68		53 73 93			5 8 5

### GENERAL DISCUSSION

Limitations of the Method. The adsorption of water vapor on glass and the collection of a small amount of unknown condensate in every measurement are two limitations which would appear to preclude its extension to significantly lower oxygen concentrations. As the oxygen concentration falls, the necessity for drying the circulating system to lower levels and the time for doing this increase. One method of reducing adsorption is indicated in the adsorption data of Razouk and Salem (29). They attribute the adsorption of water on glass to surface alkali and have shown that prolonged washing with pure water will reduce the adsorption to about one half that on unwashed glass. On the other hand, hot acid treatment for several hours followed by water washing may cause as much as a sevenfold increase in adsorption, due to the solution of surface alkali and creation of a roughened surface. The procedure indicated, therefore, is a mild detergent wash followed by prolonged washing with water. A second possibility for reducing adsorption is to coat the glass in some way for example, with a chlorosilane (11). Such treatment, if practical and effective, would not only reduce the drying time but also eliminate memory effects in the circulating system. There are also advantages to reducing adsorption in the water vapor gage, although at present adsorption in the circulating system is a greater limitation on the method.

It was never possible to free the circulating system of unknown vapors which would condense with water in the liquid nitrogen cold trap. The amount of this gas, about 0.2 liter-micron (Tables I and II), was too small for identification by the mass spectrometer; it would not be possible to sample it properly, because, if condensed and sealed off in a glass tube, there would be contamination by vapors driven from the glass during sealing. The amount found was not related to the oxygen introduced nor to the water collected, but did decrease as a given batch of hydrogen was repeatedly circulated and dried. Therefore, it was not due alone to impurities in the oxygen, nor to solution, nor trapping of hydrogen in the condensate. Presumably it was traces of hydrocarbons from the stopcock grease (Apiezon N) or traces in the original tank hydrogen absorbed by the grease and slowly released. Whatever its nature, it does impose a definite lower limit on the determination of oxygen.

This method is not alone in having limitations imposed by water vapor adsorption. Hydrogen reduction followed by the measurement of water vapor with an infrared analyzer or hygrometer is affected in the same way. Water vapor must also be eliminated in carbon reduction methods since it will be reduced, with oxygen, to carbon monoxide. In this respect chemical methods, responding to molecular oxygen only, have an advantage, but they suffer from the inconvenience of preparing and handling oxygen-sensitive reagents. This method uses simple apparatus and demands simple, though careful, manipulative skill. The sensitivity is high, and small samples can be taken.

**Applications.** This technique should be applicable to certain other analytical problems which are beyond the scope of ordinary gas analysis. Thus, through the reaction

$$H_2 + \frac{1}{2}O_2 \xrightarrow{F V} H_2O$$

small amounts of oxygen and hydrogen may be determined in each other, and in nitrogen, helium, and neon by adding excess hydrogen and oxygen, respectively. If the water vapor is condensed at the dry ice temperature, oxygen and hydrogen may also be determined in argon, krypton, carbon dioxide, and methane, although with lower sensitivity owing to the higher vapor pressure of water at this temperature. Analytical methods could also be developed on the basis of the platinum-catalyzed reaction

$$\rm CO + 1/_2O_2 \xrightarrow{Pt} \rm CO_2$$

and the condensation and measurement of carbon dioxide. Further extension is possible by including in the apparatus a nickel filament for catalyzing at  $300^{\circ}$  C. the reaction

$$CO + 3H_2 \xrightarrow{Ni} CH_4 + H_2O$$

Carbon monoxide and hydrogen could now be determined in each other and in nitrogen, inert gases, and methane. By combining the three reactions the analysis of oxygen-hydrogencarbon monoxide mixtures would be possible.

A very interesting application is to the direct determination of low concentrations of nitrogen in hydrogen and inert gases, based on the iron-catalyzed reaction

$$\begin{array}{c} & \mathrm{Fe} \\ \mathrm{N}_2 + 3\mathrm{H}_2 \xrightarrow{} 2\mathrm{NH}_3 \end{array}$$

The catalyst could in the form of an iron filament, or as granules of promoted iron-synthetic ammonia catalyst.

An obvious application is to the direct determination of moisture in solids. Dry gas would be circulated through the substance held at a definite temperature, and the water vapor condensed and measured. This approach has the advantages that small samples can be taken, provided the substance is uniform with respect to moisture content, and accurate weighings are unnecessary.

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## **Colorimetric Determination of Elemental Sulfur in Hydrocarbons**

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In the development of a rapid and accurate method for the determination of small quantities of elemental sulfur in hydrocarbons, it was found that, in acetone solution, elemental sulfur reacts rapidly and quantitatively with cyanide to give thiocyanate. Thiocyanate can be estimated colorimetrically by addition of an acetone solution of ferric chloride. The recommended procedure, based on these reactions, is sensitive to 2 p.p.m. of elemental sulfur. Peroxides, sulfides, disulfides, and mercaptans do not interfere even when present in fairly large excess. Accuracies of 98 to 99% are possible. The method is directly applicable to the analysis of petroleum fractions, and with suitable modification, it should be possible to analyze other material containing small amounts of elemental sulfur.

THE determination of small quantities of elemental sulfur dissolved in hydrocarbons or other organic solvents has been of considerable interest, and a variety of methods for this analysis has been proposed. The reactions of sulfur with metallic mercury (13, 15, 16) or metallic copper (2, 8) have been the basis of numerous procedures; however, Kiemstedt (11) reported that organic peroxides inhibit the reaction between copper and elemental sulfur leading to low results. Peroxides also react with metallic mercury giving dark precipitates which may interfere with methods based on the use of this metal. Uhrig and Levin (16) have suggested preliminary treatment of samples with aqueous ferrous sulfate to prevent such interference.

A modification of the doctor reaction, using butyl mercaptan (butanethiol) as a reagent, has been proposed by Wirth and Strong (17) for the quantitative analysis of free sulfur. However, Ball (3) has indicated that the method is susceptible to interference by peroxides and in addition gives poor results for low sulfur concentrations. Morris, Lacombe, and Lane (14) have also studied this procedure as well as one involving reactions of elemental sulfur with sulfite. They conclude that neither is suitable for analysis of sulfur concentrations below 100 p.p.m.

The polarographic reduction of sulfur appears to offer many advantages, and several papers have been published recently reporting details for such methods (9, 10). Diphenyl disulfide is the only compound so far encountered which interferes.

The reaction between sulfur and alkali cyanide has been proposed by several authors as a means for the analysis of macro quantities of elemental sulfur in various substances (1, 5, 6, 12).

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The thiocyanate formed in the reaction must be determined in the presence of an excess of cyanide; methods for accomplishing this have been proposed. Castiglioni (4) has suggested the use of the reaction followed by treatment with ferric chloride as a qualitative test for elemental sulfur, and it occurred to the authors that this might also be used as a basis for a sensitive method for the quantitative determination of the element. Investigation has shown that concentrations of sulfur as low as 2 p.p.m. in hydrocarbon solvents can be determined in such a way. Furthermore, it has been found that other sulfur-containing compounds and peroxides either do not interfere or can be readily prevented from interfering with an analysis by this method. Details of the proposed method and a discussion of the variables affecting the reactions involved are given.

## APPARATUS, REAGENTS, AND SOLUTIONS

Spectrophotometer. A Beckman Model DU spectrophotometer equipped with 1-cm. Corex cells was used for all light absorption measurements in this work.

Acetone Solvent. Fifty milliliters of water were diluted to 1 liter with technical grade acetone. Sodium Cyanide Solution. This solution was prepared by

Sodium Cyanide Solution. This solution was prepared by dissolving 0.1 gram of sodium cyanide in 100 ml. of the acetone solvent. When first prepared, the solution was cloudy but became clear upon standing for a few hours. Ferric Chloride Solution. This solution was prepared by dis-

Ferric Chloride Solution. This solution was prepared by dissolving 0.4 gram of ferric chloride hexahydrate in 100 ml. of the acetone solvent. Hydrolytic precipitation of part of the iron occurred shortly after preparation. After the precipitate had settled for 24 hours, the supernatant liquid was decanted into a dry bottle. The resulting solution was found to be stable for several weeks.

Mercuric Chloride Solution. Approximately 20 grams of mercuric chloride and 20 grams of potassium chloride were dissolved in 1 liter of distilled water.

Standard Sulfur Solution. A standard sulfur solution containing 50 p.p.m. sulfur on a weight per volume basis was prepared by dissolving 50 mg. of powdered roll sulfur in exactly 1 liter of petroleum ether. The solvent had a boiling range of 77° to 110° C. and a density of 0.73 at 25° C.

Miscellaneous Organic Compounds. The organic sulfides, disulfides, and mercaptans used in the work were obtained from Eastman Kodak Co. and used without further purification. The *tert*-butyl hydroperoxide was obtained from Union Bay State Co.; the *n*-amyl peroxide and nonyl hydroperoxide were furnished by Harry S. Mosher of Stanford University.

#### **RECOMMENDED PROCEDURE**

Analysis of Samples Containing No Mercaptans, Sulfides, or Disulfides. Place a 5-ml. sample of the solution in a 25-ml. volumetric flask. If the sample contains more than 50 p.p.m. elemental sulfur, make suitable dilutions with petroleum ether to give a sample which contains between 5 and 50 p.p.m. Add 15 ml. of the sodium cyanide solution, mix, and allow to stand for about 2 minutes. Make up to the mark with the acetone solvent, and withdraw a 5-ml. aliquot. Add exactly 5 ml. of the ferric chloride solution to the aliquot and mix. Determine the absorbance at 465 m $\mu$  against a blank which has been prepared by mixing 5 ml. of the acetone solvent with 5 ml. of the ferric chloride solution. Make absorbance readings within 10 minutes after preparation, or store the colored solutions in the dark if optical measurements are to be made at a later time. If the sample is colored, make a blank correction by carrying a 5-ml. aliquot through the procedure, with the exception that no cyanide should be added. Determine the concentration of elemental sulfur from a calibration curve which is prepared by treatment of a series of standard sulfur solutions in petroleum ether in the same way.

Analysis of Sample's Containing Sulfides, Disulfides, or Mercaptans. Take about 20 ml. of the sample and add 50 ml. of the aqueous mercuric chloride solution. Shake the mixture thoroughly. If the amount of precipitate formed is small, withdraw 5 ml. of the sample from the supernatant liquid and treat by the above procedure. If the quantity of precipitate is large, filter part of the supernatant liquid through a Büchner funnel with gentle vacuum, remove a 5-ml. sample from the filtrate, and treat by the above procedure.

#### EXPERIMENTAL

The reaction between elemental sulfur and an alkali cyanide has been shown to take place rapidly in various solvents (1, 4);

the mechanism of the reaction has been discussed in a recent publication (7). The first attempts to utilize this reaction for the quantitative analysis of small amounts of sulfur were carried out by refluxing samples dissolved in acetone over an excess of solid potassium cyanide. The acetone solution was then separated and evaporated to dryness. The resulting mixture of potassium cyanide and thiocyanate was taken up in water and the thiocyanate determined colorimetrically by addition of ferric Several objectionable features to such a procedure beion came apparent; the method was time-consuming, not as sensitive as desired, and inapplicable to samples containing high molecular weight, nonvolatile hydrocarbons. Furthermore, the results obtained were not entirely reproducible and appeared to depend upon the quantity of potassium cyanide dissolved in the solution during refluxing. This in turn depended upon the type and quantity of sample taken for analysis. For these reasons, attempts were made to carry out both the conversion of sulfur to thiocyanate and the color reaction in a single nonaqueous solvent.

Choice of Solvent. Several experiments were carried out with anhydrous acetone as the solvent, and a considerable increase in the sensitivity of the procedure was noted. This was expected on the basis of the work of Woods and Mellon (18) which has shown that the color intensity of the ferric thiocyanate complex increases as increasing amounts of acetone are added to an aqueous solution of the complex. However, it was also demonstrated that the presence of very small quantities of water caused a noticeable decrease in the intensity of the color, and if reproducible results were to be obtained, considerable care would be required to maintain anhydrous conditions throughout the analysis. Furthermore, neither sodium nor potassium cyanide is very soluble in anhydrous acetone, and solutions of ferric ion in such a solvent are rapidly decomposed giving a dark brown solution. All of these difficulties could be overcome by use of an acetone solution containing a small amount of water, and throughout the remainder of the work a solvent was used consisting of 5% of water in acetone by volume. In such a solvent small variations in water content do not appreciably affect the color intensity of the ferric thiocyanate complex. This is illustrated in Table I.

 
 Table I. Effect of Variation of Water Content on Light Absorption of Ferric-Thiocyanate Complex

Water Content of Solution, % by Volume	Absorbance at 465 m $\mu$
5.0	1.04
5.4	1.06
5.8	1.04
6.2	1.04
6.6	1.04
7.0	1.04

Iron Reagent. Solutions containing various concentrations of ferric chloride hexahydrate were prepared in 95% acetone. With all of these, rapid solution of the salt occurred followed by a slow, hydrolytic precipitation of part of the iron. After 24 hours, the precipitate could be separated by decantation or filtration to give solutions which were stable for as long as 2 months with respect to further precipitation and behavior with thiocyanate solutions. The amount of precipitate formed by the hydrolysis increased as increasing quantities of ferric chloride were taken; however, the solutions made up with larger amounts of ferric chloride also contained more iron as indicated by their color. These solutions were used to determine the optimum iron concentration for color development by addition of measured volumes of each to the acetone solvent containing measured quantities of potassium thiocyanate. The results of these experiments are shown in Table II. The decreasing light absorption at higher iron concentrations presumably results

Table II. Effect of Iron Concentration on Color Development				
FeCl <sub>3</sub> .6H <sub>2</sub> O per 100 ML Iron Beagent	Absorbance			
Grams	(465 mµ)			
0.20	0.910			
0.28	0.970			
0.40	0.973			
0.48	0.975			
0.60	0.970			
2.00	0.950			
4.00	0.930			

from an increase in acidity which is associated with the more extensive hydrolysis in the iron solutions of higher concentration. For the subsequent work, the iron reagent was prepared by dissolving about 0.4 gram of ferric chloride hexahydrate in 100 ml. of the 95% acetone. Analysis of this solution, after precipitation was complete, showed that it contained approximately 50 mg. of iron per 100 ml. of solution, which indicated that almost half of the iron originally present had precipitated.

Table 1	III.	Effect	of Th	Cyanide liocyanate	Concentration Color	on	Ferric
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Mg.	Source of S	Absorbance
0	KSCN	0.900
3.0	KSCN	0.915
3.0	S	0.910
6.0	S	0.935
9.0	s	0.955
12.0	S	0.960
15.0	KSCN	0.980
15.0	s	0.975
18.0	S	0.985
20.0	s	1.00

Effect of Cyanide Concentration. The effect of cyanide concentration upon the completeness of conversion of sulfur to thiocyanate and upon the reaction of thiocyanate with ferric ions was studied. A series of solutions containing identical quantities of sulfur as elemental sulfur and as potassium thiocyanate were treated with various volumes of a standard sodium cyanide solution. This was followed by color development with the standard iron solution. The results of these experiments are shown in Table III.

At the same cyanide concentrations, the data for solutions prepared from elemental sulfur and from potassium thiocyanate agree within the experimental error of the measurements. This indicates that quantitative conversion of the sulfur to thiocyanate is rapidly achieved even with the smallest quantity of evanide used. However, an increase in cyanide concentration results in greater light absorption with both types of samples. In all probability this results from the decrease in acidity of the solution as the cyanide concentration becomes larger. Such a change in pH undoubtedly has a considerable effect on the equilibria involved in the complex formation. Concentrations of sodium cyanide greater than 20 mg. per 25 ml. were found to cause precipitation of iron. To avoid this and yet work at near the maximum sensitivity of the method, a sodium cyanide concentration of 15 mg, per 25 ml, was chosen for conversion of sulfur to thiocyanate. It is seen from Table III that great care is not required in the preparation and addition of the cyanide reagent.

Stability of Color. A study of the stability of the ferric thiocyanate color in acetone solution revealed that a slow decrease in the color occurred when the solutions were exposed to daylight. For example, a solution which was allowed to stand for 3 hours in ordinary daylight was found to have decreased in absorbance by about 20%. Prolonged exposure to light resulted in complete loss of the red color. Solutions kept in the dark for 3 hours were found to be completely stable and gave identical values for absorbance with solutions examined immediately after preparation. The results of these studies are shown in *Table* IV.

**Calibration Curve.** A plot of absorbance against concentration of elemental sulfur was found to give a straight line passing through the origin. Beer's law was followed over a concentration range of 0 to 50 p.p.m. of elemental sulfur.

Interferences. The effect of the presence of a number of different organic compounds on the proposed analysis was investigated. The results of these tests are shown in Table V. In each case 5 ml: of a petroleum ether solution containing 0.25 mg. of sulfur and a 200-fold excess of the compound under study was used.

Ethyl, n-butyl, and benzyl mercaptan caused a marked decrease in the amount of sulfur found by the procedure. This was to be expected, inasmuch as the reaction between mercaptans and elemental sulfur is known to take place rapidly in basic solutions. Undoubtedly most of the sulfur present in these samples was converted to a disulfide in the basic cyanide solutions. It was possible to eliminate completely this interference by shaking the sample with an aqueous solution of mercuric chloride or silver nitrate prior to the addition of cyanide. The sulfur analysis was then run on an aliquot from the petroleum ether layer. With large amounts of mercaptans, filtration of the mercaptides through a Büchner funnel was necessary. Table V shows that excellent recoveries of elemental sulfur in the presence of the mercaptans studied were attained when this treatment was used.

# Table IV.Stability of Ferric Thiocyanate Color in95%Acetone

Time after Preparation, Min.	Type of Storage	Absorbance
1 25 90 180 24 hr. 1 5 15 20 30 40 60 120 170	Dark Dark Dark Dark Daylight Daylight Daylight Daylight Daylight Daylight Daylight Daylight Daylight Daylight	$\begin{array}{c} 1.10\\ 1.10\\ 1.11\\ 1.11\\ 1.10\\ 0.416\\ 1.10\\ 1.10\\ 1.10\\ 1.10\\ 1.08\\ 1.08\\ 1.07\\ 1.03\\ 0.960\\ 0.890\\ \end{array}$

The effect of four organic sulfides on the analytical procedure was investigated. With methyl, ethyl, and benzyl sulfides, essentially complete recoveries of the added sulfur were obtained. However, low results were encountered when *n*-butyl sulfide was present. Treatment of the sample containing this compound with aqueous mercuric chloride prior to the analysis removed the interference and resulted in complete recovery of the added elemental sulfur. The mercuric chloride may have removed the sulfide as a complex, thus preventing its interference; however, it seems more probable that the low recoveries in the untreated samples occurred as a result of mercaptan impurities in the *n*butyl sulfide. This was not investigated further.

Analysis of samples containing large amounts of methyl, ethyl, and phenyl disulfides gave recoveries which were low by 1 to 3%. However, theoretical results were obtained when these were treated with mercuric chloride prior to analysis. Here again, the low results may have been caused by mercaptan impurities.

The effect of hydrogen sulfide on the analysis was also investigated. A quantity of petroleum ether was saturated with the gas and then diluted fourfold with more of the solvent. Sufficient elemental sulfur was added so that 5-ml aliquots contained 0.25 mg. of the element. These were analyzed with and without

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treatment with aqueous mercuric chloride. The untreated samples gave erratic results. The low results probably arose from a decrease in the ferric ion concentration as a result of reduction by the hydrogen sulfide. In any case, the interference was readily prevented by removal of the hydrogen sulfide as mercuric sulfide. Excellent recoveries were then obtained, as illustrated in Table V.

The effect of peroxides on the proposed procedure was investigated, because this type of compound has been reported to interfere with many of the methods for determining free sulfur. Analysis of samples containing excesses of tert-butyl hydroperoxide, n-nonyl hydroperoxide, and n-amyl peroxide indicated that theoretical recoveries of sulfur could be expected when peroxides are present. As further evidence for this, a sample of known sulfur concentration was prepared in white gasoline which had been air oxidized for several hours in sunlight. Sufficient peroxides were formed by this treatment to liberate appreciable quantities of iodine from iodide. Analysis of this sample gave a complete recovery of sulfur and no peroxide interference.

Samples containing thiophane, thiophene, and large quantities of toluene and amylene were also analyzed, and these compounds were found to interfere in no way with the analysis.

### **RESULTS AND DISCUSSION**

A series of samples containing known quantities of elemental sulfur and a variety of other compounds was prepared and analyzed by the proposed procedure as a test of the method. The results of these analyses are shown in Table VI.

The data indicate that accurate analysis for elemental sulfur can be made over a concentration range of 5 to 50 p.p.m. Obviously, more concentrated samples can be analyzed by suitable

Table V.	Effect of Various Results for Eler	Compounds on nental Sulfur	Analytical
			-

		Quantity of S	Found, Mg.	•
Compound Added	Quantity Added, Mg.	Sample not treated with HgCl <sub>2</sub>	Sample treated with HgCl <sub>2</sub>	Quantity of S Taken, Mg.
None		0.250	0.250	0.250
Mercaptans Ethyl	50		0.250	0.250
n-Butyl	50	0.005 <sup>b</sup>	$0.250^{\circ}$ 0.252 $0.253^{\circ}$	0.250
Benzyl	50	0.075 <sup>c</sup>	$0.250 \\ 0.250^{a}$	0.250
Sulfides Dimethyl	50	0.248		0.250
Diethyl Di-n-butyl	50 50	0.250 0.202	0.249	$\begin{array}{c} 0.250 \\ 0.250 \end{array}$
Dibenzyl	50	0.248	0.250*	0.250
Disulfides Dimethyl Diethyl Diphenyl	50 50 50	$\begin{array}{c} 0.241 \\ 0.215 \\ 0.244 \\ 0.248 \end{array}$	$\begin{array}{c} 0.248 \\ 0.250 \\ 0.248 \\ 0.254 \end{array}$	$\begin{array}{c} 0.250 \\ 0.250 \\ 0.250 \\ 0.250 \\ 0.250 \end{array}$
Hydrogen sulfide	đ	0.254 0:252 0.110	0.250 0.250	$\begin{array}{c} 0.250 \\ 0.250 \\ 0.250 \end{array}$
Peroxides tert-Butyl hy- droperoxide	50	0.250	0.251	0.250
n-Amyl per- oxide	50	0.250		0.250
peroxide	50	0.250		0.250
Thiophane Thiophene Amylene Toluene	100 20 e f	$\begin{array}{c} 0.252 \\ 0.250 \\ 0.127 \\ 0.125 \end{array}$	$\begin{array}{c} 0.250 \\ 0.250 \\ 0.127 \\ 0.125 \end{array}$	$\begin{array}{c} 0.250 \\ 0.250 \\ 0.125 \\ 0.125 \end{array}$

<sup>a</sup> Sample treated with aqueous silver nitrate rather than mercuric chloride solution. b n-Butyl mercaptan (25 mg.) used in untreated sample.
<sup>6</sup> Benzyl mercaptan (12.5 mg.) used in untreated sample.
<sup>d</sup> Samples were approximately one quarter saturated with H<sub>2</sub>S.
<sup>e</sup> Sample contained approximately 50% anylene.
/ Sample contained approximately 50% toluene.

dilution with petroleum ether. As low as 2 p.p.m. of sulfur can be determined, although the relative error at this level of concentration would be greater than at higher concentrations. Some attempts were made to increase the sensitivity of the method further by using smaller volumes of solutions and more concentrated reagents. However, precipitation of iron was encountered under these conditions.

#### Table VI. Analysis of Samples Containing Known **Concentrations of Elemental Sulfur**

	Other Compounds	Sulfur Content, P.P.M.			
Solvent	Present	Found	Present		
Petroleum ether Petroleum ether Petroleum ether Petroleum ether Petroleum ether	···· ··· ···	5.2 10.2 15.4 20.2 50.0	5.0 10.0 15.0 20.0 50.0		
Benzene White gasoline White gasoline (oxidized)	· • • • • • •	$24.9 \\ 50.3 \\ 49.7$	$25.0 \\ 50.0 \\ 50.0 \\ 0$		
Petroleum ether	n-Butyl mercaptan	5.0	5.0		
	n-Butyl mercaptan Ethyl disulfide	9.9	10.0		
	n-Butyl sulfide Ethyl disulfide	15.4	15.0		
	n-Butyl mercaptan H <sub>2</sub> S	20.2	20.0		
	n-Butyl sulfide n-Butyl mercaptan Ethyl disulfide	49.7	50. <b>0</b>		

The method is very simple and is free from interference by compounds commonly occurring in petroleum fractions and appears to offer many advantages for the analysis of elemental sulfur in samples of this type. It seems probable that, with suitable modification, it could be applied to the analysis of other materials containing elemental sulfur.

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## Microscopic Identification of Wustite—Correction

In the captions of the figures of the article on "Microscopic Identification of Wustite" [Wells, R. G., ANAL. CHEM., 26, 715 (1954)] the last sentence should read: Original magnification  $\times 125$ , reduced 1/4 in reproduction.

## **Evaluation of Test Procedures**

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To obtain an estimate of reliability of a test method from an exchange of samples between laboratories, the data are first combed for wild results. Then a preliminary review is made to determine whether or not the reliability of the test depends on the magnitude of the variable being studied. If so, a separate estimate of reliability is made for each magnitude; if not, all the data are pooled to obtain a single estimate of reliability for the whole range of magnitudes studied. The four statistical tools used are: analysis of variance, the F test for comparing variances, Dixon's Q test for rejecting off laboratories, and Bartlett's test for homogeneity of variances. The statistical evaluation is only approximate. It gives a working estimate of the reliability of the test method, and it indicates which laboratories, if any, are not qualified to run the test without improvements in their technique. The statistical evaluation outlined has proved useful in laboratories of the Standard Oil Co. (N. J.).

RESEARCH work often depends on tests. In manufacturing, the processes are controlled, and the quality of the product is determined by testing. Both the research worker and the manufacturer want to know the reliability of the test method upon which they are depending. An approximate idea of this is usually obtained by experience. However, cases arise in which a direct determination is desirable. For example, when a new test method is being tried, or when tests are being run in several laboratories, questions arise such as: Is the new method as good as the old one? Do all laboratories give the same answer? Is the reliability of the test dependent on the magnitude of the variable being measured?

Round-Robin Tests. These questions can be answered by running a round robin of tests. Several samples are run several times each in each of several laboratories. The data are then examined to get an estimate of the reliability of the test. The results will show how the test compares with former tests, how well laboratories check one another, and how the reliability is affected by magnitude.

The results from such an exchange are not always easy to analyze. Frequently, one or two laboratories will differ radically from the others, or one or two single wild determinations will crop up, or the data may show that there is a highly significant tendency for some laboratories to do better than average on certain samples and worse than average on others. It is difficult to account for these anomalies. They make the job of estimating the reliability harder.

No matter how perverse the results, however, it is desirable to get some sort of working estimate of the reliability of the test from them. It is also desirable to learn whether the reliability depends on the magnitude of the variable being tested, and whether some laboratories need help in improving their technique. It is the purpose of this paper to suggest a method of determining these things from the results of an exchange of samples. Work by Others. Considerable work has been done on the evaluation of tests. Peakes  $(\beta)$  has shown how to decide whether a test method will distinguish between samples by using the F test. ASTM Committee E-11 on Statistical Quality Control of Materials is working on the evaluation of test procedures. The committee has made  $(\theta)$  some preliminary suggestions based on the use of control charts. However, it did not suggest any method of handling wild results in the work. Moreover, the ASTM method does not tell how to handle the situation where the reliability of the test changes with the magnitude of the result. It has been found that both of these problems crop up in practical work.

Wernimont  $(\theta, 10)$  has done considerable work on this problem. He showed (10) how to obtain separate estimates of the variability in test results due to differences between laboratories, differences between analysts in the same laboratory, day-to-day differences when the tests are run by the same analyst, and differences in replicate results on the same day by the same analyst in the same laboratory. In the method suggested here, a separate estimate of the variability due to the last two effects was not obtained. From the practical standpoint, it seemed sufficient to get an estimate of the laboratory effect and the combined effect of replicates, days, and analysts. Mitchell ( $\delta$ ) has recommended the use of control chart techniques for the control of accuracy and precision in industrial tests.



Figure 1. Evaluation of a Test Procedure

It is felt by the authors that the cost of statistical analysis is small compared to the cost involved in evaluating a test procedure. It, therefore, seems desirable to use the most efficient statistical methods available in analyzing the results. The analysis of variance technique is more efficient than the control chart techniques. High efficiency in utilizing the data is, of course, most important when the round-robin program is perforce small. In industrial work, it is sometimes desirable to get a rough estimate of the reliability of a test by running only three samples, three times each, in three laboratories. In cases such as this the authors feel that the analysis of variance will give a better estimate of reliability than control charts. When a large number of tests can be run, the control chart technique looks promising. It would seem desirable to incorporate into this technique some means of taking into account the effect of magnitude on reliability and some systematic means of combing the data for wild results.

The simplest and safest estimate of the reliability is, of course, one which is based on all of the data from the test work. However, when one or two laboratories are far out of line, or some obviously wild results are reported, this estimate of the reliability is high. It is not acceptable to the experienced tester. Although the estimate is a safe one, it is not a useful one. It is preferable, from the standpoint of the user, to reject the wild results and base his estimate of the reliability on data from laboratories which are in control. An effort is then made to bring the errant laboratories into line. In this way the round robin of test work serves two purposes: First, it gives a usable estimate of the reliability of the test method, and second, it indicates which laboratories cannot meet this reliability level without improving their technique.

#### **OUTLINE OF METHOD**

The method is not exact. It is an approximate method designed to come up with a working estimate of the reliability of a test procedure. It is offered as a step toward the development of a uniform, statistical evaluation of analytical procedures.

**Description of Terms.** The reliability (as used here) is measured in terms of the repeatability and the reproducibility.

The repeatability is a measure of the ability of the test method to check itself when it is run in one laboratory. It takes into account the effect of differences in analysts and days. The repeatability is a standard deviation. On the average, two out of three results obtained on a sample in a particular laboratory will not differ from the laboratory average for that sample by more than the repeatability.

The reproducibility is a measure of the ability of a test method to check itself when it is run in different laboratories. It takes into account all of the factors affecting the repeatability and, in addition, includes the effect of differences between laboratories. It is also a standard deviation. Two out of three results obtained in any laboratory will not differ from the over-all average (all laboratories) by more than the reproducibility.

The repeatability would be a useful estimate of test reliability, where one laboratory is being used to control a manufacturing process. The reproducibility is a more generally useful estimate of reliability which would be useful, for example, in discussing the quality of competitive products. The competitive products would very likely be tested by several different laboratories.

Statistical Tools Used. The statistical evaluation requires a knowledge of the following:

- 1. The analysis of variance technique (11).
- 2. The use of the F test for comparing variances (1).
- 3. The use of Dixon's Q test for comparing averages (2, 3).
- 4. Bartlett's test for homogeneity of variances (8).

Details on the use of these four tools can be found in the references shown and in many other textbooks on statistics. The laboratory worker will also need a desk calculating machine to speed his analysis.

Steps in the Method. The evaluation is carried out in sequence as shown in Figure 1.

1. A round robin of test work is done in several laboratories.

2. The raw data are combed to eliminate wild results.

3. A preliminary examination is made to find whether the repeatability of the method depends on the magnitude of the variable being tested.

4. The data are analyzed to obtain the repeatability and the reproducibility of the test.

When it is desirable, the second step can be deleted without interfering with the rest of the analysis. However, it has been found, particularly with newly developed tests, that some laboratories are out of control. Unless these wild data are rejected, the reliability figures obtained will not be realistic.

#### GENERAL PLAN

First, the basic data must be obtained by an exchange of samples between laboratories. Even for a rough estimate, at least three samples should be circulated to at least three laboratories. Each sample should be run three times by each laboratory. The three runs on each sample should be made on different days and by different operators, if more than one operator will be running the tests in the normal course of events. The more samples and laboratories involved, the better will be the estimate of the reliability. The results are then tabulated, and all of the totals and averages determined.

The data should then be combed to eliminate wild results.

First, look at the results obtained on each sample separately. Determine the variance within laboratories (this is the first estimate of repeatability), and look over the results to see if any single determinations differ from the laboratory sample (results obtained in one laboratory on one sample) average by more than three times the estimated repeatability. If a wild result is found, reject it, and simply substitute an averaged value. Single wild determinations are thus rejected at about the 99% confidence level. No correction is made in the degrees of freedom, since the method is only approximate. This will simplify the subsequent calculations without introducing a large error in the result. This averaged value is used as if it were a real determination in the subsequent work.

Now look over the laboratories to find if any are out of control. Compare the average result obtained by each laboratory on each sample using Dixon's Q test. If a laboratory is far out of line, all results from this laboratory should be rejected. This procedure will identify the laboratories which need help with the test procedure. The laboratories may then be compared on the basis of the spread of their results, using Bartlett's test for homogeneity of variances. If the within laboratory variance in one laboratory is much greater (99% confidence) than that obtained in the other laboratories, it should be rejected. Some improvement in technique is required before this particular laboratory is qualified to run the test.

Subsequent handling of the data hinges upon whether the repeatability of the test depends on the magnitude of the variable being measured. In some tests, the repeatability is poorer on large values than it is on smaller values, and vice versa. For example, the repeatability of the macro-Kjeldahl nitrogen determinations is poorer for high concentrations of nitrogen than it is for low concentrations. If the repeatability depends on the magnitude of the variable being measured, the data for each sample are handled separately. A separate estimate of the reliability is obtained for each sample. If enough samples have been run it may be desirable to plot the reliability estimates against the magnitude. This plot will then serve to give an estimate of the reliability for any level within the range covered by the samples.

Where the repeatability of the method is not dependent on the magnitude of the variable being tested, all of the data are handled together. A complete analysis of variance makes it possible to determine whether there is a significant laboratory by sample interaction. If the bias for a laboratory varies from one sample to another, then an "interaction" exists between laboratory and samples (12). Where such an interaction exists, an extra component must be added to the estimate of the reproducibility.

Frequently it is found that the spread between laboratories is significantly greater than that within laboratories. When this occurs, a separate component must also be added to the estimate of the reproducibility. In the ideal case the reproducibility is identical with repeatability. However, it is more common in preliminary work to find that the reproducibility is greater than the repeatability because of components contributed by differences between laboratories and by significant interaction effects. As the test is perfected, these extra components may diminish or disappear.

The best way to demonstrate the statistical evaluation of a test method is to work through an actual example. Two examples are shown here. The first represents the first round-robin results on a new test. Some of the laboratories are not in control, and a lot of data have been rejected. The second example illustrates the evaluation of a well-developed test procedure. All of the laboratories are in control, and no data are rejected.

## DETERMINATION OF CARBON ON A CATALYST

Recently it was desired to obtain an estimate of the reliability of a new method for determining the carbon on a catalyst. When the catalyst is regenerated in the normal manner, most of the carbon deposited thereon will be removed. Unfortunately, this removal is not always complete; carbon which has been entrapped within the catalyst will not be removed, even in highly efficient regenerators. A laboratory procedure was developed to determine the burnable carbon remaining on the catalyst. A portion of the catalyst sample is weighed into a combustion boat and put into a furnace under a stream of oxygen at a temperature of 1000° F. The temperature is gradually raised to  $1600^\circ$  F., and the evolved carbon dioxide is collected and weighed in an Ascarite absorber. The per cent weight of carbon collected in the absorber is reported as burnable carbon.

In order to determine the reliability of this new test, a round robin of tests was run, and the data obtained are shown in Table I.

The round-robin exchange of samples in this case consisted of sending four catalyst samples to eleven laboratories and asking each laboratory to run each sample three times. The totals and averages were determined, and the data made ready for statistical analysis.

## COMB FOR WILD RESULTS

Look at Each Sample. By examining each sample separately, an estimate of the repeatability (or replicate variance) within laboratories is made. We can then decide whether individual results in any one laboratory sample are wild. In other words, do they differ from their laboratory-sample average by more than would be expected on the basis of normal variations? The results of an analysis of variance for each sample are shown in Table II. Any results which differ from their laboratory-sample average by more than three times the repeatability, 3So, (symbols are defined in Table III) should be rejected. An inspection of the results shows that the second results obtained by Laboratory H on Sample 4 (0.39) differ from the laboratory-sample average (0.28) by more than  $3S_o$  (for this sample  $S_o = 0.035$ , so that  $3S_o = 0.105$ ). The average of the other two results (0.23) is substituted for 0.39 and appropriate changes made in the totals, and averages. Recalculating the corrected data on sample 4 shows that the sample now has a repeatability,  $S_o$ , of 0.021. There are no other wild results.

Look at Each Laboratory. The results obtained by each laboratory on each sample may now be examined to determine what laboratories, if any, are out of control. The laboratories are compared both on the basis of their average result and the spread in their results. Laboratories which are a long way off, in either respect, should be rejected. The averages can be compared using Dixon's Q test, and the spreads, using Bartlett's test for homogeneity of variances.

COMPARE AVERAGES. The averages obtained by each laboratory on each sample have been compared using Dixon's Q test (3) at the 99% confidence level. The Q ratios are shown in Table IV. The low result on sample 3 is out of line. A reexamination shows that no other laboratories should be rejected, although Laboratory H is near the rejection limit (after rejecting Laboratory G). A Q test on the over-all laboratory averages shows that no other laboratories are sufficiently high or low in over-all average to be rejected.

COMPARE SPREADS. The remaining laboratories should now be examined to determine whether some show a significantly greater spread in results than others. An analysis of variance run on the data from each laboratory is shown in Table V. The  $\chi^2$  value as determined for Bartlett's test (8) is 33.73, which

## Table I. Results Obtained on Burnable Carbon

			Samp	le No.			Labora-
Labora- tory		C	2 arbon in	3 Catalyst,	∞ <sup>4</sup>	Total	tory Average
Α	Total Av.	$     \begin{array}{r}       1.66 \\       1.68 \\       1.66 \\       \overline{5.00} \\       1.67 \\       \hline       1.67 \\       \end{array} $	$0.76 \\ 0.77 \\ 0.75 \\ \hline 2.28 \\ 0.76 \\ \hline $	0.820.810.812.440.81	$0.21 \\ 0.25 \\ 0.24 \\ \hline 0.70 \\ 0.23$	10.42	0.868
В	Total Av.	$1.60 \\ 1.58 \\ 1.57 \\ 4.75 \\ 1.58 \\ 1.58$	$0.78 \\ 0.75 \\ 0.71 \\ 2.24 \\ 0.75$	$0.83 \\ 0.83 \\ 0.75 \\ 2.41 \\ 0.80$	$\begin{array}{c} 0.24 \\ 0.22 \\ 0.24 \\ \hline 0.70 \\ 0.23 \end{array}$	10.10	0.842
С	Total Av.	$     \begin{array}{r}       1.74 \\       1.69 \\       1.65 \\       \overline{5.08} \\       1.69 \\       1.69 \\       \end{array} $	$0.75 \\ 0.76 \\ 0.81 \\ \hline 2.32 \\ 0.77 \\ 0.77 \\ 0.77 \\ 0.75 \\ 0.77 \\ 0.75 $	$\begin{array}{r} 0.81 \\ 0.80 \\ \underline{0.79} \\ 2.40 \\ 0.80 \end{array}$	$\begin{array}{c} 0.21 \\ 0.23 \\ 0.22 \\ \hline 0.66 \\ 0.22 \end{array}$	10.46	0.872
D	Total Av.	$   \begin{array}{r}     1.70 \\     1.69 \\     1.70 \\     \overline{} \\     5.09 \\     1.70 \\   \end{array} $	$0.76 \\ 0.81 \\ 0.78 \\ 2.35 \\ 0.78 \\ 0.78 \\ 0.78 \\ 0.8$	$\begin{array}{r} 0.84 \\ 0.86 \\ 0.79 \\ \hline 2.49 \\ 0.83 \end{array}$	$0.26 \\ 0.23 \\ 0.23 \\ \overline{0.72} \\ 0.24$	10.65	0.888
Е	Total Av.	$     \begin{array}{r}       1.57 \\       1.53 \\       1.58 \\       \overline{4.68} \\       1.56 \\       \end{array} $	0.70 0.67 0.70 2.07 0.69	$\begin{array}{r} 0.78 \\ 0.74 \\ 0.80 \\ \hline 2.32 \\ 0.77 \end{array}$	$\begin{array}{c} 0.20 \\ 0.20 \\ 0.22 \\ \hline 0.62 \\ 0.21 \end{array}$	9.69	0.808
F	Total Av.	$   \begin{array}{r}     1.55 \\     1.52 \\     1.50 \\     \overline{4.57} \\     1.52   \end{array} $	$0.60 \\ 0.67 \\ 0.64 \\ 1.91 \\ 0.64$	$\begin{array}{r} 0.73 \\ 0.77 \\ 0.69 \\ \hline 2.19 \\ 0.73 \end{array}$	$0.21 \\ 0.21 \\ 0.19 \\ 0.61 \\ 0.20$	9.28	0.773
G	Total Av.	$     \begin{array}{r}       1.46 \\       1.46 \\       1.44 \\       \overline{4.36} \\       1.45 \\       1.45 \\       \end{array} $	$0.68 \\ 0.75 \\ 0.73 \\ \hline 2.16 \\ 0.72 \\ \end{array}$	$0.31 \\ 0.43 \\ 0.54 \\ \hline 1.28 \\ 0.43$	$0.18 \\ 0.21 \\ 0.22 \\ 0.61 \\ 0.20$	8.41	0.701
н	Total Av.	$     \begin{array}{r}       1.65 \\       1.61 \\       1.46 \\       \overline{4.72} \\       1.57 \\       \end{array} $	$0.80 \\ 0.79 \\ 0.82 \\ \hline 2.41 \\ 0.80 \\ \hline$	$\begin{array}{r} 0.63 \\ 0.71 \\ 0.58 \\ \hline 1.92 \\ 0.64 \end{array}$	$0.26 \\ 0.39 \\ 0.20 \\ 0.85 \\ 0.28$	9.90	0.825
I	Total Av.	$     \begin{array}{r}       1.66 \\       1.62 \\       1.64 \\       \overline{4.92} \\       1.64     \end{array} $	$0.74 \\ 0.75 \\ 0.76 \\ \hline 2.25 \\ 0.75 \\ 0.75 \\ \end{array}$	$\begin{array}{c} 0.70 \\ 0.80 \\ 0.76 \\ \hline 2.26 \\ 0.75 \end{array}$	$0.26 \\ 0.22 \\ 0.23 \\ 0.71 \\ 0.24$	10.14	0.845
J	Total Av.	$1.62 \\ 1.64 \\ 1.60 \\ 4.86 \\ 1.62$	0.720.740.752.210.74	0.810.700.812.320.77	$\begin{array}{c} 0.21 \\ 0.21 \\ 0.19 \\ \hline 0.61 \\ 0.20 \end{array}$	10.00	0.833
К	Total Av.	$     \begin{array}{r}       1.47 \\       1.60 \\       1.57 \\       \overline{4.64} \\       1.55 \\       \end{array} $	$0.76 \\ 0.79 \\ 0.82 \\ \hline 2.37 \\ 0.79 \\ 0.79 \\ \hline$	0.670.730.842.240.75	$\begin{array}{c} 0.21 \\ 0.25 \\ 0.29 \\ \hline 0.75 \\ 0.25 \end{array}$	10.00	0.833
Grand Grand	total average	$52,67 \\ 1.596$	$\begin{array}{r} 24.57\\0.745\end{array}$	$\begin{array}{r} 24.27\\0.735\end{array}$	$\begin{array}{c} 7.54 \\ 0.228 \end{array}$	109.05	0.826

indicates that the variances are not homogeneous. The laboratories differ significantly in repeatability.

Inspections of the logarithms of the within-sample variances in Table V shows that sample A is the heaviest contributor to the lack of homogeneity. If the data from sample A are deleted, the  $\chi^2$  value for Bartlett's test drops to 18.35. This is not high enough to prove (with 99% confidence) that the balance of the data lack homogeneity. No further data need be rejected. However, this value for  $\chi^2$  is suspiciously high (shows a lack of homogeneity at the 98% confidence level). It is apparent that Laboratories H and K have a much higher within-sample variance than the other laboratories. From the practical standpoint the data from these laboratories were deleted. This results in a  $\chi^2$  value of only 2.11, which shows that the balance of the data are homogeneous. The decision as to what data should be rejected to eliminate nonhomogeneous variances will depend on the distribution of the variances and the judgment of the analvst.

Table II.	Repeatability	within	Laboratory	Samples
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(0	Carbon dete	ermination	ı)	
Sample No.	1	2	3	4 <sup>b</sup>
Sample average, $\%$ Variance $\times 10^3$	. 60	0.74	0,73	0.23 (0.22)
$VL^a$ Within laboratories	17.15	7.02	39.47	1.85 (0.87)
$\frac{V_o^a}{V_o^a} \text{Repeatability} = S_o = \sqrt{1}$	1.76 Vo 0.042	$\begin{array}{c} 0.66 \\ 0.026 \end{array}$	$\substack{\textbf{3,42}\\\textbf{0,058}}$	$1.20 (0.43) \\ 0.035 (0.021)$

 $^{a}$  Youden (11).  $^{b}$  The values in parentheses represent the recalculated values after rejection of the one wild value.

Table III. Nomenclature Used

Symbol

No.

- NS NL Vo VS VL VL

- Number of repeat tests on each sample in each laboratory Number of samples in the exchange program Number of laboratories in the exchange program Variance due to differences between replicate tests Variance due to differences between samples Variance due to differences between laboratories Variance due to interaction between laboratories and samples
- $\sqrt{V_o}$  = repeatability Component of variance due to differences between samples Component of variance due to differences between laboratories Component of variance due to laboratory by sample interaction So SS SL SI

Table IV. Inspection of Data to Find Laboratories with **Averages Far Out of Line** (Using Divon's () test)

	(Using Di			
Sample No.	1	2	3	4 (Corrected)
		Q V	alue <sup>a</sup>	
On highest result On lowest result	$\begin{array}{c} 0.167 \\ 0.416 \end{array}$	$\begin{array}{c} 0.182 \\ 0.533 \end{array}$	$\begin{array}{c} 0.158 \\ 0.790 \end{array}$	0.200 0.000
Revised G	Values after	Rejecting L	aboratory (	G۵

On highest result On lowest result	$\begin{array}{c} 0.067 \\ 0.176 \end{array}$	$\substack{\textbf{0.091}\\\textbf{0.334}}$	$\begin{array}{c} 0.200 \\ 0.530 \end{array}$	$0.200 \\ 0.000$
4 Value must exceed 0	670 for rol	nation at the	0007 confide	man laval (9)

<sup>b</sup> Value must exceed 0.597 for rejection at the 99% confidence level.

#### Table V. Inspection of Data to Find Laboratories with Repeatabilities Far Out of Line (Laboratory G rejected)

			(La	Doratory	G rejecte	a)				
Laboratory	A	В	С	D	$\mathbf{E}$	F (	H Correcte	I d)	J	к
Total Average	$\substack{10.42\\0.87}$	$\substack{10.10\\0.84}$	$10.46 \\ 0.87$	$\substack{10.65\\0.89}$	9.69 0.81	9.28 0.77	$\begin{array}{c} 9.74 \\ 0.81 \end{array}$	$\substack{10.14\\0.84}$	10.00 0.83	$\begin{array}{c}10.00\\0.83\end{array}$
$\begin{array}{c} \text{Variance} \times 10^3 \\ \text{Between samples} \\ \text{Within samples, } V_o \\ \text{Log } V_o \\ \text{Deviation from } \log V_o \end{array}$	$1055 \\ 0.17 \\ -3.77 \\ -0.74$	931 0.94 -3.03 0.00	1115 0.81 -3.09 -0.06	$1088 \\ 0.56 \\ -3.25 \\ -0.22$	$942 \\ 0.51 \\ -3.29 \\ -0.26$	908 0.90 -3.05 -0.02	$948 \\ 3.86 \\ -2.41 \\ +0.62$	1019 0.86 -3.06 -0.03	$1029 \\ 1.20 \\ -2.92 \\ +0.11$	$859 \\ 3.63 \\ -2.44 \\ +0.59$
	Ba	artlett's t $ x_{n-1}^2 = 2 \\ = 2 \\ = 3 $	est (8) fo 2.65 (f - 2.65 (10 3.73 (sig	r homoge 1) Σ (Lo - 1)(1.4) nificant s	neity of y og Vo - 7 131) 1 99% co	variances Log Vo) <sup>2</sup> onfidence	: level)			

Table VI. Effect of Magnitude on Repeatabilit
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(After reject	ing 4 labo	ratories)		
Sample No.	1	2	3	4
Sample average Within laboratory veriance	1.62	0.73	0.78	0.22
$10^4$ (14 degrees of freedom) Repeatability, So	$\begin{array}{c} 6.36 \\ 0.025 \end{array}$	$\begin{array}{c} 6.78 \\ 0.026 \end{array}$	$\begin{array}{c} 18.07 \\ 0.042 \end{array}$	$\substack{1.93\\0.014}$

The analysis shows which laboratories may be having trouble with their techniques. It serves to pinpoint possible sources of difficulty. A good estimate of the reliability of the test can still be obtained from the seven remaining laboratories.

Does Magnitude Affect Repeatability? Subsequent handling of the data hinges on whether the repeatability of the carbon determination depends on the amount of carbon present. This can be determined by inspection of the repeatability results in the various samples. The repeatability figures on the four samples (with data from Laboratories A, G, H, and K deleted) are shown in Table VI. It is apparent that there is no consistent trend. An F test on the two extremes shows that they are not significantly different at the 99% confidence level. In this instance we can handle all of the data together.

## Table VII. Analysis of Variance on Seven Laboratories (Laboratories A, G, H, and K rejected)

	Sum of Squares	Degrees of Free- dom	Variance	Variance Ratio
Between samples Between laboratories Laboratory by samples Replicates	$\begin{array}{r} 21.0509 \\ 0.1053 \\ 0.0445 \\ 0.0464 \end{array}$	3 6 18 56	7.0170 0.0175 0.002471 0.000828	2841ª 7.08ª 2.98ª
Total	21.2471	83		
<sup>a</sup> Significant at the 99% cont	fidence leve	el.		

Analyze All Data. The analysis of variance on all of the remaining data from the seven laboratories is shown in Table VII. The laboratory by sample interaction proved to be significant in this case. This points up the need for more work on the method. Any results obtained for repeatability and reproducibility will only be working approximations for use until the procedure is in better control. Testing the between-laboratories variance against the laboratory by sample interaction shows that it is also significant.

The components of variance due to the interaction effect and to the laboratory effect can be calculated from the data in Table VII using the equations shown by Villars (7). See Table VIII, Case B.

 $V_o = S_{o^2} = 0.000,828$ S. = 0.026 = repeatability

 $V_I = V_o + N_o \left( \frac{1}{(N_L)} \right)$  $S_{I^{2}} = 0.000,828 + 3 \left(\frac{7}{7}\right)$  $S_{I^2}$  $S_{I^2} = 0.000,469$  $S_I = 0.022 = \text{component of}$ reproducibility due to

laboratory by sample interaction

$$V_{L} = V_{I} + N_{\bullet}N_{\bullet}\left(\frac{N_{L}}{N_{L}-1}\right)$$
  

$$S_{L}^{2} = 0.0175 \text{ (Table VII)}$$
  

$$= 0.002,471 + 4(3)$$
  

$$\left(\frac{7}{7-1}\right)S_{L}^{2}$$

## Table VIII. Components of Variance<sup>a</sup>

 
 Source of Variation
 Degrees of Freedom
 Components of Variance

 Case B. Assuming that all (or most) of the laboratories, which will be using the test technique, are included in the exchange of samples

NL Between NL  $N_L - 1$  $V_{L} = V_{o} + N_{o} \frac{N_{L}}{(N_{L} - 1)} (S_{I})^{2} + N_{S}N_{o} \frac{N_{L}}{(N_{L} - 1)} (S_{L})^{2}$  $= V_{I} + N_{S}N_{o} \frac{N_{L}}{(N_{L} - 1)} (S_{L})^{2}$ laboratories  $(N_L - 1)(N_S - 1)$  $= V_{o} + N_{o} \frac{NL}{(NL - 1)} (SI)^{2}$ Laboratory by sample inter-action Replicate tests  $= S_0^2$  $N_L N_S (N_o - 1)$ (repeatability) Case C. Assuming that the laboratories involved in the exchange of samples represent only a cross section of those using the test technique  $V_L = S_{0^2} + N_{0.}S_{I^2} + N_{0}NS.S_{L^2}$ Between NL  $N_L - 1$ laboratories Laboratory by sample inter- $VI = S_0^2 + N_0 \cdot SI^2$  $(N_L - 1)(N_S - 1)$ action  $V_0 = S_0^2$  $NLNs(N_o - 1)$ Replicates a Villars (7).

 $S_L^2 = 0.001,071$  $S_L = 0.033 =$  component due to differences between laboratories

We now have all of the information required. The best estimate available for the repeatability of the method is  $S_o = 0.026$ . The best estimate of the reproducibility is given by:

Reproducibility =  $\sqrt{S_o^2 + S_I^2 + S_L^2}$ =  $\sqrt{0.000,828 + 0.000,469 + 0.001,071}$ = 0.049 = approximately 0.05%

Based on this working estimate, single carbon determinations made in any laboratory will, on the average, lie within the interval, mean value  $\pm$  reproducibility, 68% of the time. On the average, determinations will lie within the interval, mean value  $\pm$  twice the reproducibility, about 95% of the time.

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A :	N	Ā	L	Y	Т	I	С	Ā	L	С	н	Е	Μ	[]	S	Т	R	Y
-----	---	---	---	---	---	---	---	---	---	---	---	---	---	----	---	---	---	---

peatability and reproducibility of the macro-Kjeldahl method of determining nitrogen. This method is well established and in control, so that few wild results would be expected in such a survey. In this case five samples were run three times each in five laboratories (Table IX).

Comb for Wild Results. Following the suggested statistical procedure, the data were first surveyed for wild results. An analysis of variance on each sample (Table X) showed that no individual results could be rejected. Dixon's Q test on each laboratory sample showed that none of them can be discarded.

The variances within samples for each laboratory are shown in Table XI. Bartlett's test for homogeneity of variances shows that the laboratories do not differ at the 99% confidence level; therefore no data should be rejected.

**Does Magnitude Affect the Repeatability?** The next step is to determine whether the repeatability of the test depends on the concentration of nitrogen being determined. Since no data have been rejected, we can use the results in Table X. Inspection of the results shows that the repeatability is poorer as the nitrogen content goes up. Comparison of the ratio of replicate variances, F, for the extreme samples (largest and smallest nitrogen content) shows that the difference is significant.

$$F = \frac{221.3 \times 10^{-6} (10 \text{ degrees of freedom})}{2.3 \times 10^{-6} (10 \text{ degrees of freedom})} = 96.2$$



Figure 2. Reproducibility of Nitrogen Determination

Table IX. Results by Regular Macro-Kjeldahl<sup>a</sup>

			Sa	ample No.				
ra- 7		1	2 %	3 Nitrogen	4	5	Total	Average
		0.006 0.008 0.011	0.055 0.056 0.053	0.094 0.091 0.093	$0.985 \\ 0.980 \\ 0.977$	$1.323 \\ 1.351 \\ 1.329$		
ם A	Fotal Av.	0.025 0.008	$\overline{\begin{array}{c}0.164\\0.055\end{array}}$	$\begin{array}{r} \hline 0.278 \\ 0.093 \end{array}$	$2.942 \\ 0.981$	4.003 1.334	7.412	0.4941,
r	Γotal	$\begin{array}{c} 0.009 \\ 0.010 \\ 0.010 \\ \hline 0.029 \end{array}$	$\begin{array}{c} 0.061 \\ 0.059 \\ 0.060 \\ \hline 0.180 \end{array}$	$\begin{array}{c} 0.097 \\ 0.100 \\ 0.104 \\ \hline 0.301 \end{array}$	$     \begin{array}{r}       0.990 \\       0.979 \\       0.979 \\       2.948     \end{array}   $	$     \begin{array}{r}       1.342 \\       1.335 \\       1.341 \\       \overline{4.018}     \end{array} $	7.476	0.4984
Α	lv.	0.010	0.060	0.100	0.982	1.339		
T A	fotal Av.	$\begin{array}{c} 0.011 \\ 0.014 \\ 0.040 \\ 0.013 \end{array}$	$\frac{0.061}{0.062}$ 0.185 0.062	$\frac{0.099}{0.099}$ $\frac{0.295}{0.098}$	$     \begin{array}{r}       0.984 \\       0.992 \\       2.955 \\       0.985     \end{array}   $	$\frac{1.320}{1.331}$ $\overline{3.991}$ 1.330	7.466	0.4977
Т	lotal	$\begin{array}{c} 0.010 \\ 0.010 \\ 0.009 \\ \hline 0.029 \\ 0.029 \end{array}$	$ \begin{array}{r} 0.059 \\ 0.058 \\ 0.057 \\ \hline 0.174 \\ 0.059 \end{array} $	$ \begin{array}{r} 0.096 \\ 0.094 \\ 0.095 \\ \hline 0.285 \\ 0.005 \end{array} $	$\begin{array}{r} 0.974 \\ 0.981 \\ 0.983 \\ \hline 2.938 \\ 0.970 \end{array}$	$     \begin{array}{r}       1.320 \\       1.342 \\       1.335 \\       \overline{3.997} \\       1.922     \end{array} $	7.423	0.4949
£	1v.	0.011 0.012 0.012	0.058 0.061 0.062 0.062	0.095 0.097 0.096 0.097	0.979 0.999 0.977 0.992	1.332 1.301 1.355 1.331		
ר A	Fotal Av.	$\begin{array}{c} \overline{0.035} \\ 0.012 \end{array}$	$\begin{array}{c} 0.185 \\ 0.062 \end{array}$	0.290 0.097	$\overline{\begin{array}{c}2.968\\0.989\end{array}}$	$3.987 \\ 1.329$	7.465	0.4977
$\mathbf{Sample}_{A}$	total Av.	$0.158 \\ 0.0105$	$\substack{\textbf{0.888}\\\textbf{0.0592}}$	$\substack{1.449\\0.0966}$	$\substack{14.751\\0.9834}$	$\substack{19.996\\1.3331}$	37.242	0.49656
sed on data o	obtaine	d by Lake (	4).					

	Raw Data	Refined Data
Repeatability Reproducibility	$\begin{array}{c} 0.042\% \\ 0.079\% \end{array}$	${0.026\% \atop 0.049\%}$

On the raw-data basis, errors as great as 0.16% (2 × 0.079) may be expected. It is apparent that if this figure is taken as the working limit, the method is of little use in measuring carbon contents under 0.30%. For example, it would be of no use on samples such as sample 4 in the exchange program. It is felt that the estimate based on the refined data is a more realistic assessment of the potentialities of the method.

### DETERMINATION OF NITROGEN

The Esso Laboratories recently cooperated in a program for determining the reat the 99% confidence level. As indicated in Figure 1, the data from each sample should be handled separately in this case. We will get a separate estimate of the reproducibility for each sample and then try to make up a composite picture of variation of reproducibility with nitrogen content over the range studied.

The results of these calculations are shown in Table X. B. In samples 1, 4, and 5, the variance between laboratories was not significantly greater than the variance within laboratories. The two variances can therefore be pooled to give an over-all estimate of the reproducibility. In the case of samples 2 and 3, the between-laboratories variance,  $V_L$ , is significantly greater than

4	
	9
0.983	1.33
46.8	49.0
49.7	221.3
0.0070 <1.0	0.0149 <1.0
No	No
48.8 0	172.1
0.0070	0.0131
0.0070	0.0131
	$\pm$ 0.983 46.8 49.7 0.0070 <1.0 No 48.8 0 0.0070 0.0070

Table XI. Comparison of Nitrogen Determinations

			0		
Laboratory	Р	Q	R	s	т
Total Av.	$\begin{array}{c} 7.412 \\ 0.4941 \end{array}$	7.476 0.4984	$\begin{array}{c} \textbf{7.466} \\ \textbf{0.4977} \end{array}$	$\begin{array}{c} 7.423\\ 0.4949 \end{array}$	$7.465 \\ 0.4977$
Between samples	1.150	1.148	1.136	1.142	1.140
$V_o \times 10^5$ Log $V_o$	$4.89 \\ -4.310$	$\frac{1.37}{-4.863}$	$\frac{1.31}{-4.882}$	$\frac{3.02}{-4.520}$	17.19 - 3.765

Bartlett's test (3) for homogeneity of variances.  $\chi_{n^2-1} = 2.64 (5 - 1) \times 0.8493 = 9.00$  (4 degrees of freedom) This value is not significant at the 99% confidence level.

Table XII. Results by Modified Macro-Kjeldahl Method<sup>a</sup>

			200000-00 20	Sample No				
Labora- tory		1	2	3 % Nitroger	4 n	5	Total	Average
Р		$\begin{array}{c} 0.008 \\ 0.011 \\ 0.009 \end{array}$	$0.049 \\ 0.044 \\ 0.047$	0.096 0.096 0.096	0.969 0.970 0.970	$1.334 \\ 1.332 \\ 1.328$		
	Total Av.	$\begin{array}{r} 0.028 \\ 0.009 \end{array}$	$\overline{\begin{array}{c} 0.140 \\ 0.047 \end{array}}$	$\overline{\begin{array}{c} 0.288 \\ 0.096 \end{array}}$	$\overline{2.909}\\0.970$	$\overline{\begin{array}{r}3.994\\1.331\end{array}}$	7.359	0.4906
Q		$\begin{array}{c} 0.009 \\ 0.009 \\ 0.009 \\ 0.009 \end{array}$	$\begin{array}{c} 0.055 \\ 0.067 \\ 0.058 \end{array}$	0.090 0.093 0.091	$\begin{array}{c} 0.968 \\ 0.990 \\ 0.979 \end{array}$	$1.315 \\ 1.310 \\ 1.306$		
	Total Av.	$\begin{array}{c} 0.027 \\ 0.009 \end{array}$	$0.180 \\ 0.060$	$\substack{\textbf{0.274}\\\textbf{0.091}}$	$2.937 \\ 0.979$	$\substack{\textbf{3.931}\\\textbf{1.310}}$	7.349	0.4899
R		$\begin{array}{c} 0.010 \\ 0.010 \\ 0.009 \end{array}$	$0.059 \\ 0.059 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.057 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.059 \\ 0.057 \\ 0.05$	$\begin{array}{c} 0.097 \\ 0.097 \\ 0.097 \\ \hline 0.097 \\ \hline \end{array}$	$\begin{array}{c} 0.945 \\ 0.979 \\ 0.952 \\ \hline \end{array}$	$1.301 \\ 1.305 \\ 1.310 \\ 1.310$		
	Total Av.	$\begin{array}{c} 0.029 \\ 0.010 \end{array}$	$0.175 \\ 0.058$	0.291 0.097	$2.876 \\ 0.959$	$\substack{\textbf{3.916}\\\textbf{1.305}}$	7.287	0.4858
S	Total Av.	$\begin{array}{c} 0.009 \\ 0.009 \\ 0.010 \\ \hline 0.028 \\ 0.009 \end{array}$	$\begin{array}{c} 0.056 \\ 0.055 \\ 0.055 \\ \hline 0.166 \\ 0.055 \end{array}$	$\begin{array}{c} 0.093 \\ 0.091 \\ 0.091 \\ \hline 0.275 \\ 0.092 \end{array}$	$\begin{array}{r} 0.979 \\ 0.979 \\ 0.973 \\ \hline 2.931 \\ 0.977 \end{array}$	$     \begin{array}{r}       1.309 \\       1.306 \\       1.310 \\       3.925 \\       1.308 \\     \end{array} $	7.325	0.4883
Т		$\begin{array}{c} 0.014 \\ 0.014 \\ 0.014 \end{array}$	$0.063 \\ 0.064 \\ 0.064$	$0.100 \\ 0.106 \\ 0.097$	$\begin{array}{c} 0.999 \\ 0.984 \\ 1.005 \end{array}$	$1.333 \\ 1.344 \\ 1.338$		
	Total Av.	$\begin{array}{c} 0.042 \\ 0.014 \end{array}$	$0.191 \\ 0.064$	$\begin{array}{c} 0.303 \\ 0.101 \end{array}$	$2.988 \\ 0.996$	$4.015 \\ 1.338$	7.539	0.5026
	Sample total Av.	$\begin{array}{c} 0.154 \\ 0.0103 \end{array}$	$0.852 \\ 0.0568$	$\substack{1.431\\0.0954}$	$\substack{\textbf{14.641}\\\textbf{0.9761}}$	$19.781 \\ 1.3187$	36.859	0.49145
<sup>a</sup> Based	on data obtaine	ed by La	ke (4).					

Table XIII.	Modified	Nitrogen	Determination
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		Ų	•		
Sample No.	1	2	3	4	5
Sample average, % N Variance × 10 <sup>6</sup>	0.010	0.057	0.095	0.976	1.32
tories	13.2	123.2	48.7	563.0	676.0
tories	0.6	9.5	4.9	114.5	16.9
$\sqrt{V_o} = S_o = \text{first esti-}$ mate of repeatability $V_L/V_o$ ratio	0.0008 22.0	0.0031 13.0	$\begin{array}{c} 0.0022\\ 10.0 \end{array}$	0.0107 4.91	0.0041 40.0
Is $V_L$ significantly greater than $V_0$ (99% confidence)? Pooled variance $\times$ 10 <sup>6</sup> $S_L^2 \times 10^6$ Repeatability $\sqrt{S_0^2 + S_L^2} =$ reproducibility	Yes 0.8 0.0008 0.0012	Yes 7.6 0.0031 0.0041	Yes 2.9 0.0022 0.0028	No 242.6 0 0.0156 0.0156	Yes 44.0 0.0041 0.0078

## Table XIV. Comparison of Regular and Modified Method

of Determining Mitrogen						
Sample No.	1	2	3	4	5	
Average value Regular method Modified method Fetimeted reproducibility	0.010 0.010	0.059 0.057	0.097 0.095	0.983 0.976	$\substack{1.33\\1.32}$	
Regular method Modified method	$\begin{array}{c} 0.0022\\ 0.0012 \end{array}$	0.0031 0.0041	0.0033 0.0028	$0.0070 \\ 0.0156$	0.0131 0.0078	

the within-laboratory variance,  $V_{a}$ , so that separate estimates for each component should be made. The estimates can be determined from the expression:

$$V_L = S_o^2 + N_o N_S S_L^2$$

Villars (7) Case C is used here, since the five laboratories involved represent only a sample of those using the test.

The five individual estimates of the reproducibility are not particularly useful as they are. It is desirable to have some way of interpolating to get estimates of the reproducibility at intermediate nitrogen levels. Probably this can best be done by

plotting the reproducibility against nitrogen content as shown in Figure 2. Working estimates of the reproducibility of the test method can then be read off this curve.

## COMPARING TEST METHODS

When test methods are to be compared, the same calculation is made for each method, and the average values and the reproducibilities are compared.

For example, at the time the results on the nitrogen determination discussed above were made, results were also obtained using a modified macro-Kjeldahl method. The detailed results are shown in Table XII. Evaluation of this procedure shows that no results should be rejected. The results of the analysis shown in Table XIII demonstrate the anomalies frequently encountered in this sort of work. There is apparently a tendency for the repeatability to get poorer as the nitrogen content increases. In spite of this, the repeatability is much better in the sample containing 1.3% nitrogen than it is on the sample containing 1.0% nitrogen. More data on intermediate samples, or a more involved statistical analysis, are needed to explain this result.

The regular and the modified methods of determining nitrogen are compared in Table XIV. The modified method tends to give lower nitrogen values. Obviously, the method giving the best value can be determined only by running samples of known nitrogen content. There seems to be little difference between the methods from the standpoint of reproducibility.

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## Effect of Aviation Fuel Components on the Accuracy Of the Karl Fischer Electrometric Titration

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The Karl Fischer electrometric titration method for quantitatively determining water has been considered satisfactory for unleaded gasolines, light and dark oils, and, with slight modification, greases and sludges. With the advent of wider specification limits for gasoline and jet propulsion fuels, which permits the inclusion of additional naturally occurring materials, and with new or higher concentrations of fuel additives, the validity of results by this procedure is questionable. It has been theorized that finished fuels now contain materials, natural or added, which influence the accuracy of results obtained by the Karl Fischer procedure. This work was undertaken to determine if this method is practical for determining the water content of aviation fuels. The study conducted determined the effect of as little as 0.001 weight % of tetraethyllead, aromatics, olefins, mercaptans, and oxidation inhibitors on the accuracy of water determinations. Of the compounds investigated, only mercaptans appear to show any appreciable interference.

THE Karl Fischer electrometric titration is now a standard analytical procedure for the determination of moisture content of a large number of materials (8). In the majority of applications this procedure has been considered satisfactory for the accuracy required. However, there have been considerable disagreement and discussion regarding the applicability of this procedure to aviation gasoline and jet propulsion fuel. This disagreement arises from the fact that these fuels may contain quantities of materials which cause reproducible inaccurate results. For example, several members of industry believe that this method causes erroneous results for fuels containing tetraethyllead, because the lead compound reacts with, and hence consumes, the Karl Fischer reagent in much the same manner as water. Data obtained by other organizations indicate that tetraethyllead does not interfere with the accuracy of results. No data have been presented in the literature specifically on the effect of fuel components on the determination of water.

This uncertainty was of particular interest to the U.S. Air Force, which is a major consumer of aviation fuels and must operate in areas where climatic conditions could cause excess moisture in the fuel to crystallize, resulting in malfunction of equipment. Therefore, this program was undertaken to check conflicting literature and to determine other compounds that may affect the accuracy of results.

The project was conducted, keeping in mind the military fuel specifications. It was assumed that accuracy to  $\pm 0.001$  weight % water was sufficient for Air Force purposes. The Karl Fischer electrometric titration has been employed in this attempt to determine specifically the effect of several of the more predominant, and usually disputed, components of fuel blends on the accuracy of the method. Effects of aromatic, olefin, and mercaptan (thiol) compounds, tetraethyllead, and oxidation inhibitors on the accuracy of results have been investigated. Information obtained shows that fuels need not be specially treated to remove detrimental compounds before the determination of water.

#### METHOD AND APPARATUS

The method of Karl Fischer has been widely employed by research workers and has now become a routine tool for the determination of water in a wide variety of materials or products, including hydrocarbons (1, 3, 4, 11). Previous data indicated that the Karl Fischer reagent was quantitative and specific for small amounts of water. This reagent, a solution of pyridine, sulfur dioxide, and iodine in anhydrous methanol, is normally dark brown in color. A change in this color is usually evidenced at the end point. However, in most instances an electrometric procedure is preferred to the colorimetric technique because of the accuracy in detecting the end point, and because it permits determinations in dark materials where the color end point would be obscure. The electrometric method functions on the basis that an excess of the reagent will conduct current. When a slight excess of water is present, one of the electrodes becomes polarized, and the current decreases, approaching zero. Thus, the end point may be determined by a dead-stop technique.

Karl Fischer reagent, equivalent to about 3.5 mg. of water per

<sup>(12)</sup> Ibid., p. 59.

ml., was used for the titration of 25 to 40 grams of the sample. The solvent used to render the system homogeneous consisted of 1 volume of anhydrous methanol plus 3 volumes of chloroform.

The specific method, apparatus, and calculations employed have been described in detail (10).

#### DISCUSSION

Six fuels and an iso-octane were employed in this study. Four gasolines of varying aromatic and olefin content were employed to study the effect of these materials on the accuracy of the Karl Fischer technique. A single jet propulsion fuel with varying mercaptan content was used. The effect of tetraethyllead on the procedure was investigated with iso-octane as the carrier. A single gasoline containing inhibitors was employed in the investigation of inhibitor effect on the method. This was considered a sufficient number to allow conclusions to be drawn from the data obtained.

Table	I.	Effect	of	Aromatic	Concentrations	on	Water
				Determin	ation		

			Water	Tota	l Water, %
Fuel <sup>a</sup>		Water, %	Added, %	Calcu- lated	Deter- mined
Α	Av.	0.0051 0.0043 0.005	0.0040 0.0037	0.0087 0.0084	$\begin{array}{c} 0.0084 \\ 0.0079 \end{array}$
В	Av.	0.0052 0.0054 0.005	0.0038 0.0038	0.0091 0.0091	$\begin{array}{c} 0.0086 \\ 0.0088 \end{array}$
A + 17% toluene	Av.	0.0072 0.0070 0.007	0.0044 0.0041	$\begin{array}{c} 0.0115\\ 0.0112\end{array}$	$\begin{array}{c} 0.0098\\ 0.0107\end{array}$
B + 12% toluene	Av.	0.0064 0.0063 0.006	$\begin{array}{c} 0.0039\\ 0.0041 \end{array}$	$\begin{array}{c} 0.0103\\ 0.0105\end{array}$	$\begin{array}{c} 0.0111\\ 0.0103\end{array}$
<sup>a</sup> Fuel A cont 14.1% aromatics	tains 0. s and 0.	0% aromatics 9% olefins.	and 11.0%	olefins.	Fuel B contain

Aromatic Compounds. The percentage of water in samples A and B was determined in accordance with the procedure (10). To each a known amount of water was added, using absolute methanol as a carrier. The water content of each fuel was then determined to see if the difference in aromatic content of the two fuels would affect the results obtained for the total water content.

To test further the effect of the aromatic content, 12% by volume of toluene was added to each of the above fuels, and the water content was determined by the Karl Fischer method. The calculated water content, obtained by adding the percentage of water initially in the fuel to the known percentage of water injected, was compared to the water content as determined by the Karl Fischer method.

Results of the above tests are tabulated in Table I. The water concentration figures calculated in the manner given and those obtained by employing the Karl Fischer procedure agree within  $\pm 0.001\%$ , which is the limit of reproducibility of the equipment and procedure employed. Therefore, it appears that in determining moisture in aviation fuels, a high aromatic-containing sample has relatively no more effect on the accuracy of the test than a low aromatic-containing sample.

Olefin Compounds. Fuels C and D, typical fuels with a low olefin content, were selected so that the effect of the addition of small quantities of olefins would be amplified. After the initial water content of each of the fuels was obtained by the Karl Fischer method, 12% by volume of diisobutylene was added to each in order to increase the olefin content. The water content was then determined on the newly blended, higher olefin fuel. The results, presented in Table II, indicate that the concentration of the olefins used in this investigation does not tend to alter the accuracy of the Karl Fischer method. It is possible that olefinic compounds other than those in the base fuels and those added could have some influence on the data. However, this program indicates that olefins will probably be of little consequence. Because of the presence of more double bonds per molecule, diolefin materials, such as used, probably offer greater difficulty in obtaining accurate results than any mono-olefintype hydrocarbons.

Table II.	Effect of Olefin Concentration on Water						
Determination							

			Water	Total W	ater, %
Fuel <sup>a</sup>		Water, %	Added, %	Calcu- lated	Deter- mined
С	Av.	$\begin{array}{c} 0.0035 \\ 0.0042 \\ 0.004 \end{array}$	0.0036 0.0037	$\begin{array}{c} 0.0075\\ 0.0075\end{array}$	$0.0066 \\ 0.0076$
D	Av.	0.0046 0.0043 0.005	$\begin{array}{c} 0.0036\\ 0.0037 \end{array}$	$\begin{array}{c} 0.0081 \\ 0.0082 \end{array}$	0.0078 0.0089
C + 12% di- isobutylene	Av.	0.0048 0.0042 0.005	$\begin{array}{c} 0.0043 \\ 0.0044 \end{array}$	0.0088 0.0089	$\begin{array}{c} 0.0081 \\ 0.0080 \end{array}$
D + 12% di- isobutylene	Av.	0.0045 0.0042 0.004	$\begin{array}{c} 0.0040\\ 0.0042\end{array}$	0.0084 0.0086	$\begin{array}{c} 0.0080 \\ 0.0089 \end{array}$
<sup>4</sup> Fuel C conta	ins 3	5% aromat	ics and 2.5%	olefins Fue	D contain

<sup>a</sup> Fuel C contains 3.5% aromatics and 2.5% olefins. Fuel D contains 17.0% aromatics and 0.8% olefins.

Mercaptans. In view of the fact that jet propulsion fuels in present production allow a maximum of 0.005 weight % mercaptans, their effects on a typical fuel, JP-3, were investigated. Two mercaptans were used in these tests, a four-carbon and a twelve-carbon compound. Table III shows that the addition of these mercaptans to a base fuel increased the figures obtained for its water content proportionately. The addition of 0.005 weight % of either four- or twelve-carbon mercaptans gave results within the limit of reproducibility of the method, and may not actually be considered as causing an increase in the values.

Table III. Effect of Mercaptan Concentration on Water Determination

Fuel	Water, %	Average
JP-3 (sample 1691)	$\begin{array}{c} 0.0027 \\ 0.0026 \end{array}$	0.003
JP-3 (1691) + $0.005\%$ tert-butyl mercaptan	$\begin{array}{c} 0.0031\\ 0.0029\end{array}$	0.003
JP-3 (1691) $+ 0.02\%$ tert-butyl mercaptan	$0.0049 \\ 0.0055$	0.005
JP-3 (1691) + 0.005% tert-dodecyl mer- captan	$\begin{array}{c} 0.0029 \\ 0.0038 \end{array}$	0.003
JP3 (1691) + 0.02% tert-dodecyl mercap- tan	$\begin{array}{c} 0.0052\\ 0.0048 \end{array}$	0.005

However, in anticipation of higher mercaptan concentrations, a 0.02% addition was tested. Because this gave considerably higher results, which were beyond test reproducibility limits, it may be assumed that the addition of the lower concentrations caused higher results, even though within the limits of the test. There appeared to be no variations between the effect of the high and low molecular weight mercaptans on the method. For fuels in which the mercaptan concentration is known, an empirical correction probably can be applied to the apparent water content. Although not ascertained, it is theorized that other readily oxidizable or reducible sulfur compounds, if present in the fuel, will cause larger or smaller errors, respectively, in the accuracy of water content as determined by this method.

Tetraethyllead. A number of test samples were prepared using different concentrations of tetraethyllead in iso-octane. covering the range of tetraethyllead permitted in current military aviation gasoline specifications. In one series of tests iso-octane, as received from the manufacturer, was used with known quantities of tetraethyllead added. In a second series of tests the iso-octane, as received, was saturated with

Table IV. Effect on Water Determination of Addition of Tetraethyllead to Iso-octane

Ml. per Gallon Iso-octane	Water, %	Average
0	$\begin{array}{c} 0.0014 \\ 0.0013 \\ 0.0021 \end{array}$	0.002
1	0.0026 0.0017	0.002
2	0.0030 0.0005	0.002
3	0.0018 0.0020	0.002
4	0.0018 0.0019	0.002
5	0.0012 0.0015	0.001
6	0.0015 0.0014	0.002

water prior to the addition of the lead compound. Tables IV and V show the results of these tests, which indicate that tetraethyllead does not affect accuracy in water content determinations in concentrations of 0.0 to 4.6 ml. per U. S. gallon, which is the current specification range used in aviation fuels. A sample of 80-octane unleaded gasoline was obtained and the percentage of water determined before and after the addition of tetraethyllead. The results, shown in Table VI, verify the literature statement that tetraethyllead does not interfere with the Karl Fischer method.

Table V. Effect on Water Determination of Addition of Tetraethyllead to Iso-octane Saturated with Water Tetreethrillood

Ml. per Gallon Iso-octane	Water, %	Average
0	0.0067 0.0058 0.0076	0.007
3	0.0062 0.0057 0.0071	0.006
6	0.0057 0.0083 0.0070	0.007
10	0.0085 0.0075 0.0067	0.008

Oxidation Inhibitors. Present fuel specifications permit the addition of up to 1.0 pound per 5000 U.S. gallons of certain oxidation inhibitors. To obtain the ultimate effect of these materials on the method under consideration, the maximum antioxidant concentration was employed. To uninhibited high octane aviation gasoline, 2-6-di-tert-butyl-4-methylphenol, N.N'di-sec-butyl-p-phenylenediamine, and 2,4-dimethyl-6-tert-butylphenol were added individually, and the water content was determined. In comparison with tests on the base uninhibited fuel no effect on the accuracy was exhibited. Results of these tests are tabulated in Table VII. All inhibited samples appear to behave similarly under conditions of the test.

Tetraethyllead to 80-Octane Fuel						
Tetraethyllead, Ml. per Gallon 80-Octane	Water,	Average				
0	0.0047 0.0047 0.0038	0.004				
6	0.0049 0.0052	0.005				

Table VI. Effect on Water Determination of Addition of

#### CONCLUSIONS

No concentration of aromatics, olefins, tetraethyllead, or oxidation inhibitors, likely to be encountered in the near future in aviation fuels, would interfere to any appreciable extent with the accuracy of water determinations by the Karl Fischer electrometric titration method.

## Table VII. Effect of Inhibitors on Water Determination

	Water, %	Average
Fuel E <sup>a</sup>	$0.0058 \\ 0.0051$	0.006
Fuel E + 1 lb./5000 gal. (2,6- di-tert-butyl-4-methyl- phenol)	0.0056 0.0049	0.005
Fuel E + 1 lb./5000 gal. (2,4- dimethyl-6-tert-butyl- phenol)	$0.0061 \\ 0.0054$	0.006
Fuel E + 1 lb./5000 gal. (N,N'-di-sec-butyl-p- phenylenediamine)	0.0059 0.0053	0.006

<sup>a</sup> Fuel E is uninhibited high-octane aviation gasoline.

Concentration of mercaptans as high as 0.02% and as low as 0.005% cause higher values for water because of a mercaptan reaction which consumes part of the Karl Fischer reagent. However, since no current military aviation fuel specification allows more than 0.005% of mercaptans, it is unlikely that appreciable errors will arise from this source.

These tests indicate that this method is reliable and practical for determining the water content of aviation fuels manufactured under current military procurement specifications.

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## **Constant Current Source for Coulometric Titrations**

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An electromechanical servo instrument is described which is capable of maintaining electrolysis currents of any desired magnitude constant to  $\pm 0.01\%$  for periods of several hours. The control element is a converteramplifier whose input responds to very small changes in the electrolysis current, and whose output actuates a motor to position a series resistor automatically to compensate the tendency for current variation. The instrument is easily assembled from unit components which are all commercially available, and it serves excellently for all types of coulometric titrations.

T IS advantageous in coulometric titrations (4) to employ a strictly constant current so that the quantity of electricity passed can be computed simply as the product of current and electrolysis time. Several electronic instruments have been described (1, 2, 4-7) which fulfill this function, but as yet no instrument of this type appears to be commercially available. The electromechanical servo instrument described herein can be assembled very easily from components which are all commercially available, so that no special skill in the art of electronic circuitry is required, and its performance is fully equal to the best of the instruments previously described. The instrument maintains currents of any desired value constant to well within  $\pm 0.01\%$  for periods of several hours. It was designed specifically for currents ranging from about 1 to 100 ma., but by suitable selection of the power supply and the various resistors it can be used with equal precision for either smaller or larger currents.

As shown in Figure 1, the control element is a Brown Electronik amplifier and its associated motor (Type 356410-1 with 27 r.p.m. motor) which is obtainable as a unit from the Minneapolis-Honeywell Regulator Co., Wayne and Windrim Avenues, Philadelphia. The *iR* drop developed by the electrolysis current in resistor *R*-1 is opposed by the voltage of the 1.5-volt dry cell, *B*, the difference voltage constituting the input signal to the Brown amplifier. Thus when the *iR* drop differs from the battery voltage the motor operates either to increase or decrease the series resistance *R*-3 to restore balance in the amplifier input circuit, and the current is kept constant within very narrow limits. An offbalance as small as 0.05 mv. is sufficient to activate the motor when the amplifier is operated at maximum gain, and, since the *iR* drop across *R*-1 is 1.5 volts, this corresponds to a control sensitivity of  $\pm 0.003\%$ .

The value at which the current is controlled is selected by appropriate choice of resistor R-1 so that the product iR is equal to 1.5 volts. Resistance R-1 need not be known precisely because the current is measured independently by measuring with a potentiometer the voltage drop across the precision resistance R-4 and applying Ohm's law. Decade resistance boxes may be used for R-1 and R-4; but it is more convenient to use fixed, plug-in type resistors such as the Type 500 of the General Radio Co., Cambridge, Mass., which are available in a wide selection of values with an accuracy of  $\pm 0.05\%$ . With a 1.5-volt bias battery three resistors of 20, 50, and 300 ohms will provide currents of 75, 30, and 5 ma. which are sufficient for most coulometric titrations. The use of a radio-type potentiometer for R-1 is unsatisfactory because of too inconstant resistance of the sliding contact.

Alternatively, continuously variable current selection could be achieved by using the battery to power a variable voltage dividing rheostat whose output would constitute the biasing voltage. However, the use of the battery directly is not only simpler, but has the more important advantage of much better control stability because there is no current drain from the battery and consequently it maintains very constant voltage. Switch S is provided to open the battery circuit, and thus prevent battery drain, when the instrument is not in use.

The accuracy of the current measurement depends on the accuracy of R-4 and that of the potentiometer. When utmost accuracy is desired R-4 should be calibrated against a certified resistance, such as those obtainable from Leeds and Northrup Co., Philadelphia. When R-4 is selected so that the voltage drop across it is 1 volt or more, the usual type of laboratory potentiometer correct to  $\pm 0.1$  mv. will provide current readings correct to  $\pm 0.01\%$  or better.

The rectifier power supply is a General Radio Type 1204-B, whose ungrounded output is continuously variable from 0 to 300 volts with a maximum output current of 100 ma. To minimize random fluctuations in the output, the power supply is fed from a Sola constant voltage transformer. Any other power supply of similar characteristics, including a well filtered and ungrounded output, can, of course, be used. For currents not greater than about 25 ma. several large 45-volt B-batteries in series can serve instead of a line-operated power supply. The use of a relatively high voltage supply, in conjunction with the large series resistance R-2 which drops the voltage across the cell to the required range of a few volts, minimizes the tendency for current variation due to changes of the cell impedance (resistance plus back e.m.f.), and thus favors highly precise control.



In coulometric titrations using grounded line-operated vacuum tube voltmeters, or similar electronic devices, for potentiometric end-point detection the power supply usually must be left ungrounded, as otherwise coupling through ground may occur between the generator and indicator electrodes with resultant malfunctioning of the vacuum tube voltmeter. The input to the Brown amplifier must, of course, also be left ungrounded, although it is usually beneficial to ground the case of the amplifier to earth through a 5- or  $10-\mu f$ . capacitor. Also, when this supply is used

The voltage dropping resistance R-2 consists of a bank of five 6000-ohm, high wattage, resistors connected to a terminal panel in such a way that various series and series-parallel combinations can be selected by plug-in connectors to provide a range from 1200 to 30,000 ohms.

Control resistance R-3 is a 200-ohm, 10-turn Helipot potentiometer, connected directly to the motor by a short length of thickwalled rubber tubing. This serves as a simple friction drive that slips if the Helipot reaches the end of its travel, so that limit switches are not required. When the controller is turned on, the Helipot is adjusted manually so that it operates near the middle of its range. The motor and Helipot operate at 27 r.p.m. which provides amply rapid corrective action.

Optimum control action results when the voltage drop across R-3 is small compared to the total voltage output of the power supply. A value of R-3 of 200 ohms is suitable for currents ranging from about 5 to 100 ma. when the power supply output is between about 100 and 300 volts.

When the controller is turned on the power supply must always be connected to a load, because on open circuit resistor R-3would be driven to its lower limit. In actual use in coulometric titrations a dummy resistor is provided whose resistance is adjusted to match the impedance (resistance plus back e.m.f.) of the cell. By means of a single pole-double throw switch the current is directed either to the cell or the dummy resistor.

With the automatic coulometric titrator previously described (3) this switching is done automatically by a relay which also controls the timing clock. By placing a switch in one of the motor supply leads the motor and R-3 can be inactivated, but the amplifier can be left on and warmed up, during periods when it is desired to open the circuit. The switch S in series with the biasing battery should be opened when the power supply circuit is opened, as otherwise the small current drain from it detracts from the constancy of its voltage.

Because of the small time lag associated with the mechanical linkage in the servo loop, this type of controller does not have the instantaneous response of all electronic devices. However, the fact that the current tends to change only relatively slowly renders instantaneous response unnecessary. The speed of response of the present instrument is more than ample in actual practice.

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## Precision Determination of Low Concentrations of Carbon in Metals

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The determination of low concentrations of carbon in metals (<0.01%) is increasing in importance with the newer developments in metallurgy. This paper describes an extremely simplified apparatus which gives precise results. The method is applicable to a variety of metals besides iron and steels. Carbon, with a mean deviation of  $\pm 0.0002\%$ , was determined in manganese, molybdenum, copper, lead, titanium, and tin. A routine precision of  $\pm 0.0002\%$  was obtained on carbon of standard steel samples with concentrations as low as 0.0028%. The calculated lower limit of detection is 0.002 mg. of carbon which in a 1-gram sample is equivalent to 0.0002%.

THE determination of low concentrations of carbon in metals  $\mathbf{I}$  (<0.01%) has been a problem for many years and is becoming of increasing importance with the newer developments in metallurgy. Many authors have described (3, 4, 6, 8-10) complicated high vacuum equipment for the determination and have obtained good precision at the expense of maintaining and manipulating the complex high vacuum systems. Other drawbacks to these methods are the requirement for multiple loading devices in some cases, elaborate oxygen purification systems, etc.

Various attempts were made to simplify the determination. A procedure by Wells (7) utilizes the conventional resistance furnace to burn the sample in oxygen and then isolates the evolved carbon dioxide by freezing it out with liquid oxygen. The trap containing the carbon dioxide is transferred to a vacuum measuring system similar to that described by Yensen (9). A more recent modification by Hickam (1) used a mass spectrometer for the analytical part of a vacuum fusion system.

This paper describes an extremely simplified apparatus for the determination of carbon in a variety of metals. This apparatus is capable of performing the determination with precision equivalent to that which has been reported previously. The simplification is obtained in three ways: the elimination of the high vacuum requirements using only a standard rotary vacuum pump to remove the products of the preceding determination and the excess oxygen: the use of a commercially available high frequency combustion unit; and the use of a simple differential freeze-out technique (2) for isolating the carbon dioxide.

## APPARATUS

The simple schematic diagram is shown in Figure 1. The oxygen, which is the prepurified grade, is obtained from a gas cylinder through the usual reducing valve. It is further purified by passing it over copper oxide heated to about 400° C. and then through Ascarite to remove carbon dioxide and water.

The combustion unit is the standard Lindberg H-F combustion unit, H, used without modification except that the values are bypassed and the oxygen flow is regulated at the gas cylinder by means of the reducing valve and a screw clamp on the Tygon connecting the cylinder with the copper oxide tube.

The combustion products are led by Tygon tubing to the asometer, G. The usual manganese dioxide trap is eliminated. The standard gas buret, leveling bottle, L, and leveling solution (sodium sulfate plus sulfuric acid) of the Lindberg volumetric carbon determinator for the gasometer is used for convenience so that the equipment can be easily converted back for the precision determination of carbon in the higher concentrations (5). A is the four-way stopcock of the Lindberg gas buret, while B and C are precision vacuum-type stopcocks. If a special gasometer were constructed, A could be a precision three-way

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stopcock and B eliminated. The trap, T, is about 160 ml. volume and of conventional design. The one-way mercury valve, V, utilizing a fritted disk of medium porosity is placed in the system as a relief valve for a safety measure.

Vacuum-tight joints between the glass and Tygon tubing are obtained and cemented by dipping the Tygon tubing into acetone to soften it before slipping over the glass tubing.

#### PROCEDURE

The system up to stopcock B is pumped out thoroughly by flaming the cold trap, T, and the glass tubing, in order to remove any contaminants. It is advisable to flame the cold trap during the subsequent operations if the pressure in the system cannot be lowered by a reasonable time of pumping. A residual pressure of about 2 microns is more than adequate for most work.

The portions of the system before B are flushed with oxygen allowing the gas to escape through the leveling bottle, L. This flushing technique is essential and should be done between determinations. It is convenient to flush while the carbon dioxide measurement is being made so that the elapsed time of the overall measurement is not increased.



The sample, approximately 1 gram, is weighed into a standard Lindberg "cuplet," being careful not to handle the container with the fingers. It is placed in position and the apparatus swept out for a minute or two and then the leveling solution is allowed to fill the gasometer by cracking open the breech of the combustion tube. The breech is closed and the combustion is started immediately. The gas flow is controlled from the pinch clamp by watching the level of the solution in the gasometer and is adjusted that the level of the solution is stationary or advances slightly during the burning but never moves upwards. This precaution will provide sufficient oxygen to assure a complete burn without the necessity of a flowmeter. The products of combustion are swept into the gasometer, and stopcock A is turned through 90° to contain the gases in the gasometer. At this step the sample cuplet is removed to prevent overheating the gaskets in the combustion chamber. The evacuated part of the system is checked to make sure

The evacuated part of the system is checked to make sure that the residual pressure is about 2 microns of mercury and the pump is shut off. C is turned to connect the system with the pump, isolating the McLeod gage. A Dewar flask containing liquid nitrogen is placed around trap T and by manipulating A and B, the gases including the oxygen are slowly condensed in the trap. When the leveling solution has risen to the neck of the gasometer, A is turned back to connect with the oxygen system through the heater. The system is flushed with oxygen during the succeeding steps in preparation for the next run.

After a minute or two to condense the gases in the cold trap, the vacuum pump is started to remove the oxygen. It is good practice to leave B open and pump back to A until the short length of Tygon tubing starts to collapse. Then B is closed and the pumping continued until the oxygen is removed. When most of the oxygen is removed, as indicated by the sound of the pump, the three way stopcock, C, is turned to connect the trap and McLeod gage with the pump. Pumping is continued until the pressure drops to 2 microns which takes about 10 minutes. C is then turned to isolate the pump and form the measuring volume (comprised of the trap and McLeod gage). The liquid nitrogen is replaced with an alcohol slush (2) whose temperature is between  $-100^{\circ}$  and  $-105^{\circ}$  C.

This slush is prepared by adding liquid nitrogen to methanol containing a few milliliters of water in a Dewar flask and stirring vigorously. This cooling bath is of a sirupy consistency and the temperature is maintained at the desired level during the working day by periodic additions of liquid nitrogen. If the temperature falls below that desired, the alcohol will freeze, but can be rectified by adding a few milliliters of methanol. The bath made up in this manner will last for many months, requiring merely the periodic additions of liquid nitrogen to maintain the required temperature.

At this temperature (about  $-102^{\circ}$  C.) the carbon dioxide will sublime into the measuring volume where the pressure is determined with the McLeod gage. The pressure is followed until a constant value is obtained (about 12 minutes).

A blank for the determination is measured by going through the procedure without burning a sample. This is equivalent to determining the blank on the oxygen used which is the major source of the blank value. With the simple clean-up system of oxygen, the blank is still only about 2  $\gamma$  of carbon which is negligible and does not justify more elaborate precautions.

Although the amount of carbon can be calculated knowing the McLeod gage constant, volume of the system, and an approximation of the system temperature, etc., in actual practice the equation

Mg. of C = 
$$(h_1^2 - h_2^2)K$$

is used, where  $h_1 = \text{length}$  of the gas column in the sealed capillary of the McLeod gage in millimeters for the sample,  $h_2 = \text{length}$  of the gas column in the sealed capillary of the McLeod gage in millimeters for the blank, K = experimentally determined constant for the entire system. K is determined by carrying known standard samples through the procedure.

The standard was a special low-carbon stainless steel which is used as a secondary standard and has been repeatedly checked by a precision macromethod (5). The carbon content of this material is 0.038%. The variations in temperature have a negligible effect on the

The variations in temperature have a negligible effect on the value of K over the normal ambient room temperature and can be neglected. The use of the experimentally determined constant gives the most accurate results because it includes unknown variables as the temperature distribution in the apparatus, deviations from a perfect gas, precise determinations of the McLeod gage constant, precise determination of the analytical volume, and any adsorption effects in the equipment. The experimentally determined constant for the simplified apparatus is  $4.18 \times 10^{-6}$  when  $h_1$  and  $h_2$  are measured in millimeters. The theoretical constant derived from estimates of the average temperature and the measured volume of the system, assuming that carbon dioxide is a perfect gas, is  $4.3 \times 10^{-6}$ , which confirms the contention that only carbon dioxide is measured by this proceedure.

#### EXPERIMENTAL RESULTS

The accuracy of the method was proved by the determination of National Bureau of Standards standard samples. The data are presented in Table I and adequately demonstrate the accuracy of the method.

Table l	. Accuracy	of Simplified Met	hod
Sample	No. of	NBS Value, $\%$	KAPL
Designa-	Deter-		Value,
tion	minations		%
NBS 166	5	$\begin{array}{c} 0.0274 \\ 0.0114 \ (0.014)^{a} \\ 0.010^{b} \\ 0.0028 \end{array}$	0.0268
NBS 55a	7		0.0119
NBS 55c	5		0.0098
NBS 131	10		0.0028
<sup>a</sup> See Murray and <sup>b</sup> Provisional valu	Ashley (3) and e.	this text.	

NBS Sample 55a has been used by a number of the previous investigators. The results summarized by Murray and Ashley (3) show that, although the NBS recommended value is 0.014, the average value obtained by six cooperating laboratories using varied equipment and over a long period of time is 0.0114, which is in better agreement with the KAPL value of 0.0119. Stanley

Table II.	Precision of Simplified Method						
Sample Designation		Carbon, %	t	Deviation from Mean, %	Standard Deviation, %		
NBS-131 (silicon steel)		$\begin{array}{c} 0.\ 00327\\ 0.\ 00254\\ 0.\ 00271\\ 0.\ 00262\\ 0.\ 00279\\ 0.\ 00306\\ 0.\ 00301\\ 0.\ 00248\\ 0.\ 00278\\ 0.\ 00285 \end{array}$		$\begin{array}{c} 0.00046\\ 0.00027\\ 0.00010\\ 0.00019\\ 0.00002\\ 0.00025\\ 0.00020\\ 0.00033\\ 0.00033\\ 0.00003\\ 0.00003\\ \end{array}$			
NBS-55c (ingot iron)	Mean	$\begin{array}{c} 0.0028_1\\ 0.00931\\ 0.00950\\ 0.01000\\ 0.00966\\ 0.01029\\ 0.0007\\ \end{array}$	±	0.0001 0.00044 0.00025 0.00025 0.00009 0.00054	0.00025		
NBS-55a (open hearth iron)	Mean	$\begin{array}{c} 0.0097i\\ 0.01176\\ 0.01151\\ 0.01203\\ 0.01204\\ 0.01185\\ 0.01193\\ 0.01193\\ 0.01199\\ \end{array}$	Ŧ	0.00031 0.00036 0.00016 0.00017 0.00002 0.00006 0.00012	0.00039		
NBS-166 (stainless steel)	Mean	0.02767 0.02635 0.02706 0.02513 0.02766 0.02766	± +	0.00014 0.00090 0.00042 0.00029 0.00164 0.00089	0.00019		

and Yensen ( $\theta$ ) point out that the carbon content of NBS-55a obtained by the low pressure combustion method is lower (0.0109%) than the total carbon content (0.0114%). This is ascribed to the fact that an adsorbed carbon-containing component is pumped off at 600° C. When the carbon is determined by nonvacuum methods the higher value is obtained. The results of this investigation (0.0119%) confirm this observation that combustion, performed under nonvacuum conditions, produces values in good agreement with total carbon value ( $\theta$ ).

The precision of the method, as indicated by the mean deviations, are given in Table II. The data were obtained with 1gram samples without any added accelerator or flux.

General application of the method was demonstrated by determining the carbon content of pure manganese, molybdenum, copper, lead, titanium, and tin and the results are presented in Table III.

## DISCUSSION

The use of the high frequency combustion unit with an oxygen pressure somewhat above atmospheric makes possible the attainment of very high combustion temperature (>1700° C.) In the usual low-pressure systems, temperatures much in excess of 1300° C. are prohibited without elaborate precautions because of the collapsing of the furnace tube. In the methods using resistance furnaces the attainment of temperatures above  $\sim 1100^{\circ}$  C. becomes a difficult problem.

The use of the high-frequency unit also eliminates the necessity for multiple loading systems  $(\mathcal{G}, \mathcal{S})$  with the manipulation of the samples with magnets, loading buckets for nonmagnetic materials, etc. Also, the use of the cheap disposable cuplets is a tremendous advantage compared to the problems associated with crucibles, crucible liners or pretreating the combustion boats required by the other methods  $(\mathcal{G}, \mathcal{G}, \mathcal{S})$ . No evidence of excess carbon has been found by using these cuplets without previous treatment, as long as they are handled in a quantitative manner to avoid pickup of grease from the fingers, or minute shreds of paper by placing the cuplets on notebooks, table tops, or weighing the samples on paper. Another source of contamination is from cork- or rubber-stoppered containers; Bakelite closures or stoppers wrapped with aluminum foil are preferred for precision work. The necessity of properly degreasing the sample is discussed by Wells (7) and Stanley and Yensen (6).

No difficulty is encountered from oxides of sulfur. At the highcombustion temperatures in the excess of oxygen only sulfur trioxide is formed. This material is either absorbed by the leveling solution or frozen out in the cold trap. Because the carbon dioxide is measured at about  $-102^{\circ}$  C., the vapor pressure of the sulfur trioxide is negligible. Accordingly, the activated manganese dioxide trap is eliminated.

The gasometer was inserted in the system to provide flexibility in handling the combustion products. This device regulates the gas flow and makes possible the pumping out of the determination system during the combustion step. Its main function is to prevent overloading the freeze-out trap. It was assumed during the design of the system that the rapid oxygen flow rate during the combustion (about 1000 ml. per minute) would not allow sufficient time to freeze out the carbon dioxide if the gasometer was eliminated. Conversely, the gasometer made possible the use of the simplest kind of cold trap.

In addition to the high frequency combustion unit, the success of the method depends on the effectiveness for carrying excess oxygen for carbon dioxide when it is condensed in the cold trap and the selective differential freeze-out technique (2). The rapid, quantitative transfer of the carbon dioxide from the gasometer to the cold trap is due to the condensation of the excess oxygen in the cold trap. The large volume of oxygen "carries" the carbon dioxide with it in a manner analogous to a diffusion pump. Thus one is not dependent on the pure diffusion of the carbon dioxide molecules in the temperature gradient but the collisions with the larger number of oxygen molecules accelerates the rate of transfer of the carbon dioxide and makes it quantitative in a short time. Because oxygen has a vapor pressure of about 100 mm. at liquid nitrogen temperatures, it is easily removed by pumping while the carbon dioxide, water, pump oil vapors, sulfur trioxide, etc., remain frozen in the cold trap.

Table III. Carbon Content of Various metals
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Designation	No. of Deter- minations	Carbon, %	Mean Deviation, %
Electrolytic manganese	8	0.0019	+0.0002
Fused molybdenym	ă	0 0120	0.0002
Electrolytic copper	4	0.0042	0.0003
Test lead (C.P.)	$\overline{6}$	0.0004	0.0001
Crystal bar titaniuma	4	0.0179	0.0003
Tin shot (C.P.)	4	0.0016	0.0001
<sup>a</sup> 300-mg, samples using m	anganese meta	l as a bedding	material.

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The usual method of liberating the carbon dioxide for measurement is either to allow the trap to warm up to room temperature, or to replace the liquid nitrogen trap with a dry ice-acetone or Cellosolve mixture. In the first case, one must cope with all the components initially frozen out in the cold trap in the measurement. In the second case, although sulfur trioxide and the pump oil vapors will remain frozen out, sulfur dioxide, if present, and water have an appreciable vapor pressure which will produce a significant error in the pressure measurement. These considerations are pointed out by Murray and Niedrach (4) and by Horton and Brady (2). The use of the methanol slurry (2)eliminates these interferences. At  $-102^{\circ}$  C. water has a completely negligible vapor pressure ( $<15 \times 10^{-6}$  mm.) while carbon dioxide has a vapor pressure of 85 mm. Therefore, only carbon dioxide sublimes into the analytical volume whereas the water, sulfur trioxide, pump oil vapor, etc., remain frozen in the trap.

In the usual employment of the Lindberg high-frequency combustion unit, the oxygen is introduced at the top of the combustion chamber and the products of combustion are removed by the breech connection. Equally good results are obtained by reversing the gas flow, which allows for shorter Tygon connections with the rest of the apparatus because the gas control valves on the instrument are bypassed.

A modification of the procedure is to use a detachable cold trap and expand the gases, after removal of the oxygen, into a mass spectrometer (1).

Because of the high-combustion temperature attainable, no accelerator or flux need be used in most cases for millings, turnings, or drillings. Titanium and zirconium are exceptions. Complete combustion in some cases requires more time than is available if the oxygen flow is maintained at the prescribed flow rate of about 1000 ml. per minute because of the size of the gas buret. A simple modification in the procedure corrects this difficulty and indicates the flexibility of the equipment.

After the start of the ignition, the oxygen flow is regulated at the pinch clamp to supply just sufficient oxygen to support the combustion. This is accomplished by watching the liquid level in the gasometer and maintaining it stationary for the desired duration of the combustion period. If the oxygen flow rate is not fast enough, the leveling solution will start to rise in the gasometer which serves as a very convenient flowmeter.

By this means, combustion times of as long as 5 minutes were used to effect the complete oxidation of tin, copper, lead, and molybdenum. Without this precaution, the materials are incompletely oxidized and the carbon values are low and erratic. When the metals are completely converted to the corresponding oxides, there is little or no inductive coupling and rapid decrease in the temperature of the sample, as shown by its color, indicates the end of the combustion. The combustion products are then swept into the gasometer and the determination is continued.

The very reactive metals, such as titanium and zirconium, represent the opposite extreme. These metals flash-burn too quickly when burned alone and produce very erratic results. They are best handled by introducing a flux which will maintain

the combustion for about 5 minutes to ensure the complete oxidation of the carbides. This flux should alloy with the sample to slow down the oxidation and form a homogeneous melt.

After some experimentation manganese was found to be the ideal bedding material for this purpose. By itself, it burns more smoothly and at a higher temperature than iron, but more important, it forms homogeneous melts with titanium and zirconium. Also it is one of the lowest in carbon content, so that the correction required is small.

Because only a small quantity of crystal bar titanium was available, 300-mg. samples were used with 0.5 gram of electrolytic manganese as the flux. No zirconium values are reported because the available material was so high in carbon that it was outside the range covered in this paper. Nevertheless, good controllable burnings were obtained with zirconium (and titanium) on 1-gram samples plus 1 gram of manganese.

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## Quadrivalent Uranium as a Reducing Titrant

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Quadrivalent uranium has a redox potential of -0.334 volt (10) in 0.1N acid solution and is, therefore, a moderate reducing agent. It is fairly stable to atmospheric oxidation. Quadrivalent uranium, as a reducing titrant, is one of the most stable titrants that can be used for the direct titration of ferric iron. Although it can be used for several other titrations, it has no advantages over existing reagents, and, in most cases, is less convenient.

CEVERAL reagents utilizing a less common valency state of The element concerned have found wide applications in titrimetric analysis. Chromium(II) (5), titanium(III) (7), and vanadium(III) (15) salts have long been used as reducing titrants and, more recently, tungsten(III) (12), copper(III) (1), nickel(III) (13), silver(III) (20), manganese(III) (3), and molybdenum(V)(16) have been examined.

Quadrivalent uranium is one of the few elements, in an unusual valency state, which has not been examined extensively as a titrimetric reagent, although there is considerable information in the literature (8, 14) concerning the titration of uranium-(IV) by other reagents.

The only work described hitherto, in which quadrivalent uranium is used as a titrant, is by Vortmann and Binder (17),

who suggested the use of uranous sulfate as a reagent for the determination of nitrate, chlorate, chromate, manganese dioxide, and ferric iron. A visual method was used for the detection of the end point (the disappearance of a ferric thiocyanate color), and consequently only ferric iron could be determined directly. The other oxidants were determined in one of two ways:

An excess of ferrous sulfate solution was added, and the ferric iron formed was titrated with uranous sulfate

An excess of uranous sulfate solution was added, and the excess was back-titrated with ferric iron.

The present authors have extended the work of Vortmann and Binder and have examined the possibility of titrating several reducible substances. The end points in these titrations were detected potentiometrically, using a Mullard "magic-eye" potentiometric bridge (Mullard Ltd., Century House, Shaftesbury Ave., London W.C.2., England).

Although quadrivalent uranium can be used for several titrations, it has little advantage over existing reagents; in most cases, it is less convenient.

#### EXPERIMENTAL

Preparation of the Reagent. The uranous solution was prepared under the conditions described by Willard and Diehl (19) for the quantitative reduction of uranyl salts. About 43 grams of analytical reagent grade uranyl acetate were dissolved in 500 ml. of 4N hydrochloric acid and the solution was allowed to 1026

Uranyl acetate was used as starting material for the preparation of the reagent, rather than the chloride, because the chloride was not commercially available in a sufficiently pure state. Uranyl nitrate could not be used, because the nitrate ion interferes in the titration of quadrivalent uranium with dichromate (14).

(14). Stability of the Reagent. Vortmann and Binder (17) prepared their uranous sulfate solution by reduction of uranyl sulfate with zinc in sulfuric acid. The solution was stored in a stoppered dark bottle and found to be 25% oxidized after 28 days.

The present authors also stored the uranous solution in a dark bottle and fed it into the bottom of a buret by means of a siphon and two-way tap system. The solution was, therefore, open to the air. The reagent was titrated against standard potassium dichromate solution, each morning over a period of 10 days. No appreciable oxidation took place over the first 24 hours, but the titer increased steadily thereafter.



Figure 1. Variation of Reducing Power of the Uranous Reagent with pH

A reductor buret (4) using silver or lead as reductant, was not found suitable, under ordinary conditions, for maintaining the uranium solution in the quadrivalent state.

When the reagent was stored under a layer of petroleum ether (boiling point 100° to 120° C.), no oxidation took place even after several weeks. A layer of petroleum ether was also placed on top of the solution in the buret, to minimize oxidation, but this was not essential, provided that the buret was filled with fresh solution for each titration. The layer of petroleum ether, however, gave a sharp definition of the meniscus in the buret. Standardization of the Reagent. The uranous solution was

Standardization of the Reagent. The uranous solution was standardized by titration against a standard solution of potassium dichromate, prepared by weight from the oven-dried analytical reagent grade salt.

Twenty milliliters of 0.1N potassium dichromate solution were run into a 400-ml. beaker from a buret, and 10 ml. of 2N hydrochloric acid were added. The solution was stirred magnetically and titrated potentiometrically with the uranous solution, using a bright platinum indicator electrode and a saturated calomel reference electrode. The reaction was found to be somewhat sluggish at room temperature, making the end point rather indistinct, but when the titration was carried out-at 60° C., satisfactory and consistent results were obtained. Similar results were also obtained using 0.02N solutions.

## TITRATION OF VARIOUS IONS

Cerate, permanganate, and vanadate solutions were determined in a way similar to the titration of dichromate. It was again necessary to work at elevated temperatures, but  $60^{\circ}$  C. was found to be sufficient, provided the titration was performed carefully in the vicinity of the end point. In the titration of permanganate, however, low results were obtained owing to oxidation of the hydrochloric acid by the permanganate at the high temperature, with concomitant loss of chlorine. This still occurred when the hydrochloric acid medium was replaced by sulfuric acid, because there was still hydrochloric acid in the uranous solution used as titrant. When the titration was carried out in the cold, no change in the oxidation potential of the system occurred, but the permanganate was decolorized. The color change at the end point was from red to greenish yellow through yellow-brown. This gave satisfactory results if the end point was taken when the first tinge of green appeared in the solution. However, when the titration was carried out hot in the presence of 20 ml. of Zimmerman-Reinhardt preventive solution, good results were obtained.

The titration of ferric iron was more troublesome. Even at  $60^{\circ}$  C. the reaction was somewhat sluggish, causing indistinct end points and slightly high results. It was found preferable to add the titrant slowly near the equivalence point (0.1 ml. per minute), rather than to work at higher temperatures, as in the titration of quadrivalent uranium with ferric iron (14).

Ferricyanides were titrated in a way similar to ferric iron, but the reaction was even more sluggish, presumably owing to the stabilizing effect of the cyanide ions. However, provided that the titration was carried out slowly near the equivalence point, it was possible to obtain satisfactory results.

## TITRATION OF OTHER REDUCIBLE SUBSTANCES

Chlorate, persulfate, and peroxide were reduced slowly by the uranous solution even at elevated temperatures, and satisfactory end points could not be obtained. In hot solution oxidation of the hydrochloric acid occurred, which caused low results. All three radicals could, however, be determined, over a range of concentration, through ferrous ion.

A suitable excess of ferrous ammonium sulfate solution was added to a known amount of the oxidant and the mixture warmed to  $60^{\circ}$  C. for 5 to 10 minutes. The solution was made 1N with respect to hydrochloric acid, and then titrated with the uranous solution, as for ferric iron. Consistent results were obtained, and the amounts of uranous solution consumed corresponded to the calculated values.

Vortmann and Binder (17) determined nitrate in this way by boiling the nitrate solution with ferrous sulfate in the presence of hydrochloric acid in an atmosphere of carbon dioxide. An attempt was made to apply the more convenient conditions employed by Leithe (11), but the high acidity made the subsequent titration with uranous solution too sluggish. When the acidity was reduced to approximately 1N, by the addition of sodium bicarbonate, sodium carbonate, or sodium hydroxide, a satisfactory end point was still not obtainable, because the high electrolyte concentration upset the redox potentials of the system.

Finally, as the potential ranges involved in the reduction of ferric ion and dichromate were sufficiently well separated, it was possible to determine these two ions in the presence of each other.

### VARIATION OF REDOX POTENTIAL WITH ACIDITY

Searching studies of the redox potentials of the uranium(IV)uranium(VI) system have already been published (6, 9), but for the sake of completeness, the redox potential of the system was determined over a range of pH.

A suitable amount of potassium dichromate solution was titrated with uranous chloride in the usual way, and then an equal amount of uranous solution was added in excess. The acidity was adjusted to the required pH, and the potential of the system was measured using bright platinum and saturated calomel electrodes. The potential with respect to the hydrogen electrode was then calculated. This was repeated over a range of pH.

The graph obtained (Figure 1) shows a fall in potential (and therefore an increase in reducing power) with increasing pH. It

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would appear that at an even higher pH the uranous solution would be a still more powerful reductant, but as pH 2 was approached the solution became dark brown in color, and beyond this, precipitation of the uranium occurred.

## CONCLUSIONS

Quadrivalent uranium is only a moderate reducing agent in acid solution, but can be used for the direct titration of the stronger oxidants and ferric iron. Weak oxidants may be determined through their oxidation of ferrous iron. Ferric iron may also be determined in the presence of dichromate.

Quadrivalent uranium is the most stable reagent that has been used for the direct titration of ferric iron, apart from mercurous nitrate (2), but the method is less convenient and holds no advantage over those involving existing reagents.

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# Infrared Absorptiometry for Quantitative Determination of Boron Hydrides in Presence of Pentaborane

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Physical methods of determination for the boron hydrides are much easier to apply than conventional chemical methods. Of the available physical methods infrared absorption in the gas phase offers a number of advantages. Data are presented for the determination of the four component system containing diborane, tetraborane and dihydropentaborane in a relatively larger amount of pentaborane. The data are combined into equations which permit the direct calculation of the pressure in mm. for each boron hydride from measurements of the absorbance, D, at four wave lengths.

 $P_{B_{2}H_{6}} = -0.58 D_{4.06} + 0.52 D_{4.65} - 3.49 D_{5.54} + 14.81 D_{6.15}$   $P_{B_{4}H_{10}} = -3.64 D_{4.05} + 68.55 D_{4.65} - 1.45 D_{5.54} + 0.47 D_{6.15}$   $P_{B_{5}H_{9}} = -7.25 D_{4.05} + 14.65 D_{4.65} + 164.10 D_{5.54} + 0.45 D_{5.15}$   $P_{B_{5}H_{11}} = 39.86 D_{4.05} - 74.85 D_{4.65} + 2.48 D_{5.54} - 5.12 D_{6.15}$ 

At a total pressure of 100 mm. the minimum detectable amount of diborane is about 0.2 mole %; for tetraborane it is about 0.6 mole %; and for dihydropentaborane it is about 0.7 mole %.

THE quantitative and qualitative chemical determination of boron hydrides is a very difficult matter because of the similarity of the chemical properties of the compounds. With further research it may be possible to determine an individual material chemically, but at present physical methods of analysis are much easier to apply. Four techniques have been used in this laboratory and certain advantages can be claimed for each. To set infrared absorption analysis in its proper perspective with respect to these other methods, a brief review of each method is presented here.

Cryoscopic determination of pentaborane content is an excellent method for checking the quality of purified pentaborane and can probably be used as an analytical tool for material which contains as much as 20 mole % impurity. However, the method tells nothing of the kind of impurity that is present.

The mass spectrometer is an excellent method for qualitative identification of boron hydrides. It also can be made roughly quantitative, but really good analytical work requires the presence of an internal gas standard with a convenient mass such as argon (3). The difficulty then is in sample preparation and computation of results, since all significant peak heights must be related to the argon-40 peak.

A method which has been much used in this laboratory is low temperature analytical distillation. It is a time-consuming operation, and, since some of the boron hydrides are very unstable, subject to errors and perhaps impossible in a few cases.

Infrared absorption analysis suffers from none of these difficulties. The method is reasonably rapid and the sample is in the spectrophotometer a relatively short time, so that decomposition during analysis is not a serious problem. No standard gas samples need be maintained if the infrared spectrophotometer is kept in good operating condition. The technique identifies the impurities that are present except for those that occur in trace amounts, for which more data would be desirable.

#### EQUIPMENT

A conventional vacuum system consisting of a manifold, liquid nitrogen trap, oil diffusion pump, and mechanical pump was used



Figure 1. Infrared Absorption Curve of Diborane, B<sub>2</sub>H<sub>6</sub>, Obtained on Baird Double-Beam Spectrophotometer At 100.1 mm. at 25° C.



for handling the various gases used in the calibration work. A direct reading, low-pressure manometer similar to the Pearson manometer (4) was built to measure pressure in the 0 to 20-mm. range. In the laboratory model, the mercury for adjusting the height in the reference bulb was controlled by a borosilicate glass

Since some of the boron hydrides are unstable, it is important to make the determinations as soon after filling the cells as possible. With a little effort and much cooperation it was possible to fill a cell and obtain a spectrum within half an hour, during which time decomposition is negligible. This is best demon-

syringe; a micrometer was used for accurately measuring the plunger displacement. When two components were present, the gas in lower concentration was added directly to an evacuated cell and its pressure measured. The valve on the cell was closed and the vacuum manifold evacuated. Next pentaborane was vaporized into the vacuum manifold until it was near the final pressure. Then the valve on the cell was opened and the pentaborane was admitted very rapidly so as to cause as much turbulence as possible. The total pressure was read on an ordinary U-tube manometer.

 Table I. Absorbance of Pure Boron Hydrides at Various Pressures

 (Base line points at 3.50 and 5.92µ. Reference beam, air, no cell used)

Perkin Elmer Spectrum No.	Pressure, Mm. of Hg				Absorbance at			
	$B_2H_6$	B4H10	B5H9	$B_{5}H_{11}$	4.05µ	$4.65 \mu$	$5.54\mu$	6.15µ
528	1.23				0.012	0.003	0.005	0.063
1062		9.38			0.293	0.161	0.000	0.004
753		99.5			1.55	1.59	-0.010	0.0744
1064	• •	102.7			1.57	1.46	-0.007	-0.002
395			100.1		0.007	0.017	0.611	0.137
717			100.8		0.006	0.016	0.614	0.146
527			101.0		0.008	0.013	0.611	0.145
422			102.3		-0.002	0.016	0.610	0.145
733				9.58	0.256	0.015	0.008	0.010
1075	• •			10 25	0 295	<u>000</u>	0 010	0 015
723				59.5	1.40	0.056	0.053	0.030
<sup>a</sup> This sample cor	itains abou	it 1 mole 9	% of B2H6.					

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strated by data on tetraborane which is the most unstable of these boron hydrides (3). The sample used to obtain spectrum 753 (Table I) was kept at room temperature for an additional 2 hours and a new spectrum obtained. The data indicate that 2.1 mole % of the 99.5 mm. of tetraborane decomposed in this time. Since the samples used for calibration were at much lower pressures, even less decomposition would be expected. Nevertheless, the gradual accumulation of solid deposits in the cells is bothersome, and each cell was thoroughly evacuated as soon as the spectrum was obtained.

The infrared absorption instrument for this work is a doublebeam Perkin Elmer Model 21, Serial No. 161, equipped with a sodium chloride prism. Complete spectra from 2 to 15 microns were recorded for each sample using slit program 3. The spectra were run without suppression; the running time was approximately 15 minutes per spectrum. Cell-window losses were eliminated by measuring absorbance from an arbitrary base line intersecting the spectra at 3.50 and 5.92 microns, rather than by use of an evacuated cell in the reference beam. All gas cells in this work are 5.00-cm. Baird-type cells, and absorptivities are quoted for a 5.00-cm. path length.

## MATERIALS

The pentaborane was 99.9 + % pure as determined by freezing point analysis.

Diborane was purified by distillation in a Podbielniak lowtemperature distillation column of 100 theoretical plates.

Tetraborane was purified first by distillation, and then by pumping on the stirred liquid at  $-80^{\circ}$  C. until diborane disappeared from the infrared spectrum.

Dihydropentaborane was purified by distillation and pumping on the stirred liquid at  $-80^{\circ}$  C. until both tetraborane and diborane disappeared from the infrared spectrum.

## **RESULTS AND DISCUSSION**

In studies on the pyrolysis of diborane, four boron hydrides are generally found in greatest abundance: diborane, tetraborane,



At 59.5-mm. pressure at 26.5° C.

## ANALYTICAL CHEMISTRY

Table II.	Absorbance of	Mixtures	Containing	Pentaborane	and	One	Other
		Borg	on Hydride				

	(Base line poi	nts at 3.50	and $5.92\mu$	. Reference	e beam, air	, no cell u	ised)		
Perkin Elme	r	Pressure, Mm. of Hg				Absorbance at			
Spectrum No	b. $B_2H_6$	$B_4H_{10}$	B5H9	B <sub>5</sub> H <sub>11</sub>	$4.05 \mu$	4.65µ	5.54µ	$6.15\mu$	
531	1.23		99.7		0.014	0.012	0.603	0.219	
529	3.13		97.9		0.025	0.010	0.595	0.363	
530	5.13		95.3		0.038	0.009	0.574	0.486	
533	7.15		94.4		0.080	0.012	0.577	0.620	
1057		1.98	98 1		0.059	0.040	0.610	0.163	
1058		4.58	96.3		0.145	0.085	0.590	0.160	
1059		10.09	90.7		0.318	0.182	0.558	0.138	
1061		13.42	88.2		0.415	0.243	0.528	0.126	
735			97.9	1.09	0.036	0.018	0.598	0.141	
736			101.0	4.84	0.123	0.021	0.618	0.158	
1078			95.0	6.63	0.205	0.014	0.581	0.146	
737			90.4	9.57	0.251	0.025	0.559	0.133	
1079	••	••	88.7	11.80	0.344	0.019	0.551	0.142	

Table III. Absorptivities for Pentaborane and Other Boron Hydrides in Pentaborane

(Absorbance per mm. pressure per 5 cm. cell length)

	Wave Length			
$\mathbf{Compound}$	4.05µ	$4.65\mu$	$5.54\mu$	6.15µ
B2H6 B4H10 B5H9 B5H11	$\begin{array}{c} 0.0087 \pm 0.0016 \\ 0.0305 \pm 0.0011 \\ 0.00003 \\ 0.0280 \pm 0.0020 \end{array}$	$\begin{array}{c} 0.0000\\ 0.0162 \pm 0.0014\\ 0.00012\\ 0.0015\end{array}$	$\begin{array}{c} 0.0002 \\ -0.0001 \\ 0.00608 \pm 0.00006 \\ 0.0011 \end{array}$	$\begin{array}{c} 0.0679 \pm 0.0029 \\ 0.0006 \\ 0.00143 \pm 0.00004 \\ 0.0013 \end{array}$

pentaborane, and dihydropentaborane. It is true that small amounts of hexaborane, decaborane, and a solid polymer,  $(BH)_x$ , may also be present. The vapor pressure of the latter two is so small at room temperature that they do not interfere in the analysis, and less than a few tenths of 1 mole % of hexaborane are present as indicated by mass spectrometer analyses (3). Essentially, then, a four-component system is being employed.

Four simultaneous equations are needed, and these should be based on more or less characteristic absorption peaks in the interest of sensitivity. Inspection of the infrared absorption curves (the diborane curve was obtained on a Baird double-beam spectrophotometer with the cooperation of D. L. Harms and J. E. Scott) shows that pentaborane has a characteristic peak at 5.54 microns and with only slight interference by dihydropentaborane. Diborane and pentaborane are the chief absorbers at 6.15 microns; at 4.65 microns tetraborane is predominant. Dihydropentaborane absorbs at 4.05 microns, but so do diborane and tetraborane. If this absorption peak is used, it is anticipated that the dihydropentaborane determinations will not be as accurate as the others. There is another dihydropentaborane absorption peak at 8.55 microns but the location of a good  $I_0$  line is difficult because of a deposit which absorbs in the region 7 to 8 microns and is slowly laid down on the windows of the cell during repeated use. This deposit has never interfered with the 2to 6.5-micron region to date, therefore, it is felt that all bands below 6.5 microns are safe from this difficulty. The absorption due to the deposit is illustrated in the  $I_0$  line which is recorded on the curve for dihydropentaborane.

The general technique is to measure the extinction coefficients of binary mixtures of the various materials in pentaborane at the four wave lengths chosen; 4.05, 4.65, 5.54, and 6.15 microns. The total pressure was maintained near 100 mm. except for the pure sample of dihydropentaborane. Initially, two base-line points were chosen for each of the characteristic peaks used for analysis according to the method recommended by Heigl, Bell, and White (2). Considerable difficulty ensued because these points were often on the shoulders of major absorption peaks. Finally, it developed that the simplest, and most effective plan to date is to select two base-line points for all four peaks. The points so chosen are 3.50 and 5.92 microns. One peak at 6.15 microns lies outside of the base-line points, but the extrapolation

is relatively short and no difficulty is involved in obtaining consistent results. The absorbance values read from the charts are recorded in Table I for pure compounds, and in Table II for mixtures of pentaborane and one other boron hydride. Table III records the absorptivities for pentaborane and other boron hydrides when mixed with pentaborane at a total pressure of about 100 mm. In the case of pentaborane the absorptivities were determined at the various wave lengths from spectra of the pure material. These values were then used to calculate absorptivities of other boron hydrides from the mixtures. The latter were combined with coefficients calculated from data on the pure materials, and a standard deviation was determined for the whole set. The only exception to this procedure is for diborane at 6.15 microns. Since it is a small molecule, it is subject to pressure broadening, and the extinction coefficient calculated from the data for spectrum 528 of Table I is low for this reason. It is not included in the extinction

coefficient calculated for Table III.

Considerable difficulty was encountered in trying to keep tetraborane pure. The material decomposes readily and one of the products is diborane which absorbs strongly at 6.15 microns. It is probable that the extinction coefficient for tetraborane at 6.15 microns in Table III is somewhat high.

The absorptivities of Table III can be combined in a series of equations at the respective wave lengths.

$$D_{4.05} = 0.0087 P_{B_2H_5} + 0.0305 P_{B_4H_{10}} + 0.00003 P_{B_5H_4} + 0.0280 P_{B_5H_{11}}$$
(1)

$$D_{4.65} = 0.0162P_{B_4H_{10}} + 0.00012P_{B_5H_5} + 0.0015P_{B_5H_{11}}$$
(2)

$$D_{5.54} = 0.0002P_{B_2H_5} - 0.0001P_{B_1H_{16}} + 0.00608P_{B_5H_5} + 0.0011P_{B_5H_1}$$
(3)

$$D_{6.15} = 0.0679 P_{B_2 H_6} + 0.0006 P_{B_4 H_{10}} + 0.00143 P_{B_5 H_9} + 0.0013 P_{B_5 H_{11}}$$
(4)

where

D = absorbance at indicated wave length P = partial pressure in mm.

Initially, these equations were solved for each sample by a method of successive approximations. The first step is an estimation of the concentration of pentaborane from Equation 3 by ignoring other compounds. The justification for this is that pentaborane is by far the strongest absorber at 5.54 microns. The value for pentaborane is now used to help estimate tetraborane from Equation 2, then diborane is estimated from Equation 4 using all estimates made so far. The final estimate is made from Equation 1 for dihydropentaborane. Then the process is repeated using the estimates to calculate more precise values in succession again. The second approximation of each material is usually within the experimental error of the spectrophotometer.

Obviously this is a rather clumsy method of calculation. What we would like to do is to be able to solve directly for the partial pressure of each material. The equations were inverted to form a new set of equations explicit in the concentrations by the
method of Crout (1). The application of the method to Equations 1, 2, 3, and 4 results in the following series in which the pressure can be calculated directly.

$$P_{B_2H_6} = -0.58 D_{4.05} + 0.52 D_{4.65} - 3.49 D_{5.54} + 14.81 D_{6.15} (5)$$

$$P_{\mathbf{B}_{4}\mathbf{H}_{10}} = -3.64 \ D_{4.05} + 68.55 \ D_{4.65} - 1.45 \ D_{5.54} + 0.47 \ D_{6.15}$$
(6)

$$P_{\mathbf{B}_{\mathbf{5}}\mathbf{H}_{\mathbf{6}}} = -7.25 \ D_{4.05} + 14.65 \ D_{4.65} + 164.10 \ D_{5.54} + 0.45 \ D_{6.15}$$
(7)

$$P_{B_{5}H_{11}} = 39.86 \ D_{4.05} - 74.85 \ D_{4.65} + 2.48 \ D_{5.54} - 5.12 \ D_{6.15}$$
(8)

The application of these equations to unknown, impure samples of pentaborane vapor, which may contain small amounts of hexaborane not accounted for by this work, usually results in calculated pressures the sum of which is within 2% of the measured pressure. The following data illustrate the kind of analysis

Measured total pressure	e of unknown, 100.9 mm. at 27° C.
Wave Length, $\mu$	Absorbance
$\begin{array}{c} \textbf{4.05} \\ \textbf{4.65} \\ \textbf{5.54} \\ \textbf{6.15} \end{array}$	$\begin{array}{c} 0.464 \\ 0.133 \\ 0.499 \\ 0.658 \end{array}$
Compound	Calculated Pressure, Mm.
B2H6 B4H10 B8H9 B8H11	$\begin{array}{r} 7.8 \\ 7.0 \\ 80.8 \\ 6.4 \\ \hline 102.0 \end{array}$

The minimum detectable amount of diborane is about 0.2 mole %, while for tetraborane it is about 0.6 mole %, and for dihydropentaborane, it will run about 0.7 mole %.

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# **Radiochemical Assay of Cobalt-60**

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A method of determining cobalt-60 in biological materials has been found accurate within  $\pm 3\%$  in over a thousand determinations. The sample was wet-ashed, separated as cobaltic hydroxide, and electroplated as cobalt metal from a fluoborate buffer. A special methane-flow proportional counter of cylindrical geometry is used so that a maximum precision and sensitivity of detection of cobalt-60 are obtainable. Aside from the biochemical and clinical applications of this assay, the data on the quantitative electroplating of cobalt are applicable to its standard gravimetric determination.

**URRENT** interest in the chemical and biological reactions  $\lambda$  of cobalt has directed attention to the use of the isotopic tracer, cobalt-60. The recent work on vitamin B<sub>12</sub> has made this tracer of considerable biochemical and clinical importance. Cobalt-60, with a half life of 5.3 years, has many properties which should make it a very favorable isotope for experimentation. Not only is it available with high specific activity, but during decay, it emits a beta particle of 0.31 mie.v. and two gamma rays of 1.17 and 1.33 m.e.v. energy. The major difficulty in its use has been the preparation of samples for radiochemical assay. The method employed should be both sensitive and precise.

Early work on cobalt in biological systems utilized the mixture of radioactive isotopes which emitted a mixture of gamma rays, produced with the cyclotron. Sheline, Chaikoff, and Montgomery (10) simply dried their samples, but Comar and Davis (4) first subjected their material to dry-ashing, followed by counting with a dipping counter placed in a solution of the ash. Comar, Davis, and Taylor (5) utilized electrodeposition of the cobalt; because they were only detecting gamma rays, they found no advantage in the more elaborate procedure. Others have used a combination of dry-ashing and precipitating as cobalt sulfide (6).

These techniques applied to cobalt-60 either suffer from the low sensitivity of gamma ray detection or the lack of precision when beta particles are counted in dried unprocessed samples. The serious interference of both iron and the alkaline earths complicated the plating method of Comar and Davis (5), but was avoided in biological samples by an involved and time-consuming procedure.

Ballentine and Stephens (1) precipitated cobaltic hydroxide from wet-ashed material, detecting the beta emission with a highly efficient and accurate methane-flow beta counter (2). They reported an error of  $\pm 2.35\%$ , but in routine use unaccountable disagreements, as high as 25%, between duplicates are occasionally observed. An attempt to perfect a precise and dependable assay has led to the procedure described. This method has been in routine use on a thousand samples of various biological materials, covering a period of more than a year, without any of the erratic results previously experienced, and with an over-all accuracy of  $\pm 97\%$ .

The necessity of controlling a number of conditions has led to the following analytical sequence: wet-ashing with perchloric and nitric acids, collection of the cobalt carrier as cobaltic hydroxide and resolution as the chloride, electrolytic plating as cobalt metal from a potassium fluoborate buffer, and counting the beta particles in a methane-flow counter of high efficiency and reproducible geometry.

### **RESULTS AND DISCUSSION**

Ashing Procedure. The original procedure (1) employed a sulfuric-nitric acid digestion. This has been abandoned in favor of a perchloric acid digestion, because it is much smoother and more rapid. However, its main advantage is in the reduction of the amount of neutral salts resulting from the neutralization in a later step. With the sulfuric-nitric acid digestion, it was necessary to employ a large dilution to prevent both the crystallization of sodium sulfate and the production of such a high solution

density that the cobaltic hydroxide precipitates could not be collected by centrifuging. These difficulties are all avoided with the perchloric acid digestion, which should present no hazard with reasonable precautions.

Preliminary Separation of Cobalt. Cobalt carrier is routinely added to the samples before ashing. While this makes subsequent procedures easier and more accurate, it is not essential. Even with the perchloric acid digestion, the electrolyte concentration of the digest does not permit direct electroplating. Therefore, the sample is subjected to a preliminary separation from other neutral salts.



Rate of Plating and Current Density in Figure 1. **Potassium Fluoborate Buffers** 

Voltage between electrodes, 4.0 volts Per cent of cobalt deposited in 15 minutes from buffer solution containing 0.1 mg. cobalt per 10 ml. buffer Total current density. milliamperes

0

Previous experience (1) showed that the nitrosonaphthol methods were inapplicable to high electrolyte solutions, because the reagent precipitated. The precipitation as cobalt metal by reduction with sodium borohydride is described by Hoekstra (9). This reduction gives excellent recoveries on numerous occasions, but is unreliable for routine assay. With pure cobalt solutions, the very narrow range of pH and absence of oxygen necessary to achieve quantitative precipitation and to prevent resolution of the cobalt can be reproduced. However, when digests are analyzed, such control was found to be impractical. The sulfide precipitation, while simple and reproducible, involves the use of a toxic and obnoxious gas. It was found (1) that cobaltic hydroxide can be quantitatively precipitated from an alkaline perborate solution. The amorphous precipitate, used before as the counting sample, is difficult to filter from a solution of high salt content. Peptization of the semicolloidal precipitate is probably the major factor contributing to the erratic behavior of the former assay. This is completely avoided by centrifugation. Semicolloidal cobaltic hydroxide, passing through a sintered-glass filter of medium porosity, is readily and quantitatively collected by moderate centrifuging. Further, the precipitate may be readily redissolved as the chloride.

Several other elements-iron, silica, and the alkaline earths, which are the major contaminants in biological samples-also precipitate under these conditions. The alkaline earths and silica give no trouble since they are eliminated during the electrolytic plating. The amounts of iron encountered were insufficient to interfere in the assay, although iron plates along with the cobalt. Therefore, no attempt was made to eliminate the iron from the samples. Should the procedure without carrier be used, it might be necessary to employ some method of eliminating this contaminant. After resolution of the cobaltic hydroxide precipitate in 6N hydrochloric acid, the iron may be readily removed by extraction with ether.

Electrolytic Plating. Cobalt has the reputation of giving very

poor plates under the conditions, which in general were the same as those for iron, employed in the past for radiochemical assay. These plating solutions usually were alkaline ammonia solutions, used to maintain the cobalt in solution as the ammono complex. This complex is of sufficient strength to make deposition very slow, and the high electrolyte concentration leads to high current densities with etching and uneven deposition of the plate. Therefore, fluoborate was used as a complexing agent to plate from a weakly acid solution.

Figure 1 shows the relationship between the pH of the potassium fluoborate buffer and both the speed of plating and current density. The pH chosen for plating is modified by two further factors. Table I shows the resolution effect of acidic fluoborate buffers on the cobalt plate. A neutral or alkaline medium is essential to abolish this effect. However, the high current densities at a more alkaline pH lead to such a local alkaline reaction at the cathode that a copious precipitate of cobalt hydroxide is formed with concomitant severe losses. Stirring during the plating process merely augments the resolution effect in acidic buffers, while it does not avoid the precipitation of cobalt hydroxide in the neutral range. To balance these effects, a point of low current density was selected (pH 3.1) for the deposition of the major portion of the cobalt. After this has occurred, the pH may then be raised without danger of precipitation; at the same time, resolution is prevented during the removal of the electrolyte. Various

Table I.	<b>Resolution of Cobalt Plates in Potassium</b>
	Fluoborate Buffers

Buffer, pH	% Resolution
2.0	14.2
3.1	20.1
8.20	4.9 0:06

<sup>a</sup> Per cent dissolved on 30 seconds' contact with a saturated potassium fluoborate buffer. <sup>b</sup> pH obtained when 1 ml. of 1N NaOH is added to 10 ml. of plating solution of pH 3.1.



Plating of cobalt from potassium fluoborate buffer of pH 3.1 at 3.5 volts (current density between 2 and 4 ma. per square inch)



Radiation source was 0.4 m.e.v. beta radiation from cobalt-60
Brackets define operating range
A. Variation in counting rate with voltage on center wire using gain 100 and discriminator +5 volts
B. Counting rate vs. discriminator level at gain 100 and 4.3 kv.
C. Counting rate vs. relative amplifier gain at discriminator level of +5 volts and 4.3 kv. across counter



conventional siphoning methods and displacement of the electrolyte were tried, but none of these were as successful and reproducible as decreasing the hydrogen ion concentration near the end of the plating period.

The efficiency and extent of the plating process are shown in Figure 2. This experiment was carried out by plating for a prescribed time, carefully withdrawing an aliquot from the sample cup while the plating was continued, adding the carrier to the aliquot, and plating to completion. Counts were then made of the cobalt-60 in these aliquots. Quantitative plating may be carried to extremely low cobalt concentrations (less than  $5 \times 10^{-9}$  gram) without any major alteration in the procedure, permitting direct assay of many samples without the addition of carrier.

Counting Techniques. The procedure described could undoubtedly be applied to many different styles of detection systems and shapes of plating electrodes. The authors prefer methane-flow proportional counting (2). The high sensitivity to beta particles and the year-to-year stability of counting results are the most compelling reasons. Early experiments with end-on versions of the flow counter (2) showed that the bead on the center wire casts a shadow of low detection sensitivity and greatly reduces the reproducibility of the method by augmenting the effect of slight variations in the evenness of the electroplate. If

self-absorption is also to be kept to a minimum, an advantage with beta particle counting, then as large an area as possible, commensurate with a minimum volume, is desirable. This is best met by a cylindrical geometry, and, therefore, the counter and plating assembly shown in Figures 4 and 6 were constructed.

The equipment employed The equipment employed was the high voltage supply used by Higinbotham (8) (Model 400 B power supply, John Fluke Engineering Co., Springdale, Conn.) and the nonoverloading amplifier used by Bornstoin Brower and Bernstein, Brewer, and binson (3). The quantitat-Rubinson (3). ing system was either the Model 100 counting rate meter used by Elmore and Sands (7) or the decade version of the Multiscaler (Model 1060. Atomic Instrument Co., Cambridge 39, Mass.).

Figure 3 shows operation curves for the counter. Inspection reveals that at a suitable operating point with this equipment (gain, 50 to 100; voltage, 4.1 to 4.4; discriminator, +3 to +10 volts), the counter has a slope of 0.70% per hundred volts.

By the use of a standard cobalt-60 sample procured from the National Bureau of Standards, the geometry of the counting sys-tem was determined to be 49.1% when 1 mg. of carrier cobalt was present. The specific thickness of this cobalt plate is 0.0622 per square cm. The counter operates within counting mg. statistics; hence, the precision of the assay need not be limited by The background rate shielded is 14.6 the counter system. counts per minute.

#### Table II. Precision and Accuracy of Assay

Source of Error	$\pm \sigma^a$ , %
Counting, observed	0.11
Plating <sup>c</sup>	0.35
Total for all steps	3.01 3.04

<sup>a</sup> Based on ten replicates.

<sup>b</sup> Calculated as  $(\sqrt{N/N})100$  where N = total number of counts recorded per sample (predetermined count with time variable). <sup>c</sup> Replicate plates of a standard Co<sup>60</sup> solution containing as carrier 1 mg.

cobalt as chloride per sample.  $\sigma_{\text{plating}} = \pm \sqrt{\sigma^{2} \text{total} - \sigma^{2} \text{counting}}$ <sup>d</sup> Replicate assays of culture media to which were added aliquots of the standard Coso solution. 1

$\sigma_{ m ashing-precipitation}$	=	$\pm v$	$\sigma^{2}$ total	-	$\sigma^{2}_{counting}$	 $\sigma^2$ plating

The recent introduction of scintillation counters for the measurement of gamma radiation merits consideration. The primary advantage of counting gamma radiation is the absence of major self-absorption of the radiation, and consequent freedom in the nature of the sample to be counted. Often little or no sample manipulation, such as ashing or plating, is required. However, the relative sensitivity of scintillation counters is low owing to their high background counting rate. If a significant level of activity is considered to be twice the background count before correction, then the described proportional counter is 5 to 50 times more sensitive. In distinction to proportional counters, scintillation detectors do not show as well defined operational plateaus, nor do they operate within decay statistics. These characteristics limit both their long-term counting stability and their precision.

Accuracy and Precision. The use of isotopic tracers in an analytical procedure provides a ready and elegant means of

## ANALYTICAL CHEMISTRY



Figure 5. Plating Assembly Rack Showing Plating Vessels and Electrodes

checking the accuracy of a gravimetric analytical procedure. The values for the errors given in Table II represent the accuracy, as well as the precision,  $\pm 3\%$ , of procedure.

The major source of error is in the ashing and precipitation of the samples. The error of the detection and counting is limited solely by the statistical nature of the decay process, the counter operating within this statistical fluctuation. While it might be possible to reduce the error of the plating process further, this does not seem pertinent in view of the  $\pm 3.01\%$  error in the ashing and precipitation steps. The material selected for this test was a microbiological medium which had given the most erratic results in the former procedure (1). Since the over-all error is satisfactory for most tracer application, no systematic attempt has been made to lower it. The most likely source of error is loss in the separation of the cobaltic hydroxide precipitate.

A wide variety of biological materials has been assayed for cobalt-60 by this method. These materials have been predominantly mold (.Neurospora), mycelia, and homogenates, but in addition animal tissues, feces, urine, green plant tissues, bacteria, isolated B12, and proteins have been assayed. These assays are run in duplicate routinely. The variation between duplicates has been the expected range predicted from Table II. This is based on nearly a thousand individual assays.

### APPARATUS

**Plating Vessels.** The construction of the plating cups is clear from the exploded view, Figure 4, A. They are machined from 1-inch diameter 18-8 stainless steel tubing with 1/s-inch wall. The over-all length is 2.25 inches. The function of the Lucite plug is to confine the plating solution to a region of the vessel (Figure 4, B) which will be in a homogeneous electrostatic field during counting (Figure 4, C)

Plating Assembly. A variety of units may be devised to hold the vessels and anode during the plating proper. A rack to hold eight cups is shown in Figure 5. The construction is of Lucite. The 0.5-inch electrolytic copper bar anode for copper-flashing the cups is at the left end position. The plating anodes are con-structed from 14-gage platinum wire soldered to 12-gage copper bus bar and then cast in plastic. The power supply is a 6-volt storage battery. The voltage is adjusted by means of a 25ohm, 10-watt potentiometer across the batter

Counter. Figure 6 shows a cross section through the counter designed for the plating vessels of Figure 4. The sample cup, 1, designed for the plating vessels of Figure 4. The sample cup, 1, with its cobalt plate and bronze cap, 2, slides into the tapered guide cylinder, 4. A bail, 3, holds it tight against a neoprene gasket, 7, the pressure being exerted by the spring, 6, and the outer sliding sleeve, 5. Methane is passed into and out of the counter by channels and connectors, 8. The center wire, 11, is 2-mil tungsten and terminates in a 5-mm. diameter brass plumb bob, 12. This later is positioned to be completely in the recessed bob. 12. portion of the sample cup without shorting across to the cap, 2. The other end of the center wire is either welded or silver soldered to a piece of nickel wire which passes through the silver tubing, 10, in the Lucite insulator, 9. Connection is made to the amplifier and high voltage supply through an Amphenol Teflon connector, Type 82-805, 13.

### REAGENTS

Perchloric Acid, 1 to 1. Reagent grade 70% perchloric acid is diluted with an equal volume of distilled water. Both the per-

chloric and nitric acids are dispensed from automatic burets. Nitric Acid, concentrated.

Sodium Hydroxide, 9M, carbonatefree

Sodium Perborate Tetrahydrate, reagent.

Aerosol OT, 0.01%. (Fisher Scientific Co.)

Hydrochloric Acid, 6N.

Cupric Fluoborate Solution, purified. (Baker and Adamson)

Potassium Fluoborate Buffer. Fluoboric acid (0.5M) is made by diluting Baker and Adamson fluoboric acid (42 to 45%) according to the acid content on the label. Potassium hydroxide (5M)is added until the pH measured with the glass electrode is 3.1. After standing for 30 minutes, the sizable precipitate is

filtered off in a sintered-glass filter funnel of medium porosity. The reagent keeps indefinitely, but after several weeks it may require refiltering.

Sodium Hydroxide, 1.5N.

### PROCEDURE

A sample containing about 100-mg. dry weight of Ashing. tissue is placed in a 100-ml. Kjeldahl flask. To this are added 1 mg. of cobalt as cobalt chloride to act as carrier, 30 ml. of con-centrated nitric acid, and from 2 to 4 ml. of 1 to 1 perchloric acid (the amount depends on the quantity of organic matter present). A glass bead is added to prevent bumping, and the sample is boiled until the liquid remaining in the flask is no greater than the amount of perchloric acid used, and white fumes are produced. The pink color of a cobalt salt should be clearly discernible. A brown color indicates insufficient oxidation, in which case more nitric and perchloric acid should be added and the sample again boiled down

Precipitation. The ashed sample is diluted to 50 to 60 ml. with distilled water and brought carefully to boiling with frequent agitation to prevent bumping. Using 9M sodium hydroxide, the solution is made just alkaline to phenolphthalein, Using 9M sodium hy-



Flow Proportional Beta Counter for Plating Vessels

and 5 or 6 drops of the sodium hydroxide are added to give a pH of about 13. About 75 mg. of sodium perborate tetrahydrate are added immediately, and the sample is shaken well to mix, brought again to boiling, and another 25 mg. of sodium perborate are The sample is then boiled for about 30 seconds and added. allowed to cool.

The sample is transferred to a 100-ml. centrifuge tube, and the Kjeldahl flask is rinsed with an 0.01% Aerosol OT solution and then with water. The inside of the centrifuge tube is rinsed down with a small amount of the Aerosol OT solution, and the sample is centrifuged for 15 minutes. The supernatant liquid is removed by suction, using a glass filter stick (Pyrex No. 39535-10F), the inside of the tube washed down with about 10 ml. of Aerosol OT solution to wash out any remaining soluble salts, the sample again centrifuged for 15 minutes, and the supernatant liquid removed using the same filter stick. In handling the centrifuged samples, great care must be taken to prevent jarring

the tube, as the precipitate is very easily stirred up. The filter stick is left in the centrifuge tube, which is washed down with 2 ml. of concentrated nitric acid to dissolve the precipitate, a few drops being run into the inside of the filter stick. The centrifuge tube is then placed in boiling water and allowed to stand until cool, during which time 1 ml. of 6N hydrochloric acid is added to the sample. When cool, the acid is blown out of the filter stick into the centrifuge tube with compressed air, and the filter stick filled with water and blown out again, and finally rinsed off and removed. The inside of the tube is washed with about 10 ml. of Aerosol OT solution, and the sample placed in an oven at 95 to 100°C. to dry (too high a drying temperature produces an insoluble precipitate). When thoroughly dry, about 5 ml. of water are added to aid in the removal of any residual acid, and the sample is again taken to dryness, after which it can be stored indefinitely before proceeding with the analysis

Plating. A very light copper flash (1 ampere for 30 seconds) is Plating. A very light copper hash (1 ampere for 30 seconds) is applied to the inside of the stainless steel cup, using a solution of cupric fluoborate. The cup is rinsed with distilled water and dried with a clean cloth. The surface must be smooth and the plate unbroken. (In putting the Lucite plug into the bottom of the tube, care must be taken to see that all touching surfaces are clean and that the metal cap is screwed on tightly, to prevent

Some of the sample leaking into the metal cap.) The centrifuge tube containing the sample to be analyzed is rinsed with 5 ml. of saturated potassium fluoborate buffer, of

1035

pH 3.1. The tube is placed in boiling water for a few minutes, and then in cold water. The lip of the tube is lightly greased, the solution is poured into the plating cup, and then the centrifuge tube is rinsed with another 5 ml. of potassium fluoborate buffer, using 3-, 1-, and 1-ml. portions. The tube is centrifuged briefly to collect all drops of solution hanging on the sides of the tube, and this solution is added to the plating cup. The plating is carried out at 3.5 volts, which in this assembly

gives a current of 40 to 80 ma. (current density 2.1 to 4.2 ma. per square cm.). After 1.5 hours of plating, 1 ml. of 1.5N sodium hydroxide is added, and the plating is continued for another 1.5 hours. The cup is then removed from the plating setup, the solu-tion poured out quickly, and the cup rinsed with 95% alcohol and drained dry. It is then ready to be counted. The total elapsed time from start to finish of the assay is 3 days. By careful programming, 50 samples can be handled in a working week.

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# **Colorimetric Determination of Propionaldehyde**

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Aldehydes are a principal or minor product of many chemical and biochemical processes. Often, it is desirable not only to determine the amount of aldehyde(s) produced, but the specific aldehyde(s). Propionaldehyde reacts with ninhydrin in concentrated sulfuric to produce a deep red-blue color suitable for spectrophotometric analysis at a wave length of 595 m $\mu$ . The method is sensitive to 1 to 3  $\gamma$  of propionaldehyde with an accuracy within about  $\pm 5\%$  and a precision within about  $\pm 1\%$ . The method is specific for propionaldehyde in the presence of several other aldehydes and it may be used for the determination of propionaldehyde in solutions and in air.

SPECIFIC method was desired for propionaldehyde in A the presence of other aliphatic aldehydes. Numerous techniques have been proposed for the determination of micro and macro amounts of aldehydes, but no specific method for propionaldehyde was reported in the literature.

The determination of one aldehyde in the presence of another is difficult because of their closely related chemical properties; but some specificity in reaction of certain aldehydes was obtained with colorimetric methods. Because of this, various reactions were investigated that might form a specific color with propionaldehyde.

Propionaldehyde reacts with 1,2,3-triketohydrindene (ninhydrin) in the presence of concentrated sulfuric acid to yield an intense red-blue color suitable for quantitative measurement at a wave length of 595 m $\mu$ . This reaction was found to be almost specific for propionaldehyde. The chemical reactions between propionaldehyde and ninhydrin that develop color are not known but this test is similar to the reaction of ninhydrin when used as a reagent for amino acid determinations (1, 3, 5).

### **BASIC PROCEDURE**

Apparatus. Spectrophotometer, Beckman Model DU. Tubes, Lewis-Benedict, graduated at 12.5 and 25.0 ml., Corn-ing No. 7860. Flowmeter, Fisher Laboratory Model No. 11-163. Cooling bath, 25° C., any type. Pinet, 0.5 ml. Ostwald type.

Pipet, 0.5 ml., Ostwald type.

Aeration apparatus, Figure 1.

Reagents. Propionaldehyde Standard, obtained from East-man Kodak and purified by method of Smith and Bonner (4). Analyzed 99.99% by the bisulfite method.

Sulfuric Acid, specific gravity 1.84, Mallinckrodt, low nitro-

Ninhydrin Reagent, 3% solution Eastman Kodak Co. No. 2495, 1,2,3-triketohydrindene crystals in a 5% aqueous sodium bisulfite solution which is stable at room temperature. Sodium Bisulfite, 5% aqueous solution. Glycine Solution. Dissolve 5 grams of U.S.P. glycine (amino-

acetic acid) in 250 ml. of saturated sodium bicarbonate solution. Sodium Bicarbonate Solution, saturated aqueous.

Preparation of Calibration Curve. Prepare a solution of propionaldehyde standard in 5% sodium bisulfite solution to con-tain 1 mg. per ml. Transfer 0.0, 0.5, 1.0, 2.0, and 3.0 ml. of the standard solution to five 50-ml. volumetric flasks and dilute each to volume with water or bisulfite solution.

Transfer 0.5 ml. of each dilute standard to Lewis-Benedict tubes, add 0.1 ml. of distilled water, cautiously add 4.0 ml. of concentrated sulfuric acid, and mix. Stopper tubes as a protection against dilution due to water vapor and cool to  $25^{\circ}$  C. in a water bath.

Add 0.2 ml. of ninhydrin reagent and mix. Stopper tubes and allow them to stand for 1 hour at 25° C. to develop full color intensity. Dilute contents to 12.5 ml. with concentrated sul-furic acid and mix. Transfer to a 1-cm. Corex cell and read the obscributes of 505 mg, with the spectrophotometer using the absorbance at 595 m $\mu$  with the spectrophotometer, using the zero prepared standard as the blank for the zero absorbance setting of the instrument.

Plot concentration against absorbance on linear graph paper. The above standards equal 0, 5, 10, 20, and 30  $\gamma$  of propional dehyde, respectively.

Determination. The determination was made according to the described method, but the sample was prepared so that a 0.5-ml. aliquot of bisulfite solution does not contain more than 30  $\gamma$  of propional dehyde.

Applications. Formaldehyde does not react with ninhydrin to give a color complex, but inhibits the formation of color with propionaldehyde and ninhydrin (Table I). Therefore, formaldehyde must be removed prior to determination of propionaldehyde. The presence of formaldehyde can be conveniently proved by the method of Eegriwe (2).

Table I. Effect	of Formaldehyde or	n Determination
Concn. of Propion- aldehyde, γ	Concn. of Formal- dehyde, γ	Absorb- ance
20 20 20 20 20 20 20 20 20	$\begin{array}{c} 0\\ 5\\ 10\\ 20\\ 30\\ 50\\ 100\\ \end{array}$	$\begin{array}{c} 0.800\\ 0.740\\ 0.690\\ 0.600\\ 0.505\\ 0.385\\ 0.200\\ \end{array}$

The formaldehyde may be separated from the propionaldehyde effectively by passing the aldehyde vapors through a glycine (aminoacetic acid) solution which will combine the formaldehyde:

# $HCHO + NH_2CH_2COOH \rightarrow CH_2NCH_2COOH + H_2O$

n-Butyraldehyde will interfere if present in larger quantities than propionaldehyde. Equal or smaller amounts of n-butyraldehyde in relation to propionaldehyde may be determined successfully.

Determination of Propionaldehyde. IN A MIXTURE OF ALDEHYDES CONTAINING FORMALDEHYDE. Prepare an aeration ALDEHYDES CONTAINING FORMALDEHYDE. Prepare an aeration train consisting of four tubes (Figure 1). Add 20 ml. of satu-rated bicarbonate solution to  $T_1$ , 20 ml. of 5% glycine-bicarbon-ate solution to  $T_2$ , 20 ml. of 5% bisulfite solution to  $T_3$ , and 15 ml. of bisulfite solution to  $T_4$ . Prepare a sample of aldehyde mixture in sodium bisulfite solution such that a 1.0-ml. aliquot will contain 1 to 3 mg. of propionaldehyde. Add to  $T_1$  a 1.0-ml. aliquot of the prepared mixture, connect all tubes in aeration train, and adjust air-flow, delivered either by vacuum or pressure through the train at the rate of 0.2 liter

by vacuum or pressure, through the train at the rate of 0.2 liter per minute as measured by the flowmeter. Continue aeration for 3 hours. Combine, quantitatively, the contents of  $T_3$  and  $T_4$  in a 50-ml, volumetric flask and dilute to volume with water. Analyze a 0.5-ml. aliquot of the solution for propionaldehyde.

Results on the determination of propionaldehyde in mixtures of formaldehyde by aeration are given in Table II.

Table II.	Determination of Propionaldehyde in
	Formaldehyde Mixtures

Composition of	of Mixture, Mg.	Propion-
Formal- dehyde	Propion- aldehyde	aldehyde Found, Mg.
3.0 5.0 3.0 5.0 3.0 3.0	$\begin{array}{c} 0.5 \\ 0.5 \\ 1.0 \\ 1.0 \\ 2.0 \\ 3.0 \end{array}$	$\begin{array}{c} 0.48 \\ 0.47 \\ 0.99 \\ 1.00 \\ 2.05 \\ 3.02 \end{array}$

### Table III. Determination of Propionaldehyde in Aldehyde Mixtures

С	omposition of 3	Mixtures, Wt. '	%	aldehvde
Acet- aldehyde	n-Butyr- aldehyde	Isobutyr- aldehyde	Propion- aldehyde	Found, Wt. %
29.28 71.63 49.50 35.00 15.65	$19.32 \\ 0.0 \\ 20.60 \\ 0.0 \\ 5.32$	$\begin{array}{r} 22.20 \\ 0.0 \\ 10.10 \\ 21.15 \\ 30.10 \end{array}$	$\begin{array}{r} 29.20 \\ 28.37 \\ 19.80 \\ 43.85 \\ 48.93 \end{array}$	$\begin{array}{c} 30.40 \\ 28.35 \\ 19.85 \\ 43.84 \\ 49.00 \end{array}$

IN A MIXTURE OF ALDEHYDES IN THE ABSENCE OF FORMALDE-HYDE. Weigh a sample of an aldehyde mixture into a volumetric flask containing 5% bisulfite solution and dilute to volume with bi-sulfite solution. From this solution, prepare a dilute sample such that a 0.5-ml. aliquot will contain 30  $\gamma$  or less of propionaldehyde and analyze.

Data for the determination of propionaldehyde in mixtures of aldehydes other than formaldehyde are presented in Table III.



- A C E F
- Junuara-taper 24/40 glass joints Tygon tubing Flowmeter, Fisher Laboratory Model No. 11-163 Three-way stopcock Traps, 25 X 200 mm., borosilicate tube

Table IV. Determination of Propionaldehyde in Air Samples

Calculated,	Found,
γ	γ
1000	990
500	500
5000	4960
50	48

IN AIR. Pass the air containing propional dehyde vapor through a train of three tubes containing 20 ml. of glycine-bicarbonate solution in  $T_1$  and 20 ml. of 5% bisulfite solution in  $T_2$  and  $T_3$  at the rate of 0.2 liter per minute. At the end of the sampling period, combine the bisulfite solution from  $T_2$  and  $T_3$  into a volumetric flask, dilute to volume with bisulfite solution, and analyze a 0.5-ml. aliquot containing 30  $\gamma$  or less of propional dehyde by the proposed procedure.

$(30 \gamma \text{ propional dehyde})$			
Time, Min.	Absorb- ance		
$\begin{array}{c} 0 \\ 15 \\ 30 \\ 45 \\ 60 \\ 75 \\ 90 \end{array}$	0.0 0.560 0.910 1.200 1.200 1.195		

Propionaldehyde determinations in known concentrations of air samples are presented in Table IV.

### **EXPERIMENTAL**

The effects of several variables were investigated for their influence on the color formation and quantitative applications of the reaction. The factors included the absorption curve, stability and intensity of color, time and temperature of reaction, stability and concentration of ninhydrin, concentration of acid, conformity to Beer's law, and interference from other compounds. A Beckman Model DU spectrophotometer was used to measure the color. Briefly, the following procedure was employed.

An aqueous solution containing 20  $\gamma$  of propionaldehyde was transferred to a Lewis-Benedict tube, 4.0 ml. of concentrated sulfuric acid added, and the solutions were mixed and cooled to 25° C. Ninhydrin reagent was added and the reaction was allowed to stand for 1 hour for complete color development. The samples were diluted to 12.5 ml. with concentrated sulfuric acid and read by means of the spectrophotometer.

Absorption Spectrum. Spectral-absorbance curves of the redblue complex, resulting from the reaction of propionaldehyde and ninhydrin, show a maximum absorption at 595 m $\mu$ .

Transmittance and Concentration. Calibration curves were determined for propionaldehyde by plotting absorbance against concentration. The compound gave a straight-line calibration originating at zero concentration and absorbance. The color obeys Beer's law for amounts of 1 to 30  $\mu$  of propionaldehyde in the total volume of 12.5 ml.

Temperature and Time of Reaction. A correlation exists between the temperature and the length of time required for color development. Samples were treated for color development at 0°, 25°, 50°, and 100° C. At 0° C., color development was not completed for almost 3 hours; at 25° C., color development was complete in 45 to 60 minutes; at 50° C., color developed in 10 minutes; and at 100° C., the color was formed almost immediately. However, the color is not stable at the higher temperatures of 50° and 100° C. Because heat destroys the ninhydrinpropionaldehyde complex, the reagent was added after the reaction mixture was cooled to 25° C. The addition of 0.2 ml. of ninhydrin reagent to the reaction did not raise the temperature sufficiently to affect color decomposition. Total color developed in approximately 45 minutes at 25° C. and remained constant for 30 minutes. A 1-hour period for color development at 25° C. was chosen for convenience. The time of reaction at 25° C. is illustrated in Table V.

Stability of Colored Complex. Propionaldehyde was treated to develop the colored complex. The absorbances of the samples were determined at time intervals. The color was stable for only 15 minutes after the final addition of sulfuric acid, after which a gradual decrease in intensity occurred. The stability data are recorded in Table VI.

**Reagent Stability.** One of the most critical factors was the instability of aqueous solutions of ninhydrin when exposed to the air or light. Oxidation proceeded rapidly and the reagent changed in its reaction with propionaldehyde during the day. When the ninhydrin was dissolved in a 5% sodium bisulfite

solution the reagent resisted oxidation and remained stable indefinitely. The bisulfite solution of ninhydrin remained colorless compared to the rapidly darkening of pink color of the aqueous ninhydrin solution. It was believed that the bisulfite formed an addition compound with the keto groups of ninhydrin, but did not interfere in the reaction.

**Reagent Concentration.** A 0.2-ml. aliquot of 3% or higher ninhydrin in 5% bisulfite solution was optimum. Lower concentrations than 3% resulted in less color development. Higher concentration resulted in full color development, but increased the color of the blank proportionately, for ninhydrin reagent is pink in the acid-water solution.

Concentration of Acid. The optimum acid content was approximately 83 volume % in the color development reaction (Table VII). Also, the sum of volumes of acid, water, and ninhydrin reagent in the reaction mixture was critical toward color development with propionaldehyde. Table VIII illustrates that a higher intensity of color developed using 4 ml. of acid in an 83.3 volume % solution than did other amounts of acid in a similar concentration.

Time.	Absor	bances of Propional	dehyde
Min.	10 γ	20 γ	30 γ
0	0.400	0.800	1.200
5	0.400	0.800	1.200
10	0.400	0.800	1.200
15	0.400	0.795	1.200
20	0.385	0.770	1.170
30	0.350	0.745	1.160

With lower concentration of acids (70 to 75 volume %), when the samples were allowed to stand for the 1-hour reaction time and then diluted to 12.5 ml. with concentrated acid, the color rapidly gained in intensity for 3 to 5 minutes after dilution. It appeared that the reaction might take place in two steps: (1) a rearrangement of the propionaldehyde in the lower concentration of acid, and (2) coupling with ninhydrin reagent in the stronger acid concentration. In the acid range of 80 to 85 volume % when samples stood for 1 hour, no additional increase in color intensity was noted after dilution to 12.5 ml. with concentrated acid. If samples were prepared and diluted to 12.5 ml. with concentrated acid prior to the hour of development time, very little, if any, color developed.

A 4.0-ml. volume of acid, 0.6-ml. volume of aqueous sample, and a 0.2-ml. volume of reagent was chosen as the optimum reac-

Sample	Reagent	Water	Acid	Acid,	$\begin{array}{c} { m Absorb} - \\ { m ance}^a \end{array}$
Vol.,	Vol.,	Vol.,	Vol.,	Vol.,	
Ml.	Ml.	Ml.	Ml.	%	
0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5	0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2	$\begin{array}{c} 0.0\\ 0.1\\ 0.2\\ 0.3\\ 0.4\\ 0.5\\ 0.6\\ 0.7 \end{array}$	$\begin{array}{c} 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \\ 4.0 \end{array}$	$\begin{array}{c} 85.1 \\ 83.3 \\ 81.7 \\ 80.0 \\ 78.4 \\ 76.9 \\ 75.4 \\ 74.0 \end{array}$	1.100 1.200 1.170 1.140 1.080 1.070 1.040 1.020

 $^a$  Absorbance read after 1-hour reaction time and final dilution to 12.5 ml. with concentrated sulfuric acid.

Table VIII.	Optimum Ar	nount of Tota	l Acid Present
Water Vol., Ml.	Acid Vol., Ml.	Acid, Vol., %	Absorb- ance
0.6 0.8 1.0 1.2 1.4	3.0 4.0 5.0 6.0 7.0	83.3 83.3 83.3 83.3 83.3 83.3	1.080 1.200 1.170 1.060 0.920

tion mixture. This dilution gave the highest precision, reproducibility, and color intensity.

Interference. In order to determine the specificity of the ninhydrin reaction toward propionaldehyde several carbonyl compounds were tested. Aqueous bisulfite solutions of the aldehydes were prepared, each to contain 1 mg. of aldehyde, and tested. These aldehydes included formaldehyde, paraformaldehyde, acetaldehyde, paraldehyde, *n*-butyraldehyde, isobutyraldehyde, crotonaldehyde, 2-furaldehyde, benzaldehyde, vanillin, salicylaldehyde, and acrolein. They produced no color, except acrolein, which darkened in the concentrated sulfuric acid, and *n*-butyraldehyde, which gave a red-color complex with ninhydrin. The intensity of the color with *n*-butyraldehyde was much less than that of propionaldehyde and absorbed at a wave length of 470 m $\mu$ . Formaldehyde did not react with color formation, but it inhibited the color formation of propionaldehyde when the two were present together.

Because ketones have many reactions similar to aldehydes, solutions of acetone and 2-butanone were tested. 2-Butanone gave no color reaction in the proposed test. Acetone gave no color in small concentrations, but 1000  $\gamma$  of acetone gave the characteristic color of the propionaldehyde test corresponding to 4  $\gamma$  of propionaldehyde. It was thought that perhaps the acetone contained a small amount of propionaldehyde. Some acetone was subsequently purified by the method of Werner ( $\beta$ ) and supposedly all aldehydes had been removed, but this product also gave a positive test corresponding to 0.4% propionaldehyde. Therefore, acetone might interfere if present in large concentration.

Ninhydrin reagent in a neutral aqueous media was used as a characteristic test for  $\alpha$ -amino acid compounds. Several amino acids—glycine, *dl*-valine, *dl*-lysine, *l*-proline, *l*-hydroxyproline, *l*-tyrosine, and *l*-arginine—were tested and reacted negatively under the conditions of this test.

Ninhydrin used under the given conditions was specific for propionaldehyde.

## SUMMARY

A colorimetric procedure for the specific determination of propionaldehyde has been presented. Only *n*-butyraldehyde, among the aldehydes tested, reacted to give a color, which absorbed at 470 m $\mu$  as compared to 595 m $\mu$  for propionaldehyde. The method is so sensitive that the presence of acetone would not interfere in the range of concentration used.

Formaldehyde must be removed prior to the analysis since it inhibits the formation of the ninhydrin-propionaldehyde color complex.

The proposed test, adapted for the determination of propionaldehyde in air and in mixtures of other aldehydes, will quantitatively determine propionaldehyde in amounts as low as 1 to 3  $\gamma$  and has an accuracy within  $\pm 5\%$  with a precision within  $\pm 1\%$ .

### ACKNOWLEDGMENT

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# Determination of Silver with 1,2,3-Benzotriazole

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A method for the determination of silver with 1,2,3benzotriazole has been developed which proves useful when silver is determined in the presence of the interfering metals commonly encountered. Ethylenediaminetetraacetic acid is used as a complexing agent. Only silver is precipitated by 1,2,3-benzotriazole in the presence of ethylenediaminetetraacetic acid and ammonium hydroxide. No interference from metals is found; chloride, bromide, and fluoride do not interfere, but iodide forms less soluble silver iodide precipitate. Cyanide and thiosulfate dissolve the precipitate of silver benzotriazolate. From 10 to 100 mg. of silver may be determined gravimetrically or volumetrically with an average error of 0.2 mg. or less. The procedure is simple, rapid, and accurate.

SILVER is commonly determined gravimetrically or volumetrically with chloride or thiocyanate. The methods are subject to the disadvantages of lack of specificity, low molecular weight, and sensitivity to light. There are many organic reagents being suggested for the determination of macro amounts of silver; however, none of them is specific for silver. It was found that 1,2,3-benzotriazole, named silvon because of its probable specificity for silver, may be used for the determination of silver in the presence of all metals without any interference if ethylenediaminetetraacetic acid (Versenate) is used as a complexing agent.

1,2,3-Benzotriazole was first suggested as a precipitant for silver by Remington and Moyer (3) and later by Tarasevich (5). According to them copper, cadmium, iron(II), nickel, cobalt, and zinc are also precipitated by 1,2,3-benzotriazole. Curtis (1)recommended 1,2,3-benzotriazole for the determination of copper.

When an ammonical solution of the reagent is added to a slightly acid or neutral solution containing silver ions in the presence of Versenate, a precipitate is formed which, when dried at 110° C., corresponds to the formula  $AgC_{5}H_{4}N_{3}$ .



The development of a volumetric method for silver determination was based upon the fact that silver benzotriazolate (silver silvonate) is soluble in the cyanide solution. When the precipitate is dissolved in a known amount of cyanide solution, the

Table I.	Metals Precipitated by 1	,2,3-Benzotriazole
Metal	Without Versenate	With Versenate
Cu(II)	Yellowish green	No precipitate
Fe(II)	Brown precipitate	No precipitate
Ni(II)	White precipitate	No precipitate
Cd(II)	White precipitate	No precipitate
Zn(II)	White precipitate	No precipitate
Co(II)	Brown precipitate	No precipitate
Ag(I)	White precipitate	White precipitate

excess cyanide may be back-titrated by the standard silver nitrate solution using potassium iodide as the indicator.

### REAGENTS

1,2,3-Benzotriazole Solution, 2.5%. Dissolve 2.5 grams of 1,2,3-benzotriazole (Eastman Kodak Co.) in 30 ml. of concentrated ammonium hydroxide and dilute to 100 ml. with water. Standard Silver Solution. Dissolve 3.0000 grams of pure silver nitrate in 500 ml. of water and 1 ml. of concentrated nitric

acid and make up to 1 liter with water. Versenate Solution. Dissolve 400 grams of disodium dihydrogen ethylenediaminetetraacetic acid in 500 ml. of water, add a

gen ethylenediaminetetraacetic acid in 500 ml. of water, add a sufficient amount of ammonium hydroxide to dissolve the Versenate, then dilute to 1 liter with water.

Cyanide Solution, 0.05M.

Potassium Iodide Solution, 2%.

# QUALITATIVE REACTIONS

Qualitative tests were performed upon several common metals. The results are shown in Table I. As reported previously (1-3, 5, 6), 1,2,3-benzotriazole precipitates iron(II), zinc(II), cobalt(II), cadmium(II), copper(II), and nickel(II). But in the presence of ammoniacal Versenate solution, 1,2,3-benzotriazole precipitates only silver. Therefore, the reagent is probably a specific precipitate for silver when ammonium hydroxide and Versenate are employed. This may also be an excellent way to separate silver from other metals when silver is an interfering metal.

# PROPERTIES OF PRECIPITATE OF SILVER BENZOTRIAZOLATE

Stability. It was found that the temperatures from  $110^{\circ}$  to  $150^{\circ}$  C. were safe for drying the precipitate. If dried overnight at 175° C., the precipitate began to decompose. Not only did the weight of precipitate decrease, but also its color changed from white to yellowish brown. It was found that the precipitate could be dried to constant weight in 2 hours at  $110^{\circ}$  C. Unlike silver halides, the silver benzotriazolate precipitate did not show sensitivity to light.

**Composition.** 1,2,3-Benzotriazole formed precipitates with bivalent metals (1-3); the ratio was 2 to 1. However, the reagent probably forms an insoluble salt with silver in the ratio of 1 to 1. The experimental data were in good agreement with the theoretical gravimetric factor, 0.4774.

Solubility. The silver precipitate was practically insoluble in most organic solvents and acetic acid. It was very soluble in concentrated acids such as nitric, sulfuric, and hydrochloric. Cyanide and thiosulfate also dissolved the precipitate.

### EFFECT OF pH UPON PRECIPITATION

The pH range was not critical for the precipitation of silver benzotriazolate. However, the precipitation should be carried out in ammoniacal solution in order to complex the interfering metals by Versenate. When precipitation was made at the acid medium, colloidal precipitates were found. Ammonium hydroxide tended to coagulate the precipitate.

# **RECOMMENDED PROCEDURE**

Gravimetric Procedure. To a solution containing from 10 to 100 mg. of silver is added about 1 to 10 grams of disodium salt of Versenate, depending upon the amounts of interfering metals present. The solution is made neutral or slightly acid with ammonium hydroxide or nitric acid and heated to 60° to 90° C. Sufficient reagent solution, containing a slight excess of the amount necessary, is added to precipitate completely the silver present in the solution. For 10 to 100 mg. of silver, satisfactory results are obtained using 10 ml. of 2.5% reagent. The resulting precipitate is digested at 60° C. for 15 minutes and allowed to cool to room temperature. The precipitate is then filtered through a weighed, medium porosity sintered-glass crucible, washed five to six times with 10 ml. of water, and dried at 110° C. for 1 to 2 hours to constant weight. The weight of silver present in the sample is calculated by multiplying the weight of precipitate by the gravimetric factor 0.4774.

The results of several determinations of silver with the reagent, in the absence of and in the presence of interfering metals, are shown in Tables II and III. The reagent is not specific for silver (1, 2, 6); however, the combination of the reagent and Versenate make the method probably specific for silver. So far, no metals have been found causing interference. When mercury(I) ions were oxidized to mercury(II) ions, no interference was found; also univalent thallium and copper did not interfere under the conditions of determination. Table III indicates that all the interfering metals are strongly complexed by Versenate. No silver precipitate is formed with 1,2,3-benzotriazole in the presence of Versenate and iron(II). The silver ion is reduced to metallic silver by ferrous Versenate. This phenomenon was first found by Pribil *et al.* (4). Ferric Versenate complex is much more stable than ferrous Versenate complex. The equilibrium

$$FeV^{--} - e \rightleftharpoons FeV^{-}$$

is wholly displaced in favor of the ferric complex—i.e., the reducing power of the ferrous salt will be considerably increased. If the ferrous ions are oxidized to ferric ions, such interference is eliminated. The hydroxides of tin, antimony, titanium, and beryllium are insoluble even in the presence of Versenate. It is necessary to add sufficient amount of tartrate in order to complex them when they are present. The error is somewhat less than 0.2 mg. in samples containing from 10 to 100 mg. of silver and in the large amounts of foreign metals and anions.

In the study of the effect of anions on the silver determination, no interference is found from chloride, bromide, fluoride, sulfate,

	Table II. Determ	ination of S	ilver
Silver Taken, Mg.	Weight of Precipitate, Mg.	Silver Found, Mg.	Difference, Mg.
19.0519.0519.0547.6247.6247.6295.2595.2595.25	$\begin{array}{c} 40.0\\ 39.8\\ 39.5\\ 99.8\\ 99.2\\ 99.0\\ 199.6\\ 199.4\\ 199.4\\ 190.1\end{array}$	19.10 18.92 18.78 47.64 47.36 47.27 95.29 95.19 95.05	$\begin{array}{r} +0.05 \\ -0.13 \\ -0.27 \\ +0.02 \\ -0.26 \\ -0.35 \\ +0.04 \\ -0.06 \\ -0.20 \end{array}$

Table III. Determination of Silver in Presence of Foreign Metals

Metal	Form Added, 200 Mg.	Silver Taken, Mg.	Silver Found, Mg.	Dif- ference, Mg.
Hg(II) Hg(II) Pb(II) Pb(II) Pb(II) Fe(III) Fe(III) Fe(III) Fe(III) Mixture <sup>a</sup>	Hg(NO3)2 Hg(NO3)2 Hg(NO)2 PbCl2 PbCl2 Fe2(SO4)3 Fe2(SO4)3 Fe2(SO4)3	$\begin{array}{c} 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 95.25\\ 47.62\\ 47.62\\ 95.25\\ 95.25\\ \end{array}$	$\begin{array}{c} 95.48\\ 95.29\\ 95.19\\ 95.24\\ 95.36\\ 95.36\\ 95.36\\ 95.48\\ 95.36\\ 95.19\\ 47.74\\ 47.55\\ 95.15\\ 95.15\\ \end{array}$	$\begin{array}{c} +0.23\\ +0.04\\ -0.06\\ -0.01\\ -0.09\\ +0.11\\ +0.23\\ +0.11\\ -0.06\\ +0.12\\ -0.07\\ +0.33\\ -0.10\end{array}$

<sup>a</sup> The mixture contained 0.2 gram each of copper(II), cobalt(II), nickel-(II), zinc(II), and cadmium(II).

nitrate, phosphate, and acetate. The anions interfering with the precipitation are cyanide, thiosulfate, and iodide. Cyanide and thiosulfate dissolve the silver precipitate, and silver iodide is less soluble than the silver benzotriazolate.

Volumetric Procedure. Alternatively, the silver may be deter-mined volumetrically. The silver benzotriazolate precipitate is coagulated by heating and stirring, or long standing and is filtered by use of a paper-bottomed Gooch crucible. The pre-cipitate is washed with water. The precipitate is dissolved with a small amount (10 ml.) of 1 to 1 nitric acid into a 250-ml. beaker, diluted to about 50 ml. with water, then the acid is neutralized and the solution made alkaline with concentrated ammonium hydroxide. The precipitate formed, due to the neutralization, is dissolved again by adding a known amount of 0.05M cyanide solution. The excess of cyanide is back titrated by a standard silver nitrate solution using 1 ml. of 2% potassium iodide solution as indicator. The appearance of lightyellowish silver iodide indicates the end point. A blank determination is recommended.

The volumetric procedure is based upon the fact that the cyanide solution dissolves silver benzotriazolate precipitate and that silver iodide is less soluble than silver benzotriazolate. Several determinations by the volumetric procedure are shown in Table IV. The volumetric procedure has the advantages of simplicity and rapidity. It may also increase the accuracy, because contamination of the precipitate by foreign substances which do not react with cyanide will cause no error in the volumetric procedure.

### CONCLUSION

1,2,3-Benzotriazole precipitates iron(II), cobalt(II), copper(II), nickel(II), cadmium(II), zinc(II), and silver. In alkaline medium, Versenate forms very stable water-soluble complexes with all the metals mentioned except silver; therefore, 1,2,3-benzotriazole precipitates only silver in the ammonical Versenate solution. The precipitate has a high molecular weight and definite composition, and is less sensitive to light and heat.

Satisfactory results are obtained for the determination of silver with the reagent either gravimetrically or volumetrically. The volumetric procedure is preferred because of its simplicity and rapidity. Reprecipitation is not necessary because no appreci-

Dif-	Silver	Silver
ferenc	Found,	Taken.
Mg.	Mg.	Mg.
+0.0	19.08	19.05
-0.0	19.00	19.05
-0.2	18.80	19.05
+0.1	47.76	47.62
-0.1	47.52	47.62
+0.1	47.78	47.62
-0.1	95.13	95.25
+0.0	95.28	95.25
+0.1	95.36	95.25

able coprecipitation was noticed. Probably the metals are so tightly complexed by the Versenate that no appreciable amounts of free metal ions are available for coprecipitation. No attempt was made to determine silver with the reagent on a micro scale: however, it could be easily adapted to the microdetermination.

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# **Reaction of Morphine with Potassium-Mercuric Iodide**

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When morphine hydrochloride reacts in an aqueous medium with a solution of potassium mercuric iodide, a complex, having the composition [C17H19NO3.H].-[HgI<sub>3</sub>], precipitates on standing. The rate of the reaction is dependent upon the availability of HgI<sub>3</sub> anions which are supplied by the reagent in accordance with the equation:

$$\mathrm{K_{2}HgI_{4}} \rightleftharpoons \mathrm{2K^{+} + HgI_{4}^{-}} \rightleftharpoons \mathrm{2K^{+} + I^{-} + HgI_{3}^{-}}$$

A shift of the equilibrium in favor of the HgI3 ions occurs when the mercuric iodide-potassium iodide ratio is increased. Accordingly, in a series of experiments using solutions containing 0.2 mole of mercuric iodide per liter and decreasing potassium iodide concentrations the maximum product yield (98.6%) was obtained when the precipitant became saturated with respect to mercuric iodide. By this reaction as little as 1  $\gamma$  of the amine can be detected and identified, for under suitable conditions, the microcrystalline precipi-

tate formed consists of characteristic aggregates of fine, elongated needles. The ultraviolet and infrared absorption spectrum, x-ray diffraction pattern, optical rotation, and solubility behavior of the alkaloidal derivative, compared with similar properties of the free base, are of value in identification of minute amounts of the narcotic.

WHEN a mixture of mercuric iodide and potassium iodide is dissolved in water and the resulting preparation added to an aqueous solution of morphine hydrochloride, a flocculent, amorphous precipitate is formed which becomes crystalline on prolonged standing. This reaction was first reported in 1858 by the English chemist Groves (3) who, on the basis of a partial analysis, assigned to the reaction product the formula morphia- $Hg_2I_3$  (molecular weight of mercury is 100). Soon after this work was published, Valser in France used potassium iodide-mercuric iodide solutions for precipitating a number of nitrogenous bases (7, 8, 11). He included morphine in his investigations and gave to this complex the formula (morphia.HI) + (Hg<sub>2</sub>I). Finally, the alkaloidal derivative, prepared again in 1922 by François and Blanc, was assigned the formula morphine.HI.HgL (1), which composition is different from that postulated by Groves and Valser.

None of these workers characterized the complex further. Because of its importance to the forensic chemist for the identification of minute amounts of morphine, a study of the mechanism of formation and the physicochemical behavior of the alkaloidal derivative should be of both theoretical and practical value. The authors' investigations showed that the composition of the complex may be represented more correctly by the formula (morphine.H).(HgI<sub>s</sub>) and that as little as  $1 \gamma$  of the alkaloid may be detected by this reaction.

### EXPERIMENTAL RESULTS AND DISCUSSIONS

The compositions of the potassium-mercuric iodide solutions used are shown in Table I. The solutions were added slowly and with constant stirring to 500 ml. of 0.01757M aqueous solutions of morphine hydrochloride. After refrigeration for 24 hours at 5° C. the bulky, gelatinous precipitates were filtered off, dried in vacuo over phosphorus pentoxide, and the product yields obtained.

Following repeated purification (from 50% acetone) the precipitates were obtained as faintly yellowish crystalline compounds, which melted at 246.5 to 247.8° C. (corrected) to a dark brown liquid. Analyses showed that they possessed identical composition. Morphine was determined as the reineckate, the metal was precipitated as mercuric sulfide, and the halide estimated gravimetrically as silver iodide. The analytical data, recorded in Table I, justified tentatively assigning to the complex the formula (morphine.H).(HgI<sub>3</sub>). Further evidence in favor of this composition was secured by means of the following experiments.

Formation of HgI<sub>3</sub><sup>-</sup> Ions. Taurins (9) assumed that aqueous potassium iodide-mercuric iodide solutions contain both HgI<sub>3</sub><sup>-</sup> and HgI<sub>4</sub><sup>--</sup> ions and postulated that the concentration of the

Morphine Hydro $HgI_3$  ions was depleted by the addition of an excess of potassium iodide, whereas the equilibrium was shifted in favor of the  $HgI_4^-$  ions by addition of an excess of mercuric iodide. The authors'

$$2KI + HgI_{2} \rightleftharpoons K_{2}HgI_{4}$$

$$2K^{+} + I^{-} + HgI_{3}^{-} \rightleftharpoons 2K^{+} + HgI_{4}^{-}$$

$$Excess$$

$$HgI_{2}$$

$$Excess$$

## Figure 1. Equilibrium Process in Potassium-Mercuric Iodide Solutions

data strongly support the existence of this equilibrium process (Figure 1) for the product yield  $[M.H].[HgI_3]$  increased from 79.86 to 87.94% and reached 98.59% when the potassium iodide concentration in the reagent was decreased from 1.0 to 0.7 and finally reduced to 0.4 mole per liter. Apparently, the derivative is formed by interaction of the HgI<sub>3</sub> ion with the morphinonium ion produced by the virtually complete dissociation of morphine hydrochloride in water (5). The over-all reaction may be represented as follows:

Morphine.HCl 
$$\longrightarrow$$
 Morphine.H<sup>+</sup> + Cl<sup>-</sup>  
Morphine.H<sup>+</sup> + HgI<sub>3</sub>  $\rightarrow$  (Morphine.H).(HgI<sub>3</sub>)

Further data in support of this mechanism are given in Table II, which shows the influence of the potassium iodide-mercuric iodide ratio upon the rate of the reaction, and in Table III, which records the sensitivity of the reaction in the presence of equivalent amounts of morphine and morphine hydrochloride. The decreased sensitivity of the reaction when the free alkaloid, instead of its salt, is used as reactant apparently is due to the incomplete ionization of the free base in aqueous media and the sluggish supply of morphinonium ions from this system:

Morphine +  $H_2O \implies$  morphine. $H^+ + OH^-$ 

Some workers concluded that the sensitivity of this reaction

Table 1. Composition of Reagent and Morphine–Mercuric Iodide Complex											
Po	Compos	sition of ]	Reagent	Wt of		Compos (M	ition of H orphine.H	Purified C I).(HgI <sub>3</sub> ),	omplex <sup>a</sup>		Product
agent	Hgly.	KI.	H <sub>2</sub> O.	Complex.	Mer	cury	Iod	line	Mor	phine	Yield.
No.	g.	g.	ml.	G.	Found	Theory	Found	Theory	Found	Theory	%
A B C	$\begin{array}{r} 4.545 \ 4.545 \ 4.545 \ 4.545 \end{array}$	3.320 5.820 8.320	$50.00 \\ 50.00 \\ 50.00$	$\begin{array}{c} 7.513 \\ 6.702 \\ 6.085 \end{array}$	$23.08 \\ 23.01 \\ 22.95$	23.12	$\begin{array}{r} 43.73 \\ 43.85 \\ 43.63 \end{array}$	43.89	$32.84 \\ 32.65 \\ 32.57$	32.87	98.59 87.94 79.86

. <sup>a</sup> Crude product was found to contain 31.19% morphine, 23.78% mercury, and 44.63% iodine and melted to a dark brown liquid from 244° to 247° C. at a heating rate of 5° C. per minute. Hence, the composition of complex remained unchanged during purification.

Table II. Sensitivity of Potassium-Mercuric Iodide Reagents toward Morphine Hydrochloride

chloride, Mg. per Ml.	Solution A	Solution B	Solution C
0.658	Immediate heavy ppt.	Faint ppt. after 30 sec.	Slight ppt. after 3 min.
0.248	Immediate gelatinous ppt.	Faint cloudiness after 90 min.	Faint cloudiness after 5 hours
	Crystallization after 45 min. but most of ppt. still amor- phous. Crystallization com- plete after 85 min. and supernatant liquid clear	Crystallization after 4 hours	Crystallization after 9 hours
0.124	Faint opalescence after 5 min.	Faint opalescence after 4	Faint opalescence after 7
	Crystals (long, branching nee- dles) appear after 75 min.	Crystals appear after 12 hours	Crystals appear after 19 hours
0.093	Faint opalescence after 80 min.	Faint opalescence after 9 hours	No reaction
	Crystallization after 9 hours	Crystallization after 36 hours	
0.062	Faint opalescence after 3 hours Crystallization after 51 hours	No reaction	No reaction

is decreased when the reagent contains excess potassium iodide because of the solubility of the alkaloidal iodomercurate in potassium iodide solutions (8, 10). This assumption could not be confirmed by the authors, as shown in Table IV and illustrated by means of the following experiments which were carried out further to substantiate the reaction mechanism postulated.

To 1.0-ml. portions of an aqueous morphine hydrochloride solution containing 0.50 mg. of alkaloidal salt were added 0.2 ml. of solutions A, B, and C, respectively. A voluminous precipitate formed in each case immediately, but on standing for 5 minutes, this precipitate coagulated to form a few isolated gelatinous particles: so that the solution appeared now much clearer. The same experiment was repeated using 1.0 ml. of reagent in each case. Again there was observed an immediate strong cloudiness throughout the entire solution as the first few

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Solutions of	Morphine and	Morphine Hy	drochloride
Concn. of Morphine Hydrochloride, Mg./Ml.	Appearance of Crystals, Hours	Concn. of Morphine, Mg./Ml.	Appearance of Crystals, Hours
$\begin{array}{c} 0.248 \\ 0.124 \\ 0.093 \\ 0.062 \end{array}$	$0.75 \\ 1.25 \\ 8.5 \\ 48$	0.200 0.100 0.075 0.050	1.85 6.0 32 87

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### Table IV. Solubility of Morphine-Mercuric Iodide Complex in Various Solvents

Solvent	Solubility, % Wt./Vol.
Chloroform	0.004
Water	0.104
Ethanol, 95%	0.106 0,385
Acetone	0.643

drops of reagent were added, but as the addition was continued this cloudiness disappeared because the gelatinous precipitate gradually coagulated. Such an observation could easily be misinterpreted as indicating increased solubility of the alkaloidal iodomercurate in excess reagent. But in none of the experiments could the coagulated precipitate be dissolved by the subsequent addition of either 1 ml. of 10% potassium iodide or 1 ml. of precipitant.

Mercuric Iodide Reactions. In order to disprove the possibility that the ether oxygen of the alkaloid was mercurated during the reaction the following experiments were carried out.

To 0.1 gram of morphine hydrochloride dissolved in 5 ml. of water were added 100 ml. of a saturated aqueous mercuric iodide solution (45 mg. per liter). No reaction was observed. Similarly, when to 100 ml. of an aqueous 0.003M morphine hydrochloride solution (1.1274 grams per liter) 0.0003 mole (0.1364 gram) of solid red mercuric iodide was added, no reaction occurred, even when the suspension was stirred mechanically for prolonged periods of time, and the metal halide could be recovered quantitatively.

When morphine hydrogen iodide instead of morphine hydrochloride was used in these experiments, product formation occurred in each case. Thus, on addition of 100 ml. of saturated mercuric iodide solution to a solution of 0.1 gram of morphine hydrogen iodide in 5 ml. of water 2.3 mg. of material formed after 12 hours' standing. It was found to be identical to that obtained by reaction of morphine hydrochloride with potassium mercuric iodide. The same compound was isolated, when to 100 ml. of a 0.003M aqueous morphine hydrogen iodide solution (1.3479 grams per liter), 0.0003 mole of solid red mercuric iodide was added, and the suspension was stirred mechanically for several hours, the extent of product formation being a function of the reaction time.

These experiments strongly suggest that the morphine mercuric iodide complex does not form by coordination of the mercuric iodide with the oxygen of the ether linkage in the morphine molecule to give a compound of the formula shown in Figure 2. One could expect to obtain the analogous product from the reaction of morphine hydrochloride and mercuric iodide, but under the experimental conditions the alkaloidal complex forms according to the following equations.

$$\begin{array}{l} \mathrm{M.HI} \mathchoice{\longleftarrow}{\leftarrow}{\leftarrow} \mathrm{M.H^+ + I^-} \\ \mathrm{HgI}_2 + \mathrm{I^-} \Huge{\longleftarrow} \mathrm{HgI}_3^- \\ \mathrm{M.H^+ + HgI}_3^- \Huge{\longleftarrow} \mathrm{M.H.HgI}_3 \end{array}$$

This mechanism is supported further by the observation that the derivative is formed at approximately the same rate when 95 ml. of saturated mercuric iodide solution (4.5 mg. HgI<sub>2</sub>), are added to 5 ml. of an aqueous morphine hydrogen iodide solution containing 0.135 gram of the alkaloidal salt, or when 100 ml. of an equimolar (0.003M) aqueous morphine hydrochloride solution react with 0.05 ml. of aqueous potassium mercuric iodide (Solution A, Table I) containing 4.5 mg. of mercuric iodide and 3.3 mg. of potassium iodide. Evidently, in the former case the iodide ions are supplied by the morphine hydrogen iodide while in the latter case they are contributed by the potassium iodide present in the system.

Study of Ultraviolet Absorption. The ultraviolet absorption of the complex differs markedly from that of the compounds used for its synthesis as shown in Figure 3. No resemblance exists between the spectra of mercuric iodide, potassium iodide, and potassium iodide-mercuric iodide solutions and the experimental data strongly suggest that potassium iodide and mercuric iodide interact with the formation of a new compound.



Figure 2. Mode of Addition of Mercuric Iodide to Morphine Hydrogen Iodide

In the potassium iodide-mercuric iodide solutions studied a bathochromic shift of the maximum from 280 to 290 m $\mu$  occurs as the potassium iodide concentration is increased from 0.0001 to 0.0005 mole per liter. (A 0.0001*M* solution of potassium iodide was found to dissolve no more than 0.00004 mole of mercuric iodide.) This shift is evidently associated with the increased concentration of HgI<sub>4</sub><sup>-</sup> ions under these conditions (Figure 1) and the property of these ions to absorb at higher wave lengths (2).

In accordance with these interpretations the resemblance be-



Figure 3. Ultraviolet Absorption Spectra of Reagents and Reaction Product

tween the spectrum of the derivative and that of the potassiummercuric iodide solution containing the lowest potassium iodide concentration and the highest  $HgI_3^-$  ion concentration, greatly supports the formula (M.H).(HgI<sub>3</sub>) of the complex. Evidently, the  $HgI_3^-$  ions which comprise 67% by weight of the compound afford a more decisive contribution to the ultraviolet absorption pattern of the complex than does the alkaloidal base itself.

Morphine exhibits a distinct maximum at 285 m $\mu$  ( $\epsilon_{max} = 1576$ ) whereas the complex (M.H).(HgI<sub>3</sub>) shows a plateau ( $\epsilon = 6957$ ) from about 265 to 280 m $\mu$ . At 285 m $\mu$  the molar extinction coefficient of the complex is equal to 6320—i.e., about four times as great as that of the alkaloidal base. Mercuric iodide absorbs strongly at 263 m $\mu$  ( $\epsilon = 4924$ ), but no sign of a peak is observed in the spectrum of the complex at that wave length.

None of these data support the formula  $M.HI.HgI_2$  as proposed by François and Blanc, but they can all be interpreted in terms of the composition (M.H).(HgI\_3).

Nonreactivity of Hydrogen Sulfide toward Alkaloidal Derivative. When 0.0003 mole of the complex (0.2603 gram) was dissolved in 25 ml. of reagent grade acetone and a stream of dry hydrogen sulfide was bubbled slowly through the solution, no precipitation occurred. However, when red mercuric iodide



Figure 4. Electrometric Titration of Morphine-Mercuric Iodide Complex



Figure 5. X-Ray Diffraction Pattern Morphine recrystallized from water



Figure 6. X-Ray Diffraction Pattern Morphine-mercuric iodide recrystallized from 50% acetone X-Ray Diffraction Powder Data of Morphine Mercuric Iodide Complex

		$C_0 K \alpha(\lambda =$	1.790 A.)		
<i>d</i> , A.	$I/I_1$	<i>d</i> , A.	$I/I_1$	<i>d</i> , A.	$I/I_1$
10.9	100	3.63	50	2.40	20
8.94	35	3.55	1	2.37	1
8.33	55	3.47	25	2.31	20
7.58	2	3.39	2	2.27	3
6.62	30	3.30	2	2.22	3
6.09	50	3.22	15	2.18	3
5.48	45	3.10	10	2.14	20
5.10	15 B	3.00	20 B	1.98	15 B
4.53	15 B	2.92	1	1.89	2 B
4.13	5 B	2.79	10 B	1.84	3 B
3.97	15	2.67	3	1.74	3 B
3.90	15	2.60	15 B	1.71	$\tilde{2}$ $\tilde{B}$
3.78	25	2.52	3		

Table V. Optical Rotations of Morphine and Morphine Mercuric Iodide in 50% Acetone

	Conce	ntration	Observed	
Compound	wt./vol.	Mole/liter	Angle of Rotation	Specific Rotation
Morphine Morphine– mercuric iodide	$0.1860 \\ 0.5327 \\ 1.3316$	$\begin{array}{c} 0.00614 \\ 0.00614 \\ 0.01535 \end{array}$	$   \begin{array}{r}     -0.31 \\     -0.28 \\     -0.66   \end{array} $	-166.4 - 52.6 - 49.5

instead of morphine mercuric iodide was used in the experiment an immediate orange-red coloration was observed and black mercuric sulfide gradually began to precipitate. These observations do not support the formula M.HI.HgI<sub>2</sub> but again can be explained on the basis of the composition  $(M.H).(HgI_3)$ . Evidently, the HgI<sub>3</sub> ion of the complex remains undissociated in acetone and is therefore not affected by hydrogen sulfide.

Aqueous and Nonaqueous Titration of Complex. The derivative displayed only slight acidity in aqueous media and it was not possible to titrate it in pure water by means of alkali. It could be quantitatively estimated, however, in 90% (aqueous) acetone, in which solvent it exhibited a  $pK_A$  of 6.95, by means of alcoholic sodium hydroxide:

 $M.H.HgI_3 + NaOH \longrightarrow M.H.OH + Na HgI_3$ 

and in nonaqueous systems following the procedure for the determination of amine-cadmium halide complexes by means of perchloric acid ( $\beta$ ). The experimental data illustrated in Figure 4 suggest that François and Blanc's formula M.HI.HgI<sub>2</sub> is





Figure 7. Infrared Absorption Spectra of Morphine, Morphine Hydrogen Iodide, and Morphine Mercuric Iodide



Figure 8. Crystals of Morphine Hydrochloride with Potassium-Mercuric Iodide

probably incorrect, because one would expect this compound to display marked acidic properties and titratability in water.

Physicochemical Constants of Complex and Microchemical Value of the Reaction. The identification of morphine by its conversion to the mercuric iodide complex is often used as evidence in court. For this purpose, a more complete characterization of the derivative is highly desirable. Accordingly, additional characteristic properties of the alkaloidal derivative are illustrated in Figures 5, 6, and 7, which show its x-ray dif-

# ANALYTICAL CHEMISTRY

fraction pattern and infrared absorption spectrum and in Table V, which records its optical rotation in 50% aqueous acetone. Similar data are reported for the alkaloidal base.

The experimental data reported in Table V show that the observed angles of rotation are practically identical for the free base and the complex when measured on solutions of comparable molarities. However, the specific rotation of the complex is considerably smaller than that of the free base because of its much higher molecular weight. The specific rotation of the complex is not a constant but decreases slightly with increasing concentration. This phenomenon is displayed also by morphine hydrochloride, on which compound it was first observed by Hesse (4).

This reaction is extremely sensitive and may be used to identify mere traces of morphine. It is much more sensitive than the classical reaction of the alkaloid with an aqueous cadmium iodide– potassium iodide solution (Marme's reagent). When the test is carried out

under a bell jar and precautions are taken to maintain a constant humidity atmosphere, approximately 25  $\gamma$  of the substance can safely be detected and identified. The appearance of a well defined microcrystalline precipitate may take some time, but invariably the amorphous mass initially formed will gradually develop into characteristic crystal patterns whose habits may be recognized and studied under the microscope (Figures 8 and 9). When carefully carried out in a capillary tube, as little as 1  $\gamma$  of the narcotic can still be detected.



Figure 9. Crystals of Morphine Hydrochloride with Potassium-Mercuric Iodide

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# Separation of Niobium and Tantalum by Liquid-Liquid Extraction

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The limitations of existing conventional methods for the separation of niobium and tantalum make it desirable to consider any other method that offers the possibility of a separation of these two elements. Studies, using inactive niobium and tantalum as well as radioactive tracers of these elements, indicate that the separation of niobium from tantalum by liquid-liquid extraction of the hydrochloric acid solution of the elements with long-chain aliphatic and aromatic amines in organic solvents is quantitative. Further studies showed that niobium and tantalum sulfate and oxalate complexes extract with the solvated amines. However, in the case of tribenzylamine in methylene chloride, the niobium sulfate complex may be quantitatively separated from the tantalum sulfate complex if the ratio of organic to aqueous volume is maintained at 15 to 1. These methods should be valuable for rapid separations of these elements and the amine systems may be applied to industrial separations of niobium and tantalum.

THE separation of niobium and tantalum is one of the most difficult of inorganic problems. Previous methods based on repeated fractional separations are both time-consuming and often uncertain. More recently, increased interest in liquidliquid extractions led to the exploration of this technique as a more efficient method of separation for these elements.

The essential criteria for extraction are a suitable solvent and a favorable partition coefficient. Thus, one element may be extracted quantitatively while the other remains in the original medium. In laboratory scale operations, a favorable distribution coefficient is essential to keep the analytical procedure simple and the apparatus uncomplicated. Solutions of long-chain aliphatic and aromatic amines in organic solvents have been shown to be efficient acid extractants (4, 9). The use of methyldioctylamine in xylene for the separation of niobium and tantalum has been described (3). In this study, tribenzylamine, TBA, a product of Eastman Kodak Co, Rochester, N. Y., was of particular interest. It is water-insoluble and is known to form the corresponding amine acid salts which are also waterinsoluble but, in general, soluble in various organic solvents.

Table I.	Nuclear Data for Radiotanta	Radionio lum	bium and
Radio- nuclide	Production	$T_{1/2},$ Days	Characteristic Radiations
Nb <sup>95</sup> Ta <sup>182</sup>	d of Zr <sup>95</sup> Ta <sup>181</sup> (n, γ)Ta <sup>182</sup>	35 117	$\beta^-, \gamma$ $\beta^-, \gamma$

### EXPERIMENTAL

In a typical experiment, an aqueous solution containing either niobium-95 radioactive tracer or tantalum-182 radioactive tracer was extracted with an equal volume of the amine in an organic solvent. Each phase was checked for niobium-95  $\gamma$  or tantalum-182  $\gamma$  radioactivity by use of a  $\gamma$ -scintillation counter having a sodium iodide crystal. Experiments of this type showed that niobium-95-tantalum-182 tracer mixtures and nonradioactive niobium-tantalum separations could be effected by this solvent extraction method.

The nuclear properties of the two radionuclides used in these experiments are described in Table I. This information has been tabulated from data by Way *et al.* (10). Radiochemical purity of the radioactive tracer and end products of solvent extraction technique were checked by means of decay studies and absorption measurements.

The radioactive tracers were prepared as follows:

Niobium-95. Radioniobium product was obtained from the Radioisotope Sales Department, Oak Ridge National Laboratory. Decay studies and *β*-absorption measurements indicated the radiochemical purity to be 99%. Tantalum-182. Tantalum metal was bombarded in the Oak

Tantalum-182. Tantalum metal was bombarded in the Oak Ridge National Laboratory graphite reactor. After irradiation, the material was processed by dissolving the metal in concentrated nitric acid-hydrofluoric acid mixture followed by a precipitation of tantalum hydrated oxide upon the addition of concentrated ammonium hydroxide. The precipitate was then dissolved in concentrated hydrochloric acid. Aliquots were taken for each experiment.

The Tribenzylamine System. The experimental work with methyldioctylamine (3) suggested that the hydrochloric acid system would probably yield satisfactory results in the tribenzylamine extractions. In experiments with tribenzylamine, equal volumes of the sample and extracting solvent were shaken for 5 minutes. The layers were allowed to separate and the activity

Table	II.	Partition	Coefficient w in Chloroform	ith % Tribenzylamine 1ª
	% Т	BA	$K_{\rm Nb}$	$K_{\mathrm{Ta}}$
	1.2.5.5	25 5 )	$0.20 \\ 0.80 \\ 9.4 \\ 74.4$	$\begin{array}{c} 0.003 \\ 0.004 \\ 0.004 \\ 0.002 \end{array}$
	10.0	õ	72.6	0.038
<sup>a</sup> 11M	HCI	solution, equ	al volumes solvent	-aqueous phase.

of the radioactive tracers, niobium-95 and tantalum-182, was determined.

The partition coefficient for niobium varied with the hydrochloric acid concentration and per cent tribenzylamine present in the solvent are shown in Tables II and III. Quantitative separation of niobium from tantalum was effected by extracting an 11M hydrochloric acid solution of the sample with an 8% solution of tribenzylamine in chloroform or methylene chloride. Chloroform and methylene chloride were the only satisfactory solvents for tribenzylamine among those tested. A white solid precipitated upon the addition of concentrated hydrochloric acid to solutions of tribenzylamine in benzene, carbon tetrachloride, toluene, and xylene. Material balance studies showed the precipitate was amine hydrochloride. In Tables II, III, and IV

 $K_{A} = \frac{mg.A/ml. \ of \ solvent \ phase}{mg.A/ml. \ of \ aqueous \ phase} = \frac{counts/min./ml. \ of \ solvent \ phase}{counts/min./ml. \ of \ aqueous \ phase}$ 

In macro experiments using both inactive niobium, tantalum, niobium-95, and tantalum-182 radioactive tracers, solutions containing 25 mg. per ml. of tantalum were quantitatively separated from 6 mg. per ml. of niobium. In order to identify the radioactivity in each phase, radioactive decay studies were made on aqueous and chloroform phases. The half life of the aqueous phase was about 110 days which corresponded to radio-tantalum. The chloroform phase had a half life of about 31 days which was indicative of radioniobium.

Table III.	Effect of Hydrochloric Acid Concentration c	m
Extra	ction of Niobium from Tantalum by 8%	
	Tribenzylamine	

M M	Solvent	Knb	$K_{Ta}$
2	Chloroform	0.003	0.0008
3	Chloroform	0.004	0.0007
4	Chloroform	0.002	0.0002
6	Chloroform	0.003	0.0005
8	Chloroform	0.24	0.0017
9	Chloroform	5.05	0.0013
10	Chloroform	13.34	0.0028
11	Chloroform	74.38	0.0025
11	Methylene chloride	80.50	0.0023

The behavior of the niobium and tantalum oxalate complexes was similar. They were both extracted by tribenzylamine in chloroform or methylene chloride. The partition coefficients remained at 3 for niobium and 1 for tantalum with varying concentrations of tribenzylamine or added oxalic acid. There was no measurable extraction of niobium or tantalum when the solvent was benzene, carbon tetrachloride, toluene, or xylene.

The niobium sulfate complex in 0.45 to 9M sulfuric acid had a distribution coefficient of less than 0.15 when chloroform or benzene and its related compounds were employed as the solvent.  $K_{Ta}$  was less than 0.01. However, the behavior of the sulfate complexes with methylene chloride was radically different. The difference in coefficients at 4.5M sulfuric acid appeared significant enough to encourage further work on the sulfate system. With 8% tribenzylamine in methylene chloride at 4.5M sulfuric acid, niobium had a K of 0.58, whereas tantalum had a K of 0.0030. as shown in Table IV. At higher concentrations of sulfuric acid, the methylene chloride extracted the tantalum from the aqueous phase.

Increasing the concentration of tribenzylamine beyond 8% was unfavorable as the tantalum sulfate complex began to extract into the solvent. The alternative lay in a change in the volume ratio of the phases. Bush and Densen (1) showed that for a mixture of two substances having partition coefficients,  $K_a$  and  $K_b$ , the greatest fractional separation for a given extraction was attained if the ratio of the volumes of the two solvents, X and Y, was maintained at:  $V_x/V_y = \sqrt{\frac{1}{K_a K_b}}$ . Theoretically, when this relationship was applied to  $K_{Nb} = 0.58$  and  $K_{Ta} = 0.001$ , the ratio of solvent phase to aqueous phase was calculated as 41 to 1. However, a solvent ratio of 15 to 1 gave  $K_{Nb}$  equal to 99.2 while  $K_{Ta}$  remained at 0.001, thus, a quanti-

tative separation had been made. Macro experiments with niobium concentrations of 10 mg. per ml. and tantalum concentrations of 20 mg. per ml. showed 99% recovery of both elements in their separate phases.

# DISCUSSION

In the hydrochloric acid system, niobium is thought to extract into the solvent phase by the formation of an oxychloride complex of the type  $H(NbOCl_4)$  or  $H_2(NbOCl_5)$  (11).

On the other hand, neither chlorotantalates (6, 8) nor oxychloride (12) complexes of tantalum are presumed to exist in aqueous solution. This may be a possible

explanation for the lack of extraction of the tantalum into the solvent phase. Because complex ions are in equilibrium with their simple components, increased hydrochloric acid concentration would shift the equilibrium in favor of the complex and consequently increase the extractability of the niobium.

Niobium and tantalum pentoxides are both soluble in sulfuric acid. The exact configuration of these complexes are in doubt (2). However, the complexes of both niobium and tantalum, whatever their structure, are extractable to a degree by the chloroform-tribenzylamine solvent. For example, with a ratio of 15 parts of organic phase—i.e., of methylene chloride-8% tribenzylamine—to 1 part aqueous phase by volume, a quantitative separation of niobium from tantalum apparently can be made at 4.5M sulfuric acid. Although niobium can be separated from tantalum, the concentration of sulfuric acid is very critical and should be very carefully controlled at this molarity.

The use of tribenzylamine as the amine solute for the oxalic acid system results in some interesting anomalies. Both niobium and tantalum oxalato complexes (5, 7) are extracted by the chloroform and methylene chloride solvents. However, when the only change in experimental conditions is the replacement of the solvent with benzene, carbon tetrachloride, toluene, or xylene, neither niobium nor tantalum was extracted from the aqueous phase.

Material balance studies with oxalic acid show that the acid is extracted by the tribenzylamine solvated in chloroform or

Table IV.	Partition Coefficient w Concentration	vith Sulfuric Acid
$H_2SO_4$ ,		
M	KNb	$K_{Ta}$
$0.45^{a}$	0.02	0.004
4.0	0, 42	0.003
4.5	0.58	0.003
4.7	0.56	0.002
4.8	0.53	0.004
4.9	1.2	8.3
5.0	2.4	20.1
5.5	2.7	17.0
6.0	6.4	12.1
<sup>a</sup> 8% TBA in extracting equal	methylene chloride. Experi volumes solvent/aqueous phase	mental data obtained by

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methylene chloride but not in tribenzylamine solvated in carbon tetrachloride or benzene and its homologs.

These methods for the separation of niobium from tantalum should prove valuable to those interested in a rapid separation of the two metals. Of the systems reported, the separation of the two metals in the hydrochloric acid system is much more desirable for tantalum does not readily extract from any hydrochloric acid concentration. Technologically, these amine systems may be applied successfully to the industrial separation of niobium from tantalum with cheap chemicals and replace the present tedious fractionation methods.

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# Fluoride Determination by Electron Transfer Catalysis

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The marked catalytic effect of fluoride on the cerousceric electron exchange reaction suggested that this reaction could be used as a microanalytical method for fluoride determination without the necessity of removing ions which interfere in present methods. Cations in general were found to exert negligible catalytic effects. The catalytic effect of fluoride was about 900 times that of sulfate and about 370 times that of phosphate. These circumstances limit the application of the method to those samples which do not contain these ions or in which the effect of the interfering ions can be made constant. The fluoride content of bone ash has been determined by making constant the effect of interfering ions. The results of electron transfer catalysis by various negative ions are considered to be in qualitative agreement with Libby's description of the mechanism of electron transfer catalysis.

ORNIG and Libby (1) discovered a marked catalytic effect of fluoride ions on the rate of electron transfer between cerous and ceric ions in nitric acid solution and showed that the increase in electron transfer rate was proportional to the fluoride concentration. A proposed mechanism (1, 2) of electron transfer catalysis describes the placing of a small negative ion (in this case fluoride) between the positive exchanging ions, so as to form a symmetrical transition complex in which the hydration atmospheres of the positive ions are shared. This model of electron exchange catalysis suggested the possibility that the effect of fluoride on this type of electron transfer might be so specific as to permit the determination of fluoride in natural materials, without the necessity of separation of fluoride from other ions in the sample. This report presents a comparison of the catalytic effect of several ions commonly present in natural materials with that of fluoride, and indicates the limitations of fluoride analysis based upon this principle.

#### EXPERIMENTAL

The stock solution of radioactive cerium-144 was treated with hydrogen peroxide in order to convert the ion to the cerous state. The excess hydrogen peroxide was removed by heating the solu-tion on a water bath. Concentrations which would give ap-proximately 75,000 counts per minute of the radioisotope, under conditions of radioactive counting used in this method, were employed in each reaction mixture.

The reactions were carried out in 50-ml. centrifuge tubes suspended in a large, stirred bath of ethylene glycol, which was placed in a household-type deep-freeze cabinet. The tempera-ture of the bath was regulated at  $-14.2^{\circ}$  C. and rose no more than 0.4° over an 8-hour working period. At room temperature 3 ml. of 0.003M cerous nitrate hexahydrate solution in 12M nitric acid, labeled with cerium-144, were transferred to the reaction tubes and mixed with 3 ml. of an aqueous solution of the ion to be tested. After a temperature equilibrium had been established, usually by standing overnight, the reaction was started by the addition of 3 ml. of nonradioactive cerium(IV) solution (0.0003M)ceric ammonium nitrate in 6M nitric acid) at -14.2 ° C

The reaction was stopped after 160 minutes and the cerium-(IV) separated from the reaction mixture by rapid and forceful injection of 20 ml. of cold ether into the reaction mixture with a syringe. Ten minutes later, 5 ml. of the ether layer containing the cerium(IV) were removed with a cold pipet and transferred to a 25-ml. volumetric flask. The ether was removed by aeration and the flask diluted to volume with a solution containing 2 mg. per liter of nonradioactive cerium(IV). This solution acted as a carrier and diluent to impede possible adsorption of the radioactive ceric ions on container walls. The flask was allowed to stand at least 175 minutes (usually overnight) in order to permit the praseodymium-144 daughter to grow to a concentration in equilibrium with that of cerium-144. The contents of the flask were transferred to paraffined drinking cups and the radioactivity was measured (S). It is presumed that under these conditions only the energetic beta radiation from praseodymium-144 decay was measured.

Aldehydes and peroxides in untreated ether would reduce ceric ion during its extraction from the reaction mixture. The ether was satisfactorily purified in volumes sufficient for 5 days' use by washing with ceric ammonium nitrate in approximately 8M nitric acid until the color of the ceric ions remained in the wash-The ether was then washed eight times with water, or until ings. the washings were neutral, and stored in a dark bottle in the deep-freeze cabinet.

In agreement with Hornig and Libby (1), the dependable and rapid separations of the ether and aqueous phases, in the extraction of cerium(IV) from nitric acid solutions, were obtained with difficulty. The conditions specified were found by trial-anderror experiments, in which the relative volumes of ether and acid solutions were varied, to be optimal. Phase separation could not be obtained at the low temperature employed when 15 ml. of ether and 9 ml. of acid were used, or when the acid concentration was increased to 8.7M.

### RESULTS AND DISCUSSION

The results given in the figures are shown as the per cent of the exchange that has not yet occurred, plotted against time or con-

The half time of the uncatalyzed exchange reaction was found to be approximately 390 minutes (Figure 1), as compared with 220 to 224 minutes observed by Hornig and Libby (1). Since approximately 75% of the uncatalyzed exchange reaction has not yet occurred at 160 minutes, this time was the period in which the comparative catalytic effects of fluoride and other ions were examined.



In Figure 1 the y intercept is at 100% indicating zero exchange at zero time. This circumstance can be obtained only when a correction is applied to the results to allow for the apparent presence of radioactive cerium(III) ions in the ether phase. In 10 experiments, in which only cerium(III) was employed, and in which the volume changes due to mutual solubilities of ether and nitric acid solutions were observed and taken into account, 0.57  $\pm$ 0.17% of the radioactivity was found in the supernatant ether phase. This quantity represents cerium(III) in the nitric acid solution trapped in the ether, and may include also cerous ions oxidized to the ceric state. Presumably the graphical correction for zero time exchange, applied by Hornig and Libby (1), is of the same type. In the results given in Figures 2 and 3, this correction has not been applied, since comparative results only are the items of interest.

The results in Table I show that a number of ions commonly present in natural materials, in amounts in excess of those likely to be present in practical samples, do not alter the measured rate

# Table I. Ions Exhibiting No Effect on Electron Transfer between Cerous and Ceric Ions

Ion	Compound Used	Maximum Amount of Ion Tested, Mg.
Na <sup>+</sup>	NaNO3	54.0
K +	KNO3	0.06
Mg <sup>++</sup>	$Mg(NO_3)_2$	$2.0^{a}$
Fe <sup>+++</sup>	Fe(NO <sub>3</sub> ) <sub>3</sub> .9H <sub>2</sub> O	6.0
Ag <sup>+</sup>	AgNO <sub>3</sub>	6.4
1-7	NaI	0.5

Temp. -14.2° C.; time 160 minutes; HNO2 concn. 6M; total volume 9 ml. <sup>a</sup> A definite acceleration of the electron exchange was produced by 10 mg. of magnesium.



Figure 2. **Ceric Electron Exchange** 

Amounts on abscissa given as millimoles per 9 ml. of reaction mixture. These values to be multiplied by 10<sup>-3</sup> for fluoride

of electron transfer. Also 54 mg. (2.3 millimoles) of sodium, added as sodium nitrate, did not alter the catalytic effect of 1 and  $2 \gamma$  of fluoride. Silver was included in these experiments, owing to the anticipated use of silver nitrate to remove protein and chloride from blood plasma.

Figure 2 presents a comparison of the effects of some ions which were found to alter the measured rate of electron transfer. The





 A. Fluoride alone
 B. Calculated effect of fluoride in presence of 1.7 mg.
 phosphorus (as phosphate), obtained by assuming that ca-talysis by each increment of fluoride is additive to that of phosphate, and produces same effect on electron transfer as is given in AC. Observed effect of fluoride in presence of 1.7 mg.

phosphorus (as phosphate)

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high order of catalytic effect of fluoride on this example of electron transfer, discovered by Hornig and Libby (1), was confirmed. The same workers stated that chloride had a slight catalytic effect in the same direction as fluoride. However, in this work, chloride added as sodium chloride, in three trials over wide ranges of concentration, produced an apparent slight negative catalytic effect. Also, an inhibition in measured catalytic effect of fluoride was observed in the presence of chloride. A detectable inhibition was noted at 0.5mM chloride; at 1.0mM chloride, the effect of 1  $\gamma$  of fluoride was reduced by 7.8%.

Calcium ion, whose slight catalytic effect decreases in increment with increasing amount, is (except for magnesium) the only cation for which definite evidence of catalysis of the cerous-ceric electron transfer was obtained in this study. The effect of calcium and chloride on electron transfer could be controlled in practical fluoride analysis, by adjusting to a concentration above which their effects became nearly constant. The catalytic effect of 0.1 millimole of sulfate is of the order of 1/900th that of the same amount of fluoride. On this account, some materials would require removal of sulfate prior to fluoride determination by electron transfer catalysis.

The catalytic effect of 0.1 mM phosphate is approximately 1/370th that of an equal amount of fluoride (Figure 2), and the relative efficiency of phosphate increases with increasing amounts of the ion. The measured catalytic effect of phosphate on electron transfer cannot be attributed to fluoride contaminating the dibasic sodium phosphate used to supply the phosphate ion, because the observed effect was not altered by repeated treatments of the salt with concentrated nitric acid, followed by removal of the acid by evaporation at water bath temperature.

Evidence was obtained to indicate that the catalytic effects of phosphate and fluoride, when both are present, are not additive. The results given in Figure 3 show that the effects of 1 and 2  $\gamma$  of fluoride are augmented in the presence of phosphate ion equivalent to 1.7 mg. of phosphorus.

The high catalytic effect of phosphate introduces a serious interference in the application of electron transfer catalysis to fluoride analysis, since many materials, particularly those of biological origin, may contain upward of several hundred times as much phosphate as fluoride. While fluoride and phosphate may be separated by chemical manipulations and distillation (4), it would not be advantageous, on account of cost, to use electron transfer catalysis for fluoride determinations in those cases in which phosphate must be eliminated from the sample. This procedure would have found a justifiable, routine use only in the hoped for, but unrealized, circumstance that phosphate and other ions do not alter appreciably the rate of electron transfer.

Fluoride determinations by electron transfer catalysis can be made without removing the interfering ions, if the quantities and, hence, the effects of these ions can be made constant. This can easily be attained with calcified tissues, since the composition of the mineral part of a given type of calcified tissue-for example, bone—is virtually fixed by nature. Table II shows the results of fluoride determinations in bovine bone ash and gives the results of recovery of added fluoride. In this work, 5-mg. samples of bone ash were used. The fluoride contents of these samples were obtained by reference to standard curves of electron transfer, with known amounts of fluoride in the presence of the quantities of phosphorus and calcium found in 5 mg. of bone ash. These quantities of calcium and phosphorus were actually obtained by use of a solution of the same bone ash from which the fluoride had been removed by repeated treatment with nitric acid in a platinum dish, followed by removal of the excess acid by heat.

#### Table II. Analysis of Bovine Bone by Electron Transfer Catalysis

	Bone Ash	Bone Ash + 0.50 $\gamma$ Fluoride
No. of samples analyzed Fluoride found, $\gamma$ Fluoride recovered, $\gamma$ Fluoride, $\%$	$\begin{array}{r} 10 \\ 2.20 \pm 0.17^{a} \\ 0.044 \pm 0.003^{a} \end{array}$	$\begin{array}{c} 2.72 \ \pm \ 0.10^{a} \\ 0.52 \end{array}$
<sup>a</sup> Standard deviation of me	an.	

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# **Polarographic Determination of Alpha-Ketoglutaric Acid**

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I NTHE course of studies on the metabolism of plant tissues, a rapid, sensitive, and specific method for determining  $\alpha$ -ketoglutaric acid was desired. The most commonly used method for determining  $\alpha$ -ketoglutaric acid was developed by Friedemann and Haugen (4). It is based on the formation of a red-brown color when the 2,4-dinitrophenylhydrazone of  $\alpha$ -ketoglutaric acid is treated with sodium hydroxide. This method is sensitive, but it is specific for  $\alpha$ -ketoglutaric acid only when carried out under carefully defined conditions. It also suffers from the relative lack of stability of the colored compound and the range is small.

Several other procedures have been employed for determining keto acids. Clift and Cook (3) described a titration method

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based on the formation of a bisulfite addition product. Keto acids also have been determined by measuring the carbon dioxide produced on oxidation with ceric sulfate or permanganate (7, 11). These methods are not specific for  $\alpha$ -ketoglutaric acid. Chromatographic separations have been described, employing Celite (8), silica gel (5), ion exchange resins (1), and filter paper (2, 10). Procedures using silica gel columns and paper chromatograms have given good recoveries of  $\alpha$ -ketoglutaric acid, but these methods are not convenient for the determination of a single acid.

Previously, certain acids of the tricarboxylic acid cycle have been successfully determined polarographically (9, 12). The polarographic behavior of  $\alpha$ -ketoglutaric acid is such that waves suitable for quantitative analysis are obtained in solutions buffered at pH 1.8 and 8.2. The current increment observed under

ANALY	YTICAL	CHEMISTRY	

Table I.	Recovery	of	α-Ketoglutaric	Acid	from	Enzyme
		Re	action Mixtures			-

(At pH 1.8 in 0.7M potassium chloride-hydrochloric acid solution)

α-Ketoglutaric Acid Added, Micromoles	α-Ketoglutaric Acid Found, Micromoles	Recovery, %	
$\begin{array}{c} 0.0\\ 0.0\\ 100\\ 100\\ 100\\ 160\\ 160\\ 160\\ 160\\ 16$	$\begin{array}{c} 0.0\\ 0.0\\ 95.0\\ 98.7\\ 97.5\\ 159.9\\ 159.2\\ 158.7\\ 159.9\end{array}$	$\begin{array}{c} & & & & & \\ & & 95.0 \\ & & 98.7 \\ & & 97.5 \\ & & 99.9 \\ & & 99.5 \\ & & 99.2 \\ & & 99.9 \\ \end{array}$ Av. 98.5 $\pm 1.5$	

these conditions is proportional to the  $\alpha$ -ketoglutaric acid concentration.

### APPARATUS AND MATERIALS

Apparatus. The Fisher Elecdropode was employed in these studies. To facilitate determination of the limiting current, the galvanometer deflection was calibrated in terms of microamperes by substituting a 100,000-ohm Akra-ohm resistance for the polarographic cell. The galvanometer was calibrated at each of the shunt settings used. All voltages were measured versus the saturated calomel electrode.

The polarographic cell was of the H type described by Komy-athy, Malloy, and Elving ( $\beta$ ). The temperature of the cell was maintained at  $25^{\circ} \pm 0.2^{\circ}$  C. by water flowing from a thermostatically controlled constant-temperature bath.

The Beckman Model G pH meter was used in measuring pH.

Materials. Alpha-ketoglutaric acid (General Biochemicals, melting point 110-112° C.) was dissolved in water and neutralized

to bromothymol blue with 1N potassium hydroxide. The supporting electrolyte solution of pH 1.8 consisted of a 0.7M potassium chloride-hydrochloric acid solution. At pH 8.2 the electrolyte was a 1.0M ammonium chloride-ammonium hydroxide solution.

### EXPERIMENTAL

Solutions of  $\alpha$ -ketoglutaric acid were electrolyzed at pH 1.8 and 8.2 at various ionic strengths of supporting electrolyte solution. The usual polarographic procedure was followed. The mercury column of the dropping electrode was maintained at a constant height of 47.0 cm.

Capillary Constants. The weight of mercury delivered by the capillary under the conditions employed was 1.062 mg. per second. At pH 1.8 the drop time at an applied potential of -0.85 volt was 5.6 seconds and the value of  $m^{2/3}t^{1/6}$  was 1.39 mg.<sup>2/3</sup> sec.<sup>-1/2</sup> At pH 8.2 the drop time at -1.60 volts was 4.4 seconds and the value of  $m^{2/3}t^{1/6}$  was 1.33 mg.<sup>2/3</sup> sec.<sup>-1/2</sup>

Effect of Concentration, Ionic Strength, and pH. Solutions of  $\alpha$ -ketoglutaric acid give well defined polarographic waves whose values of  $E_{1/2}$  (-0.63 volt at pH 1.8 and -1.34 volts at pH 8.2) are independent of concentration over the range of 0.20 to 2.00 millimolar. The average diffusion current constant (I = i/C) $m^{2/3}t^{1/6}$ ) is 2.71  $\pm$  0.04 at pH 1.8 (10 observations) and 3.32  $\pm$ 0.07 at pH 8.2 (18 observations). A pH variation of 0.1 unit had no significant effect on  $E_{1/2}$  or  $i_d$ . At pH 8.2 a small secondary wave appeared at ionic strengths lower than 1.0; the combined wave heights were equal to that of the single wave at higher ionic strength. At pH 1.8 ionic strengths lower than 0.7 produced slight irregularities in the limiting portion of the wave.

### ANALYTICAL PROCEDURE

Select an aliquot (1 to 12 ml.) of the test solution so that the concentration of  $\alpha$ -ketoglutaric acid in the solution electrolyzed is in the range  $2 \times 10^{-4}$  to  $2 \times 10^{-3}M$ . Transfer this aliquot to a 50-ml. volumetric flask, with 35 ml. of supporting electrolyte solution, and dilute the contents to volume. Place the solution in the polarographic cell, and remove the dissolved oxygen by bubbling purified nitrogen through the solution for about 10 minutes. At pH 1.8, record the current produced over the applied voltage range of -0.35 to -0.85 volt. At pH 8.2 record the current produced over the applied voltage range of -1.05 to -1.60 volts.

Calculate the  $\alpha$ -ketoglutaric acid concentration from the equation:

 $C_m = \frac{C_s i_m}{i_s}$ 

molar concentration of  $\alpha$ -ketoglutaric acid in the

where  $C_m$ -

 $C_s$ 

sample molar concentration of  $\alpha$ -ketoglutaric acid in the standard

 $i_m$ current produced by  $\alpha$ -ketoglutaric acid in the sample i.

= current produced by  $\alpha$ -ketoglutaric acid in the standard

### SPECIFICITY

The presence of other anions is immaterial as long as they are not reducible over the range of measurement. Relatively few of the organic acids encountered in metabolic studies involving the tricarboxylic acid cycle are reducible polarographically. Of the reducible acids, only maleic acid will interfere at both pH 1.8 and 8.2. Pyruvic and cis-aconitic acids will not interfere at pH 1.8, but may interfere at pH 8.2. Fumaric acid will interfere at pH 1.8, but not at pH 8.2.

In the presence of proteins at pH 8.2, the limiting portion of the wave may be obscured by the appearance of an interfering wave at about -1.60 volts. At pH 1.8 the current produced is measured readily in the presence of protein, although the half-wave potential may be shifted to about -0.69 volt.

### APPLICATION

At pH 1.8 the polarographic method provides a rapid means for measuring the enzyme-catalyzed appearance or disappearance of  $\alpha$ -ketoglutaric acid in the absence of maleic and fumaric acids. Addition of supporting electrolyte buffered at acid pH will usually stop the enzyme reaction and the polarogram may be obtained at any convenient time. This procedure is useful especially in kinetic studies.

Table I shows the recovery of added  $\alpha$ -ketoglutaric acid from enzyme control mixtures in which the enzyme has been inactivated. Aliquots were withdrawn following incubation of the mixtures for 1 to 6 hours at 37° C. From these data it appears that  $\alpha$ -ketoglutaric acid may be determined within 3 to 4% under such conditions. The sensitivity of the method is of the order of 30  $\gamma$  per ml.

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# Determination of Sulfur by Peroxide Bomb Decomposition and Amperometric Titration

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ALTHOUGH numerous procedures have been described for the determination of micro quantities of sulfur in organic compounds, no detailed study has been reported on the use of amperometric techniques following peroxide combustion. The standard gravimetric micromethod is time-consuming and the turbidimetric procedure is subject to considerable error. For this reason, a study was undertaken to develop an amperometric titration of the sulfate resulting from a peroxide bomb combustion.

Majer (4) was the first to report an amperometric titration of sulfate in which lead nitrate was used as the titrating reagent. Since the solubility of lead sulfate varies greatly with the ionic strength of the medium, his procedure was not satisfactory for samples containing unknown quantities of salt. Spalenka (5) improved the method by specifying that the titration be performed in a medium containing 30 to 40% ethyl alcohol to repress the solubility of the precipitate. Kolthoff and Pan (3) described the optimum conditions for the amperometric titration of sulfate with lead. For a 0.001M sulfate solution, it was recommended that the medium contain at least 30% of ethyl alcohol and the potassium nitrate concentration be less than 0.1N. When this titration was applied by the authors to a system containing a high salt concentration, such as would be encountered in a peroxide fusion, the precipitation of lead sulfate was almost completely inhibited and, therefore, the method could not be adapted to the peroxide combustion. Another amperometric determination of sulfate was carried out by Heyrovský and Berezicky (1), who measured the decrease in the diffusion current of barium upon titration with sulfate. Apparently their results were inconclusive. Later Kolthoff and Gregor (2) made studies of the polarographic behavior of barium and established conditions for its determination by amperometric titration with potassium chromate.

The procedure described in this paper involves combustion of the sample in a Parr peroxide bomb, followed by precipitation of the resulting sulfate with excess barium, and an amperometric titration of the excess barium with a standard solution of potassium chromate. As the solubility product of barium sulfate is less than that of barium chromate, the precipitated barium sulfate does not redissolve in presence of small excess of chromate ions.

### APPARATUS

A Heyrovský polarograph, Model XII, manufactured by E. H. Sargent and Co., was used for the amperometric measurements. The drop time for the capillary in a 3M sodium chloride solution was 4 seconds at zero applied voltage. The cell was at room temperature. A silver-silver chloride electrode was used as the reference electrode. This was made by electrolyzing a saturated sodium chloride solution for approximately 3 minutes using a heavy gage silver wire as the anode. The large titration cell consisted of a 100-ml. wide-mouthed bottle fitted with a rubber stopper which contained openings for the silver-silver chloride electrode, a dropping mercury electrode, a nitrogen inlet and outlet tube, and the tip of a 5-ml. buret. The buret was graduated in 0.01 ml. For the microgram-scale titrations, the cell was a 50ml. centrifuge tube, which used the same components as the larger unit except that they were reduced to a smaller scale. A Parr electric ignition bomb (22-ml. capacity) was used for all combustions.

### REAGENTS

Merck's sodium peroxide and benzoic acid, and Fisher's potassium perchlorate were used for the combustion. Stock solutions of 0.01M barium chloride, 0.01M potassium chromate, and 3M sodium chloride were of Merck's reagent grade materials. The potassium chromate was standardized iodometrically with standard sodium thiosulfate solution. The other reagents, 10% sodium hydroxide solution, concentrated hydrochloric acid, sodium hypochlorite, and ethyl alcohol, also were reagent grade. The sodium hypochlorite solution was prepared by passing chlorine through a 5% solution of sodium hydroxide. Commercial sodium hypochlorite was not suitable, as it appeared to contain significant quantities of sulfate. National Bureau of Standards cystine, Merck's sulfanilamide, and Baker's sodium sulfate were used as standards.

### PROCEDURE

Combustion. To an 8- to 12-mg. sample, containing preferably 1 to 5 mg. of sulfur, are added 50 mg. of benzoic acid, 100 mg. of sodium perchlorate, and 1.5 grams of sodium peroxide. These materials are well mixed and then placed in a small paper cup which is placed in the nickel cup of a Parr macro electric bomb. The paper cup is constructed simply from a small piece of quantitative filter paper which weighs approximately 70 mg. It is used to hold the charge for combustion in the Parr electric bomb. The electric fuse wire is embedded in the charge inside the paper cup and ignited in the usual manner. After combustion, the bomb is cooled. The metal cup is placed in a small beaker, and the melt is dissolved in approximately 20 ml. of hot water. The cup is removed and washed with warm water, and the solution is transferred to a 250-ml. Erlenmeyer flask. A few glass beads are added and the solution is boiled on a hot plate for 5 minutes in order to decompose most of the peroxides. The remaining peroxides, which interfere with the amperometric titration, are decomposed by the addition of 5 ml. of sodium hypochlorite. The solution is boiled for an additional 2 minutes and then acidified by the dropwise addition of concentrated hydrochloric acid.

When sufficient acid has been added, the vigorous evolution of gases ceases and the color of the solution changes from dark gray to a clear amber. Approximately 7 ml. of concentrated hydrochloric acid are usually sufficient to decompose the excess sodium hypochlorite and to neutralize the alkaline digest. The solution is allowed to cool and is then transferred to a 50-ml. volumetric flask and diluted to the mark with distilled water. **Precipitation of Sulfate and Preparation for Titration.** A 20-

Precipitation of Sulfate and Preparation for Titration. A 20ml. aliquot of the combustion solution is placed in the large titration cell and warmed for approximately 5 minutes in a boiling water bath. Ten milliliters of the 0.01M barium chloride solution are added slowly to the aliquot, and the precipitate of barium sulfate is allowed to digest for 20 minutes. The solution is cooled, and the pH is carefully adjusted to the orange color of methyl red (ca. pH 6). In order to keep the volume to a minimum, this first is done approximately with a 10% solution of sodium hydroxide and the final adjustment is made with more dilute alkali. Thirty milliliters of ethyl alcohol are added to the solution, making a total volume of about 60 ml.

For the micro scale titration, all quantities are reduced by a factor of 10—e.g., a 2-ml. aliquot sample, 1 ml. of 0.01M barium chloride and 3 ml. of ethyl alcohol.

Amperometric Titration. The cell is connected to the titration assembly and deacrated with nitrogen for 5 minutes. The initial residual current is noted on the polarograph, which is adjusted to a sensitivity of  $5\times$  and an applied voltage of -1.35volts. The sample is titrated amperometrically with 0.01M potassium chromate. After each increment has been added, nitrogen is again bubbled through the solution for 2 minutes. Following the first sharp rise in the current, the increments of potassium chromate are limited to 1 ml. Four or five points past the first rise in current will usually be sufficient to obtain a straight line. The end point is determined graphically by plotting the galvanometer deflections (corrected for dilution effect) against the volume of titrant added. A blank determination is made to correct for any impurities in the reagents and to eliminate any effect due to the high salt concentration or other factors resulting from conditions of the determination. The same procedure is followed for the microtitration, but smaller increments (0.1 ml.) of titrant are added.

The sulfur content of the sample is calculated as follows:

Sulfur, wt. 
$$\% = \frac{V \times M \times 32.06 \times 100}{W}$$

where V = volume in milliliters of standard potassium chromate used by the sample, which equals the difference in volumes required by the sample and blank titrations; M = molarity of the standard potassium chromate; and W = weight in milligrams of the sample in the aliquot titrated.

### EXPERIMENTAL AND DISCUSSION

The paper cup arrangement for combustion in the electric ignition bomb was found to be more satisfactory than that in which the microbomb involving ignition by flame was used. During the course of these studies much more difficulty was encountered in repeatedly obtaining complete combustion with the micro flame bomb than with the electrically ignited bomb, even when micro samples were used. For that reason only the electric ignition method outlined in the procedure was followed.

Size of Sample. Using the procedure described, National Bureau of Standards cystine and Merck's sulfanilamide were analyzed. Samples ranging in sizes from 9 to 14 mg. were oxidized, and aliquots containing 0.1 to 1.5 mg. of sulfur were titrated. The results of these tests on known materials are tabulated in Table I and show an accuracy in the order of 98% of the theoretical value. In Figure 1 are recorded the data of a



Table I. Determination of Sulfur in Cystine and Sulfanilamide

	Wt. of Sulfu	ır in Aliquot, Mg.		
Sample	Present	Found <sup>a</sup>	1	Error, %
	Cystine	(26.7% Sulfur)		
1	1.50	1.47, 1.51		-0.8
2	1.36	1 33		-2.2
3	1.33	1.32.1.31		-1.1
4	1.02	1.00		-1.9
5	0.133	0.131		-1.5
			Av.	1.5
	Sulfanilamic	ie (18.6% Sulfur)		
1	1.09	1.05.1.07		-2.6
2	0.91	0.89.0.90		-1.1
3	0.176	0.173, 0.173		-1.6
			Av.	-1.8

 $^a$  Except for cystine sample 5, all results were obtained by macrotitration

Table II.	Effect	of Sodium	a Chloride or	n Titration of
Barium Cl	ıloride	Solution <sup>a</sup>	with Potassi	um Chromate

Sodium Chloride Added, Mg.	0.01 M Potassium Chromate Consumed, Ml.	Difference, Ml.
20 100 200 400 600	9.99 9.96 9.80 9.74 9.66	$ \begin{array}{r} -0.01 \\ -0.04 \\ -0.20 \\ -0.26 \\ -0.34 \end{array} $

 $^a$  Solutions contained 10 ml. of  $0.01\,M$  BaCl2, 20 ml. of H2O, and 30 ml. of ethyl alcohol.



typical experimental titration of the sulfate resulting from a cystine combustion. Curve *a* represents the microtitration of a 2ml. aliquot containing 102  $\gamma$  of sulfate sulfur from a peroxide combustion, 1 ml. of 0.01*M* barium chloride and 3 ml. of ethyl alcohol; curve *b* is the corresponding blank.

Salt Effect. To determine the effect of various concentrations of salts on the accuracy of the titration of barium with chromate, standard barium chloride solutions containing increasing concentrations of sodium chloride were used as the media. It was found that as the concentration of salt increased, the amperometric end point appeared earlier. The extent of this salt effect is seen in the data presented in Table II. Although not systematically investigated, an even more pronounced effect was caused by the presence of nitrate ion. For this reason, the presence of nitrate ions was avoided by the use of hydrochloric acid for the neutralization of the combustion products rather than nitric acid. The effect of salt on the end point of the titration is presumed to be due either to a change in the solubility of barium chromate or to a measurable amount of coprecipitation of barium ions. In any event, the blank determination, which is performed in the same manner as the unknown and with the same amounts of reagents, will compensate for these variations.

Effect of pH. Preliminary studies indicated that unless the proper pH was maintained, erratic and nonreproducible results would be obtained. This factor was studied by performing determinations on synthetic solutions which were adjusted to a selected pH value prior to the chromate titration. This synthetic solution consisted of 5 ml. of 0.01M sodium sulfate, 5 ml. of 3M sodium chloride, 10 ml. of 0.01M barium chloride, 10 ml. of water, and 30 ml. of ethyl alcohol. The pH was adjusted to the desired value, as measured with a Beckman Model G pH meter, by the careful addition of acid or alkali to the unbuffered solution. Below pH 3, no precipitate of barium chromate formed owing

to its solubility in mineral acids. At a pH between 3 to 5.5, precipitation was slow, and irregular results were obtained. In the pH range between 5.5 and 6.2, accurate and reproducible values were found. Above pH 6.5 values again became irregular and high, presumably owing to the presence of carbon dioxide from the air, resulting in the precipitation of barium carbonate. After several determinations the solutions with high pH values had a poisoning effect on the silver-silver chloride electrode.

Several buffer systems were investigated for the purpose of maintaining the pH within the designated range of 5.5 to 6.2. These included acetic acid-sodium acetate, malonic acid-sodium malonate, and succinic acid-sodium succinate. All these buffers, even in very small amounts, completely inhibited the reaction between barium and chromate ions. This was apparent from the fact that in addition to the absence of an observable precipitate of barium chromate, the current increased after the first increment of chromate had been added. In view of this unpredicted phenomenon, the pH thereafter was adjusted to the proper value without the use of buffers by simply adding acid or alkali until the solution was orange to methyl red.

Effect of Alcohol Concentration. Because the solubility of barium chromate is noticeably affected by the presence of in-

different salts, Kolthoff and Gregor (2) carried out the titration in the presence of alcohol. In order to ascertain the minimum concentration of ethyl alcohol that would be required to yield reliable results under the conditions of the determination, this factor was studied. The results obtained by titrating synthetic media similar to those used for the pH study but containing increasing concentrations of ethyl alcohol are illustrated in Figure 2. As seen in the figure, the solubility of barium chromate is appreciable when the alcohol concentration is below 50%, making the base line, and therefore the end point, uncertain. Consequently, it is recommended that the ethyl alcohol concentration be at least 50%.

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# **Determination of Formic Acid by Oxidation with Lead Tetraacetate**

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S LOW oxidation of formic acid to carbon dioxide by lead tetraacetate in aqueous acetic acid was originally reported by Grosheintz (4). More recently, Mosher and Kehr (5) have described the reaction with glacial acetic acid as solvent. The oxidation is greatly accelerated by potassium acetate, and the consequent enhanced rate of evolution of carbon dioxide makes the reaction suitable for manometric measurement of formic acid production during lead tetraacetate oxidations of carbohydra tes (6).

The present paper proposes that the accelerated reaction may have wider application for the determination of formic acid. In some respects, it offers advantages over the usual analysis by oxidation with mercuric chloride (1, 2). For example, the lead tetraacetate oxidation provides a simple, accurate, volumetric analysis (Table I, procedure 1) which is much more rapid and less tedious than the gravimetric procedure of the latter method. Further, Ahlén and Samuelson (1) have shown that

Table I.	Determination	of I	Formic	Acid
~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~		~ ~		~~~~

Procedure	HCOOH <sup>a</sup> Added, Mg.	Other Com- pounds Added, Mg. به د	HCOOH Found, Mg.	Reaction Time, Min.
1	$\begin{array}{c}1.05\\2.14\\2.14\end{array}$	 2.40 (Formaldehyde)	$1.06 \\ 2.12 \\ 2.13$	20 20 20
	2.11	5.0 (Propionic acid) 0.5 (Succinic acid)	2.11	20
2A	$\begin{array}{c} 0.080 \\ 0.16 \\ 0.33^{b} \end{array}$	0.43b (Formaldehyde)	0.077 0.16 0.33	30 30 30
2B	21.5 32.3	•••	21.3 21.4 32.1 32.7	75 90 60 75

<sup>a</sup> By alkali titration of a stock aqueous solution of formic acid. <sup>b</sup> Produced *in situ* by oxidation of 0.66 mg. of glycerol. formaldehyde interferes with the mercuric chloride method and must first be separated from the formic acid—e.g., in the analysis of sulfite waste liquors. In contrast, formaldehyde has no effect on the determination of formic acid by oxidation with lead tetraacetate (Table I). The lead tetraacetate procedure can also be used for determinations in the presence of other acids such as acetic, propionic, and succinic (Table I), and may therefore find application in the analysis of fermentation liquors.

Formic acid may be determined not only by titration of the amount of reduced lead tetraacetate but also by measuring the amount of carbon dioxide evolved. The latter procedure is especially useful in the presence of substances—e.g., glycols which consume lead tetraacetate but do not yield carbon dioxide or formic acid. The Warburg respirometer is suitable for the micro range (6) (Table I, procedure 2A), or a gas adsorption train for larger quantities of formic acid (Table I, procedure 2B). Procedures 2A and 2B are suited also to the determination of compounds, such as glycerol and oxalic acid, which yield formic acid or carbon dioxide when oxidized with lead tetraacetate.

#### EXPERIMENTAL

Reagents. Formic acid, reagent grade.

Glacial acetic acid, reagent grade.

Lead tetraacetate. A satisfactory method of preparation is given by Vogel (8).

Anhydrous potassium acetate, reagent grade.

Stopping solution: 10 grams of potassium iodide and 50 grams of sodium acetate dissolved in 100 ml. of water (3).

Standard 0.02N sodium thiosulfate. **Procedure 1.** Five milliliters of a solution of formic acid (1 to 5 mg.) in 80% acetic acid are added to 5 ml. of oxidizing solution, consisting of 100 mg. each of lead tetraacetate and potassium acetate in glacial acetic acid, in a glass-stoppered flask. A blank is prepared from 5 ml. of 80% acetic acid and 5 ml. of oxidizing solution. When the reaction has proceeded at room temperature (25° to 27° C.) for 20 to 30 minutes, 10 ml. of stopping solution is added. The yellow precipitate of lead iodide which appears may be dissolved by addition of water and the iodine is titrated with standard thiosulfate to a starch end point. The titration is equally satisfactory with the lead iodide present, but a pea-green color is given by the starch-iodine complex instead of the normal blue, and the end point is then indicated by the sudden reappearance of the bright yellow of the lead iodide.

**Procedure 2A.** A conventional constant-volume type of Warburg respirometer is used for measurement of carbon dioxide produced. Detailed descriptions of the apparatus and its manipulation are given by Umbreit *et al.* (7). The reaction temperature in the author's experiments was  $27^{\circ}$  C. One milliliter for a solution of the second state of the second state of the second state and potas-sium acetate in 90% acetic acid) is added to the vessel and 0.2 ml. of the formic acid solution (0.1 to 0.5 mg, of formic acid in 90% acetic acid) is the side of the second state acetic acid) is placed in the side arm. A blank is run simul-taneously with 1 ml. of oxidizing solution in the vessel and 0.2 ml. of 90% acetic acid in the side arm. The vessels are equilibrated for 10 to 15 minutes, the contents are mixed, and the change in pressure is observed until a constant value is attained (20 to 25 The apparatus is calibrated in the same manner by minutes). oxidizing known quantities of formic acid, or compounds such as glycerol and erythritol which yield known quantities of formic acid when oxidized by lead tetraacetate. Since the carbon dioxide produced is proportional to the change in pressure, the formic acid content of the unknown solution is determined by reference to the manometer calibration.

**Procedure 2B.** The apparatus is a gas train consisting of the reaction vessel provided with a gas-inlet and gas-outlet tube and a separatory funnel, a dry ice trap, a scrubbing bottle containing concentrated sulfuric acid, and a carbon dioxide adsorption tube containing Ascarite. In a typical experiment 10 ml. of 90% acetic acid containing 21.5 mg. of formic acid are added to the reaction vessel. The inlet tube is closed and the oxidizing solution, 25 ml. of 90% acetic acid containing 1 gram each of lead tetraacetate and potassium acetate, is introduced through the

separatory funnel. When the reaction has proceeded at room temperature for 30 to 40 minutes, the inlet tube is opened and a stream of carbon dioxide-free nitrogen is slowly flushed through the apparatus for 30 to 40 minutes, the reaction vessel being shaken occasionally. Carbon dioxide evolved is measured by the increase in weight of the ascarite tube. A blank determination is also made using the lead tetraacetate reagent and 90% acetic acid.

### ACKNOWLEDGMENT

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# Analysis of Alkyd Resins Modified with Vinyl Chloride-Acetate Copolymer

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WHEN alkyd resins are modified with vinyl chlorideacetate copolymer, they dry more rapidly and possess increased chemical and abrasion resistance. As in other types of modified alkyd resins, control is needed on the alkyd resin content as well as on the amount of modifying resin. Analysis for vinyl resins based entirely on total chlorine content does not prevent the substitution of other chlorinated resins and is affected by chlorinated plasticizers and solvents. The alkyd resin content of enamels has always been controlled by analysis for the phthalic anhydride present.

All methods for determining phthalic anhydride begin with saponification of the resin with alcoholic potassium hydroxide. Vinyl chloride-acetate resins decompose during saponification and form products which prevent determination of the phthalic anhydride content of the alkyd resin by any existing method. This

characteristic of vinyl resins necessitates an analysis based on separation of the vinyl resin from the alkyd. Alkyd resins are soluble in glacial acetic acid and aromatic hydrocarbons and partially soluble in ethyl alcohol, but vinyl chlorideacetate resins are insoluble in these materials. Use of aromatic solvents for separation would give a medium suitable for direct saponification of the alkyd resin, but consistent results could not be obtained in separations with this class of solvents. When the vehicle of the enamel was properly diluted with glacial acetic acid, then added dropwise to an excess of acetic acid, followed by considerable mechanical agitation, good quantitative separations could be obtained, except with alkyds modified with raw sovbean oil. Ethyl alcohol is an excellent precipitating medium for the separation of the vinyl resins, but alkyd resins are only partially soluble in ethyl alcohol at room temperature. If alkyd resins are first dissolved in a small amount of dioxane or methyl isobutyl ketone, they will not separate from solution when poured into alcoholic potassium hydroxide, even at low concentrations of alkali. Vinyl resins are decomposed by alkali, but a solution of 0.125Npotassium hydroxide in absolute ethyl alcohol precipitates the vinyl chloride-acetate resin without decomposition in the time required to effect separation, retaining the alkyd in solution. The separated vinyl resin must then be dried at a low temperature, since decomposition may occur at temperatures above 80° C. The alkyd resin is then present in a suitable saponifying medium for further analysis.

The vinyl chloride-vinyl acetate copolymers are available in varying proportions of the chloride to the acetate. A copolymer,

		<b>Table</b> ]	I. Resi	ults of Ana	lyses		
		Vinyl Ch Acetate Co by Gravis Separatio	lloride- polymer metric on, %	Vinyl Ch by Chlo Analysis	loride orine 5, %	Phthalic An %	hydride,
No.	Alkyd	Theoretical	Found	Theoretical	Found	Theoretical	Found
1	Castor oil, modified	32.1	$\frac{32.5}{32.3}$	29.4	29.5 29.4	23.8	23.8 23.8
2	Soybean oil, modified (low phthalic anhydride)	33.2	33.2	30.4	30.1	15.7	15.7
•	Timesed at		33.5		30.6		15.6
0	modified	34.2	33.1 33.0	31.3	$31.6 \\ 31.6$ }	•••	•••
4	Coconut oil, modified	33.2	34.0 33.5	30.4	31.7 $31.7$	•••*	

known as a medium acetate copolymer, having 89.5 to 91.5 parts of vinyl chloride combined with 2.5 to 5.5 parts of vinyl acetate (5.3 to 7.0 parts of vinyl alcohol), has the compatibility properties that make it best suited for use in vinyl-alkyd blends. The chlorine content of the vinyl chloride is known to vary, therefore, the total chlorine content of the resin serves only as an estimation of the composition of the copolymer. However, this analysis is desirable for quality control. The vinyl chloride resin can be estimated from its chlorine content, either on the enamel vehicle or on the isolated copolymer. Many methods for determining chlorine are available; most of those used with organic materials either involve special equipment or are time-consuming. To find a rapid, accurate, and simple method for total chlorine determination, suitable for routine testing and specification use to control the acetate length of the vinyl copolymer, a technique used to determine polystyrene in epoxy resins (2) was tested. This method, which involves fusion with potassium hydroxide, is not rapid, requires very little attention, and with slight modification, has proved satisfactory.

### PROCEDURE

Determination of Vinyl Chloride-Vinyl Acetate Copolymer. A dry 50-ml. Erlenmeyer flask is provided with a cork stopper and weighed. From 1 to 2 grams of the clear enamel vehicle (0.1 to 0.2 gram of vinyl resin) are poured into the flask, which is then stoppered and reweighed. Five milliliters of 1,4-dioxane are added, the sample is dissolved, and the flask is reweighed. The sample solution is poured very slowly into 200 ml. of 0.125Nalcoholic potassium hydroxide made with absolute ethyl alcohol contained in a 500-ml. glass-stoppered Erlenmeyer flask. The small sample flask is stoppered and reweighed, and the 500-ml. flask is shaken constantly for 5 minutes, preferably in a mechani-cal agitator. Without delay, the alkaline solution is filtered through a weighed fritted-glass crucible of coarse porosity, pre-viously prepared with a mat of asbestos. When all of the filviously prepared with a mat of asbestos. When all of the fil-trate has drained through, it is set aside for phthalic anhydride analysis, then the washing and transferring of the precipitate are continued with 95% ethyl alcohol. The crucible is given at least two washings with water followed by additional washing with 95% ethyl alcohol. It is then dried in an oven at  $60^{\circ}$  C. to constant weight (2 to 4 hours) and the vinyl resin content calculated on a solids basis.

% vinyl chloride-acetate copolymer =

weight of precipitate  $\times$  10,000

weight of sample  $\times$  % nonvolatile The actual weight of the sample used may be calculated in the following manner:

Following manner. If A = weight of empty flask (stoppered) B = weight of flask plus sample plus dioxane (before pouring) C = weight of flask plus residual sample (after pouring)the actual sample weight  $= \frac{B - C}{B - A} \times \text{original sample weight}$ 

Chlorinated paraffin or chlorinated diphenyl, if present in the original sample, is not precipitated by the alcohol and is present in the filtrate. Chlorinated rubber, if present, is precipitated and will appear in the crucible with the vinyl resin. It can be removed by washing with warm xylene ( $60^{\circ}$  C.) immediately after the vinyl resin has been washed with ethyl alcohol; the vinyl

resin is insoluble in xylene. Determination of Phthalic Anhydride in the Alkyd Resin. Determination of Phthalic Anhydride in the Alkyd Resin. The filtrate contains the alkyd resin. It is transferred, washing with absolute ethyl alcohol, to a 500-ml. Erlenmeyer flask and refluxed under a condenser for at least I hour in a water bath. The sample is allowed to cool to a comfortable temperature for handling, about 50° C., filtered while warm through a weighed fritted-glass crucible of medium porosity, washed, and dried in the weighted process. the usual manner.

Potassium carbonate may precipitate with the dipotassium phthalate and be a source of error. A correction is made by dissolving the weighed precipitate in about 50 ml. of distilled water that has been neutralized to the phenolphthalein end point. If the solution is alkaline to phenolphthalein, it is titrated with 0.1Nhydrochloric acid.

Weight of potassium carbonate = ml. of HCl  $\times$  normality  $\times$ 0.1382

Deduct the weight of potassium carbonate from the weight of dipotassium phthalate and calculate the phthalic anhydride, on a solids basis:

### % phthalic anhydride =

corrected weight of dipotassium phthalate  $\times$  6110

weight of sample  $\times$  % nonvolatile

The actual weight of the sample used was determined in the analysis for the vinyl resin.

If chlorinated paraffin was present in the original sample, potassium chloride is formed during saponification and affects the phthalic anhydride analysis. Its presence is indicated by dissolving the dipotassium phthalate precipitate in water and testing for chlorides by adding 6N nitric acid followed by an aqueous silver nitrate solution. Chlorinated diphenyl has no effect on the phthalic or vinyl determination. If the dipotassium phthalate contains chlorides, the phthalic anhydride should be determined by precipitation with lead acetate (1), using aliquots estimated to contain 30 to 45 mg. of phthalic anhydride.

Determination of Vinyl Chloride in Chloride-Acetate Copolymer. The enamel vehicle or the isolated copolymer separated from the alkyd resin may be used for the chlorine analysis. If there is no evidence of other chlorinated resins or plasticizers present, the determination can be made more conveniently from the vehicle of the enamel.

From 1.0 to 2.0 grams of vehicle are weighed into a nickel cru-From 1.0 to 2.0 grams of venicle are weigned into a nicker cru-cible of 30-ml. capacity. Six grams of potassium hydroxide pellets are added and the contents of the crucible dried in an oven at 110° C. for 3 hours. The fusion of the dried sample is con-ducted as for the analysis of styrenated epoxy resins (2), with the following modification: After 45 minutes of fusion, the cover is removed and the temperature gradually increased at 15-minute intervals until the fusion is complete.

Without cooling, the crucible is transferred to a 400-ml. beaker and the contents are poured into the bottom of the beaker. When cool, sufficient water is added to cover the crucible completely When and the contents are boiled for 15 minutes. The sample is filtered through a double thickness of filter paper of medium porosity into a 600-ml. beaker, and the crucible and rod are scrubbed and washed with water. The filtrate is neutralized with 6N nitric washed with water. The filtrate is neutralized with 6N nitric acid and an excess of 5 ml. of acid added for every 100 ml. of water While stirring vigorously, 20 ml. of a 5% silver nitrate volume. solution are added, and the sample is heated to boiling. When cool, it is filtered through a Gooch crucible which has been heated to  $200^{\circ}$  C., cooled, and weighed. Transfer of the precipitate and washing are done with 2% nitric acid. The crucible is dried at 110° C. for an hour followed by 15 minutes of additional drying at 200° C.

$$\%$$
 vinyl chloride =  $\frac{\text{weight of AgCl} \times 4360}{\text{weight of sample} \times \% \text{ nonvolatile}}$ 

If preferred, the volumetric method for determining chloride may be used. If a vinyl chloride-acetate copolymer of the medium acetate length was used, the vinyl chloride content of the sample should equal 89.5 to 91.5% of the copolymer found.

#### DISCUSSION

A vinyl alkyd resin made according to specification was used as A vinyl alkyd resin inade according to specification in the according as a known sample. Resin vehicle, Number 1 in Table I was made by blending 32.1 parts of Vinylite VAGH and 67.9 parts alkyd resin (nonvolatile basis). The nonvolatile content of the finished vehicle was 30.7%. The vehicles tested were also pigmented in Eacher Strategie for the finished vehicle was 30.7%. with the same formulation was used in Federal Specification TT-E-485b, then separated without difficulty by the usual high-speed

centrifuging. The vinyl chloride content of Vinylite VAGH, the copolymer used to formulate these enamels, was also determined by the fusion method. This resin, believed to contain 89.5 to 91.5% vinyl chloride, was analyzed to be 91.5 and 91.6%.

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# **Mass Spectrographic Analysis of Solids**

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THE application of mass spectroscopy to the analysis of solids has become of great interest within the last few years, and several different approaches to the problem have been made. The choice of technique and instrumentation depends greatly upon the nature of the analytical problem.

Dempster (4) was the first to discuss the analysis of traces of impurities in solids with the mass spectrograph. A double-focusing mass spectrograph, designed for mass measurements (2), a spark source (3), and photographic detection of the ions, was used for this work. Shaw and Rall (13) constructed a double-focusing mass spectrograph of the Mattauch type, with a spark source and photographic detection, for the analysis of solids. No applications to analysis using this instrument have been reported. Gorman, Jones, and Hipple (5, 9), using a modified Dempster instrument with a spark source and electrical detection, have demonstrated the quantitative possibilities of mass spectrometric analysis of solids. Their work was done with stainless steel samples, with determinations of chromium and nickel in concentrations ranging from 0.3 to 25%.

For specialized problems, ion sources may be used to simplify the instrumentation. Hickam (7, 8) studied impurities in copper by vaporizing the metal into an electron bombardmenttype ionization chamber, with time integration of the ion currents. Inghram and his coworkers (10, 11) have obtained very high sensitivities using isotopic dilution techniques and both thermal ionization and electron-bombardment ion sources.

For a general analysis of impurities in solids, the vacuum spark source has several great advantages. It is reasonably independent of such factors as relative volatility of the various impurities, differences in ionization efficiencies and nature of the material being analyzed, so that sensitivities remain high regardless of the element being determined. Contamination and background difficulties are less than for some other types of sources, as the spark is confined only to the material under analysis, and no other source parts are heated. Disadvantages of the spark source, such as the large spread in initial energies of the ions and the erratic fluctuations of current from the source, must be allowed for in the instrumentation.

The use of the spark source creates problems in the ion-detection method. The electrical detection scheme of Gorman, Jones, and Hipple used a ratio measurement to avoid the problem created by the current fluctuations due to the erratic nature of the spark. In this work they measured the ratio of the resolved ion current emerging from the magnetic analyzer to the current arriving at an electrode which served to monitor the total cur-

rent from the source. In the studies of high concentration samples, this method gave good quantitative results; but in samples of very low concentrations very high sensitivity is required in the detection system for the resolved ion current. For a typical sample, in the instrument used for the work described below. the average ion current to the final collector is of the order of  $5 \times 10^{-11}$  ampere for the singly charged peaks of the principal component. A 1 p.p.m. impurity would give a current of less than 10<sup>-16</sup> ampere, requiring a device such as an electron multiplier for detection. While this is feasible, the instrumentation to achieve maximum sensitivity in the vicinity of the large radio-frequency spark voltages becomes difficult. Photographic detection provides high sensitivity, integration of currents despite source fluctuations, and the simultaneous recording of a wide mass range: but it is less quantitative, requires the introduction of photographic plates into the vacuum system, and does not provide visual information while the source is in operation. The choice between electrical detection and photographic methods is not clear, and would depend considerably on the problems to be investigated. When quantitative results are desired, the electrical method appears preferable; when semiquantitative identifications of trace impurities are required, the photographic method has advantages.

# INSTRUMENTATION

The instrumental aspects of the work have been described in detail (6). A Mattauch-type instrument with double focusing and a spark source was constructed. The instrument was designed for either electrical recording or photographic detection; however, the photographic detection method was used in most of this work, because of the nature of the problems. Ilford Q plates have been used for all work.

The instrument is designed with a demountable source and access to the photographic plate region. The sample and plate can be changed in about 0.5 hour, by using cutoff devices in the pump lines to permit continuous operation of the pumps during this procedure, and pumping times can be as low as an hour. For electrical recording, a blank metal plate carrying an exit slit is substituted for the photographic plate and the current through this aperture strikes a collector mounted on a glass press. A "monitor" electrode between the electric and magnetic fields permits ratio measurements of the type made by Gorman, Jones, and Hipple.

### SAMPLES

Samples for this instrument are handled in the form of rods, 0.030 to 0.040 inch in diameter and 0.25 to 0.50 inch long. These are held in pin vises, so the cross-sectional shape of the rods is



unimportant. Only the tips of the rods are analyzed, as the spark is confined to this area and the amount of wearing away of the electrodes in any reasonable exposure time is small. Because the rods form part of the electrical circuit in the spark source a modification of the technique is required for insulators.

Most metals, and semiconductors like silicon and germanium, can be sawed or formed readily into rods of the desired shape. In some cases only small chips of material may be available, which are too small to be held directly in the pin vises; such material can often be forced into the ends of metal tubes with small diameters and then held in pin vises.

In the limited amount of work that has been done with insulators, the materials were ground up into a powder and packed into a metal tube 0.040 inch in diameter—gold, for example. The metal tube not only serves as a container for the powder, but also provides the necessary conducting path. With this arrangement the mass spectrum of the powder and the metal with its impurities are observed; therefore, high purity of the metal tube is desirable. Since all metals are likely to show some impurity, it probably will be necessary to choose a tube material that is free of the impurities one expects to encounter in the particular sample under study. By having several different metal tubes available, it should be possible to investigate any impurity. A run made on the empty metal tube serves as a blank. The same technique also should permit the handling of metal powders.

Contaminations on surfaces of conductors have been studied. There appears to be no simple way to study surface contaminations on insulators, or on conductors of a shape that does not permit handling in the pin vises.

Because of the high sensitivity of the instrument, surface contaminations and bulk impurities appear in the mass spectra. Careful etching of the sample is desirable to avoid unnecessary background. Surface contaminations are distinguished easily from bulk impurities as they appear strongly only in the first exposure, so there is usually no difficulty in determining impurity lines.

# MASS SPECTRA

Typical mass spectra are shown in Figure 1. Positions on the plate and line widths vary as the square root of the mass. It may be seen that nearly a 15 to 1 mass range can be covered in a single exposure. Each plate ordinarily permits six exposures, so several exposure times and magnetic fields (mass ranges) can be covered for each sample. For a 0.002-inch principal slit, the resolution of the instrument is 1500—that is,  $m/\Delta m = 1500$ , where  $\Delta m$  is the least distinguishable mass difference at mass m. This high resolution is useful in certain analytical identifications as it usually is sufficient to distinguish between and identify two lines of the same nominal mass, differing only by the small amount arising from their different packing fractions.

In Figure 1, the spectra show: (a) germanium sample with added antimony at the concentration of 6 p.p.m. (atom fraction); (b) arsenic impurity in antimony at 100.p.p.m.; (c) copper sample with 1 monolayer of gold deposited on its surface. Exposure times were 3 minutes, 30 seconds, and 1 minute, respectively. In the original plates, the antimony in germanium lines showed clearly with an exposure time of 5 seconds.

### ANALYTICAL RESULTS

A considerable variety of metals and semiconductors has been run in the machine with no important differences in the ease of handling. Many elements have appeared as impurities with no evidence of "blind spots" in the method. From these results, it appears qualitatively that, within a factor of about ten, the sensitivities for the different impurity elements in any metal will be equal. The differences arise because of different degrees of efficiency of ionization, and differences in photographic plate sensitivities for various ions. Larger sensitivity differences hold for the emission spectrograph. One of the reasons for the relatively small spread in sensitivities in the mass spectrograph is the nature of the spark (high voltage, very short "on" periods) which minimizes differences in volatility, ionization efficiencies, etc. In order to obtain semiquantitative analyses, rather than qualitative identifications or comparisons of concentrations of impurities, calibrations with standard samples are necessary as in the case of the emission spectrograph. If further work indicates that mass spectrographic sensitivities vary less than emission spectrographic sensitivities, the calibration problem for the mass spectrograph will be somewhat simplified. For many systems, standard samples could be prepared using radioactive tracers as the added impurity.



Calculations have been carried out for two systems, boron in silicon and antimony in germanium. These were chosen because samples were available covering a wide range in concentrations. Concentrations were determined from electrical resistivity measurements, with  $\sigma = nev$ , where  $\sigma$  is the electrical conductivity, nis the carrier concentration, e is the electronic charge, and vis the mobility. From this equation, n can be calculated from measurements of  $\sigma$  and published values of v. Mobilities for germanium were taken from Conwell (1), and for silicon from data obtained by Morin (12). Assuming complete ionization of the impurity centers at room temperature, the impurity concentration is equal to the carrier concentration n.

In optical spectroscopy, an internal standard is required to allow for variations in the source and in photographic plate characteristics. The most convenient internal standard in the present work has been to take a line originating from the principal component-i.e., germanium or silicon-and to refer the impurity intensity to this line. The instrumental arrangement permits six exposures on each plate, so that each sample was run for exposure times ranging from 1 second to 15 minutes. Spark conditions were kept nearly constant throughout the exposures and from one sample to another. Absorbances for the impurity line and the standard line were measured with a recording densitometer, and these absorbances were plotted as a function of exposure time. A typical plot, Figure 2, shows the expected "absorbance vs. intensity" character. If it is assumed that the source remained constant during the exposures, then the relative values of the ion currents for the two lines can be determined by the factor necessary to shift one curve into the other along the intensity (exposure) axis. This is simply the ratio of exposure times for a given absorbance in the linear portion of the curves. This procedure was followed for each sample and the same reference line was used in each case. The relative intensity values give the mass spectrographic determination of concentration (in arbitrary units). The results for boron in silicon and antimony in germanium are shown in Figures 3 and Five different samples were available for each system and in 4. most cases each sample was run twice, but not in consecutive

runs. In view of the experimental uncertainties in maintaining constancy of the spark during the exposures between samples, in determining relative intensities from the absorbance measurements, and in the mobilities used to calculate impurity concentrations, the results appear to be satisfactory. These results indicate that after calibration analyses are accurate within an uncertainty factor of 2 or 3.

The absorbance represented by the lowest concentration antimony lines was as low as could be measured on the densitometer; hence the indicated uncertainty for this sample is large. Exposure times of less than 1 second or a change to a secondary line of the impurity element are necessary to keep absorbance low enough for quantitative measurement for samples above a concentration of about  $10^{-4}$ . In the latter case a weak isotopic line would be most satisfactory, but where this is not available a multiple-charge line could be used.

Surface contaminations are distinguished readily from bulk impurities, as they show up strongly only in the first exposurei.e., in the initial sparking. Thereafter, they appear weakly as the spark gradually wears the electrode away and reaches new areas of the surface. Bulk impurities remain constant throughout the exposures.



Samples for surface contamination studies were prepared by evaporation of known quantities of appropriate metals onto the surface of clean copper wires or germanium rods. Mass spectra were obtained from samples having 0.1 and 1.0 monolayer of gold on copper, 0.1 and 1.0 monolayer of indium on copper, and a sample of germanium on which one monolayer each of indium, silver, aluminum, chromium, iron, nickel, copper, and palladium was deposited. In all these cases the lines of the deposited material were readily visible.

The number of atoms of impurity necessary for detection as a surface contamination was compared with the number necessary as a bulk impurity to give a line of the same absorbance. In the case of surface contamination, roughly  $10^{-2}$  sq. cm. of surface is sparked initially and for a one-monolayer sample this corresponds to approximately 1012 atoms of the impurity. By comparison, to obtain a line of the same absorbance but originating from a bulk impurity, an exposure of 20 seconds for an impurity concentration of  $10^{-6}$  and a total weight loss of about 0.01 mg. are required, corresponding to 10<sup>11</sup> atoms of the impurity. Very roughly the same number of impurity atoms are required in either case.

To check the feasibility of the method for insulators and for powdered samples, samples of lithium carbonate and the same lithium carbonate with 5 p.p.m. of zirconium nitrate were packed into gold tubes and run. The zirconium was detectable; so



this method is usable, but no effort was made to learn if sensitivities are as high as for metals.

### CONCLUSIONS

An instrument with photographic detection is capable of semiquantitative analyses at concentrations at least as low at  $10^{-7}$ , for most elements. The presence of background lines prevents determination of such elements as carbon, oxygen, nitrogen, and hydrogen at low concentrations. Surface contaminations as low as 0.1 monolayer are detectable. These sensitivities have been reached with photographic detection; the method undoubtedly can be made more quantitative with electrical recording, as shown by Gorman, Jones, and Hipple, but with a probable loss in sensitivity. The present limitation on sensitivity is set by faint background lines originating from traces of organic contaminants in the source chamber, and by diffuse background due to ion scattering and change of ion mass or charge in transit.

The primary usefulness of this method is for problems not suited for emission spectrographic analysis. These are for the most part cases where the emission spectrographic sensitivity is inadequate, and where general, semiquantitative results are needed. For this type of work the present method has given good results, and provides information not available by any other method.

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# Noninterference of Phosphate in an Ethylenediamine Tetraacetate **Method for Serum Calcium**

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N THE determination of calcium in leaf tissue by titration with the disodium salt of ethylenediaminetetraacetic acid (EDTA), using ammonium purpurate as indicator, Willson (5)encountered interference by orthophosphate when the ratio of phosphorus to calcium was 0.1 or greater (by weight). In cow's milk, where the ratio of phosphorus to calcium is approximately 0.8, Jenness (4) overcame the interference of phosphate by removing it with an anion exchange resin.

The authors wished to determine the calcium concentration of rat serum in which, under certain experimental conditions, the phosphorus-calcium ratio may be as high as 4. The ethylenediamine tetraacetate method of Fales (1), with minor modifications, was chosen for this purpose because it requires only 0.5 ml. of serum for each determination rather than 2 ml., as in the classical method of Fiske and Logan (2) previously in routine use in this laboratory. An investigation of the extent of the interference of phosphate in the new method was considered to be necessary. It has been found that under the specified conditions of the analytical procedure there is no interference even when phosphorus-calcium ratios are as high as 8.

## EQUIPMENT, REAGENTS, AND PROCEDURE

The recommendations of Fales (1) were followed with minor (Details of the modified procedure will be supplied exceptions. on request.) Microburets (5-ml. capacity, 0.01-ml. graduations) were used instead of pipets for addition of reagents. Aliquots of 0.5 or 1.0 ml. were diluted to 6.0 ml. with distilled water and 0.5 ml. of indicator, freshly filtered, was added. In addition, the following standard solutions were prepared: Standard Calcium Solution. Calcium acetate monohydrate

(0.44 gram), analytical reagent, was dissolved in water and diluted to 1 liter. The calcium content was found by 14 determinations in duplicate by the method of Fiske and Logan (2).

Table I. Effect of Phosphate on Analysis of Aqueous Solutions and of Serum for Calcium by Ethylenediaminetetraacetic Acid

	P-Ca Ratio	Calcium, M	g./100 Ml.ª
Solutions Studied	Calcd.	Theory b	Found
Calcium acetate c	$\begin{array}{c} 0 \\ 0.26 \\ 0.53 \\ 0.78 \\ 1.03 \\ 1.28 \\ 1.58 \end{array}$	8.45 8.45 8.45 8.45 8.45 8.45 8.45 8.45 8.45	$\begin{array}{r} 8.45 \\ 8.41 \\ 8.27 \\ 8.43 \\ 8.41 \\ 8.48 \\ 8.27 \end{array}$
Calcium chloride	$0 \\ 1.28 \\ 2.60 \\ 3.59 \\ 5.04$	5.43 5.43 5.43 5.43 5.43 5.43 5.43	$5.43 \\ 5.56 \\ 5.49 \\ 5.97 \\ 5.67$
Human serum <sup>d</sup>	$\begin{array}{c} 0.41 \\ 1.51 \\ 2.71 \\ 3.78 \end{array}$	$\begin{array}{c} 6.14 \\ 6.14 \\ 6.14 \\ 6.14 \\ 6.14 \end{array}$	$\begin{array}{c} 6.14 \\ 6.37 \\ 6.19 \\ 6.32 \end{array}$
Rat serum <sup>d</sup>	$\begin{array}{c} 0.59 \\ 1.65 \\ 3.76 \\ 5.84 \\ 8.09 \end{array}$	4.70 4.70 4.70 4.70 4.70	$4.70 \\ 4.71 \\ 4.74 \\ 4.76 \\ 4.67$

<sup>c</sup> Concentrations are given for solutions analyzed; 0.5-ml. aliquots of cal-cium acetate, calcium chloride, and human serum, and 1.0-ml. aliquots of rat serum were taken.

serum were taken.
 On basis of analysis with no added phosphate.
 Calcium acetate solution standardized by 14 determinations in duplicate
 by method of Fiske and Logan (\$).
 Phosphorus determined by method of Fiske and Subbarow (\$).

Standard Phosphate Solution. Anhydrous potassium dihydrogen phosphate (4.39 grams), analytical reagent, was dissolved in water and diluted to 1 liter after the addition of 10 ml. of 10N sulfuric acid.

Calcium Chloride Solution. An aqueous solution containing approximately 7.5 mg. of calcium per 100 ml.

All solutions were prepared with distilled water which contained only negligible amounts of calcium.

### RESULTS

Varying amounts of standard phosphate solution were added to standard calcium acetate solution, calcium chloride solution, human and rat blood serum, respectively, to provide a range of phosphorus-calcium ratios which includes values far in excess of expectation in the analysis of serum calcium. As shown in Table I. on analysis of aliquots of these solutions for calcium by the present method no evidence of interference by phosphate was disclosed.

### DISCUSSION

The method here described for analysis of calcium by titration with ethylenediaminetetraacetic acid in a photoelectric colorimeter is applicable to solutions of calcium salts-for example, diluted blood serum-which are far more dilute than the samples tested by Willson and required by his method, which relies upon natural vision for determination of the end point. The calcium concentrations in the solutions titrated (the 1.0-ml. aliquot for human serum and 0.5-ml. aliquots for the other samples were diluted to a total of 6.5 ml. for titration) ranged from 0.42 to  $0.72~\mathrm{mg.}$  per 100 ml. as compared with a range of 9.2 to 15 mg. per 100 ml. used by Willson. The authors attribute the absence of interference by phosphate in the present method to the fact that the ion products are well below the solubility product  $(K_{s,p})$  of calcium diphosphate (CaHPO<sub>4</sub>), in contrast to the conditions of Willson's experiments.

Difficulties may be encountered in the application of the present method to the analysis of urine, particularly of pathological origin, where phosphorus-calcium ratios as high as 250 are not unknown. Here the prior removal of phosphate with an anion exchange resin, as recommended by Jenness (4) in another application, may be helpful.

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# Flame Photometric Determination of Calcium in Wet-Process Phosphoric Acid

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E CONOMY in the production of wet-process phosphoric acid requires that the by-product calcium sulfate be easily filtered from the acid. Growth of the crystals of calcium sulfate to a readily filterable size is influenced greatly by the ratio of calcium ions to sulfate ions in the mother liquor (9). Control of this ratio is hampered when the time-consuming oxalate method (5) is used for the calcium determination. Iron, aluminum, and phosphorus interfere and necessitate a double precipitation of calcium oxalate, with tedious filtrations.

Flame photometric methods based upon the internal standard principle (2, 4) have been used for the determination of calcium in complex materials (1, 6, 10, 11). The direct intensity principle, with standards simulating the samples, also has been used for the determination of calcium (3, 8). The internal standard technique has the advantage of simplicity in the preparation of standards.

The flame photometric method described here-an internal standard method-cuts hours from the usual time required for the determination of calcium in wet-process phosphoric acid.

### APPARATUS AND REAGENTS

Flame Photometer. Perkin-Elmer. Model 52-C, with acetylene burner.

Shaker. Equipped to hold 250-ml. flasks.

Standard Calcium Solution. Dissolve 3.5696 grams of dried reagent calcium carbonate in 100 ml. of 1M hydrochloric acid The calcium oxide content is 2 mg. per ml. and dilute to 1 liter. Internal Standard-Strontium Chloride Solution. Dissolve

30.45 grams of the hexahydrate in distilled water and dilute to

1 liter. The strontium content is 10 mg. per ml. Cation Exchange Resin. Amberlite IR-120(H), analytical grade (Rohm & Haas Co.) or its equivalent. Treat 100 grams of resin with 5M hydrochloric acid in an ion exchange column until flame photometric tests of the eluate show an absence of sodium and calcium. Rinse the resin thoroughly and air-dry it.

Ammonium Nitrate. Analytical grade.

### STANDARDIZATION

Prepare a series of eleven concentration standards containing 50 ml. of strontium chloride solution and from 0 to 50 ml. of the standard calcium solution in 5-ml. increments. Dilute to 500 ml.

Warm up the electrical circuit of the photometer and adjust the burner to give a steady flame. Set the element selector at about 5500 A. and adjust to maximum sensitivity while atomizing the calcium chloride solution.

To calibrate the photometer, take readings on the two end concentration standards until the settings are reproducible. Then take three readings on each intermediate standard, with a recheck of the end standards after each series. Plot average readings against concentration. The standards correspond to from zero to 20 grams of calcium oxide per liter with the unknown in its specified dilution.

### ANALYSIS

Warm a 100-ml. sample of the unknown with 50 ml. of hydrochloric acid until suspended matter is dissolved. Dilute to vol-ume in a 1-liter volumetric flask. Pipet a 10-ml. aliquot into a 250-ml. flask. Dilute to 150 ml. with distilled water and add 1 250-ml. flask. Dilute to gram of IR-120(H) resin.

Shake flask and contents in the shaking machine for 15 minutes. Collect the resin on a coarse filter paper and wash it ten times with distilled water.

Dry the paper without charring and brush the resin into a platinum dish. Add 2.5 grams of solid ammonium nitrate. Heat on a hot plate until the reaction subsides, then over a Bunsen burner until organic matter is eliminated. Cool the residue, add 5 ml. of 72% perchloric acid, and evaporate just to dryness on a hot plate. Add 5 drops of concentrated hydrochloric acid and 30 ml. of distilled water. Warm to clarity, mix with 10 ml. of the internal standard solution in a 100-ml. volumetric flask, and dilute to volume.

Pour some of the unknown into the photometer and estimate the calcium content. Pour in some of the standard solution whose calcium content is nearest that of the unknown. Agreement of the scale reading with that obtained in the calibration indicates that the instrument is ready for the final measurements.

Take three alternate readings for the unknown and for the nearest standard solution. Calculate the average reading for the unknown and read the calcium content from the calibration curve.

# **EVALUATION OF METHOD**

The preponderance of phosphorus pentoxide over calcium oxide in wet-process phosphoric acid is as much as a hundredfold. The acid also contains iron, aluminum, fluorine, and sulfur as impurities.

Preliminary tests of the effects of phosphorus and of these impurities on the calcium determination were made with reagent chemicals. Lithium was the internal standard. The photometer was tuned to the diatomic band emission that occurs at about 5500 A. and which gives much greater sensitivity than the primary calcium line emission at 4226 A.

Iron, fluorine, and sulfur in their usual proportions were without effect. Aluminum depressed the calcium readings, the depression becoming more severe as the ratio of aluminum to calcium was increased. Phosphorus also interfered. Moreover, the action of aluminum and phosphorus together was synergistic.

Mitchell and Robertson (7) found that strontium lessens the interference of aluminum in the determination of calcium by the Lundegårdh flame spectrographic method. The materials with which they worked, however, apparently did not entail the combined effect of aluminum and phosphorus. Since aluminum affects the spectral emissions of strontium and calcium in the same way, strontium was viewed as a potential internal standard in the present problem.

Strontium chloride equivalent to 100 grams of strontium per liter was added to a series of five solutions containing 300 grams of phosphorus pentoxide, 4 grams of calcium oxide, and from 10 to 50 grams of aluminum oxide per liter. The emission intensities then were compared with those of similar phosphorusfree solutions. Since the factory setting of the fixed slit to transmit the lithium line emission at 6708 A. corresponds to a band emission of strontium chloride, adjustment of the slit was unnecessary.

The calcium found in the phosphorus-free solutions was uniformly 100% of that present. The calcium found in the phosphorus-containing solutions ranged from 98% of that present at 10 grams of aluminum oxide per liter to 80% at 50 grams of aluminum oxide per liter. To take advantage of the strontium standard as a means of eliminating the interference of aluminum, the phosphorus would have to be removed.

A separation by means of an ion exchange resin often proves practical for eliminating the interference of one ion in the determination of another of opposite charge. The commonly used column technique of ion exchange-with regeneration of the resin---requires an aggregate time, however, that was viewed as objectional in the present work. An alternative was sought through a batch separation of cations with a small amount of resin that then could be considered as expendable.

Calcium was separated from solution quantitatively when an aliquot equivalent to 1 ml. of wet-process acid was diluted to

Table I. Comparison of Photometric and Chemical Determination of Calcium in Wet-Process Phosphoric Acid

Pho	tometric N	fethod	Ch	Chemical Method		
Av.	Max.	Min.	Av.	Max.	Min	
1.1	4.1	4.0	4.2	4.3	4.0	
2.7	2.7	2.6	2.6	2.7	2.6	
4.1	4.1	4.0	4.0	4.0	4.0	
8.3	8.4	8.0	8.5	8.5	8.4	
2.7	2.8	2.6	2.7	2.8	2.6	
2.4	2.4	2.3	2.4	2.5	2.4	
3.7	3.7	3.5	3.8	3.8	3.7	
3.0	3.1	2.9	2.8	2.9	2.8	
3.0	3.1	2.9	3.1	3.2	3.1	
2.2	2.3	2.1	2.1	2.2	$\tilde{2}.1$	

150 ml. and shaken for 15 minutes with 1 gram of Amberlite IR-120(H) resin. The resin was collected on a paper filter, transferred to a platinum dish, and ignited with ammonium nitrate at a low temperature. The ash was digested with perchloric acid. Strontium chloride was added as an internal standard and calcium was determined photometrically. Triplicate analyses of ten samples of wet-process acid by this method are compared with analyses by the conventional chemical method in Table I.

Some commercial wet-process acids contain sodium that was added to precipitate silica. This sodium enhances the photometric reading for calcium.

Tests on synthetic samples showed that the effect of sodium on the calcium determination is linear at concentrations of sodium oxide from 0 to 4 grams per liter. When the sodium oxide content is higher, part of it must be removed. About half of it can be removed without loss of calcium by washing the resin twenty times with 0.1M hydrochloric acid. Further loss of sodium through sublimation in the ashing step lowers the sodium to less than 20% of that present in the original acid.

The flame photometric method is practically as precise as the chemical method and is faster. A single determination of calcium in wet-process phosphoric acid can be made in 1 hour, whereas the chemical method requires 5 hours. Multiple analyses by the photometric method require about one third the time required by the chemical method.

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# **Isolation and Purification of Semimicro Quantities of Morphine**

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ETHODS for the isolation of morphine from the opium poppy are numerous (3) but not satisfactory for the recovery of quantities of the order of 1 to 10 mg. The cost of production of carbon-14-labeled morphine (at least 20 mc. of carbon-14 as barium carbonate are required at a cost of \$36 per mc.) by biosynthesis (5) in amounts sufficient for tracer and metabolic studies requires a method which will give maximum yields from the limited amounts of radioactive Papaver somniferum available.

It has been shown that certain of the ion exchange resins can be applied successfully to the separation and purification of substances containing the basic nitrogen group (4). The fact that morphine contains both an N-methyl and a phenolic hydroxyl group somewhat simplifies the problem and, accordingly, several ion exchange resins were evaluated. Of the resins studied, optimum performance and maximum recoveries were obtained with Nalcite SAR (anion, polyamine) and Amberlite IRC-50 (cation, carboxylic acid). The procedure for the extraction, purification, and crystallization of morphine is given below.

### REAGENTS AND SOLUTIONS

Aqueous sodium carbonate, 10% Aqueous ammonium chloride, 20%. 1-Butanol-benzene, 1 to 1. Aqueous sodium bicarbonate, 5%.

Sulfuric acid, 0.525N. Potassium hydroxide, 2%, saturated with barium hydroxide. Aqueous methanol, 50%. Hydrochloric acid, 0.1N. Aqueous sodium hydroxide, 10%. Dihydrogen potassium-phosphate-disodium monohydrogen phosphate buffer, pH 7.0, 0.5M.

Chloroform-ethyl alcohol, 3 to 1.

Absolute ethyl alcohol.

Concentrated aqueous ammonia

# **ISOLATION AND PURIFICATION PROCEDURES**

Two grams of oven-dried (80° C.) P. somniferum capsules are ground to 40 mesh in a Wiley mill, transferred to a 40-ml. glassstoppered centrifuge tube, and mixed intimately with 10 ml. of subpered centringle tube, and initial intrinsitely with 10 mill of a 10% aqueous sodium carbonate solution. After 1 hour of quiet standing, the mixture is brought to pH 8.6  $\pm$  0.1 by the addition of 20% aqueous ammonium chloride (approximately 5 ml. being required) and extracted to 15 ml. of 1 to 1 1-butanol-benzene solution. The mixture is centrifuged to separate the organic layer, which is drawn off. The extraction is repeated twice using 10 ml. of 1-butanol-benzene solution in each case. The organic layers are combined, and shaken in succession with two 15-ml. portions of 5% aqueous sodium bicarbonate to remove pigments, many of which are present in the organic layer following the original extraction. The 1-butanol-benzene frac-tion is then shaken with 15 ml. of 0.525N sulfuric acid and washed with two 10-ml. portions of water, so as to remove the morphine and certain impurities to the aqueous phase. The procedure described permits recoveries of the alkaloid on the order of 92 to 95%; these values were checked by carrying out extractions using known amounts of morphine sulfate (1 to 20 mg. per determination) in aqueous solution. Such control extractions were carried out to the last stage (sulfuric acid) and measured spectrophotometrically at 285 and 298 m $\mu$ , respectively. In no case was less than 92% of the alkaloid recovered.

The solution obtained by the preceding extraction is next treated with 11.4 ml. of a saturated solution of barium hydroxide in 2% potassium hydroxide, and passed through a column of Nalcite SAR (hydroxide form, dimensions of  $0.8 \times 20$  cm.) at a flow rate of 0.2 to 0.4 ml. per minute. The barium-potassium hydroxide treatment removes the sulfuric acid and converts the alkaloid to its anionic form; thus facilitating its uptake by the resin. Filtration of the sulfuric acid extract treated with potassium-barium hydroxide is necessary prior to introduction to the column so that obstruction by the barium sulfate precipitate is avoided. The extract is followed by 200 ml. of 50% aqueous methanol, which treatment removes a considerable amount of pigment, as determined spectrophotometrically (7). Elution of the morphine from the column is accomplished with 0.1N hydrochloric acid, with the alkaloid being present in the first 30 ml. of effluent following the change of pH from neutrality to approximately 2.0.

Nalcite SAR has proved to be the best quaternary base resin available for separation of this type. Other strongly basic anion exchange resins were carefully evaluated and found not suitable (2) for these purposes. The large effluent volumes, required for maximum recovery (2), did not permit efficient handling of small amounts of the alkaloid.

Morphine SO4	Morphine SO <sub>4</sub> Recove	ered from Nalcite SAR
Added, Mg.	Mg.	%
4.0	4.0	100
3.7	3.65	98.8
2.0	1.97	98.5

The Nalcite effluent containing morphine is adjusted to pH 7.0 with 10% sodium hydroxide, about 3 ml. being required, and passed through a column of Amberlite IRC-50 (same dimensions as Nalcite column) which has been buffered at pH 7.0 with 0.5M dihydrogen potassium phosphate-disodium monohydrogen phosphate buffer. The experimental conditions of flow rate, washing, and elution are the same as for the Nalcite procedure.

These two resins can be used consecutively without significant loss of the alkaloid. The resins provide essentially quantitative recovery by the technique applied to the quantitative determination of the alkaloid in commercial preparations. To demonstrate this point, 4.2 mg. of morphine sulfate was applied to a Nalcite column. Morphine was determined in the effluent, which was then applied to the IRC-50, according to the outlined procedure. The results are as follows when the alkaloid was measured at 285 m $\mu$  in the Beckman spectrophotometer:

	Theory, Mg.	Found, Mg.	%Recovered
Nalcite SAR IRC-50	$\begin{array}{c} 4.2 \\ 4.2 \end{array}$	$\begin{array}{c} 4.2\\ 4.15\end{array}$	$\substack{100\\98.8}$

Washing of the column with aqueous methanol effectively removed pigment which previously had been eluted with the morphine from the Nalcite column. The final effluent is evaporated to dryness over phosphorus pentoxide; the solid material remaining is morphine suitable for crystallization. Occasionally, small amounts of pigment still contaminate it and interfere with the crystallizing procedure. In this event, the following purification procedure is recommended:

The residue is dissolved in 5 ml. of 0.1N hydrochloric acid and transferred to a glass-stoppered centrifuge tube. The mixture is adjusted to pH 8.6 with aqueous ammonia, and extracted with 15 ml. of 3 to 1 chloroform-ethyl alcohol. The organic layer is drawn off and the extraction repeated twice with 10-ml. portions of the same solvent; these are combined, and evaporated to dryness over paraffin chips. One crystallization of the residue is sufficient to obtain pure alkaloid. Crystallization is accomplished by the following method:

Crystallization is accomplished by the following method: The residue from the IRC-50 column or chloroform extraction is dissolved in 0.5 to 1.0 ml. of 0.1N hydrochloric acid. Four drops of absolute alcohol are added and the whole is mixed well. One drop of concentrated ammonia is introduced, the mixture is agitated, placed in the cold, and crystallization usually begins immediately.

# CRITERIA OF PURITY OF ISOLATED PRODUCT

1.	Carbon-nyurogen determination	С	н
2.	Calculated, % Found, % Melting point (6), 229 to 230° C.	67.3 67.6	$\substack{\textbf{6.93}\\\textbf{7.10}}$

Mixed melting point, 230° C. 3. Rf values, paper chromatography (Whatman No. 1)

Carbon hadronen determination

	Ratio	$R_f$
1-Butanol-acetic acid saturated with water	100 to 4 100 to 30	0.468 0.660

A sample of morphine base monohydrate was analyzed concurrently with each determination. The deviation of "unknown"  $R_f$  values from the reference standard in each system was  $\pm 0.01$ .

4.	Spectrophotometric Data	Isolated Product	Reference Standard
	Ultraviolet absorption in hydrochloric	acid, pH 2.0	(1)
	Absorption maximum, m $\mu$ Absorbance	$\begin{array}{c} 285 \\ 0.134 \end{array}$	$\substack{\textbf{285}\\\textbf{0.134}}$
	Ultraviolet absorption in alkaline (sodiu solution, pH 10	m h <b>ydroxide</b> )	(7)
	Absorption maximum, mµ	$\substack{298\\0.178}$	$298 \\ 0.178$

Absorbance Ratios. The ratio of absorbance at absorption maximum to absorbance at the closest minimum is useful for the characterization of a given compound as well as closely related substances (3). Analysis was made on the isolated material, the authentic sample of morphine (base) and pseudomorphine, which compound is likely to form during the extraction of morphine from alkaline media (5). Determinations carried out in the Beckman DU spectrophotometer gave:

Compound	Ratio
Morphine base monohydrate Unknown Pseudomorphine	$1.75 \\ 1.75 \\ 1.143$

### SUMMARY

The recovery of small amounts of labeled morphine from plant material requires modification of conventional extraction procedures to ensure maximum yield. The application of two ion exchange resins, Dowex II and Amberlite IRC-50, has resulted in the recovery of morphine of high purity. The technique presented may also be applied to the quantitative determination of the alkaloid in pharmaceutical preparations, animal tissues, and plant material.

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# Decay and Growth Tables for the Naturally Occurring Radioactive Series

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THE tables which follow are intended primarily for use by the analytical chemist. While in no sense complete, they have been compiled in an effort to reduce or eliminate much of the tedious and duplicative labor involved in the analysis of radioactive materials.

The tables are divided into three sections devoted to the three naturally occurring radioactive series. The nuclear data are those compiled by the National Bureau of Standards (2).

The decay of each radiosotope is given in one or more appropriate time units except where the half life of a particular nuclide is either too long or too short to be of analytical importance.

The growth of families is given in relative disintegration rates, which are readily determined by standard counting methods, rather than in numbers of atoms. The criterion throughout has been utility to the analyst. Thus, where the growth of a family is either too slow to be useful, or is effectively complete in a few hours, it has not been computed.

Although the uncertainty of the half lives and the precision of present-day counting techniques do not warrant more than three significant figures in most cases, values have been computed to four or five significant figures to permit reasonably accurate interpolation.

The equations used for calculating growth and decay were adapted from those originally proposed by Rutherford (3) and Bateman (1).

### SYMBOLS

$N_0$	= number of atoms of the parent (first member) of
$T_{1}, T_{2}, T_{n}$	a family at an arbitrary time, $t = 0$ = half life of the first, second, <i>n</i> th member of a family

Table I.

Mode of Decay

Isotope

Th 282

Ra 228

Ac<sup>228</sup>

Th 228

Ra 224

↓ Rn<sup>220</sup>

↓ -Po<sup>216</sup>

At216\_

→ Pb212

-Bi<sup>212</sup>∢

↓

Po212

T1 208

T

Pb<sup>208</sup>∢

Synonym

ThC'

ThC<sup>4</sup>

ThD

α

8-

Stable

Table II.       Thorium Series (4n)									
<b>m</b> .		(Dec	ay in mir	utes and	hours)				
Min.	Ac <sup>228</sup>	Th <sup>228</sup>	Ra <sup>224</sup>	Rn <sup>220</sup>	Pb <sup>212</sup>	Bi212	T} 208		
0 1 2 3 4 5	$\begin{array}{c} 1.0000\\ 0.9981\\ 0.9962\\ 0.9943\\ 0.9925\\ 0.9906 \end{array}$	1.0000	1.0000 0.9999 0.9997 0.9996 0.9995 0.9993	$\begin{array}{c} 1.0000\\ 0.4662\\ 0.2174\\ 0.1013\\ 0.0472\\ 0.0220 \end{array}$	$\begin{array}{c} 1.0000\\ 0.9989\\ 0.9978\\ 0.9967\\ 0.9956\\ 0.9946 \end{array}$	$\begin{array}{c} 1.0000\\ 0.9886\\ 0.9774\\ 0.9662\\ 0.9552\\ 0.9443 \end{array}$	$\begin{array}{c} 1.0000\\ 0.7996\\ 0.6394\\ 0.5113\\ 0.4089\\ 0.3269 \end{array}$		
6 7 8 9 10	$\begin{array}{c} 0.9888\\ 0.9869\\ 0.9851\\ 0.9831\\ 0.9814 \end{array}$		$\begin{array}{c} 0.9992 \\ 0.9991 \\ 0.9989 \\ 0.9988 \\ 0.9988 \\ 0.9987 \end{array}$	$\begin{array}{c} 0.0103 \\ 0.0048 \\ 0.0022 \\ 0.0010 \\ 0.0005 \end{array}$	$\begin{array}{c} 0.9935 \\ 0.9924 \\ 0.9913 \\ 0.9902 \\ 0.9892 \end{array}$	$\begin{array}{c} 0.9336 \\ 0.9229 \\ 0.9124 \\ 0.9020 \\ 0.8917 \end{array}$	$\begin{array}{c} 0.2614 \\ 0.2090 \\ 0.1672 \\ 0.1337 \\ 0.1069 \end{array}$		
20 30 40 50	$\begin{array}{c} 0.9630 \\ 0.9451 \\ 0.9274 \\ 0.9101 \end{array}$		$\begin{array}{c} 0.9974 \\ 0.9960 \\ 0.9947 \\ 0.9934 \end{array}$	0.0000	$0.9784 \\ 0.9678 \\ 0.9573 \\ 0.9470$	$\begin{array}{c} 0.7952 \\ 0.7091 \\ 0.6324 \\ 0.5639 \end{array}$	$\begin{array}{c} 0.0114 \\ 0.0012 \\ 0.0001 \\ 0.0000 \end{array}$		
Hours									
$1 \\ 2 \\ 3 \\ 4 \\ 5$	$\begin{array}{c} 0.8931 \\ 0.7976 \\ 0.7123 \\ 0.6362 \\ 0.5681 \end{array}$	0.9999 0.9999 0.9999 0.9998 0.9998	0.9921 0.9843 0.9765 0.9688 0.9611		0.9367 0.8774 0.8219 0.7698 0.7211	$\begin{array}{c} 0.5029 \\ 0.2529 \\ 0.1272 \\ 0.0639 \\ 0.0322 \end{array}$			
6 7 8 9 10	$\begin{array}{c} 0.5074 \\ 0.4532 \\ 0.4047 \\ 0.3614 \\ 0.3228 \end{array}$	0.9998 0.9997 0.9997 0.9996 0.9996	$\begin{array}{c} 0.9535 \\ 0.9460 \\ 0.9385 \\ 0.9311 \\ 0.9237 \end{array}$		$\begin{array}{c} 0.6755\\ 0.6327\\ 0.5927\\ 0.5552\\ 0.5200 \end{array}$	$\begin{array}{c} 0.0162 \\ 0.0081 \\ 0.0041 \\ 0.0021 \\ 0.0010 \end{array}$			
$11 \\ 12 \\ 13 \\ 14 \\ 15$	$\begin{array}{c} 0.2883 \\ 0.2575 \\ 0.2299 \\ 0.2053 \\ 0.1834 \end{array}$	0.9995 0.9995 0.9995 0.9994 0.9994	$\begin{array}{c} 0.9164 \\ 0.9092 \\ 0.9020 \\ 0.8948 \\ 0.8878 \end{array}$		$\begin{array}{c} 0.4871 \\ 0.4563 \\ 0.4274 \\ 0.4003 \\ 0.3750 \end{array}$	$\begin{array}{c} 0.0005\\ 0.0003\\ 0.0001\\ 0.0001\\ 0.0000 \end{array}$			
16 17 18 19 20	$\begin{array}{c} 0.1638 \\ 0.1463 \\ 0.1306 \\ 0.1167 \\ 0.1042 \end{array}$	$\begin{array}{c} 0.9993 \\ 0.9993 \\ 0.9993 \\ 0.9993 \\ 0.9992 \\ 0.9992 \end{array}$	0.8807 0.8738 0.8669 0.8600 0.8533		$\begin{array}{c} 0.3512 \\ 0.3290 \\ 0.3082 \\ 0.2887 \\ 0.2704 \end{array}$				
21 22 23 24	$\begin{array}{c} 0.0931 \\ 0.0831 \\ 0.0742 \\ 0.0663 \end{array}$	$0.9991 \\ 0.9991 \\ 0.9990 \\ 0.9990 \\ 0.9990$	$0.8465 \\ 0.8398 \\ 0.8332 \\ 0.8266$		$0.2533 \\ 0.2373 \\ 0.2222 \\ 0.2082$				

$\lambda_n$	-	decay constant of the nth mem-
		ber of a family ( $\lambda_n = 0.69315/$
		$T_n$ )
$N_0\lambda_1$	-	activity (disintegration rate) of
		the parent of a family at $t = 0$
$N_1\lambda_1, N_n\lambda_n$	=	activities of the first and $n$ th
		members of a family at any time
$\alpha_0$	=	alpha activity of the parent at $t = 0$
$\alpha_t$	=	total alpha activity of the chain
		at any time
$\beta_t$	=	total beta activity of the chain at
		any time

## **GROWTH AND DECAY EQUATIONS**

Relative activity of a single radioactive species:

$$\frac{N_1\lambda_1}{N_0\lambda_1} = e^{-\lambda_1 t} \tag{1}$$

Activity of the first decay product relative to the initial activity of a parent which was pure at t = 0:

$$\frac{N_2\lambda_2}{N_0\lambda_1} = \frac{\lambda_2}{\lambda_2 - \lambda_1} \left( e^{-\lambda_1 t} - e^{-\lambda_2 t} \right)$$
(2)

•••	α	4.0	$1.39 \times 10^{10}  \mathrm{Y}$	5.0×10 <sup>-11</sup> Y <sup>-1</sup>
MsTh1	β -	0.002	6.7 Y	$\begin{bmatrix} 0 & 1035 \text{ Y}^{-1} \\ 0 & 000283 \text{ D}^{-1} \end{bmatrix}$
MsTh2	β-	1.6	6.13 H	$\begin{bmatrix} 2.7138 \text{ D}^{-1}\\ 0.1131 \text{ H}^{-1} \end{bmatrix}$
RdTh	α	$\begin{bmatrix} 72\% & 5.4 \\ 28\% & 5.3 \end{bmatrix}$	1.90 Y	$\begin{bmatrix} 0.3648 & Y^{-1} \\ 0.00100 & D^{-1} \end{bmatrix}$
$\mathbf{Th}\mathbf{X}$	α	$[{}^{95\%}_{5\%}{}^{5.7}_{5.4}$	3.64 D	0.1904 D <sup>-1</sup> 0.0079 H <sup>-1</sup>
Tn	α	6.3	54.5 S	$[ \begin{smallmatrix} 0 & .7631 & M^{-1} \\ 0 & .0127 & S^{-1} \end{smallmatrix} ]$
ThA	$\begin{bmatrix}\alpha\\(0.014\%\beta^{-})\end{bmatrix}$	6.8 (?)	0.158 S	$[ \substack{263.2 \cdot M^{-1} \\ 4.3870 \ S^{-1} }$
••••	α	7.8	0.0003 S	2300 S <sup>-1</sup>
ThB	β-	$[\frac{88\%}{12\%} \frac{0.3}{0.6}$	10.6 H	$\begin{bmatrix} 1 . 5694 D^{-1} \\ 0 . 0654 H^{-1} \end{bmatrix}$
ThC	$\begin{bmatrix} 66.3\% \beta^{-} \\ 33.7\% \alpha \end{bmatrix}$	$\begin{bmatrix} 2.3 \\ 1\% 5.6 \\ 2\% 5.8 \\ 70\% 6.0 \\ 27\% 6.1 \end{bmatrix}$	60.5 M	$\begin{bmatrix} 0.6874 \text{ H}^{-1} \\ 0.0115 \text{ M}^{-1} \end{bmatrix}$

8.8

1.8

Thorium Series (4n)

Half Life

 $3.04\times10^{\,-7}\,\mathrm{S}$ 

3.1 M

. . .

Decay Constants

Energies in Mey.

ACA
 IIIn K

 $2.3 \times 10^{6} \, \mathrm{S}^{-1}$ 

[13.416 H<sup>-1</sup> 0.2236 M<sup>-1</sup>

# Table III. Thorium Series (4n)

			(De	ecay in da	ys)			
Time, Days	Ra <sup>228</sup>	A c 228	Th228	Ra <sup>224</sup>	Pb <sup>212</sup>	Time Days	, Ra <sup>228</sup>	Th <sup>228</sup>
0 1 2 3 4 5	$\begin{array}{c} 1.0000\\ 0.9997\\ 0.9994\\ 0.9992\\ 0.9989\\ 0.9989\\ 0.9986 \end{array}$	$\begin{array}{c} 1.0000\\ 0.0663\\ 0.0044\\ 0.0003\\ 0.0000 \end{array}$	$\begin{array}{c} 1.0000\\ 0.9990\\ 0.9980\\ 0.9970\\ 0.9960\\ 0.9950 \end{array}$	$\begin{array}{c} 1.0000\\ 0.8266\\ 0.6833\\ 0.5648\\ 0.4669\\ 0.3859 \end{array}$	$\begin{array}{c} 1.0000\\ 0.2082\\ 0.0433\\ 0.0090\\ 0.0019\\ 0.0004 \end{array}$	50 51 52 53 54 55	$\begin{array}{c} 0.9859 \\ 0.9856 \\ 0.9854 \\ 0.9851 \\ 0.9848 \\ 0.9845 \end{array}$	$\begin{array}{c} 0.9513 \\ 0.9503 \\ 0.9494 \\ 0.9484 \\ 0.9475 \\ 0.9465 \end{array}$
6 7 8 9 10	0.9983 0.9980 0.9977 0.9975 0.9972		0.9940 0.9930 0.9920 0.9910 0.9901	$\begin{array}{c} 0.3190 \\ 0.2637 \\ 0.2180 \\ 0.1802 \\ 0.1489 \end{array}$	0.0001 0.0000	56 57 58 59 60	$\begin{array}{c} 0.9843 \\ 0.9840 \\ 0.9837 \\ 0.9834 \\ 0.9831 \end{array}$	$\begin{array}{c} 0.9456 \\ 0.9446 \\ 0.9437 \\ 0.9427 \\ 0.9418 \end{array}$
11 12 13 14 15	0.9969 0.9966 0.9963 0.9960 0.9958		$\begin{array}{c} 0.9891 \\ 0.9881 \\ 0.9871 \\ 0.9861 \\ 0.9851 \end{array}$	$\begin{array}{c} 0.1231 \\ 0.1018 \\ 0.0841 \\ 0.0695 \\ 0.0575 \end{array}$		61 62 63 64 65	$\begin{array}{c} 0.9829 \\ 0.9826 \\ 0.9823 \\ 0.9820 \\ 0.9817 \end{array}$	$\begin{array}{c} 0.9409 \\ 0.9399 \\ 0.9390 \\ 0.9380 \\ 0.9371 \end{array}$
16 17 18 19 20	$\begin{array}{c} 0.9955\\ 0.9952\\ 0.9949\\ 0.9946\\ 0.9943 \end{array}$		$\begin{array}{c} 0.9841 \\ 0.9832 \\ 0.9822 \\ 0.9812 \\ 0.9802 \end{array}$	$\begin{array}{c} 0.0475\\ 0.0393\\ 0.0325\\ 0.0268\\ 0.0222 \end{array}$		66 67 68 69 70	$\begin{array}{c} 0.9815\\ 0.9812\\ 0.9809\\ 0.9806\\ 0.9804 \end{array}$	$\begin{array}{c} 0.9362 \\ 0.9352 \\ 0.9343 \\ 0.9334 \\ 0.9324 \end{array}$
21 22 23 24 25	0.9941 0.9938 0.9935 0.9932 0.9929		0.9792 0.9783 0.9773 0.9763 0.9753	$\begin{array}{c} 0.0183 \\ 0.0152 \\ 0.0125 \\ 0.0104 \\ 0.0086 \end{array}$		71 72 73 74 75	$\begin{array}{c} 0.9801 \\ 0.9798 \\ 0.9795 \\ 0.9792 \\ 0.9790 \end{array}$	$\begin{array}{c} 0.9315 \\ 0.9306 \\ 0.9296 \\ 0.9287 \\ 0.9278 \end{array}$
26 27 28 29 30	0.9927 0.9924 0.9921 0.9918 0.9915		$\begin{array}{c} 0.9743 \\ 0.9734 \\ 0.9724 \\ 0.9714 \\ 0.9705 \end{array}$	$\begin{array}{c} 0.0071 \\ 0.0058 \\ 0.0048 \\ 0.0040 \\ 0.0033 \end{array}$		76 77 78 79 80	0.9787 0.9784 0.9781 0.9779 0.9776	$\begin{array}{c} 0.9269 \\ 0.9259 \\ 0.9250 \\ 0.9241 \\ 0.9232 \end{array}$
31 32 33 34 35	0.9913 0.9910 0.9907 0.9904 0.9901		$\begin{array}{c} 0.9695\\ 0.9685\\ 0.9676\\ 0.9666\\ 0.9656\\ \end{array}$	0.0027 0.0023 0.0019 0.0015 0.0013		81 82 83 84 85	0.9773 0.9770 0.9767 0.9765 0.9762	$\begin{array}{c} 0.9222 \\ 0.9213 \\ 0.9204 \\ 0.9195 \\ 0.9186 \end{array}$
36 37 38 39 40	0,9898 0,9896 0,9893 0,9890 0,9887		$\begin{array}{c} 0.9647 \\ 0.9637 \\ 0.9627 \\ 0.9618 \\ 0.9608 \end{array}$	$\begin{array}{c} 0.0011\\ 0.0009\\ 0.0007\\ 0.0006\\ 0.0005 \end{array}$		86 87 88 89 90	0.9759 0.9756 0.9754 0.9751 0.9748	$\begin{array}{c} 0.9176 \\ 0.9167 \\ 0.9158 \\ 0.9149 \\ 0.9140 \end{array}$
41 42 43 44 45	0.9884 0.9882 0.9879 0.9876 0.9873		$\begin{array}{c} 0.9598 \\ 0.9589 \\ 0.9579 \\ 0.9570 \\ 0.9560 \end{array}$	$\begin{array}{c} 0.0004\\ 0.0003\\ 0.0003\\ 0.0002\\ 0.0002\\ 0.0002 \end{array}$		91 92 93 94 95	$\begin{array}{c} 0.9745 \\ 0.9743 \\ 0.9740 \\ 0.9737 \\ 0.9734 \end{array}$	$\begin{array}{c} 0.9131 \\ 0.9121 \\ 0.9112 \\ 0.9103 \\ 0.9094 \end{array}$
46 47 48 49 50	0.9870 0.9868 0.9865 0.9862 0.9859		0.9551 0.9541 0.9532 0.9522 0.9513	$\begin{array}{c} 0.0002 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \end{array}$		96 97 98 99 100	0.9732 0.9729 0.9726 0.9723 0.9723 0.9721	0.9085 0.9076 0.9067 0.9058 0.9049

Activity of the second decay product relative to the initial activity of a parent which was pure at t = 0:

$$\frac{N_{3}\lambda_{3}}{N_{0}\lambda_{1}} = \lambda_{2}\lambda_{3} \left[ \frac{e^{-\lambda_{1}t}}{(\lambda_{1} - \lambda_{2})(\lambda_{1} - \lambda_{3})} + \frac{e^{-\lambda_{1}t}}{(\lambda_{2} - \lambda_{1})(\lambda_{2} - \lambda_{3})} + \frac{e^{-\lambda_{1}t}}{(\lambda_{3} - \lambda_{1})(\lambda_{3} - \lambda_{2})} \right]$$
(3)

Activity of the *n*th member of the decay chain relative to the initial activity of a parent which was pure at t = 0:

$$\frac{N_n\lambda_n}{N_0\lambda_1} = (-1)^{n-1} (\lambda_2 \dots \lambda_n) \times \begin{bmatrix} \frac{e^{-\lambda_1 t}}{(\lambda_1 - \lambda_2) (\lambda_1 - \lambda_3) (\dots) (\lambda_1 - \lambda_n)} + \dots \\ \dots + \frac{e^{-\lambda_n t}}{(\lambda_n - \lambda_1) (\lambda_n - \lambda_2) (\dots) (\lambda_n - \lambda_{n-1})} \end{bmatrix}$$
(4)

# USE OF THE TABLES

Fraction Remaining After a Given Time. EXAMPLE 1. What fraction of radium-223 remains after 15 days?

# Table IV. Thorium Series (4n)

(Growth of decay products from initially pure parent.<sup>a</sup> Parent, thorium-228)

Valid 1 hour after purification, assuming 100% retention of radon-220.
 Assumes 100% counting yield of all betas.

Table V. Thorium Series (4n)

# (Growth of decay products from initially pure parent. Parent, thorium-228)

Time, Days	Ra <sup>324</sup>	Pb212	$\alpha_t/N_0\lambda_1$	$\beta_t/N_0\lambda_1$
0 1 2 3 4 5	$\begin{array}{c} 0.0000\\ 0.1733\\ 0.3165\\ 0.4345\\ 0.5319\\ 0.6123 \end{array}$	$\begin{array}{c} 0.0000\\ 0.0878\\ 0.2280\\ 0.3578\\ 0.4679\\ 0.5593 \end{array}$	$\begin{array}{c} 1.0000\\ 1.5986\\ 2.1666\\ 2.6508\\ 3.0535\\ 3.3861 \end{array}$	$\begin{array}{c} 0.0000\\ 0.2204\\ 0.5929\\ 0.9404\\ 1.2356\\ 1.4809 \end{array}$
6 7 8 9 10	0.6786 0.7332 0.7781 0.8151 0.8456	$\begin{array}{c} 0.6349 \\ 0.6972 \\ 0.7485 \\ 0.7907 \\ 0.8255 \end{array}$	$\begin{array}{r} 3.6603 \\ 3.8861 \\ 4.0718 \\ 4.2245 \\ 4.3503 \end{array}$	$\begin{array}{r} 1.6835 \\ 1.8506 \\ 1.9882 \\ 2.1015 \\ 2.1950 \end{array}$
11 12 13 14 15	$\begin{array}{c} 0.8705 \\ 0.8910 \\ 0.9078 \\ 0.9214 \\ 0.9325 \end{array}$	$\begin{array}{c} 0.8540 \\ 0.8775 \\ 0.8967 \\ 0.9124 \\ 0.9252 \end{array}$	$\begin{array}{r} \textbf{4.4533} \\ \textbf{4.5372} \\ \textbf{4.6060} \\ \textbf{4.6619} \\ \textbf{4.7072} \end{array}$	$\begin{array}{r} 2.2717 \\ 2.3345 \\ 2.3861 \\ 2.4283 \\ 2.4626 \end{array}$
16 17 18 19 20	$\begin{array}{c} 0.9416 \\ 0.9488 \\ 0.9547 \\ 0.9594 \\ 0.9631 \end{array}$	$\begin{array}{c} 0.9356 \\ 0.9440 \\ 0.9508 \\ 0.9563 \\ 0.9606 \end{array}$	$\begin{array}{r} 4.7437 \\ 4.7729 \\ 4.7968 \\ 4.8156 \\ 4.8299 \end{array}$	$\begin{array}{r} 2.4905 \\ 2.5130 \\ 2.5314 \\ 2.5462 \\ 2.5577 \end{array}$
21 22 23 24 25	$\begin{array}{c} 0.9660 \\ 0.9682 \\ 0.9698 \\ 0.9710 \\ 0.9719 \end{array}$	0.9640 0.9667 0.9687 0.9702 0.9713	$\begin{array}{r} 4.8410 \\ 4.8493 \\ 4.8554 \\ 4.8596 \\ 4.8622 \end{array}$	2.5669 2.5741 2.5795 2.5836 2.5865
26 27 28 29 30	$\begin{array}{c} 0.9724 \\ 0.9726 \\ 0.9727 \\ 0.9725 \\ 0.9723 \end{array}$	$\begin{array}{c} 0.9720 \\ 0.9724 \\ 0.9726 \\ 0.9726 \\ 0.9726 \\ 0.9724 \end{array}$	4.8635 4.8637 4.8631 4.8617 4.8597	$\begin{array}{r} 2.5884 \\ 2.5896 \\ 2.5901 \\ 2.5901 \\ 2.5896 \\ 2.5896 \end{array}$
31 32 33 34 35	0.9719 0.9714 0.9708 0.9701 0.9694	$\begin{array}{c} 0.9721 \\ 0.9717 \\ 0.9711 \\ 0.9705 \\ 0.9699 \end{array}$	$\begin{array}{r} 4.8572 \\ 4.8543 \\ 4.8511 \\ 4.8476 \\ 4.8439 \end{array}$	$\begin{array}{r} 2.5888 \\ 2.5876 \\ 2.5862 \\ 2.5846 \\ 2.5829 \end{array}$
36 37 38 39 40	0.9687 0.9679 0.9671 0.9663 0.9654	$\begin{array}{c} 0.9692 \\ 0.9684 \\ 0.9676 \\ 0.9668 \\ 0.9659 \end{array}$	$\begin{array}{r} 4.8399 \\ 4.8359 \\ 4.8317 \\ 4.8274 \\ 4.8230 \end{array}$	2.5810 2.5789 2.5768 2.5746 2.5724
41 42 43 44 45	0.9645 0.9636 0.9627 0.9618 0.9609	0.9651 0.9642 0.9633 0.9624 0.9615	4.8185 4.8140 4.8094 4.8048 4.8002	2.5701 2.5677 2.5653 2.5629 2.5605
46 47 48 49 50	$\begin{array}{c} 0.9599 \\ 0.9590 \\ 0.9581 \\ 0.9571 \\ 0.9562 \end{array}$	$0.9605 \\ 0.9596 \\ 0.9587 \\ 0.9577 \\ 0.9568 \\ 0$	4.7955 4.7908 4.7861 4.7814 4.7767	$\begin{array}{r} 2.5580 \\ 2.5556 \\ 2.5530 \\ 2.5506 \\ 2.5481 \end{array}$

### Table VI. Thorium Series (4n)

(Growth of decay products from initially pure parent.<sup>a</sup> Parent, radium- 224)

 $\begin{array}{l} (\mathrm{Ra}^{224}) \; \frac{N_1\lambda_1}{N_0\lambda_1} = e^{-\lambda_1 t} \\ (\mathrm{Rn}^{229}) \; \frac{N_2\lambda_2}{N_0\lambda_1} = 1.00017 \; (e^{-\lambda_1 t} - e^{-\lambda_2 t}) \\ (\mathrm{Po}^{216}) \; \frac{N_2\lambda_3}{N_0\lambda_1} = 1.00017 \; e^{-\lambda_1 t} - 1.00308 \; e^{-\lambda_2 t} + 0.00291 \; e^{-\lambda_2 t} \\ (\mathrm{Pb}^{212}) \; \frac{N_4\lambda_4}{N_0\lambda_1} = 1.13823 \; e^{-\lambda_1 t} + 0.00143 \; e^{-\lambda_2 t} - 1.13966 \; e^{-\lambda_4 t} \\ (\mathrm{Bi}^{212}) \; \frac{N_5\lambda_5}{N_0\lambda_1} = 1.15151 \; e^{-\lambda_1 t} - 0.00002 \; e^{-\lambda_2 t} - \\ 1.25947 \; e^{-\lambda_4 t} + 0.10798 \; e^{-\lambda_5 t} \\ (\mathrm{Tl}^{208}) \; \frac{N_6\lambda_6}{N_0\lambda_1} = 1.15219 \; e^{-\lambda_1 t} + 0.00001 \; e^{-\lambda_2 t} - \\ 1.26564 \; e^{-\lambda_4 t} + 0.11381 \; e^{-\lambda_5 t} - 0.00037 \; e^{-\lambda_6 t} \\ \frac{\alpha_t}{N_0\lambda_1} = 4.15185 \; e^{-\lambda_1 t} - 2.00327 \; e^{-\lambda_2 t} + 0.00291 \; e^{-\lambda_5 t} - \\ 1.25947 \; e^{-\lambda_4 t} + 0.10798 \; e^{-\lambda_5 t} \\ \frac{\beta_t \; b}{N_0\lambda_1} = 3.05387 \; e^{-\lambda_1 t} + 0.00143 \; e^{-\lambda_2 t} - 3.24033 \; e^{-\lambda_4 t} + \\ 0.18540 \; e^{-\lambda_5 t} - 0.00037 \; e^{-\lambda_5 t} \end{array}$ 

Valid 1 hour after purification, assuming 100% retention of radon-220 and its decay products.
 Assumes 100% counting yield of all betas.

# Table VII. Thorium Series (4n)

(Growth of decay products from initially pure parent.

	Parent, ra	dium-224)	
Time, Hours	Pb212	$\alpha_t/N_0\lambda_1$	$\beta_{\ell}/N_0\lambda_1$
0 1 2 3 4	$\begin{array}{c} 0.0000\\ 0.0617\\ 0.1204\\ 0.1748\\ 0.2253\end{array}$	1.0000 2.9936 3.0087 3.0328 3.0594	$\begin{array}{c} 0.0000\\ 0.0878\\ 0.2096\\ 0.3425\\ 0.4758\end{array}$
5 6 7 8	$\begin{array}{c} 0.2721 \\ 0.3155 \\ 0.3557 \\ 0.3928 \end{array}$	$3.0856 \\ 3.1098 \\ 3.1315 \\ 3.1505$	$\begin{array}{c} 0.6044 \\ 0.7262 \\ 0.8402 \\ 0.9464 \end{array}$
9 10 11 12 15 18 21 24	$\begin{array}{c} 0.4271\\ 0.4588\\ 0.4880\\ 0.5149\\ 0.5832\\ 0.6355\\ 0.6749\\ 0.7036\end{array}$	3.1667 3.1803 3.1914 3.2002 3.2137 3.2111 3.1956 3.1698	$\begin{array}{c} 1.0449\\ 1.1361\\ 1.2203\\ 1.2981\\ 1.4962\\ 1.6488\\ 1.7644\\ 1.8498\end{array}$
30 36 42 48 54 60 66 72	$\begin{array}{c} 0.7369\\ 0.7472\\ 0.7425\\ 0.7284\\ 0.7082\\ 0.6846\\ 0.6590\\ 0.6326\end{array}$	3.0953 3.0006 2.8944 2.7823 2.6681 2.5543 2.4425 2.3336	$\begin{array}{c} 1.9514\\ 1.9873\\ 1.9805\\ 1.9462\\ 1.8948\\ 1.8331\\ 1.7657\\ 1.6956\end{array}$
78 84 90 96 102 108 114 120	$\begin{array}{c} 0.\ 6061\\ 0.\ 5798\\ 0.\ 5542\\ 0.\ 5053\\ 0.\ 5053\\ 0.\ 4822\\ 0.\ 4600\\ 0.\ 4388\end{array}$	$\begin{array}{c} 2.2283\\ 2.1268\\ 2.0294\\ 1.9360\\ 1.8467\\ 1.7613\\ 1.6797\\ 1.6018 \end{array}$	$\begin{array}{c} 1.6249\\ 1.5549\\ 1.4863\\ 1.4197\\ 1.3554\\ 1.2935\\ 1.2341\\ 1.1773\end{array}$
126 132 138 144	$\begin{array}{c} 0.4185 \\ 0.3992 \\ 0.3807 \\ 0.3630 \end{array}$	$1.5275 \\ 1.4565 \\ 1.3889 \\ 1.3244$	$1.1229 \\ 1.0709 \\ 1.0213 \\ 0.9739$

The answer is read directly from Table XVI. It is 0.3952.

EXAMPLE 2. What fraction of bismuth-210 remains after 7 days, 3 hours, and 14 minutes? By virtue of the fundamental equation for ex-

ponentials

$$e^{(a+b)} = e^a e^b \tag{5}$$

the decay of bismuth-210 for the desired time is the product of its decay in 7 days (0.3677 from Table X), in 3 hours (0.9823 from Table IX), in 10 minutes (0.9990 from Table IX), and in 4 minutes (0.9996 from Table IX)—i.e.,

(0.3677) (0.9823) (0.9990) (0.9996) = 0.3607

EXAMPLE 3. What fraction of thorium-228 will remain after 1 year?

Since the tables do not give decay values beyond 100 days, it is necessary either to make use of the decay constant given in Table I or to use Equation 5, thus:

Fraction remaining after 1 year  $= e^{-0.3648} = 0.6943$ , or,

Fraction remaining after 100 days = 0.9049 (Table III)

Fraction remaining after 65 days = 0.9371 (Table III)

Fraction remaining after 365 days =  $(0.9049)^3(0.9371) = 0.6943$ 

Differential Decay. Mixtures of nuclides are sometimes encountered which cannot conveniently be resolved by chemical methods. Various means have been employed to determine the amount of each species present, but one of the most generally useful is the technique of differential decay.

EXAMPLE 4. A mixture is known to contain only radium-223 and radium-224 in equilibrium with their decay products. When counted, the activity of the mixture is found to have decayed to half its original value in 5 days. What fraction of the original activity was due to radium-224 and its decay products?

# Table VIII. Uranium-Radium Series (4n + 2)

					,
Isotope	Synonym	Mode of Decay	Energies, Mev.	Half Life	Decay Constants
U238	UI	α	4.2	4.51 × 10° Y	$1.54 \times 10^{-10}  \mathrm{Y}^{-1}$
Th234	UX1	β-	$\begin{bmatrix} 56\% & 0.2 \\ 44\% & 0.1 \end{bmatrix}$	24.10 D	$\begin{bmatrix} 0.02876 \text{ D}^{-1} \\ 0.0012 \text{ H}^{-1} \end{bmatrix}$
Pa234	UX2	$\begin{bmatrix} \beta^{-} \\ (0.12\% \text{ IT}) \end{bmatrix}$	$[ \begin{smallmatrix} 90\% & 0.5 \\ 10\% & 1.2 \\ (0.4) \end{smallmatrix}$	1.14 M	0.6080 M <sup>-1</sup>
Pa234ª	$\mathbf{U}\mathbf{Z}$	β-	2.3	6.7 H	0.1035 H <sup>-1</sup>
↓U234	UII	α	4.8	267,000 Y	0.0000026 Y -1
↓ Th <sup>280</sup>	Io	α	$\begin{bmatrix} 75\% & 4.7 \\ 25\% & 4.6 \end{bmatrix}$	80,000 Y	0.0000087 Y -1
Ra <sup>226</sup>	•••	α	$\begin{bmatrix} 93\% & 4.8 \\ 7\% & 4.6 \end{bmatrix}$	1,620 Y	0.000428 Y <sup>-1</sup>
₩ Rn <sup>222</sup>	Em	α	5.5	3.825 D	$\begin{bmatrix} 0.1812 \text{ D}^{-1} \\ 0.00755 \text{ H}^{-1} \end{bmatrix}$
Po <sup>218</sup>	RaA	$\begin{bmatrix} \alpha \\ (0.04\% \beta^{-}) \end{bmatrix}$	6.0 (?)	3.05 M	0.2273 M <sup>-1</sup>
-Pb <sup>214</sup>	RaB	β-	0.7	26.8 M	0.02586 M <sup>-1</sup> 1.5518 H <sup>-1</sup>
←At <sup>218</sup>		$\begin{bmatrix} \alpha \\ (0.04\% \beta^{-}) \end{bmatrix}$	6.7 (?)	2.0S	0.3466 S <sup>-1</sup>
¥ Rn <sup>218</sup>	•••	α	7.1	0.019 S	36.5 S <sup>-1</sup>
Bi214	RaC	$\begin{bmatrix} \beta^- \\ (0.04\% \alpha) \end{bmatrix}$	23% 3.2 77% 1.7 5.5	19.7 M	$\begin{bmatrix} 0.03519 \text{ M}^{-1} \\ 2.1111 \text{ H}^{-1} \end{bmatrix}$
Po <sup>214</sup>	RaC'	α	7.7	0.000164 S	4,227 S <sup>-1</sup>
-+T1210	RaC"	β-	1.8	1.32 M	0.5251 M <sup>-1</sup>
Pb <sup>210</sup> ←	RaD	β-	0.03	22 Y	0.03151 Y -1
B;210	PoF	μ β <sup>-</sup>	$\begin{bmatrix} 92\% & 1.1 \\ 8\% & 1.2 \end{bmatrix}$	4 85 D	<b>0.1429 D</b> <sup>-1</sup>
	nab	(10-59% ~)	(5.0)	1.00 2	L0.00595 H <sub>1-</sub>
Po <sup>210</sup>	RaF	a a	5.3	38.4 D	$\begin{bmatrix} 0.005008 \text{ D}^{-1} \\ 1.8279 \text{ Y}^{-1} \end{bmatrix}$
Tl <sup>208</sup> ←	•••	β-	1.5	4.23 M	0.1639 M <sup>-1</sup>
→Pb <sup>206</sup> <sup>a</sup> Isomer.	RaG	Stable		•••	

		Table I	X. Urani	ium-Radiu	ım Series	(4n + 2)		
Time, Min.	Th234	Pa234	Rn <sup>222</sup>	Po <sup>218</sup>	Pb214	Bi214	<b>Tl</b> 210	Bi210
0 1 2 3 4 5	1.0000 0.9999	$\begin{array}{c} 1.0000\\ 0.5444\\ 0.2964\\ 0.1614\\ 0.0879\\ 0.0478\end{array}$	1.0000 0.9999 0.9997 0.9996 0.9995 0.9994	$\begin{array}{c} 1.0000\\ 0.7967\\ 0.6348\\ 0.5057\\ 0.4029\\ 0.3210 \end{array}$	$\begin{array}{c} 1.0000\\ 0.9744\\ 0.9496\\ 0.9253\\ 0.9017\\ 0.8787\end{array}$	1.0000 0.9654 0.9320 0.8998 0.8687 0.8387	$\begin{array}{c} 1.0000\\ 0.5915\\ 0.3499\\ 0.2069\\ 0.1224\\ 0.0724 \end{array}$	1.0000 0.9999 0.9998 0.9997 0.9996 0.9995
6 7 8 9 10	0.9998	$\begin{array}{c} 0.0260\\ 0.0142\\ 0.0077\\ 0.0042\\ 0.0023 \end{array}$	0.9992 0.9991 0.9990 0.9989 0.9987	$\begin{array}{c} 0.2557 \\ 0.2038 \\ 0.1623 \\ 0.1293 \\ 0.1030 \end{array}$	0.8562 0.8344 0.8131 0.7923 0.7721	0.8097 0.7817 0.7547 0.7285 0.7034	$\begin{array}{c} 0.0428 \\ 0.0253 \\ 0.0150 \\ 0.0089 \\ 0.0052 \end{array}$	0.9994 0.9993 0.9992 0.9991 0.9990
20 30 40 50	$\begin{array}{c} 0.9996 \\ 0.9994 \\ 0.9992 \\ 0.9990 \end{array}$	0.0000	$\begin{array}{c} 0.9975 \\ 0.9962 \\ 0.9950 \\ 0.9937 \end{array}$	$\begin{array}{c} 0.\ 0106\\ 0.\ 0011\\ 0.\ 0001\\ 0.\ 0000 \end{array}$	$\begin{array}{c} 0.5962 \\ 0.4603 \\ 0.3554 \\ 0.2744 \end{array}$	$\begin{array}{c} 0.\ 4948 \\ 0.\ 3480 \\ 0.\ 2448 \\ 0.\ 1722 \end{array}$	0.0000	0.9980 0.9970 0.9960 0.9950
Hours								
$1 \\ 2 \\ 3 \\ 4 \\ 5$	$\begin{array}{c} 0.9988 \\ 0.9976 \\ 0.9964 \\ 0.9952 \\ 0.9940 \end{array}$		0.9924 0.9850 0.9776 0.9703 0.9629		$\begin{array}{c} 0.2119 \\ 0.0449 \\ 0.0095 \\ 0.0020 \\ 0.0020 \\ 0.0004 \end{array}$	$\begin{array}{c} 0.1211 \\ 0.0148 \\ 0.0018 \\ 0.0002 \\ 0.0000 \end{array}$		0.9940 0.9882 0.9823 0.9765 0.9706
6 7 8 9 10	$\begin{array}{c} 0.9928 \\ 0.9916 \\ 0.9904 \\ 0.9893 \\ 0.9881 \end{array}$		$\begin{array}{c} 0.9557 \\ 0.9485 \\ 0.9414 \\ 0.9343 \\ 0.9273 \end{array}$		0.0001 0.0000			$\begin{array}{c} 0.9649 \\ 0.9592 \\ 0.9535 \\ 0.9478 \\ 0.9422 \end{array}$
11 12 13 14 15	$\begin{array}{c} 0.9869 \\ 0.9857 \\ 0.9845 \\ 0.9833 \\ 0.9822 \end{array}$		0,9203 0,9134 0,9065 0,8997 0,8929					$\begin{array}{c} 0.9366 \\ 0.9310 \\ 0.9255 \\ 0.9200 \\ 0.9146 \end{array}$
16 17 18 19 20	$\begin{array}{c} 0.9810 \\ 0.9798 \\ 0.9786 \\ 0.9775 \\ 0.9763 \end{array}$		0.8862 0.8795 0.8729 0.8663 0.8598					0.9091 0.9038 0.8983 0.8931 0.8877
21 22 23 24	$\begin{array}{c} 0.9751 \\ 0.9739 \\ 0.9728 \\ 0.9716 \end{array}$		$\begin{array}{c} 0.8533 \\ 0.8470 \\ 0.8405 \\ 0.8343 \end{array}$					0.8824 0.8772 0.8720 0.8668

# Table X. Uranium-Radium Series (4n + 2)

Time, Davs	Th284	Rn <sup>222</sup>	Pb210	Bi210	Po <sup>210</sup>	Time, Dava	Th284	Ph210	<b>Bi210</b>	Poli
0 1 2 3 4 5	$\begin{array}{c} 1.0000\\ 0.9716\\ 0.9441\\ 0.9173\\ 0.8913\\ 0.8661 \end{array}$	$\begin{array}{c} 1.0000\\ 0.8343\\ 0.6960\\ 0.5806\\ 0.4844\\ 0.4041 \end{array}$	1.0000 0.9999 0.9998 0.9997 0.9997 0.9996	$\begin{array}{c} 1.0000\\ 0.8668\\ 0.7514\\ 0.6513\\ 0.5646\\ 0.4894 \end{array}$	1.0000 0.9950 0.9900 0.9851 0.9802 0.9753	50 51 52 53 54 55	$\begin{array}{c} 0.2374 \\ 0.2307 \\ 0.2241 \\ 0.2178 \\ 0.2116 \\ 0.2056 \end{array}$	$\begin{array}{c} 0.9957\\ 0.9956\\ 0.9955\\ 0.9954\\ 0.9953\\ 0.9953\\ 0.9953\end{array}$	0.0008 0.0007 0.0006 0.0005 0.0004 0.0004	0.7785 0.7746 0.7707 0.7669 0.7630 0.7592
6 7 8 9 10	$\begin{array}{c} 0.8415 \\ 0.8176 \\ 0.7945 \\ 0.7719 \\ 0.7501 \end{array}$	$\begin{array}{c} 0.3371 \\ 0.2813 \\ 0.2346 \\ 0.1957 \\ 0.1633 \end{array}$	$\begin{array}{c} 0.9995 \\ 0.9994 \\ 0.9993 \\ 0.9992 \\ 0.9991 \end{array}$	$\begin{array}{c} 0.4242 \\ 0.3677 \\ 0.3188 \\ 0.2763 \\ 0.2395 \end{array}$	0.9704 0.9655 0.9607 0.9559 0.9512	56 57 58 59 60	0.1998 0.1941 0.1886 0.1832 0.1781	0.9952 0.9951 0.9950 0.9949 0.9948	$\begin{array}{c} 0.\ 0003\\ 0.\ 0003\\ 0.\ 0003\\ 0.\ 0002\\ 0.\ 0002 \end{array}$	0.7554 0.7517 0.7479 0.7442 0.7404
$11 \\ 12 \\ 13 \\ 14 \\ 15$	$\begin{array}{c} 0.7288 \\ 0.7081 \\ 0.6880 \\ 0.6685 \\ 0.6496 \end{array}$	$\begin{array}{c} 0.1362 \\ 0.1137 \\ 0.0948 \\ 0.0791 \\ 0.0660 \end{array}$	0.9991 0.9990 0.9989 0.9988 0.9988	$\begin{array}{c} 0.2076 \\ 0.1800 \\ 0.1560 \\ 0.1352 \\ 0.1172 \end{array}$	0.9464 0.9417 0.9370 0.9323 0.9276	$     \begin{array}{r}       61 \\       62 \\       63 \\       64 \\       65 \\     \end{array} $	$\begin{array}{c} 0.1730 \\ 0.1681 \\ 0.1633 \\ 0.1587 \\ 0.1542 \end{array}$	0.9947 0.9947 0.9946 0.9945 0.9944	$\begin{array}{c} 0.0002 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \end{array}$	0.7368 0.7331 0.7294 0.7258 0.7221
16 17 18 19 20	$\begin{array}{c} 0.6312 \\ 0.6133 \\ 0.5959 \\ 0.5790 \\ 0.5626 \end{array}$	$\begin{array}{c} 0.0551 \\ 0.0459 \\ 0.0383 \\ 0.0320 \\ 0.0267 \end{array}$	$\begin{array}{c} 0.9986 \\ 0.9985 \\ 0.9984 \\ 0.9984 \\ 0.9983 \end{array}$	$\begin{array}{c} 0.1016 \\ 0.0881 \\ 0.0763 \\ 0.0662 \\ 0.0574 \end{array}$	0.9230 0.9184 0.9138 0.9092 0.9047	66 67 68 69 70	$\begin{array}{c} 0.1498 \\ 0.1456 \\ 0.1415 \\ 0.1374 \\ 0.1335 \end{array}$	$\begin{array}{c} 0.9943 \\ 0.9942 \\ 0.9941 \\ 0.9941 \\ 0.9940 \end{array}$	$\begin{array}{c} 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0000 \end{array}$	0.7185 0.7149 0.7114 0.7078 0.7043
21 22 23 24 25	$\begin{array}{c} 0.5466 \\ 0.5311 \\ 0.5161 \\ 0.5014 \\ 0.4872 \end{array}$	$\begin{array}{c} 0.0222\\ 0.0186\\ 0.0155\\ 0.0129\\ 0.0108 \end{array}$	0.9982 0.9981 0.9980 0.9979 0.9978	$\begin{array}{c} 0.\ 0497\\ 0.\ 0431\\ 0.\ 0374\\ 0.\ 0324\\ 0.\ 0281 \end{array}$	0.9002 0.8957 0.8912 0.8867 0.8823	71 72 73 74 75	$\begin{array}{c} 0.1298 \\ 0.1261 \\ 0.1225 \\ 0.1190 \\ 0.1157 \end{array}$	0.9939 0.9938 0.9937 0.9936 0.9935		$\begin{array}{c} 0.7008 \\ 0.6973 \\ 0.6938 \\ 0.6903 \\ 0.6869 \end{array}$
26 27 28 29 30	$\begin{array}{c} 0.4734 \\ 0.4600 \\ 0.4469 \\ 0.4343 \\ 0.4220 \end{array}$	$\begin{array}{c} 0.0090 \\ 0.0075 \\ 0.0063 \\ 0.0052 \\ 0.0044 \end{array}$	0.9978 0.9977 0.9976 0.9975 0.9974	$\begin{array}{c} 0.0243 \\ 0.0211 \\ 0.0183 \\ 0.0158 \\ 0.0137 \end{array}$	0.8779 0.8735 0.8692 0.8648 0.8605	76 77 78 79 80	$\begin{array}{c} 0.1124 \\ 0.1092 \\ 0.1061 \\ 0.1031 \\ 0.1002 \end{array}$	0.9935 0.9934 0.9933 0.9932 0.9931		$\begin{array}{c} 0.6834 \\ 0.6800 \\ 0.6766 \\ 0.6732 \\ 0.6699 \end{array}$
31 32 33 34 35	$\begin{array}{c} 0.4100 \\ 0.3984 \\ 0.3871 \\ 0.3761 \\ 0.3654 \end{array}$	$\begin{array}{c} 0.0036\\ 0.0030\\ 0.0025\\ 0.0021\\ 0.0018 \end{array}$	0.9973 0.9972 0.9972 0.9971 0.9970	$\begin{array}{c} 0.0119 \\ 0.0103 \\ 0.0089 \\ 0.0078 \\ 0.0067 \end{array}$	0.8562 0.8519 0.8477 0.8434 0.8392	81 82 83 84 85	$\begin{array}{c} 0.0973 \\ 0.0946 \\ 0.0919 \\ 0.0893 \\ 0.0868 \end{array}$	0.9930 0.9929 0.9929 0.9928 0.9928 0.9927		$\begin{array}{c} 0.6665 \\ 0.6632 \\ 0.6599 \\ 0.6566 \\ 0.6533 \end{array}$
36 37 38 39 40	$\begin{array}{c} 0.3551 \\ 0.3450 \\ 0.3352 \\ 0.3257 \\ 0.3165 \end{array}$	$\begin{array}{c} 0.0015\\ 0.0012\\ 0.0010\\ 0.0009\\ 0.0007 \end{array}$	0.9969 0.9968 0.9967 0.9966 0.9966	$\begin{array}{c} 0.0058\\ 0.0051\\ 0.0044\\ 0.0038\\ 0.0033 \end{array}$	0.8350 0.8308 0.8267 0.8226 0.8185	86 87 88 89 90	$\begin{array}{c} 0.0843 \\ 0.0819 \\ 0.0796 \\ 0.0773 \\ 0.0751 \end{array}$	0.9926 0.9925 0.9924 0.9923 0.9923		$\begin{array}{c} 0.6500 \\ 0.6468 \\ 0.6436 \\ 0.6404 \\ 0.6372 \end{array}$
41 42 43 44 45	$\begin{array}{c} 0.3075 \\ 0.2988 \\ 0.2903 \\ 0.2821 \\ 0.2741 \end{array}$	$\begin{array}{c} 0.0006 \\ 0.0005 \\ 0.0004 \\ 0.0003 \\ 0.0003 \end{array}$	$\begin{array}{c} 0.9965 \\ 0.9964 \\ 0.9963 \\ 0.9962 \\ 0.9961 \end{array}$	$\begin{array}{c} 0.0029\\ 0.0025\\ 0.0021\\ 0.0019\\ 0.0016\end{array}$	0.8144 0.8103 0.8063 0.8022 0.7982	91 92 93 94 95	$\begin{array}{c} 0.0730 \\ 0.0709 \\ 0.0689 \\ 0.0670 \\ 0.0651 \end{array}$	0.9922 0.9921 0.9920 0.9919 0.9918		$\begin{array}{c} 0.6340 \\ 0.6308 \\ 0.6277 \\ 0.6245 \\ 0.6214 \end{array}$
46 47 48 49 50	$\begin{array}{c} 0.2663 \\ 0.2588 \\ 0.2514 \\ 0.2443 \\ 0.2374 \end{array}$	$\begin{array}{c} 0.0002 \\ 0.0002 \\ 0.0002 \\ 0.0001 \\ 0.0001 \end{array}$	$\begin{array}{c} 0.9960 \\ 0.9960 \\ 0.9959 \\ 0.9958 \\ 0.9957 \end{array}$	$\begin{array}{c} 0.0014 \\ 0.0012 \\ 0.0010 \\ 0.0009 \\ 0.0008 \end{array}$	0.7942 0.7903 0.7863 0.7824 0.7785	96 97 98 99 100	$\begin{array}{c} 0.0632 \\ 0.0614 \\ 0.0597 \\ 0.0580 \\ 0.0564 \end{array}$	0.9917 0.9917 0.9916 0.9915 0.9914		$\begin{array}{c} 0.6183 \\ 0.6152 \\ 0.6121 \\ 0.6091 \\ 0.6060 \end{array}$
#### Table XI. Uranium-Radium Series (4n + 2)

(Growth of decay products from initially pure parent.<sup>a</sup> Parent, radium-226)  $(\operatorname{Ra}^{226}) \frac{N_1 \lambda_1}{N_0 \lambda_1} = e^{-\lambda_1 t}$  $(\mathrm{Rn}^{222}) \frac{N_2 \lambda_2}{N_0 \lambda_1} = 1.00001 \ e^{-\lambda_1 t} - 1.00001 \ e^{-\lambda_2 t}$ (Po<sup>218</sup>)  $\frac{N_3\lambda_3}{N_0\lambda_1} = 1.00001 \ e^{-\lambda_1 t} - 1.00056 \ e^{-\lambda_2 t} + 0.00055 \ e^{-\lambda_3 t}$ (Pb<sup>214</sup>)  $\frac{N_4\lambda_4}{N_0\lambda_1} = 1.00001 \ e^{-\lambda_1 t} - 1.00545 \ e^{-\lambda_2 t} - 0.00007 \ e^{-\lambda_2 t} +$  $0.00552 \ e^{-\lambda_4 t}$ (Bi<sup>214</sup>)  $\frac{N_{s\lambda_{s}}}{N_{s\lambda_{s}}} = 1.00001 \ e^{-\lambda_{1}t} - 1.00906 \ e^{-\lambda_{2}t} + 0.00001 \ e^{-\lambda_{3}t} + 0.00001 \ e^{-\lambda_{3}t}$  $0.02084 \ e^{-\lambda_4 t} - 0.01179 \ e^{-\lambda_5 t}$ (Po<sup>214</sup>)  $\frac{N_{6}\lambda_{6}}{N_{0}\lambda_{1}} = \frac{N_{5}\lambda_{5}}{N_{0}\lambda_{1}}$  $(Pb^{210}) \frac{N_{7}\lambda_{7}}{N_{6}\lambda_{1}} = 1.01378 \ e^{-\lambda_{1}t} + 0.00048 \ e^{-\lambda_{2}t} - 1.01428 \ e^{-\lambda_{7}t}$ (Bi<sup>210</sup>)  $\frac{N_{3\lambda_8}}{N_{0\lambda_1}} = 1.01379 \ e^{-\lambda_1 t} - 0.00179 \ e^{-\lambda_2 t} - 1.01489 \ e^{-\lambda_7 t} +$  $0.00286 \ e^{-\lambda_{s}t}$ (Po<sup>210</sup>)  $\frac{N_9\lambda_9}{N_0\lambda_1} = 1.01402 \ e^{-\lambda_1 t} + 0.00005 \ e^{-\lambda_2 t} - 1.03267 \ e^{-\lambda_1 t} -$  $0.00010 \ e^{-\lambda_8 t} + 0.01868 \ e^{-\lambda_9 t}$  $\frac{\alpha_t}{N_b\lambda_1} = 5.01405 \ e^{-\lambda_1 t} - 3.00958 \ e^{-\lambda_2 t} + 0.00056 \ e^{-\lambda_3 t} + 0.02084 \ e^{-\lambda_4 t} - 0.01179 \ e^{-\lambda_5 t} - 1.03267 \ e^{-\lambda_7 t} - 0.00010 \ e^{-\lambda_5 t} + 0.01868 \ e^{-\lambda_5 t}$  $\frac{\beta_t b}{N_0 \lambda_1} = 4.02759 \ e^{-\lambda_1 t} - 2.01582 \ e^{-\lambda_2 t} - 0.00006 \ e^{-\lambda_3 t} + 0.02636 \ e^{-\lambda_4 t} - 0.01179 \ e^{-\lambda_5 t} - 2.02917 \ e^{-\lambda_7 t} + 0.00286 \ e^{-\lambda_5 t}$ 

<sup>a</sup> Valid 1 hour after purification, assuming 100% retention of radon-222 and its decay products.
 <sup>b</sup> Assumes 100% counting yield of all betas.

#### Table XII. Uranium-Radium Series

(Growth of decay products from initially pure parent. Parent, radium-226)

Time, Days	<b>R</b> n <sup>222</sup>	Pb210	Bi210	Po210	$\alpha_t/N_0\lambda_1$	$\beta_t/N_0\lambda$
0 1 2 3 4 5	$\begin{array}{c} 0.0000\\ 0.1657\\ 0.3040\\ 0.4193\\ 0.5156\\ 0.5959 \end{array}$	0.0000 0.0000 0.0001	0.0000	0.0000	$\begin{array}{c} 1.0000\\ 1.4892\\ 1.9054\\ 2.2525\\ 2.5422\\ 2.7838 \end{array}$	0.0000 0.3194 0.5979 0.8304 1.0243 1.1861
6 7 8 9 10	0.6628 0.7187 0.7653 0.8042 0.8367	$\begin{array}{c} 0.0002 \\ 0.0003 \\ 0.0003 \\ 0.0004 \\ 0.0005 \end{array}$	0.0000 0.0001 0.0001 0.0002 0.0002		2.9853 3.1536 3.2939 3.4110 3.5086	$\begin{array}{r} 1.3211 \\ 1.4337 \\ 1.5277 \\ 1.6062 \\ 1.6716 \end{array}$
$11 \\ 12 \\ 13 \\ 14 \\ 15$	0.8637 0.8863 0.9052 0.9209 0.9340	$\begin{array}{c} 0.0005\\ 0.0006\\ 0.0007\\ 0.0008\\ 0.0009 \end{array}$	$\begin{array}{c} 0.0002\\ 0.0002\\ 0.0003\\ 0.0004\\ 0.0004\\ 0.0004 \end{array}$		3.5901 3.6580 3.7147 3.7619 3.8014	1.7263 1.7719 1.8100 1.8417 1.8683
16 17 18 19 20	$\begin{array}{c} 0.9449 \\ 0.9541 \\ 0.9617 \\ 0.9680 \\ 0.9733 \end{array}$	$\begin{array}{c} 0.0010\\ 0.0010\\ 0.0011\\ 0.0011\\ 0.0012 \end{array}$	$\begin{array}{c} 0.0005\\ 0.0006\\ 0.0007\\ 0.0007\\ 0.0007\\ 0.0007\end{array}$		3.8344 3.8618 3.8847 3.9038 3.9197	$\begin{array}{r} 1.8905 \\ 1.9090 \\ 1.9245 \\ 1.9374 \\ 1.9482 \end{array}$
21 22 23 24 25	0.9777 0.9814 0.9845 0.9871 0.9892	$\begin{array}{c} 0.0013\\ 0.0014\\ 0.0015\\ 0.0016\\ 0.0017\end{array}$	$\begin{array}{c} 0.0008\\ 0.0009\\ 0.0010\\ 0.0011\\ 0.0011\\ 0.0011 \end{array}$	0.0000 0.0001 0.0001 0.0001	3.9331 3.9442 3.9535 3.9612 3.9675	1.9573 1.9649 1.9712 1.9766 1.9810
26 27 28 29 30	$\begin{array}{c} 0.9910 \\ 0.9925 \\ 0.9937 \\ 0.9948 \\ 0.9956 \end{array}$	$\begin{array}{c} 0.0018 \\ 0.0018 \\ 0.0019 \\ 0.0020 \\ 0.0021 \end{array}$	$\begin{array}{c} 0.0012\\ 0.0013\\ 0.0014\\ 0.0015\\ 0.0015\\ 0.0015 \end{array}$	$\begin{array}{c} 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \end{array}$	3.9728 3.9774 3.9811 3.9842 3.9868	$\begin{array}{c} 1.9848 \\ 1.9880 \\ 1.9906 \\ 1.9929 \\ 1.9948 \end{array}$
31 32 33 34 35	0.9963 0.9969 0.9974 0.9979 0.9982	$\begin{array}{c} 0.0022\\ 0.0023\\ 0.0024\\ 0.0024\\ 0.0024\\ 0.0025 \end{array}$	0.0016 0.0017 0.0018 0.0019 0.0019	$\begin{array}{c} 0.0001\\ 0.0001\\ 0.0001\\ 0.0001\\ 0.0001\\ 0.0001 \end{array}$	3.9890 3.9908 3.9923 3.9936 3.9947	$\begin{array}{c} 1.9964 \\ 1.9978 \\ 1.9990 \\ 2.0000 \\ 2.0009 \end{array}$
36 37 38 39 40	0.9985 0.9987 0.9989 0.9991 0.9993	$\begin{array}{c} 0.0026 \\ 0.0027 \\ 0.0028 \\ 0.0029 \\ 0.0030 \end{array}$	$\begin{array}{c} 0.0020\\ 0.0021\\ 0.0022\\ 0.0023\\ 0.0023\\ 0.0024 \end{array}$	$\begin{array}{c} 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0001 \\ 0.0002 \end{array}$	3.9956 3.9963 3.9969 3.9974 3.9978	2.0016 2.0023 2.0028 2.0034 2.0038
45 50 60 70 80 90 100	0.9997 0.9998 0.9999 0.9999 0.9999 0.9999 0.9999 0.9999	$\begin{array}{c} 0.0034\\ 0.0038\\ 0.0047\\ 0.0055\\ 0.0064\\ 0.0072\\ 0.0081 \end{array}$	$\begin{array}{c} 0.0028\\ 0.0032\\ 0.0041\\ 0.0049\\ 0.0058\\ 0.0067\\ 0.0075\\ \end{array}$	$\begin{array}{c} 0.0002\\ 0.0003\\ 0.0005\\ 0.0006\\ 0.0009\\ 0.0012\\ 0.0015 \end{array}$	$\begin{array}{c} 3.9992 \\ 3.9997 \\ 4.0001 \\ 4.0003 \\ 4.0005 \\ 4.0007 \\ 4.0010 \end{array}$	2.0055 2.0067 2.0086 2.0103 2.0120 2.0137 2.0154

#### Table XIII. Uranium-Radium Series (4n + 2)

(Growth of decay products from initially pure parent. Parent, lead-210)  $\cdot (Pb^{210}) \frac{N_1 \lambda_1}{N_0 \lambda_1} = e^{-\lambda_1 t}$ 

(Bi<sup>210</sup>)  $\frac{N_2\lambda_2}{N_2\lambda_1} = 1.00060 \ (e^{-\lambda_1 t} - e^{-\lambda_2 t})$ 

(Po<sup>210</sup>)  $\frac{N_{s\lambda_{s}}}{N_{s\lambda_{s}}} = 1.01815 \ e^{-\lambda_{1}t} + 0.03634 \ e^{-\lambda_{2}t} - 1.05449 \ e^{-\lambda_{3}t}$ 

Time, Days	Bi <sup>210</sup>	Po210	Time, Days	Bi210	Po <sup>210</sup>
0	0.0000	0.0000	40	0.9939	0.1517
ĩ	0 1332	0 0003	41	0 9942	0 1559
$\hat{2}$	0 2485	0 0013	42	0 9945	0 1601
จี	0 3484	0.0027	43	0 0047	0 1643
4	0.04254	0.0047	44	0.0050	0 1694
** E	0.4304	0.0011	44	0.9930	0.1004
5	0.5105	0.0071	40	0.9951	0.1720
6	0.5755	0.0098	46	0.9952	0.1767
7	0.6320	0.0128	47	0.9953	0.1808
8	0.6809	0.0160	48	0.9954	0.1848
9	0.7233	0.0194	49	0.9955	0.1889
10	0.7601	0.0230	50	0.9955	0.1929
11	0.7919.	0.0268	51	0.9955	0.1969
12	0.8195	0.0306	52	0.9955	0.2009
13	0.8434	0.0346	53	0.9955	0.2049
14	0.8640	0.0387	54	0.9955	0.2088
15	0.8820	0.0429	55	0.9955	0.2127
10	0 9075	0.0471	20	0.0054	0.0167
10	0.8978	0.0471	50	0.9954	0.2107
17	0.9109	0.0514	57	0.9954	0.2205
18	0.9226	0.0007	28	0.9953	0.2244
19	0.9328	0.0601	59	0.9953	0.2283
20	0.9415	0.0645	60	0.9952	0.2321
21	0.9491	0.0689	61	0.9952	0.2359
22	0.9556	0.0733	62	0.9951	0.2397
23	0.9612	0.0777	63	0.9950	0.2435
24	0.9661	0.0821	64	0.9950	0.2472
25	0.9704	0.0866	65	0.9949	0.2510
06	0.0740	0.0010	66	0.0048	0.9547
20	0.9740	0.0310	67	0.9948	0.2347
21	0.9772	0.0904	07	0.9948	0.2084
28	0.9799	0.0998	08	0.9947	0.2621
29	0.9822	0.1042	69	0.9946	0.2657
30	0.9843	0.1086	70	0.9945	0.2694
31	0.9860	0.1130	71	0.9944	0.2730
32	0.9875	0.1174	72	0.9944	0.2766
33	0.9888	0.1217	73	0.9943	0.2802
34	0.9899	0.1261	74	0.9942	0.2837
$\overline{3}\overline{5}$	0.9909	0.1304	75	0.9941	0.2873
26	0.0016	0 1347	76	0.0040	0.9009
00 27	0.9910	0.134/	10	0.9940	0.2908
37	0.9924	0.1499	70	0.9940	0.2943
00	0.9929	0.1432	10	0.9939	0.2978
39	0.9934	0.14/5	(9)	0.9938	0.3013
40	0.9999	0.1917	80	0.9937	0.3049

Let  $C_0$  represent the original count obtained, let  $R_1$  be the fraction of  $C_0$  due to radium-223 and its decay products, and let  $R_2$ be the fraction due to radium-224 and its decay products. In 5 days, radium-223 decays to 0.7339 of its original value (Table XVI) and radium-224 decays to 0.3859 of its original value (Table III). Therefore,

# $C_0 = 1.0000 R_1 + 1.0000 R_2$

 $0.5 C_0 = 0.7339 R_1 + 0.3859 R_2$ 

The two equations are solved simultaneously, and it is found that  $R_1 = 0.3279 C_0$  and  $R_2 = 0.6721 C_0$ . The results give a measure of the relative activity of each isotope and its decay products in the mixture at the time of the first measurement. A determination of the absolute amount of each isotope depends upon the radioactive equilibrium constant (see below), the nature and energy of the radiation counted, the self-absorption of the sample, and the geometry of the counter, subjects which are beyond the scope of this discussion.

**Radioactive Equilibrium.** When a radioisotope decays to a radioactive daughter whose half life is shorter than that of its parent, a condition of radioactive equilibrium will eventually be attained in which the daughter product is decaying as rapidly as it is being produced. The mixture then decays with the half life of the parent.

The ratio of daughter activity to parent activity is given by the expression

Isotope	Synonym	Mode of Decay	Energ Met	ies, 7,	Half Life	Decay Constants
U <sup>285</sup>	AcU	α	$\begin{bmatrix} 20\% \\ 00\% \end{bmatrix}$	4.6	$8.8 imes10^8$ Y	$7.9  imes 10^{-10}  \mathrm{Y}^{-1}$
¥ Th <sup>281</sup>	UY	β-	L0070	4.4 0.2	25.65 H	$\begin{bmatrix} 0.6486 \text{ D}^{-1} \\ 0.0270 \text{ H}^{-1} \end{bmatrix}$
Pa <sup>231</sup>	•••	α	$\begin{bmatrix} 58\% \\ 25\% \\ 3\% \\ 13\% \end{bmatrix}$	$5.0 \\ 4.9 \\ 4.8 \\ 4.7$	34,300 Y	0.0000202 Y -1
Ac <sup>227</sup>	•••	$\begin{bmatrix} \beta^{-} \\ (1.2\% \ \alpha) \end{bmatrix}$	(•	0.02 4.9)	22.0 Y	$\begin{bmatrix} 0.0315 \text{ Y}^{-1} \\ 0.0000863 \text{ D}^{-1} \end{bmatrix}$
-Th <sup>227</sup>	RdAe	α	$\begin{bmatrix} 24\% \\ 22\% \\ 7\% \\ 25\% \\ 22\% \end{bmatrix}$	$     \begin{array}{r}       6.1 \\       6.0 \\       5.9 \\       5.8 \\       5.7 \\     \end{array} $	18.6 D	0.0373 D <sup>-1</sup> 0.00155 H <sup>-1</sup>
Fr <sup>223</sup>	AcK	β-		1.2	21 M	[1.9804 H <sup>-1</sup> 0.0330 M <sup>-1</sup>
↓ →Ra <sup>223</sup>	AcX	α	$\begin{bmatrix} 41\% \\ 35\% \\ 17\% \\ 7\% \end{bmatrix}$	$5.7 \\ 5.6 \\ 5.5 \\ 5.4$	11.2 D	$\begin{bmatrix} 0.0619 \text{ D}^{-1} \\ 0.00258 \text{ H}^{-1} \end{bmatrix}$
¥ Rn²19 	An	α	$\begin{bmatrix} 69\% \\ 15\% \\ 12\% \\ 4\% \end{bmatrix}$	$     \begin{array}{r}       6.8 \\       6.6 \\       6.4 \\       6.2 \\     \end{array} $	3.92 S	10.61 M <sup>-1</sup>
Po 215	AcA	$\begin{bmatrix} \alpha \\ (0.0005\% \beta^{-}) \end{bmatrix}$		7.4 (?)	0.00183 S	22,800 M <sup>-1</sup>
-Pb <sup>211</sup>	AcB	β-	$\begin{bmatrix} 20\% \\ 80\% \end{bmatrix}$	$0.5 \\ 1.4$	36.1 M	1.1520 H <sup>-1</sup> 0.0192 M <sup>-1</sup>
At215		a		8.0	10-4 S	416,000 M <sup>-1</sup>
→Bi <sup>211</sup>	AcC	$\begin{bmatrix} \alpha \\ (0.32\% \beta^{-}) \end{bmatrix}$	$\begin{bmatrix} 84\% \\ 16\% \end{bmatrix}$	6.6 6.3 (?)	2.16 M	$\begin{bmatrix} 19.25 \text{ H}^{-1} \\ 0.3209 \text{ M}^{-1} \end{bmatrix}$
-T1207	AcC"	β-		1.5	4.76 M	$\begin{bmatrix} 8.736 \text{ H}^{-1} \\ 0.1456 \text{ M}^{-1} \end{bmatrix}$
Po211	AcC'	α		7.4	0.52 S	79.98 M <sup>-1</sup>
Pb <sup>207</sup>	AcD	Stable				

Table XIV. Uranium-Actinium Series (4n + 3)

# Table XV. Uranium-Actinium Series (4n + 3)

(Decay in minutes and hours)												
Time, Min.	Th231	$\mathrm{Th}^{227}$	Fr223	Ra <sup>228</sup>	Pb211	Bi211	Time, Hours	Th231	Th 227	Fr223	Ra <sup>223</sup>	Pb211
0 1 2 3 4 5	$\begin{array}{c} 1,0000\\ 0,9995\\ 0,9991\\ 0,9986\\ 0,9982\\ 0,9977 \end{array}$	1.0000	$\begin{array}{c} 1.0000\\ 0.9675\\ 0.9361\\ 0.9057\\ 0.8763\\ 0.8479 \end{array}$	1.0000	$\begin{array}{c} 1.0000\\ 0.9810\\ 0.9623\\ 0.9440\\ 0.9261\\ 0.9085 \end{array}$	1,0000 0.7255 0,5263 0.3819 0,2770 0.2010	6 7 8 9 10 11	$\begin{array}{c} 0.8504 \\ 0.8276 \\ 0.8056 \\ 0.7841 \\ 0.7632 \\ 0.7428 \end{array}$	$\begin{array}{c} 0.9907 \\ 0.9892 \\ 0.9877 \\ 0.9861 \\ 0.9846 \\ 0.9831 \end{array}$	0.0000	$\begin{array}{c} 0.9846 \\ 0.9821 \\ 0.9796 \\ 0.9771 \\ 0.9745 \\ 0.9720 \end{array}$	$\begin{array}{c} 0.0010 \\ 0.0003 \\ 0.0001 \\ 0.0000 \end{array}$
6 7 8 9 10	$\begin{array}{c} 0.9973 \\ 0.9968 \\ 0.9964 \\ 0.9959 \\ 0.9955 \end{array}$	0.9997	$\begin{array}{c} 0.8204 \\ 0.7937 \\ 0.7679 \\ 0.7430 \\ 0.7189 \end{array}$	0.9996	$\begin{array}{c} 0.8912 \\ 0.8742 \\ 0.8576 \\ 0.8413 \\ 0.8253 \end{array}$	0.1458 0.1058 0.0767 0.0557 0.0404	$12 \\ 13 \\ 14 \\ 15 \\ 16$	$\begin{array}{c} 0.7230 \\ 0.7038 \\ 0.6850 \\ 0.6668 \\ 0.6489 \end{array}$	0.9815 0.9800 0.9785 0.9770 0.9755		$\begin{array}{c} 0.9695 \\ 0.9670 \\ 0.9645 \\ 0.9621 \\ 0.9596 \end{array}$	
20 30 40 50	$\begin{array}{c} 0.9910 \\ 0.9866 \\ 0.9822 \\ 0.9778 \end{array}$	$\begin{array}{c} 0.9995 \\ 0.9992 \\ 0.9990 \\ 0.9987 \end{array}$	$\begin{array}{c} 0.5168 \\ 0.3715 \\ 0.2671 \\ 0.1920 \end{array}$	$\begin{array}{c} 0.9991 \\ 0.9987 \\ 0.9983 \\ 0.9979 \end{array}$	$\begin{array}{c} 0.6811 \\ 0.5621 \\ 0.4639 \\ 0.3829 \end{array}$	$\begin{array}{c} 0.0016 \\ 0.0001 \\ 0.0000 \end{array}$	17 18 19 20	$\begin{array}{c} 0.6317 \\ 0.6148 \\ 0.5985 \\ 0.5825 \end{array}$	$\begin{array}{c} 0.9739 \\ 0.9724 \\ 0.9709 \\ 0.9694 \end{array}$		$\begin{array}{c} 0.9571 \\ 0.9546 \\ 0.9522 \\ 0.9497 \end{array}$	
Hours 1 2 3 4 5	$\begin{array}{c} 0.9734 \\ 0.9474 \\ 0.9221 \\ 0.8975 \\ 0.8736 \end{array}$	$\begin{array}{c} 0.9984 \\ 0.9969 \\ 0.9954 \\ 0.9938 \\ 0.9923 \end{array}$	$\begin{array}{c} 0.1380 \\ 0.0190 \\ 0.0026 \\ 0.0004 \\ 0.0001 \end{array}$	$\begin{array}{c} 0.9974 \\ 0.9949 \\ 0.9923 \\ 0.9897 \\ 0.9872 \end{array}$	$\begin{array}{c} 0.3160 \\ 0.0998 \\ 0.0316 \\ 0.0100 \\ 0.0032 \end{array}$		21 22 23 24	$\begin{array}{c} 0.5669 \\ 0.5518 \\ 0.5371 \\ 0.5228 \end{array}$	0.9679 0.9664 0.9649 0.9634		0.9473 0.9448 0.9424 0.9400	

$$K_{\bullet} = \frac{\lambda_2}{\lambda_2 - \lambda_1} = \frac{T_1}{T_1 - T_2}$$
(6)

or, for a three-member chain, the ratio of the activity of the third member to that of the first member is

$$K_{\epsilon} = \left[\frac{T_1}{T_1 - T_2}\right] \left[\frac{T_1}{T_1 - T_1}\right]$$
(6a)

If the half lives of the decay products are very much smaller than that of the parent,  $K_{\bullet}$  is very nearly equal to unity.

The time required for the first decay product to reach 99.9% of equilibrium is approximately 10  $K_sT_2$ .

EXAMPLE 5. How long does it take for thorium-234 to reach 99.9% of equilibrium in a sample of uranium-238? How much thorium-234 is present in a microcurie of uranium-238 30 days after purification?

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Time, Days	Th281	Ac <sup>227</sup>	Th <sup>227</sup>	Ra <sup>222</sup>	Tin Day	ne, 75 Ac <sup>227</sup>	$\mathrm{Th}^{227}$	Ra <sup>223</sup>
0 1 2 3 4 5	$\begin{array}{c} 1.0000\\ 0.5228\\ 0.2733\\ 0.1429\\ 0.0747\\ 0.0391 \end{array}$	1.0000 0.9999 0.9998 0.9997 0.9997 0.9996	$\begin{array}{c} 1.0000\\ 0.9634\\ 0.9282\\ 0.8942\\ 0.8615\\ 0.8300 \end{array}$	1.0000 0.9400 0.8836 0.8306 0.7807 0.7339	50 51 52 53 54 55	0.9957 0.9956 0.9955 0.9954 0.9953 0.9953	$\begin{array}{c} 0.1552 \\ 0.1495 \\ 0.1440 \\ 0.1387 \\ 0.1337 \\ 0.1288 \end{array}$	$\begin{array}{c} 0.0453\\ 0.0426\\ 0.0400\\ 0.0376\\ 0.0354\\ 0.0332 \end{array}$
6 7 8 9 10	$\begin{array}{c} 0.0204 \\ 0.0107 \\ 0.0056 \\ 0.0029 \\ 0.0015 \end{array}$	0.9995 0.9994 0.9993 0.9992 0.9991	$\begin{array}{c} 0.7996 \\ 0.7704 \\ 0.7422 \\ 0.7151 \\ 0.6889 \end{array}$	$\begin{array}{c} 0.6898 \\ 0.6484 \\ 0.6095 \\ 0.5729 \\ 0.5385 \end{array}$	56 57 58 59 60	0.9952 0.9951 0.9950 0.9949 0.9948	$\begin{array}{c} 0.1241 \\ 0.1195 \\ 0.1152 \\ 0.1109 \\ 0.1069 \end{array}$	$\begin{array}{c} 0.0312 \\ 0.0294 \\ 0.0276 \\ 0.0260 \\ 0.0244 \end{array}$
11 12 13 14 15	$\begin{array}{c} 0.0008 \\ 0.0004 \\ 0.0002 \\ 0.0001 \\ 0.0001 \end{array}$	0.9991 0.9990 0.9989 0.9988 0.9988	$\begin{array}{c} 0.6637 \\ 0.6394 \\ 0.6160 \\ 0.5935 \\ 0.5718 \end{array}$	$\begin{array}{c} 0.5062 \\ 0.4758 \\ 0.4473 \\ 0.4204 \\ 0.3952 \end{array}$	61 62 63 64 65	0.9947 0.9947 0.9946 0.9945 0.9944	$\begin{array}{c} 0.1030 \\ 0.0992 \\ 0.0956 \\ 0.0921 \\ 0.0887 \end{array}$	$\begin{array}{c} 0.0229\\ 0.0216\\ 0.0203\\ 0.0190\\ 0.0179 \end{array}$
16 17 18 19 20	0.0000	0.9986 0.9985 0.9984 0.9984 0.9983	$\begin{array}{c} 0.5509 \\ 0.5307 \\ 0.5113 \\ 0.4926 \\ 0.4746 \end{array}$	$\begin{array}{c} 0.3715 \\ 0.3492 \\ 0.3282 \\ 0.3085 \\ 0.2900 \end{array}$	66 67 68 69 70	$\begin{array}{c} 0.9943 \\ 0.9942 \\ 0.9941 \\ 0.9941 \\ 0.9940 \end{array}$	$\begin{array}{c} 0.0855\\ 0.0823\\ 0.0793\\ 0.0764\\ 0.0736\end{array}$	$\begin{array}{c} 0.0168\\ 0.0158\\ 0.0149\\ 0.0140\\ 0.0131 \end{array}$
21 22 23 24 25		0.9982 0.9981 0.9980 0.9979 0.9978	$\begin{array}{c} 0.4572 \\ 0.4405 \\ 0.4244 \\ 0.4089 \\ 0.3939 \end{array}$	$\begin{array}{c} 0.2726 \\ 0.2563 \\ 0.2409 \\ 0.2264 \\ 0.2128 \end{array}$	71 72 73 74 75	0.9939 0.9938 0.9937 0.9936 0.9935	$\begin{array}{c} 0.0709 \\ 0.0683 \\ 0.0658 \\ 0.0634 \\ 0.0611 \end{array}$	$\begin{array}{c} 0.0124 \\ 0.0116 \\ 0.0109 \\ 0.0103 \\ 0.0096 \end{array}$
26 27 28 29 30		0.9978 0.9977 0.9976 0.9975 0.9974	$\begin{array}{c} 0.3795 \\ 0.3656 \\ 0.3522 \\ 0.3394 \\ 0.3269 \end{array}$	$\begin{array}{c} 0.2001 \\ 0.1881 \\ 0.1768 \\ 0.1662 \\ 0.1562 \end{array}$	76 77 78 79 80	0.9935 0.9934 0.9933 0.9932 0.9931	0.0589 0.0567 0.0547 0.0527 0.0507	$\begin{array}{c} 0.0091 \\ 0.0085 \\ 0.0080 \\ 0.0075 \\ 0.0071 \end{array}$
31 32 33 34 35		0.9973 0.9972 0.9972 0.9971 0.9970	$\begin{array}{c} 0.3150 \\ 0.3035 \\ 0.2924 \\ 0.2817 \\ 0.2714 \end{array}$	$\begin{array}{c} 0.1468 \\ 0.1380 \\ 0.1297 \\ 0.1219 \\ 0.1146 \end{array}$	81 82 83 84 85	0.9930 0.9929 0.9929 0.9928 0.9928 0.9927	$\begin{array}{c} 0.0489 \\ 0.0471 \\ 0.0454 \\ 0.0437 \\ 0.0421 \end{array}$	$\begin{array}{c} 0.0067 \\ 0.0063 \\ 0.0059 \\ 0.0055 \\ 0.0052 \end{array}$
36 37 38 39 40		0.9969 0.9968 0.9967 0.9966 0.9966	$\begin{array}{c} 0.2614 \\ 0.2519 \\ 0.2427 \\ 0.2338 \\ 0.2252 \end{array}$	$\begin{array}{c} 0.1077 \\ 0.1013 \\ 0.0952 \\ 0.0895 \\ 0.0841 \end{array}$	86 87 88 89 90	$\begin{array}{c} 0.9926\\ 0.9925\\ 0.9924\\ 0.9923\\ 0.9923\\ 0.9923\end{array}$	$\begin{array}{c} 0.0406 \\ 0.0391 \\ 0.0377 \\ 0.0363 \\ 0.0349 \end{array}$	$\begin{array}{c} 0.0049 \\ 0.0046 \\ 0.0043 \\ 0.0041 \\ 0.0038 \end{array}$
41 42 43 44 45		$\begin{array}{c} 0.9965 \\ 0.9964 \\ 0.9963 \\ 0.9962 \\ 0.9961 \end{array}$	$\begin{array}{c} 0.2170 \\ 0.2091 \\ 0.2014 \\ 0.1940 \\ 0.1869 \end{array}$	$\begin{array}{c} 0.0791 \\ 0.0743 \\ 0.0699 \\ 0.0657 \\ 0.0617 \end{array}$	91 92 93 94 95	0.9922 0.9921 0.9920 0.9919 0.9918	$\begin{array}{c} 0.0337\\ 0.0324\\ 0.0312\\ 0.0301\\ 0.0290 \end{array}$	$\begin{array}{c} 0.0036\\ 0.0034\\ 0.0032\\ 0.0030\\ 0.0030\\ 0.0028 \end{array}$
46 47 48 49 50		0.9960 0.9960 0.9959 0.9958 0.9958 0.9957	$\begin{array}{c} 0.1801 \\ 0.1735 \\ 0.1672 \\ 0.1611 \\ 0.1552 \end{array}$	$\begin{array}{c} 0.0580 \\ 0.0545 \\ 0.0513 \\ 0.0482 \\ 0.0453 \end{array}$	96 97 98 99 100	0.9917 0.9917 0.9916 0.9915 0.9914	$\begin{array}{c} 0.0279 \\ 0.0269 \\ 0.0259 \\ 0.0250 \\ 0.0250 \\ 0.0241 \end{array}$	$\begin{array}{c} 0.0026\\ 0.0025\\ 0.0023\\ 0.0022\\ 0.0022\\ 0.0021 \end{array}$

# Table XVI. Uranium-Actinium Series (4n + 3)(Decay in days)

Table XVII. Uranium-Actinium Series (4n + 3)

(Growth of decay products from initially pure parent.<sup>a</sup> Parent, actinium-227)

 $\begin{array}{ll} (\mathrm{Ac}^{227}) & \frac{N_{1}\lambda_{1}}{N_{0}\lambda_{1}} = e^{-\lambda_{1}t} \\ (\mathrm{Th}^{227}) & \frac{N_{2}\lambda_{2}}{N_{0}\lambda_{1}} = 0.99029 \ (e^{-\lambda_{1}t} - e^{-\lambda_{2}t}) \\ (\mathrm{Fr}^{223}) & \frac{N_{2}\lambda_{2}}{N_{0}\lambda_{1}} = 0.012 \ (e^{-\lambda_{1}t} - e^{-\lambda_{2}t}) \\ (\mathrm{Ra}^{2123}) & \frac{N_{2}\lambda_{3}}{N_{0}\lambda_{1}} = 1.00369 \ e^{-\lambda_{1}t} - 2.48912 \ e^{-\lambda_{2}t} + 0.00002 \ e^{-\lambda_{3}t} + 1.48541e^{-\lambda_{3}t} \\ (\mathrm{Rn}^{219}) & \frac{N_{4}\lambda_{4}}{N_{0}\lambda_{1}} = \frac{N_{3}\lambda_{3}}{N_{0}\lambda_{1}} \\ (\mathrm{Po}^{215}) & \frac{N_{4}\lambda_{3}}{N_{0}\lambda_{1}} = \frac{N_{3}\lambda_{3}}{N_{0}\lambda_{1}} \\ (\mathrm{Pb}^{211}) & \frac{N_{6}\lambda_{6}}{N_{0}\lambda_{1}} = 1.00370 \ e^{-\lambda_{1}t} - 2.49248 \ e^{-\lambda_{2}t} - 0.00002 \ e^{-\lambda_{2}t} + 1.48875 \ e^{-\lambda_{3}t} + 0.00007 \ e^{-\lambda_{6}t} \\ (\mathrm{Bi}^{211}) & \frac{N_{3}\lambda_{7}}{N_{0}\lambda_{1}} = 1.00370 \ e^{-\lambda_{1}t} - 2.49268 \ e^{-\lambda_{2}t} - 0.00002 \ e^{-\lambda_{2}t} + 1.48895 \ e^{-\lambda_{3}t} + 0.00007 \ e^{-\lambda_{6}t} \\ (\mathrm{T1}^{207}) & \frac{N_{3}\lambda_{6}}{N_{0}\lambda_{1}} = 1.00370 \ e^{-\lambda_{1}t} - 2.49268 \ e^{-\lambda_{2}t} - 0.00003 \ e^{-\lambda_{2}t} + 1.48995 \ e^{-\lambda_{3}t} + 0.00007 \ e^{-\lambda_{6}t} \\ (\mathrm{T1}^{207}) & \frac{N_{3}\lambda_{6}}{N_{0}\lambda_{1}} = 1.00370 \ e^{-\lambda_{1}t} - 2.49313 \ e^{-\lambda_{2}t} - 0.00003 \ e^{-\lambda_{2}t} + 1.48995 \ e^{-\lambda_{3}t} + 0.00008 \ e^{-\lambda_{6}t} \\ \frac{\alpha_{t}}{n_{0}\lambda_{1}} = 5.01707 \ e^{-\lambda_{1}t} - 10.95034 \ e^{-\lambda_{2}t} + 0.00002 \ e^{-\lambda_{2}a}t + 5.94518 \ e^{-\lambda_{3}t} + 0.00007 \ e^{-\lambda_{6}t} \\ \frac{\alpha_{t}}{\alpha_{0}} = 418.089 \ e^{-\lambda_{1}t} - 912.529 \ e^{-\lambda_{2}t} + 0.002 \ e^{-\lambda_{2}a}t + 495.432 \ e^{-\lambda_{3}t} + 0.00014 \ e^{-\lambda_{6}t} \\ \frac{\beta_{t}}{N_{\lambda_{1}}} = 2.01939 \ e^{-\lambda_{1}t} - 4.98561 \ e^{-\lambda_{2}t} - 0.01205 \ e^{-\lambda_{2}a}t + 2.97813 \ e^{-\lambda_{4}t} + 0.00014 \ e^{-\lambda_{6}t} \\ \frac{\alpha_{t}}{N_{\lambda_{1}}} = 3.01939 \ e^{-\lambda_{1}t} - 4.98561 \ e^{-\lambda_{2}t} - 0.01205 \ e^{-\lambda_{2}a}t + 2.97813 \ e^{-\lambda_{4}t} + 0.00014 \ e^{-\lambda_{6}t} \\ \frac{\alpha_{t}}{N_{\lambda_{1}}} = 2.01939 \ e^{-\lambda_{1}t} - 4.98561 \ e^{-\lambda_{2}t} - 0.01205 \ e^{-\lambda_{2}a}t + 2.97813 \ e^{-\lambda_{4}t} + 0.00014 \ e^{-\lambda_{6}t} \\ \frac{\alpha_{t}}{N_{\lambda_{1}}} = 2.01939 \ e^{-\lambda_{1}t} - 4.98561 \ e^{-\lambda_{2}t} - 0.01205 \ e^{-\lambda_{2}a}t + 2.97813 \ e^{-\lambda_{4}t} + 0.00014 \ e^{-\lambda_{6}t} \\ \frac{\alpha_{t}}{N_{\lambda_{1}}} +$ 

Table XVIII.	Uranium-Actinium	Series	(4n	+	3	)
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(Growth of decay products from initially pure parent. Parent, actinium-227)

Time, Days	Th227	Ra <sup>223</sup>	$\alpha_t/N_0\lambda_1$	$\alpha_t/\alpha_0$	$\beta_t/N_0\lambda_1$	Time, Days	Th <sup>227</sup>	Ra <sup>223</sup>	$\alpha_t/N_0\lambda_1$	$\alpha_t/\alpha_0$	$\beta \iota / N_0 \lambda_1$
0 1 2 3 4 5	$\begin{array}{c} 0.0000\\ 0.0361\\ 0.0710\\ 0.1045\\ 0.1368\\ 0.1679 \end{array}$	$\begin{array}{c} 0.0000\\ 0.0018\\ 0.0057\\ 0.0113\\ 0.0186\\ 0.0274 \end{array}$	$\begin{array}{c} 0.0120 \\ 0.0553 \\ 0.1054 \\ 0.1615 \\ 0.2229 \\ 0.2890 \end{array}$	$\begin{array}{c} 1.0000\\ 4.6052\\ 8.7808\\ 13.457\\ 18.577\\ 24.087\end{array}$	$\begin{array}{c} 0.0000\\ 0.0154\\ 0.0229\\ 0.0341\\ 0.0486\\ 0.0660 \end{array}$	50 51 52 53 54 55	$\begin{array}{c} 0.8324 \\ 0.8379 \\ 0.8432 \\ 0.8484 \\ 0.8533 \\ 0.8581 \end{array}$	$\begin{array}{c} 0.6804 \\ 0.6905 \\ 0.7002 \\ 0.7096 \\ 0.7188 \\ 0.7278 \end{array}$	3.5657 3.6113 3.6556 3.6985 3.7403 3.7807	$\begin{array}{c} 297.15\\ 300.94\\ 304.63\\ 308.21\\ 311.69\\ 315.06 \end{array}$	$1.3720 \\ 1.3921 \\ 1.4116 \\ 1.4305 \\ 1.4489 \\ 1.4668$
6 7 8 9 10	$\begin{array}{c} 0.1979 \\ 0.2268 \\ 0.2546 \\ 0.2814 \\ 0.3072 \end{array}$	$\begin{array}{c} 0.0374 \\ 0.0487 \\ 0.0609 \\ 0.0741 \\ 0.0880 \end{array}$	$\begin{array}{c} 0.3593 \\ 0.4330 \\ 0.5098 \\ 0.5892 \\ 0.6708 \end{array}$	$\begin{array}{r} 29.938\\ 36.084\\ 42.485\\ 49.103\\ 55.901 \end{array}$	$\begin{array}{c} 0.0860\\ 0.1084\\ 0.1328\\ 0.1591\\ 0.1869 \end{array}$	56 57 58 59 60	0.8627 0.8671 0.8713 0.8754 0.8793	$\begin{array}{c} 0.7364 \\ 0.7449 \\ 0.7530 \\ 0.7610 \\ 0.7687 \end{array}$	$3.8201 \\ 3.8582 \\ 3.8951 \\ 3.9310 \\ 3.9657$	$318.34 \\ 321.51 \\ 324.59 \\ 327.58 \\ 330.48$	$1.4842 \\ 1.5010 \\ 1.5174 \\ 1.5333 \\ 1.5487$
11 12 13 14 15	$\begin{array}{c} 0.3321 \\ 0.3561 \\ 0.3791 \\ 0.4014 \\ 0.4228 \end{array}$	$\begin{array}{c} 0.1027 \\ 0.1179 \\ 0.1336 \\ 0.1497 \\ 0.1662 \end{array}$	$\begin{array}{c} 0.7542 \\ 0.8390 \\ 0.9249 \\ 1.0117 \\ 1.0990 \end{array}$	$\begin{array}{r} 62.849 \\ 69.917 \\ 77.077 \\ 84.305 \\ 91.580 \end{array}$	$\begin{array}{c} 0.2161 \\ 0.2465 \\ 0.2779 \\ 0.3102 \\ 0.3431 \end{array}$	61 62 63 64 65	$\begin{array}{c} 0.8831 \\ 0.8868 \\ 0.8903 \\ 0.8936 \\ 0.8969 \end{array}$	0.7762 0.7834 0.7904 0.7972 0.8038	$\begin{array}{r} 3.9994 \\ 4.0320 \\ 4.0637 \\ 4.0943 \\ 4.1240 \end{array}$	$333.28 \\ 336.00 \\ 338.64 \\ 341.19 \\ 343.66$	$1.5637 \\ 1.5782 \\ 1.5923 \\ 1.6059 \\ 1.6191$
16 17 18 19 20	$\begin{array}{c} 0.4434 \\ 0.4633 \\ 0.4824 \\ 0.5009 \\ 0.5186 \end{array}$	$\begin{array}{c} 0.1829 \\ 0.1999 \\ 0.2170 \\ 0.2342 \\ 0.2515 \end{array}$	$1.1866 \\ 1.2742 \\ 1.3618 \\ 1.4491 \\ 1.5359$	98.880 106.19 113.48 120.76 127.99	$\begin{array}{c} 0.3766 \\ 0.4105 \\ 0.4447 \\ 0.4791 \\ 0.5136 \end{array}$	66 67 68 69 70	$\begin{array}{c} 0.9000 \\ 0.9030 \\ 0.9059 \\ 0.9087 \\ 0.9114 \end{array}$	$\begin{array}{c} 0.8102 \\ 0.8164 \\ 0.8224 \\ 0.8282 \\ 0.8338 \end{array}$	$\begin{array}{r} \textbf{4.1527} \\ \textbf{4.1805} \\ \textbf{4.2074} \\ \textbf{4.2334} \\ \textbf{4.2586} \end{array}$	$346.06 \\ 348.37 \\ 350.62 \\ 352.79 \\ 354.89$	1.6319 1.6443 1.6563 1.6679 1.6792
21 22 23 24 25	$\begin{array}{c} 0.5357 \\ 0.5522 \\ 0.5681 \\ 0.5834 \\ 0.5981 \end{array}$	$\begin{array}{c} 0.2688 \\ 0.2860 \\ 0.3032 \\ 0.3203 \\ 0.3372 \end{array}$	$1.6221 \\ 1.7075 \\ 1.7921 \\ 1.8757 \\ 1.9583$	$135.17 \\ 142.29 \\ 149.34 \\ 156.31 \\ 163.19 \\$	$\begin{array}{c} 0.5481 \\ 0.5826 \\ 0.6170 \\ 0.6512 \\ 0.6851 \end{array}$	71 72 73 74 75	$\begin{array}{c} 0.9140 \\ 0.9165 \\ 0.9189 \\ 0.9212 \\ 0.9234 \end{array}$	$\begin{array}{c} 0.8393 \\ 0.8446 \\ 0.8497 \\ 0.8546 \\ 0.8594 \end{array}$	$\begin{array}{r} 4.2830 \\ 4.3066 \\ 4.3294 \\ 4.3514 \\ 4.3728 \end{array}$	$356.92 \\ 358.88 \\ 360.78 \\ 362.62 \\ 364.40$	1.6901 1.7007 1.7109 1.7208 1.7304
26 27 28 29 30	$\begin{array}{c} 0.6123 \\ 0.6259 \\ 0.6391 \\ 0.6518 \\ 0.6640 \end{array}$	$\begin{array}{c} 0.3540 \\ 0.3706 \\ 0.3871 \\ 0.4033 \\ 0.4193 \end{array}$	2.0397 2.1199 2.1988 2.2764 2.3526	169.97 176.66 183.23 189.70 196.05	$\begin{array}{c} 0.7187 \\ 0.7520 \\ 0.7849 \\ 0.8174 \\ 0.8494 \end{array}$	76 77 78 79 80	$\begin{array}{c} 0.9255 \\ 0.9276 \\ 0.9295 \\ 0.9314 \\ 0.9332 \end{array}$	$\begin{array}{c} 0.8640 \\ 0.8685 \\ 0.8727 \\ 0.8770 \\ 0.8810 \end{array}$	$\begin{array}{r} 4.3934 \\ 4.4133 \\ 4.4326 \\ 3.4512 \\ 4.4691 \end{array}$	$366.11 \\ 367.77 \\ 369.38 \\ 370.93 \\ 372.43$	$1.7396 \\ 1.7485 \\ 1.7572 \\ 1.7656 \\ 1.7737$
31 32 33 34 35	$\begin{array}{c} 0.6757 \\ 0.6871 \\ 0.6980 \\ 0.7085 \\ 0.7186 \end{array}$	$\begin{array}{c} 0.4351 \\ 0.4506 \\ 0.4658 \\ 0.4808 \\ 0.4955 \end{array}$	2.4274 2.5008 2.5727 2.6430 2.7119	$\begin{array}{r} 202.28\\ 208.40\\ 214.39\\ 220.25\\ 225.99 \end{array}$	0.8809 0.9119 0.9424 0.9724 1.0018	81 82 83 84 85	$\begin{array}{c} 0.9350 \\ 0.9367 \\ 0.9383 \\ 0.9399 \\ 0.9414 \end{array}$	0.8849 0.8887 0.8923 0.8959 0.8993	$\begin{array}{r} 4.4865 \\ 4.5030 \\ 4.5195 \\ 4.5351 \\ 4.5502 \end{array}$	373.87 375.25 376.62 377.93 379.18	1.7815 1.7890 1.7963 1.8034 1.8102
36 37 38 39 40	$\begin{array}{c} 0.7283 \\ 0.7377 \\ 0.7468 \\ 0.7555 \\ 0.7638 \end{array}$	$\begin{array}{c} 0.5099 \\ 0.5240 \\ 0.5378 \\ 0.5513 \\ 0.5846 \end{array}$	2.7793 2.8451 2.9095 2.9723 3.0336	$231.61 \\ 237.10 \\ 242.46 \\ 247.69 \\ 252.80$	$\begin{array}{c} 1.0306 \\ 1.0588 \\ 1.0865 \\ 1.1136 \\ 1.1041 \end{array}$	86 87 88 89 90	$\begin{array}{c} 0.9428 \\ 0.9442 \\ 0.9455 \\ 0.9468 \\ 0.9480 \end{array}$	0.9026 0.9057 0.9088 0.9117 0.9156	$\begin{array}{r} 4.5648 \\ 4.5789 \\ 4.5925 \\ 4.6056 \\ 4.6182 \end{array}$	380.40 381.57 382.71 383.80 384.85	$1.8168 \\ 1.8232 \\ 1.8294 \\ 1.8352 \\ 1.8409$
41 42 43 44 45	0.7719 0.7797 0.7872 0.7944 0.8013	$\begin{array}{c} 0.5775 \\ 0.5901 \\ 0.6024 \\ 0.6144 \\ 0.6262 \end{array}$	3.0933 3.1516 3.2084 3.2637 3.3176	257.78 262.63 267.37 271.98 276.46	$\begin{array}{c} 1.1660 \\ 1.1912 \\ 1.2158 \\ 1.2399 \\ 1.2634 \end{array}$	91 92 93 94 95	$\begin{array}{c} 0.9492 \\ 0.9503 \\ 0.9514 \\ 0.9525 \\ 0.9535 \end{array}$	$\begin{array}{c} 0.9174 \\ 0.9200 \\ 0.9226 \\ 0.9251 \\ 0.9275 \end{array}$	$\begin{array}{r} 4.6304 \\ 4.6422 \\ 4.6536 \\ 4.6645 \\ 4.6751 \end{array}$	385.87 386.85 387.80 388.71 389.59	1.8464 1.8517 1.8568 1.8618 1.8666
46 47 48 49 50	$\begin{array}{c} 0.8080 \\ 0.8145 \\ 0.8207 \\ 0.8266 \\ 0.8324 \end{array}$	0.6376 0.6487 0.6596 0.6702 0.6804	3.3700 3.4210 3.4706 3.5189 3.5657	$280.83 \\ 285.08 \\ 289.22 \\ 293.24 \\ 297.15$	$1.2863 \\ 1.3086 \\ 1.3303 \\ 1.3514 \\ 1.3720$	96 97 98 99 100	$\begin{array}{c} 0.9544 \\ 0.9554 \\ 0.9563 \\ 0.9571 \\ 0.9579 \end{array}$	0.9298 0.9320 0.9341 0.9362 0.9382	4.6853 4.6951 4.7046 4.7137 4.7225	390.44 391.26 392.05 392.81 393.54	$1.8712 \\ 1.8757 \\ 1.8800 \\ 1.8841 \\ 1.8881$

Table XIX. Uranium-Actinium Series (4n + 3)(Growth of decay products from initially pure parent.<sup>4</sup> Parent, thorium-227)

<sup>a</sup> Valid 1 hour after purification, assuming 100% retention of radon-219 and its decay products. <sup>b</sup> Assumes 25-kev. threshold and 100% counting yield for all countable betas.

$$K_{\bullet} = \frac{4.51 \times 10^9}{\left(4.51 \times 10^9 - \frac{24.1}{365}\right)} = 1.0000 \text{ (data from Table VIII)}$$

Time for 99.9% equilibrium = (10) (1.0000) (24.1) = 241 days Because of its extremely long half life, the decay of uranium-238 is negligible. Since  $K_e = 1.0000$  and the decay of thorium-234 in 30 days is 0.4220 (Table X), solution of Equation 2 yields

$$\frac{N_2\lambda_2}{N_0\lambda_1} = 1 - e^{-\lambda_2 t} = 0.5780 \text{ microcurie}$$

Growth of Decay Products. EXAMPLE 6. How much polonium-210 is present in a sample of lead-210 15 days after purification? The answer is read directly from Table XIII. The activity of polonium-210 will be 4.29% of the initial activity of the lead-210.

EXAMPLE 7. Assuming 100% retention of radon-219, how much will the alpha activity of the actinium-227 chain increase during the first 36 hours after purification? From Table XXI the ratio of the alpha activity at 36 hours to the initial alpha activity is 0.0795/0.0120 = 6.625.

EXAMPLE 8. Assuming 100% radon retention, what is the beta activity of the radium-226 chain relative to the activity of

#### Table XX. Uranium-Actinium Series (4n + 3)

(Growth of decay products from initially pure parent. Parent, thorium-227)

Time, Days	Ra <sup>223</sup>	$\alpha_t/N_{0\lambda_1}$	$eta_t/N_0\lambda_1$	Time, Days	Ra <sup>223</sup>	$lpha_t/N_{0\lambda_1}$	$eta \iota / N_0 \lambda_1$
0 1 2 3 4 5	$\begin{array}{c} 0.0000\\ 0.0589\\ 0.1121\\ 0.1600\\ 0.2031\\ 0.2417\end{array}$	$\begin{array}{c} 1.0000\\ 1.1968\\ 1.3746\\ 1.5326\\ 1.6724\\ 1.7952 \end{array}$	$\begin{array}{c} 0.0000\\ 0.1133\\ 0.2202\\ 0.3165\\ 0.4030\\ 0.4804 \end{array}$	50 51 52 53 54 55	$\begin{array}{c} 0.2761 \\ 0.2687 \\ 0.2614 \\ 0.2542 \\ 0.2471 \\ 0.2401 \end{array}$	$\begin{array}{c} 1.2600 \\ 1.2245 \\ 1.1898 \\ 1.1557 \\ 1.1223 \\ 1.0896 \end{array}$	$\begin{array}{c} 0.5529 \\ 0.5380 \\ 0.5233 \\ 0.5089 \\ 0.4947 \\ 0.4808 \end{array}$
6 7 8 9 10	$\begin{array}{c} 0.2760 \\ 0.3066 \\ 0.3335 \\ 0.3572 \\ 0.3779 \end{array}$	$\begin{array}{r} 1.9025 \\ 1.9955 \\ 2.0754 \\ 2.1432 \\ 2.1998 \end{array}$	$\begin{array}{c} 0.5495 \\ 0.6109 \\ 0.6651 \\ 0.7127 \\ 0.7543 \end{array}$	56 57 58 59 60	$\begin{array}{c} 0.2333 \\ 0.2266 \\ 0.2201 \\ 0.2136 \\ 0.2073 \end{array}$	$\begin{array}{c} 1.0576\\ 1.0262\\ 0.9956\\ 0.9657\\ 0.9365\end{array}$	$\begin{array}{c} 0.4672 \\ 0.4538 \\ 0.4406 \\ 0.4278 \\ 0.4152 \end{array}$
11 12 13 14 15	$\begin{array}{c} 0.3958 \\ 0.4111 \\ 0.4241 \\ 0.4350 \\ 0.4438 \end{array}$	2.2463 2.2835 2.3121 2.3330 2.3467	$\begin{array}{c} 0.7903 \\ 0.8212 \\ 0.8473 \\ 0.8691 \\ 0.8870 \end{array}$	61 62 63 64 65	$\begin{array}{c} 0.2012 \\ 0.1952 \\ 0.1893 \\ 0.1836 \\ 0.1780 \end{array}$	$\begin{array}{c} 0.9080 \\ 0.8802 \\ 0.8531 \\ 0.8266 \\ 0.8009 \end{array}$	$\begin{array}{c} 0.4029 \\ 0.3909 \\ 0.3791 \\ 0.3676 \\ 0.3564 \end{array}$
16 17 18 19 20	$\begin{array}{c} 0.4509 \\ 0.4562 \\ 0.4601 \\ 0.4626 \\ 0.4639 \end{array}$	$2.3540 \\ 2.3555 \\ 2.3517 \\ 2.3430 \\ 2.3300 $	$\begin{array}{c} 0.9012 \\ 0.9121 \\ 0.9200 \\ 0.9251 \\ 0.9277 \end{array}$	66 67 68 69 70	$\begin{array}{c} 0.1725\\ 0.1672\\ 0.1620\\ 0.1570\\ 0.1521 \end{array}$	$\begin{array}{c} 0.7758 \\ 0.7514 \\ 0.7276 \\ 0.7045 \\ 0.6821 \end{array}$	$\begin{array}{c} 0.3455\\ 0.3349\\ 0.3245\\ 0.3144\\ 0.3045 \end{array}$
21 22 23 24 25	$\begin{array}{c} 0.4640 \\ 0.4631 \\ 0.4612 \\ 0.4585 \\ 0.4551 \end{array}$	2.3132 2.2928 2.2694 2.2431 2.2144	$\begin{array}{c} 0.9280 \\ 0.9262 \\ 0.9226 \\ 0.9173 \\ 0.9105 \end{array}$	71 72 73 74 75	$\begin{array}{c} 0.1473 \\ 0.1426 \\ 0.1381 \\ 0.1337 \\ 0.1294 \end{array}$	$\begin{array}{c} 0.6602 \\ 0.6390 \\ 0.6183 \\ 0.5983 \\ 0.5788 \end{array}$	$\begin{array}{c} 0.2949 \\ 0.2856 \\ 0.2765 \\ 0.2677 \\ 0.2591 \end{array}$
26 27 28 29 30	$\begin{array}{c} 0.4510 \\ 0.4463 \\ 0.4410 \\ 0.4353 \\ 0.4292 \end{array}$	2.1836 2.1509 2.1165 2.0808 2.0438	$\begin{array}{c} 0.9023 \\ 0.8929 \\ 0.8825 \\ 0.8711 \\ 0.8588 \end{array}$	76 77 78 79 80	$\begin{array}{c} 0.1252\\ 0.1212\\ 0.1172\\ 0.1134\\ 0.1097 \end{array}$	$\begin{array}{c} 0.5599 \\ 0.5416 \\ 0.5238 \\ 0.5065 \\ 0.4897 \end{array}$	$\begin{array}{c} 0.2508 \\ 0.2427 \\ 0.2348 \\ 0.2271 \\ 0.2197 \end{array}$
31 32 33 34 35	$\begin{array}{c} 0.4227 \\ 0.4159 \\ 0.4088 \\ 0.4015 \\ 0.3940 \end{array}$	2.0059 1.9671 1.9277 1.8878 1.8475	$\begin{array}{c} 0.8459 \\ 0.8323 \\ 0.8181 \\ 0.8035 \\ 0.7885 \end{array}$	81 82 83 84 85	$\begin{array}{c} 0.1061 \\ 0.1026 \\ 0.0992 \\ 0.0960 \\ 0.0928 \end{array}$	$\begin{array}{c} 0.4735\\ 0.4577\\ 0.4425\\ 0.4277\\ 0.4133 \end{array}$	$\begin{array}{c} 0.2125 \\ 0.2055 \\ 0.1988 \\ 0.1922 \\ 0.1858 \end{array}$
36 37 38 39 40	$\begin{array}{c} 0.3863 \\ 0.3785 \\ 0.3706 \\ 0.3627 \\ 0.3547 \end{array}$	1.8069 1.7662 1.7255 1.6848 1.6443	$\begin{array}{c} 0.7732 \\ 0.7576 \\ 0.7419 \\ 0.7260 \\ 0.7100 \end{array}$	86 87 88 89 90	$\begin{array}{c} 0.0897 \\ 0.0867 \\ 0.0838 \\ 0.0810 \\ 0.0783 \end{array}$	$\begin{array}{c} 0.3994 \\ 0.3860 \\ 0.3729 \\ 0.3603 \\ 0.3481 \end{array}$	$\begin{array}{c} 0.1796 \\ 0.1736 \\ 0.1678 \\ 0.1622 \\ 0.1567 \end{array}$
41 42 43 44 45	$\begin{array}{c} 0.3467 \\ 0.3386 \\ 0.3306 \\ 0.3226 \\ 0.3147 \end{array}$	$\begin{array}{r} 1.6039 \\ 1.5639 \\ 1.5242 \\ 1.4849 \\ 1.4461 \end{array}$	$\begin{array}{c} 0.6940 \\ 0.6779 \\ 0.6619 \\ 0.6459 \\ 0.6301 \end{array}$	91 92 93 94 95	$\begin{array}{c} 0.0756\\ 0.0731\\ 0.0706\\ 0.0682\\ 0.0659 \end{array}$	$\begin{array}{c} 0.3363 \\ 0.3248 \\ 0.3137 \\ 0.3030 \\ 0.2926 \end{array}$	$\begin{array}{c} 0.1514 \\ 0.1463 \\ 0.1414 \\ 0.1366 \\ 0.1319 \end{array}$
46 47 48 49 50	$\begin{array}{c} 0.3068 \\ 0.2990 \\ 0.2913 \\ 0.2837 \\ 0.2761 \end{array}$	$1.4077 \\ 1.3699 \\ 1.3328 \\ 1.2960 \\ 1.2600 \\ 1$	$\begin{array}{c} 0.6143 \\ 0.5987 \\ 0.5832 \\ 0.5679 \\ 0.5529 \end{array}$	96 97 98 99 100	$\begin{array}{c} 0.0636 \\ 0.0615 \\ 0.0594 \\ 0.0573 \\ 0.0554 \end{array}$	$\begin{array}{c} 0.2826 \\ 0.2728 \\ 0.2634 \\ 0.2543 \\ 0.2456 \end{array}$	0.1274 0.1231 0.1189 0.1148 0.1109

pure radium-226 6 days, 6 hours after purification? Although the growth factors are not given for fractional days after the first 5 days, a linear interpolation will produce an answer which is correct to better than 0.5%. From Table XII, the beta growth factors of radium-226, 6 and 7 days after purification, are 1.3211 and 1.4337, respectively. The average increase between these two times is 0.0047 per hour, so the increase in 6 hours is 0.0282. The beta growth factor for 6 days, 6 hours is 1.3211 + 0.0282 = 1.3493.

EXAMPLE 9. A mixture of thorium-227 and thorium-228 is purified and a sample is alpha-counted periodically. At 24 and 120 hours the counts are 10,000 and 20,000 counts per minute, respectively. Assuming 100% retention of the radon isotopes, calculate the counts due to each thorium isotope at the time of purification.

This problem is treated in the same manner as in Example 4. The growth factors are found in Tables V and XX. Let A be the number of counts at zero time from thorium-227 and B the number of counts from thorium-228, at zero time.

	Thorit	1m-228	Radiu	m-226	Actiniu	ım-227
Hours	$\alpha_t/N_0\lambda_1$	$\beta t/N_0\lambda_1$	$\alpha_t/N_0\lambda_1$	$\beta_l/N_0\lambda_1$	$\alpha_t/N_0\lambda_1$	$\beta_t/N_0\lambda_1$
0 1 2 3 4 5 6	$\begin{array}{c} 1.0000\\ 1.0234\\ 1.0472\\ 1.0711\\ 1.0952\\ 1.1195\\ 1.1441 \end{array}$	$\begin{array}{c} 0.0000\\ 0.0003\\ 0.0015\\ 0.0037\\ 0.0070\\ 0.0113\\ 0.0165 \end{array}$	$\begin{array}{c} 1.0000\\ 1.0160\\ 1.0363\\ 1.0580\\ 1.0798\\ 1.1021\\ 1.1238\end{array}$	$\begin{array}{c} 0.0000\\ 0.0048\\ 0.0167\\ 0.0308\\ 0.0454\\ 0.0601\\ 0.0747 \end{array}$	$\begin{array}{c} 0.0120\\ 0.0136\\ 0.0153\\ 0.0169\\ 0.0186\\ 0.0203\\ 0.0221 \end{array}$	$\begin{array}{c} 0.0000\\ 0.0104\\ 0.0118\\ 0.0121\\ 0.0122\\ 0.0123\\ 0.0123\\ 0.0124 \end{array}$
7 8 9 10 11 12	1.1688 1.1937 1.2187 1.2438 1.2690 1.2943	$\begin{array}{c} 0.0228\\ 0.0299\\ 0.0378\\ 0.0464\\ 0.0558\\ 0.0658 \end{array}$	$1.1454 \\ 1.1668 \\ 1.1881 \\ 1.2092 \\ 1.2302 \\ 1.2511 $	$\begin{array}{c} 0.0892\\ 0.1035\\ 0.1178\\ 0.1320\\ 0.1460\\ 0.1600 \end{array}$	$\begin{array}{c} 0.0238\\ 0.0256\\ 0.0273\\ 0.0291\\ 0.0309\\ 0.0327 \end{array}$	$\begin{array}{c} 0.0125\\ 0.0126\\ 0.0127\\ 0.0129\\ 0.0130\\ 0.0131 \end{array}$
13 14 15 16 17 18	1.31971.34511.37061.39601.42141.4469	$\begin{array}{c} 0.0764 \\ 0.0875 \\ 0.0992 \\ 0.1112 \\ 0.1238 \\ 0.1367 \end{array}$	$1.2718 \\ 1.2924 \\ 1.3128 \\ 1.3330 \\ 1.3530 \\ 1.3729 $	$\begin{array}{c} 0.1738\\ 0.1876\\ 0.2012\\ 0.2147\\ 0.2282\\ 0.2415 \end{array}$	$\begin{array}{c} 0.0345\\ 0.0363\\ 0.0382\\ 0.0400\\ 0.0419\\ 0.0438 \end{array}$	$\begin{array}{c} 0.0133\\ 0.0134\\ 0.0136\\ 0.0138\\ 0.0138\\ 0.0139\\ 0.0141 \end{array}$
19 20 21 22 23 24	$1.4723 \\ 1.4977 \\ 1.5230 \\ 1.5482 \\ 1.5734 \\ 1.5986$	$\begin{array}{c} 0.1499 \\ 0.1635 \\ 0.1773 \\ 0.1915 \\ 0.2058 \\ 0.2204 \end{array}$	1.3927 1.4124 1.4319 1.4512 1.4703 1.4892	$\begin{array}{c} 0.2547 \\ 0.2679 \\ 0.2809 \\ 0.2938 \\ 0.3067 \\ 0.3194 \end{array}$	$\begin{array}{c} 0.0456 \\ 0.0475 \\ 0.0495 \\ 0.0514 \\ 0.0533 \\ 0.0553 \end{array}$	$\begin{array}{c} 0.0143\\ 0.0145\\ 0.0147\\ 0.0147\\ 0.0149\\ 0.0152\\ 0.0154 \end{array}$
26 28 30 32 34 36	$1.6486 \\ 1.6982 \\ 1.7474 \\ 1.7961 \\ 1.8443 \\ 1.8921$	$\begin{array}{c} 0.2501 \\ 0.2803 \\ 0.3111 \\ 0.3422 \\ 0.3735 \\ 0.4050 \end{array}$	$1.5268 \\ 1.5639 \\ 1.6004 \\ 1.6364 \\ 1.6718 \\ 1.7067$	$\begin{array}{c} 0.3446\\ 0.3694\\ 0.3938\\ 0.4179\\ 0.4416\\ 0.4650 \end{array}$	$\begin{array}{c} 0.0592 \\ 0.0632 \\ 0.0672 \\ 0.0713 \\ 0.0754 \\ 0.0795 \end{array}$	$\begin{array}{c} 0.0159 \\ 0.0164 \\ 0.0169 \\ 0.0175 \\ 0.0181 \\ 0.0187 \end{array}$
38 40 42 44 46 48	$\begin{array}{c} 1.9393\\ 1.9859\\ 2.0319\\ 2.0774\\ 2.1223\\ 2.1666\end{array}$	$\begin{array}{c} 0.4365 \\ 0.4680 \\ 0.4995 \\ 0.5308 \\ 0.5619 \\ 0.5929 \end{array}$	1.7411 1.7749 1.8083 1.8411 1.8735 1.9054	$\begin{array}{c} 0.4880\\ 0.5106\\ 0.5330\\ 0.5550\\ 0.5766\\ 0.5979 \end{array}$	$\begin{array}{c} 0.0837\\ 0.0880\\ 0.0923\\ 0.0966\\ 0.1010\\ 0.1054 \end{array}$	$\begin{array}{c} 0.0193\\ 0.0200\\ 0.0207\\ 0.0214\\ 0.0222\\ 0.0229 \end{array}$
52 56 60 64 68 72	$\begin{array}{r} 2.2533\\ 2.3376\\ 2.4195\\ 2.4989\\ 2.5761\\ 2.6508\end{array}$	$\begin{array}{c} 0.6541 \\ 0.7141 \\ 0.7728 \\ 0.8301 \\ 0.8860 \\ 0.9404 \end{array}$	1.9677 2.0281 2.0868 2.1437 2.1990 2.2525	$\begin{array}{c} 0.6397 \\ 0.6801 \\ 0.7194 \\ 0.7575 \\ 0.7944 \\ 0.8304 \end{array}$	$\begin{array}{c} 0.1143\\ 0.1234\\ 0.1327\\ 0.1422\\ 0.1518\\ 0.1615 \end{array}$	$\begin{array}{c} 0.0246\\ 0.0263\\ 0.0281\\ 0.0300\\ 0.0320\\ 0.0341 \end{array}$
80 88 96	$2.7936 \\ 2.9276 \\ 3.0535$	$1.0448 \\ 1.1431 \\ 1.2356$	$2.3550 \\ 2.4514 \\ 2.5422$	0.8989 0.9635 1.0243	$\begin{array}{c} 0.1814 \\ 0.2019 \\ 0.2229 \end{array}$	$\begin{array}{c} 0.0386 \\ 0.0434 \\ 0.0486 \end{array}$
108 120	$3.2278 \\ 3.3861$	1.3641 1.4809	$2.6685 \\ 2.7838$	1.1 <b>088</b> 1.1861	$\begin{array}{c} 0.2554 \\ 0.2890 \end{array}$	0.0569 0.0660

10,000 = 1.1968 A + 1.5986 B

20,000 = 1.7952 A + 3.3861 B

A = 1597 counts per minute from thorium-227 at zero time

B = 5060 counts per minute from thorium-228 at zero time

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Table XXI. Growth of Alpha and Beta Activities from Initially Pure Parents during First 120 Hours

Parent

# Magnesium Determination in Bovine Blood Serum

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A STUDY was made to compare the three most widely used methods for the determination of magnesium in serum. Magnesium values in calf serum were determined following the procedures outlined by Kunkel *et al.* (8), Simonsen *et al.* (12), and Hoffman (7). The first method (8) employs a dye, Titan yellow, which forms a colored lake with magnesium hydroxide. The intensity of this lake can be measured by a photoelectric colorimeter. The second method (12) employs precipitation of magnesium as magnesium ammonium phosphate from the calcium-free filtrate, and the colorimetric measurement of the phosphorus in the precipitate. The third method (7) employs the precipitation of magnesium with 8-quinolinol and the quinoline concentration is estimated colorimetrically as ferric quinoline.

Because some modifications have been made and also because it is the principal method employed in the authors' laboratory, the first method is briefly discussed here. However, for more details the publications of Heagy (5), Kunkel *et al.* (8), and Orange and Rhein (11) should be consulted.

To 4 ml. of a tungstic acid filtrate, add 1 drop of alcoholic methyl red indicator, sufficient 0.1N sodium hydroxide to change the indicator color from red to yellow, 1 ml. of 0.1% polyvinyl alcohol, 1 ml. of 0.05% Titan yellow solution, 2 ml. of 1.5N sodium hydroxide, and sufficient deionized water to make the total volume to 10 ml. The resulting colored solution is read against a blank at 540 m $\mu$ .

To test the accuracy of the methods, recovery experiments were performed for the Titan yellow and 8-quinolinol methods. The Titan yellow method gave an average recovery value of 100.3% with a standard deviation of  $\pm 4.2$  (see Table I), and the 8-quinolinol method gave an average recovery value of 92.2% (three values, range 82.3 to 98.8%).

Table I.	<b>Recovery from</b>	Known	Amounts	of	Magnesium
	Added to	5 1 Ml. o	f Serum		

Calf No.	Mg in Serum, γ	$\mathrm{\substack{Mg}\ Added,}\ \gamma$	${f Mg} { m Calcd.,} {f \gamma}$	Mg Found, γ	Recovery,
X 569	20.2	10	30.2	29.7	98.3
X 569	12.1	10	22.1	19.0	86.0
N 2780	12.9	10	22.9	23.5	102.6
X 569	12.5	10	22.5	22.7	100.9
N 614	28.9	10	38.9	38.5	99.0
N 572	14.5	10	24.5	24.4	99.6
N 173	18.3	10	28.3	29.3	103.5
N 572	15.4	10	25.4	25.7	101.2
N 173	19.4	10	29.4	29.6	100.7
N 3158	16.4	4	20.4	21.1	102.9
Mixture	11.6	10	21.6	21.5	99.5
Mixture	11.2	10	21.2	21.6	101.9
Mixture	11.7	10	21.7	22.7	104.6
Mixture	10.6	10	20.6	21.5	104.4
					Av. 100.3ª
Hoffman	(7)				
$\mathbf{X}$ 589	14.8	10	24.8	20.4	82.3
$\mathbf{X}$ 582	2.9	10	12.9	12.7	98.4
$\mathbf{X}$ 580	15.0	10	25.0	24.7	98.8
<sup>a</sup> Stand	ard deviatio	on, 4.2.			

A further test of the reproducibility of the two methods was performed by determining the serum magnesium values in 23 samples by both methods. Basing the Titan yellow values as 100%, the 8-quinolinol values, in comparison, were  $84.9 \pm 12.33\%$ , the range being 24.8 to 140.8% (see Table II). The 15% discrepancy in some part can be accounted for by the loss of magnesium hydroxyquinoline in the washing process. The 8-quino-linol method appeared somewhat nonreproducible and unreliable and the breakage of the special tubes used in the method was so excessive that this method was discontinued.

In comparing the Titan yellow and the ammonium phosphate methods, 94 samples were analyzed. In 70 samples (Group 1) the magnesium ammonium phosphates were left for precipitation for 2 hours as suggested by Simonsen *et al.* (12); in 24 samples (Group 2) the solutions were left overnight. Samples in Group 1 gave an average value of  $90.1 \pm 14.8\%$ , and the samples in Group 2 gave an average value of  $101.6 \pm 6.0\%$  as compared to the Titan yellow values based at 100%. The average values for these comparisons are shown in Table II. The Titan yellow method was chosen for routine estimation of serum magnesium for its ease, rapidity, and reproducibility.

 Table II. Average Magnesium Serum Values from Three

 Methods

		Method	
No. of Detns.	Titan yellow, mg. %	MgNH4PO4, mg. %	Hydroxyquinolate, mg. %
22 70	2.02	1 624	1.62
24	1.82	1.85%	
<sup>a</sup> Magnesium am <sup>b</sup> Magnesium am	m <b>oni</b> um phosphat monium phosphat	te precipitated fo e precipitated ov	r 2 hours. ernight.

There has been some controversy as to whether calcium interfered in the determination of magnesium by the Titan yellow method (2, 3, 6, 9). Therefore, magnesium content in serum was determined in two ways: directly in serum and in a calcium-free filtrate. In 15 samples the average values were 1.54 mg. % for the calcium-free filtrate and 1.47 mg. % for the direct serum. In the calculations of these values two standard curves were employed. The calcium-free filtrate values were read against a standard curve containing no calcium, while the direct serum values were read against a standard curve containing  $100\gamma$  of calcium per 10 ml.

The 0.07 mg. % difference in the two different methods of estimating the magnesium concentration is negligible for routine analysis; however, for more accurate results in the direct serum determination, an appropriate amount of calcium must be added to the blank and standard solutions. When the transmittance readings, for the direct serum, were read against the standard curve containing no calcium, the values averaged 1.31 mg. % (see Table III).

Table III.	Serum	Magnesium	Concent	trations	Using
Calcium-F	ree and	Calcium-Cor	ntaining	Deprote	einized
		Filtrate		•	

		LINCIACO	
Calf No.	Calcium-Free Filtrate, Mg. %	Direct Serum <sup>a</sup> , Mg. %	Direct Serum <sup>b</sup> , Mg. %
N 574 N 575 N 576 N 580 N 2783 N 3158 N 3158 N 3158 N 574 N 578 X 580 N 576 N 577 N 577 N 577 N 578 N 576 N	$\begin{array}{c} 2.30\\ 1.47\\ 2.08\\ 1.37\\ 1.53\\ 0.39\\ 1.55\\ 1.75\\ 1.32\\ 1.41\\ 1.68\\ 1.08\\ 1.76\\ 1.70\end{array}$	$\begin{array}{c} 2 & 11 \\ 1 & 28 \\ 2 & 06 \\ 1 & 20 \\ 1 & 38 \\ 0 & 37 \\ 0 & 96 \\ 1 & 37 \\ 1 & 27 \\ 0 & 94 \\ 1 & 52 \\ 0 & 85 \\ 1 & 46 \\ 1 & 28 \end{array}$	$\begin{array}{c} 2.28\\ 1.43\\ 2.22\\ 1.36\\ 1.53\\ 0.48\\ 1.20\\ 1.51\\ 1.40\\ 1.20\\ 1.65\\ 1.00\\ 1.65\\ 1.02\\ 1.42\end{array}$
Av.	1.54	1.31	1.47

<sup>a</sup> Value determined using blank and standard containing no calcium which was also used for determining values in column 1. <sup>b</sup> Value determined using blank and standard solutions containing added calcium similar in amount to that found in serum. To prove that the excess ammonium and oxalate ions, introduced in precipitating calcium, had no effect, 1 ml. of 1% ammonium oxalate  $[(NH_4)_2C_2O_4.H_2O]$  was added to the 10 ml. of blank and all known solutions. No significant difference in the ammonium oxalate added standards and the other standards were found.

In working with a standard curve containing a definite amount of calcium, it is likely that an error would be introduced, as all samples will not contain the same amount of calcium. Probably this accounts for the 0.07 mg. % difference in the aforementioned values. This finding, that the amount of calcium found in bovine serum does interfere with the estimation of magnesium by the Titan yellow method, is in contrast to that found by Gilliam (3), Garner (2), and Ludwig and Johnson (9).

Whole blood values of magnesium in cattle were reported by two investigators to be approximately the same as that of serum (1, 7), while Green and Macaskill (4) reported that two thirds of the magnesium was contained in the corpuscles while one third was located in the plasma. In this experiment a measured amount of whole blood from young calves was laked by the addition of water and magnesium was estimated in the manner described by Kunkel *et al.* (8). Orange and Rhein (11) stated that iron in the solution of ashed whole blood cells interfered in the estimation of magnesium; however, since in this method samples are not ashed, the small amount of iron is probably removed during deproteinization with tungstic acid (10). In making the comparison 27 samples were divided into three groups.

1. Those with values of serum magnesium over 2.00 mg. %.

2. Those with values of serum magnesium between 2.00 and 1.00%.

3. Those with values of serum magnesium under 1.00 mg. %.

Group	No. of Analyses	Av. Serum, Mg. %	Av. Whole Blood Mg, Mg. %	Ratio
1	13	2.27	2.27	1:1
$\overline{2}$	- 9	1.53	1.94	1:1.3
3	5	0.76	1.39	1:1.8

The tabulated results indicate that the drop in magnesium content of the red corpuscles takes place more slowly than in serum. This is in agreement with the work done by Tufts and Greenberg (13). The calves in Group 1, which would be considered normal, had a ratio of 1:1 or the same amount of magnesium in their serum as in whole blood. This is in agreement with the published work of Kunkel *et al.* (8) and Eveleth (1).

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# **Determination of Ring Content of Aromatic Petroleum Fractions**

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ROMATIC fractions from petroleum have been analyzed by density-temperature coefficient and molecular weight (5), refractive index-temperature coefficient and molecular weight (3), and numerous other correlations of physical properties and structure. Recently Martin and Sankin (7) described a method for determining aromatics in petroleum concentrates using characterization functions derived from molecular weight, density, and specific dispersion. These authors pointed out that it should be possible to eliminate the specific dispersion factor and to substitute refractive index in its place. This is highly desirable because measurement of refractive dispersion is difficult, whereas refractive index can be determined with ease.

#### DERIVATION OF EQUATIONS

Trial attempts to find a group of functions which would give satisfactory calculation of aromatic content indicated that the refractive index did not correlate directly with the aromatic content. Using the "characteristic factors" of Smittenberg (9), it was found that the correlation curves of aromatic ring content,  $R_A$ , with  $M\Delta n$  were logarithmic in form rather than linear and that there was an appreciable spread of the values. This spread was later found to be due to total ring content.

It was found by trial that the factor  $M\Delta r_i$ , which is derived from the refractivity intercept,  $r_i$ , could be correlated with total ring content,  $R_T$ , in combination with the factor  $M\Delta d$ . In these equations, M = molecular weight $\Delta d = d_4^{20} - 0.8510$  $\Delta n = n_D^{20} - 1.4750$  $\Delta r_i = \left(n_D^{20} - \frac{d_4^{20}}{2}\right) - 1.0495$  $\Delta r_i = \Delta n - \frac{\Delta d}{2}$ 

 $R_T$  = total number of rings

 $R_A$  = number of aromatic rings

By plotting  $M\Delta r_i$  versus  $M\Delta d$  for the data given in Table I for a series of aromatic petroleum concentrates and alkyl aromatics, a chart is obtained which has isobaric lines for total ring content. This chart is shown in Figure 1.

Determination of the slope of the  $R_T$  lines in Figure 1 permits calculation of relative values of the two factors. The reciprocal of the slope is incorporated into the expression:

$$R_T = aM(b\Delta r_i + \Delta d) + C$$

which is of the form derived by Van Nes and Van Westen (10) for relating  $M \Delta n$  and  $M \Delta d$  to  $R_T$ . 1/slope = b in this equation. It was found that b = -0.67. Therefore,

$$R_T = aM(\Delta d - 0.67 \Delta r_i) + C$$

			·····	1	,	
	$R_A$	$R_T$	$n^{20}_{\ \mathrm{D}}$	d20	M	Ref.
	-	Ligh	t Cracked	Gas Oil		
Whole aromatic Adsorption fraction I II III	$2.27 \\ 1.91 \\ 2.41 \\ 2.81$	$2.61 \\ 2.37 \\ 2.57 \\ 2.94$	$\begin{array}{r}1.6163\\1.5862\\1.6249\\1.6404\end{array}$	$\begin{array}{r}1.0217\\0.9848\\1.0314\\1.0518\end{array}$	196) 196 196 208	
	F	ractions	from Web	ster Crude	,	
400-922° F. 400-500° F. 500-600° F. 600-700° F. 700-800° F. 800-922° F.	$     \begin{array}{r}       1.68 \\       1.22 \\       1.25 \\       1.40 \\       1.49 \\       1.91 \\       \end{array} $	2.95 1.58 2.09 2.64 3.81 4.10	$\begin{array}{r} 1.5474 \\ 1.5283 \\ 1.5413 \\ 1.5458 \\ 1.5511 \\ 1.5539 \end{array}$	$\begin{array}{c} 0.9689 \\ 0.9230 \\ 0.9506 \\ 0.9666 \\ 0.9808 \\ 0.9889 \end{array}$	289 167 193 244 331 408	(7)
		ight Tul	ha Oila (65	0.0000 F		
Fact Taxas	2 04	2 20	1 5649	0-730° F.,	- 207	
Michigan Webster Mirando	$1.79 \\ 1.69 \\ 1.58$	$     \begin{array}{r}       3.29 \\       2.62 \\       3.08 \\       3.43 \\     \end{array}   $	$1.5042 \\ 1.5449 \\ 1.5478 \\ 1.5568$	$0.9857 \\ 0.9575 \\ 0.9742 \\ 0.9876$	304 299 284	
	J	Traction	s from Bor	neo Crude		
IV V VI	$1.30 \\ 1.38 \\ 1.08$	$3.05 \\ 3.26 \\ 3.56$	${}^{1.5352}_{1.5492}_{1.5502}$	$\begin{array}{c} 0.9671 \\ 0.9742 \\ 0.9804 \end{array}$	$281 \\ 305 \\ 350 \end{pmatrix}$	(10)
		Α	lkyl Tetra	lins		
Tetralin 1-Methyl Tetralin 1-Ethyl Tetralin 1-Butyl Tetralin 1-Amyl Tetralin	$\begin{array}{r} 1.00 \\ 1.00 \\ 1.00 \\ 1.00 \\ 1.00 \\ 1.00 \end{array}$	2.00 2.00 2.00 2.00 2.00 2.00	1.54151.53531.53181.52181.52181.5178	$\begin{array}{c} 0.9694 \\ 0.9582 \\ 0.9528 \\ 0.9342 \\ 0.9271 \end{array}$	$ \begin{array}{c} 132\\ 146\\ 160\\ 188\\ 202 \end{array} $	(4)
		Alky	vl Naphth	alenes		
1-C7-Naphthalene 1-C9-Naphthalene 1-C11-Naphthalene 2-C9-Naphthalene 2-C11-Naphthalene	2.00 2.00 2.00 2.00 2.00 2.00	2.00 2.00 2.00 2.00 2.00 2.00	$\begin{array}{r} 1.5583 \\ 1.5472 \\ 1.5394 \\ 1.5460 \\ 1.5382 \end{array}$	$\begin{array}{c} 0.9500\\ 0.9367\\ 0.9280\\ 0.9304\\ 0.9220\end{array}$	$\begin{array}{c} 225 \\ 253 \\ 281 \\ 253 \\ 281 \\ 253 \\ 281 \end{array}$	(1)

Table I. Alkyl Aromatics and Petroleum Aromatic Concentrates Used to Develop Equations

By plotting  $M(\Delta d - 0.67 \Delta r_i)$  versus  $R_T$  for the same set of data used in Figure 1, a straight line is obtained. The slope of this line is set equal to a and the intercept at the point where  $M(\Delta d - 0.67 \Delta r_i) = 0$  is set equal to C. This plot is shown in Figure 2. The expression for calculating  $R_T$  than becomes:

 $R_T = 0.060 \ M(\Delta d - 0.67 \ \Delta r_i) + 1.10$ By substitution of  $\Delta n - \frac{\Delta d}{2}$  for  $\Delta r_i$ , this equation becomes:

$$R_T = 0.080 \ M(\Delta d - 0.50 \ \Delta n) + 1.10 \tag{1}$$



Showing isobaric  $R_T$  lines for materials in Table I

Attempts to relate the same characteristic factors to aromatic ring content failed. Trial of a series of known characteristic factors was also unsuccessful. It was found, however, that a new factor could be written which would give the desired results. For convenience, this factor is called the "density intercept." It is expressed as  $M \Delta d_i$ , where:

$$\Delta d_i = \left( d_4^{20} - \frac{n_D^{20}}{2} \right) - 0.1135$$
$$\Delta d_i = \Delta d - \frac{\Delta n}{2}$$

Plotting  $M \Delta d_i$  versus  $M \Delta n$  for the same series of data used in developing the  $R_T$  expression permitted calculation of b' in the expression

$$R_T = a'M(\Delta d_i + b'\Delta n) + C'$$

This is again similar to the expression derived by Van Nes and Van Westen (10).



Table II.	Deviations from the Direct Method						
Quantity	Average Deviation	Maximum Deviation	Method				
RA RT RA RT	$\begin{array}{c} 0.13 \\ 0.12 \\ 0.11 \\ 0.14 \end{array}$	$\begin{array}{c} 0.24 \\ 0.29 \\ 0.26 \\ 0.33 \end{array}$	Sp. disp. Sp. disp. This method This method				

b' was found to be equal to -2.25. Plotting  $M(\Delta d_i -2.25 \Delta n)$  versus  $R_A$  showed that a' = -0.040. The expression was therefore rewritten to change the signs and C' was then found to be 0.58. The expression becomes:

$$R_A = 0.040 \ M(2.25 \ \Delta n - \Delta d_i) + 0.58$$

By substitution of  $\Delta d - \frac{\Delta n}{2}$  for  $\Delta d_i$ , this equation becomes:

$$R_A = 0.040 \ M(2.75 \ \Delta^n - \Delta d) + 0.58 \tag{2}$$

From the values of  $R_T$  and  $R_A$  other desired values of carbon distribution and ring content can be calculated as described by Martin and Sankin (7).

#### **TESTING METHOD**

Martin and Sankin (7) presented data for a series of aromatic petroleum fractions used in testing their method. There were complete data available for 17 of these fractions.  $R_A$  and  $R_T$  were calculated from the data on elemental analysis and hydrogenation ("direct method") (6, 10). The values of  $R_A$  and  $R_T$ were then calculated by the method of Martin and Sankin and from Equations 1 and 2 derived in this work.

Table II shows the deviation of this method and the specific dispersion method from the direct method data.

It can be seen that the deviations for these 17 fractions are approximately equal to those of the specific dispersion method. These fractions range from highly aromatic light cracked gas oil fractions to heavy lubricating oils. The molecular weights range from 167 to 408.

Martin and Sankin (?) also presented elemental analyses for 15 additional aromatic concentrates. From these data, one may calculate:

$$(3R_A + R_N) = M_0(0.0833 C_0 - 0.496 H_0)$$

where  $R_A$  = aromatic ring content

 $R_N =$  naphthenic ring content

- $M_0$  = molecular weight  $C_0$  = per cent carbon × 10<sup>-2</sup>
- $H_0 = \text{per cent barbon} \times 10^{-2}$

The values of  $(3R_A + R_N)$  [or  $(2R_A + R_T)$ ] were calculated for all 32 examples, using the direct method data, the dispersion method, and the method derived in this paper. The results, expressed as deviations from the direct method, are shown in Table III.

It can be seen that the errors of this method are approximately similar to those of the dispersion method.

#### APPLICATION OF METHOD

This method is not as precise for the majority of petroleum products as the n-d-M method of Van Nes and Van Westen (10). The n-d-M method was developed for use with distillate fractions rather than with aromatic concentrates; it gives erratic results when applied to such concentrates. The method described in this paper fills a need for a procedure which can be applied to petroleum aromatics without requiring difficult experimental measurements.

In the study of arc interruption in transformer oil (8), the

Table	III.	Deviations	of	(3 <b>R</b> <sub>A</sub>	+	$\boldsymbol{R}_N$ )	from	the	Direct	
			$-\mathbf{M}$	ethod						

	Dispersion Method	This Method	
Average Maximum	0.24 0.67	$\substack{\textbf{0.20}\\\textbf{0.64}}$	
from theory of errors	0.29ª	0.26%	
<sup>a</sup> 0.29 = $\sqrt{(2 \times 0.13)^2 + (0.12)^2}$ <sup>b</sup> 0.26 = $\sqrt{(2 \times 0.11)^2 + (0.14)^2}$			

Table IV.	Results	Obtained	on	Transformer	Oils	by	Various	Methods
-----------	---------	----------	----	-------------	------	----	---------	---------

						$R_A$			$R_T$	
М	$n_{\rm D}^{20}$	$d_4^{20}$		S Type	n-d-M	Disper- sion	This work	n-d-M	Disper- sion	This work
				Fractions from Oxidized	Transform	er Oil				
302 315 353 340 293 442	$\begin{array}{c} 1.5034 \\ 1.5173 \\ 1.5170 \\ 1.5350 \\ 1.5552 \\ 1.5334 \end{array}$	$\begin{array}{c} 0.9068 \\ 0.9272 \\ 0.9217 \\ 0.9459 \\ 0.9822 \\ 0.9447 \end{array}$	124 134 134 152 184 146	Percolate Percolate Rafinate (MeOH) Extract (MeOH) Extract (MeOH)	$0.70 \\ 0.96 \\ 1.11 \\ 1.48 \\ 1.57 \\ 1.73$	$\begin{array}{c} 0.89 \\ 1.05 \\ 1.10 \\ 1.35 \\ 1.65 \\ 1.47 \end{array}$	$\begin{array}{c} 0.85 \\ 1.09 \\ 1.21 \\ 1.53 \\ 1.63 \\ 1.76 \end{array}$	2.432.672.572.733.133.20	2.11 2.57 2.59 2.97 3.22 3.53	2.11 2.49 2.50 2.87 3.24 3.38
				Fractions from Arced T	ransformer	Oil				
308 300 275 262	$\substack{1.5069\\1.5220\\1.5247\\1.5442}$	$\begin{array}{c} 0.9005 \\ 0.9138 \\ 0.9303 \\ 0.9525 \end{array}$	$130 \\ 142 \\ 148 \\ 173$	Percolate Percolate Percolate Percolate	$0.96 \\ 1.34 \\ 1.13 \\ 1.48$	0.98 1.12 1.15 1.41	$1.05 \\ 1.38 \\ 1.25 \\ 1.51$	$2.02 \\ 1.79 \\ 2.30 \\ 2.27$	$1.94 \\ 2.13 \\ 2.34 \\ 2.53$	$1.93 \\ 2.04 \\ 2.30 \\ 2.50$
	Aromatic Concentrates from Transformer Oil									
267 270 285	$\substack{1.4948\\1.5303\\1.5252}$	0.8939 0.9438 0.9365	110 152 147	Silica gel + Celite (naphthenic) Silica gel only Silica gel (arced oil)	$0.54 \\ 1.12 \\ 1.08$	$0.70 \\ 1.19 \\ 1.16$	$0.70 \\ 1.22 \\ 1.18$	$2.14 \\ 2.57 \\ 2.57 \\ 2.57$	$1.87 \\ 2.57 \\ 2.52$	$1.80 \\ 2.51 \\ 2.48$

n-d-M method has been applied to the whole oil (2). However, silica gel percolation of these arced oils yields aromatic concentrates which do not lend themselves to analysis by the n-d-Mmethod. Furthermore, studies of oxidation of transformer oil require fractionation by silica gel adsorption and again the aromatic portions are such that the n-d-M method gives erratic results.

Some of these aromatics are described in Table IV. The aromatics in this table were separated either by percolation through silica gel or by washing the aromatics through a silica gel column with methanol. It can be seen that the agreement between results obtained by this method and the dispersion method are good.

This method is not intended for use with concentrates having a value of  $R_A$  less than 0.9, or a value of  $R_T$  less than 1.2. Concentrates of this nature are better represented by the *n*-d-*M* method.

This method may give incorrect results when applied to alkyl aromatics. It is intended primarily for aromatic concentrates.

# ANALYTICAL PROCEDURE

Concentrate the aromatics by any suitable method, such as silica gel adsorption.

Determine  $n_{30}^{\circ}$ ,  $d_4^{\circ\circ}$ , and molecular weight. Calculate:  $\Delta d = d_4^{\circ\circ} - 0.8510$ ;  $\Delta n = n_{30}^{\circ} - 1.4750$ .

Substitute these values in:

$$R_T = 0.080 \ M(\Delta d - 0.50 \ \Delta n) + 1.10 \tag{1}$$

$$R_A = 0.040 \ M(2.75 \ \Delta n - \Delta d) + 0.58 \tag{2}$$

The following equations may be used to calculate other desired quantities:

$$\kappa_N = \kappa_T - \kappa_A \tag{3}$$

$$C_T = 0.070 M + 0.3 R_A + 0.1 R_T \tag{4}$$

$$C_A = 4.0 \ R_A + 2 \tag{5}$$

$$C_R = 4.0 R_T + 2$$
 (6)

$$\% C_A = \frac{100 C_A}{C_T}$$
(7)

$$\% C_{R} = \frac{100 C_{R}}{C_{T}}$$
(8)

$$\% C_N = \% C_R - \% C_A \tag{9}$$

$$\% C_P = 100 - \% C_R \tag{10}$$

Equations 3 to 10 are based on Equations 7 to 13 of (7).

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#### CONCLUSIONS

Equations have been derived to express the ring content and carbon distribution of aromatic petroleum concentrates in terms of readily measured physical properties. Only refractive index, density, and molecular weight are required. The deviations in known concentrates are equivalent to those found in other methods for aromatic concentrates requiring more elaborate experimental data.

The method is based on "characteristic factors" of hydrocarbons and involves  $M\Delta n$ ,

 $M \Delta d$ , and  $M \Delta r_i$ , as well as a new "characteristic factor" defined as the density intercept  $M \Delta d_i$ .

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# Argentometric Determination of Halides Using the Dead-Stop End Point

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THE dead-stop end point has been repeatedly used where a reversible couple appears or disappears at the equivalence point (3, 4, 7, 13). A second type is to be found where the same substance undergoes both oxidation at the anode and reduction at the cathode (6, 11). Both of these types use inert electrodes, usually platinum. As has been discussed (12), the dead-stop phenomenon is based on electrochemical cell behavior with the result that the presence of a metal ion in solution should initiate electrolysis with electrodes made of the same metal. This paper discusses the use of silver electrodes and silver nitrate solution as a dead-stop technique. Some of the theoretical aspects of this system have been discussed by Bradbury (2).

The argentometric analysis of halide mixtures by potentiometric and amperometric methods has been reviewed (9). Clippinger and Foulk (5) used the dead-stop end point for the argentometric determination of halides. Platinum electrodes were used and nitrite ion was required as a "depolarizer." Salomon (10) was the first to report a galvanometric titration using silver electrodes and silver nitrate solution to titrate chloride. This essentially was the first dead-stop technique, but the experiment was a demonstration, and the details are not clear. No work on mixtures of halides was reported. Data were given to show that the current increased from a very small value at the equivalence point.

### EXPERIMENTAL

Apparatus. The electrical circuit described by Wernimont and Hopkinson (14) is the basic requirement. The Sargent Model III polarograph or the Fisher Elecdropode may be used. In any case a galvanometer with a sensitivity of 0.01  $\mu$ a. per mm. of scale is desirable. The electrodes were silver wires fused to copper leads and sealed into glass tubing with De Khotinsky cement. The seal was protected with Glyptal for water resistance, particularly in the presence of ammonium hydroxide. The use of silver foil electrodes did not improve the end point detection (2). A stirring device is necessary, since the solution must be stirred continuously during the titration.

stirred continuously during the titration. **Reagents.** Standard silver nitrate solution was prepared from reagent grade silver nitrate which had been ground and dried at 110° C. for 2 hours. Potassium bromide used in this work was analyzed by the A.C.S. revised specification method (1) and was found to contain chloride equivalent to 1 mg. of potassium chloride per gram. Sodium chloride and potassium iodide were reagent grade and were dried 2 hours at 110° C.

(1) and was found to contain chloride equivalence of 1.1.5. or potassium chloride per gram. Sodium chloride and potassium iodide were reagent grade and were dried 2 hours at 110° C.
General Procedure. Weigh a sample which will require 15 to 50 ml. of 0.1M silver nitrate solution into a 250-ml. beaker. Make the volume to 100 ml. with water, add 5 ml. of 2% dextrin solution, and add the required acid or other reagent. Start the stirrer, dip the electrodes into the solution, and apply 10 mv. between the electrodes. Add silver nitrate solution from a buret at such a rate that drops are visible. The current first increases,

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then decreases, and reaches a minimum at the end point followed by an increase after the end point. In the vicinity of the end point, time for equilibration must be allowed after the addition of each drop. Usually 1 to 2 minutes are necessary. With mixed halides a slower rate of addition is required, and experience is necessary to find the correct end point which is the first permanent increase in current. After use the electrodes must be soaked in concentrated sodium thiosulfate solution and stored in water. Failure to do this results in a surface film on the silver which must be removed by scraping with a knife.

#### RESULTS

The determination of chloride alone was done in the presence of 5 drops of concentrated nitric acid, or 10 drops of glacial acetic acid. The latter made the end point somewhat easier to detect. Since silver chloride is appreciably soluble, the end point was not sharp in any case (2). Some typical results are listed in Table I.

The determination of bromide alone was done in the presence of 5 drops of concentrated nitric acid, 10 drops of glacial acetic acid, or 5 ml. of 1M ammonium carbonate solution as recommended by Kolthoff (8). The end points were easier to detect than those with chloride, but experience is necessary. The acetic acid medium gave the best results as shown by Table II.

The determination of iodide alone was tried in distilled water only, in the presence of 5 ml. of concentrated ammonium hydrox-

Table I. Titration of Chloride with 0.1M Silver Nitrate

HNO3 I	Medium	HOAc Medium				
NaCl taken, g.	NaCl found, g.	NaCl taken, g.	NaCl found, g.			
$\begin{array}{c} 0.1780\\ 0.2553\\ 0.2648\\ 0.2295\\ 0.2321\\ 0.2330\\ 0.2642\\ 0.1881\\ 0.2530\\ \end{array}$	$\begin{array}{c} 0.1787\\ 0.2548\\ 0.2643\\ 0.2293\\ 0.2317\\ 0.2329\\ 0.2649\\ 0.1878\\ 0.2534\\ \end{array}$	$\begin{array}{c} 0.2367\\ 0.2546\\ 0.2337\\ 0.2074\\ 0.2486\end{array}$	$\begin{array}{c} 0.2367\\ 0.2545\\ 0.2343\\ 0.2071\\ 0.2482 \end{array}$			

 Table II.
 Titration of Bromide with 0.1M Silver Nitrate

 HOAa Medium
 HNO: Medium

HUAC I	vredium	III IN O3 IVIEUIUIII			
KBr taken, g.	KBr found, g.	KBr taken, g.	KBr found, g.		
$\begin{array}{c} 0.5666\\ 0.5308\\ 0.4636\\ 0.4399\\ 0.4167\\ 0.5707\\ 0.4788\\ 0.4625\\ 0.3832\\ 0.3664 \end{array}$	$\begin{array}{c} 0.5663\\ 0.5307\\ 0.4634\\ 0.4396\\ 0.4162\\ 0.5703\\ 0.4779\\ 0.4617\\ 0.3830\\ 0.3669\\ \end{array}$	$\begin{array}{c} 0.3408\\ 0.3595\\ 0.4556\\ 0.3335\\ (NH_4)_2CO\\ 0.2896\\ 0.3351\\ 0.3218\\ 0.2889\\ 0.2934\\ 0.4744\\ \end{array}$	0.3408 0.3388 0.4547 0.3326 3 Medium 0.2883 0.3330 0.3330 0.3208 0.2856 0.2939 0.4744		

Table III. Titration of Iodide with 0.1M Silver Nitrate

$H_2O$	Only	(NH <sub>4</sub> ) <sub>2</sub> CO <sub>2</sub> Medium				
KI taken, g.	KI found, g.	KI taken, g.	KI found, g.			
0.6599	0.6571	0.5132	0.5116			
0.7714	0.7714	0.5158	0.5149			
0.5557	0.5576	0.7043	0.7024			
0.4873	0,4870	0.5042	0.5034			
0.6113	0.6121	0.4142	0.4123			
0.6337	0.6343	0.4906	0.4884			
0.6182	0.6184	0.5481	0.5480			
0.8061	0.8054	0 4263	0.4249			
0.6412	0.6409	0.5355	0.5342			
NH4OH	Medium	0.3672	0.3661			
0.3717	0 3898	HOAc	Medium			
0.5041	0.5161	0.5020	0.5017			
0.4729	0.4797	0.4225	0.4213			
0.5230	0.5373	0.5556	0.5552			
0 7443	0 7454	0 5191	0 5199			
0.5104	0.5241	0.4817	0.4813			
0.8133	0.8171					
0.5258	0.5356					
0 5163	0 5285					
0.5948	0.6017					

Titration of Bromide with 0.1M Silver Nitrate Table IV. Acetic Acid Medium, 1.0 Gram Sodium Chloride Present

KBr Taken, G.	KBr Found, G.
$\begin{array}{c} 0.5666\\ 0.5308\\ 0.4636\\ 0.4399\\ 0.4167\\ 0.5707 \end{array}$	$\begin{array}{c} 0.5671 \\ 0.5309 \\ 0.4642 \\ 0.4397 \\ 0.4171 \\ 0.5710 \end{array}$

ide, 10 drops of glacial acetic acid, and 5 ml. of 1M ammonium carbonate solution (8) added to the water. Only the results in ammonium hydroxide medium were poor. The end point was easily detected, although varied current fluctuations were found in the vicinity of the end point. The results are listed in Table III.

Since the previous results indicated that the acetic acid medium was the only one in which all three halides gave good results, a check was made to see if bromide could be determined in the presence of chloride in this medium. For this purpose 1 gram of sodium chloride and 10 drops of glacial acetic acid were added to the medium. In order to minimize coprecipitation the reagent must be added slowly, and time for equilibration must be allowed near the end point. The current break is easily detected after a few trial observations. Some results are shown in Table IV.

Since the determination of bromide in the presence of chloride was successful, the determination of iodide, bromide, and chloride in a mixture of all three was tried. For this determination about 0.4 gram of each halide was taken, and 10 drops of glacial acetic acid were added to the solution. By experiment it was found that 10 ml. of 2% dextrin solution gave better results than the 5 ml. previously used. Also by experiment it was found that the titration must be done almost dropwise to minimize coprecipitation and to make the end point detection possible. The end point breaks were not abrupt, and care was necessary to detect the minima. Some results are shown in Table V. Ammonium carbonate, ammonium hydroxide, and mixed media which had been recommended (8, 9) gave less satisfactory results than the acetic acid medium. A typical titration plot is shown in Figure 1.

### NATURE OF THE END POINT

As was suggested in the introduction, no current was expected to flow until a slight excess of silver ion was present. However, a current did flow which went through a maximum when about one half of the silver nitrate had been added as shown in Figure 1. It was also observed that silver halide was adsorbed on the surface of the silver cathode during the titration and that the surface cleared in the vicinity of the end point particularly when the precipitated silver halide coagulated. One reasonable explanation for the observed current during the titration is the reduction of adsorbed silver halide at the cathode and electrolytic dissolution of the silver anode to form silver halide:

Table V. Titration of Mixed Halides with 0.1M Silver Nitrate-Acetic Acid Medium

<b>KI</b> , 1	Meq.	KBr,	Meq.	NaCl,	Meq.	Total	, Meq.
Taken	Found	Taken	Found	Taken	Found	Taken	Found
$\begin{array}{c}1.200\\0.741\end{array}$	$\begin{array}{c}1.209\\0.743\end{array}$	$1.628 \\ 1.435$	1.642 1.462	$1.441 \\ 1.788$	$1.479 \\ 1.785$	$\begin{array}{c} 4.269 \\ 3.964 \end{array}$	$\begin{array}{c} 4.330 \\ 3.990 \end{array}$
$0.529 \\ 0.798 \\ 0.44$	0.532	$0.580 \\ 1.084 \\ 1.506$	0.583 1.110	3.287 2.801	3.295 2.768	$4.396 \\ 4.683 \\ 4.112$	4.410 4.678
$0.844 \\ 0.692 \\ 0.920$	$0.842 \\ 0.692 \\ 0.915$	$1.326 \\ 1.302$	$1.321 \\ 1.307$	3.489 3.166	3.479 3.168	$\frac{4.113}{5.507}$ 5.394	5.492 5.390
$0.897 \\ 0.634 \\ 1.213$	$0.900 \\ 0.638 \\ 1.211$	$1.209 \\ 1.176 \\ 2.284$	$1.210 \\ 1.172 \\ 2.275$	$1.404 \\ 2.777 \\ 1.857$	1.404 2.792 1.855	$3.510 \\ 4.587 \\ 5.354$	$3.504 \\ 4.602 \\ 5.341$



Typical Titration of a Halide Mixture with Figure 1. Silver Nitrate in Acetic Acid Medium

Cathode. 
$$AgX + e \rightarrow Ag + X^{-}$$
  
Anode.  $Ag - e + X^{-} \rightarrow AgX$ 

The adsorption of silver halide would limit the current up to the mid-point of the titration, and the concentration of halide ion would limit the current in the last half. The concentration of silver ion would limit the current beyond the equivalence point.

#### CONCLUSION

In a medium containing 10 drops of glacial acetic acid per 100 ml. of water, iodide, bromide, and chloride can be titrated in succession with silver nitrate solution, using silver electrodes with 10 mv. applied, by observing the dead-stop end points. Time for equilibration is necessary.

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# **Polarography of Salicylic Acid**

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THE polarographic reduction of benzoic acid has been reported by von Stackelberg and Stracke (5). They observed that the reduction occurred in two steps with half-wave potentials of -1.94 and -2.46 volts vs. S.C.E. Elving and Hilton (1) did not find a reduction wave for benzoic acid using lithium chloride and hydroxide as supporting electrolytes.

It was suspected that the location of the hydroxyl group adjacent to the carboxyl group would make salicylic acid more easily reducible than benzoic. However, according to a paper published in 1949 (4), salicylic acid was not found to be reducible in alkaline solutions of tetramethylammonium hydroxide. With tetramethylammonium iodide as supporting electrolyte, one reduction was observed at -1.66 to -1.83 volts, depending upon the concentration of salicylic acid. This reduction was attributed by the authors to hydrogen ions rather than to salicylic acid itself.



Effect of electrolyte

This work was undertaken to determine if, under proper conditions, salicylic acid could be reduced at the dropping mercury electrode, and if the reduction wave would be suitable for analytical purposes. Preliminary experiments indicated that one reduction occurred at -2.39 volts vs. S.C.E. in neutral and alkaline salicylate solutions, with certain quaternary ammonium compounds as supporting electrolytes. Only one reduction was observed for benzoic acid and that was a poorly defined wave at about -2.7 volts. Other hydroxybenzoic acids were not found to be reducible below -2.8 volts. No attempt was made to devise a specific analytical method, but studies were made of some of the variables, which would be expected to affect the polarographic reduction of salicylic acid, in order to facilitate the establishment of such a procedure.

#### APPARATUS AND REAGENTS

E. H. Sargent Model III polarograph.

The polarographic *H*-cell was constructed with a 1.0*M* tetrabutylammonium chloride anode separated from the sample by an agar plug, 1.0*M* with tetrabutylammonium chloride (2). Beckman Model M pH meter.

Quaternary ammonium hydroxides, for use as supporting electrolytes, were prepared from the corresponding iodides by reaction with silver oxide. The iodides were synthesized from the tertiary amines and alkyl iodides, and were purified by repeated recrystallization.

Salicylic acid and related compounds were the best grades available commercially. Aqueous 0.02M solutions were prepared of all the acids, with an equivalent amount of tetrabutylammonium hydroxide added in each case to increase the solubility. A 0.02M solution of methyl salicylate was made in ethylene glycol monomethyl ether.

#### PROCEDURE

Portions of the 0.02M solution of the compound being studied were pipetted into 10-ml. volumetric flasks, the supporting electrolyte was added, and the volumes were adjusted to 10 ml. with distilled water. About 2 ml. of the solution was transferred to the cell, and after removal of oxygen by scrubbing with inert gas, polarographic measurements were made at intervals of 0.03 volt, beginning at -1.8 volts. Potentials were calculated from the voltages by correcting for the potential of the anode vs. S.C.E. and for the internal resistance of the cell (about 1000 ohms).

### RESULTS

Salicylic acid was reduced at the dropping mercury electrode, and yielded one well-formed curve, having a half-wave potential of -2.39 volts vs. S.C.E. The type of curve formed, using three different quaternary ammonium hydroxides as supporting electrolytes, is shown in Figure 1. No maxima were observed.

When n, the number of electrons involved in the reduction of salicylic acid with methyltributylammonium hydroxide as supporting electrolyte, was calculated by substitution in the Ilkovič equation, a value of 2.0 was obtained. Data used to make the calculation were  $i_d/c$ , 3.47  $\mu$ a. per millimole per liter; the capillary constants,  $m^{2/3} = 1.1$  and  $t^{1/6} = 0.98$  at -2.6 volts; and the diffusion coefficient of salicylic acid, calculated (3) to be 7.32  $\times 10^{-6}$ .

#### EFFECTS OF VARIABLES

No effect was observed on either the half-wave potential or wave height for the reduction of 0.004M salicylate in 0.1Mmethyltributylammonium hydroxide, adjusted to various values of pH from 6.8 to 12.7 by the addition of acetic acid.

Over the range of concentration of salicylate investigated, 0.001M to 0.012M, the half-wave potential remained constant. When diffusion currents were plotted against the corresponding



Figure 2. Polarography of Other Benzoic Acids

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concentrations, a straight line was formed, the slope of which was 3.47  $\mu a$ . per millimole per liter,

Figure 1 shows the reduction of salicylic acid with three different quaternary ammonium hydroxides as supporting electrolytes. The reduction potential of tetramethylammonium hydroxide is too low to allow a distinguishable curve for salicylic acid, which explains the failure of Korshunov et al. (4) to observe a reduction for the acid. The diffusion current is somewhat greater with methyltributyl- than with tetrabutylammonium hydroxide. This unexplained difference in wave heights with the two supporting electrolytes was also observed in the reduction of methyl salicylate, but not in the reduction of sodium ions. Although satisfactory polarograms were obtained at 0.05 to 0.1M concentrations of supporting electrolyte, they were better at 0.2M.

The half-wave potential remained constant over the temperatures range studied, 7° to 35° C., while the diffusion current increased about 2% per degree.

Presence in the solution of oxygen or sodium ions, both more easily reduced than salicylic acid, was found to have no effect on the net diffusion current of salicylic acid.

# **REDUCTION OF COMPOUNDS RELATED TO SALICYLIC ACID**

Attempts were made to reduce benzoic acid and the two isomers of salicylic acid using tetrabutylammonium hydroxide as supporting electrolyte, because it is reduced at the highest potential of any known ion, and, therefore, allowed the widest span of study possible. The results, as shown in Figure 2, reveal no reduction of m- or p-hydroxybenzoic acid. Benzoic acid gives only a poorly defined wave at about -2.7 volts, the height of which is roughly half that of the curve for an equal concentration of salicylic acid. The two-step reduction of benzoic acid as reported by von Stackelberg and Stracke (5) was not observed in this investigation. It may be that one of the reduction curves found in this earlier work was caused by the reduction of hydrogen ions, because the authors also reported that acetic acid, which is generally recognized as polarographically nonreducible, was reduced at -1.99 volts.

Related compounds, o- and p-aminobenzoic acid, were not found to be reducible. The methyl ester of salicylic acid is more easily reduced than the acid itself, having a half-wave potential of about -2.15 volts in a 30% ethylene glycol monomethyl ether and 70% water solution which is 0.2M with tetrabutylammonium hydroxide. The diffusion current for the reduction of methyl salicylate is about 50% greater than that obtained with the same concentration of salicylic acid under the same conditions.

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# Quantitative Determination of Nitric Oxide and Nitrous Oxide by **Infrared Absorption**

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HEMICAL methods for the analysis of nitric oxide and A nitrous oxide are time-consuming (6-8). Mass spectrometric methods have been applied to this problem, so that by various schemes one can analyze gas mixtures containing any or all of the following compounds: carbon monoxide, carbon dioxide, water, nitrogen, nitrous oxide, nitrogen dioxide, and either nitric oxide or oxygen (2-4). This note concerns an infrared method for the analysis of mixtures of nitric oxide and nitrous oxide in samples which may also contain carbon monoxide, nitrogen, and hydrogen. If oxygen is present, only nitrous oxide can be determined, since nitric oxide cannot exist in the presence of oxygen.

#### EXPERIMENTAL

A Perkin-Elmer Model 12A spectrometer equipped with rock A Perkin-Elmer Model 12A spectrometer equipped with rock salt optics was used to obtain all analytical data. The Perkin-Elmer Model 21 spectrophotometer was employed to obtain all qualitative spectra. The gas cell used had a path length of approximately 9.5 cm. All samples were passed through an Ascarite scrubbing train before admission to the gas handling system; this removed any adventitious carbon dioxide and nitrogen dioxide. The absorbance of the evacuated sample cell was used to correct all absorbance data was used to correct all absorbance data.

#### MATERIALS

The nitrous oxide was obtained from the Matheson Co. Its spectrum showed no definitely spurious absorption bands when compared to published data (5). However, nitric oxide is a weak absorber, and it is possible to miss its presence as an

impurity in small quantities. This was checked by obtaining the spectrum of a sample of nitrous oxide at approximately 40-cm. pressure, then adding oxygen to the cell containing this nitrous oxide until a total pressure of approximately 84 cm. was attained. The resultant spectrum had no new bands. If nitric oxide were present, the addition of oxygen would result in new bands owing to the strong absorber nitrogen dioxide formed in the cell. It was assumed that nonabsorbing gases such as nitrogen were not present (mass spectrometer results indicate that the

nitrogen content may have been approximately 1%). The nitric oxide also was from the Matheson Co. and was found to contain nitrous oxide and nitrogen dioxide as impurities. The latter gas was eliminated by scrubbing with Ascarite. The Ascarite-scrubbed nitric oxide still contained nitrous oxide as an impurity. The nitrous oxide content was determined by spectral analysis using the nitrous oxide calibration curve and was found to be 4.4 mole %. A correction for this amount of impurity was applied to all calibration curves and synthetic samples. Dry tank nitrogen was used as the diluent in setting up the working The analytical points used in the analysis are indicated curves. in Figure 1, which is a spectrum of the Ascarite-scrubbed nitric oxide containing the nitrogen dioxide impurity.

#### CALIBRATION AND METHOD

Both nitric oxide and nitrous oxide are light gases and subject to strong pressure broadening effects and do not obey Beer's law; consequently, working curves were constructed using procedures previously described (1). The points on the calibration curve for nitric oxide were obtained by blending known amounts of nitric oxide (Ascarite-scrubbed) with enough nitrogen to attain a cell pressure of approximately 80 cm. of mercury. The same procedure was followed with nitrous oxide (Ascarite-scrubbed). In

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setting up the nitric oxide calibration curve the partial pressures were corrected to take care of 4.4% nitrous oxide, which was the impurity. An evacuated cell correction was applied to all the data and in Figures 2 and 3 the calibration curves are reproduced.

The frequency of the analytical point used for nitric oxide is at the transmission minimum at approximately 1908 cm.<sup>-1</sup> of the Rbranch of the 1876 cm.<sup>-1</sup> fundamental. For nitrous oxide the transmittance minimum at approximately 1275 cm.<sup>-1</sup> of the P branch of the 1285 cm.<sup>-1</sup> fundamental absorption band is used as the analytical point.



Figure 1. Infrared Spectrum of Tank Nitric Oxide Containing Nitrous Oxide as an Impurity

All samples are pressurized with nitrogen to approximately 80 cm. of mercury cell pressure and then analyzed. As can be seen from Figures 2 and 3, the absorptivities of nitric oxide and nitrous oxide are very different, and hence one must generally analyze at a high partial pressure of sample to get nitric oxide data, then cut pressure in order to obtain nitrous oxide data.



Total cell pressure is approximately 80 cm. of mercury in a 9.5-cm. cell

The absorbance of nitrous oxide at the nitric oxide point was checked and found to be zero. There should be no nitric oxide absorption at the nitrous oxide point. Because carbon monoxide might be present in some samples, its absorbance was evaluated at the nitric oxide point and also found to be zero.

# PROCEDURE AND CALCULATIONS

It is advisable to study samples at high initial partial pressures and to reduce the sample partial pressures as necessary. This reduction is accomplished in each case by diluting with nitrogen, keeping a cell pressure of 80 cm. of mercury. When an acceptable absorbance is obtained for one of the gases, the appropriate calibration curve is used to get the partial pressure of that component. This is repeated for the second gas at an appropriate sample partial pressure. The partial pressures of the components

Table I. Analyses of Synthetic Nitric Oxide-Nitrous Oxide Gas Mixtures

			Partial	Pressures	Comp	% osition
Synthetic		NO	N <sub>2</sub> O	$NO + N_2O$	NO	N <sub>2</sub> O
1	Theoret. Obsd. Diff.	$16.5 \\ 16.8 \\ 0.3$	$\begin{array}{c} 6.3\\ 6.2\\ 0.1 \end{array}$	$\substack{22.8\\23.0\\0.2}$	$72.4 \\ 73.0 \\ 0.6$	$27.6 \\ 27.0 \\ 0.6$
2	Theoret. Obsd. Diff.	$\begin{array}{c} 20.7\\21.0\\0.3\end{array}$	5.4 5.7 0.3	$26.1 \\ 26.7 \\ 0.6$	79.3 78.7 0.6	$20.7 \\ 21.3 \\ 0.6$
3	Theoret. Obs <b>d</b> . Diff.	$\begin{array}{c}2.6\\2.5\\0.1\end{array}$	$\begin{array}{c} 20.1 \\ 19.6 \\ 0.5 \end{array}$	22.7 22.1 0.6	$\begin{array}{c} 11.4\\ 11.3\\ 0.1 \end{array}$	88.6 88.7 0.1
4	Theoret. Obsd. Diff.	$4.2 \\ 4.5 \\ 0.3$	$\begin{array}{c} 5.4\\ 5.5\\ 0.1\end{array}$	$9.6 \\ 10.0 \\ 0.4$	$\substack{43.6\\45.0\\1.4}$	$56.4 \\ 55.0 \\ 1.4$
5	Theoret. Obsd. Diff.	$\substack{16.9\\16.4\\0.5}$	3.3 3.6 0.3	$\begin{array}{c} 20.2\\ 20.0\\ 0.2 \end{array}$	$83.3 \\ 82.0 \\ 1.3$	16.7 18.0 1.3
6	Theoret. Obsd. Diff.	$\begin{smallmatrix}16.2\\16.1\\0.1\end{smallmatrix}$	$3.3 \\ 3.7 \\ 0.4$	$19.5 \\ 19.8 \\ 0.3$	$82.9 \\ 81.3 \\ 1.6$	$17.1 \\ 18.7 \\ 1.6$
7ª	Theoret. Obsd. Diff.	$1.8 \\ 2.0 \\ 0.2$	$3.8 \\ 3.7 \\ 0.1$	5.6 5.7 0.1	$8.6 \\ 9.9 \\ 1.3$	$18.6 \\ 18.3 \\ 0.3$
Av. erro	or	0.3	0.3	0.4	1.0	0.9

On the basis of total pressure (80 cm.) the estimated error (95% confidence) for NO is  $\pm0.8\%$  and the estimated error (95% confidence) for N=O is  $\pm1.0\%$ .

<sup>a</sup> Synthetic 7 also contained 13.1% CO and 59.7% N<sub>2</sub>.



Total cell pressure is approximately 80 cm. of mercury in a 9.5-cm. cell

are recalculated to a common total sample pressure, and the per cent composition of each component is thereby determined. In Table I are given the results of a set of synthetic samples prepared to assess the accuracy of this method.

#### ACKNOWLEDGMENT

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# **Rapid Determination of Nitrates and Nitrites**

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THE purpose of this paper is to present a rapid and sensitive method for the quantitative determination of either nitrite nitrogen or nitrogen in a mixture of nitrate and nitrite ions, as is often desired in plant and soil analysis.

The procedure utilizes a reaction which was originally suggested by Griess (3) for the estimation of nitrous acid and was subsequently modified and improved by Ilosvay (5), Holbourn (4), Morpeth (7), Rider and Mellon (8), and others. The reaction, still widely used for the determination of nitrites in waters and many other diverse materials, consists of diazotization of sulfanilic acid by the nitrite ion and subsequent coupling with 1naphthylamine to form a red dye. If the nitrate ion is to be determined, it is first reduced to nitrite by powdered zine and manganese(II) sulfate, as proposed by Bray (1). Bray also combined the reagents into a single dry powder to simplify the procedure and to increase greatly the stability of the reagent. This powder reagent has been used extensively as a qualitative test for nitrate in plant tissue.

#### REAGENTS

Standard Nitrate Solution. Dissolve 0.607 gram of sodium nitrate in 1 liter of water for a stock solution of 100 p.p.m. of nitrogen.

Acetic Acid Solution. Dilute 20 ml. of glacial acetic acid to 100 ml.

**Powder Mixture** [As prepared by Bray (1)]. One-hundred grams of barium sulfate (dried at 110° C.), 10 grams of manganese-(II) sulfate monohydrate, 2 grams of finely powdered zinc, 75 grams of powdered citric acid, 4 grams of sulfanilic acid, and 2 grams of 1-naphthylamine.

Grind any coarse materials to a fine powder. Mix the manganese(II) sulfate, powdered zinc, sulfanilic acid, and 1-naphthylamine separately with portions of barium sulfate. Then mix thoroughly all ingredients including the barium sulfate and eitric acid. Use extreme care to have room, table top, and equipment free of nitrate and nitrite. Store the powder in a blackened bottle, since light affects the 1-naphthylamine. The reagent is stable for many months in a bottle painted on the outside with black paint.

If the determination of nitrite in the presence of nitrate is desired, omit the powdered zine and manganese(II) sulfate.

The citric acid, besides contributing to the acidity of the solution, complexes iron which interferes when present in large quantities. The barium sulfate acts as a carrier, zinc and manganese-(II) sulfate reduce the nitrate, and the sulfanilic acid and 1naphthylamine are used directly in forming the dye. For best analytical results the powder should be made up several days before it is to be used.

# EXPERIMENTAL

Effect of Order of Addition of Reagents. Several workers have advised that the sulfanilic acid be added several minutes before the 1-naphthylamine, ostensibly because any unreacted nitrite still present is partially destroyed by reaction with the amino group when the coupling agent is added. However, since a proportionate amount of nitrite is destroyed in the standard as well as in the unknown, simultaneous addition results only in a slight loss of sensitivity.

Effect of pH. As shown by Rider and Mellon (8), the reaction is fairly sensitive to pH changes. The pH curve given by those workers was found to hold generally for the conditions of this determination—that is, maximum color development is obtained between the approximate pH values of 1.7 and 3.0. Since the dye is more stable and the over-all reaction (with simultaneous addition of reagents) is faster at the lower pH values, a pH level of about 2.0 is recommended. For this purpose a 20% acetic acid solution gives approximately the correct pH and is a medium from which the powder is easily centrifuged. Effect of Temperature. No effect over the range of normal laboratory temperatures was found. Excessively high or low temperatures are not recommended (6).

Effect of Light. The powder should be kept in a black bottle until ready for use. During the color development, ordinary artificial laboratory lighting appears to have no effect, but direct sunlight should be avoided.

Stability of the Powder Reagent. One major advantage of the powder reagent is its great comparative stability. If kept in a blackened, air-tight bottle, the dry, properly compounded powder is good for at least 2 to 3 years.

Time of Contact of Powder Reagent with Solution. The zinc must be separated from the solution after color development because the reducing action of the zinc will cause the color to fade. Equal color development was found for shaking periods ranging from 20 to 90 seconds; therefore, a constant 1-minute shaking time (before beginning of centrifugation) is recommended. Filter paper may be used instead of the centrifuge to remove the powder. However, filter paper partially adsorbs the dye, decreasing greatly the sensitivity and accuracy of the test.

Effect of Amount of Powder Reagent Added. The powder should be in excess, but too much excess is also harmful. Constant color development was obtained when the amount of powder used was between 0.1 and 0.8 gram; therefore, careful measurement of the powder in a 0.4-gram scoop is recommended.

Interfering Ions. Several ions interfere with the reaction. An excellent table and a discussion of these interferences are given by Rider and Mellon (8). Iron is complexed by the citric acid and copper does not interfere appreciably up to  $10\gamma$ . This would cover the range usually encountered in soil and plant material extracts. It has been found that aluminum does not interfere up to at least 500 times the concentration of the nitrate.

Table I.	<b>Recovery of Added</b>	Nitrate in Soi	ls and Plants
Extract	Added, P.P.M.	Found, P.P.M.	Recovered, %
Soil 1	$\begin{smallmatrix}&0\\10\\20\end{smallmatrix}$	$\begin{array}{c} 16.2\\ 25.6\\ 37.3\end{array}$	94.0 105.5
Soil 2	0 10 20	$14.8 \\ 24.1 \\ 34.8$	93.0 100.0
Alfalfa	0 6	$\begin{array}{c} 3.4\\ 9.3 \end{array}$	98.3
Oat	$\begin{array}{c} 0\\ 2\\ 4\end{array}$	2.0 3.8 5.7	90.0 92.5

#### RESULTS

This method is easily sensitive to 0.05 p.p.m. of nitrogen as nitrate in solution, and the standard curve is conveniently plotted between 0.2 and 1.0 p.p.m. in steps of 0.2. More concentrated solutions should be diluted to this range. Duplicates can be reproduced in this range within  $\pm 5\%$ .

Varying quantities of standard nitrate solution were added to soil and plant material extracts which were then analyzed by the recommended procedure. The results are given in Table I.

### RECOMMENDED PROCEDURE

Procure a representative sample of the material to be tested, and obtain a colorless extract of it. An extraction with 0.1N hydrochloric acid is recommended for soils or ground plant material. Strongly colored plant-material extracts may be decolorized by a

method such as that described by Frear (2). Solutions which are strongly acid or alkaline should be neutralized, and any interfering ions present should be removed or complexed.

To 1 ml. of the test solution in a 12-ml. centrifuge tube, add 9 ml. of the acetic acid solution. With a small measuring scoop add 0.3 to 0.5 gram of the powder reagent, stopper, shake for 50 to 60 seconds, and centrifuge until the supernatant liquid is clear (about 4 or 5 minutes at 4000 r.p.m.). Decant the white film on top and pour the clear red solution into an absorption cell. Transmittancy may be read conveniently in a filter photometer with a green filter or in a spectrophotometer at 520 m $\mu$ .

Convert transmittancy readings to amounts of nitrogen with a standard curve established with known solutions. The standard curve should cover the range from 0.2 to 1.0 p.p.m. in solution in the absorption cell, or 2 to 10 p.p.m. in the sample solution.

#### PRECAUTIONS

All glassware, table tops, etc., must be kept scrupulously clean. Reagents should be nitrite- and nitrate-free. X-ray grade barium sulfate is satisfactory if thoroughly dried. Rubber stoppers used to close the tubes during shaking must be washed carefully. As reported by Warington (9), solutions and reagents unduly exposed to laboratory air may pick up small amounts of nitrite. Distilled water must be checked frequently for contamination.

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# **Device for Measuring Changes of Optical Transmittance with Temperature**

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HE relative purity of a compound is invariably associated with a physical constant. A melting point or melting range is customarily used for solids and this value is most often determined by the capillary tube technique. The melting point obtained by this method is not thermodynamically defined, for it is neither the liquid-solid equilibrium temperature under constant atmospheric pressure nor the triple point. However, it is extremely convenient and universally employed. Reproducible results are obtained and no difficulties are encountered if the sample to be tested is relatively pure, of definite composition, and actually melts below 230° C.

Frequently samples are encountered whose melting point cannot be determined reproducibly by the capillary tube method. These solids can be classified in one or more of the following categories: hydrates; high melting-i.e., above 250° C.; compounds which decompose before or at their melting point; amorphous waxes, asphalts, or plastics; and vegetable oils or fats. In each of these categories special techniques must be used. In testing these chemicals, operators may get results that differ as much as several degrees from each other. Morton (8) lists the factors that affect melting point values. Included among these are rate of heating, thickness of the capillary wall, size of the sample, and stem corrections. If capillary tube technique is employed for these special samples, different manipulative techniques are necessary. For compounds which decompose or melt above 250° C. the capillary tube must be either submerged when the temperature of the bath is just a few degrees below the melting value, or as an alternative, the capillary must be heated at a rate of 10° to 20° C. per minute, constantly, rather than slowing to the usually prescribed 1° to 2° C. per minute near the melting point. A ball and ring test (1) is often employed to test asphalts. Fats present special problems; an allotropic form may result after the sample is melted and drawn up into the capillary tube (5); hence the filled tube must be left in the icebox over night.

Variations to avoid the capillary tube method were employed by Dennis and Shelton (2), who introduced the copper bar, and by Johns (4), who used an aluminum block upon which the sample, placed between two watch glasses, was heated.

Attempts have been made to avoid the human equation by

making this determination automatic. Perhaps the first of these was made by Dubosc (3) and a later one by Wick and Barchfeld (11). At this time true automatic equipment was not available. Kardos ( $\theta$ ) used a Kofler ( $\gamma$ ) apparatus and measured the melting point by attaching a recorder to a photoelectric cell and noting the change in current in milliamperes. More recently Müller and Zenchelsky (9) made a fully automatic instrument based upon the sudden increase of reflected light from the surface of the melted sample to a photoelectric cell. This instrument has been able to attain a precision of  $\pm 0.3^{\circ}$  C.

The meltometer (10) here described reduces to fixed and reproducible values the variables contributing to the melting point



Figure 1. Meltometer



Figure 2. Schematic Diagram of Meltometer



Figure 3. Diagram of Microfurnace

errors. These variables are sample size and rate of rise of temperature. The meltometer is fully automatic, portable, and extremely versatile. It can be used to measure melting points, decomposition points, softening points, melting ranges, and arbitrary melting points as represented by amorphous waxes, asphalts, and plastics. Since samples can be run over again, it is possible to determine mixed melting points and allotropic forms. Studies of eutectic mixtures can be made, as well as molecular weight determinations.

With the recorder the following investigations are possible: optical transmittance against time and temperatures, sublimation rate with temperatures, temperature-spectral absorption, and temperature-birefringence.

The meltometer utilizes the increase or decrease in the transmittance of unfiltered, polarized, or monochromatic light through a heated sample to signal that a change of phase has occurred in the sample. This intelligence may be recorded with sample temperature to permit continuous study of the phase change with temperature or it may be used to lock the pyrometer indicator to permit a fixed value of temperature to be indicated for a particular change in optical transmittance. In the latter operation the instrument is completely automatic.

### DESCRIPTION OF INSTRUMENT

The meltometer is self-contained, consisting of a microfurnace attached to a cabinet (Figures 1 and 2).

Microfurnace. The microfurnace, which can be tilted, houses the stabilized light source, the melting point block, filters, shutter, and phototube (Figure 3). The cover can be removed readily for the purpose of placing the sample (sandwiched between two thin microscope cover slips) into the gold-plated brass block. The heater coil is embedded within the block, and a Chromel-Constantan thermocouple is located on the inner surface of the block adjacent to and in thermal symmetry with the sample. An aperture in the block permits a beam from the light source to pass through the sample and to the phototube. The small volume of the melting point block, 0.166 cubic inch, permits rapid approach to equilibrium temperature at heater currents selected by the heater control knob. A rate of rise of temperature of 0.5° C. per minute at any temperature within the range of the instrument is reached within 15 minutes from the time the heater is turned on.

Cabinet. The cabinet encloses the meters, power supply, and amplifier (Figure 4). The optical transmittance of the sample is indicated on the meter relay and the temperature of the sample is indicated on the locking-type pyrometer. The automatic action of the meltometer is initiated by a pair of relay contacts in the optical transmittance indicating meter relay. The position of the contacts may be manually adjusted by means of knurled knobs projecting through the meter relay window. One is an upper limit and the other a lower limit optical transmittance contact. When the pointer of the meter relay touches either contact, three actions occur at once: (1) The pyrometer indicator is locked by electrostatic action at the temperature indicated at that instant and remains at that indication until released manually; thus, no overshoot is noted; (2) the heater is disconnected from the power supply and cools rapidly, readying the melting point block for another determination; and (3) an indicator lamp signals the operator that the determination is completed.

# **OPERATION OF INSTRUMENT**

The meter relay is adjusted at the start to indicate zero transmittance (-50) and 100% transmittance (+50) by means of the zero adjust knob and the adjustable light shutter, respectively. The sample is introduced between two clean cover glasses by

EATER\_CONTRO HEATER HEATER CONTROL PILOT-HEATER HEATER SWIT 6 ļę 5 🖬 5.6 226 METER RELAY \*\*\* ZERO \*\*\*\* AZERO MAG 1946 LINE CORD Figure 4. Wiring Diagram of Meltometer

Table 1. Comparison of Meltometer values with Known Melting Points					
Compound	Ambient % Trans.	Rate of Temp. Rise, ° C./Min.	% Change in Transmittance of Amb. Trans.	Known Melting Pt., °C.	Meltometer Melting Pt., °C.
Naphthalene Naphthalene Benzoic acid Benzoic acid <i>p</i> -Nitrobenzoic acid <i>p</i> -Nitrobenzoic acid	15 7 7 10	2 3 5 1 2	100 220 110 110 110 220	80.2280.22122.12122.12242.4242.4242.4	81 80 123 123 241 242
Succinic acid	••	$\tilde{2}$	175	189-190	189

Table I.	Comparison	of Meltometer	Values wit	h Known:	Melting	Points
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means of a dentist's amalgam carrier, which was found most suitable for transferring fixed small amounts of powdered materials, and placed in the block. The cover is closed and the optical transmittance of the sample is now indicated on the meter relay. This value is noted so that in repeated determinations the sample size (transmittance) may be duplicated. The sensitivity is now adjusted by opening (or closing) the adjustable shutter until the meter relay indicates zero center. The manual setting of the meter relay contacts may now easily be determined.

If the behavior of the sample is known, the heater control may be set for a suitable rate of rise and the meter relay contact may be set to close at a specific per cent change in transmittance from ambient transmittance. Unknowns may be run at a high rate of rise with contacts placed out of range of scale to determine a suitable rate of rise and a suitable setting for the change-intransmittance contacts. One electrometer tube is used to amplify the phototube current.

A melting range may be found by first setting the meter relay contact at about +20. This permits the instrument to shut off at the first indication of change in light transmittance. The temperature reading obtained will correspond to the lower value of the range. Without disturbing the sample, the meter relay contact can now be set at +50 and the heater turned on again. The second temperature reading obtained will be the upper value of the range.

The reproducibility and accuracy of the instrument were determined by testing samples of known and unknown melting points.

Crystalline Compounds. Table I gives a comparison of hand book values and meltometer values over a range of 160° C. The time-temperature-optical transmittance behavior of naphthalene, a sharp-melting compound, is shown in Figure 5. Each value of Table II was obtained by a different operator who had no instructions other than those supplied with the instrument.

Nonmelting Crystalline Solids. As the sample decomposed, the percentage transmittance decreased and the indicating needle approached 0% transmittance (-50). Meltometer values for *dl*-methionine were darkening at 276° and decomposing at 287° C. (literature values are 278° and 283° C., respectively).

Amorphous Solids. Asphalt softening point value could be ob-

	Table II.	Automatic	Melting Point D	eterminations	
Compound	Ambient % Trans.	Rate of Temp. Rise, °C./Min.	% Change in Transmittance of Amb. Trans.	Known Melting Pt., °C.	Meltometer Melting Pt., ° C
Acetanilide	17	3.5	220	114	114.5
	18	5	220	114.5	114.5
	25	7	220		115
	28	5	220	•••	114.5
$\beta$ -Aminocaproic acid	13	4	220	206	207
	19	8	220		207
Upjohn I	8	0.5	220	1984	198
	8	0.5	$\bar{2}\bar{3}\bar{0}$		199
	8	0.5	190		197
	8	0.5	220		199
	Ř	0 5	220		197
	š	0.5	220		107
	ŏ	0.5	220		100
	10	0.5	220	• • •	109
	10	0.5	220		198
	12	0.5	230		195
	12	0.5	230		196
	12	0.5	230		197
	12	0.5	230		199
	13	0.5	210		197
	23	0.5	220		200
	25	0.5	230		200
Upjohn II	8	0.5	220	190.5ª	186
-	9	0.5	220		187
	10	0.5	220		188
	11	0 5	220		180
	12	0.5	220		180
	19	0.5	220		100
	12	0.5	220	• • •	100
	10	0.5	220		190
	19	0.5	220	• • •	194
Upjohn III	9	0.5	220	195.54	194
	13	0.5	220		196
	16	0.5	220		196
	25	0.5	220		201
	29	0.5	220		202
Upiohn V	8	0.5	230	1954	194
	13	Ŏ.5	220		196
Uniohn VI	7	0.5	220	175 54	179
Opjoint VI	<u>.</u>	0.5	220	110.00	170
	11	0.0	220		173
	11	0.5	220		173
	12	0.5	220	• • •	174
	14	0.5	220		175
	19	0.5	220		175



Figure 5. Optical Transmittance-Temperature-Time Record of Compound with Sharp Melting Point Melting and solidification points are clearly shown

tained by placing the sample between cover slips just above the aperture. The head was tilted and at the softening point the sample viscosity suddenly decreased, filled the space, and thus shut off the light beam. Values were reproducible to  $\pm 1^{\circ}$  C. No comparisons with ball and ring values were available.

The softening point of fats could also be determined within 1° C. with the apparatus, but no consistent results could be obtained by the capillary method, as the majority of fats would supercool and would not set to a crystalline solid, once melted.

Plastics likewise could not be compared, but Figure 6 illustrates the behavior of a resin which discolored near its melting point. The curve clearly shows the phenomenon which might pass unobserved with ordinary methods.



Softening point is clearly shown

The periphery of samples melted first, indicating a negative temperature gradient from the heater coil to the central aperture. To determine the symmetry of this gradient, thermocouples were placed on opposite sides of the aperture (test positions A and B in Figure 7) at the distance which gave closest agreement between known melting points and melting points measured with the instrument. The thermocouples were made of No. 30 copper and constantan wires and were connected to a Leeds and Northrup Type K2 potentiometer through a selector switch. Readings were taken as the temperature of the block was raised and lowered between ambient temperature and 290° C. at a rate of change of 2° C. per minute. The maximum temperature differential was 1° at 290° C. with no perceptible differential below 206° C.

The reproducibility and accuracy of the instrument were found to be  $\pm 1\%$  of scale. The original meltometer had a range of 300° C., but it was soon apparent that other ranges and greater accuracies were required for some applications. Accordingly, the Illinois Testing Laboratories supplied for further tests pyrometer ranges as follows: 0-100°, 0-150°, 0-300°, 0-500°, 105-210°, and 205-310° C. Accuracies of 0.05° C. with ranges of 15° C. may be obtained by the use of recorders, as supplied by the Brown Instrument Co., division of Minneapolis Honeywell Regulator Co.



Figure 7. Diagram of Melting Point Block

Temperature-optical transmittance studies conveniently reveal the identifying phase changes of most compounds, and, in addition, provide information most useful to the physicist and the chemist.

#### ACKNOWLEDGMENT

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# **Rapid Estimation of Ethylenes with Mercuric Acetate**

# **By Nonaqueous Titration**

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$$H_{g}(CH_{3},CO,O)_{2} + CH_{3}OH \rightleftharpoons H_{g} OCH_{3} + CH_{3}CO,OH OCH_{3} + CH_{3}CO,OH OCH_{3}$$
(1)

The direct formation of mercury addition products as above has been utilized by Marquardt and Luce (4, 5) and also by Martin (6) for determination of a number of unsaturated compounds containing terminal double bonds. Marquardt and Luce have described two separate procedures for estimation of styrene and several styrene derivatives. Martin has used a somewhat different procedure and determined styrene and some of its derivatives as well as several other monomers. Hornstein (3) has utilized Martin's method for determination of rotenone.

The present paper describes a modification of the above procedures, which offers a simpler and quicker method for determination of some ethylene compounds. The sample is allowed to react with a known excess of mercuric acetate and then the unreacted acetate is directly estimated by nonaqueous titration.

The author has found (2) that mercuric acetate can be very accurately estimated by Palit's method (7) of glycolic titration using propylene glycol-chloroform (1 to 1) as the titration medium and thymol blue as indicator. A standard solution of hydrochloric acid in the same solvent medium as above is used for titration, and the end point (yellow to pink) is extremely sharp. Mercuric chloride may be precipitated out in the course of the titration, but it does not interfere with the detection of the correct end point. Perchloric acid cannot be used for titration of mercuric acetate, and hydrochloric acid must be used.

When titrating with hydrochloric acid, not only does the excess unreacted mercuric acetate react with the acid, but the mercury addition product also takes up one equivalent of acid as follows:

Hence, the difference between the milliequivalents of mercuric acetate taken and the milliequivalents of acid used in titration directly gives the amount of unsaturation in millimoles.

### REAGENTS

Mercuric acetate, approximately 0.25N solution in methanol. About 20 grams of mercuric acetate are dissolved in 500 ml. of methanol with the addition of 1 ml. of glacial acetic acid, and filtered. The solution is standardized by titration in propylene glycol-chloroform (1 to 1) solvent medium with 0.1N glycolic hydrochloric acid, using thymol blue as indicator.

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Hydrochloric acid, 0.1N solution in propylene glycol-chloroform (1 to 1), prepared by adding 8 to 9 ml. of concentrated hydrochloric acid in 1 liter of the mixed solvent. The acid is standardized as follows. About 0.2 gram of mercuric oxide (analytical reagent) is accurately weighed out and dissolved in 5 ml. of glacial acetic acid by gentle heating and then evaporated almost to dryness. The residue is taken up with 25 ml. of propylene glycol-chloroform and titrated with hydrochloric acid, using thymol blue as indicator (1 ml. of 0.1N hydrochloric acid = 0.01083 gram of mercuric oxide).

Mixed solvent, prepared by mixing propylene glycol and chloroform in equal volumes. The solvents should be neutral to thymol blue.

Thymol blue, 0.2% solution in ethyl alcohol.

#### PROCEDURE

About 2 millimoles of the unsaturated compound are treated with 20. to 25 ml, of the mercuric acetate solution in a glassstoppered bottle or conical flask and allowed to stand at room temperature (about 30° C.) for 10 to 30 minutes. The reaction mixture is then diluted with about 25 ml. of propylene glycolchloroform (1 to 1) and the sample is titrated with glycolic hydrochloric acid, using thymol blue as indicator. At the end point, the indicator shows a sharp color change from yellow to pink.

To test the accuracy of the present method, the samples of the unsaturated compounds were simultaneously estimated by the alkalimetric method of Luce and Marquardt (5); the analytical data are represented in Table I.

	Table I. A	nalytical Dat	a
Compound	Time of Reaction, Minutes	% Found by Marquardt- Luce Method	% Found by Present Method
Styrene Cyclohexene Allyl alcohol Allyl acetate Vinyl acetate	$5-10 \\ 10-15 \\ 30 \\ 30 \\ 30 \\ 30 \\ 30 \\ 30 \\ 30 \\ 3$	99.4,99.7 96.7,96.3 97.7 88.1,76.3 33.2,28.4	99.2,99.6 96.6,96.9 98.2,98.2 97.4,96.8 96.9,97.5,97.0

#### CALCULATION

 $\frac{(a-b) \times N}{1000} \times M$  = amount of unsaturated compound in

grams, where a is the acid titer milliliters for blank (mercuric acetate solution); b, the acid titer for the sample; N, the normality of the acid; and M, the molecular weight of the ethylenic compound.

#### DISCUSSION

The method seems to be particularly useful for analysis of some unsaturated esters like vinyl acetate and allyl acetate, for which the methods of Marquardt and Luce (4, 5) and of Martin (6) are not suitable, since under the conditions used by them, the esters may undergo hydrolysis and lead to erroneous results. With vinyl acetate and vinyl benzoate Martin (6) obtained about twice the theoretical value. This arises from the fact that his method measures not only the acetic acid liberated in the course of the mercury addition reaction, but also an equivalent amount of acid liberated as a result of the hydrolysis of the vinyl esters. The alkalimetric method of Marquardt and Luce gives low and erratic results with vinyl acetate and allyl acetate for the same reason. Methyl acrylate and methyl methacrylate cannot be estimated with mercuric acetate by any of these methods, as they do not quantitatively react with mercuric acetate under the conditions employed.

Mercury addition products are, in general, unstable toward halogen acids. In some cases, they are so very susceptible to acidity that titration with hydrochloric acid is not possible. This has been observed with  $\alpha$ -methylstyrene, cinnamic acid, and diisobutylene, where the addition products undergo quick decomposition in the course of titration with hydrochloric acid. In other cases that have been studied, the decomposition is very slow, and at the end point, the acid color of the indicator persists for several minutes, so that no difficulty is involved in detecting the correct end point.

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# Determination of Cyclohexane-Type Naphthenes by Analytical Dehydrogenation

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THE increase in the use of catalytic reforming processes to improve motor gasoline and to produce benzene, toluene, and xylenes has stimulated interest in analytical procedures for determining naphthenes (cycloparaffins) in the gasoline boiling range, since naphthenes are converted to aromatics in their forming process. Most naphthenes in this range are cyclopentane or cyclohexane derivatives.

A method for determining cyclohexane-type naphthenes in petroleum naphthas has been described by Rampton (4). This method consists of dearomatizing the petroleum fraction; selectively dehydrogenating the reactable cyclohexanes in the saturates over a platinum catalyst to the corresponding aromatics (gem-substituted cyclohexanes, such as 1,1-dimethylcyclohexane, are not dehydrogenated); determining the aromatics produced; and calculating the amount of reactable cyclohexanes present.



Figure 1. Glass Dehydrogenation Apparatus

The successful application of this method and some modifications of the apparatus and procedure are reported here.

#### APPARATUS MODIFICATIONS

The modified apparatus, employed to obtain the data herein reported, is shown in Figure 1. The sample reservoir is graduated to aid in measuring the feed rate, and is water-jacketed to prevent the vapor pressure of low boiling samples from interfering with the feed rate. The reactor tube is inserted into a solid of aluminum alloy, which is heated by electrical windings. The reactor tube is inserted into a solid block This block is insulated and uniform temperature is maintained by a pyrometer controller actuated from a thermocouple in the alloy block. Several reactor tubes can be installed block. Several reactor tubes can be installed in the same block, thus increasing the efficiency of operation. The feed inlet tube has been redesigned to assure a continuous flow of sample to the preheater, which is a short section of 4-mm. borosilicate glass baseds incorporated within the reactor tube. These changes probeads incorporated within the reactor tube. These changes pro-vide a more constant vapor flow through the catalyst bed than is provided with dropwise feed. No hydrogen is passed through the unit during operation, as additional hydrogen may tend to reverse the reaction and limit the rate of throughput. The modifications permit higher feed rates and result in essentially complete dehydrogenation in one pass.

#### CATALYST TREATMENT

The catalyst was prepared in accordance with the detailed instructions given by Rampton (4). In this work Darco Corp. activated carbon, 10- to 20-mesh, was used as the catalyst support. When a new catalyst is flushed with hydrogen, the flow must be continued until no more hydrochloric acid is given off as evidenced by a test for chlorides in the exit gases. This step is essential if a highly active catalyst is to be obtained. It has been found in another laboratory (3) that considerable time is saved by hydrogenating the catalyst at 425° C., then reverting to 325° C. for operation. These workers have also been successful in regenerating at this higher temperature some catalysts which had been contaminated by sulfur compounds. The activated catalyst must be maintained in a hydrogen atmosphere, especially at elevated temperatures.

### **PROCEDURE CHANGES**

Samples may be dearomatized either by large scale acid absorption similar to the ASTM method (1) or by adsorption on silica gel. The authors have found it convenient to dearomatize by acid absorption. If this method is used, the raffinates must be percolated through a small amount of silica gel—e.g., 1 part of gel for 2 to 5 parts of sample—to remove any sulfur contaminants before dehydrogenation. Using samples prepared in this manner and maintaining the catalyst in a hydrogen atmosphere, it has been possible to maintain two tubes of active catalyst for 18 months. During this time 50 volumes of hydrocarbons per volume of catalyst have been charged, with no decrease in catalyst activity. Catalyst activity is checked daily, by dehydrogenating pure cyclohexane which is relatively difficult to dehydrogenate. A 97% or higher conversion to benzene is an acceptable activity test.

#### RATE STUDIES

Rate studies were made to determine the effect of these apparatus modifications and procedure changes on the conversion of cyclohexanes to aromatics. Figure 2 shows that for feed rates up to 20 ml. per hour (equivalent to 1 volume of sample to 3 volumes of catalyst per hour), the amount of dehydrogenation is unaffected by rate and is essentially quantitative. Above 20 ml. per hour the conversion tends to decline; however, it is still at least 97% up to 40 ml. per hour.

In addition to reducing operation time, the increased feed rate reduced the amount of ring opening in the methylcyclopentane molecule. While the opening of cyclopentane rings does not affect the analysis of cyclohexanes, which is the primary object of this procedure, it is undesirable if the saturates remaining after dehydrogenation are to be analyzed further.

Infrared analysis of the saturated hydrocarbons remaining after dehydrogenation of a pure hydrocarbon blend containing 35% cyclohexane, 15% methylcyclopentane, and three paraffins proved that methylcyclopentane was lost by ring opening to form methylpentanes. Data in the following table show that at a rate of 20 ml. per hour the loss of methylcyclopentane was reduced to about 5% in the case investigated.

Rate, Ml./Hour	Methylcyclopentane Loss, %
6	50
12	12
20	5

By operating at 20 ml. per hour, a yield of dehydrogenated material sufficient for analysis is obtained in 1 hour.

#### **DISCUSSION OF RESULTS**

Runs made on known blends of six- and seven-carbon hydrocarbons which consisted of the major components present in narrow boiling gasoline fractions showed deviations from the known values of about 1%. Table I shows the composition of ten blends and the deviation of the experimental results from the known values. Where dehydrogenation analyses were carried out in duplicate, the reproducibility was about 0.5%.

Table I.	Dehydro	ogenat	ion for	Determ	ining
Cyclo	hexanes i	in Hyd	lrocarbo	on Blene	ds

Blend Composition, Number,

Par- Naphthenes			Theoretical %	
affins	Cyclopentanes	Cyclohexanes	Cyclohexanes	Deviations <sup>a</sup>
3	Methylcyclo-			
3	pentane Methylcyclo-	Cyclohexane	49.2	+0.2, +0.8
	pentane	Cyclohexane	43.0	+2.2, +2.8
3	Methylcyclo- pentane	Cyclohexane	32.5	+0.8
3	Methylcyclo-	Couldhanna	40.0	1 * 0
4	Methylcyclo-	Cyclonexane	42.9	+1.0
1	pentane	Cyclohexane	38.2	-0.3
1		hexane	62.7	+2.4
1	•••	Methylcyclo- beyane	81.8	+06+06
<b>2</b>	4 dimethylcyclo-	Methylcyclo-		10.0, 10.0
1	4 dimethylcyclo-	nexane Methvlcvclo-	63.8	+0.7, +0.8
1	pentanes	hexane	15.8	-1.3
1	• • •	hexane	21.9	+1.0, +1.1
			Average deviati	on 1.1

<sup>a</sup> Experimental value minus theoretical value.

# ANALYTICAL CHEMISTRY

The data obtained from runs with naphtha fractions in the sixto eight-carbon range were compared with infrared analyses. Table II compares cyclohexanes by dehydrogenation of the whole sample to the sum of the individual cyclohexanes by infrared analysis of a series of distillate fractions. The agreement between cyclohexanes determined by these two independent methods was generally better than 1%. This comparison could not be extended beyond the eight-carbon range because infrared methods have not been extended beyond this range.

Table II. Comparison of Cyclohexanes by Dehydrogenation and Infrared Methods in Straight-Run Naphtha Fractions

Boiling Bange	Carbon No.	Cyclohexanes,		
°C.	Range	Dehydrogenation	Infrareda	Difference
60-80	C6-C7	28 37 19.5	27.7 36.7 20.5	+0.3 +0.3 -1.0
60-92 60-105	C6-C7 C6-C8	35.5 24 40.5	$     \begin{array}{r}       36.0 \\       24.4 \\       40.1 \\       20.1     \end{array} $	-0.5 -0.4 +0.4
80-90	C6-C7			$-0.9 \\ -0.9 \\ +1.9$
90-105	C7-C8	9 54.5 53.5	$8.2 \\ 54.4 \\ 53.6$	$^{+0.8}_{-0.1}$
105-130	C7-C8	$55 \\ 54.5 \\ 32$	$\begin{array}{c} 54.8\\52.9\\33.1\end{array}$	$^{+0.2}_{+1.6}_{-1.1}$
			Average diffe	erence 0.7

<sup>a</sup> Summation of analyses of a series of distillate fractions.



Figure 2. Effect of Rate on Dehydrogenation on Cyclohexanes

The experimental values for cyclohexanes by dehydrogenation, given in Tables I and II, were calculated from the aromatic content of the dehydrogenation product back to an original sample basis using the formula given by Rampton (4). The aromatic contents of the products were determined by ASTM method (1) although any method yielding aromatic contents with an accuracy of  $\pm 1\%$  is satisfactory.

The accuracy achieved in the range studied indicates that dehydrogenation of cyclohexane rings is practically the only reaction occurring, and there is no appreciable amount of interfering reactions such as cracking or isomerization—for example, isomerization of alkylcyclopentanes to form cyclohexanes would have produced high values for cyclohexanes. To establish the amount of cracking, several samples of the exit gases were analyzed by the mass spectrometer. These gases contained about 98% hydrogen, produced by dehydrogenation of cyclohexanes, and less than 2% hydrocarbons, showing that very little cracking occurs.

This method has already proved to be a valuable analytical tool for the determination of cyclohexane type naphthenes in the sixto eight-carbon range. By using fractional distillation and proper choice of cut points, cyclohexane, methylcyclohexane, and total  $C_8$  cyclohexanes (excluding 1,1-dimethylcyclohexane) have been determined in petroleum fractions.

In addition to its use in the lower molecular weight range, the dehydrogenation method may be extended to the  $C_9$  range and higher. Decahydronaphthalene, a dicyclic, has been quantitatively dehydrogenated in the presence of *n*-decane. There are, however, two complications to be considered when the method is applied to the higher range: The possible number of *gem*-substituted cyclohexane isomers increases rapidly, so that the reactable cyclohexanes should not be confused with total cyclohexanes, and there is at present no independent method for checking the ac-

curacy of results. The few results obtained so far have appeared to be logical, but further investigation is needed.

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# High Temperature Refractometry with an Abbe-Type Instrument

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ALTHOUGH refractive index measurements have been used for many years for the characterization of petroleum waxes, there has been little uniformity in testing temperatures. Many wax technologists have used 176° F. (80° C.) since 1929, following the suggestion of Ferris, Cowles, and Henderson (3), who reported refractive indices at that temperature in connection with their work on the composition of paraffin wax. Until recently 176° F. was generally acceptable, since it fulfilled a primary requisite of maintaining any available petroleum wax in the molten state.

Lately, however, many petroleum waxes are being produced that melt in the neighborhood of  $200^{\circ}$  F. and, consequently,  $176^{\circ}$  F. is no longer adequate for general wax refractometry.

The temperature  $212^{\circ}$  F. is suggested herewith as a suitable temperature for refractive index measurements, since it exceeds the melting point of any current petroleum wax product and it is conveniently attainable by the use of low pressure steam as a heating medium. The adaptation and use of an Abbé-type refractometer for  $212^{\circ}$  F. refractometry is described below.

#### APPARATUS

**Refractometer.** The instrument shown in Figures 1 and 2 was manufactured by the Valentine Technical Instrument Corp. and is designated as the Improved Precision model. In order that temperatures of the order of 212° F. could be registered, a Saybolt viscosity thermometer with a range 204° to 218° F., graduated in 0.2° F. divisions, was installed in the jacket well. A special housing to protect this thermometer from breakage was made by welding a brass pipe extension to the guard originally supplied



Figure 1. Refractometer Setup

with the refractometer. The thermometer was checked against one calibrated by the National Bureau of Standards, and was found to agree, after emergent stem correction, within  $0.1^{\circ}$  at  $212^{\circ}$  F.

Temperature-Control System. The water jacket of the refractometer was connected to a source of low pressure steam and colinked with a 1-liter water tank in such a way that either water from the tank or steam could be admitted to the jacket. The water tank was equipped with a 500-watt immersion heater. Water from the jacket outlet was recirculated, but the steam exhaust was vented to the drain.



Figure 2. Refractometer Setup

In preparing the instrument for use, the tank water was heated from room temperature to 200° F. while being circulated through the jacket to raise the prism temperature gradually, thus avoiding the damage by thermal shock that is likely to result from too rapid heating. When the temperature had reached 200° F. or above, over a period of at least 30 minutes, the water was shut off and steam at about 1-pound gage pressure was turned on. After 5 minutes of steam flow with the temperature remaining constant  $(\pm 0.1^{\circ} \text{ F.})$ , the instrument was considered to be at temperature equilibrium and ready for index determinations.

The jacket temperature in this apparatus held well within  $\pm 0.1^{\circ}$  F. for an hour or more. Although occasional drift of 0.1° or 0.2° was observed, it was too slow to be significant during

any one index determination. In any case, however, it is advisable to record the jacket temperature with each determination.

Insulation of Refractometer. Initial potentiometric temperature measurements made on the uninsulated refractometer indicated a temperature differential of  $6^{\circ}$  to  $8^{\circ}$  F. between the steam-heated jacket and the sample. In order to minimize local cooling and to provide satisfactory temperature control, the prism mountings were insulated as described below.

Slabs of cork 0.25 inch thick were cut to fit the sides of the prism mountings, extending 0.25 inch beyond the perimeters. Slabs for the top, bottom, front, and back were cut to the width of the mountings. Thus, when the pieces were in place, the overlap of the side insulation furnished a surface for cementing the abutting It was, of course, necessary to slabs covering the other areas. make suitable openings for the light path, for the heating medium connections, for the thermometer well, and for the spindle. The covering for the entire left or spindle side of both prism mountings was a single slab, pieced together around the spindle, and fixed rigidly to the ton prism mounting insulation. The covering of rigidly to the top prism mounting insulation. The covering of the front, bottom, and right side of the lower mounting was fastened to, and moved with, the mounting when it was opened or closed. The cork slabs were joined with Armstrong's No. 299 waterproof cement. Although this cement is slow drying, it is resistant to water. The cork covering was coated with a thin layer of asbestos cement, which was in turn painted with a thin slurry of 1:1 alundum powder and 40° Bé, sodium silicate solution. This coat, when dry, served to form a strengthening crust over the structure. A final coat of white paint was applied to the finished insulation.



Light Source. A sodium are lamp was used as the light source, as it permitted the removal of the compensating prisms, and thus allowed more room for insulation and for positioning the prisms. Furthermore, it afforded an advantage in standardization, since most published values are given for the D line of so-dium.

#### ADJUSTMENT OF THE REFRACTOMETER

It is standard practice to construct refractometers to read accurately at 68° F., and it follows that readings made at any other temperature are subject to a temperature correction. When, however, an instrument is to be used routinely for determinations of index at a temperature differing considerably from 68° F., it is advantageous to readjust the alignment of the prisms with respect to the refractive index scale in order to read correct values directly. The adjustment was made by reference to a glass test piece:  $n_D^{20} = 1.5000$ . According to information furnished by the manufacturer, the refractometer was subject to a deviation of  $-0.7 \times 10^{-5}$  in index reading for every degree (centigrade) rise in temperature. Therefore, if the instrument is to read correctly at 212° F., it should register a value of 1.50053 for the test piece at 77° F., which happened to be the temperature of the instrument when it was adjusted in these experiments.

The adjustment was accomplished by the following procedure:

The refractometer was placed in reverse to the normal position. The body of the instrument was tilted slightly toward the operator, and the lower prism mounting was opened to an angle of about  $110^{\circ}$  with the upper mounting. The test piece was fixed to the exposed face of the upper prism with a drop of monobromonaphthalene, the polished side against the prism and the polished end downward. A square of white paper was placed against the source, placed above, into the lower end of the test piece. A sharp field division appeared in the telescope. The field division was set at the center of the cross hairs.

The scale arm was loosened by turning the set screw that grips the arm to the spindle. The arm was then shifted with reference to the spindle until the hair line observed through the microscope coincided with the reading 1.50053. The arm was set in this position by tightening the set-screw.

The accuracy of the setting was tested by proceeding as in a normal refractive index determination—that is, a series of readings was taken, until at least four were recorded that agreed within 0.0002. Since the averaged readings fell somewhat more than 0.0002 below the desired reading of 1.50053, a fine adjustment was made by setting the hairline of the microscope at 1.50053 on the scale, and shifting the cross hairs in the telescope to bring their center to the field division. The cross hairs were shifted by turning the Allen set-screw on the side of the telescope barrel.

An alternative and more direct method of adjustment can be used where suitable index standards are available with known refractive index at 212° F. (The Sun Oil Co. has supplied *n*-Hexadecane and *cis*-Decalin as standards for cooperative tests, but arrangements are in progress to transfer this service to one of the nationally known research institutions.)

The refractometer, placed in normal position, is raised to working temperature, and the standard substance is placed in the space as in normal testing. The field division is placed at the cross hair intersection as accurately as possible and the sector scale is adjusted as described above. The correct setting for the sector scale can be calculated as follows:

Scale reading 
$$= n_{\rm D}^{212} + c (212 - t)$$

where,

t = observed jacket temperature, ° F.

c = temperature coefficient of refractive index

The value 0.00025 is applicable to pure *n*-hexadecane or cis-Decalin if (212 - t) is not over 15° F.

#### JACKET AND SAMPLE TEMPERATURES

Tests with Water. The sample temperature was determined under test conditions by relating the refractive index of water, as observed in the modified instrument, to the corresponding temperature derived from published data.

The data of Tilton and Taylor (6) are considered to be of the highest order of dependability but are limited to the range 59° to 140° F. On the other hand, the data presented in the Chemical Rubber Publishing Co. Handbook (4) cover the range from 57° to 212° F., although the source is not given. The two sets of data, portions of which are plotted in Figure 3, are, however, in fair agreement with each other in the range covered by both. The maximum disagreement at any point is 0.0001. This is within the limit of accuracy of ordinary laboratory work, although it may be a significant deviation for precision work. The agreement is such, however, that the use of the handbook data at 212° F. as a standard appeared justified.

Test data were obtained with a 30% heart cut from redistilled water. In each of three tests, index readings were started as soon as the prisms were closed on the sample, and were continued without pause, with an alternating up and down approach to the cross hairs, as long as enough water remained to give a readable field division. Average readings after temperature equilibrium was established, were, respectively, for the three tests: 1.31769, 1.31767, and 1.31765. The average of these, 1.31767, was accepted as the determined value of  $n_D^{c}$ . The jacket temperature was 213.8° F.

The sample temperature was computed from the above data by the equation:

$$t = 212 + \frac{n_{\rm D}^t - n_{\rm D}^{212}}{c}$$

where

c =temperature coefficient of index

=  $-0.000164/^{\circ}$  F. This was calculated from stated values for  $n_{\rm D}^{208.4^{\circ}}$  and  $n_{\rm D}^{212^{\circ}}$ 

Substituting the value determined in the instrument for  $n_{\rm D}^t$ , the calculation is:

$$t = 212 + \frac{1.31767 - 1.31783}{-0.000164} = 213.0^{\circ}$$
 F.

Since the jacket temperature was  $213.8^{\circ}$  when the data were taken, a gradient of  $-0.8^{\circ}$  F. is demonstrated between the jacket and the sample.

The sample temperature can also be determined graphically by extrapolation of the  $n_D - t$  curve, Figure 3, to intercept the coordinate  $n_D = 1.31767$ . The lines meet at 212.9° F. as double circled in Figure 4, which is a detail of Figure 3, including only the area near 212° F. By this method the temperature gradient is shown to be  $-0.9^{\circ}$  F.

Correction of the jacket temperature by  $-0.8^{\circ}$  or  $-0.9^{\circ}$  to derive a true sample temperature is suggested by these data, but such a refinement is regarded as unwarranted in view of the uncertainty of the accuracy of the reference data.



Tests with Pure Hydrocarbons. The accuracy of results obtained with this modified Abbé refractometer was established by testing samples of *n*-hexadecane and *cis*-Decalin representing the approximate extremes in refractive index encountered in petroleum waxes. The indices of these samples were determined by use of an Eykman refractometer giving results estimated to have accuracy better than 0.0002. Refractive indices at 212° F. were calculated by the following equations which were derived

from a number of index observations on the Eykman instrument over a range of temperatures:

For *n*-hexadecane (75° to 216° F.)

$$n = {}_{\rm D}^{t_{\rm o}} F 1.45044 - 0.0002317t(^{\circ} F.)$$

For cis-Decalin (156° to 288° F.)

$$n_{\rm D}^{\rm roo\,F.} = 1.49697 - 0.0002364t - 0.00000028t^2$$

Values for  $n_{\rm D}^{212}$  calculated by these equations are, respectively, 1.4013 and 1.4456.



Figure 5. Refractive Indices in Study of Petroleum Waxes

Refractive indices of the standards were determined with the Abbé instrument by each of four operators. Results for each sample agreed within 0.0002. The averaged results are given below, and compared with the Eykman values.

	Refractive Index, 212° F.		
	n-Hexadecane	cis-Decalin	
Eykman refractometer Abbé refractometer Deviation	1.4013 1.4014 +0.0001	1.4456 1.4454 -0.0002	

The deviations are of opposite sign, indicating that average results are accurate.

On the basis of the results obtained with water and with hydrocarbons, it was concluded that the temperature of the insulated jacket may be accepted as the sample temperature.

#### PROCEDURE FOR ROUTINE TESTING

One or 2 drops of liquid sample (or an equivalent amount of solid wax) is placed in the sample space between the prisms. Temperature equilibrium is established in about 2 minutes. Several individual index readings are then taken in the customary manner, and at least four of these, agreeing within 0.0002, are averaged to give a value for  $n_{\rm b}^{\rm t}$ .

The refractive index,  $n_D^{212}$ , is calculated from the equation:

$$n_{\rm D}^{2^{12}} = n_{\rm D}^t + c(t - 212)$$

where

t = jacket temperature in ° F. (corrected for emergent stem)

c = temperature coefficient of index for the sample

For hydrocarbon waxes, the value of c is 0.000214 per ° F. Corrections to 212° F. can be determined more conveniently by use of a table listing the calculated corrections, including emergent stem and temperature coefficient, for all thermometer readings from 210° to 214° F. at intervals of 0.1°.

#### DISCUSSION

This modified refractometer has been used routinely for over 2 years with no difficulties other than occasional damage to the prism insulation or deterioration of rubber tube connections. Originally it was thought that the prisms would loosen or become clouded under high temperature conditions, making index readings difficult, but neither condition has yet been encountered.

Figure 5 illustrates one method of employing refractive indices in the study of petroleum waxes. Values for normal paraffin hydrocarbons fall on or slightly below the diagonal line. This line was established from literature data (1, 2, 5) calculated to 212° F. The points are for substantially oil-free petroleum waxes, and the position of each, with respect to the line, gives an approximate idea of the composition. Those close to the line are rich in straight-chain compounds, while many of the others are essentially free of normal paraffins, and presumably of highly branched or cyclic structure.

#### ACKNOWLEDGMENT

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# Spectrophotometric Determination of Vicinal Glycols

# Application to the Determination of Ribofuranosides

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STANDARD method for the determination of vicinal glycol groups in organic compounds is the oxidation of a weighed sample by a known amount of periodate followed by a volumetric determination of the excess periodate (3). The work reported in this paper demonstrates that the consumption of periodate by vicinal glycol groups may be followed spectrophotometrically by using the absorption band of metaperiodate which has a maximum at about 223 m $\mu$  (2). The advantages of this spectrophotometric determination of periodate consumption over the usual technique are twofold. First, the rate of the oxidation reaction may be followed readily and the completion of the reaction is ascertained easily. Second, the determination of vicinal glycol groups may be carried out on about  $10^{-8}$  to  $10^{-6}$  mole of sample. It is feasible, therefore, to apply the method to the quantitative determination of such groups in materials which have been separated by paper chromatography. Recently, a method for carrying out such determinations colorimetrically was described by Adelberg (1). This colorimetric method involves considerable treatment of the sample prior to development of the final color and appears to be less accurate than the procedure described here.

In applying this procedure to the quantitative determination of vicinal glycol groups, several factors must be considered. One of these factors is the absorption of light by the products of the reaction and the reactants. The products formed in this reaction are iodate ion and compounds which may contain carbonyl or carboxyl groups. Fortunately, iodate ion has an extinction coefficient which is at most one tenth that of metaperiodate in the region 220 to 240 m $\mu$  and introduces no large error in the determination. Unconjugated carbonyl and carboxyl groups also do not interfere, but difficulties may be encountered in oxidations where products with conjugated carbonyl or carboxyl groups are formed. In contrasting this procedure to the standard volu-

metric procedure, formic acid is oxidized to carbon dioxide and water by metaperiodate. This appears to be a photochemical reaction which occurs in the light beam of the spectrophotometer.

The spectrophotometric determination of vicinal glycol groups was carried out on a variety of compounds. The data obtained with several ribofuranosides are summarized in Table I. The strong purine or pyrimidine absorption of the compounds listed in the table does not interfere with the determination. In addition, the absorption spectra of the ribosides apparently are sufficiently like those of the corresponding dialdehydes produced in the oxidation so that results of reasonable accuracy are obtained.

#### EXPERIMENTAL

**Materials.** A 0.1*M* stock solution of sodium metaperiodate (G. Frederick Smith Chemical Co., Columbus, Ohio) was prepared and standardized by the usual procedure (3). A portion of this solution was diluted to a concentration of  $10^{-4}M$ . The ribofuranosides, with the exception of the adenosine, were commercial samples (Schwarz Laboratories, Inc., Mount

### Table I. Spectrophotometric Determination of Periodate Consumption by Ribofuranosides

	Wave	Moles $(\times 10^7)$	Mole of P		
Compound	$m\mu^a$	of Riboside	Added	Consumed	Ratio
Adenosine Adenosine-5'-	227	3.63	4.94	3.69	0.98
acid	227	0.97	1.98	1.02	0.95
Guanosine	224	3.06	4.94	3.08	0.99
Cytidine	227	4.10	4.94	3.84	1.07
Uridine	230	4.10	4.94	4.09	1.00
a Wave longth	at which d	sepperance of	opriodete	was followed	

<sup>b</sup> Moles of riboside to moles of periodate consumed.

Vernon, N. Y.). The adenosine was a sample of material carefully purified in this laboratory. An aqueous solution of known concentration was prepared from each one of the ribosides.

Distilled water which contains no appreciable amount of periodate oxidizable impurities was used for preparing all solutions.

**Procedure.** A Beckman Model DU spectrophotometer was used for measuring absorbancies.

Four quartz absorption cells with a 1.00-cm. light path are filed with water and absorbance readings taken using one of the cells (No. 1) as a blank. Any absorbance differences for the cells 2, 3, and 4 are emptied, dried, and filled in the following manner: Equal and known volumes of solution containing the oxidizable sample are added to cells 2 and 4 and the same volume of water is put in cell 3. Similarly, equal and known volumes of  $10^{-4}M$  periodate solution—ca. 20 to 100% in excess—are added to cell 3 and 4, while an equal volume of water is added to cell 2. The solutions in the cells are mixed and their absorbancies are measured at 5- to 10-minute intervals against cell 1 as The absorbance of cell 4 subtracted from the sum of a blank. the absorbancies of cells 2 and 3 represents the decrease in absorbance due to consumption of periodate by the sample. When this absorbance difference becomes constant in 60 to 90 minutes, the reaction is considered complete. This difference divided by the absorbance of cell 3 at zero time is equal to the fraction of the known amount of added periodate that is consumed in oxi-

dizing the vicinal glycol groups. The absorbance of cell 3 is followed in order to correct for the slow change in absorption which takes place in  $10^{-4}M$  solutions of metaperiodate. This change presumably is due to changes in temperature, pH, etc. (2).

The ribonucleosides are oxidized at a rapid rate at concentrations of  $10^{-4}M$ . With substances which are oxidized at inconveniently slow rates (hours or days for complete oxidation) in such concentrations—i.e., ethylene glycol and  $\alpha$ -methylglucoside—it is desirable to alter the above procedure. In such cases the reaction may be carried out at concentrations about twentyfold higher in absorption cells with a 0.5-mm. path length. An alternative procedure, which also has proved useful, is to carry out the oxidation in much more concentrated solution, remove aliquots at desired intervals, and dilute to a convenient volume for the absorbance readings.

In applying this spectrophotometric method to compounds which have been eluted from a paper chromatogram, it is necessary to apply a correction for the periodate-oxidizable substances which are eluted from the paper along with the desired material. This correction can be made readily by eluting a blank piece of paper of known area and determining spectrophotometrically the amount of periodate consumed by the eluate. A correction then may be applied to the amount of periodate consumed by the sample based on the area of paper from which the sample was eluted.

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# Determination of Adsorbed Moisture on Uranium and Uranium Oxide

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 $\Lambda$  LTHOUGH this method was intended to determine only the order of magnitude of the per cent of moisture adsorbed on uranium and uranium oxide (U<sub>3</sub>O<sub>8</sub>) during treatment prior to weighing, it was evaluated more precisely because of its general applicability to other similar problems.

Probably the most important advantage of the Karl Fischer reagent is its ability to determine rapidly and accurately very small quantities of water. However, when the sample to be analyzed is insoluble in alcohol or the Karl Fischer reagent, or will react with the reagent, as is the case with a number of inorganic salts and oxides (5), a direct titration with Karl Fischer reagent would seem to be unsatisfactory. Recently (7), the Karl Fischer titration has been employed for the determination of moisture in gases by passing a measured amount of gas through a known amount of reagent.

A modification of this technique was utilized to overcome the inherent disadvantages of direct titration of insoluble uranium and uranium oxide. The sample was placed in a quartz tube and heated, and the evolved moisture swept into a specially designed titration cell where it reacted with the reagent. The dead-stop end-point technique of Foulk and Bawden (1, 3) was used for detecting the end point of the titration.

#### REAGENTS

Absolute methanol. No attempt was made to desiccate or further purify the methanol.

Karl Fischer reagent, prepared in the usual manner with 269 ml. of pyridine, 84.7 grams of iodine, 667 ml. of absolute methyl alcohol, and 64 grams of liquid sulfur dioxide.

Standard water solution, prepared by the dilution of a known amount of water with absolute methanol.

Sodium tartrate dihydrate, reagent grade. Barium chloride dihydrate, reagent grade. Magnesium perchlorate, anhydrous.

#### APPARATUS

As only small amounts of water were to be determined, the apparatus described has been used successfully to keep contamination from atmospheric moisture to a minimum prior to and during the titration.

The initial pressure of the tank nitrogen, used as a sweep gas, was controlled by a low range (0 to 10 pounds per square inch) pressure regulator. Three drying towers of anhydrous magnesium perchlorate were used for preliminary drying of the sweep gas; remaining traces of moisture were removed by a dry ice-acetone trap. Stopcocks on either side of the cold trap permitted fine control of the gas flow rate.

The essential part of the heating tube was composed of 25-mm. quartz tubing, 65 cm. in length. A standard taper 29/42 male joint was sealed to one end of the tube to serve as a sample port. An 8-mm. diameter side arm, 5 cm. from the sample port, allowed introduction of the sweep gas into the heating tube. A 15-cm. section of 8-mm. tubing was sealed to the exhaust end of the heating tube. Although the dimensions of the quartz tube are not critical, the ground-glass joints must be located at sufficient distances from the furnace to prevent volatilization of the joint lubricant when the tube is heated.

The quartz tube was heated by a 12-inch tube furnace, whose temperature was controlled by a variable autotransformer. Temperatures below 300° C. were read from a thermometer placed in the tube; higher temperatures were indicated by a thermocouple placed outside the tube in the heated zone. The position in the tube of platinum boats  $(3 \times 3^{/}_{5} \times 3^{/}_{5})$  inches), which we have the tube of platinum boats  $(3 \times 3^{/}_{5} \times 3^{/}_{5})$  inches).

The position in the tube of platinum boats  $(3 \times 3/_5 \times 3/_8$  inches), which were used as sample containers, was adjusted with a steel rod which had a small hook at one end for removing the sample boats.

The titration cell was a combination of earlier designs used by Roman and Hirst (7) and Zerban and Sattler (8) and consisted of two parts joined by a standard taper joint. The buret entrances (standard taper female joints), gas inlet, and gas outlet were sealed to the top part of the cell, which was held permanently in position. The gas outlet was protected by a drying tube containing magnesium perchlorate. Platinum electrodes, sealed through standard taper male joints, were inserted into two female joints projecting from the side of the bottom part of the cell, facilitating the removal of the electrodes for cleaning. The gas inlet tube extended nearly to the bottom of the titration vessel to ensure complete absorption of moisture by the solution and simultaneously provide stirring. A similar titration vessel, used for standardization of Karl Fischer reagent against a standard water-methanol solution, contained a side arm, with a ground-glass stopper, for pipetting the standard water solution into the cell.

Karl Fischer reagent and methanol were contained in 2-liter reservoirs, connected by ground-glass joints to 50-ml. automatic burets. This assembly was protected from atmospheric moisture by magnesium perchlorate drying tubes. Standard taper male joints were sealed onto the buret tips to allow an airtight seal to the top of the titration cell. This assembly is shown in Figure 1.

All connections in the apparatus between the cold trap and titration cell were ball-and-socket joints. The electrical circuit used for the dead-stop titration was simi-

The electrical circuit used for the dead-stop fitration was similar to that shown by Mitchell and Smith (4).

Water, Mg.	Vol. K.F., Ml.	Reagent Strength. Mg. H <sub>2</sub> O/Ml. K.F
Wa	ter-Methanol Sta	ndard
99.7 99.7 99.7	$\begin{array}{c} 30.02 \\ 30.17 \\ 30.07 \end{array}$	$     \begin{array}{r}       3 & 32 \\       3 & 30 \\       3 & 32     \end{array}   $
Sod	ium Tartrate Dib	ydrate
Water Evolved <sup>a</sup> , Mg.		
$83.0 \\ 84.9$	$24.95 \\ 25.55$	3 33 3 32

#### EXPERIMENTAL PROCEDURE AND RESULTS

Although, in preliminary titrations of reagent versus methanol, the forward and back titrations gave identical results, the forward titration was used in all cases to establish the end point. As it was realized that some overtitration might occur, the ratio of reagent to methanol was determined periodically, and was known at all times to within  $\pm 0.7\%$ . A more accurate knowledge of the ratio was unnecessary, because the amount of methanol used to back-titrate was never more than 0.1 ml.

Standardization of Karl Fischer Reagent with Standard Water-Methanol Solution. Procedures similar to those described by Mitchell and Smith (2) were employed in the preparation and use of a water-methanol standard to standardize the Karl Fischer reagent. Values obtained by standardization of the reagent in this manner are listed in Table I.

Standardization of Karl Fischer reagent by direct addition of sodium tartrate dihydrate to the cell is not recommended when using a cell similar to the one used here. As the only stirring action present results from the sweep gas, this titration is so lengthy that, after a few unsuccessful attempts, it was given up as impractical.

Standardization of Karl Fischer Reagent with Sodium Tartrate Dihydrate. Neuss, O'Brien, and Frediani (6) tested a number of crystalline hydrates for use as water standards and found that sodium tartrate dihydrate proved outstanding. The procedure for the standardization was as follows:

Approximately 10 ml. of the stock methanol solution was added to the cell and nitrogen allowed to bubble through at about 15 to 30 ml. per minute. This solution was titrated to the end point with Karl Fischer reagent, and allowed to stand for about an hour with nitrogen flowing to check the apparatus for moisture. The gas flow was increased to about 60 to 70 ml. per minute, and a platinum boat, containing approximately 0.5 gram of sodium tartrate

#### ANALYTICAL CHEMISTRY

dihydrate, was immediately inserted into the quartz tube. The gas flow was readjusted to its original rate, and the temperature of the furnace adjusted to 140° C. The moisture evolved from the sample was swept into the titration cell, where it was absorbed in a slight deficiency of reagent (as calculated from the theoretical amount of water present); the titration was then completed with the reagent. To ensure complete removal of water from the apparatus, the analysis was not terminated until the equilibrium was maintained for 30 minutes of gas flow. The sample was removed from the apparatus, cooled in a desiccator, and weighed. The moisture loss on heating was then calculated from the change in weight. The sodium tartrate used in these determinations contained the theoretical amount of water (15.66%) as determined by drying to constant weight at 150° C.

Results of two standardizations of the reagent with sodium tartrate dihydrate are given in Table I. They were obtained the day before standardization with the water-methanol standard. The agreement is good enough to indicate that standardization of the reagent with the dihydrate is feasible. Only two values for reagent strength, obtained using the evolution procedure, were compared with standardization against the watermethanol solution because of the time required for each determination. All subsequent standardizations were carried out in this manner to prove the indicated feasibility of the method, yielding results differing only by the expected decrease of reagent strength with time.

**Blanks.** In order to demonstrate the ability of the system to remain moisture-free under operating conditions for extended periods of time, nitrogen was allowed to bubble through an equilibrated solution of methanol and Karl Fischer reagent. No change in the end point occurred during 5-hour periods at furnace temperatures between  $25^{\circ}$  and  $900^{\circ}$  C.



Figure 1. Titration Assembly

Moisture could be introduced into the system when the heating tube was opened to allow introduction of the sample. The amount of this contamination was determined by inserting empty boats into the tube and following the general procedure described for standardization. The amount of reagent necessary to titrate the moisture introduced into the system under the conditions of temperature and humidity in this laboratory was invariably equivalent to 0.3 mg. of water. All values presented have been corrected for this blank, where applicable.

Effect of Nitrogen Flow Rate. Karl Fischer reagent was standardized against sodium tartrate dihydrate at different nitrogen flow rates to determine the effect on water recovery. Results are given in Table II. At the slowest flow rate, a large amount of water initially condensed on the cooler portions of the quartz tube and on the connecting tube, but was subsequently carried into the titration cell by the dry nitrogen with no apparent loss in recovery. At the most rapid flow rate, the tendency of the solution in the cell to froth was pronounced, and discouraged attempts to use a more rapid gas flow. A flow rate of 15 to 30 ml. per minute was selected as optimum.

Determination of Adsorbed Moisture on Uranium Metal and Uranium Oxide. A procedure similar to that used for reagent standardization with sodium tartrate was used to analyze uranium metal and uranium oxide for adsorbed moisture. For each determination, an accurately weighed sample of approximately 2 grams was used. The strength of the reagent (milligrams of water per milliliter of reagent) was determined the day previous to each analysis. Immediately before analysis, the uranium metal was cleaned with dilute nitric acid, washed with water and acetone, and dried with a stream of air filtered through glass wool. The oxides were obtained by ignition of pure samples, of the source indicated in Table III, for 3 hours at 850° C.; they were then stored in a desiccator containing anhydrous magnesium perchlorate until analyzed. The results of these analyses are shown in Table III. Because of the small volumes of reagent used and the comparatively large blank, these results probably do not have an absolute accuracy better than 0.01%.

To ascertain the extent of water recovery, several samples of uranium oxide, obtained by ignition of the metal, were mixed with small amounts of barium chloride dihydrate and analyzed for moisture. The results are given in Table IV. Analysis of the barium salt by drying to constant weight at 150 ° C. yielded a water content of 14.7%. Because the spike recovery is very good there is no reason for believing that the small amounts of water evolved from these samples were not being recovered.

Table II.	Effect of Nite	rogen Flow Rate on	Water Recovery
Detn.	Flow Rate Ml./Min.ª	Time for Analysis, Hours	Mg. H <sub>2</sub> O per Ml. K.F. <sup>b</sup>
1	4	7	3.34
2	12	5	3.36
3	40	4	3.33
<sup>a</sup> Diamete <sup>b</sup> Only one	er of gas inlet was e of these determin	approximately 1 mm. ations was made per day,	in the order 2, 1, 3.

#### DISCUSSION

The amount of moisture adsorbed on uranium metal and uranium oxide was usually in the order of 0.02%. Oxides obtained from ignition of uranyl sulfate gave a higher result, about 0.1%. These results would have been more accurate had a weaker reagent, having a capacity of about 1 mg. water per ml., been used. However, the authors were primarily interested in the order of magnitude of adsorbed moisture. Furthermore, the reagent strength used permitted better evaluation of this method of standardization and analysis.

The method should be useful for determining sorbed or combined moisture evolved from many materials which are insoluble under normal titration conditions, or which will react with the reagent. Samples which decompose to form water, or which yield reactive volatile decomposition products when heated, could not be analyzed for adsorbed or combined moisture by this

Table III.	Determination of Per Cent of H <sub>2</sub> O on Uranium
	Metal and Uranium Oxide

Sample	Source	Temp. of Detn., °C.	Vol. of Reagent, Ml.	Reagent Strength, Mg. H <sub>2</sub> O/Ml. K.F.	H <sub>2</sub> O, %
U metal		115	0.05	3.70	0.01
U metal		450	0.03	3.69	0.01
U metal		425	0.03	3.69	0.01
$U_3O_8$	U metal	860	0.11	3.90	0.02
$U_3O_8$	U metal	860	0.05	3.78	0.01
$U_3O_8$	U metal	860	0.12	3.78	0.02
$U_3O_8$	$UO_2(NO_3)_2$	860	0.23	3.72	0.04
$U_3O_8$	$UO_2(NO_3)_2$	860	0.25	3.72	0.04
$U_3O_8$	$UO_2SO_4$	860	0.84	3.72	0.13
$U_3O_8$	$UO_2SO_4$	860	0.48	3.72	0.08
$U_3O_8$	$UO_2SO_4$	860	0.46	3.71	0.08
$U_3O_8$	$UO_2SO_4$	860	0.51	3.71	0.09
$U_3O_8$	$UO_2F_2$	860	0.06	3.72	0.01
$U_3O_8$	$UO_2F_2$	860	0.04	3.72	0.01

#### Table IV. Determination of Water on Uranium Oxide Spiked with Barium Chloride Dihydrate<sup>a</sup>

U <sub>3</sub> O <sub>8</sub> ,	$\operatorname{BaCl_2 \cdot 2H_2O}_{\operatorname{Added}}$	Vol. K.F. <sup>b</sup> ,	H2O Detd.,	Calcd. H <sub>2</sub> O from Spike,	$H_2O$ from $U_3O_8$ ,
$\begin{array}{c} 2.3718 \\ 2.2792 \\ 2.3541 \end{array}$	8.6 8.7 6.9	$0.49 \\ 0.56 \\ 0.39$	$0.078 \\ 0.093 \\ 0.062$	0.056 0.057 0.044	$0.02 \\ 0.04 \\ 0.02$
<sup>a</sup> Furn <sup>b</sup> 3.78	ace temperature of mg. water per ml.	840° C. Karl Fische	er reagent.		

method. By proper adjustment of temperature, separate determinations of adsorbed and combined water might be possible.

#### RECOMMENDATIONS

Although the method gave satisfactory results, experience indicates that certain modifications would increase the speed and accuracy of the analysis.

A. If a back-flushing system similar to that in the familiar Dumas nitrogen procedure were incorporated in the apparatus, the blank arising from moisture introduced when the sample is inserted might be eliminated.

inserted might be eliminated. B. The addition of a stopcock on the bottom of the titration cell would permit the cell to be drained, avoiding the opening of the system to the atmosphere between titrations.

C. Minimizing the length of glass tubing between the furnace and titration cell, or winding this tubing with insulating material, heating tape, or Nichrome wire, would result in a more rapid transfer of moisture.

D. Routine standardization of the reagent with a water-methanol standard would be much more rapid than standardization by the evolution procedure with sodium tartrate dihydrate; the latter could be used periodically to check the efficiency of the apparatus.

E. Tests conducted in this laboratory since completion of this program indicate that the moisture content of argon, as supplied commercially, is sufficiently low possibly to preclude the necessity for drying towers and cold trap.

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#### 82. **Trinitronaphthalenes**

Contributed by SIEN MOO TSANG, Research Division, American Cyanamid Co., Bound Brook, N. J., and W. C. McCRONE, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.



Structural Formulas for Trinitronaphthalenes Described

### 1,2,5-TRINITRONAPHTHALENE 1

INE needles of form II are obtained on recrystallization from methyl or ethyl alcohol. However, on continued stirring of the solution at room temperature, a solution phase transformation occurs and small, equant monoclinic crystals of form I are obtained. Well-formed crystals are also obtained from thymol on a microscope slide.

CRYSTAL MORPHOLOGY Crystal System. Monoclinic.



**Fusion Preparation** Figure 1. of 1,2,5-Trinitronaphthalene





Figure 2. Orthographic Projection of Typical Crystal of 1,2,5-Trinitronaphthalene



Figure 3. **Fusion Preparation** of 1,3,8-Trinitronaphthalene





Figure 4. Orthographic Projection of Typical Crystal of 1,3,8-Trinitronaphthalene

Form and Habit. Equant from ethyl alcohol showing prisms

 [10], clinopinacoid [010], and clinodome [011].
 Interfacial Angles (Polar). 110 Λ 110 approximately 28°. Beta Angle. 95°. Optical Properties 95°

Refractive Indices (5893 A.; 25° C.).  $\alpha = 1.487 \pm 0.002$ ; = 1.807 ± 0.01;  $\gamma = 1.809 \pm 0.01$ . Optic Axial Angles (5893 A.; 25° C.).  $2V = (-)10^{\circ}$ ; 2E =в

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Optic Axial Plane. 001.

Acute Bisectrix.  $\alpha = a$ . Extinction.  $\beta \wedge c = 5^{\circ}$  in obtuse  $\beta$ . FUSION DATA. 1,2,5-Trinitronaphthalene melts with neither decomposition nor sublimation at 112–113° C. It crystallizes spontaneously on cooling from a large number of randomly oriented nuclei to give fairly large single crystals of form II (Figure 1). An optic axis interference figure showing  $2E = (-) 65^{\circ}$  with little or no dispersion is easily obtained. It has not been possible to obtain form I from form II by seeding a fusion preparation at any temperature above room temperature. Fairly well-formed crystals are produced in the zone of mixing during a thymol mixed fusion; a profile angle of 115° is fairly common.

#### 1,2,5-TRINITRONAPHTHALENE II

1,2,5-Trinitronaphthalene(II) is the form normally obtained on recrystallization from either solution or fusion. These crystals show an optic axial angle, 2E, of (-) 65° with little or no dispersion.

### **1,3,8-TRINITRONAPHTHALENE**

Excellent crystals of 1,3,8-trinitronaphthalene are obtained from nitromethane, chloroform, ethyl alcohol, and acetic acid. It is insoluble in water

- CRYSTAL MORPHOLOGY
- Crystal System. Monoclinic.

Form and Habit. Stubby prisms from nitromethane showing the prism {110}, basal pinacoid {001}, and usually the ortho pinacoid {100}

Interfacial Ángles (Polar).  $110 \wedge 1\overline{10} = 128^{\circ}$ . Beta Angle. 125°

OPTICAL PROPERTIES

- Refractive indices (5893 A.; 25° C.).  $\alpha = 1.602 \pm 0.002$ ; =  $1.747 \pm 0.005$ ;  $\gamma = 1.84 \pm 0.01$ . Optic Axial Angles (5893 A.; 25° C.). 2V = (-) 88°.
  - Dispersion. r > v.

Acute Bisectrix.  $\alpha \Lambda c = 30^{\circ}$  in acute  $\beta$ .

1,3,8-trinitronaphthalene sublimes below the FUSION DATA. melting point to give a few small equant monoclinic crystals lying usually on the clinopinacoid  $\{010\}$ . The melting point is  $218-219^\circ$  with little, if any, decomposition. Crystallization occurs with little supercooling and although rapid at first, the rate of crystallization is essentially zero at room temperature. The patterns of shrinkage cracks and gas bubbles are characteristic (Figure 3). Conoscopic observations may show an optic axis at the edge of the field. A thymol mixed fusion shows a variable acute profile angle; all refractive indices are greater than that of the thymol melt.

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The work described was performed under contract betwe Cornell University and the Office of Scientific Research and L velopment during World War II. Alfred T. Blomquist was tec nical representative of OSRD Section B-2-A supervising progress of this work.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

# SCIENTIFIC COMMUNICATION

# Sealed-Tube Combustion for Determination of Carbon. Hydrogen, and Nitrogen

OBGANIC compounds can be burned in a sealed quartz tube in pure oxygen together with a bit of metallic copper as shown in Figure 1; water, carbon dioxide, and nitrogen from the sample are the only gaseous compounds formed. Any nitrogen oxidecopper oxide compounds formed (2) are decomposed as the excess of oxygen diffuses into the copper. The capillaries are filled as described for the filling of Carius tubes (1).

The gases can be used for isotopic analysis in a mass spectrometer or counter or they can be determined in the apparatus shown in Figure 2: After the apparatus has been completely evacuated and leveling bulb G lowered so that the mercury level is situated below C, the sample tube, placed in tube C as shown, is broken by turning glass fork D. G is raised until the mercury level is situated in the graduated tube, A, and gas volume and mercury pressure (10 to 30 mm.) are read. G is raised, stopcock F is opened, and the gas volume is read at atmospheric pressure. Potassium hydroxide is drawn in through E and the gas volume is read again. The third reading gives the nitrogen, the second carbon dioxide plus nitrogen, and the first water plus carbon dioxide plus nitrogen.

A few analyses carried out with 0.1- to 0.2-mg. samples gave



 $\boldsymbol{C}$ Capillary sealed off

b. Constriction c. Metallic copper b. Constriction d. Point where capillary is sealed off Tubes used in first experiments had a total length, when sealed off, of 120 mm and an inner diameter of 3 mm.

an accuracy somewhat less good than that of the Pregl methods on the 5-mg. scale. It is, however, expected that this will be changed after a thorough investigation of the method.

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Figure 2. Apparatus

- Measuring tube Ground joint with silicone lubricated with silicone grease and tightly fixed with strong
- springs Side tube for capillary with С. D.
- sample Glass fork for holding and breaking capillary Stopcock of measuring tube Stopcock of comparison  $\frac{E}{F}$
- tube Leveling bulb Point where tubes A and LG. H.
- H. Point where tubes A and L widen
  K. Rubber stopper cemented into ground joint with Krönigs glass cement. Inner bore lubricated with silicone grease

with silicone grease L. Comparison tube M. Expansion room for com-bustion gases N. Glass collar for catching mercury drops Hg. Mercury In the apparatus used for the first analyses the volume of the upper measuring capillary was 0.2 ml. and the total volume of the graduated part was 25 ml. The volume of room M was about 150 ml.

Modified Amperometric Titration Apparatus Which Eliminates Agar Bridge. W. J. Seagers and H. A. Frediani, Chemical Control Division, Merck & Co., Inc., Rahway, N. J.

A METHOD for eliminating the agar bridge in the conventional amperometric titration apparatus (3) without the use of a mercury pool has been developed in this laboratory. The reference electrode in the modified apparatus is a platinum electrode of large area whose potential relative to that of the rotating platinum electrode is maintained by an external potential source.





The present apparatus has two advantages over previously described setups. The platinum anode eliminates the difficulties usually enountered in the use of an agar bridge as the means of electrical contact between the anode and cathode compartments. This makes maintenance simple. The potential source is such that changes from one set of titration conditions to another require only a simple adjustment. This makes the instrument versatile and eminently suitable for investigational use.

#### APPARATUS

The circuit diagram is shown in Figure 1. All parts are available from radio supply houses (Table I). The tolerances of all parts are not critical, so that reasonable substitutions can be made.

The zero-shunt circuit (1) powered by battery  $B_1$  is necessary for the utilization of the full sensitivity of the microammeter, since a relatively large initial current may flow through the circuit unlike most applications of the conventional apparatus. The potentiometers,  $R_1$  and  $R_2$ , are adjusted so that the microammeter may be shunted and its range extended to 0 to 200 and 0 to 300  $\mu$ a. in addition to the 0- to 100- $\mu$ a. range.

A diagram of the cell used is shown in Figure 2. The anode, A, is a 0.05-mm. platinum sheet  $15 \times 50$  mm. curved to fit the inside of the cell. The rotating platinum electrode, C, is of conventional construction using No. 18 platinum wire but with the



Figure 2. Diagram of Cell and Electrodes

A. Sheet platinum anode B. Cone of stirring motor C. Rotating platinum electrode addition of two stirring paddles immediately above the electrode itself. At 400 to 700 r.p.m., the stirring is sufficiently efficient so that current readings may be made within a few seconds after each addition of reagent yet meter needle fluctuations are negligible. The glass cell, designed for easy draining and rinsing, is 60 mm. in diameter and 120 mm. long (dimensions are not critical) with a 2-mm. bore stopcock at the bottom. The rotating electrode is driven by a suitable adjustable speed motor shown as B.

In order to select the potential applied to the electrodes, the conventional method ( $\mathscr{P}$ ) is used with the exception that the rotating platinum cathode and the sheet platinum anode are used in place of the dropping mercury electrode and saturated calomel electrode when recording the polarogram.

# Table I. Parts List

М.	0-100 µa. microammeter, Welsh Scientific Co., Model 451
S1. S2.	Single-pole single-throw toggle switches
$S_2$ .	Three-position two-circuit rotary switch
$B_1, B_2,$	1.5-volt 4FH Burgess dry cells
$R_1$ .	500-ohm wire-wound potentiometer
$R_2$ .	300-ohm wire-wound potentiometer
$R_3$ .	100-ohm wire-wound potentiometer
$R_4$ .	1500-ohm 0.5-watt carbon resistor
R5.	5000-ohm wire wound potentiometer
V.	0-3 volts d.c. voltmeter. Welsh Scientific Co., Model 451

For continuous routine use on a single type of titration—i.e., chloride with silver nitrate—the cost of the apparatus may be decreased by eliminating the voltmeter and substituting a 5000ohm adjustable power resistor for potentiometer  $R_3$ . The potential applied to the electrodes is adjusted by moving the contact of the power resistor until a voltmeter inserted across the electrodes indicates the proper potential (for chloride with silver nitrate under the usual conditions, 4, this potential is 1.1 volts). The batteries may be eliminated and the instrument made lineoperated by the use of a suitable rectifying system if desired.

This routine instrument has given excellent results for the determination of chloride in such varied products as corn distiller's dried grains, saline suspensions of cortisone, and procaine penicillin. The instrument built according to the circuit diagram has proved to be an excellent tool for research and versatile enough for performing many varied amperometric titrations.

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- Inexpensive Conductivity-Determining Equipment. Robert M. Creamer and D. Harry Chambers, U. S. Bureau of Mines, College Park, Md.

DURING experiments with nonaqueous electrolytes it became necessary to measure the conductivity of many solutions. Experiments conducted with several signal sources and detecting devices, when used in conventional bridge circuits, indicated that a vacuum-tube oscillator source and a sensitive alternating current vacuum-tube voltmeter gave excellent results. These instruments have been recommended before for conductivity determinations but their cost has been high. Recently instruments in an unassembled form have been made available at a very reasonable cost (Heath Co., Benton Harbor, Mich., Electronic Instrument Co., Brooklyn 11, N. Y., and others). These instruments provide good precision, ease of operation, and excellent flexibility in determining conductivities.

The vacuum-tube voltmeter has the following advantages: insensitivity to room noise, wide frequency sensitivity, sensitivity

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adjustable to the demands of the bridge, high internal resistance which permits high resistances to be measured, relatively simple construction, and precise null indication at relatively high minimum signal at bridge balance. The voltmeter must be reasonably sensitive to achieve good precision. With a meter that has a full scale sensitivity of 0.01 volt, it has been found possible to compare resistances from about 100 to greater than 10<sup>6</sup> ohms with a precision within 0.1% with a Leeds & Northrup 7651 potentiometer at 1000 cycles. When resistances of  $5 \times 10^4$ ohms or greater are compared a Wagner ground should be used to reduce null reading and increase precision. A kit-type vacuumtube voltmeter has given excellent service without any changes in circuitry.



The less sensitive multipurpose vacuum-tube voltmeters can be used if a conventional amplifier such as is shown in Figure 1 is also employed. This will allow the sensitivity of the voltmeter to be increased by a factor of 100. This amplifier requires a meter of  $10^{\circ}$  ohms or higher internal resistance as an indicator.

Because the vacuum-tube voltmeter responds to 60-cycle as well as 1000-cycle voltages, relatively high precision can be obtained if the bridge is energized by one half of a small, centertapped, 2.5-volt, radio filament transformer. A series of conductance determinations made at this frequency checked those made at 1000 cycles to within the limits of precision of the determination, if an alternating current vacuum-tube voltmeter was used.



If a higher or variable bridge energizing frequency is required, an audio-frequency signal generator can be used to energize the bridge. These generators usually have low harmonic content in their outputs; thus the bridge is easier to balance because of lower residual null voltage. They are also rapidly variable over a wide frequency range, if this is required. These instruments are available unassembled at reasonable cost, and usually require 1099

few changes for conductivity work. Most of these devices cannot be directly connected to a low-resistance slide-wire. Much better operation will be achieved if a small, adjustable "line-tovoice coil" radio transformer is used to match the signal source and bridge. The transformer should be adjusted for maximum bridge voltage. If a low-resistance slide-wire is used and the signal generator has much less than 0.02-watt output, it may be necessary to add a power-output stage as described by Sulzer [*Electronics*, 25, 95 (January 1952)] and shown in Figure 2. This may replace the existing output circuit if 25 ma. at 200 to 300 volts and 6.3 volts at 0.45 ampere are available.

Instruments of the type described have been found useful in laboratory work [Phillips, J. P., J. Chem. Educ., 30, 70 (1953)]. Assembly is facilitated by the comprehensive directions that accompany the instruments and by the fact that both are relatively simple. The accuracy possible with their use depends on the accuracy of other bridge components. These two instruments, however, are useful with apparatus capable of very precise determinations. Thus, accuracy is easily obtainable at reasonable cost.

Use of Surface Active Agents in Breaking Emulsions Formed in Vegetable Extractions. C. L. Dunn and R. H. Earle, Jr., Hercules Experiment Station, Hercules Powder Co., Wilmington, Del.

IN DETERMINING insecticide residues in various foodstuffs, the material to be analyzed is frequently extracted in a finely divided state with a suitable solvent. An effective device for this operation is a Waring Blendor or similar apparatus. Many materials when so treated form very stable emulsions, and solvent extract recoveries are poor. Large quantities of salts, such as sodium sulfate, are sometimes effective in breaking these emulsions, but frequently the emulsions are resistant to such treatment. It has been found in this laboratory that surfactants can be used in some cases to break the emulsion. Preliminary information is given here for the use of other workers faced with similar problems.

The use of surfactants in breaking oil-water emulsions was reported by Lawrence and Killner [J. Inst. Petroleum, 34, 821-56 (1948)]. In light of their findings the following surfactants were evaluated for demulsification properties:

Anionic. Allied Chemical's Nacconol N.R.S.F., Monsanto's Santomerse 3, Ninol Laboratories' Toximul 300, and Atlantic Refining's Ultrawet K.

Cationic. Armour's Armac HT, Arquad 14, and Arquad 2HT. Nonionic. Hercules' Synthetics C-71.

The chemical compositions of the surfactants used are not readily available. In general, the anionic detergents are alkyl aryl sulfonates; the cationics are quaternary ammonium salts; and the nonionic is an alkaryl-alkylene oxide adduct.

In most cases separation was obtained to a greater or lesser degree. Ultrawet K with sodium sulfate was most satisfactory under the conditions tried. Typical data are given in Table I.

Further investigation might have revealed a more efficient demulsification system, but needs were met by this system. Separation was obtained in 5 to 10 minutes with a 40 to 75% recovery of extraction solvent. Longer standing did not materially increase the recovery. Centrifugal separation of untreated emulsions gave low and variable recoveries in addition to being time-consuming and laborious.

**Extraction and Demulsification Technique.** The emulsionbreaking technique was applied to three different foods which formed stable emulsions during extraction: potatoes, canned field peas, and peeled oranges.

The samples were pulped in an appropriate solvent using a stainless steel stirrer fitted with sharp vanes and driven by a 1/30-hp. Eclipse Model ARL air stirrer. With the stirrer on,

Table I. Demulsification Using Surfactants

Material	Sample, Grams	Solvent	Solvent, Ml.	Na2SO4, Grams	Ultrawet (25% Solids), Ml.	Extract Recovered, Ml.
Canned field peas	500	Benzene	1500	250	7	1100
Peeled						1100
oranges	1500	Heptane	1500	400	15	1100
Potatoes	1000	Benzene	2000	400	10	750

anhydrous sodium sulfate was added and thoroughly dispersed. The stirrer was removed and the Ultrawet solution gently blended in with a spatula. (Vigorous stirring resulted in formation of an unbreakable emulsion.) After standing for several minutes, the emulsion broke. The clear supernatant was decanted, washed with distilled water, and dried over anhydrous sodium sulfate. The amount of Ultrawet needed to break the emulsion appeared to be somewhat critical and had to be determined experimentally for each material.

Preparation of Ultrawet Solution. As total chlorine determination was to be used as a measure of insecticide content of the extracts, it was desirable to use a chloride-free solution of Ultrawet K. The most satisfactory method of preparing this material was by passage through an ion exchange column containing IRA 400 (Rohm and Haas). Using a column 4 cm. in diameter and 45 cm. long, two passes (the column regenerated between passes) were sufficient to purify 1 liter of Ultrawet K solution containing 25% solids. After the second pass, the column could be used without regeneration for preliminary chloride removal from an untreated batch of solution.

# **Precision Metal and Glass Syringe with Teflon Gaskets.** C. V. Robinson, New England Center Hospital, Boston, Mass.

THIS syringe was developed for use with a special vacuum system for the assay of radioactive water [Robinson, C. V., *Rev. Sci. Instr.*, 22, 353 (1951)]. In the procedure it is necessary to deliver 0.2 ml. of water into a partial vacuum drop by drop with good control. It is also desirable to deliver as nearly as possible the entire amount and to be able to clean and dry the syringe readily. The ordinary type of syringe retains 60 to 70 mg. of water in the hub of the needle and is difficult to control unless it is heavily greased; in this case it is difficult to clean and decontaminate. The syringe described here has a smooth, controllable action of adjustable resistance, delivers nearly the entire sample except for what is in the bore of the needle, requires no grease, and may be readily taken apart, cleaned, and dried.

The syringe consists of three principal parts: the barrel, 1, the combined needle and plug, 2, and the plunger, 3 (see Figures 1 and 2). In the syringe shown, the barrel is a piece of precision-bore borosilicate glass tubing (manufactured by Fischer & Porter Co., Hatboro, Pa.) of 0.1875 inch bore and 2 inches long. The useful capacity is 0.7 ml. and the motion is 0.221 inch per 0.1 ml. The needle-and-plug assembly consists of part 2A made by silver-soldering together the stainless steel tube of a 3-inch No. 22 needle and the brass plug proper, the brass flange, 2B, the brass nut, 2C, and the Teflon (tetrafluorethylene resin manufactured by Du Pont) gasket, 2D. This assembly is inserted into the barrel and screwed together tightly enough so that the expansion of the gasket holds the plug firmly in place. Reasonable care must be exercised, as overtightening will break the glass. The plunger, 3A, similarly has a sleeve, 3B, nut, 3C, and Teflon gasket, 3D, and is tightened to give the desired amount of resistance. The plug or plunger is tightened by applying small wrenches to the nut and to the flat surfaces which have been filed at the end of the threaded portion (see Figure 1).

Teflon is suitable as the gasket material because it is readily deformed, is hydrophobic and chemically inert, and has a greasy quality, so that it slides smoothly over glass.

Several tests were made to compare the properties of this type of syringe with the standard 1-ml. tuberculin syringe. The pre-





Figure 2. Precision Syringe Over-all length 17.5 cm.

cision syringe was found to deliver all but 16 mg. of water, 14 mg. of which were in the bore of the needle. The tuberculin syringe with the same size of needle, 3-inch No. 22, was found to have a 90-mg. residue at the end of delivery, 13 mg. of which were in the syringe, 62 mg. in the hub of the needle, and 15 mg. in the bore of the needle.

A plunger-stop was provided for one of the precision syringes to test the reproducibility of delivery. A series of six deliveries gave an average of  $0.4071 \pm 0.00023$  gram, with a standard deviation of 0.57 mg, for a single delivery.

Teflon needle caps are provided for these syringes, so that samples may be stored in them (see Figure 2). Evaporation rates at room temperature were measured on a pair of capped precision syringes and on a pair of tuberculin syringes whose tips were waxed shut. Each syringe contained about 0.4 ml. of water. The average rate of evaporation for the tuberculin syringes was found to be 2 mg. per day. When the precision syringes were adjusted for moderate plunger resistance, the average rate was found to be 0.6 mg. per day and, when adjusted for high resistance, less than 0.1 mg. per week.

A number of syringes of this type have been in use in this laboratory over the past year, not only for use with the tritium assay system but also for directly sampling cerebrospinal fluid from catheters connected into the subarachnoid space and for storing these samples prior to assay. The syringes may be autoclaved with no other ill effect than loosening of the gaskets, which can be corrected by subsequent tightening under sterile conditions. Refrigeration seems to offer no difficulty even when ice crystals are formed. Stainless steel construction and handtightening could be incorporated into the design without major difficulty. Although only three sizes have been built to date— 0.7-ml., 1.0-ml., and 5-ml. capacity—it should be practical to extend the range of sizes to smaller and larger syringes.

The plunger and barrel parts of the 0.7-ml. syringe provide a nicely controllable pipetter which requires no grease and is sufficiently air-tight even when adjusted for moderate resistance.

For pistons of 3-mm. diameter or less it is preferable, because of the simpler construction, to make the plunger a solid metal rod and use a loose-fitting metal barrel with a Teflon packing and a packing nut to make the seal. A stainless steel piston 2.3 mm. in diameter made in this way [Chapman-Andresen, C., and Robinson, C. V., *Compt. rend. trav. Lab. Carlsberg, Ser. chim.*, 28, 343 (1953)] has proved to be satisfactory for fairly rapid delivery of several microliters of water, adjustable to  $\pm 0.01 \ \mu$ l. In this type of construction a much smoother action is obtained if the plunger is polished lengthwise than if it is polished around.



- ACCURATE—Weighing is by the substitution method. To weigh an object in the pan, built-in weights on the same beam as the sample pan are mechanically removed from the beam by turning dials on the front panel. The projected scale has the same values whether one gram or 200 grams is being weighed. Weights are constructed of a high chrome-nickel alloy and are well within Class S tolerances.
- **FAST**—All weights from 200 grams to 100 mg are operated by dials on the front panel. The total of the weights employed is read directly from the counters on the panel. Values from 100 mg to 1/10 mg are read on the projected scale at the right of the counters.

An adjustable air damping system stops the swing of the beam in the shortest practical time without creeping.

**EASE OF OPERATION**—The optical system provides high magnification and sharp definition of the projected scale. It is easy to read 1/10 mg on the vernier and possible to estimate 1/20 mg. The zero adjustment is optical and controlled by a knob on the case.

A single lever controls the beam arrest, pan arrest and light.





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## ASTM Mass Spectrometer Session Draws Record Attendance

NEW ORLEANS.—Attendance figures here at the Jung Hotel clearly indicate the ASTM E-14 Committee Meeting on Mass Spectrometry has reached the status of "mecca" for national experts in the field. From listening to 48 papers in 4 days (May 24 to 28) one gathered the impression that there is a definite trend by manufacturers to produce smaller, portable units at lower cost with performance characteristics comparable to larger laboratory installations. Smaller spectrometers are now being designed specifically for monitoring and control of process gases, with rugged construction capable of continuous service.

Although the principle of applying combined electrical and magnetic fields for improved ion beam focusing was reported almost 20 years ago, Consolidated Engineering has now introduced a small commercial instrument to exploit recent advances in techniques. The cycloidal analyzer, as described by C. F. Robinson and L. G. Hall, is free of aberration due to cross-talk, has substantially higher dispersion than a sectortype instrument, and will make a clean separation between two peaks when there is no valley present between them.

To evaluate adequately the nonmagnetic radio-frequency principle of mass selection, research workers at Phillips Petroleum have constructed a 5-stage tube and have studied the operating variables. T. C. Wherry and F. W. Karasek said the RF principle appears attractive because it promises to give a mass spectrometer of simple construction and rugged nature. Data indicate the tube is capable of sufficient resolution to handle many hydrocarbon analyses encountered in a petroleum refinery. Suitable accuracy was also obtained during the analysis of a gas mixture containing 64.8% hydrogen, 3.0% helium, 9.0% methane, 20.1% nitrogen, and 3.7 argon.

Beckman Instruments now has a mass spectrometer (RF linear decelerator) which features resolution to 100 and a dynamic range of 2000 to 1, reported W. Donner. Stressing mass spectra and stability data, Donner said the cracking pattern stability for both *n*-butane and propane was within 1%; that sensitivity stability is in the range of 2%. This performance is obtained without a temperature controlled source. Elimination of any magnet gives a compact spectrometer tube especially suited for process applications where size is an important consideration.

At Esso Laboratories in Baton Rouge, K. P. Lanneau, L. H. Lane, and R. J. Vick have been evaluating the new ion resonance spectrometer manufactured by General Electric. From a performance point of view, Lanneau said-it has excellent sensitivity and resolution up to mass 100 for application to gaseous compounds. It can be used to reproduce mass spectra on a cathode ray oscillograph, and is well suited for analysis of regenerator vent gases in the refinery (CO<sub>2</sub>, CO, O<sub>2</sub>, N<sub>2</sub>). The instrument has unusually high resolution in the low mass range, should offer interesting possibilities in the field of atomic energy. Spectra show complete resolution between mass peaks for helium and D<sub>2</sub> (m = 0.04 atomic mass unit).

cases. He said he believed the method

was the most precise for mobility stud-

limitations which paper chromatog-

raphy can overcome. For instance,

Herbert A. Sober told that recent work

by himself and associates indicates that

large protein molecules can be separated

by a cellulose ion exchanger developed

by them. In addition, they find the

method often aids them to have a higher

resolving power than in electrophoresis.

either one of the techniques according to

William R. Carroll, NIH, should realize

that the two techniques are complemen-

Analysts trying to specialize with

Electrophoresis, however, has certain

#### Pros and Cons of Paper Chromatography Weighed at NIH Symposium

ies

WASHINGTON.—Instrument makers, designers, and users gathered here at the National Institutes of Health May 24 to 26 for the Fourth Annual Research Equipment Exhibit. In addition to the exhibit, there were four symposia on instrumentation techniques and the latest developments in instrument research.

Most heavily attended symposium was on electrophoresis and chromatography. Symposium chairman, Henry G. Kunkel, opened the session, with a discussion of the advantages and disadvantages each technique offered. He pointed out that for the present he favored reliance on boundary electrophoresis for definitive conclusions in most

Dean Burk (seated) and George L. Hobby, both of National Cancer Institute, look over new electrostatic electron microscope produced by Farrand Optical Co.

Kent M. Wight, National Cancer Institute, and Lawrence R. Crisp, NIH, discuss compact polarograph made by Patwin Instruments. Precalibrated electrodes are used for faster analyses of routine samples



VOLUME 26, NO. 6, JUNE 1954



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#### NEWS

Robert J. Koegel (left). National Cancer Institute, explains features of rotary Kieldahl digestion apparatus (American Instrument Co.) he helped design, to Elizabeth G. Frame, NIH, and Leonard H. Bolz, National Bureau of Standards. Several changes in the instrument were incorporated at the suggestion of the Committee on Microchemical Apparatus of the ACS Division of Analytical Chemistry



tary and should not be divorced from one another. Rather, they should be used to cross reference each other, with each contributing its obvious advantages to the final conclusion.

In the symposium on methodology and instrumentation in microanalysis, Alsoph H. Corwin, Johns Hopkins, discussed the design and construction of a new microbalance. He hopes that it will eliminate many of the previous sources of error which have occurred. It is housed in a double sheath of aluminum, is free from relative humidity and temperature changes, and has incorporated a special device which makes it free from vibration. Balance uses specially fabricated materials and is designed and constructed by techniques developed by him over a period of 20 years. He has taken especial pains in constructing the balance to incorporate strain-relief devices.

An electronic device which can obtain data on the configuration of individual molecules was described by Raold Wangsness, Naval Ordnance Laboratory, White Oak, Md. Wangsness says he uses the technique of nuclear magnetic resonance to measure quadruple moments of molecules. Comments by others after the paper had been delivered said the apparatus was about "one step removed from visualization of the actual molecule."

Some of the instruments on display at the exhibit included a recording spectrophotometer by Beckman Instruments. It automatically records the light absorption spectra of a sample and enables the operator to make a closer determination of the chemical composition of the sample. Farrand Optical showed an improved electron microscope which permits magnification to 200,000-plus diameters, gives high resolution, and costs \$12,500 to build.

#### **Glycerol Determination**

The Uniform Methods Committee of the American Oil Chemists' Society has recommended that:

The acetin method for glycerol, Ea 4-38, and the dichromate method for glycerol, Ea 5-38, be removed from AOCS official methods.

The sodium periodate method for glycerol, Ea 6-51, be retained as the only AOCS official method for glycerol, with minor changes.

The recommendations were approved by the society at its April 1954 meeting.

#### 100,000th Scientific Instrument

Commemorating the production of the 100,000th instrument—a recording spectrophotometer—by Beckman instruments, Inc., South Pasadena, Calif., Arnold O. Beckman, president, presented a gold nameplate to R. Francis Faull, manager of the California Research Corp.'s La Habra Laboratory, where the instrument will be used in research on petroleum hydrocarbons.

#### **Exhibit of Physical Society**

The exhibition of the Physical Society opened April 8 at the Imperial College of Science and Technology, London, England. Among the scientific instruments and apparatus was the "flying spot" microscope which uses television to magnify to an extent that rivals the electron microscope. The pitch indicator developed by Kelvin Hughes gives the pilot an exact reading of the nose elevation of his aircraft during take-off. Photographic representations of the electron density in a crystal were shown by the Crystallographic Department of the Cavendish Laboratory, Cambridge. A micro hardness tester for rubber

#### ANALYTICAL CHEMISTRY

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#### **Royal Pan**

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What has happened is that at long last we have found some stretch in the chains that lock graininess and light sensitivity in mutual bondage. We can make an emulsion that gives less graininess without yielding sensitivity, or we can have sensitivity without paying in graininess. The latter seemed the better choice for the first commercial application, which we call *Kodak Royal Pan Film*.

Its graininess is perhaps even a little less than that of the highly satisfactory Kodak Super Panchro-Press Film, Type B (fastest Kodak film hitherto offered the press photographer, a connoisseur of emulsion speed), but at the practical gamma of 0.7 it has twice the speed. Exposure Index is 200 for daylight, 160 for tungsten. Its characteristic curve combines the best features of Kodak Super-XX Panchromatic Sheet Film and Kodak Super Panchro-Press Film, Type B. There is more exposure latitude, more development latitude, more detail in the dark areas, more detail in the light areas, less need for dodging in printing.

If your work, business, or hobby has been crying out for faster sheet film, all you need do now is send someone out to the nearest Kodak dealer for some Kodak Royal Pan Film. There might be several press photographers at the counter ahead of him.

#### **Microprint reader**

Now on sale is the *Kodagraph Microprint Reader*, a sleek and unobtrusive instrument intended to create enthusiasm for the microprint idea among those who have had doubts about the comfort of reading microprint day in and day out. This one takes any size of microprint card from  $8\frac{1}{2}$ " x 14" down and thus involves no commitment to any one size. Anyone interested enough to drop a post card to Eastman Kodak Company, Industrial Photographic Division, Rochester 4, N. Y., will be directed to the nearest dealer who can demonstrate why this reader is worth \$300.

#### **Texture in food**

This is the newest form of *Myverol Distilled Monoglycerides*.

Monoglycerides are fats having one fatty acid chain per glycerine molecule instead of the usual three.



The monoglyceride business has been excellent lately. Now we find that in the form of these beads barely visible individually to the naked eye, our monoglycerides have caught the fancy of certain segments of the ice cream trade.

We didn't invent monoglycerides. (Nature did, probably about the same time she invented fats in general.) What we do is use our unique molecular stills to concentrate and purify monoglycerides from the reaction mixtures in which they are formed. The reason they're selling so well seems to be the fact that in imparting various desirable textural characteristics to foods like baked goods, margarine, shortening, dessert toppings, and confectionery, a little distilled monoglyceride goes a lot farther than the undistilled reaction mixtures which would otherwise have to suffice.

There are numerous physical and chemical variations of Myverol Distilled Monoglycerides that we can furnish for free trial in response to inquiries directed to Distillation Products Industries, Rochester 3, N. Y. (Division of Eastman Kodak Company).

#### Advice on diffraction

The atomic number of chromium is 24, of copper 29, of molybdenum 42. *K*-emission from targets of one or another of these elements is com-

monly used for x-ray diffraction work. The higher the atomic number, the shorter the wavelength of the K-radiation. It takes more photographic silver to stop—and therefore respond to—short-wavelength photons than those of longer wavelength. Thus it comes about that for chromium K-radiation, as example, there is considerably less difference between the speeds of our slowest and fastest x-ray films than there is for the K-radiation from molybdenum.

Kodak Industrial X-ray Film, Type K is our fastest for x-ray diffraction. Like most x-ray films, it has emulsion on both sides. There is also Kodak Single-Coated X-ray Film. It has a little more than half the speed of Type K for chromium K-radiation but less than a quarter of Type K speed for molybdenum K-radiation. (The profusion of K's here is purely coincidental; we can clear up the confusion, if any, by sending you our free chart "Kodak Films for X-ray Diffraction.") There may be instances where an x-ray diffractionist needs this higher speed for the shorter-wavelength radiation but is inconvenienced by the parallax associated with the presence of two images on opposite sides of the base.

Though we doubt this combination of circumstances comes up frequently, we can perform a slight but perhaps useful service by showing a couple of easy ways to remove one of the images. Economically this has advantages over the tricky and expensive business of launching a special, extra-silver-rich, single-coated film. Researchers with modest budgets who regard film as the least expensive and most versatile x-ray receptor for diffraction patterns will agree, we hope.

Write Eastman Kodak Company, X-ray Division, Rochester 4, N. Y., for a reprint on removing the second image, for a copy of the x-ray diffraction film selection chart, or for the address of the nearest Kodak X-ray Dealer. It is he who can sell you 25 feet of 35mm Kodak Industrial X-ray Film, Type K for \$2.61 or 100 sheets of 4" x 5" Kodak Single-Coated X-ray Film for \$7.43.

Prices quoted are subject to change without notice.

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(Nash and Thompson), will test the hardness of a specimen of rubber  ${}^{3}/_{16}$  inch in diameter, a nucleonic weight gage (Electronic Instruments) uses radioisotopes to control the weight of tobacco used in cigarette-making machines, and the unit developed by Mullard Ltd., for use with a resistance furnace temperature controller, will automatically control the temperature of the furnace from 1° to 1000°C. over short or long periods.

The small analog computer built of standardized component units exhibited by Elliot Brothers (London), Ltd., has an accuracy of 0.1% as against 1% for most existing small analog computers. A direct-reading spectrometer for determining the composition of an alloy was shown by Hilger and Watts.

A number of exhibits were especially concerned with the development of British nuclear industry. The Atomic Energy Authority showed a small scale working model of an electromagnetic pump which may be used for pumping liquid metals such as mercury or sodium through the breeder reactor being built at Dounreay, in order to draw off the heat. Equipment used for surveying for radioactive materials from aircraft was shown by Edison Swan Electric Co.

CARL

A range of scintillation phosphors for use in scintillation' counters was shown by Isotope Developments, Ltd., and a number of radiation counters of different shapes and sizes have been designed for both industrial and personnel use.

#### Southwest Regional Meeting

The Dallas-Fort Worth Section of the AMERICAN CHEMICAL SOCIETY will be host to members during the Tenth Southwest Regional Meeting, to be held in Fort Worth December 2 to 4. The region embraces Texas, Louisiana, Arkansas, Oklahoma, and New Mexico.

#### ANALYST'S CALENDAR

- American Chemical Society. 126th National Meeting, New York, N. Y., September 12 to 17.
- Seventh Annual Analytical Symposium. Minneapolis, Minn., June 18 and 19.
- Sixth Annual Oak Ridge Summer Symposium on Modern Analytical Chemistry. Oak Ridge, Tenn., August 23 to 27.
- Sixteenth Midwest Regional Meeting, American Chemical Society, Omaha, Neb., November 4 to 6, 1954.

Papers are invited for presentation in the fields of analytical chemistry, biochemistry, chemical education, industrial and agricultural chemistry, organic chemistry, petroleum chemistry, and physical and inorganic chemistry. Information on title, author's name, and subject matter must be received not later than September 1. Two copies of a 200 word abstract must be received not later than October 1. General correspondence regarding the technical program should be sent to Harold Jeskey, Southern Methodist University, Dallas, Tex. Chairman of the section on analytical chemistry is C. T. Kenner, Southern Methodist University, Dallas, Tex.

#### Microchemistry and Microchemical Instrumentation

A symposium on Microchemistry and Microchemical Techniques will be held September 17, 1954, in connection with the Instrument Society of America First International Instrument Exposition, at Convention Hall in Philadelphia. The purpose of the symposium is to show that microchemistry has now come of age and supplies a useful and much needed technique. Historical develop-

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ment and newest progress in various phases will be presented with emphasis on instrumentation. The symposium should serve as a review of past and present accomplishments as well as an idea of what may be expected in the future.

Opening of Symposium and Introductory Remarks. H. K. ALBER, Chairman, Arthur H. Thomas Co., Philadelphia, Pa.

Microchemical Techniques. A. A. BENE-DETTI-PICHLER, Honorary Chairman, Queens College, Flushing, N. Y.

Quantitative Organic Microanalysis. AL STEVERMARK, Hoffmann-La Roche Inc., Nutlev. N. J.

Physicochemical Methods in Microanalysis. JOHN MITCHELL, JR., Experiment Sta-tion, E. I. du Pont de Nemours & Co., Wilmington, Del.

KONTES

Microanalysis in the Petroleum Industry. HARRY LEVIN, Texas Oil Co., Beacon, N. Y. Chemical Microscopy (Fusion Methods). WALTER C. MCCRONE, Armour Research

Foundation, Chicago, Ill. Discussion and showing of moving pictures

depicting microchemical techniques.

The committee for the symposium consists of Arthur H. Hale representing the Affiliated Analytical Group, Delaware Section, AMERICAN CHEMICAL SOCI-ETY; Jos. Alicino, representing the Metropolitan Microchemical Society, New York; Wm. C. Ellenbogen representing the Analytical and Microchemical Group, Philadelphia Section, AMERI-CAN CHEMICAL SOCIETY; Richard Zahner representing the South Jersey Section. AMERICAN CHEMICAL SOCIETY; Herbert K. Alber, Chairman.

Members of the sponsoring groups or sections may obtain free preregistration tickets from their representatives on the committee

#### Summer Courses at University of Padua

The University of Padua will hold summer courses at Bressanone (Dolomites zone), Italy, including a series of lectures at the Center of Polarography.

July 26 to 30. Production and Use of Radioactive Isotopes.

August 2 to 6. Theoretical and Practical Polarography.

August 9 and 10. Chromatography.

August 11. Electrophoresis.

August 12. Spectrophotometry. August 13. Electronics.

Information is available from the University of Padua, Via L. Loredan, Padua, Italy.

#### Zeitschrift für Physikalische Chemie

The May 1954 issue of the Zeitschrift für Physikalische Chemie, Neue Folge (Volume 1, Nos. 1/2) contains papers by Abel, Bandow, Block, Bonhoeffer, Gonser, Hauffe, Kahlweit, Rahmel, Schulz, Schwab, Strehlow, and Suhrmann. It

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is published by Akademische Verlagsgesellschaft m. b. H., Holbeinstrasse 25–27, Frankfurt-am-Main, Germany.

#### NEW BOOKS

Chemistry of the Lanthanons. R. C. Vickery. vii + 280 pages. Academic Press, Inc., 125 East 23rd St., New York, N. Y., 1953. \$6.00. Reviewed by CHARLES V. BANKS, Iowa State College, Ames, Iowa.

The material presented was taken primarily from the literature prior to 1952, although several references are included to work published during 1952.

After four short chapters entitled "Historical Development," "Modes of Occurrence," "Structure, Spectroscopy and Paramagnetism," and "Isotopic Constitution, Radioactivity and Valency," the author discusses at great length (8 chapters, 132 pages) the subject of separational techniques. The older separational techniques are discussed in great detail, while only 4 or 5 pages are devoted to each of the newer techniques of ion exchange and solvent extraction methods.

Chapter 13 gives a rather complete discussion of the properties of the lighter

lanthanons, which includes a discussion of the oxides, hydroxides, carbonates, oxalates, halides, nitrates, sulfates, sulfides, phosphates, cyanides, perchlorates and complex compounds. Chapter 14 is devoted to a brief discussion (5 pages) of the properties of the heavy lanthanons.

Analytical methods for the rare earths which are based on oxidationreduction, average atomic weight, neutralization, magnetic susceptibility, absorption spectroscopy, emission spectroscopy, luminescence, fluorescence, flame photometry, polarography, ion exchange, and radioactivity are discussed in Chapter 15.

Uses and applications of the lanthanons are briefly discussed in Chapter 16 and yttrium is discussed in the appendix.

The book is well written, but has several typographical errors.

Chromatographic Methods of Inorganic Analysis. F. H. Pollard and J. F. W. McOmie. viii + 192 pages. 26 figures. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y., 1953. \$5.50. Reviewed by LOUIS B. ROCKLAND, Fruit and Vegetable Chemistry Laboratory, Western Utilization Research Branch, U. S. Department of Agriculture, Pasadena, Calif.

Ostensibly a coverage of general chromatographic methods for inorganic analysis, the book is concerned largely with the paper chromatography of inorganic ions. The emphasis on paper chromatography is not necessarily objectionable, because of the great utility of these techniques for both qualitative and quantitative inorganic analysis. The authors have presented an excellent summary of procedures for the estimation of inorganic ions based on their own work as well as contributions from other groups in the field. The text is well organized and written in a manner that should be acceptable to students as well as research workers.

A brief but adequate history of the development of chromatography is presented, although the reviewer was disappointed not to find reference to the paper by H. W. Hermance [Bell Labs. Record, 16, 370 (1938)] describing the use of paper as a medium for microanalysis of inorganic ions. As several other very recent books on paper chromatography have developed considerable space to literature reviews, it is creditable that the authors have minimized extraneous material and have



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#### NEWS

prepared the first practical book concerned with inorganic analysis by paper chromatography techniques. Extensive tables of chromatographic solvents and  $R_I$  values as well as detailed descriptions of procedures are presented. Workers in other fields of endeavor may find interest in the discussion of cellulose powder chromatographic columns.

An unusual feature of the book is the presentation of two schemes for qualitative inorganic analysis using filter paper chromatography procedures exclusively. These schemes of analysis should find interest among teachers of chemistry as well as workers dealing with problems of separation and identification of short half-life radioisotopes. Detailed descriptions are also presented for the quantitative estimation of molybdenum, boron, gold, and uranium as individual ions, and copper, iron, aluminum, titanium, vanadium, uranium, bismuth, antimony, and cadmium in various types of mixtures. Other brief sections are concerned with chromatography on media other than cellulose and a review of the recent work on electromigration and electrochromatography of inorganic ions

One of the few shortcomings of this volume is the omission of data on the sensitivities of the procedures employed for the detection of anions and cations. The reversal of the letters identifying the chromatograms on the colored frontispiece may confuse the casual reader. One other minor oversight was the mislabeling of Figure 5b as an illustration of the test tube chromatography procedure referred to in the text.

While this book may ultimately prove to be an interim report on the development of chromatographic procedures for the separation and estimation of inorganic ions, it comes as an excellent introduction to a rapidly growing field of interest.

#### Handbuch der Analytischen Chemie. W. Fresenius and G. Jander. Springer-Verlag, Berlin, Göttingen, Heidelberg, Germany, 1953. Band Va $\beta$ . xiii + 370 pages. DM 72. Band VIII b $\gamma$ , xiii + 118 pages. DM 24. Reviewed by HANS STOL-TEN, General Anilinè & Film Corp.

Volume  $Va\beta$  is a continuation of Part Three of the "Handbuch der Analytischen Chemie" dealing with quantitative determinations and methods of separation. It treats the elements of the fifth main group, and this section is specifically concerned with the element phosphorus.

In keeping with the encyclopedic nature of this work, the treatment of the subject matter is extremely comprehensive. The exhaustive series of analytical methods for the determination of phosphorus are all-encompassing. The chemical gravimetric and volumetric procedures are accompanied by all the applicable instrumental techniques. These include macro, semimicro, and micro quantitative determinations. The analyses include all the phosphoric, phosphorus, and hypo- acids in addition to all the phosphates, phosphine, and elemental phosphorus.

The value of this series as a reference work is enhanced by the orderly and logical method of presentation. A discussion of the eternally important problem of sampling precedes the description of the analyses. The latter are introduced by a statement of the specific chemical reaction employed followed by the preparation of reagents, the analytical procedure, and observations covering sensitivity, precision, interferences, and limitations. Each basic method is accompanied by all the pertinent literature references. This volume may be considered a catalog of the literature relating to phosphorus as well as a catalog of analytical methods.

The "Handbuch der Analytischen Chemie" should attain that stature for analytical chemists which Beilstein occupies for the organic chemists.

The elements of the eighth subgroup are treated in Band III b $\gamma$  in the same manner as phosphorus. This volume is equally divided between the individual determinations of platinum, palladium, iridium, rhodium, osmium, and ruthenium and the separations of each of these metals from all, or any combination of, the others. More emphasis is placed on the physicochemical and instrumental methods of analysis.

A section is devoted to problems, under the heading "Special Cases."

#### Determination of Dysprosium in Holmium by Radio-Activation Analysis. G. Phillips and F. W. Cornish, Chemistry Division, Atomic Energy Research Establishment, London, England, 1953.

The dysprosium content of specpure holmium oxide has been determined by radio-activation analysis without chemical separation. The composite decay curve for the sample after neutron irradiation has been resolved by calculation from the experimental points. Dilution of the sample by grinding with sucrose in the solid state has been shown to lead to an inhomogeneous mix compared with aqueous dilution. Two series of experiments have been carried out using aqueous dilutions; liquid counting gave  $11.71 \pm 0.09\%$  dysprosium in Ho<sub>2</sub>O<sub>3</sub>, while solid counting gave  $11.66 \pm 0.16\%$ dysprosium in Ho<sub>2</sub>O<sub>3</sub>. The error limits refer to standard deviation of the mean.

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Infrared spectra of single fibers and crystals can be run with aid of hood on infrared microscope being adjusted by Vincent J. Coates



Infrared microscope for fiber and crystal studies, and beneath it, a 10meter gas cell for making analyses of trace components. Martha Lester is the operator

VOLUME 26, NO. 6, JUNE 1954

Over-all view of Perkin-Elmer laboratory. Tables in foreground are used as work surfaces for sample preparation, changing prisms, checking spectra

## LABORATORY OF THE MONTH

#### Laboratory setup enables prospective user to evaluate use of infrared instrumentation

**I** N THIS ERA of new instruments and methods, instrument manufacturers are finding that the customer must be shown the value of expensive equipment before he buys. Before a company or research laboratory is willing to invest a sizable sum of money in new instruments, it wants to be convinced that the instrument will prove a real asset to its analytical or quality control laboratory.

About two years ago, Perkin-Elmer Corp. set up a complete infrared laboratory to demonstrate to prospective customers how infrared instrumentation could be used to solve their particular problems. Since virtually every prospect for an infrared spectrometer is found to have some specific problem in mind for the instrument, it is the primary purpose of the laboratory to investigate the application of infrared analysis to prospective customer problems. Occasionally there are requests for flame photometer or electrophoretic analyses. Considerable time is also spent in developing new infrared analytical techniques and instruments, investigating improvements in existing instruments or accessories, and preparing data for papers delivered at technical meetings by Perkin-Elmer staff members.

At latest count, the laboratory submitted over 600 problem analysis reports last year. These run the gamut of practically all industries concerned with organics—food and drugs, through plastics, paints, explosives, and petroleum. About 60% of the analyses are conducted on solids, 35% on liquids, and 5% on gases. Over 25% of the requests are for quantitative analyses.



For further information, circle number 34 A on Readers' Service Card, page 41 A



Harry Hausdorff (left), director of the laboratory, explains the intricacies of a specific analysis to visiting chemist



Recent advance in infrared solids analysis is the potassium bromide press. Helene Sternglanz pours a mixture of sample and potassium bromide into the die, which is subsequently evacuated and pressed to form an infrared-transparent pellet

Equipment includes a small oven for drying specimens, a balance, and an exhaust hood. In the foreground is a glass-topped table for viewing and matching spectra

> Pure compounds, solvents, and samples are stored in the cabinet to the left. Crystal prisms for different regions of the infrared spectrum are in the center cabinet. To the right is a file of reference spectra including over 5000 spectra run off in the laboratory and 2000 API spectra





## Coleman's Ounce of Prevention Protects Clarity of Schenley Whiskies

Schenley was the first distiller to use the Coleman Photo-Nephelometer for routine testing of whiskey samples from the bottling line. In a recent ad in Time, Schenley said:

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Write for folder—"Nephelometry: A Short Cut to Precision Chemical Analysis." Dept. A., Coleman Instruments, Inc., Maywood, Ill.





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#### Michigan Symposium on Instrumentation for Industrial Hygiene included technical papers, reviews, exhibits, demonstrations

Not very long ago, the word "instrumentation" was more than likely to suggest the New York Philharmonic or the Boston Symphony Orchestra even to scientists and technologists. Since instrumentation has become recognized as a distinct branch of both science and technology, it has even acquired special emphasis in a variety of fields. So wide and extensive is this branch of knowledge that one not infrequently encounters instrument symposia devoted to a special field of science.

#### Instrumentation for **Industrial Hygiene**

We participated recently in a Symposium on Instrumentation for Industrial Hygiene at the School of Public Health of the University of Michigan. under the sponsorship of the University's Institute of Industrial Health and School of Public Health and some nine cooperating agencies in the field of public health, industrial health, and instrumentation. This symposium had four distinct objectives: (1) to bring together instrument makers and users of instruments in the field of industrial hygiene; (2) to make known what is needed and what is available; (3) to exhibit home-assembled devices and to exchange information and ideas on equipment and (4) to gather material for an Encyclopedia for Instrumentation for Industrial Hygiene.

All sessions and exhibits were held in the stately and impressive halls and chambers of the Horace Rackham Building from May 24 to 27.

A series of general sessions was devoted to comprehensive review addresses. In addition, there were technical sessions and papers devoted to specific types of instrumentation. The emphasis throughout was on instrumentation rather than a compilation of data obtained with instruments.

Nearly 100 instrument manufacturers were represented in well chosen exhibits. In addition, nearly 100 homeassembled instruments were exhibited by some 25 individuals, companies, universities, or research agencies. These represented instrumental developments for special problems, and, in most cases, were devices for which no appropriate instrument is commercially available. In our opinion, the value of such exhibits and demonstrations is becoming appreciated more and more by the scientific public. It has been an old and well established practice at national meetings of the American Medical Association and a similar, though more extensive, exhibit at the Dyson Perrins Laboratory in Oxford was one of the outstanding features of the 1952 International Congress of Analytical Chemistry.

The preparation of the encyclopedia is now under way. This will include not only all the papers presented at the symposium, but descriptions and illustrations of all instruments which were exhibited. It is likely that some of the papers will also appear in various scientific journals.

The subjects discussed at the symposium were, almost without exception, of direct interest to the analytical chemist. In many respects, it may be said that industrial hygiene is largely concerned with the analysis of toxic or hazardous materials. There are certain features which are more particularly significant to the industrial hygienist. Automatic recording, the initiation of alarms, or actual control of environmental conditions such as ventilation by Ralph H. Müller



is more important than it would be for the average analyst. Some problems, such as proper illumination or noise levels, are obviously of more interest to the physicist.

#### **Topics Discussed**

For the present, it may be helpful to list the topics which were discussed, because it may require something of the order of a year before the papers are published or collected ultimately in the encyclopedia. The review addresses were as follows:

Concepts and Purposes of the Sympo-um. William G. Frederick. sium.

Sampling and Analyzing Air for Con-taminants in Work Places. Leslie Silverman.

Laboratory Type Instrument of Specific Application to Industrial Hygiene. Hervey B. Elkins.

Instruments for Measuring Air Velocity and Metering Air. Knowlton J. Caplan. Instruments for Measuring Sound and

Vibration. Charles R. Williams. Instruments Specifically Designed for tmospheric Pollution Evaluation. Atmospheric

George D. Clayton. Home-Assembled Instruments. Warren

A. Cook.

Instruments for Measuring Ionizing Radiations. Karl Z. Morgan. Instruments for Measuring Ultraviolet, Visible and Lefenet Figure 1.

Visible, and Infrared Energy. Hugh Archer.

The technical papers included:

Instruments for Sampling and Analyzing Organic Vapors in Air. E. M. Adams. Developments in the Sampling of Air-Borne Dust. Theodore F. Hatch.

The Calibration of Gas, Vapor, and ust Instruments. Their Accuracy and Dust Instruments. Their Acc Sensitivity. E. W. Gilliland. Electrometric Instruments.

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#### INSTRUMENTATION

Instrumentation for Particle Sizing. Melvin W. First.

Absorption and Emission Spectroscopy. E. J. Rosenbaum.

Meteorological Measurements in Air Pollution Studies. E. Wendell Hewson. Filter Collecting Media. Leslie A.

Chambers. Application of Electronics to Home-Made Instruments. An Elementary

Made Instruments. An Elementary Treatment. Ralph H. Müller.

The Construction of Automatic Continuous and Intermittent Sampling Devices. William L. Wilson.

The Calibration of Airflow Measuring Instruments. George M. Hama.

Devices for Stack Sampling and the Measurement of Air Velocities in Stacks. Richard B. Engdahl.

Vibration Measuring Instruments and Their Application to Noise Control. H. C. Hardy.

Intermittent Sound Measurement. A Challenge. H. H. Scott.

Alpha Hazard Monitoring Instruments. Duncan A. Holaday.

Calibration of Instruments for Measuring Ionizing Radiation. Harold O. Wyckoff.

Infrared Measurement. W. G. Hazard. Ultraviolet Measurement. Rudolph Nagy.

The Measurement of Light. W. Glenn Pracejus.

It was amply evident from this meeting that the subject requires the combined talents of the physician, the industrial engineer, the analytical chemist, the toxicologist, and the instrument designer. While all but the latter are required to define the problem, it is largely the responsibility of the instrument designer to provide equipment which will maintain a safe, healthy, and efficient environment for the worker in industry.

When equipment can be designed which records the concentration of a given hazardous substance, and which also has the means for controlling or correcting the conditions that give rise to the hazard, we have the beginning of completely automatic correction. It is obvious that one cannot attain complete security unless the system also includes periodic self-calibration and "fail-safe" features.

#### Automation

Today, one hears a great deal about automation. Apparently, this is intended to apply to the state of affairs in which instrumentation is carried to its logical conclusion. Conditions are not merely measured by instruments, but the output of the latter is used to control a process. It is a complete closed-loop servo-system in which definitive instructions are supplied to the machine, by manual dial settings, or by punched card, or by tape-recorded

#### INSTRUMENTATION

instructions. From the analytical standpoint, if chemical analysis of the product is proceeding continuously, and at high speed, and if these results are used to control all process variables, we have complete automation.

As we have remarked frequently, there is no need or place for statistical quality control in such cases. Statistical considerations appeal principally to the legal, business, or administrative mind. Obviously, our present practices of grab or sample analysis require statistical treatment, but until instrumental inspection of the complete output is attained, we shall have to be addicted to these practices.

In a recent discussion on sampling, our good friend Beverly Clarke made the humorous and shrewd observation that 100% sampling is the ideal. Perhaps his audience was a bit too willing to accept the belief that one must settle for a much smaller sample. In these days, when more and more measurements of high precision are being made in microsecond intervals, the possibility of analyzing the entire output becomes increasingly likely. One rapidly approaches that feeling of security expressed by Mark Twain's "Christian faith and four aces." Although statistical considerations are becoming increasingly popular, there are some very fundamental and serious doubts being raised against their indiscriminate use. Einstein has become increasingly worried about probability considerations and has expressed the uncomfortable feeling in the hunch that "God does not roll dice."

#### Interpretation

There is no denying the fact that interpretation becomes increasingly difficult as information becomes more meager. There was a time when the manufacture of carbon granules for use in telephone transmitters was so poorly understood that very thorough and profound statistical studies were involved in order to maintain reasonable production. Those studies were justifiably famous, but they became less and less important as extensive research into the nature of the carbonization process was made. A crude analogy to what we are discussing is afforded by the field of high-speed computing. An analog computer possesses whatever precision we build into its components. A digital computer, which does nothing but counting, has absolute precision.



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Automatic analysis of up to 10 components in a sample in 10 minutes is possible with The Perkin-Elmer Corp.'s Multi-Component Analyzer. The instrument presents its analytical results on an electric typewriter, a counter, or a punched card device and in a form suitable for automatic computing. It is applicable wherever a large number of routine analyses are conducted on more than one component of a mixture. Instrument time is shortened to nearly 50% that required by usual manual spectrometer analyses, according to Perkin-Elmer, and chances for human error are reduced.



The instrument consists of a standard single-beam infrared spectrometer attached to a programming control unit which sequences through 10 predetermined analytical wave lengths and 10 corresponding slit widths selected for the components to be analyzed. The sample cell is automatically shifted into and out of the infrared beam to give quantitative readings. For each of the 10 wave lengths, the signal is allowed to reach equilibrium and the transmittance value measured with the cell out of the optical path and then with the sample moved into the beam.

Photometric accuracy in the 15 to 35% transmission range is  $\pm 0.002$  absorbancy unit; in the 35 to 60% range,  $\pm 0.001$  absorbancy unit. Quantitative analyses are reported to have been made with an average error of less than 0.1% absolute and a relative error of less than 0.2% of the amount of material present.

For calibration purposes, wave length

indexing and zero setting check points may be selected by means of push buttons at the end of the automatic cycle. 1

#### **X-Ray Diffraction Scales**

Transparent scales for the rapid reading of powder camera films are offered by N. P. Nies for three camera diameters and a selection of radiations. Five or six scales of slightly different length are furnished for each radiation to provide for film shrinkage variation up to 1%.

Scales now available for camera diameters of 57.3 or 143.2 mm. are d values for Co, Cr, Cu, and Fe radiations; for camera diameter of 114.6 mm., dvalues for Co, Cr, Cu, Fe, and Mo radiations and sin<sup>2</sup> theta. **2** 

#### **Organic Chemicals**

Now available from Bios Laboratories are the following chemicals: Aziminonaphthalene  $\beta$ -Benazaldoxime Benzopinacol Camphylamine Cardol Digoxin 3,5-Diiodosalicylic acid Methionol Pyridine-4-aldehyde 1-Tyrosylglycylglycine **3** 

#### Aldehyde Reagent

Azobenzenephenylhydrazinesulfonic acid is now available from Techniservice. The reagent is useful in the detection of aldehydes in acetals, in the differentiation of aliphatic and aromatic aldehydes, and in the preparation of derivatives of aldehydes and ketones. **4** 

#### **Immersion Heater**

Fisher Scientific Co.'s Glo-Quartz heater is a wand of fused quartz, less than 1/2 inch in diameter, encasing a 400-watt heating coil. Because of the resistance of quartz to thermal shock, a red-hot heater can be placed directly in a reaction flask without cracking or breaking. And it will not burn out when not under liquid. Fisher reports that the heater is an excellent source of infrared radiation.

The unit can be used to build gas heating chambers, in small high-temperature baths, in table-top vaporizers or steam generators, in gas or liquid pipelines, or in microfurnaces. It reportedly will bring 250 ml. of water to boiling in 3 minutes. In a corrosive system, Glo-Quartz plus a thermoregulator and relay provides automatic control where other immersion heaters cannot be used. 5

#### **Die for Infrared Analysis**

A die that is said to make infrared analysis of solids as easy as that of



liquids is being marketed by The Perkin-Elmer Corp. With use of the die both quantitative and qualitative analyses have been made of amino acids, complex organic solids.

and various commercial products.

A small quantity of sample and pure potassium bromide are squeezed together under high pressure in the evacuated die to form clear disks of the bromide-sample mixture. These disks are transparent to the entire infrared spectrum. They are 13 mm. in diameter.

The die features a split cone construction so that the disks can be removed without fracturing or chipping. In addition to the die, a laboratory press of 20,000 pounds capacity and a small vacuum pump are required to fabricate the disks. **6** 

#### **Polarizing Microscopes**

A new series of polarizing microscopes for chemical analysis has been announced by Bausch & Lomb Optical Co. The instruments feature the ballbearing, low-position, fine-focusing stand previously available only with B&L Dynoptic laboratory instruments. The precentered nosepiece has repeat automatic settings. Standard equipment is three achromatic objectives—  $4 \times, 10 \times, \text{ and } 21 \times.$ 

The microscopes have a precentered, revolving stage with 1° graduations and vernier readings to 6 minutes of arc. The Polaroid analyzer is of the flip-in type. Eyepieces are  $5 \times$  and  $7.5 \times$ 

For further information, see coupon on page 41 A

Huygenian, with crosshairs and focusing eve lenses. Instruments can be furnished with an integral substage illuminator instead of the standard mirror. 7

#### Densitometer

A semiautomatic densitometer, Model DSA-102, for paper chromatography and electrophoresis, has been designed by Matthew Laboratories to permit quantitative evaluation of any size of paper strip or sheet without cutting. Curves are plotted directly on a screw driven table, eliminating the need for writing down values.

Interchangeable slit and circular apertures for high resolution scanning are provided. The signal from the red or blue sensitive phototube is read by means of a stable d.c. amplifier. Adjustments are provided to balance out dark currents and background light and give full scale meter readings. 8

#### **Drop Counter**

A new drop counter, manufactured by Microchemical Specialties Co., has a photoelectric cell connected to a timer for accurately counting from one to 400 drops at each setting. Drops fall directly from glass tip into collecting tube without touching any part of the counter assembly. The timer automatically indexes the collector to the next position after the set number of drops has been delivered into a tube.



The unit eliminates the need for attendance during fraction collection, according to the manufacturer. Ion exchange or chromatographic columns may be used by employing a stopcock to control the flow rate. 9

5956

#### **Pipet Control**

Micropet, a pipet control that eliminates mouth pipetting and accidental imbibing of toxic or infectious liquids, is being sold by National Instrument Co. It is fabricated of anodized aluminum and other corrosion resistant materials.

To use the control, a pipet is slipped into it and liquid is drawn into the pipet by collapsing and releasing a rubber bulb. Liquid is dispensed by depressing a release plunger. A delayed action

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#### **NEW PRODUCTS**

mechanism in the plunger permits the discharge to be controlled from a rapid to a barely perceptible flow. **10** 

#### **Microscope Viewer**

The Micro-Viewscope, distributed by William J. Hacker & Co., Inc., combines in one unit a  $75 \times$  and  $500 \times$  microscope with enclosed illumination and projection elements that render an image on an 8-inch ground glass screen. The instrument has a mechanical stage that offers control for centering or for scanning details of a slide and is equipped for photomicrography.

Micro-Viewscope is claimed to give relief from eyestrain and facilitates discussion by a number of observers who are studying material together on the ground glass.

#### **Organic Chemicals**

Eleven organic chemicals have been added to the list of materials offered by Distillation Products Industries. Among these is squalene, distilled by



Guardian RESEARCH

Where rigid laboratory specifications for control must be met, Tygon R-3603 flexible plastic laboratory tubing is specified by research laboratories the world over.

Tygon R-3603 is chemically inert, completely non-toxic and non-oxidizing. Glass clear, Tygon R-3603 offers complete solution visibility. Its mirror-smooth walls offer minimum restriction to flow. Tygon R-3603 while extremely tough and durable is flexible as a string. It is easily connected and is adaptable for branching lines with Tygon "Y" fittings. Tygon R-3603 is normally available in sizes ranging from .120" I.D. -2" I.D. Specify the genuine . . . the quality tubing that is branded — Tygon R-3603. Available at your laboratory supply house.

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DPI from the liver oil of the basking shark as a colorless liquid with a boiling point of 330° C. at atmospheric pressure and believed to be 90 to 95% pure. Other new organic chemicals are:

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5-Chloro-3-nitro-o-phenylenediamine

(p-Chlorophenyl)acetonitrile

(p-Chlorophenyl)acetonitr

- N,N'-Dimethylcarbanilide
- 2-Hydroxymethyl-2-methyl-1,3-propanediol

12

- Phenyl selenide
- 4-Picoline *n*-Propyl nitrate

Tri-*n*-propylamine

#### Oxygen Analyzer

A direct-reading dissolved oxygen analyzer claimed to operate as simply as a conventional pH meter has been developed by Dorber Co. The amperometric type instrument employs a rotating platinum electrode with a stable electronic system which automatically translates diffusion current into units of dissolved oxygen concentration. It determines dissolved oxygen from 0 to 10 p.p.m. within 5% accuracy for most aqueous solutions. Operation is on 115-volt a.c. current, and is not affected by power line fluctuations.



The instrument is not subject to interference by dissolved iron, calcium, magnesium, sulfides, nitrites, or organic matter, according to the manufacturer. Samples can be colored or turbid. 13

#### **Photometric Scanner**

A scanner for measuring the amount and distribution of materials on paper strips from electrophoresis and paper chromatography work is being sold by Research Specialties Co. The Model 2001 device is for use in Beckman Model DU spectrophotometers.

The scanner is designed so that the paper strip can be moved past the slit window of the spectrophotometer for readings at intervals corresponding to the desired slit widths. By plotting optical density against distance for successive readings, a graph is obtained

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195-D

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Type 908-F Synchronous Motor **Dial Drive** 

shown attached to Type 1304-B Beat-Frequency Oscillator

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With this unique drive, any desired portion of the oscillator frequency range is automatically swept in repetitive cycles. Installing takes but a minute. The unit operates from 115-volt. 60-cycle power. When done, a flip of a switch disengages the drive from the oscillator shaft

Oscillators equipped with this drive can be used with recording equipment to record conveniently and automatically the frequency response of the network under study.

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Type 908-P1 Drive - takes 50 sec to cover one-frequency decade, or 15 sec/octave

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Prices Available on Request

General Radio Co., 275 Mass. Ave., Cambridge 39, Mass. Please send copy of June 1954 G-R EXPERIMENTER describing the Type 1304-B Beat-Frequency Oscillator. Name ..... 589 Company ..... Street ..... City ... 

For further information, circle number 47 A on Readers' Service Card, page 41 A

representing the quantitative and configurational aspects of the pattern.

The indicator dial of the apparatus is marked for 1- or 2-mm. paper movements. An adapter is available permitting the scanner to be used in a Beckman Model B spectrophotometer.

#### **Multiple Stirrer**

Multiple stirrers with three or six spindles and constant temperature baths are being marketed by Labline, Inc. Originally developed for ASTM D 665 turbine oil rusting tests, the units can be used for other multiple applications where solutions must be stirred at constant speed and temperature.

The bath is controlled with a hydraulic thermostat. An induction motor drives the spindles by means of an endless neoprene-covered nylon belt. Tall form beakers of 400-cc. capacity are used. 15

#### Laboratory Labels

A label that is claimed to withstand the rigors of laboratory use has been developed by Avery Adhesive Label Corp. The Kum-Kleen label is made from a plastic impregnated stock that is impervious to oils, greases, and water and is resistant to soap, acids, alkalies, and other chemicals.

The labels are self-adhesive and easily removed. They are reported to withstand temperatures of over 200° F. for 2 hours, or may be frozen without damage. They are available in three sizes in sheet form. 16

#### Aluminum Nitrate

A pure aluminum nitrate reagent for use as the salting out agent in the fluorometric determination of uranium is available from Fisher Scientific Co.

Uranium content of the chemical is reported as approximately 0.0000001%.

#### **Radioactive Versene**

Carbon-14 tagged ethylenediaminetetraacetic acid, or Versene, is now being synthesized by Abbott Laboratories for research use. Because of its physical and biological stability, the material is expected to be of value as a tool for the study of food, enzymes, metabolism, reaction rates, metallic activation and deactivation, and for other purposes.

The compound may be doubly labeled with most metallic ions in addition to the carbon-14 on the methylene carbon



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atom. For instance, iron-55, iron-59, copper-64, and zinc-63 can be incorporated into the molecule. **18** 

#### **API Hydrocarbon Samples**

Now available as American Petroleum Institute Standard samples of 5 ml. in borosilicate glass ampoules are: 2,2dimethyl-3-ethylpentane, 4-methyl*trans*-2-hexene, 1-methylcyclohexene.

17

#### MANUFACTURERS' LITERATURE

**Recording Spectrometer.** Folder of 4 pages describes and gives specifications of recording spectrometer with range from 2100 to 7000 A. Leeds & Northrup Co. **20** 

Raman Spectrometer. Four-page data sheet describes recording Raman spectrographic equipment for rapid analysis in industrial laboratories. Minneapolis-Honeywell Regulator Co. (*Data Sheet* 10.16-8a). **21** 

Infrared Spectrophotometer. Sixpage brochure describes features and lists specifications of double-beam instrument for fast, efficient infrared analysis. The Perkin-Elmer Corp. (Model 21). 22

**Electrical Instruments.** Catalog tabulates data and prices for microammeters, milliammeters, ammeters, voltmeters, and other instruments and accessories. Burlington Instrument Co. (*Cat. N-1*). **23** 

Furnaces. Catalog of 33 pages describes and gives specifications of furnaces, ovens, and control instruments for heat treating and general laboratory work. K. H. Huppert Co. 24

Raman Spectrography Equipment. British-made spectrographs for use in photographic work or as direct reading instruments with Raman spectra, as well as accessories, described in 20-page booklet. Hilger & Watts, Ltd. (*Cat. CH334/2*). 25

Viscometers. Booklet of 21 pages briefly discusses rheology and describes use of viscometer that operates by measuring force required to rotate a spindle in a fluid. Brookfield Engineering Laboratories, Inc. (Solutions to Sticky Problems). 26

Adsorbents for Chromatography. Pamphlet of 8 pages discusses properties and application of acid, nonalkaline, and basic aluminum oxides with constant properties for use as adsorbents in

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#### NEW PRODUCTS

chromatographic analysis. Alupharm Chemicals. 27

**Electrophoresis Apparatus.** Fourpage bulletin discusses use of Englishmade apparatus for continuous electrophoretic separation of complex mixtures on filter paper. Shandon Scientific Co. **28** 

Fraction Collectors. Eight-page booklet describes automatic fraction collectors for column chromatography and discusses method of using them. Shandon Scientific Co. 29

**Molecular Distillation Apparatus.** Six-page folder presents features, performance information, and specification data for two portable high vacuum distilling units marketed by Bronwill Scientific, Inc. (*Models C and P*). **30** 

**Electrolytic Desalting Apparatus.** Description of apparatus for removal of inorganic salts from solutions in paper chromatography applications presented in 2-page bulletin. A. S. Aloe Co. **31** 

Laboratory Furniture. Layout kit contains 28-page catalog of sectional furniture, cardboard models of furniture, and scaled layout sheets for planning laboratory furniture arrangement. Labline, Inc. (Cat. F-153). 32

**Thermocouples.** Technical data and prices for thermocouples, packing glands, and protection tubes given in catalog of 16 pages. Conax Corp. (*Cat. 1530*). **33** 

**Thermocouples.** Pyrometer accessory catalog contains selection information on thermocouples, protection tubes, and accessories. West Instrument Corp. **34** 

**Combustion Tubes.** Six-page folder describes and lists dimensions of impervious, vitrified refractory tubes for industrial laboratory use. Coors Porcelain Co. **35** 

**Organic Chemicals.** Folder lists prices in U. S. dollars for organic research chemicals available from British company. Organic Research Chemicals, Ltd. **36** 

Photographic Materials. Catalog describes photographic materials and light filters for laboratory use. Eastman Kodak Co. **37** 

**Ethanolamines.** Bulletin of 40 pages presents information on properties, specifications, methods of analysis, uses, and handling for monoethanolamine, diethanolamine, and triethanolamine and contains a bibliography *listing 381 refer*ences. Jefferson Chemical Co., Inc. **38** 

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Instrument Repairs. A 16-page catalog describes repair service for balances, microscopes, colorimeters, spectrometers, and other scientific instruments. A. J. Griner Co. **42** 

**Ovens.** Information on line of laboratory ovens, sterilizers, incubators, and related equipment presented in 36-page catalog. Precision Scientific Co. (*Cat.* 331). **43** 

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Determination of the Gamma Isomer Content of Benzene Hexachloride by CI-36 Isotope Dilution Method, Craig, Taylor and Brown; Anal. Chem., 25, 1661, (1953).

Penicillin Assay by Isotope Dilution Methods, Craig and Harris; C. & E. News, Sept. 28, 1953, p. 3962.

Estimation of Naphthalene in Cold Tar Products by Isotopic Dilution Analysis, W. S. McDonald and H. S. Turner; Chemistry and Industry, 1952, 1001.

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