ANALYTICAL CHEMISTRY
Walter J. Murphy, Editor

"Communications to the Editor"

MANY of the innovations added to ANALYTICAL CHEMISTRY over the past five years are the result of the active participation of the Advisory Board. The editor welcomes this opportunity to acknowledge publicly and with warm appreciation the interest and valuable assistance rendered by its members.

At the most recent meeting of the board, the suggestion was made by I. M. Kolthoff that a section "Communications to the Editor," devoted exclusively to scientific matters, be added to the journal. We look with favor on this proposal, although we realize full well that such a department may add further trials and tribulations to the editor's daily life. Our sole objective is to publish a journal that meets the needs and requirements of analytical chemists the world over. A few more responsibilities are welcomed by a loyal staff who derive much personal satisfaction from the services rendered the profession of analytical chemistry.

Many research projects are pursued for years before the results are published. This is especially true of many forms of research conducted in colleges and universities. A thesis for a doctorate usually is published after the candidate has received the degree, and frequently publication is delayed for several years. During the course of such research discoveries often are made which are of wide interest to workers in the field. Occasionally research in fields other than analytical will disclose information of a fundamental nature that is of definite interest and value to analytical chemists.

During a research study for an advanced degree frequently new reagents for qualitative detection and quantitative determination of inorganic and organic constituents are discovered, classical techniques are greatly improved, and new physicochemical principles are used in quantitative analyses. These are but a few examples of many instances where the literature could be enhanced by a "Communications to the Editor" section. After such discoveries have been made considerable time may elapse before an exhaustive study is completed and sufficient results are compiled to warrant preparation of a detailed manuscript. We believe readers of ANALYTICAL CHEMISTRY will welcome an opportunity to be informed of new discoveries which can be appropriately reported in the new department in the form of a brief but exact "Communication to the Editor." This new column will adhere to the high scientific standards maintained throughout the journal. It is not intended to take the place of "Notes on Analytical Procedures" or "Aids for the Analyst," each of which serves a most useful purpose.

Scientists in other fields of chemistry often obtain results that are indirectly of great significance to analytical chemists. ANALYTICAL CHEMISTRY is usually not the most suitable medium for the publication of studies on solubilities, oxidation-reduction reactions, acid-base reactions, catalyzed and induced reactions, properties of precipitates, etc. Yet readers of ANALYTICAL CHEMISTRY would welcome an opportunity to get acquainted with the main results of such studies, which are appropriate for the new column, especially when their importance to analytical chemistry is clearly emphasized.

The new column offers speedy publication of new discoveries of major analytical importance. We must ask, however, that those who avail themselves of this opportunity use it with discretion and always with the rights of others in mind. In order to maintain high quality, communications will be handled in a manner similar to that employed for more lengthy manuscripts. Frankly, the success or failure of this column will depend upon the speed with which contributions are reported on by reviewers and the care and wisdom they use in making their recommendations. Our highly favorable experiences of the past indicate that we will have the loyal support of the army of reviewers in this new editorial venture. We have said time and again, and once more we repeat, that we believe no profession is more conscious of ethics than is the chemical profession. The structure of scientific publications would have crumbled years ago if scientists had not lived up to high ethical standards.

Many proffered communications will be rejected, just as many manuscripts are rejected, for one or more reasons. Of those who may receive rejection slips, we ask forbearance and a spirit of understanding. One of the most pleasant duties of an editor is to write a letter accepting a manuscript or communication; perhaps his most unpleasant duty is to reject one, for the editor appreciates the time and energy expended in the research work done and the effort devoted to the writing of a manuscript. No great writer has not produced his quota of relatively mediocre work; no great actor is outstanding in every part he portrays. Similarly, a chemist must expect, at times, to receive critical analyses of his results. No one is endowed with perfection, and editors are not exempt from inclusion among the fallible.

Thus we offer still another service to our authors and readers in our effort to provide a positive and dynamic approach to scientific and technical journalism in the broad fields of analytical chemistry. This is your journal and is edited to serve the best interests of analytical chemists.

DOUBLE BOND INDEX

A Correlation Useful for Classification of Aromatics and Olefins in Petroleum Fractions

M. R. LIPKIN, ALBERT SANKIN, AND C. C. MARTIN
Sun Oil Company, Norwood, Pa.

Double bond index is defined by the equation:

$$DBI = \frac{(\delta - 98)(M + 17)}{3190\ Q}$$

where δ is specific dispersion for the F and C lines of hydrogen, M is molecular weight, and Q is the number of double bonds per molecule. Double bond index is useful in identifying the predominant type of aromatic or olefinic hydrocarbon in a petroleum fraction, regardless of the presence of paraffins and naphthenes. The data necessary are specific dispersion; molecular weight, which can be obtained either from density and mid-boiling point or from viscosities at 100° and 210° F.; and number of double bonds per molecule, which can be calculated from per cent hydrogen before and after complete hydrogenation of the sample. At present this correlation is regarded as a research tool and not as a routine analytical method because the determination of number of double bonds is time-consuming.

RECENT research in hydrocarbon analysis has been directed toward the determination of quantity (*3, 8, 9, 15, 16, 26*) and type (*6, 14, 18, 27, 31, 32*) of olefins and aromatics in petroleum. For this purpose specific dispersion has been a most useful property because it is nearly constant for paraffins and naphthenes, increases with increasing unsaturation, and varies considerably depending on the position of the double bonds relative to each other.

In the gasoline boiling range, the quantity of aromatics may be determined from the specific dispersion with correction for olefin content by using the method of Grosse and Wackher (*9*) or the method of Groennings (*8*). These methods are limited to gasolines of low end point which contain no dicyclic aromatics. Many investigators (*6, 14, 27, 31, 32*) have recommended the use of specific dispersion for the identification of type of hydrocarbon because of its dependence on the number of double bonds and the configuration of the molecule. Thorpe and Larsen (*27*) have developed a simple system for the calculation of specific dispersion from a knowledge of structure, which enables one to calculate accurate specific dispersions for most olefins and aromatics and to explain the wide differences in specific dispersion between types such as anthracenes and phenanthrenes, which are both condensed tricyclic aromatics. Specific dispersion itself and the constants developed by Thorpe and Larsen are both particularly useful for identification of pure compounds but of limited value for mixtures such as petroleum fractions.

The average specific dispersion (for the F and C lines of hydrogen) for paraffins and naphthenes has previously been reported as about 98.3 (*27, 31*). Von Fuchs (*30*) has recently called attention to the fact that at that time only paraffins and monocyclic naphthenes were considered, and that polycyclic naphthenes are now believed to have specific dispersions systematically lower than 98. He has measured specific dispersions as low as 95 on medicinal white oils. The data available to the authors indicate that the average for naphthenes present in petroleum may be as low as 96 or 97, and that a few naphthenes may have specific dispersions as low as 94. There are relatively few data available on the specific dispersions of polycyclic naphthenes, and as those specific dispersions are not usually lower than 96, the earlier value 98 has been used in the derivation of the present correlation.

A property called specific dispersion per double bond is used in this paper. Specific dispersion per double bond is defined as the specific dispersion of sample minus 98 divided by the average number of double bonds per molecule. Number of double bonds

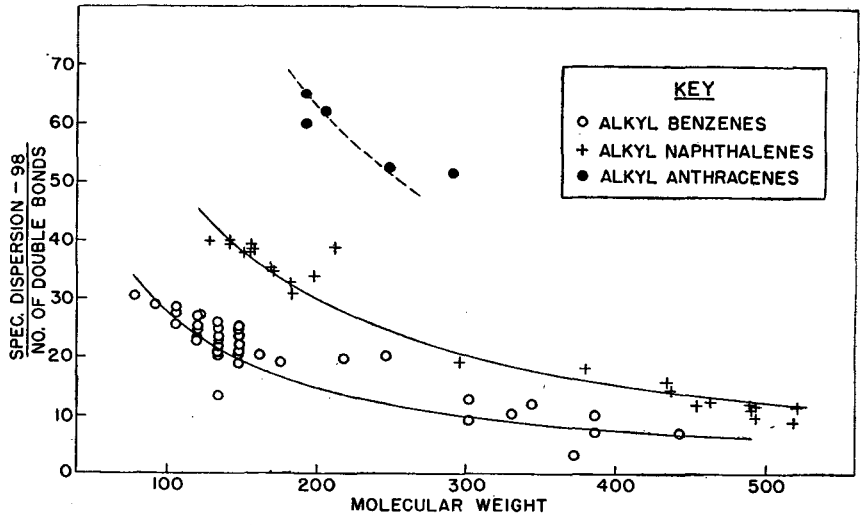


Figure 1. Specific Dispersion per Double Bond for Aromatics

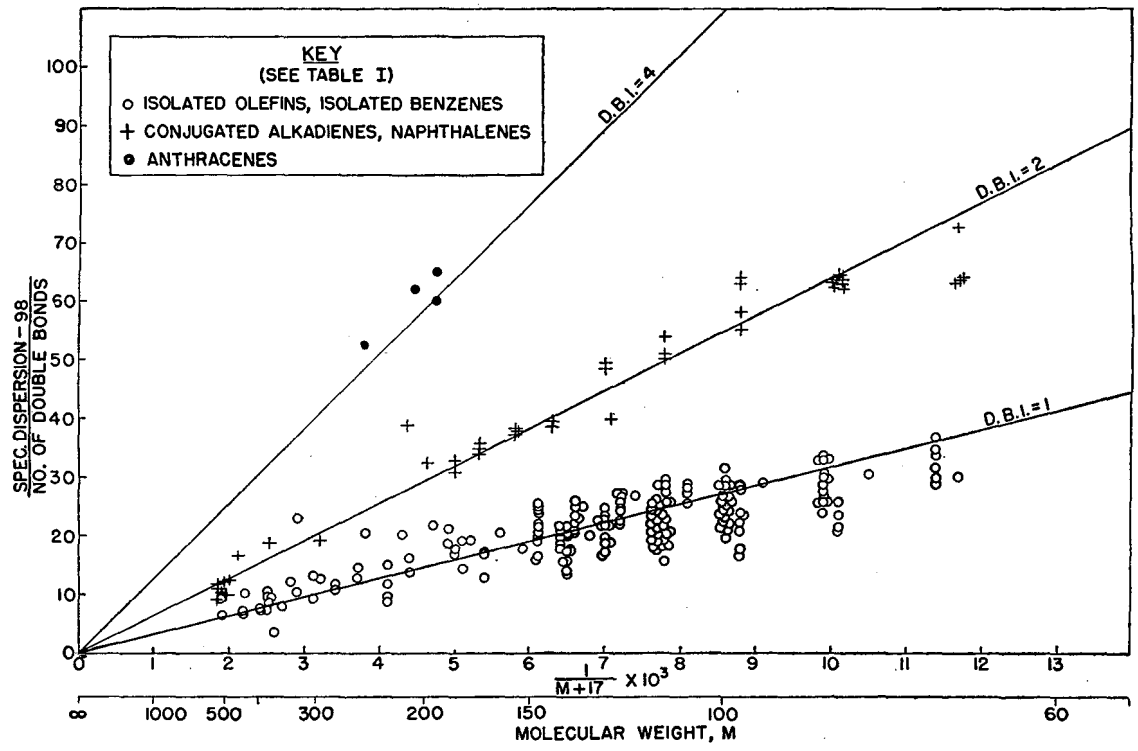


Figure 2. Classification of Unsaturated Hydrocarbons

as used in this paper refers to both olefinic and aromatic unsaturation, as determined by the quantity of hydrogen that can be added to the hydrocarbon molecules. All aromatics and olefins are included in the general classification of unsaturated hydrocarbons.

Specific dispersion per double bond gives a more definite classification of unsaturated hydrocarbons than does specific dispersion itself. Using specific dispersion per double bond, most unsaturated hydrocarbons fall into definite classes and such types as mono-olefins and alkyl benzenes fall into the same class although they are of widely different specific dispersions.

Several terms must be defined for use in subsequent discussion. Aromatics and olefins are treated as if they consist entirely of single and double bonds. A double bond separated from all other double bonds by at least two single bonds is known as an "isolated" double bond. When two or more double bonds are mutually conjugated or adjacent, there is an interaction which makes it necessary to consider this group of double bonds as a single "unsaturated system." The word "isolated" is also used in describing the separation of such unsaturated systems by at least two single bonds. Thus the group name "isolated benzene hydrocarbons" includes such compounds as benzene, diphenylmethane, 3-phenyl-1-propene, and 1,4-dihydronaphthalene, but excludes such compounds as naphthalene, biphenyl, styrene, and 1,2-dihydronaphthalene.

DERIVATION OF DOUBLE BOND INDEX

Figure 1 shows a plot of specific dispersion per double bond against molecular weight for alkyl benzenes, naphthalenes, and anthracenes. The curves drawn for these series show that specific dispersion per double bond decreases with increasing molecular weight. These curves are converted to straight lines which intersect at the origin by plotting specific dispersion per double bond against reciprocal of the quantity, molecular weight plus 17. On such a plot most other homologous series of unsaturated hydrocarbons also show straight lines converging at the origin.

Figure 2 shows a plot of specific dispersion per double bond against reciprocal of molecular weight plus 17 for several important groups of unsaturated hydrocarbons. Most of these hydrocarbons are divided into two groups with the specific dispersion per double bond of the higher group about twice that of the lower at the same molecular weight. The lower group includes cyclic and noncyclic mono-olefins, isolated polyolefins, and isolated substituted benzenes. The higher group contains noncyclic conjugated diolefins and naphthalenes. Anthracenes fall in a third group with the specific dispersion per double bond about four times that of the lowest group at the same molecular weight.

For convenience, the relation shown in Figure 2 is expressed in terms of double bond index (DBI), which is defined as the ratio of specific dispersion per double bond of sample to specific dispersion per double bond for the same molecular weight at the line representing the lowest group. The equation for double bond index is:

$$\mathrm{DBI} = \frac{\delta - 98}{Q} \div \frac{3190}{M + 17} = \frac{(\delta - 98)(M + 17)}{3190\,Q} \qquad (1)$$

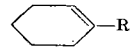
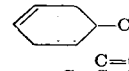
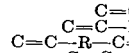
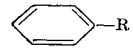
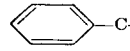
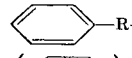
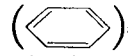
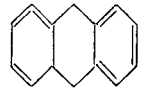
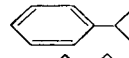
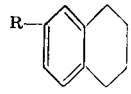
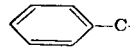
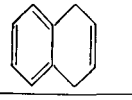
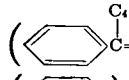
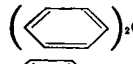
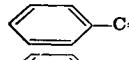
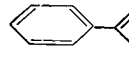
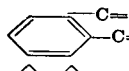
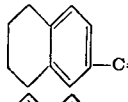
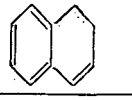
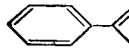
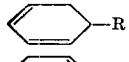
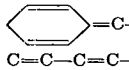
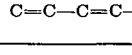
where δ = specific dispersion for the F and C lines of hydrogen

$$\left(\frac{n_F^{20} - n_C^{20}}{d_4^{20}} \times 10^4\right)$$

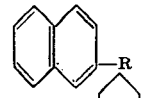
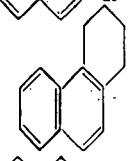
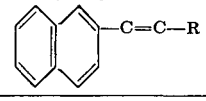
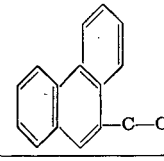
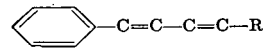
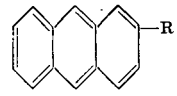
M = approximate molecular weight, and Q = number of double bonds per molecule.

Double bond index is a more convenient property than specific dispersion per double bond because, for a given unsaturated system, it does not vary with molecular weight. For instance, a mono-olefin of 100 molecular weight and a mono-olefin of 500 molecular weight have very different specific dispersions per double bond, but both have double bond indexes of 1.

Table I. Double Bond Indexes

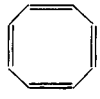
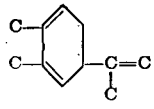
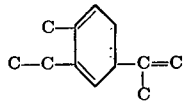
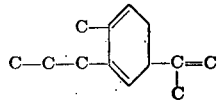
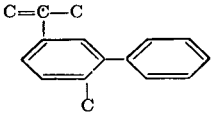
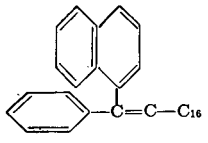
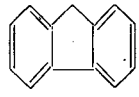
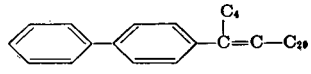
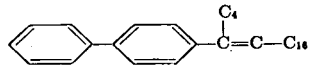
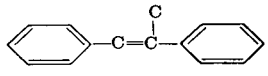
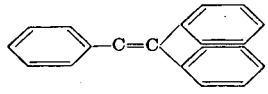
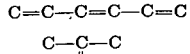
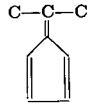
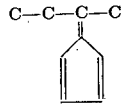
Type[a]	Carbon Structure Example[b]	No. of Compounds	Av. DBI	Av. Dev.	Group Average DBI	Av. Dev.	Plotted in Figure	References
Isolated olefins					0.9	0.1		
Cyclenes		41	0.8	0.1			2	*27, 33*
Alkenyl cyclenes		3	0.9	0.1			2	*27*
Alkenes		135	0.9	0.1			2	*1, 4, 27, 33*
Alkadienes		5	0.8	0.1			2	*27, 33*
Alkatrienes		2	0.9	0			2	*27*
Alkenyl cyclane		1	1.1	...			2	*27*
Alkylidene cyclanes		6	1.0	0.1			2	*27*
Isolated benzenes					1.1	0.1		
Alkyl benzenes		47	1.1	0.1			2	*1, 21, 23, 27, 33*
Alkenyl benzene		1	1.0	...			2	*2*
Diphenyl or substituted diphenyl alkanes		9	1.0	0.1			2	*7, 21, 23, 27*
Triphenylalkane		1	1.1	...			2	*27*
9,10-Dihydroanthracene		1	1.2	...			2	*27*
Cyclyl benzene		1	1.0	...			2	*27*
Cyclano benzenes		9	1.2	0.1			2	*27*
Cyclenyl phenyl alkane		1	1.1	...			2	*27*
Cycleno benzene		1	0.9	...			2	*27*
Conjugated benzene olefins					1.5	0.2		
Two isolated alkenyl benzenes		1	1.3	...			3	*21*
1,1-Diphenyl alkenes		4	1.4	0.1			3	*27*
Alkenyl benzenes		8	1.5	0.3			3	*1, 21, 27*
Cyclenyl benzene		1	1.5	...			3	*27*
Dialkenyl benzene		1	1.7	...			3	*27*
Alkenyl cyclano benzenes		2	1.7	0.1			3	*21*
Cycleno benzene		1	1.7	...			3	*27*
Biphenyls		3			1.5	0.1	3	*7*
Conjugated olefins					1.9[c]	0.1		
Cyclodienes		10	1.4	0.3			3	*7, 27, 33*
Alkylidene cyclenes		3	1.9	0			3	*7, 27*
Alkylidene 2,5-cyclohexadienes		8	1.8	0.1			3	*27*
Alkadienes		20	2.0	0.1			2	*1, 27, 33*

for Unsaturated Hydrocarbons

Type[a]	Carbon Structure Example[b]	No. of Compounds	Av. DBI	Av. Dev.	Group Average DBI	Av. Dev.	Plotted in Figures	References
Naphthalenes					2.0	0.1		
Alkyl naphthalenes		17	2.0	0.1			2	*21, 27*
Cyclano naphthalenes			2.0	0			2	*27*
Conjugated alkenyl naphthalenes		4	2.0	0.2			2	*21, 27*
Phenanthrenes		1			2.5	...	3	*27*
Conjugated benzene diolefins		4			3.2	0.1	3	*13*
Anthracenes		5			4.4	0.3	2	*27*

[a] "Isolated" is used in place of "nonconjugated, nonadjacent." The generic names of the subgroups follow the system used by Egloff (*7*). This system differs from the "Definitive Report of the Commission of the Reform of the Nomenclature of Organic Chemistry" translated by Patterson in 1933 (*24*) in that it substitutes "cyclane. cyclyl, cyclene, and cyclenyl" for "cycloalkane, cycloalkyl, cycloalkene, and cycloalkenyl," respectively, and also adds the words "cyclano" and "cycleno" as defining the radicals formed by the fusion of a cyclane or a cyclene, respectively, with another ring structure.
[b] Where there is only one compound in a subgroup, the example of carbon structure shows the compound for which data were available.
[c] This average excludes conjugated cyclodienes, as there is a definite trend in this subgroup.

Table II. Double Bond Indexes of Several Unusual Hydrocarbons, Some of Which Show Effect of Internal Mixing

		Empirical Formula	Specific Dispersion	DBI	Reference
1		C_8H_8	228	1.2	*7*
2		$C_{11}H_{16}$	173	1.3	*27*
3		$C_{12}H_{16}$	168	1.3	*27*
4		$C_{13}H_{20}$	161	1.3	*27*
5		$C_{16}H_{18}$	222	1.5	*27*
		$C_{24}H_{46}$	208	1.8	*21*
7		$C_{13}H_{16}$	288	1.8	*27*
8		$C_{38}H_{60}$	177	1.9	*27*
9		$C_{34}H_{52}$	197	2.1	*27*
10		$C_{15}H_{14}$	325	2.1	*27*
11		$C_{20}H_{16}$	374	2.4	*27*
12		C_6H_8	331	2.4	*27*
13		C_8H_{10}	341	3.1	*34*
14		C_9H_{12}	322	3.2	*34*

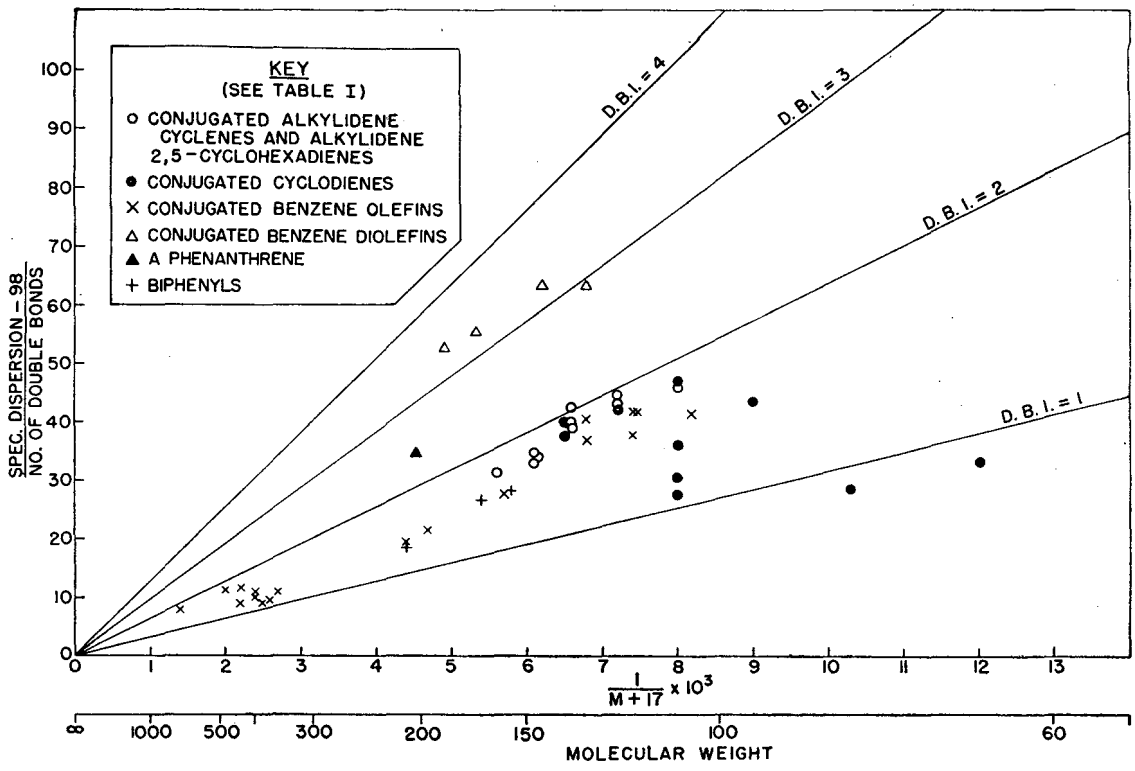


Figure 3. Classification of Unsaturated Hydrocarbons

Lines of constant double bond index equal to 1, 2, and 4 have been drawn on Figure 2 to demonstrate the classification of some of the unsaturated hydrocarbons. There are several groups of unsaturated hydrocarbons not shown in Figure 2, which have double bond indexes intermediate between these lines, and these are discussed below.

DOUBLE BOND INDEXES OF PURE HYDROCARBONS

Tables I and II show the double bond indexes for all unsaturated hydrocarbons (with the exception of acetylenes), on which experimental specific dispersions are available.

In Table I, 359 compounds are divided into nine major groups, many of which are further divided into subgroups. The nomenclature used is, in general, the same as that used by Egloff (*7*).

The average double bond indexes for the various groups and the average deviations within each group are shown in this table. The data for individual compounds are plotted in Figures 2 and 3. Table II shows double bond indexes for several unusual hydrocarbons which cannot readily be classified in Table I.

Mono-olefins and isolated polyolefins, which behave like mono-olefins, have double bond indexes varying from 0.8 to 1.1 and an average double bond index of 0.9. Alkyl benzenes and all other isolated benzene aromatics have double bond indexes varying from 0.9 to 1.2 with an average value of 1.1. Thus, isolated olefin double bonds and isolated benzene rings have nearly equal double bond indexes. Figure 4 shows a plot of double bond index against molecular weight for all the isolated benzenes. There is a distinct trend toward higher double bond index with increasing number of substitutions on the aromatic ring as evidenced by pentaethyl- and hexaethylbenzene. Benzene itself has a double bond index of 0.9 and most of the compounds are grouped between 1.0 and 1.2.

When a benzene ring is conjugated with an olefin bond or a second benzene ring, the average double bond index is about 1.5 as shown for the conjugated-benzene olefins or styrene type hydrocarbons and for the biphenyls. There is, however, considerable variation in the conjugated benzene olefin group, as shown in Figure 3.

Conjugated diolefins have double bond indexes near 2.0 except for the lower molecular weight members of the conjugated cyclodiene series. 1,3-cyclopentadiene and 1,3-cyclohexadiene have double bond indexes of 0.8 but there is a definite trend toward 2.0 as side chains are added to these rings (see Figure 3). Thorpe and Larsen (*27*) have already noted that as far as specific dispersion is concerned, the parent compounds behave like benzene, and the substituted compounds, more like conjugated diolefins. Unfortunately, there are no conjugated alkenyl cyclenes on which specific dispersion data are available, but because of the alkylidene cyclenes and the alkadienes, a double bond index near 2.0 for conjugated diolefins is fairly well established. Naphthalene derivatives also have double bond indexes of 2.0, so that the naphthalene system and the conjugated diolefin system have equal double bond indexes.

The only alkyl phenanthrene on which specific dispersion has been reported shows a double bond index of 2.5. Four conjugated benzene diolefins show an average of 3.2. The anthracenes have an average double bond index a little above 4.0, which means that their presence in a hydrocarbon mixture should be easily detected.

Table I shows that the average deviation within any major group is usually less than 10% of the double bond index for the group and that the differences between major groups are sufficient to make double bond index useful in the analysis of complex hydrocarbon mixtures.

Table I shows the double bond indexes of nine major unsaturated systems. The double bond index of a hydrocarbon containing two or more isolated unsaturated systems is the average of the indexes of the individual systems weighted according to the number of double bonds in each system. Many of the compounds in Table I contain two or more isolated unsaturated systems with equal double bond indexes. For such a hydro-

carbon the double bond index is, of course, equal to that of each of the isolated systems. Compounds 2, 3, and 4 in Table II illustrate, however, the phenomenon of internal mixing—that is, the presence in the same molecule of two isolated unsaturated systems with different double bond indexes. The resultant double bond index of 1.3 may be explained by double bond indexes of 1.5 for the two double bonds in the ring and 0.9 for the chain double bond.

Compounds 8 to 12 show that a linear conjugation of three unsaturated groups increases double bond index in contrast to the effect of branched conjugation of three unsaturated groups, as illustrated by the 1,1-diphenyl alkenes and the alkylidene 2,5-cyclohexadienes shown in Table I. Compound 1 in Table II, cyclo-octatetraene, has a double bond index of 1.2 which is evidence of its similarity to a benzene type aromatic. No experimental specific dispersions are available for adjacent diolefins.

EXPERIMENTAL

For pure hydrocarbons of known structure the only experimental work necessary for determining double bond index is the measurement of specific dispersion. For unknown hydrocarbons, petroleum fractions, or other hydrocarbon mixtures, specific dispersion, molecular weight and number of double bonds per molecule must be experimentally determined. The following procedures may be used.

Specific Dispersion. The dispersion ($n_F - n_C$) may be determined on an Abbe refractometer or a more accurate instrument such as the Pulfrich. Specific dispersion is equal to dispersion times 10^4 divided by density obtained at the same temperature. If direct determination of dispersion is impossible because of lack of equipment or darkness of sample, specific dispersion may be calculated by the Lipkin-Martin equation (*17*) from refractive index for the sodium *D* line, density, and molecular weight.

Molecular Weight. This property may be calculated from 50% boiling point and density by the method of Mills, Hirschler, and Kurtz (*22*), or from viscosities at 100° and 210° F. by the method of Hirschler (*11*).

Number of Double Bonds per Molecule. Number of double bonds, *Q*, may be calculated from molecular weight and per cent hydrogen determined on the sample before and after complete hydrogenation, using the following equation:

$$Q = \frac{M\,(H_h - H_o)}{2.016\,(100 - H_h)} \qquad (2)$$

where M is molecular weight before hydrogenation, H_o is weight per cent of hydrogen before hydrogenation, and H_h is weight per cent of hydrogen after hydrogenation.

Hydrogen determinations may be made by combustion methods (*10*, *25*, *28*) or from the correlation of Cauley and Delgass (*5*) using aniline point, gravity, and boiling point. Per cent hydrogen is used rather than per cent carbon, because the presence of even small quantities of nonhydrocarbons causes serious error in number of double bonds calculated from per cent carbon.

A procedure for hydrogenation of petroleum fractions used in this laboratory is as follows: A ratio of approximately 1 part by weight of wet Raney nickel catalyst to 2 parts of sample is used. (Raney nickel is stored under water to prevent its oxidation.) The catalyst is removed from the container, the excess water is allowed to drain off, and the catalyst is weighed while wet. The catalyst is washed in a beaker with several portions of absolute methanol, and each washing is decanted through a Büchner funnel. After the third washing the catalyst is transferred to the Büchner funnel and the excess solvent sucked off until the catalyst begins to get hot because of the oxidation of the nickel. A small portion of sample is immediately added and the catalyst and sample are mixed. The excess sample is then drawn off the Raney nickel by suction and the catalyst is rapidly added to the sample in a beaker and stirred well. It is important that the catalyst be well dispersed in the sample in order to get efficient hydrogenation. Failure to disperse the catalyst is usually due to incomplete removal of water in the alcohol washing procedure.

The sample-catalyst mixture is transferred to the hydrogenation apparatus and hydrogenated at 200° C. and 2000 pounds' pressure for 3 hours. After hydrogenation the sample is filtered to remove the catalyst. (In some cases the quantity of sample available for hydrogenation may be too small to permit washing the catalyst after the alcohol washings. In such cases alcohol will be present in the hydrogenated sample and must be removed by washing the sample with water and drying it over calcium chloride.)

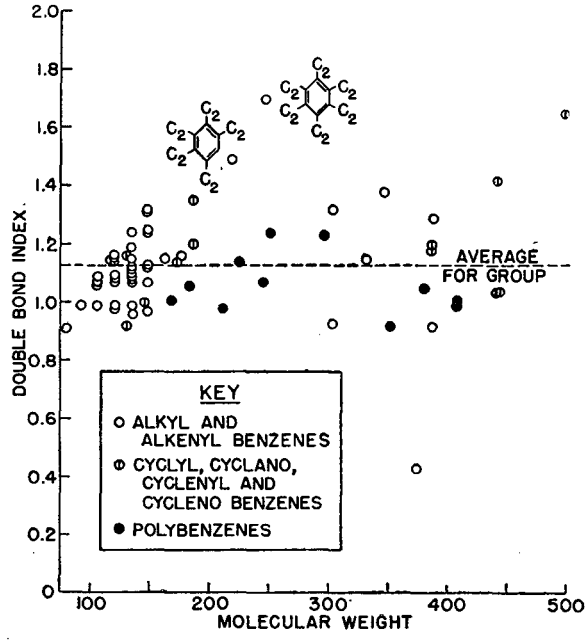


Figure 4. Double Bond Index *vs.* Molecular Weight for Isolated Benzene Aromatics

If the sample is not completely hydrogenated after one hydrogenation, the procedure should be repeated with fresh batches of catalyst until no unsaturation remains. In general, gasoline samples can be hydrogenated in one treatment, but heavier samples such as lubricating oils may require three or more hydrogenations. Aromatic concentrates in particular may require many hydrogenations.

Specific dispersion has been used to measure the completeness of hydrogenation. The *F-C* specific dispersion for paraffins and naphthenes is 98 or less. However, this method is somewhat insensitive for fractions of high molecular weight, such as lubricating oils, because two units in specific dispersion may be equivalent to as much as 5% unsaturation. Refractive index is more sensitive to unsaturation, and a more accurate method for determining complete hydrogenation is to obtain refractive index before and after an additional hydrogenation. If there is no change in refractive index, the sample is completely hydrogenated. Infrared and ultraviolet absorption methods for determining completeness of hydrogenation are being investigated.

ERROR IN DETERMINATION OF DOUBLE BOND INDEX

Double bond index is not sensitive to errors in molecular weight. When Equations 1 and 2 are combined for the calculation of double bond index, (M + 17) appears in the numerator and M appears in the denominator, with the result that any error in molecular weight is nearly canceled.

The error in double bond index due to specific dispersion is proportional to experimental error in measuring specific dispersion divided by the increment (δ − 98). Assuming an error of 2 in specific dispersion, the corresponding error in double bond index will be less than 10% for all samples having specific dis-

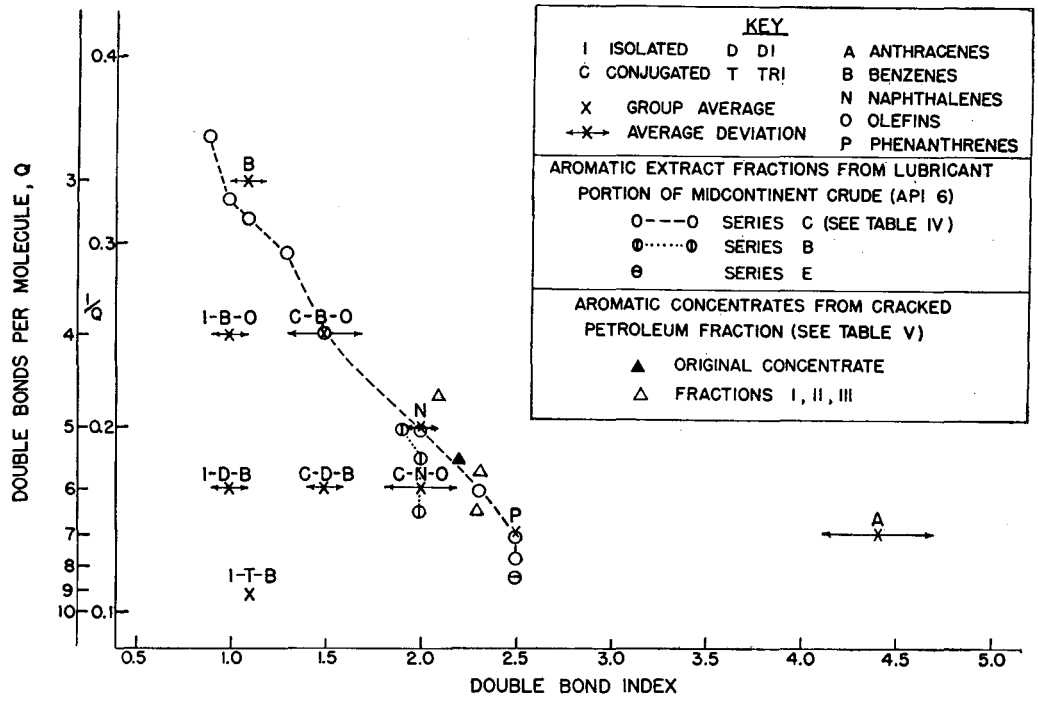


Figure 5. Hydrocarbon Map for Aromatic Concentrates

persions higher than 120. For samples of specific dispersion below 120, another significant source of error lies in the use of 98 as the average specific dispersion for all paraffins and naphthenes (see Equation 1). For such samples there are two alternative methods for obtaining double bond index accurately within 10%: (1) obtaining accurate specific dispersions before and after hydrogenation and substituting the specific dispersion of the hydrogenated sample for 98 in Equation 1, or (2) concentrating the unsaturates so that the specific dispersion is greater than 120.

Table III. Permissible Deviation in Per Cent Hydrogen to Give Double Bond Index Accurate to ±10%

Double Bonds per Molecule	Molecular Weight 100	200	300	400	500
0.25	±0.02	±0.01	....	....	....
0.50	0.04	0.02	±0.01	±0.01	....
0.75	0.06	0.03	0.02	0.01	±0.01
1.00	0.08	0.04	0.02	0.02	0.01
1.50	0.12	0.06	0.04	0.03	0.02
2.00	0.16	0.08	0.05	0.04	0.03
4.00	0.32	0.16	0.11	0.08	0.06
6.00	....	0.24	0.16	0.12	0.09
8.00	....	....	0.21	0.16	0.13
10.00	....	....	0.27	0.20	0.16
12.00	....	....	....	0.24	0.19

Lastly, the experimental accuracy of double bond index is dependent upon the accuracy of per cent hydrogen determination (see Equation 2), which is in turn dependent upon the combustion method used. Therefore, Table III is presented to show the limits within which per cent hydrogen may vary without causing an error in double bond index greater than 10%. The permissible deviation in per cent hydrogen varies with both Q and molecular weight. In general, for samples having two or more double bonds per molecule, routine combustion methods (*10*, *28*) with an accuracy of 0.05% are satisfactory. For samples composed of large quantities of saturated hydrocarbons and therefore a small average number of double bonds per molecule, more time-consuming combustion methods (*28*) with accuracies of 0.01% must be used. As a general rule either the more accurate per cent hydrogen methods should be used or the unsaturates should be concentrated for all hydrocarbon mixtures having specific dispersions less than 120.

DOUBLE BOND INDEX OF MIXTURES

The experimentally determined double bond index of a hydrocarbon mixture is a measure of the proportion of the various types of double bonds present. For example, double bond index of 1.5 for a mixture of alkyl benzenes and alkyl naphthalenes would indicate 50% benzene double bonds and 50% napthhalene double bonds and, therefore, a ratio of 5 moles of benzenes to 3 of naphthalenes.

Calculations show that the double bond index of a mixture does not depend upon the concentration of unsaturated hydrocarbons because saturated hydrocarbons make no contribution to double bond index. Thus, the double bond index can be experimentally determined either on the original mixture or on the concentrated unsaturates, providing the concentration of unsaturated hydrocarbons in the original mixture is sufficiently great to permit accurate determinations.

Specific dispersion is nearly additive on a weight per cent basis, whereas double bonds per molecule and molecular weight are, of course, additive on a mole per cent basis. When all components are of the same molecular weight, weight per cent equals mole per cent and there is no discrepancy between experimental double bond index and the theoretical double bond index (which could be calculated if the percentage of each type of double bond in the mixture were known). For ordinary petroleum fractions there is no appreciable discrepancy, and calculations show that the discrepancy should not exceed 5% of the double bond index even for unusual hydrocarbon mixtures.

If acetylenes were present in a hydrocarbon mixture, the experimental double bond index would be extremely low, as acetylenes have low specific dispersions and an acetylenic bond would calculate as two double bonds.

APPLICATION TO PETROLEUM

When hydrocarbon analysis methods are used on petroleum fractions, the effect of nonhydrocarbons must be considered. The concentration of nonhydrocarbons varies greatly from one sample to another, and little is known about their structures. Grosse and Wackher (*9*) have discussed the effect of nonhydrocarbons on specific dispersion. However, for double bond index the effect of nonhydrocarbons on calculated number of double bonds may be more serious because the nonhydrocarbons are usually removed during catalytic hydrogenation. The authors believe that, for aromatic fractions containing not more than 1 to 2% of nonhydrocarbon elements, the interference is not serious.

In petroleum fractions double bond index can be used to classify the unsaturated hydrocarbon types because it is dependent upon the relative positions of both olefinic double bonds and aromatic rings. Double bond index is most easily interpreted when used in conjunction with other analytical tools such as specific dispersion, bromine number (*12*), rings per molecule (*18, 29*), or number of double bonds per molecule.

The double bond indexes of the various unsaturated hydrocarbon types can be plotted against number of double bonds per molecule (Q) to provide a hydrocarbon map useful for the analysis of mixtures of unsaturated hydrocarbons. For using such a map, the Q for a petroleum fraction must be determined fairly accurately. Reference to Equation 2 shows that the calculation of an accurate value for Q requires a reasonably accurate molecular weight, even though an accurate molecular weight is not necessary for the calculation of double bond index. For highly aromatic samples physical property methods frequently give more accurate values for the molecular weight after hydrogenation than before hydrogenation. For such samples number of double bonds per molecule may be calculated from the following equation:

$$Q_h = \frac{M_h\,(H_h - H_o)}{2.016\,(100 - H_o)} \qquad (3)$$

where M_h is molecular weight after hydrogenation, and the other symbols are the same as in Equation 2. [If Q_h is used for calculating double bond index, it is necessary to substitute $(M_h - 2\,Q_h)$ for M in the numerator of Equation 1.]

Figure 5 shows a double bond index *vs.* reciprocal of Q hydrocarbon map for use with aromatic concentrates. The average double bond indexes for various aromatic hydrocarbon groups are plotted along with arrows indicating the average deviations of the groups. The reciprocal of Q is used, so that interpolation between the groups is linear. This map is particularly useful because the location of a sample on the map depends only upon number of double bonds per molecule and the positions of those double bonds and not upon molecular weight. However, the map cannot be used for petroleum fractions containing saturated hydrocarbons because saturates would decrease Q without affecting double bond index. Aromatics may be concentrated from a petroleum fraction by use of silica gel (*16*).

Figure 5 also shows a number of aromatic fractions extracted from Midcontinent crude by A.P.I. Research Project 6 at the National Bureau of Standards (*19*). The corresponding data are shown in Table IV. The A.P.I. 6 workers extracted the aromatics from the Midcontinent crude, distilled the extract portion, and selected five narrow boiling fractions known as samples A, B, C, D, and E. These samples were successively extracted to obtain 30 or 40 fractions from each sample. The last few fractions in each series contain some saturated hydrocarbons which would interfere with the use of the hydrocarbon map. A few representative fractions were completely hydrogenated at the Bureau of Standards to obtain additional information on their composition. On these fractions, three from Series B, nine from Series C, and one from Series E, the authors have calculated number of double bonds per molecule and double bond indexes from their data as shown in Table IV. There is some uncertainty in these calculated values because some of the data were interpolated rather than experimentally determined and also because the original fractions contained small quantities of nonhydrocarbons which were removed during the hydrogenation. Equation 3 was used to calculate number of double bonds per molecule from molecular weights experimentally determined on the hydrogenated fractions and percentages of hydrogen determined on both the original and the hydrogenated fractions.

Table IV. Double Bond Indexes of Aromatic Fractions Extracted from Midcontinent Crude (*19*)

	Boiling Range (° C.) at 1 Mm. Hg	No. of C Atoms per Molecule	Q_h[a]	DBI
Series B	198–204			
Fraction				
12		22.8	6.5	2.0
16		23.5	5.5	2.0
19		23.9	5.0	1.9
Series C	214–218			
Fraction				
1		22.5	7.8	2.5
7		23.5	7.1	2.5
13		24.7	6.1	2.3
20		25.6	5.0	2.0
26 and 27		27.2	4.0	1.5
30		27.7	3.4	1.3
33		28.3	3.2	1.1
35		28.5	3.1	1.0
37		28.9	2.8	0.9
Series E	251–258			
Fraction 1		26.7	8.4	2.5

[a] Q_h is number of double bonds per molecule calculated by Equation 3 using molecular weight of hydrogenated fraction. These molecular weights were experimentally determined; most of the molecular weights of original fractions were interpolated.

The points for Series C are joined by a dashed line in Figure 5 to show the trend in double bond index with changing number of double bonds. This dashed line passes from phenanthrenes to naphthalenes to benzenes with no significant deviations. Thus, there is no evidence of appreciable quantities of anthracenes, isolated polybenzenes, or conjugated polybenzenes in these fractions. The low Q on the last fraction, C-37, is probably due to the presence of some saturated hydrocarbons. Fraction C-1 probably contains some tetracyclic aromatics. The three fractions from Series B differ somewhat from Series C in type of aromatics and may contain small amounts of isolated or conjugated polybenzenes. Fraction E-1 probably contains tetracyclics.

Thus double bond index confirms the conclusion of Mair, Willingham, and Streiff (*20*) that in this crude the aromatic rings present in any one molecule are usually condensed together, but also shows that the tricyclic aromatics are not anthracenes. Additional information on polycyclic aromatics is needed to fill out the hydrocarbon map.

Table V shows the application of double bond index to a cracked petroleum product of 250° to 350° C. (480° to 660° F.) boiling range. The double bond indexes of the original sample containing 58% saturated hydrocarbons and of the aromatic fraction concentrated by silica gel (*16*) are both 2.2. This agreement is evidence that saturated hydrocarbons have no effect upon the double bond index of a mixture.

Table V. Analysis of Cracked Petroleum Fraction Boiling 250–350° C.

	n_D^{20}	d_4^{20}	$n - d/2$	50% B.P., ° C.	Approx. Mol. Wt.[a]	δ	Bromine No.[b]	% H	Q_h[c]	DBI	Rings per Mol.[d]	Volume %
Original	1.5190	0.8979	1.0700	302	(228)	189	8	11.42	3.1	2.2	...	
Hydrogenated	1.4653	0.8499	1.0404	290	228	96	0	13.87			1.6	
Aromatic concentrate	1.6163	1.0217	1.1054	314	(196)	289	19	8.0	5.5	2.2	2.3	42% of original
Hydrogenated	1.4829	0.8939	1.0360	271	200	97	0	13.10			2.3	
Aromatic fraction I	1.5862	0.9848	1 0938	297	(196)	255	21	8.9	4.6	2.1	2.1	31% of aromatic concentrate
Hydrogenated	1.4793	0.8830	1.0378	260	193	98	0	13.26			2.0	
Aromatic fraction II	1.6249	1.0314	1.1092	320	(196)	300	20	7.8	5.7	2.3	2.4	36% of aromatic concentrate
Hydrogenated	1.4855	0.8998	1.0356	276	203	97	0	13.00			2.4	
Aromatic fraction III	1.6404	1.0518	1.1145	345	(208)	317	22	7.3	6.5	2.3	2.6	33% of aromatic concentrate
Hydrogenated	1.4885	0.9071	1.0350	288	212	97	0	13.05			2.6	

[a] Molecular weights estimated from density and mid boiling point by method of Mills, Hirschler, and Kurtz (*22*). Data for aromatic concentrates fell outside range of correlation, so molecular weights on these concentrates are only approximate.
[b] Bromine numbers obtained by method of Johnson and Clark (*12*).
[c] Q_h is number of double bonds per molecule calculated by Equation 3 using molecular weight of hydrogenated fraction.
[d] Number of rings per molecule determined on hydrogenated fractions by Waterman method (*29*) using specific refraction and molecular weight and on aromatic concentrates by Lipkin-Martin method (*18*) using density and density coefficient.

When the 2.2 double bond index and 5.5 double bonds per molecule of the aromatic concentrate are plotted on the hydrocarbon map, Figure 5, a mixture of naphthalenes and phenanthrenes is indicated. These data together with the 2.3 rings per molecule show that there may be 25 to 40% tricyclics present. However, the data on this one fraction are not sufficient to prove that these two aromatic types are the only ones present in considerable concentration. To determine whether the position on the map is caused by averaging several aromatic types, the aromatic concentrate was further separated on silica gel into three fractions (Table V). Although the physical properties of these fractions are considerably different, the double bond indexes range only from 2.1 to 2.3 and when the fractions are plotted on Figure 5 they fall close to a line joining the naphthalene and phenanthrene groups. Thus aromatic fraction I probably contains little but naphthalenes while fractions II and III contain about 40 and 70% phenanthrenes in mixture with naphthalenes.

To check these conclusions the number of rings per molecule was calculated on the aromatic fractions by the method of Lipkin and Martin (*18*) and on the hydrogenated aromatic fractions by the method of Vlugter, Waterman, and van Westen (*29*). These calculations show 0 to 10%, 40%, and 60% tricyclics, respectively, on fractions I, II, and III, which check very well with the above analyses from double bond index. Incidentally, the excellent agreements between rings per molecule calculated by the two methods (see Table V) show that there is little or no naphthene ring in the aromatic molecules (*18*).

It is interesting to note that the double bond indexes of these fractions show no evidence of the presence of anthracenes, isolated polybenzenes, or conjugated polybenzenes, the same conclusion as was reached previously concerning the A.P.I. 6 aromatic fractions.

In this discussion the bromine numbers of the fractions have not been considered. The bromine numbers by the Johnson and Clark method (*12*) vary only between 19 and 22 for the aromatic fractions. Their significance is uncertain because so few aromatics or nonhydrocarbons in this boiling range have been investigated. If the bromine numbers could be entirely attributed to olefinic double bonds, as many as 25% of the aromatic molecules could be aromatic olefins. Further study of the meaning of bromine number in this range is necessary.

The application of double bond index to a number of petroleum distillate fractions has been described. Double bond index should also be useful on residual oils, although the hydrogenation and determination of per cent hydrogen may be difficult. Residual oils may also contain large quantities of nonhydrocarbons.

Double bond index can be applied to the solution of many problems in hydrocarbon analysis, such as the determination of the predominant type of olefin or aromatic in mixture with saturated hydrocarbons, the detection of the conjugated diolefins in mixture with mono-olefins and isolated diolefins, and the detection of naphthalenes in straight-run gasoline fractions.

LITERATURE CITED

(1) Am. Petroleum Inst., Research Project 44 at National Bureau of Standards, Selected Values of Properties of Hydrocarbons as of Dec. 31, 1946.
(2) Auwers, K., Roth, W. A., and Eisenlohr, F., *Ann.*, **373**, 267 (1910).
(3) Bond, G. R., IND. ENG. CHEM., ANAL. ED., **18**, 692 (1946).
(4) Campbell, K. N., and Eby, L. T., *J. Am. Chem. Soc.*, **63**, 2683 (1941).
(5) Cauley, S. P., and Delgass, E. B., *Oil Gas J.*, **45**, No. 12, 166 (1946).
(6) Deanesly, R. M., and Carleton, L. T., IND. ENG. CHEM., ANAL. ED., **14**, 220 (1942).
(7) Egloff, G., "Physical Properties of Hydrocarbons," New York, Reinhold Publishing Corp., Vol. I (1939), II (1940), III (1946), and IV (1947).
(8) Groennings, S., IND. ENG. CHEM., ANAL. ED., **17**, 361 (1945).
(9) Grosse, A. V., and Wackher, R. C., *Ibid.*, **11**, 614 (1939).
(10) Hindin, S. G., and Grosse, A. V., *Ibid.*, **17**, 767 (1945).
(11) Hirschler, A. E., *J. Inst. Petroleum*, **32**, 133 (1946).
(12) Johnson, H. L., and Clark, R. A., ANAL. CHEM., **19**, 869 (1947).
(13) Klages, A., *Ber.*, **40**, 1768 (1907).
(14) Kok, W. J. C. de, and Waterman, H. I., *Chimie & Industrie*, **44**, 91 (1940).
(15) Kurtz, S. S., Mills, I. W., Martin, C. C., Harvey, W. T., and Lipkin, M. R., ANAL. CHEM., **19**, 175 (1947).
(16) Lipkin, M. R., Hoffecker, W. A., Martin, C. C., and Ledley, R. E., *Ibid.*, **20**, 130 (1948).
(17) Lipkin, M. R., and Martin, C. C., IND. ENG. CHEM., ANAL. ED., **18**, 433 (1946).
(18) *Ibid.*, ANAL. CHEM., **19**, 183 (1947).
(19) Mair, B. J., Willingham, C. B., and Streiff, A. J., *J. Research Natl. Bur. Standards*, **21**, 565 (1938).
(20) *Ibid.*, **21**, 581 (1938).
(21) Mikeska, L. A., *Ind. Eng. Chem.*, **28**, 970 (1936).
(22) Mills, I. W., Hirschler, A. E., and Kurtz, S. S., *Ibid.*, **38**, 442 (1946).
(23) Neyman-Pilat, E., and Pilat, S., *Ibid.*, **33**, 1382 (1941).
(24) Patterson, A. M., *J. Am. Chem. Soc.*, **55**, 3905 (1933).
(25) Simmons, M. C., ANAL. CHEM., **19**, 385 (1947).
(26) Thorne, H. M., Murphy, W., and Ball, J. S., IND. ENG. CHEM., ANAL. ED., **17**, 481 (1945).
(27) Thorpe, R. E., and Larsen, R. G., *Ind. Eng. Chem.*, **34**, 853 (1942).
(28) Tunnicliff, D. D., Peters, E. D., Lykken, L., and Tuemmler, F. D., IND. ENG. CHEM., ANAL. ED., **18**, 710 (1946).
(29) Vlugter, J. C., Waterman, H. I., and van Westen, H. A., *J. Inst. Petroleum Tech.*, **18**, 735 (1932).
(30) von Fuchs, G. H., private communication.
(31) von Fuchs, G. H., and Anderson, A. P., *Ind. Eng. Chem.*, **29**, 319 (1937).
(32) Ward, A. L., and Fulweiler, W. H., IND. ENG. CHEM., ANAL. ED., **6**, 396 (1934).
(33) Ward, A. L., and Kurtz, S. S., *Ibid.*, **10**, 559 (1938).
(34) Willstätter, R., and Heidelberger, M., *Ber.*, **46**, 517 (1915).

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Quantitative Analysis with the X-Ray Spectrometer

Accuracy and Reproducibility

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Preliminary performance tests are reported for the Norelco Geiger-counter x-ray spectrometer with respect to reproducibility of measurements and accuracy in quantitative analysis of powder mixtures. Specimen preparation is discussed, and the necessity for avoiding large crystallites in the powder is indicated. The reproducibility with the automatic recorder appears not to be good enough for precise quantitative work. Manual counting in a single scanning usually permits the determination of quartz, in mixtures containing more than 10% quartz, with an average accuracy of about ±5% of the absolute amount present. Higher accuracy can be attained by several scannings.

BECAUSE the x-ray powder diffraction pattern is a characteristic physical property of a crystalline compound, it lends itself readily to the analytical problem of identification of the substance, both as the pure compound and as a component of a powder mixture. Although this useful application of diffraction patterns was recognized by early workers (*4*), it was not until the latter half of the 1930's that suitable methods for its routine application were worked out. Clark and Reynolds (*1*) in 1936 developed a method for quantitative analysis of mine dusts for quartz that utilized the x-ray powder patterns. Then, in 1938, qualitative identification by diffraction methods was put on a routine basis by Hanawalt, Rinn, and Frevel (*3*).

The advent of the Norelco Geiger-counter x-ray spectrometer (*2*) appeared to offer new possibilities for the analysis of powder mixtures. In addition to speedy qualitative identification procedures, it makes possible intensity measurements of high accuracy, which are so necessary in quantitative studies.

This laboratory has been interested recently in the whole problem of precise quantitative analysis with the spectrometer, and has adapted to the recording spectrometer the internal standard technique of Clark and Reynolds (*1*) for quartz determination in industrial dusts. Details and procedures have been reported (*5*). The fundamental problem of the precise quantitative analysis of powder mixtures in general is not a simple one. This communication reports preliminary results on reproducibility and accuracy for measurements made with the Norelco spectrometer. Many of the studies have been made on quartz because of the laboratory's special interest in dust analysis.

Early investigations had to do with the reproducibility of measurements made on the instrument. A so-called standard sample of quartz (designated as NAP) was supplied with the instrument. It was soon found that the instrument reproduced the angular positions of the diffraction lines of the quartz pattern with good accuracy, but that intensity reproducibility was poor. If the standard sample of quartz was moved 1 mm. or so on the sample holder, the intensity of its strongest line at 3.33 Å. might differ by 20% from the previous value, all other factors being held constant. Other quartz samples were available, and they were found to give intensity values differing among themselves and from that of the standard sample. Under constant instrumental conditions, of course, any pure compound should show the same intensity for a given line of its pattern regardless of the source of the compound. The above observations initiated the investigation of several compounds for constancy of line intensity, reproducibility of specimen preparation, and instrument stability.

SPECIMEN PREPARATION

An important part of the problem of reproducibility has to do with specimen preparation. The observations with the standard quartz sample obviously point to nonuniformity of the specimen. It was early realized that specimens flowed on a microscope slide, with the aid of a trace of binding agent, could not be used for serious work, as they are almost invariably too thin for optimum diffraction intensities, and successive specimens are not of uniform thickness. In the present studies all specimens were mounted by pressing the powder gently into the cavity of an ordinary microscope drop slide (cavity depth 0.8 mm.) by means of a plane glass plate. This sufficed for all measurements except certain high-angle measurements on quartz, where it was necessary to use a drop slide with cavity depth of 1.75 mm. in order to have a sample with effectively infinite thickness.

Another important factor affecting reproducibility is the statistical distribution of crystallite sizes in the specimen. Theoretical and experimental studies, to be reported elsewhere, have indicated that a maximum crystallite size of less than 5 microns is needed for good reproducibility in the case of quartz and presumably for most mineral silicates as well. In fact, it appears that many difficulties in specimen preparation previously attributed to preferred orientation effects are actually the result of the presence of too large crystallites in the material. If the material is not fine enough to yield measurements of good accuracy, it must be ground to the proper fineness by ball milling or other means. It is not claimed that the method of mounting described above does not lead to some orientation effects, but merely that it leads to reproducible specimens of a given material.

INSTRUMENT STABILITY AND STANDARDIZATION

After considerable work had been done it became obvious that it was necessary to standardize the spectrometer from day to day in order to get comparable results. The instrument exhibited a drift (variable from day to day) such that a truly standard sample would give a different intensity value from day to day, although measurements of the sample taken in any single day showed excellent constancy. This standardization can be done by adjusting slightly the slit-height wedge, so that the intensity of a selected line of the standard has the same value from day to day. Rather than readjust the instrument each day, a simpler procedure is to measure the intensity of the standardizing substance and calculate a factor for use on subsequent measurements. Three measurements made at, say, the beginning, middle, and end of the day, suffice for calculation of the factor to be used on all measurements during that day.

The present studies have been made without any elaborate changes in the instrument. It was received with a damping and amplitude control for the signal to the recorder consisting of a Daven attenuator (terminal impedance 500/500) with five 1000-ohm steps of resistance in series. This was replaced about February 1, 1947, with a control designed after the one used at the laboratories of the American Cyanamid Company, Stamford,

Table I. Reproducibility of Quartz Intensity Measurements

(G.-C. spectrometer using recorder)

G.-C. Trace No.	Sample	No. of Specimens	Line, Å.	No. of Traces	Scanning Motor Speed, R.P.M.	Average Deviation from Mean, % I = area	Average Deviation from Mean, % I = height	Greatest Deviation from Mean, % I = area	Greatest Deviation from Mean, % I = height	Remarks
20	NAP	1	3.33	8	1	3.18	3.11	6.87	6.26	Orginal instrument
306	NAP	1	1.81	6	1	..	0.98	..	2.69	American Cyanamid control added
307	NAP	1	1.81	8	1	..	2.83	..	4.40	
308	IHFM	1	1.81	6	1	..	2.28	..	4.05	
309	IHFM		1.81	6 (rotating)	1	..	1.21	..	2.07	
318-319	IHFM	1	1.81	12	0.25	2.92	2.05	7.01	6.38	
398-399	IHFM	10	3.33	1 each	0.25	3.18	1.63	8.41	3.63	Counting circuits stabilized
428-429	IHFM	10	3.33	1 each (rotating)	0.25	2.21	1.10	4.84	3.69	

Table II. Reproducibility of Quartz Intensity Measurements

(G.-C. spectrometer using counting technique)

Date	Sample	Line Measured, Å.	No. of Detns.	Average Deviation from Mean, % I = area	Average Deviation from Mean, % I = height	Greatest Deviation from Mean, % I = area	Greatest Deviation from Mean, % I = height	Remarks
11/13-19/46	IHFM	3.33	6	1.07	1.14	2.14	2.54	
	IHFM	4.25	6	3.20	2.30	4.99	3.61	
	IHFM	1.81	6	1.3	0.49	2.14	1.48	
11/15/46-1/20/47	IHFM	3.33	20	0.90	0.76	2.40	2.35	(3 results from first line included in 20)
1/28/47	IHFM	3.33	3	0.18	0.57	0.27	0.87	
2/13/47	IHFM	3.33	3	0.84	0.67	1.25	1.03	
5/21/47	IHFM	3.33	10	1.08	0.54	1.93	1.31	Counting circuit stabilized
6/5/47	IHFM	3.33	4	...	0.48	...	0.70	
3/18/47	HS	3.33	10	2.1	1.84	4.30	4.25	
3/31/37	NCCP	3.33	10	1.17	0.76	2.24	1.04	
3/20/47	HS	3.33	10	10.1	12.9	25.7	25.0	<325-mesh but >5μ

Conn. The latter control has a Daven attenuator (250/250) with six 120-ohm resistances in series. Between each pair of resistances are 500-mfd. condensers to ground, which can be connected as needed. An additional 5000-ohm resistance can be placed in series to smooth very high backgrounds. For use with this control the range of the Brown recorder was changed from 0–50 to 0–10 mv. These changes led to distinct improvement in recorder reproducibility. More recently the improved performance to be expected by stabilizing the counting circuits of the instrument has been pointed out, and some of the measurements were made with a Sola transformer stabilizing the counting circuit. A few measurements have been made using a rotating sample holder, but the results are not entirely conclusive as to whether it possesses advantages for such studies.

REPRODUCIBILITY WITH AUTOMATIC RECORDING

Table I summarizes a few results on quartz intensities when the automatic recorder is used. All results presented should be considered in the light of attempting to utilize a single measurement on a specimen as the basis of an analysis. The average and greatest deviations expressed in percentage are then a measure of the expected accuracy of a quantitative analysis based on a single measurement. Inasmuch as in all but special cases, direct analysis is impossible because of the effects of absorption on the intensities, it is necessary to use an internal standard technique. Most analyses then entail the measurement of two lines, a line of the unknown substance and a neighboring line of the standard. The ratio of the intensity of the unknown line to that of the standard line is determined, and the unknown content read from a curve on which this ratio has been plotted against percentage of the unknown for a series of known mixtures. For an analysis based on a single measurement of each line the ratio may be in error by as much as the sum of the greatest possible deviations of the two individual line measurements. These can be rather sizable when the recorder technique is used.

The original reproducibility with the recorder (G.-C. Trace 20) is very unsatisfactory. There is improvement after the addition of the American Cyanamid control (Trace 306 and later ones). Slower scanning speed does not indicate any noticeable improvement, and the advantage of the rotating sample is inconclusive in this set of data. All the studies except the last two were made on a single specimen which was not touched or disturbed in any way during the series of traces, all of which were made under as nearly identical conditions as possible. The last two studies show some results with the recorder on a series of specimens of the same sample of quartz. Reproducibility in this case is also a check of the reproducibility of mounting successive specimens of the same lot of material. If the instrument reproducibility is about the same as in previous studies it is evident that specimen preparation is rather good. The data of Table I also suggest that the heights are a more consistent measure of the intensity than the areas. Results with the recorder, however, are not considered good enough for precise quantitative work, inasmuch as the deviations become larger where a substance is measured in the presence of another component.

REPRODUCIBILITY USING COUNTING TECHNIQUE

Table II presents data on the reproducibility of quartz intensities measured by counting technique. All studies except the last one were made on material sized to <5μ. The lines have been manually scanned at 0.05° intervals, counting in the neighborhood of 5000 counts for points around the top of the peak, and enough counts at the base line to locate it with the same absolute error. After the data had been plotted, the areas beneath the curves were calculated as the product of the height and width at half maximum intensity. Each determination was made on a separately prepared specimen of the sample. The increased reproducibility of the counted results is strikingly evident. It is also evident that the reproducibility of specimen preparation is remarkably good. The poorer reproducibility of the 4.25 Å. line is expected because of its smaller multiplicity. In general, the superiority of heights over areas for intensity measurements seems to be established (but see data on sodium chloride below). The various samples of finely ground quartz show approximately the same reproducibility. Where larger particles are present, however, the reproducibility is much poorer, as is illustrated by the last study on Hot Springs quartz (HS, <325-mesh but >5μ). The average intensity of the 3.33 Å. line of quartz based on heights and expressed in arbitrary units is 353.7 for sample IHFM, 348.4 for sample HS, and 355.7 for sample NCCP. The average of these is probably the most reliable value for high purity samples of quartz. If sample IHFM is weighted twice in averaging, as it is based on twice as many determinations, the value is 352.4. Thus various samples of high purity quartz from different sources are shown to have a reproducible constant intensity for the 3.33 Å. line within approximately ±1%. The corresponding measurement for the coarser sample (HS > 5μ) is 309.7. In addition to the poorer reproducibility with the coarser material there is also a decrease in the intensity observed, which is probably at least partially the effect of extinction in the larger crystallites.

Table III lists similar intensity measurements for sodium chloride. Samples of reagent grade sodium chloride were reduced to a fine state of subdivision by 2- and 4-hour grinds in a motor-driven mullite mortar. The present data were taken before the necessity for standardizing the instrument daily was fully realized, and it

is believed the deviations would be somewhat less if they were repeated. The most remarkable observation here is that the areas proved more consistent than the heights as a measure of intensity. This is just the reverse of that for quartz, and is believed to be related to the fact that investigators have experienced difficulty in attempting to grind sodium chloride to a fine state of subdivision. The samples are apparently mixtures of very fine and relatively coarse particles. Some samples actually showed slight broadening of the line; hence the results on heights alone would be expected to be less reliable. Because of the effects of line broadening, intensities based on heights alone are satisfactory only for crystallite sizes above the colloidal range (>1000 Å.). All studies of substances containing appreciable amounts of material in the colloidal range must be based on areas, unless all samples can be expected to have identical size distributions for the component in question.

ANALYTICAL PROCEDURES

The superior reproducibility of the counting technique over the recorder is demonstrated in the studies with pure quartz, and it has been concluded that for precise quantitative analysis the counting technique is needed. Further substantiation is given by the observation that the accuracy of measurement falls off if the same material is measured in a mixture. This fact is illustrated, as well as one of the steps in the preparation of the standard curve, by the data of Table IV, which represent a portion of the data for the standard curve for quartz determination in dust using calcium fluoride as the standardizing substance. The technique employed is essentially that of Clark and Reynolds (*1*) modified for use with the Geiger-counter spectrometer.

These are the data for the mixture designated KS-9, representing the 60% quartz point on the standard curve. It is a mechanical mixture of 60% quartz and 40% calcite, to which has been added 25% calcium fluoride (fluorite). This determination has been based entirely on heights of the 3.33 Å. quartz line and the 3.16 Å. fluorite line. Other determinations of points on the curve have been made by both areas and heights, and the results based on heights alone are better. Reproducibility of the line height for quartz is not nearly as good as it was when the pure material was being studied, as has been found to be the case generally. In this instance the fluorite line was measured with better general accuracy than the quartz line. This is just chance, as other sets of data are just reversed in this respect. The ratio for this point on the curve is 4.19, with an average percentage deviation from the mean of 3.34%. The greatest deviation from the mean of the 14 determinations is 7.4%. These can be interpreted in terms of an actual analysis of a 60% quartz sample as follows: Once in 14 measurements one can expect a ratio which in an actual analysis would lead him to report the result from the curve as 55.6% quartz or 64.4% quartz, depending on which side of the average it occurred. On the average, however, the analysis made by a single determination of the ratio would give a result of 58.0 or 62.0%.

The curve is a straight line down to 10% quartz and has a slight curvature between 10 and 0%. It does not seem possible that this curvature is real. However, others have observed a similar curvature at low percentages in setting up standard curves for various analyses. Further work on this point is contemplated. In using this curve in the analysis of samples containing more than 10% quartz, it is felt that results will rarely be off farther than ±10%, and usually not more than ±5%, of the absolute amount of quartz present.

Table III. Reproducibility of Sodium Chloride Intensity Measurements

(G.-C. spectrometer using counting technique)

Sample, Hours	Line Measured, Å.	No. of Detns.	Average Deviation from Mean, % I = area	Average Deviation from Mean, % I = height	Greatest Deviation from Mean, % I = area	Greatest Deviation from Mean, % I = height
M-2	1.989	3	0.27	1.75	0.41	2.58
B-2[a]	1.989	3	3.3	4.72	5.00	7.17
EA-2	1.989	3	1.47	2.59	2.19	3.88
All 2 and 4	1.989	9	1.3	2.16	2.87	4.23

[a] This sample of reagent grade NaCl always gave an extremely rough and pitted surface. Microscopic examination showed presence of foreign material. Poorer reproducibility from such a sample is evident in percentage deviation.

Table IV. Analysis of Data for 14 Determinations of 60% Quartz Point on Standard Quartz Analysis Curve

(Standardizing substance, CaF_2)

Count	SiO_2, 3.33 Å. line I = height	SiO_2, 3.33 Å. line Deviation from mean	CaF_2, 3.16 Å. line I = height	CaF_2, 3.16 Å. line Deviation from mean	Ratio of Intensities HQ/HF	Ratio of Intensities Deviation from mean
1	137.0	−3.4	32.2	−1.3	4.25	+0.06
2	142.5	+2.1	33.2	−0.3	4.29	+0.10
3	148.0	+7.6	34.0	+0.5	4.35	+0.16
4	135.0	−5.4	34.8	+1.3	3.88	−0.31
5	136.5	−3.9	34.0	+0.5	4.01	−0.18
6	143.5	+3.1	33.3	−0.2	4.31	+0.12
7	136.0	−4.4	33.7	+0.2	4.04	−0.15
8	143.5	+3.1	33.3	−0.2	4.31	+0.12
9	139.7	−0.7	32.1	−1.4	4.38	+0.19
10	137.0	−3.4	32.7	−0.8	4.18	−0.01
11	138.3	−2.1	33.3	−0.2	4.15	−0.04
12	134.0	−6.4	33.9	+0.4	3.95	−0.24
13	145.6	+5.2	34.0	+0.5	4.28	+0.09
14	148.5	+8.1	33.9	+0.4	4.38	+0.19
Av.	140.4	±4.2	33.5	±0.6	4.19	±0.14
Av. deviation, %		2.99		1.79		3.34
Greatest deviation, %						7.4

Table V. Analyses for Test Mixtures for Quartz

Mixture No.	Diluent	Quartz, % Actual	Quartz, % Analyzed	Mixture No.	Diluent	Quartz, % Actual	Quartz, % Analyzed
1	Al_2O_3	2	3.3	6	MgO	40	42.5
2	MgO	5	5.0	7	NaCl	60	58.7
3	MgO	10	10.0	8	$CaCO_3$	60	55.7
4	NaCl	15	15.5	9	$CaCO_3$	80	71.2
5	Bentonite	35.6	33.3	10	Al_2O_3	90	95.5

Table VI. Analysis of Known Mixture Containing 60% Quartz Done in Quintuplicate

Count	Ratio, HQ/HF	Quartz from Curve, %
1	4.01	57.0
2	4.09	58.3
3	4.19	60.0
4	4.46	63.3
5	4.35	62.0
Av.	4.22	60.1 ± 0.9[a]

From average ratio, 4.22, percentage quartz is 60.3.

[a] Probable error.

The results of some measurements of known mixtures, set forth in Table V, represent the kind of accuracy to be expected from a single manual scanning of the quartz and fluorite lines in an analysis. For quartz in industrial dusts, in studies of the silicosis hazard, this accuracy is entirely adequate.

The attainment of higher accuracy simply demands more determinations. If one can afford the time to make two separate counts of the ratio, the accuracy will be improved; and if one can afford to do the ratio determination in quintuplicate, a very satisfactory result may be expected. Table VI gives the results of an analysis of a known mixture containing 60% quartz, the analysis being done in quintuplicate. Whether one takes the average of the five percentages as read from the curve, or the value based on the average of the five ratios, the result is very satisfactory. In dust analysis work this additional accuracy is not essential, but there are many cases in which it would be well worth the additional time it requires.

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LITERATURE CITED

(1) Clark, G. L., and Reynolds, D. H., IND. ENG. CHEM., ANAL. ED., 8, 36–40 (1936).
(2) Friedman, H., *Electronics*, 18, 132–7 (1945).
(3) Hanawalt, J. D., Rinn, H. W., and Frevel, L. K., IND. ENG. CHEM., ANAL. ED., 10, 457–512 (1938).
(4) Hull, A. W., *J. Am. Chem. Soc.*, 41, 1168–75 (1919).
(5) Klug, H. P., Alexander, L., and Kummer, E., *J. Ind. Hyg. Toxicol.*, in press.

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Determination of Gamma-Benzene Hexachloride by Partition Chromatography

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A partition chromatographic method for determining the gamma isomer content of benzene hexachloride products is described. The partition solvents are nitromethane and *n*-hexane and the supporting medium is silicic acid. The method is sufficiently convenient and rapid for routine production analysis, and requires no expensive equipment or specially trained personnel. The accuracy is about 2% based upon the actual gamma isomer content of synthetic mixtures of the isomers. The procedure appears to be adaptable to the determination of some of the other isomers and related constituents.

COMMERCIAL benzene hexachloride, produced by the chlorination of benzene, is a complex mixture containing at least five space isomers of 1,2,3,4,5,6-hexachlorocyclohexane, together with other closely related chlorinated materials (*5*, *6*). It is known that the insecticidal activity of benzene hexachloride products resides principally in the gamma isomer of hexachlorocyclohexane (*6*). Heretofore, no completely satisfactory method has been available for the determination of this constituent.

Direct methods for determining the biological activity of insecticidal preparations are, of course, applicable to this material, but their accuracy is subject to statistical limitation, and the methods are time-consuming and require special laboratory facilities and stringent control of technique and environmental conditions. Methods based on infrared absorption have been described by Daasch (*2*) and by Kauer, DuVall, and Alquist (*3*). The infrared methods require expensive equipment, considerable preliminary calibration, and a detailed qualitative knowledge of the composition of the material to be analyzed. The only other method which has been reported for the analysis of benzene hexachloride products is the cryoscopic method of Bowen and Pogorelskin (*1*). This method, as described, requires substantial quantities of highly purified gamma isomer, and involves the methods of precision thermometry.

The method described below involves the separation of the gamma isomer from the other constituents by partition chromatography. A column of a solid (such as silicic acid) is used to support a solvent phase (nonmobile solvent), through which is flowed a second immiscible solvent (mobile solvent) in which the sample is initially dissolved. A continuous partition between the two solvents takes place as the sample is washed down through the column, and a progressive separation of the constituents of the sample occurs.

Partition chromatography was first employed for the separation of acetylamino acids by Martin and Synge (*4*), who also proposed a theory for the method. Other applications of the method and the theory were recently reviewed by Synge (*7*). Ramsey and Patterson (*5*) applied the procedure to the separation and identification of the constituents in commercial benzene hexachloride; they employed nitromethane and *n*-hexane as the partition solvents and silicic acid as the supporting medium.

The present authors have established the conditions and detailed procedures required for the quantitative determination of the gamma isomer content and have demonstrated that the method can be made of good precision and accuracy, and is sufficiently convenient and rapid for routine production analysis. The partition chromatographic method is also applicable to the determination of the other constituents present in commercial benzene hexachloride and can be conveniently adapted to the analysis of the gamma isomer content of the usual formulations. The method is an absolute one, requiring no empirical calibration. It does not require expensive equipment and materials or specially trained personnel.

REAGENTS

n-Hexane, commercial grade. Nitromethane, commercial grade.

Mobile solvent (*n*-hexane saturated with nitromethane). Add approximately 900 ml. of the *n*-hexane to 50 ml. of the nitromethane contained in a 1-liter separatory funnel and agitate vigorously for 5 minutes. Allow the excess nitromethane to separate out, draw off from the mobile solvent, and store for future use.

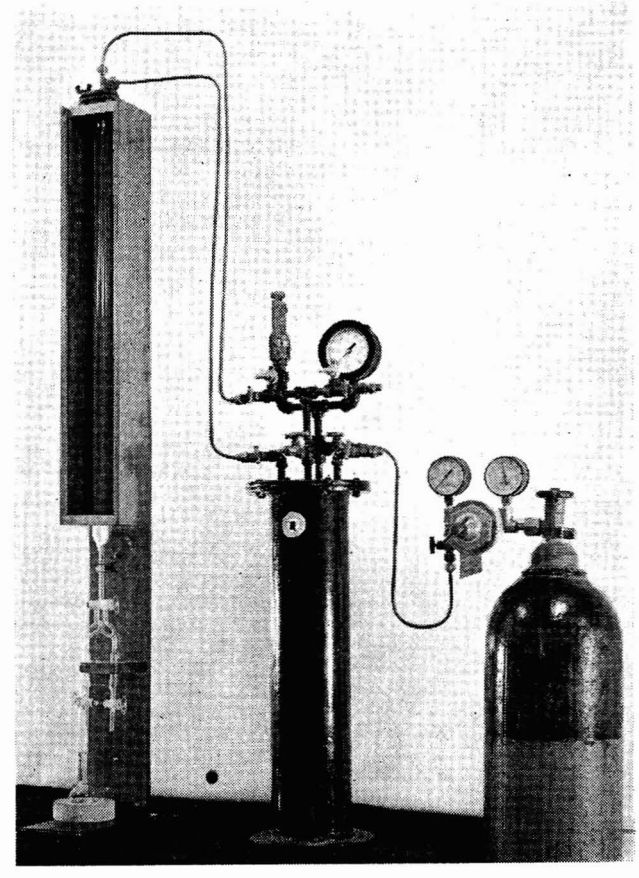

Figure 1. Partition Chromatographic Apparatus

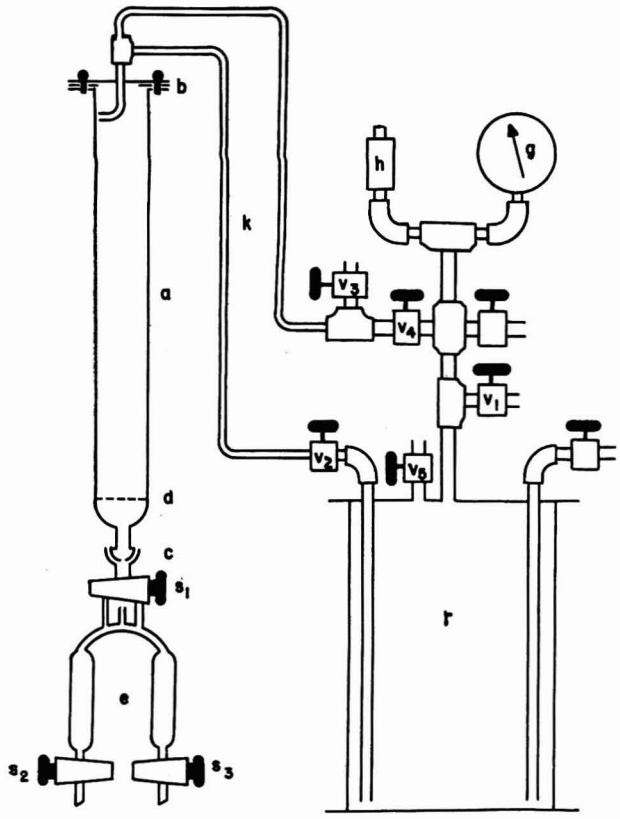

Figure 2. Schematic Diagram of Apparatus

a. Partition chromatographic column, Pyrex pipe, 32 inches long × 1 inch inside diameter
b. Flanged glass pipe fitting (Corning) on upper end of *a* with Koroseal gasket
c. Pyrex ball and socket joint, size 18/7
d. Porous plate supported by glass wool
e. Pyrex fraction collector, 10-ml. capacity in each side arm
g. Pressure gage, 0 to 30 pounds, 3.5-inch, single bronze tube
h. Safety valve, 50 pounds, side outlet
k. Connecting lines, 1/4 inch copper tubing
r. Mobile solvent reservoir, 8-liter capacity
s. Stopcocks, 2-mm. Pyrex
v. Valves, Hoke type, 1/4-inch

Miscellaneous Apparatus

Pyrex flasks, round-bottomed, long-necked, 100-ml. capacity for collection and evaporation of partition fractions
Pyrex flasks, round-bottomed, long-necked, 200-ml. capacity for collection and evaporation of the gamma isomer cut
Wood rack for storing flasks used in collection of partition fractions
Gas pressure regulator and reducing valve, Hoke-Phoenix type, for nitrogen
Nitrogen, water pumped

Silicic acid (precipitated), analytical reagent grade. A suitable grade may be obtained, for example, from Mallinckrodt Chemical Works.

APPARATUS AND PROCEDURE

The apparatus developed for the partition chromatographic analysis of benzene hexachloride samples is shown in Figures 1 and 2. The enclosed wooden column support with a removable Plexiglas plate on the front side is desirable, considering the length of the column and the fact that it is operated under pressure. For the routine application of the method, the pressure reservoir for the partition solvent is of great advantage in permitting the uninterrupted addition of the solvent to the top of the silicic acid column. As shown in Figures 1 and 2, multiple connections may be provided, so that more than one column can be operated from the same solvent supply. The multiple solvent evaporator (Figure 5) permits the removal of the solvent concurrent with the collection of successive fractions from the column, so that immediate observation of solids content is obtained. A wooden rack is a convenience for holding in order the many flasks used in each determination.

Charging the Solvent Reservoir (Figure 2). Charge the solvent reservoir, *r*, by adding about 6 liters of the mobile solvent through v_5, keeping v_1 closed and v_3 and v_4 open. Apply pressure to the reservoir by closing v_2, v_3, v_4, and v_5, adjusting the pressure-reducing valve on the nitrogen gas cylinder to about 8 pounds' gage pressure, and carefully opening v_1.

Preparation of Partition Chromatographic Column (Figure 2). Weigh out 100 ± 0.5 grams of silicic acid and transfer it to a large mortar. Measure out 55 ml. of nitromethane and add it in successive 10-ml. portions to the silicic acid, thoroughly mixing after each addition by grinding with the pestle. After all the nitromethane has been added and mixed, add 300 ml. of the mobile solvent in 100-ml. portions, mixing after each addition. Transfer the resultant slurry to a 600-ml. beaker.

Carefully disconnect and remove the clean and dry glass partition column, *a*, from its support and insert the glass wool plug and the porous plate support, *d*, in the bottom of the column. The amount of glass wool used should be just sufficient to support the silicic acid column, as excessively large plugs tend to decrease the sharpness of separation. Stopper the bottom of the partition column with a small cork.

Stir the slurry of silicic acid and the mobile solvent and pour it from the beaker into the glass column, holding down the porous plate and glass wool plug with a glass rod. When about half of the slurry has been added, remove the glass rod and pour in the remainder of the slurry. Replace the charged partition column in its support and connect the top of column *b* to the reagent and pressure lines, *k*, by means of the flanged glass pipe fitting.

Remove the cork stopper from the bottom of the column and drain the excess mobile solvent under pressure from the column by opening v_4. During the removal of the excess solvent, the silicic acid tends to pack down in the column ahead of the solvent level. When the level of the mobile solvent just reaches the top of the silicic acid layer, release the pressure in the column by closing v_4 and opening v_3. Care must be taken to prevent the splitting, cracking, or drying of the silicic acid column which may result if the solvent is allowed to drain below the level of the silicic acid layer. Such defects seriously impair the efficiency of the column. The partition column is now ready for use.

Preparation of Sample (Figure 3). Benzene hexachloride products are conveniently classified for analysis on the basis of the gamma isomer content as follows:

Low gamma samples, <5% gamma isomer content
Medium gamma samples, >5% <15% gamma isomer content
High gamma samples, >15% gamma isomer content

Figure 3. Sample Preparation Apparatus

a. Kohlrausch type flask, to contain 100 ml. at 20° C.
b. Büchner funnel, fritted disk type, medium porosity, Pyrex, 34-mm. inside diameter
c. Vacuum connection

The sample preparation involves the complete extraction of the gamma isomer from the bulk of the other constituents present in the sample. By variation of the sample weight, the optimum amount of gamma isomer is secured for addition to the chromatographic column.

Crush and thoroughly mix the sample by means of a mortar and pestle. Weigh into a tared 125-ml. Erlenmeyer flask an amount of the ground sample sufficient to provide approximately 0.2 to 0.3 gram of the gamma isomer after extracting and aliquoting. Add 25 ml. of the mobile solvent to the flask and heat just to boiling on the electric hot plate. (Caution—fire.) Stopper the flask with a cork and agitate for 5 minutes. Cool to room temperature and decant the supernatant solution through the Büchner funnel into the Kohlrausch flask, employing gentle suction. Repeat the hot extraction step on the residue in the flask employing this time 10 ml. of the mobile solvent. (This second hot extraction may be omitted on low gamma samples, where the quantity of gamma isomer to be extracted is small; and on high gamma material, where the sample may be almost completely soluble.) Wash the residue and flask with five 10-ml. portions of the cold mobile solvent, decanting each wash through the Büchner funnel into the Kohlrausch flask. Remove the Büchner funnel and vacuum connection from the Kohlrausch flask and dilute the contents to the 100-ml. mark with the mobile solvent. Mix thoroughly and transfer a 25-ml. aliquot of the solution to the prepared partition chromatographic column.

Operation of Column (Figure 2). Disconnect the flanged glass fitting carrying the solvent and pressure lines from the top of column *b*. Measure out and carefully allow a 25-ml. aliquot of the sample solution to flow onto the top of the silicic acid column by means of a volumetric pipet, being careful that the flow of sample solution does not disturb the silicic acid layer. Reconnect the solvent and pressure lines to the top of the column and drive the sample solution into the column by closing v_2 and v_3 and opening v_4. When the level of the sample solution reaches the top of the silicic acid layer, release the gas pressure by closing v_4 and opening v_3. Place a 100-ml. graduated cylinder at the bottom of column at *c*, to collect the effluent.

Wash down the walls of the glass column once with approximately 10 ml. of the mobile solvent by opening v_3 and v_2. Drive the wash solution into the column by closing v_2 and v_3 and opening v_4 until the level of the wash solution reaches the top of the silicic acid layer. Release the pressure by closing v_4 and opening v_3, and add about 50 ml. of the mobile solvent to the column by opening v_2. Close v_3 and start partition separation with a continuous flow of solvent to the column from the reservoir. Control the rate of solvent flow by adjusting the gas pressure in the reservoir so that the effluent flow from the column is 4 to 5 ml. per minute.

Allow 100 ml. of the mobile solvent to flow through the column into the graduated cylinder. Stop the flow by closing v_2, remove the graduated cylinder, and attach the fraction collector, *e* (see also Figure 4), to the ball joint, *c*. Close stopcocks s_2 and s_3, open s_1 to the left vessel, open v_2, and continue the partition separation. Collect approximately 10 ml. (to mark) in the left vessel, then turn s_1 to the right vessel. While the right vessel is filling, drain the left vessel through s_2 into a 100-ml. round-bottomed long-necked flask labeled fraction 1. When the right vessel is filled, turn s_1 to the left vessel and drain the right vessel through s_3 into a second 100-ml. flask, labeled fraction 2. Continue the collection of effluent fractions, labeling each in succession until the separation of the gamma isomer is completed. (Usually 30 to 40 10-ml. fractions are required.)

Removal of Mobile Solvent from Effluent Fractions. While the partition column is still operating, begin the recovery of the gamma isomer by transferring the round-bottomed flasks containing the effluent fractions from the rack to the solvent evaporator (Figure 5). Connect each flask by means of a cork stopper to the two-way stopcock, *b*, on the vacuum line, place the flasks in the water bath (60° C.), and apply vacuum. When the solvent evaporation is complete, carefully release the vacuum by turning stopcock *b* to open the line, remove the flasks, and replace them in their proper positions in the rack.

IDENTIFICATION OF GAMMA ISOMER FRACTIONS

The residues in the flasks are examined for the gamma isomer fraction. Recognition of these fractions is not difficult when once observed. Under ideal conditions each component of the benzene hexachloride sample partitioned occurs in a distinct and successive series of effluent fractions, separated from other components by two or more flasks which contain no solid matter. Frequently, a small amount of oily material which crystallizes slowly on prolonged standing occurs in some of the intermediate flasks.

The first solids that appear in the series of effluent fractions are the higher chlorinated products—i.e., hepta- and octachlorocyclohexanes. These are followed by more or less oily residues and then by a series of fractions containing the alpha isomer, characterized by its flaky or fluffy appearance. The amount of the alpha isomer then diminishes until two or three fractions occur which contain very little solid material and perhaps some oily substance. These indicate the end of the alpha isomer cuts. The next cut which follows after the alpha isomer is the gamma isomer, which increases in amount with succeeding fractions and is characterized by the thin rosette or fanlike crystal forms spreading out from the larger central masses. The amount of gamma isomer in each cut diminishes rapidly in the latter fractions until several empty flasks are secured. These indicate the end of the gamma isomer cut. The material which is identified as the gamma isomer is recovered by dissolving the solids in each flask in *n*-hexane and quantitatively transferring the solutions to a weighed 200-ml. round-bottomed long-necked flask. The *n*-hexane is evaporated and the weight of the combined gamma isomer fractions obtained.

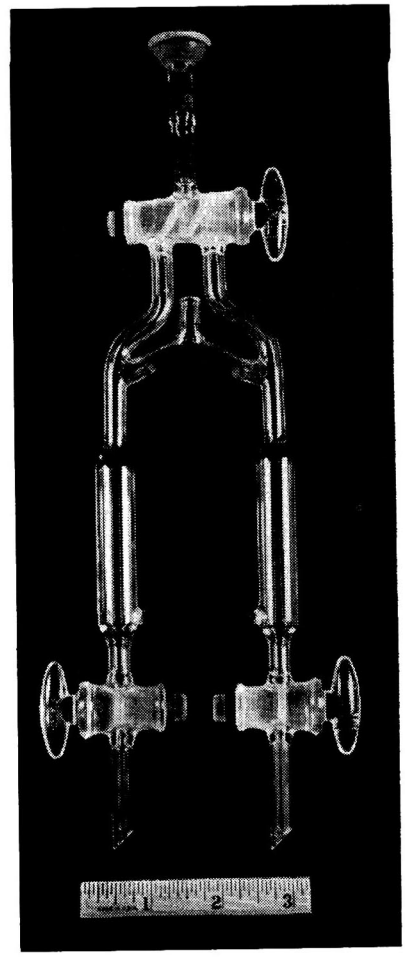

Figure 4. Fraction Collector

Table I. Melting Point Data for Typical Gamma Fractions

Sample No.	Melting Point, ° C.	Mixed Melting Point, ° C.
1	113–114	113–114
2	112–114	112–113
3	110–113	112–114
4	111–114	112–114
5	111–114	112–114

CALCULATIONS

$$\% \text{ gamma isomer} = \frac{\text{weight of gamma found in 25-ml. aliquot}}{\text{weight of total sample taken for analysis}} \times 400$$

RECOVERY OF SOLVENTS

A considerable proportion of the solvents employed in these determinations may be collected and purified by distillation for reuse by saving all the extraction and wash solutions and by placing a cold trap in the vacuum line from the water bath evaporator employed for stripping the solvent from the various fractions.

RESULTS AND DISCUSSION

During the preliminary development of the method the various partition fractions of the benzene hexachloride samples were identified by recrystallization and measurement of the melting

Table II. Partition Chromatographic Analysis of Synthetic Samples for Gamma Isomer Content

Sample No.	Formulated Composition in Weight Per Cent: Octachlorocyclohexane	Heptachlorocyclohexane	Benzene Hexachloride Isomers: α	β	γ	Wt. % Gamma Isomer Found	Relative % Error
S-1	0	0	0	0	100	100	0.0
S-2	0	0	80.0	5.0	15.0	15.2	1.33
S-3	0	0	65.0	5.0	30.0	30.2	0.66
S-4	1.2	4.8	67.8	16.1	10.1	10.3	1.98
S-5	0.9	4.1	65.9	15.0	14.1	14.2	0.71
S-6	2.1	6.5	58.2	13.0	20.2	20.4	0.99
S-7	3.2	8.8	47.9	9.7	30.4	30.7	0.99
S-8	4.2	10.2	38.8	6.1	40.7	40.4	0.74
S-9	5.3	14.1	29.6	2.1	48.9	48.2	1.43

points as described by Ramsey and Patterson (*5*). In the final stages of development, the identity and purity of the alpha, beta, and gamma isomer fractions were determined by the melting points and mixed melting points with the pure isomers, and by comparison of the x-ray diffraction patterns with those reported by Kauer, DuVall, and Alquist (*3*). Because of the complex physical nature of such isomer mixtures, it was recognized that such tests are not necessarily sufficient criteria of the purity of the gamma fractions. However, assuming no great abnormalities exist, the melting point data for some typical combined gamma fractions secured from the partition column and presented in Table I are an indication of the probable purity of these fractions. The mixed melting point data were secured on mixtures of equal parts of the gamma fractions with the pure gamma isomer (melting point 113–114° C. corrected).

In order to demonstrate the probable accuracy of the method, a number of synthetic mixtures were prepared from the pure benzene hexachloride isomers and related products. The formulation of these samples and the results secured are presented in Table II. Considering the difficulties involved in the blending and sampling of such mixtures for analysis, the results are excellent and show an accuracy of about 2% of the actual gamma isomer content of the synthetic mixtures.

As a further test of the accuracy of the method, a 50% dust base formulation was prepared from a benzene hexachloride stock sample which analyzed as 34.6% gamma isomer content. On analysis of the well mixed formulation, the gamma isomer content found was 17.4% as compared to an anticipated value of 17.3%.

The precision of the method is indicated by the data presented in Table III, which lists the results of duplicate analysis of typical samples. It appears from these data that the precision of the method is about the same as the maximum relative error observed in the analysis of the synthetic samples.

As a check on the efficiency of the partition separation in an analysis, the melting point of the gamma fraction may be secured. In general, the melting point values as determined by the capillary tube method fall in the range of 110° to 114° C.

The total time required for the routine analysis of gamma isomer content by this method is about 3 hours. Under normal conditions, a trained laboratory technician can operate two columns simultaneously and thus perform four analyses in an 8-hour shift.

While the experimental work has been confined to the development of a quantitative method for the gamma isomer content, a good separation of the other constituents, particularly those preceding the gamma isomer fraction, has been observed. The method has been used in the determination of the hepta- and octachlorocyclohexanes, and of the alpha and beta isomers in these mixtures, but the accuracy of these determinations has not yet been established.

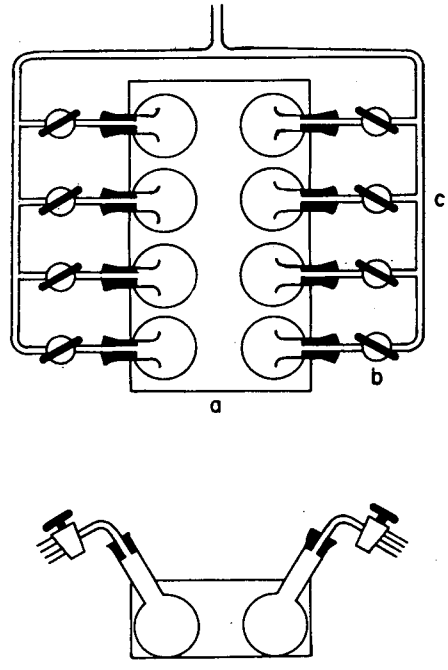

Figure 5. Multiple Solvent Evaporator

a. Metal water bath, 10 × 15 × 2 inches, heated by thermostatically controlled electric hot plate
b. Stopcock, 2-mm. Pyrex
c. Multiple vacuum connection

Table III. Precision of Partition Chromatographic Method

Sample No.	Type	% Gamma Isomer Found: 1	2	% Deviation from Mean
10	Low	0.92	0.89	1.66
11	Medium	10.2	10.2	0.00
12	Medium	10.5	10.7	0.94
13	Medium	11.0	11.0	0.00
14	Medium	11.7	11.9	0.85
15	Medium	12.1	12.1	0.00
16	Medium	13.3	13.5	0.75
17	Medium	13.5	13.1	1.50
18	Medium	13.8	14.0	0.72
19	Medium	14.1	13.8	1.08
20	High	15.4	15.5	0.32
21	High	36.1	36.2	0.14
22	High	36.5	36.2	0.41
23	High	37.2	37.1	0.13
24	High	39.8	40.1	0.37
25	High	45.0	45.8	0.88

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Bowen, C. V., and Pogorelskin, M. A., ANAL. CHEM., **20**, 346 (1948).
(2) Daasch, L. W., *Ibid.*, **19**, 779 (1947).
(3) Kauer, K. C., DuVall, R. B., and Alquist, F. N., *Ind. Eng. Chem.*, **39**, 1335 (1947).
(4) Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **35**, 1361 (1941).
(5) Ramsey, L. L., and Patterson, W. I., *J. Assoc. Official Agr. Chem.*, **29**, 337 (1946).
(6) Slade, R. D., *Chemistry & Industry*, **64**, 314 (1945).
(7) Synge, R. L. M., *Analyst*, **71**, 256 (1946).

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Direct Estimation of Penicillin G in Small Broth Samples

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A method is presented for the estimation of penicillin G in broths by chromatographic adsorption. The method is specific for penicillin G, samples require no preliminary purification, and only 100 to 200 units of penicillin are required for an analysis. The application of aqueous chromatography to resolution of mixtures of the other known penicillins is described.

PENICILLIN, as produced by mold growth, consists of a mixture of several antibiotics (*10*). At present, five antibiotics of the penicillin class are known: Δ^2-pentenylpenicillin (F), *n*-amylpenicillin (dihydro F), *n*-heptylpenicillin (K), benzylpenicillin (G), and *p*-hydroxybenzylpenicillin (X). Each has its own specific biological activity and properties. The penicillin being produced at the present time is largely penicillin G. In order to increase yields of this penicillin, precursors, such as phenylacetic acid and β-phenylethylamine, have been added to media to increase the amount of benzylpenicillin produced. It is obvious that any analytical method allowing the easy and rapid analysis of broth samples for penicillin G would be of value in studying the effects of such precursors.

The methods for the determination of penicillin G so far described in the literature are not particularly applicable to the analysis of broth samples. The gravimetric determination (*9*), based on the insolubility of the *N*-ethylpiperidine salt of the penicillin in certain solvents, requires reasonably pure penicillin G in milligram quantities. The recently described polariscopic–ultraviolet absorption method is applicable to crystalline and amorphous penicillin preparations (*5*), whereas the method employing infrared analysis requires crystalline samples (*1*). Although the microbiological differential assay (*6*) can be used to analyze broths, it is limited by the assumption that only three known penicillins are present in any sample. The value of the countercurrent distribution method (*2, 3*) in analyses for penicillin G depends greatly upon the composition of the samples analyzed. The method is of limited value for samples containing appreciable proportions of penicillins F and dihydro F.

The method described in this paper has evolved from a study of the adsorption chromatography of penicillins in aqueous solutions. It is based on the fact that, on a column of Super Filtrol (an acid-treated bentonite), penicillin G is more strongly adsorbed under given conditions than any other known penicillin and may be eluted as a separate fraction. The method is particularly applicable to fermentation broths and is not affected by the number or types of penicillins occurring in the sample to be analyzed. The method is not intended for use on purified samples, for which the more accurate physical and chemical methods are available.

EXPERIMENTAL

The chromatograms obtained when single penicillins are adsorbed and eluted on columns of Super Filtrol are shown in Figure 1. The columns were prepared in the manner described under procedure. It is seen that the sequence in which the penicillins are eluted is: X and dihydro F (first eluted), F, then G (last eluted). A tendency for dihydropenicillin F to be adsorbed more strongly than penicillin X is indicated by the longer "tail" of the elution band of the former penicillin. Penicillin K appears to be largely, if not entirely, inactivated upon adsorption. When a mixture of penicillins containing penicillin G is chromatographed, the latter is adsorbed more strongly than the other penicillins and is eluted as a separate elution band. Penicillin F is the only known penicillin which is sometimes not completely separated from penicillin G by this procedure (Figure 2). The extent of overlapping of the bands of the two penicillins is rarely greater than that illustrated, and the leading edge of the penicillin G elution curve may be extrapolated easily to the abscissa to facilitate calculation of recovery.

Although penicillin G could be separated from other penicillins in a "synthetic" mixture, it was necessary to determine how the separation would be affected by extraneous materials normally found in broths before the method could be applied to broth analysis. To this end, the penicillin in steep liquor broth was inactivated by adjusting to pH 2 and heating in a steam bath for an hour. The broth was then cooled, adjusted to pH 4.6 with alkali, and added to known mixtures of penicillins to the extent of 25% by volume. These samples were then chromatographed in the normal manner, and the inactivated broth was found to have no effect on the resolution. Although the inactivated broth was undoubtedly not identical to untreated broth, the absence of effect indicated that broth components would not noticeably de-

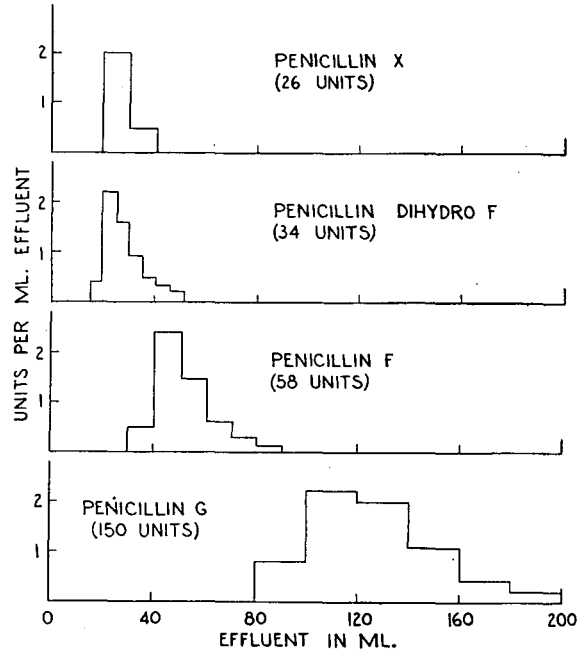

Figure 1. Chromatograms of Single Penicillins on Super Filtrol Columns at pH 4.6

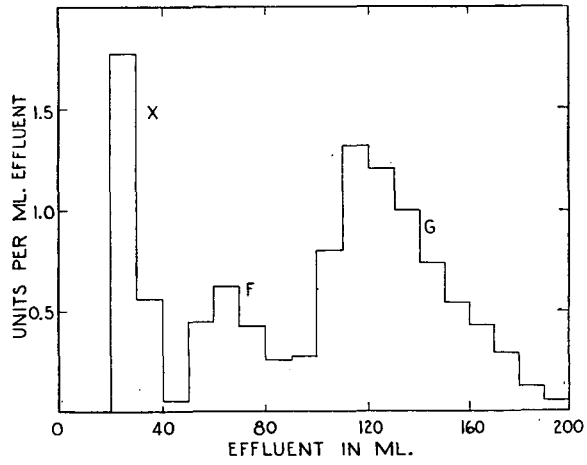

Figure 2. Chromatogram of a Mixture of Penicillins X, F, K, and G on a Super Filtrol Column

Column of pH 4.6 contained 4 grams of Super Filtrol and 4 grams of Celite 545. Penicillins adsorbed: X, 23 units; F, 27 units; K, 37 units; G, 84 units

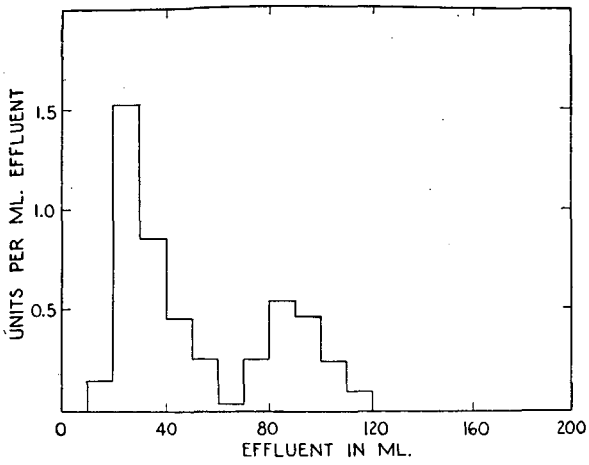

Figure 3. Chromatogram of a Steep Liquor Broth Sample

Column of pH 4.6 contained 4 grams of Super Filtrol and 4 grams of Celite 545. Penicillin adsorbed was 73 units from steep liquor broth, 28% of which was found to be penicillin G

crease the efficiency of the adsorbent. This is, in fact, found to be the case, provided that a new column is used for each analysis. Repeated use of a column for broth analyses, however, leads to decreased resolution, probably due to saturation of the adsorbent with nonpenicillin broth components.

Figure 3 shows a typical chromatogram obtained with a sample of steep liquor broth containing no penicillin G precursor. Although the total penicillin applied to the column was only 73 units, a clear-cut analysis was possible. The first band consisted of a mixture of penicillins other than G, and penicillin G was recovered as an almost completely separated band.

The adsorptive capacity of Super Filtrol has been found to vary from batch to batch. This means that each batch of Super Filtrol to be used in the estimation of penicillin G must be tested to determine the amount of adsorbent required for satisfactory resolution. This may easily be done by the method of trial and error. For example, for four different lots of adsorbent, amounts ranging from 4 to 6.5 grams gave satisfactory separation of penicillin G, equal amounts of Celite 545 being used in each case. Figure 4 gives a comparison of chromatograms for a mixture of penicillins X, F, K, and G obtained with columns of two different lots of Super Filtrol. Figure 4 shows the resolution obtained with a column containing 4 grams of one lot, that obtained with 4 grams of a second lot, and that obtained with an increased amount (6.3 grams) of the second lot.

REAGENTS AND MATERIALS

Super Filtrol, obtained from the Filtrol Corporation, Los Angeles, Calif.

Celite 545, obtained from Johns-Manville Corporation, New York, N. Y.

Phosphoric Acid, 50%.

Phosphate Buffer, 1 *M*, pH 6.1, containing 114.4 grams of potassium monobasic phosphate and 28.6 grams of potassium dibasic phosphate per liter.

Potassium Monobasic Phosphate. Solution, 0.05 *M* (pH about 4.6).

Penicillins. Pure sodium penicillin G is required as a standard. The penicillin G used in these experiments was identical in potency with a standard sample of crystalline penicillin G from the U. S. Food and Drug Administration. Corn steep liquor broth containing a small proportion of penicillin G, or broth from a synthetic medium (which contains no penicillin G), may be used as a second standard.

Chromatogram Tubes. Sections of Pyrex tubing, 14 mm. in inner diameter and about 22 cm. in length, are used. A constriction at one end gives an opening of approximately 2 mm., and glass wool is tamped above the constriction as a base for the adsorbent.

A pressure liquid-feed system is used to control the rate of flow of eluent.

PROCEDURE

Preparation of Samples. Column samples should be 1 ml. or less in volume and should contain between 50 and 200 units of penicillin. A standard solution of sodium penicillin G may be prepared with pH 6.1 buffer, providing the latter is not of sufficient

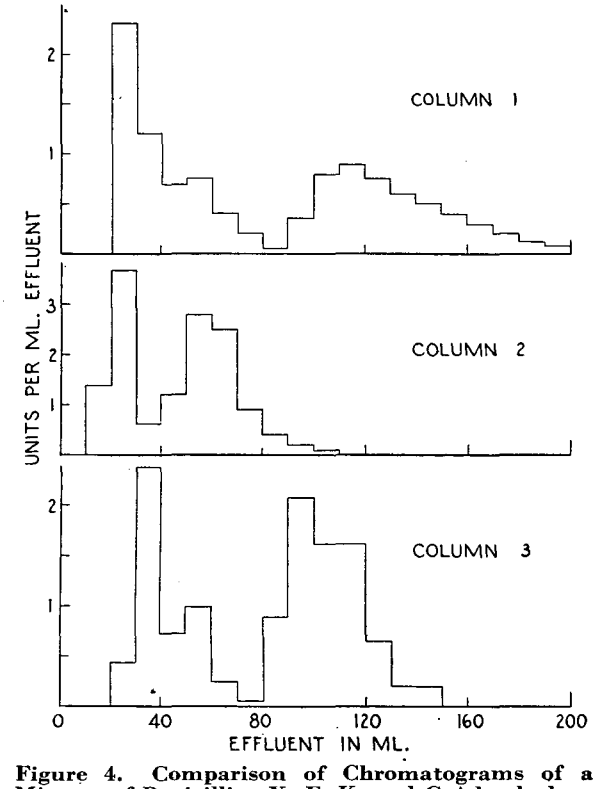

Figure 4. Comparison of Chromatograms of a Mixture of Penicillins X, F, K, and G Adsorbed on Columns of Two Different Lots of Super Filtrol

Column 1. 4 grams of Super Filtrol A and 4 grams of Celite 545
Column 2. 4 grams of Super Filtrol B and 4 grams of Celite 545
Column 3. 6.3 grams of Super Filtrol B and 6.3 grams of Celite 545
Penicillin mixture consisted of 28 units X, 20 units F, 21 units K, and 83.5 units G

strength to affect the pH of the column. For example, 1% (0.07 *M*) phosphate buffer, pH 6.1, may be employed, as 1 ml. of this solution does not have a noticeable effect on the column pH. Such a solution of known penicillin G concentration may be kept for some time and makes unnecessary the preparation of a new solution for each test. Broth samples are prepared as follows: The mycelium is strained from the liquid by drawing the latter into a pipet, the tip of which is covered with a small amount of cotton. As soon as the column (described below) is ready for use, the broth liquid is adjusted to pH 4.6 with 50% phosphoric acid and diluted, if necessary, with 0.05 *M* potassium monobasic phosphate so as to contain between 50 and 200 units of penicillin per ml. One milliliter of this broth liquid is then applied to the column and an aliquot of the same solution is immediatley diluted with appropriate buffer for assay by the *Staphylococcus aureus* cup plate method (*4*, *8*).

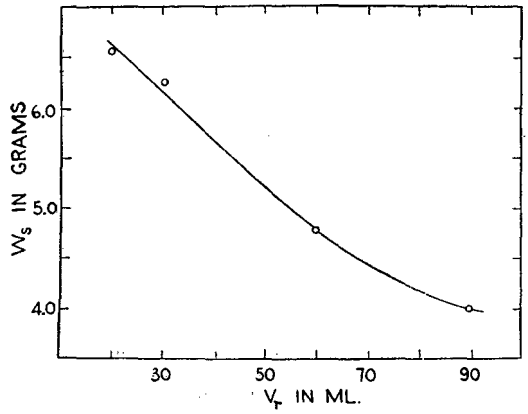

Figure 5. Relationship between W_s and V_r

Column contained 4 grams of adsorbent and 4 grams of Celite 545

Preparation of Columns. Four grams of Super Filtrol and 4 grams of Celite 545 are mixed with 20 ml. of 0.05 *M* phosphate solution and the uniform slurry is poured into a glass tube previously plugged with glass wool. Air pressure (approximately 6 cm. of mercury) is immediately applied at the top of the tube to pack the adsorbent column. This method of packing has been found to yield more uniform columns than dry packing, but pressure must be applied to prevent differential settling. When the top of the column is about to become dry, more phosphate solution is added. The column is then washed with phosphate, using a pressure liquid-feed system. This operation usually requires about 8 hours when an effluent flow rate of 1 to 2 ml. per minute is employed. When a column is left overnight, the pressure is shut off and a piece of rubber tubing is fitted over the bottom of the column tube and closed with a pinchcock. Previously washed columns left in this manner require about an hour of additional washing before use, as the pH of the column tends to decrease.

Chromatographic Procedure. As soon as the effluent from the bottom of an adsorption column has the same pH as the solution being fed to the top, the column is ready for use. Air pressure is applied to the top of the tube to force the excess phosphate solution through the adsorbent. When no solution is present on the top of the column, the air pressure is released, and the previously prepared 1-ml. sample is pipetted onto the column. The sample is forced into the adsorbent by air pressure, and is washed in by two 1-ml. portions of phosphate solution. The tube above the column is then filled with the phosphate solution and the pressure liquid-feed system connected. This is regulated to cause an effluent flow rate of exactly 1 ml. per minute. All operations are carried out at room temperature (25° to 30° C.).

Effluent is collected from the time the sample is placed on the column. A total volume of 180 to 200 ml. is caught. The size of the effluent fractions may be varied as desired; chromatograms shown in this paper were obtained with 10-ml. fractions. Smaller fractions (5 ml. or less) may be taken to obtain a more accurate estimate of the resolution attained. If it is desired to decrease the number of fractions, larger portions (50 ml. or more) may be caught after penicillin G begins to be eluted. If this procedure is used, however, small fractions (10 ml. in volume, for example) must be taken at the initial and final stages of elution of penicillin G to ensure separation of penicillin G from other adsorbed penicillins and elution of all the penicillin G. The fractions are adjusted as collected to about pH 6 by the addition of 1 ml. of 1 *M* phosphate buffer and are then ready for assay, without further dilution, by the cup plate method. When the adjusted effluent fractions cannot be assayed immediately, they may be kept in the refrigerator for at least 48 hours without noticeable loss in penicillin content.

Standardization and Calculations. Satisfactory separation of penicillin G from the other penicillins is normally obtained when the retention volume of penicillin G (the volume of effluent collected before penicillin G is first observed in the effluent) is 70 to 80 ml. If 4 grams of Super Filtrol are not sufficient to cause satisfactory separation, more adsorbent must be used. The Super Filtrol–Celite 545 ratio is maintained at unity to allow easy flow of eluent through the columns.

V_r, the retention volume of penicillin G for a column containing 4 grams of a given lot of Super Filtrol and 4 grams of Celite 545, was determined for each of a number of Super Filtrol lots. Also determined was W_s, the amount of each lot of adsorbent required for satisfactory resolution. In Figure 5, W_s is plotted against V_r for several Super Filtrol lots. Such a curve is of value because, once V_r is determined for a batch of Super Filtrol, W_s may be read directly from the curve.

The average recovery of adsorbed penicillin G varies generally from 75 to 90%, depending upon the particular lot of Super Filtrol being employed for adsorption. The value used in calculating the amount of penicillin G in unknown samples must be based on experiment and is obtained by adsorbing known amounts of penicillin G on several columns. This figure should be checked occasionally by control runs. Assuming a recovery of 79% of penicillin G, for example, the penicillin G content of a broth sample is calculated as follows:

$$\%\ \text{G} = \frac{\text{units of G in effluent}}{\text{total units of penicillin in sample}} \times \frac{100}{0.79}$$

The average recovery of penicillin G, for the lot of Super Filtrol used to the greatest extent in this laboratory, was 79%. Table I

Table I. Recovery of Penicillin G from Mixtures of Penicillins

Penicillin Mixture	Penicillins in Mixture: G, Units	X, Units	F, Units	K, Units	G Recovered, Units	Actual G Found (Assuming 79% Recovery), %
In buffer solution						
1	83.5	52	12	40	61	92
2	83.5	52	12	40	61	92
3	58.5	31	23	37	46	100
4	150	..	..	138	118	100
5	167	..	..	...	134	101
Added to inactivated steep liquor broth						
6	0	33	24	36	0	...
7	25	50	..	36	17	87
8	100	33	..	36	81	102
9	134	17	..	18	103	97
10	150	..	..	...	123	104

Table II. Recovery of Penicillin G Added to Broths

Broth	G in Broth, Units	G Added, Units	G Recovered, Units	Recovery of Total G, %	Actual G (Assuming 79% Recovery), %
Steep liquor[a]	55	83.5	131	88	112
Steep liquor[b]	5	83.5	77	87	110
Synthetic medium	0	83.5	64.7	77	98

In each case, broth was from *P. chrysogenum* Q176 fermentation.

[a] Stirred jar fermentation.
[b] Shaken flask fermentation.

gives some typical recoveries obtained with samples containing from 0 to 100% penicillin G. The variation in the values is due at least in part to the method of assay, the accuracy of which is assumed to be 10%. Recoveries of penicillin G added to broth samples are given in Table II. The high recovery obtained in one of the examples may have been due to error in the estimation of the amount of penicillin G initially present in the broth. Because this value was relatively large, error in its estimation would affect appreciably the calculated recovery of the added penicillin. The last column of each table shows the percentage of actual penicillin G found, assuming 79% recovery. The found values are generally within 10% of the correct value.

Table III. Estimation of Penicillin G in Broths and Commercial Samples

Penicillin Sample	G Found, %	G Determined by Other Methods, %[a]	%[b]	%[c]
Commercial penicillins				
Firm A	21	21	19	23
Firm B	83	..	88	91
Firm C	83	..	74	..
Firm D	102	..	107	..
Processing samples				
Firm E				
Broth	69	60	73	..
Solvent extract	65	77	...	..
Buffer	71	..	80	..
Laboratory broths				
Steep liquor medium	29	..	30	..
Synthetic medium	0[d]	..	0[e]	..

[a] Results obtained from firms involved.
[b] Results obtained with method of Higuchi and Peterson (*6*).
[c] Results obtained with modified Craig distribution method (*3*).
[d] No penicillin G detected.
[e] Calculated on assumption that only penicillins F, dihydro F, and K present. If penicillins X, K, and G are assumed present, calculations show 13% G.

To secure an approximate check of the accuracy of the results obtained with the adsorption method, several samples were chromatographed for which penicillin G values had been obtained with two other methods, the microbiological differential assay (*6*) and a modified Craig procedure (*2, 7*). Table III lists the values found for a few commercial penicillins and broth samples. Reasonable agreement occurs among the values obtained with the different methods of analysis.

AQUEOUS CHROMATOGRAPHY OF OTHER PENICILLINS

Although the use of Super Filtrol columns was developed particularly for the estimation of penicillin G, the columns are capable of resolving mixtures of other penicillins as well. The degree of resolution obtained for mixtures of penicillins more easily eluted than penicillin G varies considerably with different batches of the adsorbent.

The resolution of penicillin mixtures is aided by the fact that penicillin K is usually entirely inactivated upon adsorption. Whenever penicillin K is eluted, its recovery is very small and its elution band just precedes that of penicillin G. Comparison of chromatograms obtained with mixtures of penicillins X and G and of X, K, and G shows no significant differences. In each case, there is a portion of effluent of zero penicillin content (within the sensitivity of the assay) between the elution bands of penicillins X and G, and penicillin K does not appear in the effluent. The fact that penicillin K does not interfere with the resolution of other penicillins is inferred not only from the absence of a penicillin K elution band but also because the recoveries of penicillins X, F, and G are to all purposes the same with or without penicillin K in the mixtures.

Figure 2 shows a normal chromatogram for a mixture of penicillins X, F, K, and G. Three elution bands are obtained, penicillin K having been inactivated on the column. The separation of the penicillins, although not complete, is ample for the analysis of the mixture.

Dihydropenicillin F is adsorbed slightly more strongly than penicillin X (Figure 1). Although negligible resolution of a mixture of penicillins X, F, and dihydro F was obtained with a standard column, it was possible partially to resolve these penicillins on a larger column containing 8 grams of adsorbent and 4 grams of Celite. However, poor recoveries of the penicillins resulted (80% X, 40% F, and 36% dihydro F). Normal recoveries on standard columns are: penicillin X, 97%; F, 83%; dihydro F, 74%.

The two least adsorbed penicillins, X and dihydro F, may be chromatographed in fairly large amounts without overloading the columns. In this way, it was possible to detect 5% penicillin G in a supposedly pure preparation of dihydro F when 730 units of the latter were adsorbed for the analysis.

ADSORPTION UNDER OTHER CONDITIONS

The adsorption of penicillins on columns of Super Filtrol at pH 6.1 was investigated, the eluent being 0.07 *M* phosphate buffer. The increase in pH from 4.6 to 6.1 caused a marked decrease in the adsorptive capacity of the adsorbent, rapid elution, and poor resolution.

As a solution of potassium monobasic phosphate has no buffering capacity, the effect was determined of buffered solutions upon the length of time required to prepare columns and upon the degree of resolution of mixtures of penicillins. Aqueous 0.05 *M* solutions of sulfuric, hydrochloric, nitric, and phosphoric acids were adjusted to pH 4.6 with pyridine. The procedure of column preparation and adsorption of penicillins was carried out in the normal manner, using the buffered solutions instead of the unbuffered phosphate. None of the penicillins was eluted by 180 ml. of buffer when the solutions of sulfate, chloride, and nitrate were employed. A normal chromatogram was, however, obtained with the phosphate buffer. The effect of acetate buffer was also determined; although the penicillins were eluted, poor separation was obtained. From these data, it appears that phosphate may be necessary for resolution and elution of penicillins on columns of Super Filtrol. Although the time required for the preparation of a column is decreased by 1 or 2 hours when unbuffered phosphate wash solution is replaced by the pyridine phosphate buffer, the use of the latter is discouraged by its unfavorable odor. The effect on resolution of varied buffer salts concentrations was not investigated.

The rate at which the phosphate solution is forced through a column containing adsorbed penicillin affects the degree of resolution. Reproducible results are obtained only when the same flow rate is used in all experiments. The effluent flow rate adopted was 1 ml. per minute; greater rates caused less satisfactory resolution.

LITERATURE CITED

(1) Barnes, R. B., Gore, R. C., Williams, E. F., Linsley, S. G., and Peterson, E. M., ANAL. CHEM., **19**, 620 (1947).
(2) Craig, L. C., *J. Biol. Chem.*, **155**, 519 (1944).
(3) Craig, L. C., Hogeboom, G. H., Carpenter, F. H., and du Vigneaud, V., *Ibid.*, **168**, 665 (1947).
(4) Foster, J. W., and Woodruff, H. B., *J. Bact.*, **47**, 43 (1944).
(5) Grenfell, T. C., Means, J. A., and Brown, E. V., *J. Biol. Chem.*, **170**, 527 (1947).
(6) Higuchi, K., and Peterson, W. H., ANAL. CHEM., **19**, 68 (1947).
(7) Mortimer, D. C., M.S. thesis, University of Wisconsin, 1947.
(8) Schmidt, W. H., and Moyer, A. J., *J. Bact.*, **47**, 199 (1944).
(9) Sheehan, J. C., Mader, W. J., and Cram, D. J., *J. Am. Chem. Soc.*, **68**, 2407 (1946).
(10) Winsten, W. A., and Spark, A. H., *Science*, **106**, 192 (1947).

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Conductometric Titration of Chloride in Sea Water and Marine Sediments

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Two applications are presented of conductometric titration equipment described previously. In the titration of sea water, differences between duplicate runs average less than one fifth those of the conventional Knudsen titration, although the time per sample is doubled. In analysis of marine sediment, the accuracy is greater than is actually necessary and the time per sample is one fifth that of the Mohr titration.

CHLORIDE is the most abundant of the ions present in sea water; hence its quantitative determination is useful in many applications connected with the study of the sea.

Perhaps the most fruitful of these applications is in determining the density of sea water. Precise tables (*2*) are available for computing the salinity and the density of sea water from its chlorinity and temperature, when chlorinity is defined as the weight of total halides, expressed as chloride, contained in 1 kg. of sea water.

Although other procedures have been developed, the Knudsen method remains the most widely used by oceanographic laboratories for the routine determination of chlorinity. This modification of the Mohr method uses special volumetric glassware calibrated directly in chlorinity units.

The normal deviation between duplicate titrations by this method is about 0.05%, which causes uncertainties in the density of the same order of magnitude as that caused by a temperature error of 0.05° C. Because oceanographic temperature measurements are good to ±0.01° C., the accuracy of density measurement could be considerably improved if chlorinity data were more accurate. In many instances, such as the calculation of deep sea currents and the preparation of standard comparison sea water, such increased accuracy is important.

The method described in this paper has been found to give duplicate checks to 0.01%. The time required is approximately 8 minutes per complete titration as compared to 4 minutes by the Knudsen method.

Another oceanographic application utilizing chloride analyses is the determination of the water content of marine sediments. The direct procedure of weighing a sample wet and weighing again after oven-drying is often erroneous because of evaporation of water from the sample during storage. A method which has proved more reliable is that of titrating the soluble chloride in a sample and calculating its water content from an assumed chlorinity value. Inasmuch as this value can be estimated within ±1%, the water content values also have this accuracy, which is normally sufficient.

The standard procedure in such titrations is to leach out soluble chloride by repeated washings of the sediment. The Mohr titration can then be used on the clear filtrate.

The procedure described in this paper is convenient because the presence of the sediment in the chloride solution does not interfere with its conductometric titration. This eliminates the need for leaching and reduces the analysis time from 25 to approximately 5 minutes per sample.

EQUIPMENT

The electrical equipment used for measuring relative conductance changes during titrations (*1*) consists essentially of a regulated 1000-cycle source and an off-balance Wheatstone bridge In this type of circuit, the conductance changes register directly on a microammeter. The sensitivity is such that 0.02% change in conductance is easily detectable.

Two conductance cells are used to compensate for temperature rise and any other incidental changes not directly caused by the titration. Each cell consists of a 1-liter wide-mouthed reagent bottle with two pure silver electrode disks sealed to opposite sides of the cell. Construction details are given in (*1*).

PROCEDURE

In titrating sea water, the standard Knudsen 15-ml. automatic pipet and the Knudsen automatic buret calibrated directly in chlorinity units were used. For sediment titrations, a sample (about 10 grams) was dried, weighed, and remoistened with distilled water. It was then transferred into the titration cell and diluted to volume. Coarse sand particles and shell fragments were retained in the weighing dish, as such comparatively coarse particles cause the meter to flicker when they approach the electrode surfaces too closely while being stirred. An ordinary 25-ml. buret was used for these titrations.

Titration. The electrical apparatus including the stirrers is turned on and adjusted as described in (*1*). After a few minutes for the apparatus to stabilize, the conductance of the compensat-

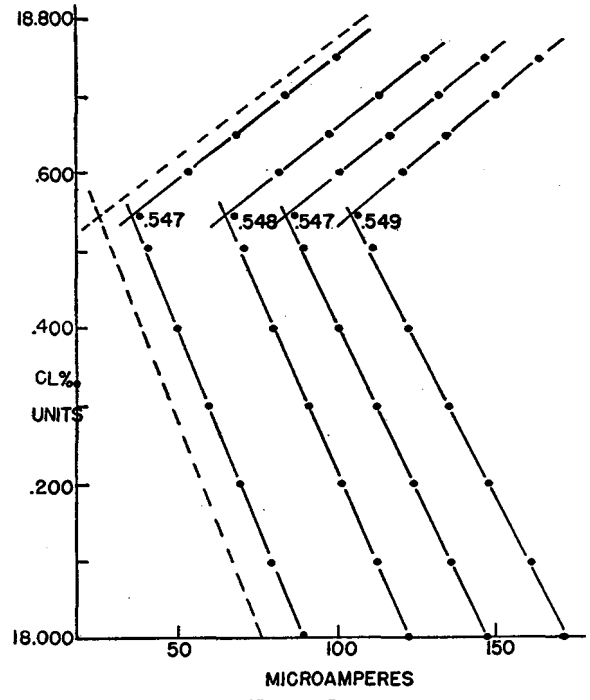

Figure 1

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Table I. Routine Determination of Chlorinity of Sea Water

Bottle No.	Conductometric Method 1st	2nd	Av.	Knudsen Method 1st	2nd	Av.	Difference between Duplicates Cond.	Knudsen
527	18.822	18.827	18.825	18.83	18.81	18.32	0.005	0.020
730	18.908	18.903	18.906	18.905	18.895	18.90	0.005	0.010
550	18.924	18.924	18.924	18.885	18.895	18.89	0.000	0.010
187	18.957	18.958	18.958	18.955	18.945	18.95	0.001	0.010
106	18.998	18.999	18.999	18.97	18.97	18.97	0.001	0.000
526	18.341	18.342	18.342	18.345	18.325	18.335	0.001	0.020
321	18.430	18.433	18.432	18.425	18.41	18.418	0.003	0.015
895	18.445	18.439	18.442	18.43	18.41	18.42	0.006	0.020
401	18.403	18.402	18.403	18.41	18.39	18.40	0.001	0.020
969	18.770	18.771	18.771	18.77	18.765	18.768	0.001	0.005
290	18.739	18.734	18.737	18.72	18.705	18.713	0.005	0.015
463	18.330	18.331	18.331	18.315	18.295	18.305	0.001	0.020
896	18.321	18.324	18.323	18.335	18.31	18.323	0.003	0.025
495	18.333	18.335	18.334	18.33	18.31	18.32	0.002	0.020
197	18.329	18.327	18.328	18.325	18.31	18.318	0.002	0.015
900	18.612	18.614	18.613	18.61	18.60	18.605	0.002	0.010
452	18.773	18.774	18.774	18.755	18.75	18.753	0.001	0.005
296	18.835	18.833	18.834	18.83	18.81	18.82	0.002	0.020
420	18.880	18.876	18.878	18.885	18.87	18.878	0.004	0.015
211	18.917	18.920	18.919	18.905	18.90	18.903	0.003	0.015
						Av.	0.0025	0.014

ing cell is made equal to that of the titration cell by adding to it a sample similar to the unknown and diluting to nearly the same volume. Now the bridge knob is set at its electrical center (previously marked on the dial) and a small amount of water or sodium chloride is added to bring the meter to balance. This adjustment need not be repeated unless samples of widely differing concentration are being titrated. Differences in conductance as high as 25% between the two cells do not cause serious meter drift. The equipment is now ready for the titrations.

The first few drops of reagent are added in order to determine the direction in which the meter will deflect. The balance point of the bridge registers as a maximum meter reading, but the response is not linear within 150 microamperes of balance; hence the needle is set 150 microamperes below balance on the side giving decreasing readings as reagent is added, as shown in Figure 1. In titrations of sea water, or when the approximate end point is known, no readings need be plotted until the end point is approached.

The buret level is now carefully adjusted to a graduation mark within approximately 10% of the end point and this value is plotted against the corresponding meter reading. Subsequent readings are plotted every 0.100 chlorinity unit until the meter readings begin to deflect permanently upscale, indicating that the end point has been passed. Readings are now taken at intervals of 0.050 because of the greater changes encountered after the end point. Three or four points before and after the end point are sufficient to determine its value accurately. As the buret levels were adjusted to graduation marks, buret reading errors are minimized.

Table I is a comparison of a number of duplicate titrations of sea water chlorinity. The average difference between duplicates by the Knudsen method is 5.6 times as great as that of the conductance titrations. Inasmuch as identical glassware and techniques were used in both methods, the greater precision of the conductance titrations is due entirely to the difference in end-point indicators.

Comparison between average values for each bottle shows higher results by the conductance method. The average difference is +0.013. The first Knudsen run agrees better with the conductometric average than the second (+0.008), probably because the technician making the first runs was more experienced than the one making the second runs.

In sediment titrations, where the approximate end-point value is not known, the silver nitrate is added more cautiously. As the end point is approached, the meter needle begins deflecting upscale momentarily with each drop of reagent added. These needle deflections become more pronounced until finally a permanent deflection is observed. After the end point, readings can be plotted for each drop. As with sea water, three or four points before and after the end point are sufficient to establish its value.

In Table II comparative data between the oven-drying method and the conductance titration are shown. In many cases the water contents are higher by the conductance titration method and in a few cases the values are considerably higher, indicating partial drying of the samples during storage.

DISCUSSION

In Figure 1, the abscissa values are proportional to the per cent conductance change occurring in the solution during titration. A conductance change of 1% is equivalent to a meter deflection of approximately 70 microamperes. Before the end point is reached, the conductance of the titration mixture decreases rather slowly. Immediately upon passing the end point the conductance rises with added silver nitrate at a rate three times as great as it previously decreased. If one calculates the conductance changes which should occur on the basis of simple electrolyte theory, including changes in ionic activities, the dotted curve in Figure 1 results. This is seen to follow very closely the observed changes.

Three major effects are present before the end point; silver ion is precipitated as rapidly as it is added, nitrate ion replaces chloride in solution, and the titration mixture is being diluted by the reagent. All of these cause the conductance to decrease. As soon as the end point is passed, silver and nitrate ions are added but none are removed. Because the reagent is considerably more concentrated than the titration mixture, the dilution effect is overcome and the conductance rises rapidly. The tendency of points near the end point to round off may be due either to the finite solubility product of silver chloride or the change in the ionic species adsorbed by the silver chloride. Before the end point, chloride is predominantly adsorbed. As the end point is approached, the chloride concentration becomes low enough to cause removal of the adsorbed ions. Immediately after the end point, silver ions are adsorbed until the precipitate becomes saturated, whereupon the line assumes its true slope. Neither of these mechanisms will cause an erroneous end point if values near the end point are ignored in drawing the intersecting lines.

Table II. Water Content of Marine Sediments

Depth below Surface *Inches*	Water, Oven Method %	Water, Chloride Method %
0- 3	76	76
3- 6	68	72
6- 9	69	69
9-12	67	67
12-15	60	64
15-18	62	62
18-21	60	60
21-24	58	59
24-27	57	57
27-30	55	56
30-33	55	55
33-36	55	55
36-39	53	54
39-42	54	54

SUMMARY

Conductometric titrations offer an accurate and convenient method for chloride determination. In the titration of sea water, duplicate runs agreed to 0.01%, about five times the precision attained by the standard Knudsen titration, although 8 minutes are required per sample as compared to 4 minutes for the Knudsen method.

The technique is also useful in titrating soluble chloride in marine sediments, as the presence of the sediment in the titration mixture does not interfere. In this case, the titration time is reduced to 5 minutes from the 25 minutes required if the Mohr titration is applied.

LITERATURE CITED

(1) Anderson, L. J., and Revelle, R. R., ANAL. CHEM., 19, 264 (1947).

(2) Knudsen, Martin, "Hydrographical Tables," Copenhagen, G. E. C. Gad, 1901.

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Determination of Chlorogenic Acid in Coffee

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A new method for the rapid and precise determination of chlorogenic acid in green and roasted coffee is based upon the absorption of chlorogenic acid at 324 millimicrons. The chlorogenic acid is extracted from flaked coffee samples with water, and for green coffee the ultraviolet absorption is measured directly on the extract. For roasted coffee, the absorption is measured on the extract before and after precipitation of the chlorogenic acid with basic lead acetate. The Slotta and Neisser iodometric oxidation method for chlorogenic acid has been investigated and improved.

CHLOROGENIC acid, which occurs in green coffee to the extent of 5 to 8%, is one of the major water-soluble constituents of the bean. Its importance in coffee chemistry has long been recognized, and considerable attention has been devoted to it in the literature.

The compound was first isolated by Gorter (*7*), who found it widely distributed in the leaves and seeds of numerous plants. Freudenberg (*6*) and Fischer (*5*) described the structure of the compound as the quinic acid ester of caffeic acid.

Charaux (*4*) was one of the first workers to attempt the measurement of chlorogenic acid in plant materials. His method was based upon the separation of the acid by lead precipitation, regeneration of the free acid, saponification, and extraction of the resulting caffeic acid with ether. The caffeic acid was then weighed directly. This method can have only preparative value because of losses during saponification and ether extraction.

Hoepfner (*8*) in 1932 developed a method based upon the red color formed when chlorogenic acid is treated with nitrous acid. Plücker and Keilholz (*12*) questioned the reliability of this method for roasted coffee, because catechol and protocatechuic acid, likely to be present in the roasted product, give red colors under the conditions of the reaction. These authors devised an indirect method based upon the hydrolysis of the chlorogenic acid, extraction of the resulting caffeic acid with ether, and a final estimation by the nitrite color reaction. Efforts to carry out these steps quantitatively in this laboratory were unsuccessful.

Jurany (*9*) proposed a method based upon the separation of chlorogenic acid as its lead salt, regeneration of the free acid, and a final estimation of the chlorogenic acid by optical rotation or titration with alkali. These methods cannot be considered valid in view of the presence in coffee of isomers of chlorogenic acid which have different rotatory powers and different neutralization equivalents, as shown by recent work in this laboratory (*3*).

Slotta and Neisser (*15*) recognized the unreliability of the results obtained by earlier methods and developed a new technique for measuring chlorogenic acid. This method consists of a Soxhlet water extraction of a defatted sample, lead precipitation, regeneration with hydrogen sulfide, and estimation of the liberated chlorogenic acid by its reaction with alkaline hypoiodite.

Early experience with the Slotta and Neisser method revealed certain weaknesses and indicated the need for modifications. Therefore, the initial efforts in the current investigation were directed toward improvement of the method.

The manipulative difficulties inherent in the Slotta and Neisser method made it desirable to devise a new analytical procedure for chlorogenic acid. It was found that chlorogenic acid has intense light absorption at 324 millimicrons. Additional work was undertaken in order to develop an analytical method for chlorogenic acid based upon ultraviolet absorption measurements. This paper reviews briefly the recommended modifications of the Slotta and Neisser iodometric method and presents a new ultraviolet absorption method for chlorogenic acid.

Subsequent to the development of this ultraviolet method, Rudkin and Nelson (*13*) reported a maximum absorption at about 325 millimicrons of a mixture of phenolic compounds, including chlorogenic acid, which they found in sweet potatoes.

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PURIFICATION AND PROPERTIES OF CHLOROGENIC ACID

Three samples of chlorogenic acid were isolated from green coffee by two different methods and purified by repeated crystallization to serve as analytical standards. Sample 1 and a further recrystallized fraction, No. 2, were isolated through the potassium caffeine complex, while sample 3 was separated by solvent partition. As chlorogenic acid is not available commercially, an extraction and purification procedure is included in this paper. The isolation of chlorogenic acid from coffee through the potassium caffeine complex is a modification, by Kremers (*10*) of this laboratory, of previously reported methods (*6*, *7*).

Extract 1 kg. of flaked green coffee by stirring with 6 liters of 70% isopropanol for about 3 hours, filter on a Büchner funnel, and extract the residue again for a short time with 6 liters of 70% isopropanol. Concentrate the total extract by vacuum distillation to 2.4 liters and cool to about 5° C. for several hours. Remove the precipitated fats by filtering with a filter aid. Concentrate the filtrate under vacuum to about 300 ml. Dissolve in this concentrate, by heating if necessary, 8 grams of potassium acetate and 20 grams of caffeine. Add 300 ml. of 95% ethanol and keep at about 5° C. for 2 days for crystallization of the crude complex.

Collect the crude complex by filtration on a Büchner funnel and wash with a little 50% ethanol. Dissolve the complex in the minimum volume of hot water and add sufficient 95% ethanol with stirring to initiate precipitation. Keep the solution at 5° C. overnight, collect the reprecipitated complex by filtration, and dry at room temperature. About 70 grams of complex are obtained from 1 kg. of coffee.

Add 100 grams of the complex in small portions to 225 ml. of hot water containing 24 grams of tartaric acid, cool for several minutes, and remove the potassium bitartrate by filtration. Extract the filtrate with chloroform in a liquid-liquid extractor for about 30 hours, or until the caffeine is completely removed. Keep the aqueous phase at 5° C. for 1 day for crystallization of the chlorogenic acid. Collect the acid by filtration, wash with cold water, and dry at 70° C. in a vacuum oven. The chlorogenic acid as obtained melts at 203–206° C.

Recrystallization from water two or three times gives a pure product, as indicated by Table I. The samples as used were dried for 16 hours in a vacuum oven at 70° C. and were found to contain 0.5 molecule of water of crystallization, which was measured by drying to constant weight in vacuum over phosphorus pentoxide at 135° to 140° C. A weight loss of 2.4 to 2.7% was observed; the theoretical moisture content for the hemihydrate is 2.48%. The presence of 0.5 molecule of water of crystallization in the oven-dried samples is further verified by titration data, which show a neutralization equivalent of 360 to 367; the calculated value for the hemihydrate is 363. A titration curve for sample 1 is shown in Figure 1. For uniformity of comparative data, all the chlorogenic acid values on coffee samples reported herein are expressed in terms of the anhydrous compound, $C_{16}H_{18}O_9$, molecular weight 354.

IODOMETRIC OXIDATION METHOD FOR CHLOROGENIC ACID

Reagents. Petroleum ether, C.P., boiling point 20–40° C.

Lead acetate, C.P., neutral, saturated water solution.

Iodine solution, 0.1 *N*, prepared as directed by A.O.A.C. (*2*, 43.21).

Sodium thiosulfate solution, 0.1 *N*, prepared as directed by A.O.A.C. (*2*, 43.28).

Starch indicator, 1%, prepared as directed by A.O.A.C. (*2*, 6.3).

Sulfuric acid, 2 *N*.

Sodium hydroxide, 1 *N*.

Filter paper, Green's fluted No. 488½, 18.5 cm.; Whatman's No. 1, 5.5-cm. and 12.5-cm.

Celite filter aid, Johns-Manville, No. 545.

Asbestos, acid-washed medium fiber.

Procedure. Prepare the sample by grinding through a 1-mm. screen on a laboratory Wiley mill and flaking on a 3-roll Lehmann chocolate mill. Determine moisture by drying a 2-gram sample in a 70° C. vacuum oven for 16 hours.

Defat a 4-gram sample of the flaked green coffee by washing with three 25-ml. portions of petroleum ether, allow the residue to stand about 5 minutes or until most of the solvent has evaporated and transfer to a 2-liter volumetric flask. For roasted coffee weigh 4 grams of the flaked sample directly into the volumetric flask, add 1600 ml. of water, and extract 20 minutes with occasional shaking. Dilute to volume, mix, and filter by suction through No. 1 Whatman paper on a 12.5-cm. Büchner funnel. Discard the first 50 ml. of filtrate, transfer exactly 1 liter to a 2-liter distilling flask, and concentrate under vacuum at 20° to 30° C. to about 75 ml.

Transfer to a 250-ml. beaker, keeping the total volume at about 100 ml. Add slowly with stirring 2 ml. of neutral lead acetate solution, and allow to stand overnight.

Add 1 gram of Celite, stir thoroughly, and filter through a thin asbestos bed in a 5.5-cm. Büchner funnel. Wash three times with 15 ml. of water, or until the volume of filtrate and washings is about 150 ml. Do not allow the bed to dry, as this makes deleading difficult. Transfer the lead precipitate and bed to the same 250-ml. beaker used for the precipitation, keeping the volume at about 80 ml. While stirring continuously with a mechanically driven glass stirrer, add slowly 2 ml. of 2 *N* sulfuric acid, and continue stirring for 20 minutes or until the chlorogenate has decomposed. Filter through a No. 1 Whatman paper in a 5.5.-cm. Büchner funnel, and wash with four 20-ml. portions of water. Neutralize the filtrate and washings with 2 *N* sodium hydroxide to a pH of 6 to 7, transfer to a 200-ml. volumetric flask, and make to volume.

Transfer 50 ml. to a glass-stoppered Erlenmeyer flask and add 25 ml. of 0.1 *N* iodine solution. Immediately, with constant stirring, add 25 ml. of 0.1 *N* sodium hydroxide dropwise from a buret over a 3-minute period. Stopper the flask, and allow to stand in a dark place for 1 hour. During this period the temperature should be 25° ± 2° C. At the end of the reaction period add 10 ml. of 2 *N* sulfuric acid, and after 5 minutes titrate with standard 0.1 *N* sodium thiosulfate solution, using starch indicator.

Run a blank titration on 25 ml. of the iodine solution, using 50 ml. of water in place of the 50-ml. aliquot of sample. Calculate the per cent of anhydrous chlorogenic acid present in the coffee (dry basis) by the following equation:

$$\% \text{ chlorogenic acid} = \frac{(\text{volume of } Na_2S_2O_3 \text{ blank} - \text{volume of } Na_2S_2O_3 \text{ sample}) \times N\ Na_2S_2O_3 \times 3.36}{\text{dry weight of sample titrated}}$$

$$\text{The factor } 3.36 = 100 \times \frac{\text{milliequivalent weight of chlorogenic acid}}{\text{iodine equivalent}}$$

Table I. Properties of Pure Chlorogenic Acid[a]

Sample No.	Melting Point Corrected, °C.	Optical Rotation, $[\alpha]_D^{20}$	Neutralization Equivalent	Iodine Equivalent, Atoms per Mole	Wave Length of Maximum Absorption, mμ	$E_{1\text{ cm.}}^{1\%}$
1	207–9	−39.8	352	10.55	324	532
2	206–7	−37.7	357	10.55	324	522
3	207–8	−38.0	354	10.55	324	525

[a] Values calculated on an anhydrous weight basis.

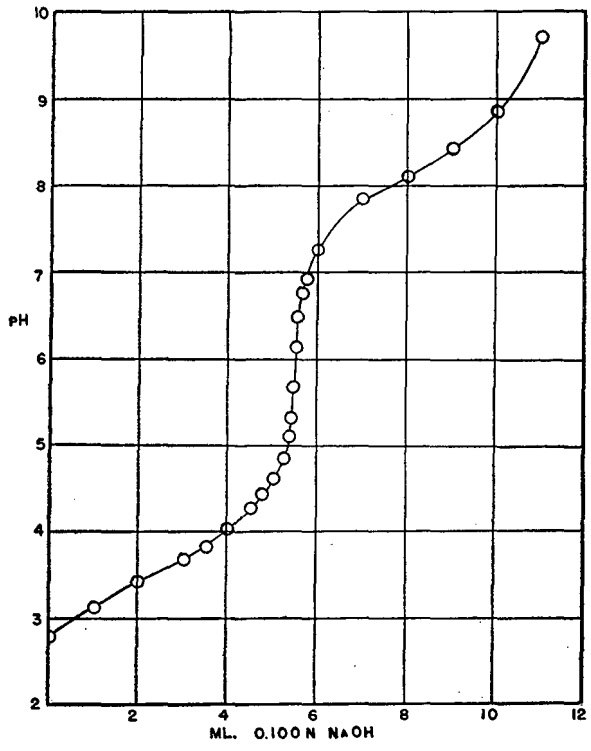

Figure 1. Titration Curve for Chlorogenic Acid Hemihydrate

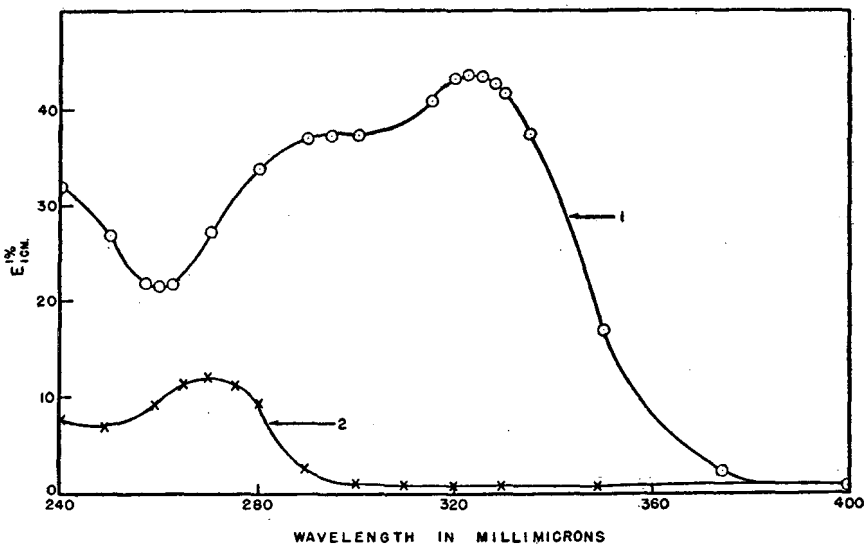

Figure 2. Ultraviolet Absorption of Green Coffee Extract

1. Original water extract
2. Filtrate from lead precipitate of water extract, deleaded

Conditions Affecting the Reaction between Chlorogenic Acid and Iodine. The Slotta and Neisser iodometric

Table II. Effect of Chlorogenic Acid Level upon Iodine Titration[a]

No. of Titrations	Excess Iodine	Chlorogenic Acid Taken	Chlorogenic Acid Found	
	%	*Mg.*	*Mg.*	%
2	90	47.8	41.1	88.0
2	106	41.0	40.5	98.7
2	145	34.2	34.1	99.7
6	188	27.3	28.2	103.4
6	282	20.5	21.6	105.1
2	425	13.7	15.9	116.1
2	810	6.8	9.2	135.3

[a] Values calculated using ratio of 10.55 atoms of iodine per mole of chlorogenic acid.

Table III. Effect of pH upon Reaction of Chlorogenic Acid and Iodine

pH before Adding Iodine	pH during Iodine Reaction	Chlorogenic Acid Found (34.1 Mg. Added)	
		Mg.	%
3.3	10.9	34.1	100.0
7.2	11.0	34.3	100.6
8.1	11.0	33.8	99.1
9.7	11.0	31.6	92.7
11.6	11.1	24.0	70.4

Table IV. Effect of Temperature upon Iodine Reaction

Determinations	Temperature	Chlorogenic Acid Taken	Chlorogenic Acid Found	
	°C.	*Mg.*	*Mg.*	%
3	18–20	34.1	30.5	89.3
3	25–27	34.1	34.3	100.7
3	35–40	34.1	35.6	104.3

method (*15*) is based upon the oxidation of chlorogenic acid with iodine in alkaline solution. The solution to be analyzed is mixed with a measured amount of standardized iodine, then hypoiodite is generated by adding sodium hydroxide. Following a given reaction period, the solution is acidified and the liberated excess is back-titrated with standard thiosulfate solution. The theoretical aspects of this reaction and its application in the measurement of catechol and its isomers, 1,2-naphthoquinone-4-sulfonic acid, adrenaline, tyrosine, and thiamine have been presented by Slotta and Neisser (*14*, *16*). Their work indicated that under specified conditions *o*-diphenols such as chlorogenic acid react with about 10 atoms of iodine and showed that the hypoiodite oxidation involves the aromatic ring only without affecting the side chain.

Current observations have shown that exact control of conditions during the iodine reaction are necessary for precise measurements. Reliable results are obtained when: (*a*) the amount of chlorogenic acid in the aliquot treated with iodine is 30 to 40 mg. (see Table II), (*b*) about 150% excess iodine is present (see Table II), (*c*) the pH of the solution prior to the addition of the iodine is between 3 and 8 (see Table III), (*d*) the sodium hydroxide (25 ml., 0.1 *N*) is added over a period of 3 minutes, and the final pH is between 10.9 and 11.1, (*e*) the temperature is 25° ± 2° C. (see Table IV), and (*f*) the reaction time is 1 hour.

Under these arbitrary conditions, 1 molecule of anhydrous chlorogenic acid reacts with 10.55 atoms of iodine. This figure is an average of 20 titrations on a pure chlorogenic acid sample. If one or more of the factors listed in the preceding paragraph is varied, the amount of iodine consumed per mole of chlorogenic acid will also vary.

Conditions Affecting Formation and Decomposition of Lead Chlorogenate. The precipitation of chlorogenic acid by means of lead acetate has been used by many workers as a means of separating the acid from other soluble constituents of coffee. In using this step in an analytical procedure, it is essential to employ the conditions that will give quantitative precipitation and recovery of the chlorogenic acid and at the same time provide optimum specificity. The conditions known to affect the lead precipitation and recovery of chlorogenic acid are: (*a*) concentration of chlorogenic acid and of excess lead, (*b*) pH of the solution, (*c*) time and temperature of precipitation, (*d*) method of collecting the precipitate, and (*e*) method of regenerating the lead salt.

Early work indicated that several hours were required for complete precipitation of chlorogenic acid by lead under the conditions of the Slotta and Neisser method. In the later work on the ultraviolet absorption method, it was found that by suitable manipulations essentially complete lead precipitation from dilute solutions could be made in about 1 hour. This was achieved by the use of basic lead acetate in the presence of potassium acetate, and by heating the lead-treated solution briefly, then cooling to about 0° C., with constant stirring, for the remaining period. These conditions were not applied to the iodometric method.

Regeneration of the chlorogenic acid from the lead salt has been one of the most difficult steps in the iodometric procedure. The substitution of sulfuric acid for hydrogen sulfide as the reagent for decomposing the lead chlorogenate improves both the precision and convenience of this step. The sulfuric acid should be added in a limited excess (25 to 100%), inasmuch as a large excess results in an apparent loss of chlorogenic acid. High temperatures during deleading in acid solution also result in low recovery.

ULTRAVIOLET ABSORPTION METHOD FOR CHLOROGENIC ACID

Reagents. Petroleum ether, C.P., boiling point 20° to 40° C.
Potassium acetate, C.P., crystalline ($CH_3COOK.3H_2O$).
Lead acetate, C.P. basic dry powder, saturated water solution.
Filter paper, Whatman No. 1, 11-cm. Green's Fluted No. 488½, 18.5-cm.

Procedure. Prepare the sample for analysis by grinding and flaking. Determine its moisture content by drying 2 grams to constant weight in a 70° C. vacuum oven. Defat a 2-gram sample of the flaked green coffee by washing with three 25-ml. portions of petroleum ether, boiling point 20° to 40° C. Allow the residue to stand about 5 minutes, or until most of the solvent has evaporated. Transfer to a 1-liter volumetric flask. For roasted coffee weigh 2 grams of the flaked sample directly into a 1-liter volumetric flask.

Add about 800 ml. of distilled water and shake occasionally for 20 minutes. Dilute to volume, shake thoroughly, and filter through a Büchner funnel, using a No. 1 Whatman filter paper. Discard the first 25 to 50 ml. of filtrate and collect about 150 ml.

Dilute the filtrate of both the green and roasted coffee 1 to 10 with water and measure the optical density at 324 millimicrons. Calculate the per cent chlorogenic acid in green coffee using an $E^{1\%}_{1\ cm.}$ value of 526 for anhydrous chlorogenic acid.

Analysis of roasted coffee requires a lead precipitation step. Place 100 ml. of the roasted coffee extract in a 200-ml. volumetric flask. Add 1 gram of potassium acetate and 2 ml. of saturated basic lead acetate solution with swirling. Place the flask in a steam or boiling water bath for 5 minutes, then transfer to an ice bath at about 0° C., and stir mechanically for 1 hour with a glass stirrer. Make to volume, shake thoroughly, and filter through a fluted filter paper. Discard the first 25 to 50 ml. of filtrate and collect about 25 ml. Take the density reading of this filtrate without dilution at 324 millimicrons. Subtract one fifth of the density reading of the lead filtrate from the density reading of the 1 to 10 diluted original extract. Calculate the per cent chlorogenic acid using this corrected density value.

The absorption measurements reported herein were made with a Beckman quartz spectrophotometer, Model DU, using 1-cm. quartz cells and a slit width of 0.3 to 0.5 mm.

Within the limits used, 5 to 20 mg. per liter, the ultraviolet absorption of chlorogenic acid in water solution follows Beer's law of direct proportionality between absorption and concentration, and molecular extinction values of potassium chlorogenate and potassium caffeine chlorogenate are identical with those of chlorogenic acid. The effects of the presence in coffee of isomeric forms of chlorogenic acid and of free caffeic acid upon the absorption have been neglected, all measurements being made with pure chlorogenic acid as the standard.

Preparation of Sample. The importance of proper preparation for analysis of a plant material such as coffee cannot be

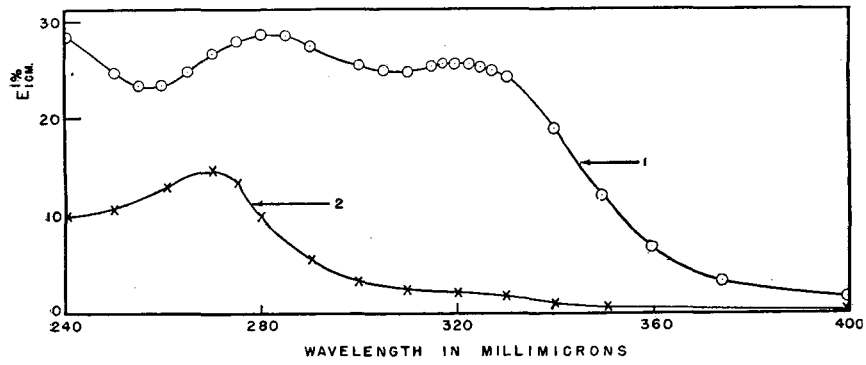

Figure 3. Ultraviolet Absorption of Roasted Coffee Extract

1. Original water extract
2. Filtrate from lead precipitate of water extract, deleaded

overemphasized. Extensive investigations in this laboratory have shown that accurate analytical values on green coffee can be obtained only on samples in which most of the cell structure has been broken. It is recommended that, for the analysis of any water-soluble constituent of green and roasted coffee, all samples be milled to a particle thickness of 50 to 100 microns. A 3-roll chocolate mill, cereal flaking mill, or any mill giving comparable grinding can be used for this purpose.

Extraction and Precipitation of Chlorogenic Acid. Several different techniques have been used in previous methods for extracting the chlorogenic acid from coffee. Analyses with both the iodometric and ultraviolet methods show that a 500 to 1 batch extraction gives higher and more reproducible chlorogenic acid values than a 10 to 1 batch extraction or Soxhlet extraction.

A slight turbidity in the dilute water extracts of green coffee can be avoided by petroleum ether extraction. This solvent does not remove any substances which absorb light in the range of 324 millimicrons. Turbidity is not encountered in the water extracts of roasted coffee.

Experimental results on pure solutions show that basic lead acetate is a better precipitating agent than the neutral salt and that effective precipitation can be obtained by heating the lead-treated solution for several minutes in a steam bath and then stirring in an ice bath for 1 hour. The addition of potassium acetate lowers the solubility of the lead chlorogenate. By taking advantage of these factors it is possible to precipitate in 1 hour 98.5% of the chlorogenic acid in 100 ml. of solution containing 15 mg. of pure chlorogenic acid. The solubility of lead chlorogenate in pure solution would account for essentially all of the material remaining in solution in lead-treated green coffee extracts which absorbs at 324 millimicrons. Excess lead acetate does not interfere with the absorption. Under comparable conditions, 0.2 mg. of chlorogenic acid is in solution after the lead precipitation of pure solutions, and 0.3 mg. of apparent chlorogenic acid is in solution after the precipitation of the green coffee extracts. The basic lead acetate removes 98.0% of the material absorbing at 324 millimicrons from green coffee extracts. These data, shown in Figure 2, indicate that the total ultraviolet absorption at 324 millimicrons of aqueous green coffee extract is as reliable a measurement of chlorogenic acid as a method based upon the absorption before and after lead precipitation.

The observations made on roasted coffee extracts show that an appreciable fraction of the material absorbing light at 324 millimicrons remains in solution after treatment with basic lead acetate. The ultraviolet absorption spectrum of a roasted coffee extract is shown in Figure 3. The soluble fraction contains the equivalent of 0.8 mg. of chlorogenic acid or 8.4% of the total of 9.4 mg. in 100 ml. of solution. After the correction factor for the solubility of lead chlorogenate (0.2 mg.) is applied, there remains in solution about 6% of the total material, calculated as chlorogenic acid, absorbing at 324 millimicrons. It is not known whether this represents a modified chlorogenic acid, some entirely different substance formed during roasting, or an interference with the precipitation of lead chlorogenate. These observations indicate that lead precipitation should be included in the procedure for roasted coffee.

Factors Affecting Light Absorption of Chlorogenic Acid. The absorption of chlorogenic acid remains constant over the pH ranges 2.4 to 5.9 in solutions buffered with citric acid–disodium phosphate mixtures. In a single experiment the absorption of the acid in pure solution was found to decrease by about 18% at 324 millimicrons when the pH was raised above 8; this change is characteristic of phenolic compounds. It is also possible that the decrease is a secondary effect of air oxidation in the slightly alkaline solution. No change in the absorption at 324 millimicrons was observed in pure solutions or coffee extracts held at pH 4 to 6 for 1 to 3 days at room temperature in diffused daylight.

Precision and Specificity of Ultraviolet Method. A comparatively high degree of precision is attainable with the ultraviolet absorption method because of the simplicity of the manipulative procedure and the reproducibility of the final light measurement. The statistical methods of Moran (*11*) and the A.S.T.M. (*1*) have been applied to the calculation of the precision of the ultraviolet method. The value of $\pm 2\sigma$ has been used in calculating the LU_{95} value (limit of uncertainty for 95 out of 100 determinations). A correction factor of 0.869 has been applied in these calculations as recommended by the A.S.T.M. (*1*). On the basis of the determinations shown in Table V, the LU_{95} for chlorogenic acid by direct ultraviolet readings on water extracts of green coffee is $\pm 0.24\%$. When the determination includes lead precipitation as used for roasted coffee, the corresponding LU_{95} value is $\pm 0.10\%$.

There is some variation between the chlorogenic acid values found by the ultraviolet and iodometric methods. The ultraviolet absorption figures, as shown in Table V, are 5% higher than the iodometric values for green coffee and 17% lower for roasted coffee. These variations reflect the different types of specificity of the two methods. The ultraviolet absorption procedure is the more reliable of the two methods for both green and roasted coffee, as it is based on a highly specific measurement. It has also been observed in this laboratory that pyrolyzed products of sucrose,

Table V. Comparison of Ultraviolet and Iodometric Methods for Determining Chlorogenic Acid in Coffee

	Ultraviolet Absorption Method		
	Direct reading	By difference before and after lead precipitation[a]	Iodometric Method[b]
	Per Cent (Dry Basis)		
Green Bogota coffee	7.69	7.59	7.41
	7.69	7.59	7.46
	7.77	7.72	7.41
	7.83	7.81	7.46
	7.88	7.87	7.46
	7.93	7.85	7.41
Av.	7.80	7.74	7.43
Roasted blended coffee	4.86	4.58	5.75
	4.88	4.58	5.77
	4.83	4.56	5.61
	4.84	4.56	6.09
	4.82	..	..
	4.73	..	..
	4.78	..	..
	4.83	..	..
	4.80	..	..
	4.78	..	..
Av.	4.82	4.57	5.81

[a] Correction of 0.2 mg. added for solubility of lead chlorogenate.
[b] Correction of 3.0 mg. added for solubility of lead chlorogenate.

comparable to those present in roasted coffee, react with iodine under the conditions used in the iodometric method for chlorogenic acid.

The water-soluble alkaloids, proteins, and sucrose found in water extracts of green coffee apparently do not interfere with the direct ultraviolet absorption method. The absorption at 324 millimicrons of a solution containing pure chlorogenic acid, trigonelline, caffeine, sucrose, and the nondiffusible fraction of green coffee extract, which together represent about 90% of the water-soluble portion of flaked green coffee, is almost identical with the absorption of the same amount of chlorogenic acid in pure solution, as shown by the absorption curves in Figure 4.

Dialysis experiments indicate that the high molecular weight components of green coffee extract do not interfere with the absorption measurements. The fraction of the water extract which diffuses through cellophane contains essentially all of the material which absorbs light at 324 millimicrons.

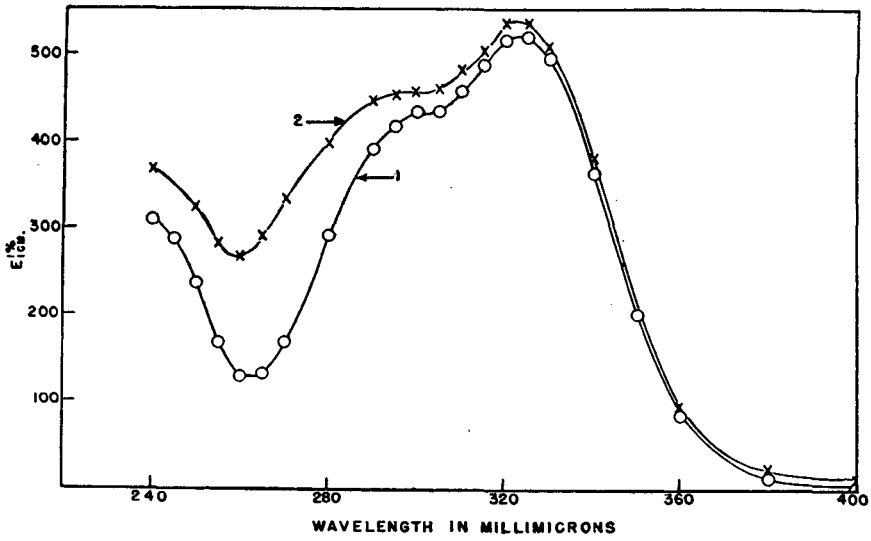

Figure 4. Absorption Curves of Chlorogenic Acid

1. **Pure chlorogenic acid hemihydrate**
2. **Mixture of chlorogenic acid hemihydrate, caffeine, trigonelline, sucrose, and nondiffusibles in green coffee water extract**

Known amounts of pure chlorogenic acid added to the extraction mixture of green coffee and water can also be measured quantitatively by direct absorption measurement. For example, the recovery of 142.2 mg. of chlorogenic acid added to 2-gram samples of green coffee was 142.5, 142.5, 140.5, and 140.0 mg. for four separate extractions.

These observations indicate that the ultraviolet absorption method is more precise and specific and at the same time much more rapid than any existing procedure for measuring chlorogenic acid in coffee.

ACKNOWLEDGMENT

These methods for measuring chlorogenic acid were developed as a part of the program of fundamental research on the chemistry of coffee sponsored by the Maxwell House and Sanka Divisions of General Foods Corporation. The authors acknowledge with gratitude the interest and encouragement of R. E. Kremers and H. M. Barnes of this laboratory.

LITERATURE CITED

(1) Am. Soc. Testing Materials, "Manual on Presentation of Data," third printing, Philadelphia, 1941.
(2) Assoc. Official Agr. Chem., "Official and Tentative Methods of Analysis," 6th ed., Washington, D. C., 1945.
(3) Barnes, H. M., Feldman, J. R., and White, W. V., "Isochlorogenic Acid from Coffee," presented before Division of Organic Chemistry at the 111th Meeting of AM. CHEM. SOC., Atlantic City, N. J., April 1947.
(4) Charaux, C., *J. Pharm. Chim.*, **2**, 292–8 (1910).
(5) Fischer, H. O. L., and Dangschat, G., *Ber.*, **65B**, 1037 (1932).
(6) Freudenberg, K., *Ibid.*, **53B**, 232 (1920).
(7) Gorter, K., *Ann.*, **358**, 327 (1907); **359**, 217 (1908); **379**, 110 (1911).
(8) Hoepfner, W., *Chem.-Ztg.* **56**, 991 (1932).
(9) Jurany, H., *Z. anal. Chem.*, **94**, 225 (1933).
(10) Kremers, R. E., personal communication.
(11) Moran, R. F., IND. ENG. CHEM., ANAL. ED., **15**, 361 (1943).
(12) Plücker, W., and Keilholz, W., *Z. Untersuch. Lebensm.*, **68**, 97–109 (1934).
(13) Rudkin, G. O., and Nelson, J. M., *J. Am. Chem. Soc.*, **69**, 1470 (1947).
(14) Slotta, K. H., and Neisser, K., *Ber.*, **71B**, 1611 (1938).
(15) *Ibid.*, p. 1616.
(16) *Ibid.*, p. 1984.

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Determination of Bromine Addition Numbers

An Electrometric Method

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BROMINE addition is widely used in the petroleum industry to measure the olefinic unsaturation of hydrocarbon materials, and a number of methods have been proposed for this determination. One of the most common is the Francis method (*1*) and its modifications by Mulliken and Wakeman (*4*) and Lewis and Bradstreet (*3*). The original Francis method used an excess of a potassium bromide–bromate reagent in acid solution as the brominating agent, and the excess was determined iodometrically. In the modifications of the method, the excess of the brominating reagent and the time allowed for bromination are carefully controlled in order to avoid high results as a consequence of substitution of bromine in the hydrocarbon. These procedures have all been found somewhat time-consuming, and considerable trouble is encountered in determining the proper excess of reagent, particularly when the samples are colored.

Kaufmann (*2*) has proposed a method which involves the use of a bromine solution in methyl alcohol saturated with sodium bromide

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An electrometric method for the determination of bromine addition numbers has been developed which is particularly suited for routine work. The procedure involves direct titration with the stable potassium bromide-bromate reagent. A catalyst is used to increase the reaction rate, and cooling is employed to reduce substitution to a minimum. The method is believed to have a number of advantages: Only a single, stable, standard reagent is used; the presence of dark colored materials causes no interference; substitution is reduced to a minimum; and the method is rapid and particularly suited for routine analyses.

as the brominating reagent. However, Uhrig and Levin (*6*) have shown that unless considerable care is taken to control the excess of reagent, high results are obtained. The latter workers titrate directly with a solution of bromine in glacial acetic acid and determine the end point by the appearance of the yellow color of the excess bromine. By avoiding a large excess of bromine the possibility of substitution is greatly reduced, and good results are obtained. The main disadvantages of this procedure are that the reagent requires frequent standardization with sodium thiosulfate, and the end-point detection becomes difficult with colored materials.

This paper describes a method for the determination of bromine addition which has been in use in these laboratories for several months and has given excellent results even in the hands of unskilled operators. It involves a direct titration with the stable potassium bromide–bromate reagent. The end point is determined by the dead-stop electrometric technique, so that the presence of colored materials does not interfere. A catalyst is used to increase the reaction rate and cooling is employed to reduce substitution to a minimum.

The method is believed to have a number of advantages over those previously reported—only a single, stable, standard reagent is required; the presence of dark colored materials causes no interference; errors due to substitution are reduced to a minimum; and the method is rapid and particularly well suited for routine analyses.

APPARATUS

Figure 1 shows a general view of the apparatus. The dead-stop technique is employed with an electric eye tube to detect the flow of current at the end point.

Electrometric Apparatus. The electrode stand and platinum electrodes from a Fisher Senior titrimeter were used in this work. Figure 2 shows a circuit diagram of the amplifying unit used. The potential applied across the platinum electrodes is approximately 0.5 volt.

Titration Apparatus. Figure 3 shows a schematic diagram of the apparatus used for routine work. The titration vessel, *A*, is approximately 12 cm. high and 4.5 cm. in diameter and is jacketed so that ice water may be circulated to cool the contents. A two-way stopcock at the bottom of the cell is used to fill the vessel with titration solvent and empty it after the titration is complete. The titration solvent is precooled in glass coils immersed in the large 9.4-liter (2.5 gallon) Dewar flask, *B*. Ice is used for cooling, and the cold water from the Dewar is also used to cool the titration cell. Tygon tubing is used for all the flexible joints. The titration solvent is prepared in 5-gallon lots and stored below the laboratory bench. It is transferred from the storage bottle to the 2-liter container, *C*, by applying a suction at *D*. From *C* the solvent flows by gravity through the cooling coil to the titration cell.

REAGENTS

Titration Solvent. The following are mixed in the indicated volumetric proportions:

Glacial acetic acid, C.P.	80
Methanol, C.P.	7
Carbon tetrachloride, C.P.	15
Sulfuric acid, 6 *N*	2
Alcoholic mercuric chloride solution (10 grams of C.P. mercuric chloride per 100 ml. of 95% ethanol)	2

Potassium Bromide–Bromate, 0.626 *N*. Exactly 17.42 grams of dried C.P. potassium bromate and 70 grams of C.P. potassium bromide are dissolved in distilled water and diluted to exactly 1 liter.

PROCEDURE

The sample is weighed or pipetted into the titration vessel which has been filled with 110 ml. of the cooled titration solvent. A sample size is chosen according to the following table:

Estimated Bromine No.	Approx. Wt. of Sample, Grams
>200	0.2
20–200	0.5
1-20	2

If the sample is viscous and difficult to transfer or the solubility in the solvent is poor, a few milliliters of carbon tetrachloride may be used to dissolve and transfer the sample.

The stirring motor is started. Before beginning the titration the temperature of the solution should be less than 5° C. The electric eye control is adjusted so that the eye is very nearly

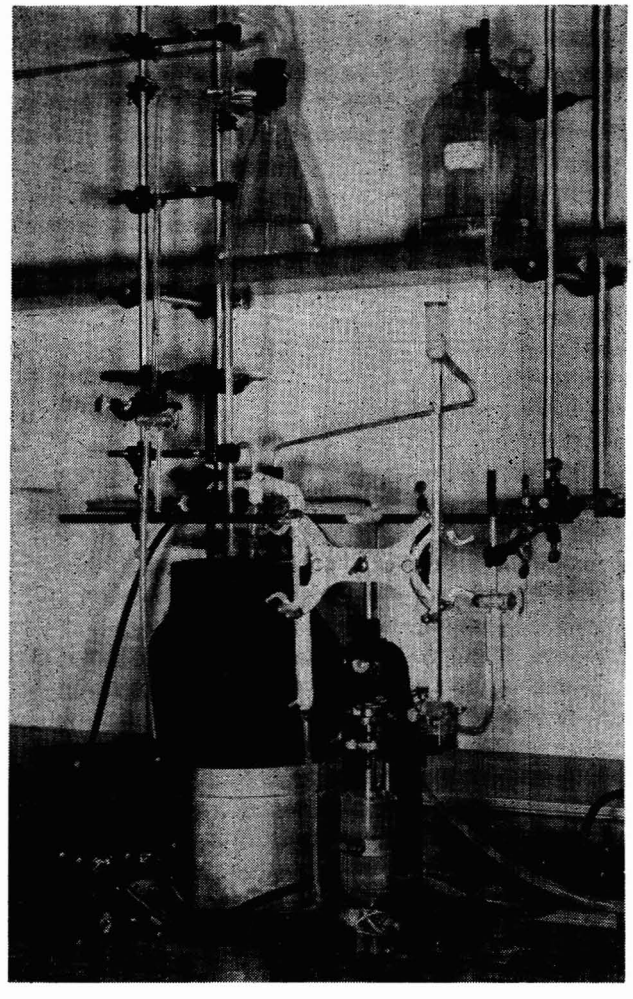

Figure 1. Electrometric Bromine Number Apparatus

Table I. Bromine Numbers by Electrometric Method Obtained at 0° to 5° C. on Pure Compounds

Compound	Bromine Number Theory	Found	% Deviation from Theory
1-Octene	142.5	140	1.8
		140	1.8
		141	1.0
		142	0.4
1-Decene	114.0	113	0.9
		114	0.0
		111	1.8
		111	1.8
1-Octadecene	63.3	63.2	0.2
		63.7	0.6
		64.6	1.8
		62.0	2.1
		62.0	2.1
Styrene	153.5	152	1.0
		152	1.0
		152	1.0
α-Methylstyrene	135.3	133	1.7
		133	1.7
Diisobutylene	142.5	142	0.4
		142	0.4
Tetraisobutylene	71	61	14.0
		62	13

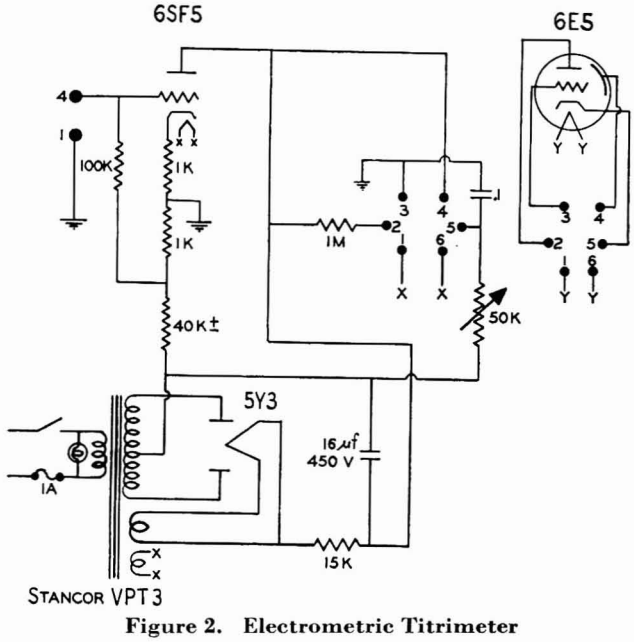

Figure 2. Electrometric Titrimeter

closed. The standard reagent is added dropwise at first, and if the eye does not flicker appreciably, the rate of addition may be increased until the eye opens. Small additions are then made until the eye remains open for 30 seconds or longer. If more than 15 ml. of reagent are required, the analysis should be repeated using a smaller sample.

The bromine number of a material is defined as the grams of bromine absorbed per 100 grams of sample and may be calculated by the use of the following equation:

$$\text{Bromine No.} = \frac{\text{ml. of standard reagent} \times \text{normality of standard reagent} \times 8}{\text{weight of sample}}$$

If the normality of the potassium bromide-bromate is exactly 0.626,

$$\text{Bromine No.} = \frac{\text{ml. of standard reagent} \times 5}{\text{weight of sample}}$$

DISCUSSION

In choosing a solvent for an electrometric titration involving hydrocarbon compounds, a number of factors had to be considered. The material had to be a reasonably good solvent for aromatic and paraffinic hydrocarbons, as well as a conductor, and have a freezing point lower than 0° C. As it was considered highly desirable to use the stable potassium bromide–bromate reagent, a solvent had to be chosen which would tolerate a reasonable amount of this without forming two phases. The solvent also had to be acidic enough to cause a fairly rapid release of bromine from the mixture of potassium bromide and potassium bromate. The solvent described under Reagents fits these requirements reasonably well. Its freezing point is approximately −1° C., and it has sufficient conductivity to cause no difficulty in the detection of the small flow of current at the end point. Approximately 15 ml. of reagent can be added without separation of phases. If more than this is required a smaller sample must be chosen. A small amount of dilute sulfuric acid is incorporated in the solvent in order to hasten the release of bromine from the bromide-bromate reagent. Without this, the reaction is slow enough to make the titration impractical.

The recommended solution is a reasonably good solvent for hydrocarbons. With some materials of high molecular weight solubility trouble is sometimes encountered, but usually this can be overcome by solution of the sample in 5 or 10 ml. of carbon tetrachloride and slow addition of this solution to the solvent. This reduces the water tolerance to perhaps 10 ml. and a sample size must be chosen which will require less than this amount of reagent. In a few cases it has been found necessary to run the titration at room temperature in order to obtain complete solution of the sample. However, most samples encountered in the petroleum industry are readily soluble and give no trouble.

Highly branched olefins were found to give evidence of some substitution even with the direct titration procedure when titrations were made at room temperature. Thus, diisobutylene gave bromine numbers ranging from 144 to 154 compared with a theoretical of 142.5 and the end points in titrating this material were very poorly defined. It has been shown (*5*) that the rate of halogen substitution reactions is greatly reduced at low temperatures, while the rate of the addition reaction is not materially affected, so that by cooling the reaction mixture to 0° to 5° C. this difficulty is overcome. This precaution is probably not necessary for many or most samples encountered. However, it is simpler for routine work to construct the apparatus so that all samples are run at low temperatures.

Lewis and Bradstreet (*3*) have suggested the use of metal salts as catalysts in the addition of bromine to olefinic double bonds.

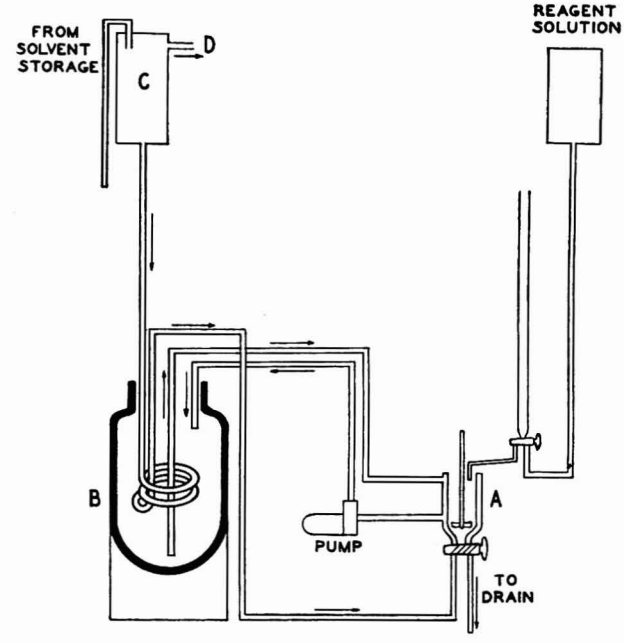

Figure 3. Titration Apparatus

Table II. Bromine Numbers by Electrometric Method Obtained at 0° to 5° C. on Diluted Samples of Diisobutylene

Sample	Theory	Found	% Deviation from Theory
Toluene, C.P.	0.0	0.08	...
Highly treated kerosene	0.0	0.09	...
White gasoline	0.0	0.06	...
Diisobutylene in toluene, wt. %			
17.2	24.5	24.2	1.2
		24.3	0.8
6.7	9.6	9.7	1.0
		9.9	3.1
3.3	4.8	5.0	4.2
		5.0	4.2
Diisobutylene in highly treated kerosene, wt. %			
78.3	111.6	110	1.5
		112	0.3
37.7	53.7	54.1	0.7
		54.9	0.7
18.5	26.4	25.9	1.0
		25.5	3.5
7.07	10.1	10.1	0.0
		10.3	2.0
3.72	5.3	5.3	0.0
		5.4	1.9
Diisobutylene in gasoline, wt. %			
80.4	114.6	114	0.8
		114	0.8
40.9	58.3	57.8	1.0
		58.8	0.7
20.7	29.5	29.5	0.0
		28.9	2.0
8.35	11.9	12.2	2.5
		12.0	0.8
4.20	6.0	6.0	0.0
		6.1	1.7

and a number of these were examined. It was found that mercuric chloride was particularly effective for increasing the reaction rate, and when this salt was present the titration could be completed in a much shorter time.

Table I shows the results obtained by this procedure on a number of organic compounds. The first three were obtained from the Connecticut Hard Rubber Company and had a guaranteed minimum purity of 95%. The styrene and α-methyl styrene were Dow products of 99.5% purity and the diisobutylene and tetraisobutylene were Eastman White Label compounds. None of the materials was further purified.

In order to determine the applicability of the procedure and apparatus for routine work, a series of samples was prepared from diisobutylene and various hydrocarbon solvents. These were analyzed by a semiskilled operator who was unaware that the samples were test materials. The data in Table II show an average deviation of 1.5% from the theoretical values.

In general, the results by this method have been found to be superior to results obtained by the Lewis and Bradstreet procedure when analyses are made by operating personnel on a routine basis, particularly when highly branched olefinic compounds are being analyzed. The amount of judgment required in in the use of this procedure is considerably less than with most other methods, and the time required per analysis is low. For most samples, the titration can be completed in 2 to 3 minutes.

LITERATURE CITED

(1) Francis, A. W., *Ind. Eng. Chem.*, **18**, 821 (1926).
(2) Kaufmann, H. P., *Z. Untersuch. Lebensm.*, **51**, 3 (1926).
(3) Lewis, J. B., and Bradstreet, R. B., IND. ENG. CHEM., ANAL. ED., **12**,387 (1940).
(4) Mulliken, S. R., and Wakeman, R. L., *Ibid.*, **7**, 59 (1935).
(5) Technical Advisory Committee, Petroleum Administration for War, *Rept.* ZAL-1 (9), 1945.
(6) Uhrig, K., and Levin, H., IND. ENG. CHEM., ANAL. ED., **13**, 90 (1941).

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Preformed Vitamin A in Mixed Feeds

Chemical Estimation by Chromatographic Methods

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Preformed vitamin A, after being added to mixed feeds, may be separated from interfering substances such as the carotenoids and the oxidation products of vitamin A and then estimated by the Carr-Price reaction. A chromatographic adsorption method using a mixture of magnesia and Celite as the adsorbent and petroleum ether as the solvent is used. The procedure also effects a separation of the alcohol and ester forms of vitamin A. Identification of all the constituents is aided by their fluorescence in ultraviolet light. Recovery from mixed feeds ranged from 84 to 98%.

THE wide use of fish liver oils as an ingredient of mixed feeds has stimulated study of methods for estimating preformed vitamin A in these feed products. Reports indicate that physicochemical methods may be applied, providing certain obstacles can be overcome. One of the main difficulties involves the separation of vitamin A from interfering substances occurring as constituents of other ingredients of the feed. These substances may interfere with either spectrophotometric methods based upon measuring absorption in the region 325 to 328 mμ or colorimetric methods for measuring the intensity of the blue color at 618 to 620 mμ given by the Carr-Price reaction.

Impurities have been removed in a number of ways. One of the earliest methods, which consisted of saponifying the material and then estimating the vitamin A in the unsaponifiable fraction, is readily adaptable for natural vitamin A products such as liver oils. However, when vitamin A is added to feedstuffs, particularly plant materials, all the interfering substances are not removed with the saponifiable fraction, and the oxidation products of vitamin A do not appear to be removed by saponification. Some constituents mask absorption in the ultraviolet, while others produce colors not characteristic of vitamin A when Carr-Price reagent is used.

Chromatographic adsorption methods have been used in the preparation of extracts of vitamin A in order to free them of interfering substances. Floridin earth (*1*), calcium phosphate (*3*), sodium carbonate (*5*), charcoal (*6*), bone ash (*8*), alumina (*13*),

and magnesia (*16*) have been used as adsorbents. Some of the materials retain vitamin A and pass the remaining substances; the vitamin A present is then estimated by difference. Other adsorbents pass the vitamin A and retain the interfering substance. This latter type of adsorption was found the more desirable for this work.

Alumina and magnesia proved to be satisfactory adsorbents for the separation of vitamin A from feed extracts. Of the two, magnesia was chosen because it has less affinity for the vitamin.

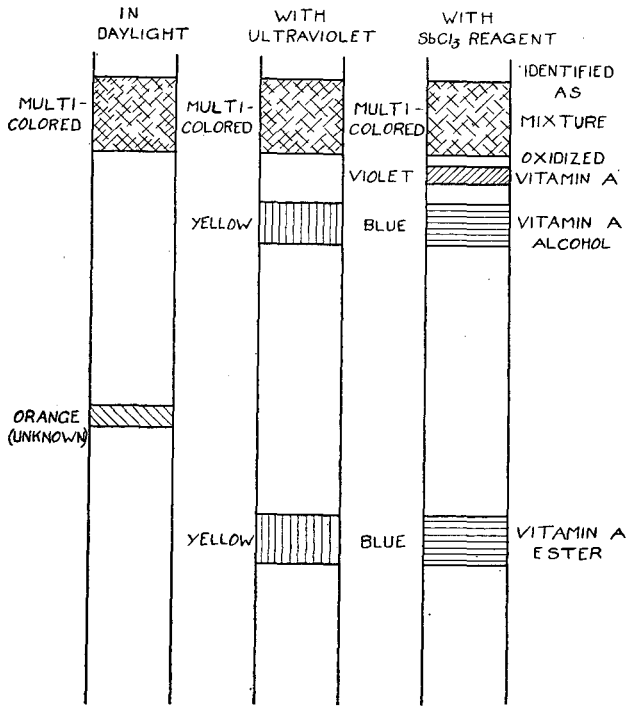

Figure 1. Diagrammatic Representation of Chromatogram of Feed Extract

Wilkie and DeWitt (*16*) used magnesia for the estimation of vitamin A in oleomargarine and their method was adapted for this study. A mixture of magnesia and Celite in the ratio of 1 to 2 when used as the adsorbent, forms an excellent chromatogram with vitamin A and an extract of feedstuffs. It also distinguishes between the alcohol and ester forms of the vitamin (*6, 9, 14, 15*) as well as their oxidation products (*4*).

PROCEDURE

Materials and Apparatus. Petroleum Ether (Merck benzin), purified by shaking with anhydrous sodium sulfate and then with activated charcoal. The manufacturer's product was found to have a fluorescent substance that was adsorbed on magnesia. This made purification necessary (*7*). The solvent was then distilled and a fraction that boiled between 39° and 48° C. was used for extraction and chromatography.

Ethyl Ether, U.S.P., made peroxide-free by distilling over iron wire and discarding the first and last 10%. It was stored over 15% sodium hydroxide.

Magnesium Oxide (Baker's c.p. light powder) and Celite (Johns Manville No. 545) were mixed together carefully with the aid of a screen in the ratio of two Celite to one magnesia by weight. The mixture was tested to pass vitamin A and retain carotenoids.

Since the completion of this work the authors have been informed that the J. T. Baker Chemical Company no longer makes a "light powder" c.p. magnesium oxide. However, the U.S.P. grades of magnesium oxide in the "light powder" or "extra light powder" types were found to be as useful as the c.p. grades. The extra light powder is more retentive than the light powder, but the former allows the solvent to pass through very slowly.

Chloroform, c.p., anhydrous.

Antimony Trichloride Reagent, a saturated solution of antimony trichloride in chloroform.

Ultraviolet Light Source, Stroblite, consists of a GE-2.5-watt argon bulb and a Kopp glass filter U.V. No. 41, obtained from Scientific Supplies Company, Seattle, Wash.

Preparation of Column. Soft-glass tubes, 40 cm. long and 18 mm. in diameter, are used. A cotton plug is inserted in the bottom and the tube is filled with about 4.5 grams of the adsorbent mixture which is packed down to a height of about 10 cm. The length of the column used will depend on the amount of non-vitamin A material present in the extracts. A cotton plug is pushed down on top of the adsorbent and over this about 0.5 cm. of anhydrous sodium sulfate is added. Slight pressure hastens the progress of the solvent through the column (*12*).

Method. Twenty-five grams of feedstuff are Soxhlet-extracted for 4 hours with purified petroleum ether. The extract is concentrated to 25 ml. by use of vacuum and mild heat. An aliquot of this extract, containing 5 to 40 units of vitamin A, is pipetted onto a column previously wetted with petroleum ether. The column is developed and the vitamin A ester eluted in the first fraction with more petroleum ether. In cases where vitamin A alcohol is being eluted, solutions of 5% chloroform in petroleum ether are used. The fluorescent vitamin A fractions are followed down the column with the aid of the ultraviolet light.

Eluates are concentrated to a small volume by evaporation in vacuo. Aliquots are pipetted into colorimeter tubes and evaporated to near dryness, and 1 ml. of chloroform is immediately added. The tubes are then placed in an Evelyn colorimeter and the blue color is developed by adding 10 ml. of antimony trichloride reagent.

The colorimeter was calibrated with dogfish liver oil which had been standardized with a Beckman quartz spectrophotometer. All work was carried out under a red darkroom light or with low actinic glassware. An absolute darkroom was necessary for use of the ultraviolet light, which was used only momentarily to locate the bands. With experience, this was kept at a minimum.

DISCUSSION

The chromatogram, under the ultraviolet light, in daylight, and when sprayed with antimony trichloride reagent, is indicated in Figure 1. Under ultraviolet the adsorbent which forms the background is a dark purple, almost black. Both the ester and the alcohol forms of vitamin A fluoresce as a bright yellowish band on the columns. Under similar conditions various colors have been noted for this fluorescence (*11, 13, 15, 16*).

Oxidized vitamin A was obtained by aerating a sample of vitamin A oil in a stability apparatus (*10*) until the addition of antimony trichloride reagent gave a purple color. These oxidation products of vitamin A (*4*) did not exhibit a fluorescent band on the column with ultraviolet light and were left with the remaining pigments on the upper section of the column. The pigments of the feedstuff extracts show a variety of colored bands with ultraviolet light. Many of these pigments, some of which are carotenoid in nature, form distinct visible bands in daylight (see Figure 1).

Proof and identification of the various bands of vitamin A and oxidized vitamin A, alone or in combination with the feedstuff extracts, are established by extruding the developed column carefully onto a glass plate and spraying or painting it with antimony trichloride reagent (see Figure 1). A plastic medicinal atomizer is used to spray on the antimony trichloride reagent. Vitamin A bands turn a bright blue immediately, oxidized vitamin A develops a violet color in a few seconds, and the carotenoid pigments turn a variety of colors. Of interest here is the fact that the blue color that forms with carotene lasts for 2 to 3 minutes, whereas that formed with vitamin A lasts only about 30 seconds.

An unidentified pigment of alfalfa offered some difficulty when alfalfa-containing feedstuff extracts were chromatographed. It appeared as an orange band between the vitamin A ester and the vitamin A alcohol. This pigment did not fluoresce in the ultraviolet, did not turn any color with antimony trichloride reagent, and could not be extracted with 90% aqueous methanol. Eluted solutions were distinctly yellow, but small traces of it did not interfere with the accuracy of the vitamin A determination. Careful elution eliminated this pigment altogether.

Recovery experiments showed a column efficiency ranging from

Table I. Recovery of Vitamin A by Chromatography

Potency of Oil, Units/G.	Feed Oils Alone: Units added to column	Feed Oils Alone: Units recovered from column	Feed Oils Alone: Recovery, %	Extracts of Feed with Feed Oils[a]: Type of feed	Units added to gram of feed	Units recovered per gram of feed	Recovery, %	Recovery[b], %
7350	34.2	34.0	99.4	.....	...	...	..	...
5850	269.7	281.2	104.3	.....	...	...	..	...
2650	...	...	...	Mill run	23.9	22.1	92.5	...
2650	...	...	...	Mill run + 5% alfalfa	23.3	22.0	94.4	...
2960	...	...	...	Mixed feed A	21.5	20.2	94.0	...
1650	...	...	...	Mixed feed B	4.75	4.66	98.1	...
1630	...	...	...	Mixed feed B	3.40	3.09	90.9	...
9720	60.8	55.0	90.3	.....	...	...	..	...
9720	...	...	...	Mixed feed B	40.3	34.8	86.4	95.6
9720	...	...	...	Mixed feed C	3.07	2.57	83.8	92.8
9720	...	...	...	Mixed feed C	8.35	7.20	86.4	95.6
9160	55.0	51.2	92.2	.....	...	...	..	...
9160	...	...	...	Mixed feed C	3.52	3.30	94.1	102.1
9160	...	...	...	Mixed feed C	8.18	7.58	92.7	100.5
3350	40.8	38.8	95.1	.....	...	...	..	...
3350	...	...	...	Mixed feed C	2.96	2.65	93.1	97.9
3350	...	...	...	Mixed feed C	8.00	7.08	88.6	93.2
Av.			96.3				91.2	96.8

[a] Oil mixed with feed and extracted with petroleum ether. Aliquots containing from 5 to 40 units to chromatographic columns.
[b] Based on chromatographic assay of oil.

Table II. Effect of Saponification on Recovery of Vitamin A from Chromatogram

Sample	Units Recovered Using Whole Extract	Units Recovered Using Saponified Extract
1	15.0	14.7
2	18.7	16.2
3	19.4	15.1
4	9.1	8.2
5	4.4	3.8
6	3.3	3.9

84 to 104%. The recovery of vitamin A was determined by adding an aliquot of a petroleum ether solution of a vitamin A oil to the column and then eluting immediately. Eluates were concentrated and measured with antimony trichloride reagent. A second aliquot of the same solution was used as a control. The recovery of vitamin A, when oils alone were used, is included in Table I. The average recovery is 96.3%.

Recovery tests also were made of vitamin A in feedstuffs (Table I). A vitamin A oil was added to the feedstuff, at levels roughly corresponding to those found in practical rations, and the feed was immediately Soxhlet-extracted. The recovery in these cases averaged 91.2%. When chromatographic assays were applied to oils used in fortifying the feed, it was possible to calculate the probable loss in operations other than the chromatography. This is shown in Table I as a calculated recovery of 96.8%.

Inasmuch as whole and saponified extracts of feeds have been used in chromatographic work (*3, 5, 16*), it seemed advisable to determine the effect of saponification on the recovery of vitamin A from the column.

Some samples of feed extracts containing vitamin A oils were saponified and then chromatographed; the whole extract was used as a control. Aliquots of the extract were pipetted into a flask and saponified with 0.5 *N* alcoholic potassium hydroxide, the mixture was refluxed and extracted with ethyl ether. The ethyl ether was washed with water, removed under vacuum, and replaced by petroleum ether. The solutions were then chromatographed.

Results of the comparison of the saponified extracts with the whole extracts are shown in Table II. The recovery when the whole extracts were used was somewhat better than when saponified extracts were used. Saponification does not seem to offer any improvement over the use of the whole extract. Thus, it is advisable to use the whole extract for chromatographic analysis, as the saponification technique has been shown to be subject to error (*2*) and saponification is believed to contribute to losses if followed by chromatography (*5*). This has been attributed to the instability of vitamin A alcohol. Saponification does not remove oxidized vitamin A, as it still appears in the same relative position on the column when antimony trichloride reagent is sprayed on the extruded column.

Studies were made to evaluate the applicability of the chromatographic method for determining the vitamin A content in practical rations and for following the disappearance of the vitamin during storage. For convenience, test conditions were selected which greatly accelerated the rate of destruction.

A sample of mixed commercial laying mash containing large quantities of yellow corn and alfalfa, and which had no preformed vitamin A included in it, was used. One-half per cent of three different feeding-blend oils of an approximate potency of 1500 units per gram was added to the mash and mixed in thoroughly by hand. The potency of the mixed mashes was calculated to be 7.5 units per gram. In addition, a sample of feed, with 0.5% cottonseed oil added, was treated in the same manner as a blank. Into small, thin cotton bags, 25-gram portions of the mash were packed loosely, and a pair of bags of each sample was immediately Soxhlet-extracted with petroleum ether. The remainder of the bags were set away for storage under conditions that greatly accelerated the rate of destruction. The samples were analyzed periodically by putting the bags directly into the Soxhlet apparatus and extracting with petroleum ether. The extracts were concentrated and chromatographed.

The quantities of vitamin A found in the feeds at the various times of the test are presented in Table III. The amounts of vitamin A found ranged from 8.2 down to 1.4 units per gram. The low values were determined with the same ease as were the initial values. The results demonstrate the ability of the method to determine small quantities of vitamin A in feeds, as well as to follow its disappearance under test conditions. Negligible readings were obtained from the blank samples.

In further tests, a number of commercial feeds containing fish oils were analyzed for vitamin A. Breeders and laying mashes, dog meals, calf pellets, and rabbit pellets were studied. The ingredients of the feeds varied widely in both type and amount, and for that reason offered a good test of the versatility of the method.

Table III. Vitamin A Recovery

(Demonstration of application of chromatographic method in following destruction of preformed vitamin A in mixed feeds under accelerated conditions[a])

Stored, Days	Lot 1: Units/g.	Lot 1: %[b]	Lot 2: Units/g.	Lot 2: %[b]	Lot 3: Units/g.	Lot 3: %[b]
0	7.2		7.0		7.2	
	7.2	96	8.2	101	7.0	95
5	6.2		4.0		3.7	
	6.2	83	3.8	52	3.8	51
8	4.2		4.2		4.4	
	3.6	52	4.0	55	4.3	59
12	3.6	48	...	...	...	..
13	...	..	4.0		4.1	
			3.7	51	4.0	53
18	...	..	3.1			
			2.9	40	...	..
19	3.4					
	3.0	43	...	...	...	..
20	...	..	...	...	2.5	
					2.7	35
28	...	..	2.5			
			2.2	32	...	..
30	2.0					
	2.4	29	...	...	...	..
34	...	..	...	...	1.8	
					1.4	21

[a] Feeds compounded to contain 7.5 units per gram.
[b] Percentage figures calculated from average of two values and based on original estimated potency of 7.5 units per gram.

The dog meal contained no green feed and extracts were chromatographed easily. The rabbit pellets were a little more difficult to analyze, inasmuch as 35% of their ingredients were alfalfa products and it became necessary to use a longer chromatographic column in this case. However, despite the varied feeds and conditions, results obtained were in good agreement with the expected potency.

SUMMARY

A mixture of one part magnesia and two parts Celite by weight makes a satisfactory adsorbent for the chromatographic estimation of vitamin A. Petroleum ether is a satisfactory solvent for the elution of vitamin A ester, and 5% chloroform in petroleum ether for the alcohol form. The Carr-Price reaction determines vitamin A in the eluates. The adsorbent separates carotenoid pigments, vitamin A oxidation products, vitamin A alcohol, and vitamin A esters. Identification of these constituents is aided by their fluorescence in ultraviolet light.

Recovery of vitamin A added to a chromatographic column ranged from 90 to 104%. When added to mixed feeds, recovery ranged from 84 to 98%. Recovery of added vitamin A is not improved by saponifying the petroleum ether extracts of the feeds.

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LITERATURE CITED

(1) Awapara, J., Mattson, F. H., Mehl, J. W., and Deuel, *H. J., Jr., Science*, 104, 602 (1946).
(2) Benham, G. H., *Can. J. Research*, 22B, 2, 21 (1944).
(3) Brew, W., and Scott, M. B., IND. ENG. CHEM., ANAL. ED., 18, 46 (1946).
(4) Castle, D. C., Gillam, A. E., Heilbron, I. M., and Thompson, H. W., *Biochem. J.*, 28, 1702 (1934).
(5) Cooley, M. L., Christiansen, J. B., and Schroeder, C. H., IND. ENG. CHEM., ANAL. ED., 17, 689 (1945).
(6) De, N. K., *Indian J. Med. Research*, 25, 17 (1937).
(7) Graff, M. M., O'Connor, R. T., and Skau, E. L., IND. ENG. CHEM., ANAL. ED., 16, 556 (1944).
(8) Mann, B. T., *Analyst*, 68, 233 (1934).
(9) Reed, G., Wise, E. C., and Frundt, R. J. L., IND. ENG. CHEM., ANAL. ED., 16, 509 (1944).
(10) Sanford, F. B., Harrison, R. W., and Stansby, M. E., *Com. Fisheries Rev.*, 8, 3, 16 (1946).
(11) Sobotka, H., Kann, S., and Winternitz, W., *J. Biol. Chem.*, 152, 635 (1944).
(12) Strain, H. H., "Chromatographic Adsorption Analysis," New York, Interscience Publishers, 1945.
(13) Swain, L. A., Fisheries Research Board of Canada, *Progress Rept.* 49, 13 (1941).
(14) Swain, L. A., *J. Fisheries Research Board Can.*, 6, 1131 (1943).
(15) Wilkes, J. B., IND. ENG. CHEM., ANAL. ED., 18, 702 (1946).
(16) Wilkie, J. B., and DeWitt, J. B., *J. Assoc. Official Agr. Chem.*, 28, 174 (1945).

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Determination of Trace Impurities

In High-Purity Magnesium and Calcium

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Sensitive photometric methods for trace impurities have been applied to the analysis of high-purity magnesium, magnesium alloys, and calcium. Manganese is determined as permanganic acid, following periodate oxidation; iron as the thiocyanate complex; nickel as the tetravalent complex with dimethylglyoxime; copper by carbon tetrachloride extraction of the carbamate complex; and silicon by reduction of silicomolybdate to molybdenum blue.

THE production of high-purity magnesium by the Pidgeon ferrosilicon process has been described by Pidgeon and Alexander (*12*). Staub (*21*) described the reduction of calcium from its oxide by means of aluminum, in furnaces designed for the ferrosilicon process for magnesium. Standard methods of analysis (*2*), being designed to test conformity to commercial specifications, are not always applicable to metals produced by these processes. Aside from the importance of sensitivity, the lower concentrations of impurities tend to minimize interference of one impurity on the determination of another. It therefore appeared desirable to investigate possible modifications of existing methods and applicability of others. Improved sensitivity, adequate accuracy, and reasonable speed were the principal considerations.

Typical compositions of the metals are given in Table I. The present work is restricted to the determination of small amounts of manganese, iron, nickel, copper, and silicon, most of which affect the metallurgical and chemical properties of the metals. Further work on other impurities will be published at a later date.

EXPERIMENTAL PROCEDURE

Standard solutions of magnesium, calcium, aluminum, and zinc were prepared by dissolving the pure metals in hydrochloric acid. Standard iron solution was prepared by dissolving ferrous ammonium sulfate crystals in water containing a few milliliters of hydrochloric acid. The objections to this salt as a primary standard are well known (*7*), but the uncertainty of its composition may be ignored where only microgram quantities are involved. Standard nickel and copper solutions were prepared by dissolving the respective sulfates in water containing a few milliliters of hydrochloric acid, and standardizing the nickel solution gravimetrically with dimethylglyoxime. Standard silicon solution was prepared from sodium metasilicate crystals and standardized gravimetrically. (This solution was prepared fresh daily.) Two standard manganese solutions were prepared; one for use in determining the element, and another, more concentrated, for studying manganese interference in the determination of the other impurities. Both were prepared from standardized potassium permanganate solutions by reduction with sulfur dioxide, in the presence of sulfuric acid. The solutions were then boiled to expel excess sulfur dioxide.

Each method was tried first on pure solutions of the ion to be determined. Effects of the presence of other constituents were then studied. There was some difficulty in finding sufficiently pure samples for reference blanks, to which known amounts of the various impurities were to be added. Generally, the purest available material was used, and blank differences were assumed

Table I. Typical Compositions of High-Purity Magnesium, Magnesium Alloys, and Calcium

Metal	Al	Zn	Mn		Fe	Ni	Cu	Si	Mg
	%	%	%	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	%
Magnesium alloys	0 to 10	0 to 4	0.1 to 2.5	..	20	10	10	50	88–99
Magnesium	....	....	....	20	20	5	5	40	99.95+
Calcium	....	....	....	20	20	5	5	30	0.5–2.5

to be due to the presence of the impurity under study in the reference sample. Where a reaction was suspected between the reagent and an ion other than that being determined, both ions were varied to determine the extent of the interference. In this case, the foreign ion was supplied from varying batches of material to avoid the possibility of the two ions varying in constant ratio.

APPARATUS

All photometric readings were made on a Coleman Universal spectrophotometer, using light paths of 1.3 and 4.0 cm.; and on a Hilger Spekker absorptiometer, equipped with Ilford spectrum filters, and using light paths of 1.0 and 4.0 cm. Although the former instrument gives finer selectivity of wave lengths, the latter is generally more sensitive.

DETERMINATION OF MANGANESE

Most methods for this element involve its oxidation to permanganic acid, followed by colorimetric or volumetric determination (*19*). With suitable precautions, this oxidation may be applied to trace amounts. Oxidizing agents generally used are sodium bismuthate, ammonium persulfate (with silver nitrate), or potassium periodate.

Potassium periodate is the preferred reagent for the colorimetric determination of traces of manganese (*17*). Although the persulfate-silver reaction is generally adequate, its use with calcium-rich materials often leads to precipitation of what appears to be calcium sulfate.

The low amounts of manganese involved necessitate the use of concentrated sample solutions. Nitric acid solutions give the highest possible concentration of magnesium or calcium ions without danger of precipitation.

Effect of Acidity. An acid concentration of approximately 14 ml. of free nitric acid per 100 ml. of solution appears to give the best results. Lower acidities retard the reaction, while higher acidities cause the color to fade. This corresponds to the sulfuric acid acidity recommended by Richards (*13*). Precise control of acidity is not easily attained in dissolving magnesium in nitric acid, because of the evolution of lower oxides of nitrogen. This reaction may be repressed in favor of hydrogen evolution by adding the acid slowly, in the presence of a large excess of water. Acidity adjustment with ammonia and nitric acid is possible, but this unduly increases concentration of salts in the solution.

Inasmuch as the determination of manganese as a trace impurity is required only in pure magnesium and calcium (see Table I), the effects of other alloying elements (aluminum and zinc) were not investigated.

Procedure. PURE MAGNESIUM. Weigh 5 grams of coarse turnings into a 400-ml. beaker, add 100 ml. of water, and tilt the beaker to allow the sample to slide to one side. Add, in small increments, 40 ml. of nitric acid (specific gravity 1.42) to the side of the beaker away from the sample. The rate of addition should be sufficient to maintain a slow, steady reaction, with a minimum evolution of oxides of nitrogen. After all the acid has been added, warm gently, if necessary, to complete solution of the sample, then boil vigorously to remove oxides of nitrogen.

Remove from heat, add 0.5 gram of potassium metaperiodate crystals, and stir to dissolve. Boil gently for 10 to 15 minutes, then keep warm until color is fully developed, adding more periodate if necessary. Cool, transfer to a 100-ml. volumetric flask, and dilute to the mark.

Determine optical density in a 4.0-cm. absorption cell, using green light at 530 mμ.

PURE CALCIUM. Place 100 ml. of water in a 400-ml. beaker. Carefully add 5 grams of coarse turnings, and more water if the reaction becomes too vigorous. When the reaction is complete, add 30 ml. of nitric acid (specific gravity 1.42), mix well to dissolve, and boil free of lower oxides of nitrogen.

Remove from heat, and continue as with pure magnesium.

CALIBRATION. Measure into 400-ml. beakers quantities of standard manganese solution containing 0, 50, 100...up to 500 micrograms of manganese (0 to 100 p.p.m. on a 5-gram sample). Dilute to about 80 ml., add 14 ml. of nitric acid (specific gravity 1.42), and boil free of lower oxides of nitrogen.

Remove from heat, and continue as with pure magnesium. Plot optical densities against micrograms of manganese. The resulting curve will be a straight line, passing through the origin. The same calibration is used for both magnesium and calcium samples.

DETERMINATION OF IRON

Many methods for determining traces of iron are listed by Sandell (*16*), but few are as rapid and sensitive as the thiocyanate method.

Hydrochloric acid was chosen to give maximum solubility of the magnesium and calcium salts. Nitric acid might have been used, but it was thought best to avoid the possibility of reactions between thiocyanate and nitric acid (*16*). Hydrogen peroxide is used to oxidize the iron, and the excess removed by boiling. Although the presence of small amounts of this oxidant is known to improve stability of the ferric-thiocyanate complex (*11*), it was thought better for control work to remove all excess peroxide.

Possible interferences from magnesium, aluminum, zinc, manganese, copper, and calcium were investigated, and found to be insignificant for the quantities involved (Table II). Possibility of loss of ferric chloride in boiling was also studied, and found to be negligible, provided the solution is not allowed to evaporate to crystallization.

Variations in thiocyanate concentration affected both the slope of the working curve and the magnitude of its intercept on the density axis (Figure 1). This was out of proportion with the

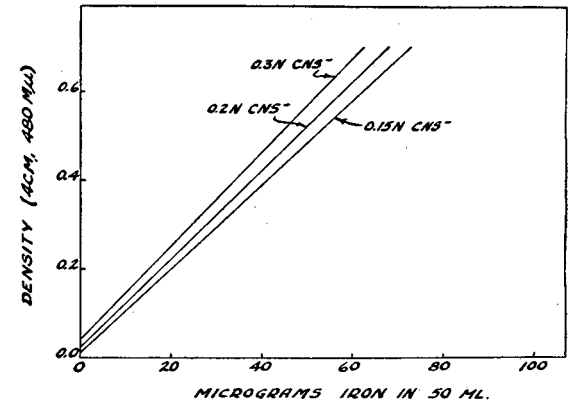

Figure 1. Effect of Thiocyanate Concentration

Table II. Determination of Iron with Thiocyanate

Fe Taken	Other Ions Present	Fe Found
Micrograms	*Mg.*	*Micrograms*
10	850 Mg, 100 Al, 30 Zn, 20 Mn	11.5
20	850 Mg, 100 Al, 30 Zn, 20 Mn	20.0
30	850 Mg, 100 Al, 30 Zn, 20 Mn	29.5
40	850 Mg, 100 Al, 30 Zn, 20 Mn	41.0
20	0.1 Cu	19.5
20	1000 Ca	21.0
40	1000 Ca	41.5
60	1000 Ca	60.5
20	(Ferrous salt, oxidized with H_2O_2, boiled)	20.0
20	(Ferric salt, not boiled)	20.0
30	(Boiled dry, redissolved in water)	5.0

Table III. Colorimetric Determination of Nickel with Dimethylglyoxime

(Readings taken at 445 mμ)

Ni Taken *Micrograms*	Other Ions Present *Mg.*	Ni Found *Micrograms*
13	850 Mg, 100 Al, 30 Zn, 20 Mn	14
23	850 Mg, 100 Al, 30 Zn, 20 Mn	24
33	850 Mg, 100 Al, 30 Zn, 20 Mn	33
23	850 Mg, 100 Al, 30 Zn, 20 Mn, 0.1 Cu	24
23	850 Mg, 100 Al, 30 Zn, 20 Mn, 0.1 Fe	23
20	1000 Ca (modified procedure)	20
40	1000 Ca (modified procedure)	39

apparent blank correction due to iron in the reagents, thus suggesting appreciable light absorption by the thiocyanate itself.

Procedure. Special Reagents. Hydrogen peroxide, 15%, prepared by diluting 30% reagent grade.

Potassium or ammonium thiocyanate, 1 *N*, standardized and checked frequently. Solutions used should be within 3% of theoretical concentration (*6*).

Pure Magnesium and Its Alloys. Weigh 5 grams of coarse turnings into a 400-ml. beaker, cover with 50 ml. of water, and dissolve by the careful addition of 40 ml. hydrochloric acid (specific gravity 1.18). Warm gently, if necessary, to complete solution. Cool, transfer to a 100-ml. volumetric flask, and dilute to the mark. Aliquots of this sample solution may be used for the determination of nickel and copper as well as iron.

Pipet 20 ml. (equivalent to 1-gram sample) into a 150-ml. beaker, and dilute to about 30 ml. Prepare a blank, containing 8 ml. of hydrochloric acid, and dilute to about 30 ml. Add 1 ml. of hydrogen peroxide, and boil 5 to 10 minutes, keeping volume from going below about 15 ml. Cool, transfer to a 50-ml. volumetric flask, add 10 ml. of thiocyanate solution, and dilute to the mark.

Determine optical density as soon as possible, in a 4-cm. absorption cell, using blue-green light at 480 mμ.

Pure Calcium. Place 50 ml. of water in a 400-ml. beaker. Carefully add 5 grams of coarse turnings, and more water if the reaction becomes too vigorous. When the reaction is complete, add 25 ml. of hydrochloric acid (specific gravity 1.18), mix well, and warm gently to dissolve. Cool, transfer to a 100-ml. volumetric flask, and dilute to the mark. Continue as with magnesium, except that the blank should contain only 5 ml. of hydrochloric acid.

Calibration. Measure into 150-ml. beakers quantities of standard iron solution containing 0, 10, 20. . . . up to 100 micrograms of iron (0 to 100 p.p.m. on a 1-gram sample). Dilute to about 30 ml., and add 1 ml. of hydrochloric acid and 1 ml. of hydrogen peroxide. Boil, and continue as with magnesium. Plot optical densities against micrograms of iron. The resulting curve will in general not pass through the origin. An offset curve is then drawn through the origin. The same curve is used for both magnesium and calcium samples.

DETERMINATION OF NICKEL

Dimethylglyoxime and related dioximes are the favorite colorimetric reagents for nickel (*5*).

In the A.S.T.M. method (*2*), the α-furil dioxime complex is extracted with *o*-dichlorobenzene, but control of the amount of tartaric acid used is critical, and copper must first be removed. Passamaneck (*9*) precipitates nickel with dimethylglyoxime, and dissolves the precipitate in pyridine, to give a yellow color. This method was tried, but the separation of a few micrograms of nickel from a gram of magnesium or calcium was found to be slow and sometimes incomplete. Sandell and Perlich (*18*) extract nickel dimethylglyoxime with chloroform, and determine the separated nickel as the tetravalent color complex with dimethylglyoxime (*14*). In this work the latter method is applied, without preliminary separation.

Comparatively high concentration of citrate is required to prevent precipitation of the large amounts of aluminum present in some magnesium alloys. The buffering effect of ammonium citrate necessitates the use of additional ammonia to attain the optimum pH for the color reaction.

The spectral transmission curve of nickelic dimethylglyoxime shows a minimum at about 445 mμ, with an isobestic point nearby and a lesser minimum at about 543 mμ (*8*). The latter wave length is generally used, because of possible iron interference in the blue. For higher sensitivity, the blue wave *length is preferable*, and iron interference has been shown to be negligible, in concentrations likely to be found in high-purity magnesium and calcium (Table III). Copper interference was also found to be insignificant.

Color Development and Stability. A time study was made to determine the optimum wave length for color stability (Figure 2). In the range 440 to 465 mμ, maximum absorption appears to be at 450 mμ, but stability is apparently attained more rapidly at 445 mμ. The best combination of stability and sensitivity is attained by reading at the latter wave length, between 2 and 5 minutes after mixing.

Application to Calcium. In attempting to apply this procedure to calcium, a precipitate of calcium citrate was obtained on duplicating the conditions used for magnesium. This was overcome by reducing the citrate concentration. This results in diminished buffer action, so less ammonia may be used. Under these conditions, the precipitation of calcium citrate is retarded sufficiently to allow photometric readings in a clear solution. The working curve produced by these conditions coincides with that drawn up under the conditions used with magnesium.

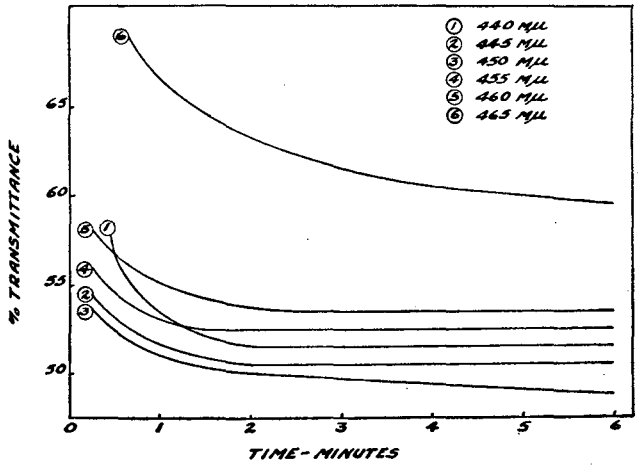

Figure 2. Variation of Nickelic-Dimethylglyoxime Color with Time

Procedure. Special Reagents. Ammonium citrate, dibasic, 250 grams per liter. Dimethylglyoxime, 1 gram in 100 ml. of ethanol.

Pure Magnesium and Its Alloys. Pipet 20 ml. of the chloride solution prepared for iron determination (equivalent to 1-gram sample) into a 50-ml. volumetric flask. Prepare a blank by evaporating 8 ml. of hydrochloric acid (specific gravity 1.18) down to about 1 ml., transferring to a 50-ml. volumetric flask, and diluting to 20 ml. Add 20 ml. of citrate solution, then bromine water, dropwise, until solution is just yellow. Add 5 ml. of ammonium hydroxide (specific gravity 0.90), then 1 ml. of dimethylglyoxime solution, and dilute to the mark.

Determine optical density, between 2 and 5 minutes after addition of the dimethylglyoxime, in a 4-cm. absorption cell, using blue light at 445 mμ.

Pure Calcium. Proceed as with magnesium, but use only 5 ml. of hydrochloric acid in preparing the blank, and only 2 ml. of citrate solution. Dilute to about 40 ml. after adding the bromine, then just enough ammonium hydroxide to destroy the bromine color, plus 1 ml. excess.

Calibration. Measure into 50-ml. volumetric flasks quantities of standard nickel solution containing 0, 10, 20. . . . up to 60 micrograms of nickel (0 to 60 p.p.m. on a 1-gram sample). Dilute to about 20 ml., and add 1 ml. of hydrochloric acid (specific gravity 1.18). Add citrate, and continue as with pure magnesium.

Plot optical densities against micrograms of nickel. The resulting curve will in general not pass through the origin. An offset curve through the origin is then drawn.

Table IV. Determination of Copper

(Readings at 435 mμ)

Cu Taken *Micrograms*	Other Ions Present *Mg.*	Cu Found *Micrograms*
10	1000 Mg	11
40	1000 Mg	41
31	1000 Mg, 100 Al	33
11	1000 Mg, 30 Zn	11.5
21	1000 Mg, 25 Mn	21
21	1000 Mg, 100 Al, 30 Zn, 25 Mn	20
0	1000 Mg, 0.1 Ni	11
40	1000 Mg, 0.1 Ni	48
30	1000 Mg, 0.1 Fe	29
10	1000 Ca (modified procedure)	10.5
40	1000 Ca	40
20	1000 Ca, 0.1 Mn	21

DETERMINATION OF COPPER

Of the many reagents available for colorimetric copper determination, dithizone and sodium diethyldithiocarbamate appear to be favorites (*15*). Both were tried with magnesium and calcium, but greater specificity and simplicity of application favored the latter. The hydrobromic acid method (*22*) was also tried but found to be insufficiently sensitive.

Haywood and Wood (*6*) have applied the carbamate method in aqueous solution to magnesium alloys, gum acacia being added as the protective colloid. The range of copper contents covered (0.0 to 0.1%) is considerably higher than that considered in this investigation. Nevertheless, satisfactory results were obtained by this method down to less than 10 p.p.m. of copper in pure magnesium, with only a change in sample concentration. When this was applied to alloys, a turbidity appeared, apparently due to the action of zinc. When ammonia was added in an attempt to eliminate this interference (*10*), magnesium hydroxide precipitated. Sandell's extraction procedure with carbon tetrachloride (*15*) was then attempted, and satisfactory results were obtained.

Interferences. Magnesium, aluminum, zinc, manganese, and iron gave no interference, when increased amounts of citrate and ammonia were used as in the method for nickel in magnesium (see Table IV). Nickel does interfere, 10 parts of nickel being roughly equivalent to 1 of copper, thus allowing the use of a simple correction. When the procedure is applied to calcium samples, the quantities of citrate and ammonia used must be reduced (as with the nickel method), to retard the precipitation of calcium citrate. Under these conditions, some samples produce a pinkish extract, apparently due to manganese (*15*). This has little effect on the absorption in violet light (Table IV). Although the manganese content of magnesium alloys is much higher than that of pure calcium, the large amount of citrate used in former case tends to prevent manganese interference (*10*).

Effect of Citrate on Copper Color. From the foregoing, it follows that the citrate ion acts both as complexing agent and as a buffer. A further function of the citrate ion is evident on plotting working curves with varying amounts of citrate and ammonia (Figure 3). These suggest that the citrate ion plays a major part in the copper-carbamate reaction, conformity to Beer's law being affected by the citrate concentration. The difference between the straight-line portions of the two curves may be due, in part, to copper in the citrate. However, when parallel curves are drawn through the origin, it is clear that the two curves coincide up to about 25 micrograms of copper in 10 ml. of carbon tetrachloride.

In the procedures which follow, bromine is added to ensure that all the copper is in the divalent state. The procedures are then identical with those for nickel, up to the point where the color reagent is added.

Procedure. Special Reagents. Ammonium citrate, the same solution as used for the nickel method. Sodium diethyldithiocarbamate, 1 gram in 100 ml. of water.

Pure Magnesium and Its Alloys. Pipet 20 ml. of the chloride solution prepared for iron determination (equivalent to 1-gram sample) into a 100-ml. separatory funnel. Prepare a blank by evaporating 8 ml. of hydrochloric acid (specific gravity 1.18) down to about 1 ml., transferring to a 100-ml. separatory funnel, and diluting to 20 ml. Add 20 ml. of citrate solution, then bromine water, dropwise, until solution is just yellow. Add 5 ml. of ammonium hydroxide (specific gravity 0.90), then 1 ml. of carbamate reagent, and exactly 10 ml. of carbon tetrachloride. Shake vigorously for about 2 minutes, and allow layers to separate.

Draw off enough of the carbon tetrachloride layer to give a reading in a 1.0- or 1.3-cm. absorption cell, passing the extract through a dry, coarse filter paper. Determine optical density at 435 mμ. Apply a correction for nickel, subtracting one tenth the nickel content from the apparent copper content.

Pure Calcium. Proceed as with magnesium, but apply the same modifications regarding citrate and ammonia additions, and dilution, as applied in determining nickel in calcium.

Calibration. Measure into 100-ml. separatory funnels quantities of standard copper solution containing 0, 10, 20.... up to 60 micrograms of copper (0 to 60 p.p.m. on a 1-gram sample). Dilute to about 20 ml., and add 1 ml. of hydrochloric acid (specific gravity 1.18). Add citrate, and continue as with pure magnesium. Repeat the entire procedure, using the quantities of citrate and ammonia used with calcium.

Plot optical densities against micrograms of copper for both sets of data. The resulting curves will be of the form indicated in Figure 3. Draw a straight line through the origin parallel to the straight portions of the two curves. These should coincide up to about 25 micrograms of copper. The final working curve for magnesium samples is this straight line extended to about 60 micrograms of copper. For calcium samples, the curved portion of the final graph is offset from the preliminary curve by a constant amount parallel to the concentration axis.

DETERMINATION OF SILICON

Colorimetric methods for this element are generally based on the reaction between soluble silicic acid and molybdate ion to form a yellow heteropoly acid (*1*, *22*). Although this color can be estimated photometrically to give a measure of the silicon concentration, greater sensitivity may be attained by reducing the molybdenum in the complex to molybdenum blue (*4*, *6*).

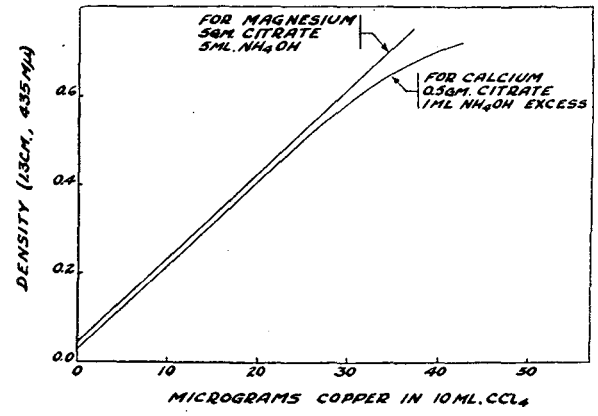

Figure 3. Effect of Ammonium Citrate on Copper Color

The method then resolves itself into three steps: solution of the sample in such manner that all the silicon is converted to soluble silicic acid; adjustment of acidity to the point where the silicomolybdate complex will form; and reduction, under such conditions that only that portion of the molybdenum in the complex is affected. In investigating the first step, alloys of known silicon content were used instead of standard silicon solution. These were either spectrographic standards (*3*) or samples standardized spectrographically against those standards.

Conditions for Magnesium. In Stenger's procedure (*22*), magnesium alloys are dissolved in sulfuric acid, the reaction vessel being kept cool to prevent loss of silicon hydride. Boric acid is also added, probably to prevent loss of silicon due to fluorides (*20*). With alloys of high purity, boric acid does not appear to

Table V. Determination of Silicon

Si Present	Other Ions Present	Conditions	Si Found
Micrograms	*Mg.*		*Micrograms*
20	1000 Mg	With boric acid	20
30	850 Mg, 100 Al, 30 Zn, 20 Mn	With boric acid	29
20	1000 Mg	Without boric acid	20
30	850 Mg, 100 Al, 30 Zn, 20 Mn	Without boric acid	32
26	1000 Mg	Dissolved cool	26
26	1000 Mg	Dissolved hot	16
10	1000 Ca	NaOH-HCl procedure	9
20	1000 Ca	NaOH-HCl procedure	21
40	1000 Ca	NaOH-HCl procedure	38

be necessary (Table V). However, poor recoveries of silicon resulted when the solutions were allowed to heat up in the process of dissolving the sample, presumably due to loss of hydride. High-purity alloys, containing less than 100 p.p.m. of silicon, were found to leave no insoluble residue, thus eliminating the necessity of fusion with sodium carbonate (*22*).

Effect of Acidity. The reaction between silicic acid and molybdate ion appears to work best at an acid concentration of approximately 0.1 *N* (*1*). The color failed to develop when attempts were made at much higher acidities, while the use of less acid led to incomplete solution of the sample.

Reduction Conditions. Sodium sulfite has been used as reducing agent in the production of molybdenum blue from silicomolybdic acid (*4*), but its use requires precise pH control. If stannous chloride is used, the solution must be made strongly acid after the silicomolybdate yellow color has fully developed; otherwise, a deep blue solution results, probably due to conversion of some of the excess molybdate to molybdenum blue. In the strongly acid solution, the resulting blue density is proportional to silicon concentration.

Application to Calcium. Attempts were made to apply this method to calcium, but results were widely erratic. (Hydrochloric acid was substituted for sulfuric, to prevent precipitation of calcium sulfate.) The loss of hydrides in the vigorous reaction of dissolving calcium in water was apparently responsible. Attempts to slow down the reaction by cooling gave no better results. The use of an alkaline solution was found to be necessary to assure quantitative conversion of silicon to soluble silicates. This led to silica contamination from the glassware, both with Pyrex and with Corning alkali-resisting glass, necessitating the use of platinum. Once the sample was dissolved, conditions of the procedure for magnesium were essentially duplicated, except that hydrochloric replaced sulfuric acid.

Procedure. Special Reagents. Sodium hydroxide, 1 *N*, stored in a hard rubber or wax-lined bottle. Hydrochloric acid, 1 *N*. Sulfuric acid, 1 to 4, freshly prepared. Ammonium molybdate, 80 grams per liter, filtered.

Stannous chloride, 1%. Dissolve 1 gram in 2 ml. of hydrochloric acid (specific gravity 1.18) and dilute to 100 ml. Prepare fresh every 2 or 3 days.

Pure Magnesium and Alloys. Weigh 2 grams of coarse turnings into a 400-ml. beaker, cover with 100 ml. of water, and tilt the beaker to allow the sample to slide to one side. Place in a cold water bath, and dissolve by the slow addition of 27.5 ml. of sulfuric acid (1 to 4) to the side of the beaker away from the sample. The rate of addition should be sufficiently rapid to keep a slow, steady reaction going, without overheating. When the reaction subsides after all the acid has been added, warm gently to complete solution. Cool, transfer to a 200-ml. volumetric flask, and dilute to the mark. Pipet 50 ml. (equivalent to 0.5-gram sample) into a 100-ml. volumetric flask. Prepare a blank, containing 1 ml. of sulfuric acid (1 to 4), diluted to 50 ml., in another 100-ml. flask. Add 5 ml. of molybdate solution, and allow to stand 10 to 15 minutes. Add 10 ml. of sulfuric acid (1 to 4), and allow to stand 2 or 3 minutes. Add 2 ml. of stannous chloride solution, dilute to the mark, and allow to stand 10 to 15 minutes.

Determine optical density in a 4-cm. absorption cell, using red light at 650 mμ.

Pure Calcium. Using a plastic pipet, place 5 ml. of 1 *N* sodium hydroxide in a 75-ml. platinum crucible, and dilute to 25 ml. Add 0.5 gram of coarse turnings, and heat to boiling. stirring with a platinum or hard-rubber rod to assist solution. When reaction is complete, carefully add 5 ml. of hydrochloric acid (1 to 1), with continuous stirring. Remove from heat, and adjust with 1 *N* hydrochloric acid and 1 *N* sodium hydroxide, until just acid to litmus, then add 5 ml. of 1 *N* hydrochloric acid excess. Prepare a blank, containing the same quantity of sodium hydroxide in another platinum crucible. Adjust acidity as with the sample, using only 1 *N* acid and alkali. Cool and transfer to 100-ml. volumetric flasks. Add 5 ml. of molybdate solution, and allow to stand 10 to 15 minutes. Add 10 ml. of hydrochloric acid (specific gravity 1.18), and allow to stand 2 or 3 minutes. Add 2 ml. of stannous chloride solution, dilute to the mark, and allow to stand 10 to 15 minutes.

Determine optical density in a 4-cm. absorption cell, using red light at 650 mμ.

Calibration. Measure into 100-ml. volumetric flasks quantities of standard silicon solution containing 0, 5, 10. . . up to 50 micrograms of silicon (0 to 100 p.p.m. on a 0.5-gram sample). Add 1 ml. of sulfuric acid (1 to 4) and dilute to 50 ml. Continue as with magnesium.

Plot optical densities against micrograms of silicon. The resulting curve will, in general, not pass through the origin. An offset curve is then drawn through the origin. If hydrochloric acid is used instead of sulfuric acid, and the calcium procedure is followed, the resulting curve through the origin will coincide.

SUMMARY

Sensitive photometric methods have been applied for the determination of microgram quantities of manganese, iron, nickel, copper, and silicon in high-purity magnesium, magnesium alloys, and calcium. Effects of interferences from other elements likely to be present have been studied. The methods have been used on a routine basis for the past year, with satisfactory results.

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LITERATURE CITED

(1) Adams, M. F., Ind. Eng. Chem., Anal. Ed., **17**, 542 (1945).
(2) Am. Soc. Testing Materials, Committees E-2 and E-3, "Methods of Chemical Analysis of Metals," p. 163, Philadelphia, American Society for Testing Materials, 1946.
(3) Am. Soc. Testing Materials, Committee E-2, "Report on Standard Samples for Spectrochemical Analysis," Philadelphia, American Society for Testing Materials, 1947.
(4) Boyle, A. J., and Hughey, V. V., Ind. Eng. Chem., Anal. Ed., **15**, 618 (1943).
(5) Diehl, H., "Applications of the Dioximes to Analytical Chemistry," Columbus, Ohio, G. Frederick Smith Chemical Co., 1940.
(6) Haywood, F. W., and Wood, A. A. R., "Metallurgical Analysis," p. 110, London, Adam Hilger, 1944.
(7) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," rev. ed., p. 595, New York, Macmillan Co., 1945.
(8) Mitchell, A. M., and Mellon, M. G., Ind. Eng. Chem., Anal. Ed., **17**, 380 (1945).
(9) Passamaneck, E., *Ibid.*, **17**, 257 (1945).
(10) Patridge, R. F., *Ibid.*, **17**, 422 (1945).
(11) Peters, C. A., MacMasters, M. M., and French, C. L., *Ibid.*, **11**, 502 (1939).
(12) Pidgeon, L. M., and Alexander, W. A., *Trans. Am. Inst. Mining Met. Engrs.*, **159**, 315 (1944).
(13) Richards, M. B., *Analyst*, **55**, 554 (1930).
(14) Rollet, A. P., *Compt. rend.*, **183**, 212 (1926).
(15) Sandell, E. B., "Colorimetric Determination of Traces of Metals," p. 217, New York, Interscience Publishers, 1944.
(16) *Ibid.*, p. 262.
(17) *Ibid.*, p. 312.
(18) Sandell, E. B., and Perlich, R. W., Ind. Eng. Chem., Anal. Ed., **11**, 309 (1939).
(19) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., p. 562, New York, D. Van Nostrand Co., 1939.
(20) *Ibid.*, p. 802.
(21) Staub, P. H., *Chem. Met. Eng.*, **52**, No. 8, 94 (August 1945).
(22) Stenger, V. A., *Proc. Am. Soc. Testing Materials*, **44**, 754 (1944).

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Preparation of Organolithium Reagents

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An improved procedure for the preparation of methyl-, ethyl-, isopropyl-, *n*-amyl-, and phenyllithium is presented. Advantages of the procedure are due chiefly to the use of lithium sand and an apparatus of special design which allows all operations to be carried out in an inert atmosphere of 98% helium. The general assembly and details of construction of the apparatus are shown.

SINCE the observation by Ziegler and Colonius (*10*) that organolithium reagents could be prepared directly from the organo halide and lithium, and the many refinements and extensions of this method of preparation by Gilman *et al.* (*6–8*), these reagents have become much more available and useful to the organic chemist. However, for synthetic work, their preparation on a larger scale than that given by the above authors is usually desirable.

The apparatus and methods given in the literature (*1, 2, 6*), when applied on a 0.5- to 1.0-mole basis, were tedious and in some cases unsatisfactory. Hence, a modified method was developed, using the apparatus and procedure given below, which uniformly gave good yields of organolithium reagents, in some cases better than those previously reported (*7, 8*).

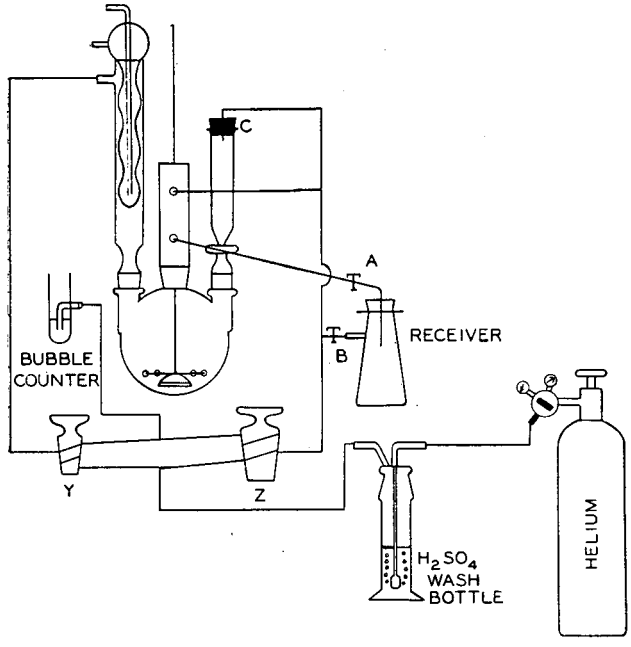

Figure 1. Assembly of Apparatus

The advantages of this method are due chiefly to the use of lithium sand (*3, 4, 9*) and a simple filtration device which allows all operations to be conducted in an inert atmosphere. Because coating of the lithium in large-scale runs became pronounced and in some cases had a deleterious effect, it was found advantageous to use finely divided lithium. The methods for comminuting lithium ordinarily used—i.e., cutting into small pieces (*8*) or rasping (*6, 7*)—proved impractical with the 10-gram (or more) portions of lithium used. Lithium sand, however, prepared by melting lithium in liquid petrolatum and cooling with stirring, was very satisfactory. The method of collecting the reaction product by filtration through the stirrer shaft, described below, prevented losses from exposure of the very reactive organolithium reagent to the air.

Helium (98%), rather than nitrogen, was used as the inert atmosphere for the reaction in order to prevent nitride formation with the finely divided lithium (*6*). Because of its low price, helium makes an economical inert atmosphere for this type of work.

As shown in Table I, highly successful results were obtained in the preparation of methyl-, ethyl-, isopropyl-, *n*-amyl-, and phenyllithium. However, when the same method was applied to the preparation of benzyllithium from benzyl chloride in ether, it failed to give any organolithium reagent. As observed by Ziegler and Dersch (*11*), the products were bibenzyl plus a small amount of unreacted benzyl chloride.

Table I. Yields of Organolithium Reagents

RX Compound	Solvent	Yield RLi, %
CH_3I[a]	Et_2O	95
C_2H_5Br[b]	Petroleum ether	92
Iso-C_3H_7Cl[c]	Petroleum ether	88
n-$C_5H_{11}Cl$[d]	Petroleum ether	90
C_6H_6Br[b]	Et_2O	98

R = alkyl or aryl radical.

[a] Merck, purified by treating with Hg.
[b] Merck, reagent, redistilled.
[c] Eastman, pure, used directly.
[d] Paragon, pure, purified by washing with concentrated sulfuric acid, drying over calcium chloride, and fractionating. The fraction boiling between 105° and 107° was used.

Calculation of the yield of organolithium reagent was based on simple acid titration of an aliquot after decomposition with water. Although this method is known to give high results in some cases (*5*) due to the cleaving action of certain organolithium compounds on ether, the error is negligible with methyl- and phenyllithium because of their comparative stability in ether (*7, 8*). These were the only two reagents prepared in ether. The others were prepared in petroleum ether where high results from acid titration are not encountered.

APPARATUS

The general assembly of the apparatus is pictured in Figure 1.

It is essentially a hollow-shaft stirrer carrying a sintered-glass filter at the bottom with a chamber sealed about the upper part of the stirrer shaft, so that gas or liquid may be introduced or withdrawn from the shaft. The stopcocks are so arranged that the helium may bubble through the solution or exert a pressure on its surface. Inasmuch as a pressure of several pounds of helium is used, it is necessary to clamp all parts securely. During the preparation of the organolithium compound, stopcocks *Y* and *Z* remain in the position shown, thus causing the helium to bubble through the solution. To effect filtration, *Y* and *Z* are reversed and the helium pressure forces the solution through the stirrer shaft and into the receiver. All solutions are introduced into the dropping funnel by removing stopper *C*. When receivers are changed, pinchclamps *A* and *B* are closed, and any type of receiver may be used in place of the filter flask by merely providing the necessary inlet and outlet connections.

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The first apparatus designed is illustrated in Figure 2. The details of construction are obvious and require no special comment.

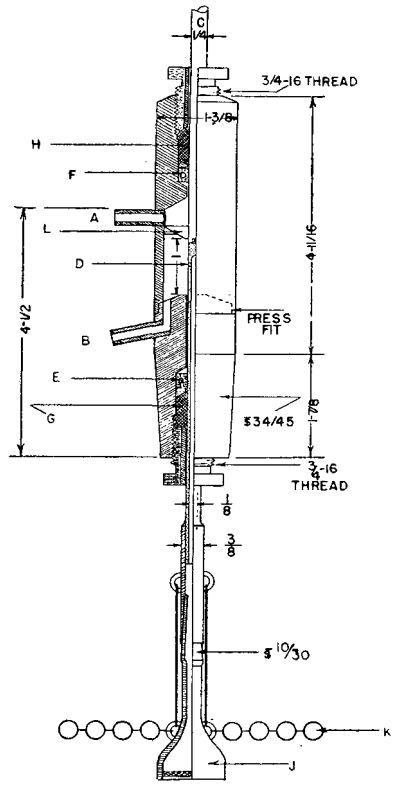

Figure 2. Diagram of Apparatus

A. Helium tube
B. Filtrate outlet tube
C. Stirrer shaft
D. 2 holes, 1/16 inch diameter
E, F. Nice No. 402-2 ball bearings 1/4 × 11/16 inch
G, H. Graphite-lead-asbestos packing
J. Ace filter tube 8610, 25-mm. diameter and porosity A (Ace Glass Co., Vineland, N. J.)
K. Hirschberg type stirrer of 18-gage Chromel wire
L. Baffle

Stainless steel was used except for the ball bearings, which are of high-chrome carburized steel, and the stirrer shaft, which is brass. The packing nuts are also bushed with brass. The packing is a lead-asbestos-graphite type. Ball bearings are essential under the conditions of unfavorable lubrication encountered. It is necessary to disassemble the apparatus after use to clean and lubricate the ball bearings. The weight of the unit is sufficient to hold it tight in the standard-taper joint against 0.1 atmosphere pressure differential. The modification of the glass filter tube (*J*, Figure 2) by sealing on a male 10/30 joint and two glass loops requires no special comment.

The use of packing is always somewhat objectionable. The apparatus was later modified and a lower seal of the type illustrated in Figure 3 was used. Although this is more difficult to construct, it constituted a considerable improvement. The exposed ball bearing is also much easier to clean.

The authors constructed an all-glass apparatus in which the sealing action was effected by very small clearance between the stirrer shaft and its bearing. This method was satisfactory for a few runs, but because of lack of lubrication, the wear was very rapid and excessive leakage soon developed. Rubber seals are rapidly destroyed by solvent action.

PROCEDURE

The apparatus (Figure 1) was swept out with a stream of helium which was continued at a moderate rate throughout the preparation. Liquid petrolatum (200 ml.), previously heated to 250° with lithium, and 10.4 grams (1.5 grams atoms) of clean lithium were then added to the three-necked flask and heated until the lithium was melted, after which the solution was allowed to cool to room temperature with rapid stirring. The liquid petrolatum was then filtered from the finely divided lithium by reversing the flow of helium and was collected in a 500-ml. filter flask for re-use in subsequent preparations. Occasional plugging of the sintered-glass filter was relieved by reversing the helium flow to backwash the filter. The lithium was washed with 100 ml. of the solvent to be used in the preparation (either absolute ether or dry, unsaturate-free Skellysolve A, boiling point 28° to 38° C.) which was also removed by filtration and collected in a 500-ml. filter flask.

To the washed, finely divided lithium were now added 50 ml. of solvent and, with rapid stirring, several milliliters of the alkyl (or aryl) halide in order to initiate the reaction. The remainder of the 0.5 mole of halide, dissolved in 300 ml. of solvent, was added over the course of 1.5 hours, and the dropping funnel was rinsed with 50 ml. of solvent after the addition was completed. Stirring was continued another hour and, by reversing the helium flow, the reaction mixture was then filtered from excess lithium and lithium halide and collected in a narrow-mouthed 500-ml. graduated cylinder which served as receiver in place of the filter flask. The residue was stirred with 100 ml. of fresh solvent which also was filtered into the receiver, bringing the total volume of filtrate to about 500 ml.

After thorough mixing, the solution was allowed to stand for several hours in the tightly stoppered receiver to permit the insoluble material that passed through the filter to settle. To determine the yield of organolithium reagent, two 5-ml. portions were removed and decomposed with water, and the lithium hydroxide formed (equivalent to the organolithium compound present) was titrated with standard hydrochloric acid. In most cases, the organolithium reagent solution was slightly less than 1 *N*. The yields are given in Table I.

The procedure had to be modified slightly in the case of ethyllithium because of its limited solubility in petroleum ether. One half the quantities of lithium and ethyl bromide were used, 300 ml. of petroleum ether were added before the reaction mixture was filtered, and both the petroleum ether reaction mixture and wash portion were filtered hot. This resulted in a solution that was 0.29 *N* at room temperature (27° C.). On cooling, it deposited crystals of ethyllithium which burst into a vivid violet flame when exposed to the air. Phenyllithium was prepared with 2.8 grams of lithium, 15.7 grams of phenyl bromide, and 60 ml. of ethyl ether.

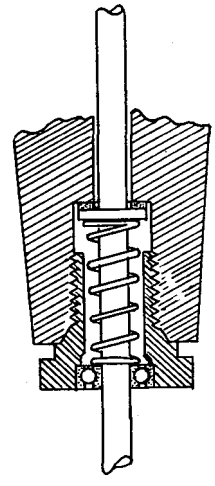

Figure 3. Seal

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LITERATURE CITED

(1) Adams, R., Wolff, H., Cain, C. K., and Clark, J. H., *J. Am. Chem. Soc.*, **62**, 1772 (1940).
(2) Anker, R. M., and Cook, A. H., *J. Chem. Soc.*, **1941**, 323.
(3) Bartlett, P. D., Swain, C. G., and Woodward, R. B., *J. Am. Chem. Soc.*, **63**, 3229 (1941).
(4) Friedlander, Paul, *Fortschr. Teerfarben Fabrikation*, **17** 125 (1930).
(5) Gilman, H., and Haubein, A. H., *J. Am. Chem. Soc.*, **66**, 1515 (1944).
(6) Gilman, H., Langham, W., and Moore, F. W., *Ibid.*, **62**, 2327 (1940).
(7) Gilman, H., Moore, F. W., and Baine, O., *Ibid.*, **63**, 2479 (1941).
(8) Gilman, H., Zoellner, E. A., and Selby, W. M., *Ibid.*, **55**, 1252 (1933).
(9) Ziegler, K., German Patent 512,882 (1930).
(10) Ziegler, K., and Colonius, H., *Ann.*, **479**, 135 (1930).
(11) Ziegler, K., and Dersch, F., *Ber.*, **64B**, 448 (1931).

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Determination of Carotene in Alfalfa and Cereal Grasses

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The photochemical destruction of carotene in the presence of chlorophyll was negligible during carotene analyses under normal conditions of laboratory illumination. Destruction was appreciable, however, when the samples were placed directly in sunlight or left unprotected for several hours on the laboratory table. A method of carotene analysis is proposed which eliminates the present laborious extraction procedure for removing carotene from fresh plant tissue. It involves the inactivation of carotene-destroying enzymes by blanching or autoclaving, drying at low temperatures, and the use of a simplified procedure for extraction of carotene from the dried material. The advantages and disadvantages of the method are discussed.

PEPKOWITZ (*3*) has shown that sunlight may cause considerable destruction of carotene in solutions that also contain chlorophyll. He reported that extracts of cooked vegetables lost more carotene than did extracts of raw vegetables when exposed to sunlight. His experiments, however, were not designed to show the amount of carotene that will be destroyed during actual carotene analyses. It is desirable that the extent of this loss be known, so that the need for preventive measures may be considered.

This paper evaluates the photochemical destruction of carotene that occurs during actual carotene determinations in both fresh and dried plant tissue. It is also concerned with a procedure for eliminating the use of the Waring Blendor and for reducing sampling error in the determination of carotene in fresh plant tissue.

DESTRUCTION OF CAROTENE BY LIGHT

Five-gram samples of blanched (1 minute with steam) and unblanched fresh alfalfa leaves were analyzed by the method of Wall and Kelley (*5*), except that they were not minced prior to comminution. The blanched samples were included in the experiment because of the report by Pepkowitz that carotene destruction is greater in extracts of cooked than raw vegetables. Part of the samples were comminuted in a Waring Blendor and extracted near the windows of the laboratory on a clear day. Although the samples were not placed directly in the rays of the sun, the laboratory table was brightly illuminated. During 2 hours between blending and chromatographing the samples were not protected from light. The remainder of the samples were held in a refrigerator until night, when they were comminuted and analyzed; the only light source was a 25-watt Mazda lamp equipped with a Wratten Series O photographic darkroom filter. As a further precaution, extractions were made in amber glass separatory funnels. The results of the experiment are presented in Table I.

Table I. Effect of Sunlight on Determination of Carotene in Fresh and Dehydrated Alfalfa

Treatment	Carotene	Destruction
	γ/g. dry wt.	%
Unblanched leaves, dark	848	...
Unblanched leaves, sunlight	793	6.5
Blanched leaves, dark	877	...
Blanched leaves, sunlight	850	3.1
Dehydrated meal, dark	265	...
Dehydrated meal, sunlight	258	2.6

The effect of sunlight on the determination of carotene in dehydrated alfalfa meal by the simplified procedure of Silker, Schrenk, and King (*4*) was also studied because the length of exposure to sunlight is much shorter than with other methods.

Sixty milliliters of a 33% solution of acetone in Skellysolve B were added to 1-gram samples of meal. After standing in the dark overnight, the samples were filtered and washed with Skellysolve B on a Büchner funnel. The filtrates were transferred to beakers and concentrated on a steam plate in a hood to about 40 ml. The solutions were then chromatographed on a 1 to 2 mixture of magnesia (Micron brand No. 2641) and Supercel. The carotene was eluted from the adsorbent with a 4% solution of acetone in Skellysolve B. Carotene concentration was measured at 4360 Å. with a Beckman spectrophotometer. The unprotected samples analyzed by this method were exposed to light for about 45 minutes, during the filtration and evaporation procedures. The samples which were protected from light were analyzed at night, using the darkroom filter described above.

The results of this experiment also appear in Table I.

Using the method of Silker, Schrenk, and King with alfalfa meal samples, the effect of quality and intensity of radiation on the photochemical destruction of carotene was studied. The extracts after filtration were transferred to 600-ml. clear Pyrex beakers and exposed to various types of radiation, as shown in Table II. After exposure, the extracts were concentrated and chromatographed without further protection from light, as the data of Table I indicate that little destruction occurs during the latter steps.

It is apparent from Table I that, under normal conditions of illumination in the laboratory, the destruction of carotene by sunlight was negligible during analysis. Table II, however, shows that photochemical destruction was appreciable when extracts were placed directly in the rays of the sun or left unprotected for several hours on the laboratory table. Ultraviolet light caused no destruction during a 30-minute exposure. Light from a Mazda lamp was not detrimental. Thus, it appears that precautions need to be taken to prevent photochemical destruction only if sunshine falls directly upon the samples.

The carotene value for a blanched sample (Table I) was somewhat higher than the value for the corresponding unblanched sample. This confirms the data of Zscheile and Whitmore (*6*), who found that carotene was more easily and more thoroughly extracted from blanched than from unblanched alfalfa leaves.

Table II. Effect of Intensity and Type of Light on Destruction of Carotene in Extracts of Dehydrated Alfalfa Meal

Treatment	Exposure Time[a]	Carotene	Loss during Exposure
	Hours	*γ/g. dry wt.*	%
Normal lab. illumination (day)	0	283	0
	2	270	4.6
	3	248	12.4
Direct sunlight	0.25	194	31.5
Ultraviolet lamp (1 foot)	0.5	286	0
100-watt mazda lamp (3 feet)	1	279	1.2

[a] Does not include exposure during concentration of solutions.

ESTIMATION OF CAROTENE OF FRESH PLANT TISSUE

One of the most widely used methods of extracting carotene from fresh plant tissue is that of Moore and Ely (*2*), which con-

Table III. Effect of Temperature and Time of Drying

(Comparison of Moore-Ely extraction procedure with a proposed method for determination of carotene in fresh alfalfa)

Method of Extraction	Drying Temp.	Drying Time	Carotene	Loss by Drying
	° C.	Hours	γ/g. dry wt.	%
Moore-Ely extraction	...	..	374	0
Proposed method	36	22	356	4.8
	50	4	348	7.0
	65	2.5	353	5.6
	65	4	350	6.4
	65	6	332	11.2
	80	2	323	13.6
	80	4	302	19.3
	80	6	299	20.1
	100	1	315	15.8
	100	2	306	18.2

Table IV. Determination of Carotene in Fresh Plant Tissue

Sample	Moore-Ely Extraction	Proposed Method[a]
	γ per gram dry weight	
Alfalfa 1	428	403
Alfalfa 2	440	421
Wheat grass 1	457	501
Wheat grass 2	600	601
Brome grass 1	510	538
Brome grass 2	601	566
Oat grass	404	400

[a] Dried at 65° C. for 4 hours.

sists of comminuting the sample in a Waring Blendor with a mixture of ethanol and petroleum ether. There are certain disadvantages to this method as it now exists. Splashing during comminution may result in some loss of solution. It is also necessary to subject the comminuted solution to a laborious extraction procedure to remove the carotene completely from the ethanol phase. For this reason, the number of determinations that one operator can perform in a day is small.

Mitchell and Hauge (*1*) have reported that an autoclaved sample of alfalfa which was dried in a Despatch oven at temperatures below 33° C. had lost essentially no carotene when analyzed by a method similar to that of Moore and Ely. The experiments described below were performed to show the feasibility of determining the carotene of fresh plant tissue by blanching or autoclaving to inactivate the carotene-destroying enzyme system, drying at low temperatures, and extracting the carotene from the dried residue by the simplified procedure of Silker *et al.*

Samples of whole alfalfa were wrapped in towels, autoclaved for 5 minutes at 5 pounds' pressure in a horizontal steam sterilizer, and dried in a Despatch oven under various conditions of time and temperature. The dried samples were ground to pass through a 20-mesh screen and were analyzed by the method of Silker, Schrenk, and King. Grinding to 20-mesh has been found adequate in this laboratory, although some workers prefer finer grinding (*6*). Ten 5-gram samples of the fresh material were also analyzed by the extraction procedure of Moore and Ely as employed in the method of Wall and Kelley, to serve as a basis for comparison. Ten determinations were made because of the large sampling error that occurs with such a nonuniform material as fresh whole alfalfa.

The data presented in Table III show that both time and temperature of drying affected the amount of carotene that was destroyed. A long drying period at low temperature resulted in less destruction than a short drying period at the higher temperatures. Long drying periods are to be avoided, however, because they increase materially the time required to complete the determination. Drying for 4 hours at 65° C. appears to be optimum, for at higher temperatures the amount of destruction increased rapidly and became appreciable. With some samples, depending upon size of sample and type of plant material, the drying time cannot be reduced much below 4 hours.

Inactivation of the carotene-destroying system of the plant tissue by blanching or autoclaving is an essential operation. Unless this is done, extensive carotene destruction will occur during drying. In conjunction with the experiment reported in Table III, an unblanched sample was also dried at 65° C. for 4 hours. Carotene destruction in this case amounted to 31.5%, compared to 6.4% for the blanched sample.

The carotene content of a variety of fresh plant materials was determined by both the proposed method and the Moore-Ely extraction procedure. Values are presented in Table IV for comparative purposes.

The chief disadvantage of the proposed method is the length of time required to complete the analysis. Because of the 4-hour drying period and the necessity of allowing the sample to stand overnight in contact with the extracting solution, the determination cannot be completed in one day. However, during much of this time the analyst is free to carry on other work, so that the actual amount of time devoted to the determination is comparable with that for other methods—it is estimated that about 80 minutes of actual working time are required to make a single determination by the proposed method, while about 90 minutes are needed by the Wall and Kelley procedure.

The method will be of especial value to those who have a large number of fresh samples requiring attention at a given time and a limited amount of technical help to care for them. After blanching and drying, the samples can be held for several weeks at freezing temperatures without loss of carotene.

The proposed method greatly reduces sampling error (Table V). By other methods the plants are finely chopped and mixed, and because the leaves contain much more carotene than the stems, slight variation in sampling the chopped tissue may result in exceedingly poor checks between duplicates. Furthermore, the determination usually cannot be repeated if the analytical technique is faulty. With the proposed method, on the other hand, much more uniform mixing and sampling of the dried material can be achieved. There will also be a reserve supply of material available if it becomes necessary to repeat the determination because of analytical failures. The slight destruction of carotene that may occur during drying is a small price to pay for the increased precision that is obtained.

Table V. Precision of Moore-Ely Extraction Procedure and Proposed Method

Moore-Ely Extraction			Proposed Method		
Individual analysis	Av.	Maximum variation	Individual analysis	Av.	Maximum variation
γ/g. dry wt.	γ/g. dry wt.	γ	γ/g. dry wt.	γ/g. dry wt.	γ
Alfalfa					
360			376		
360			379		
433			383		
446			383		
457			384		
470	421	110	388	382	12
Oat grass					
371			396		
378			397		
414			400		
452	404	81	405	400	9

LITERATURE CITED

(1) Mitchell, H. L., and Hauge, S. M., *J. Biol. Chem.*, **164**, 543 (1946).
(2) Moore, L. A., and Ely, R., IND. ENG. CHEM., ANAL. ED., **13**, 600 (1941).
(3) Pepkowitz, L. P., *J. Biol. Chem.*, **149**, 465 (1943).
(4) Silker, R. E., Schrenk, W. G., and King, H. H., IND. ENG. CHEM., ANAL. ED., **16**, 513 (1944).
(5) Wall, M. E., and Kelley, E. G., *Ibid.*, **15**, 18 (1943).
(6) Zscheile, F. P., and Whitmore, R. A., *Ibid.*, **19**, 170 (1947).

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Determination of Chlorine Dioxide and Other Active Chlorine Compounds in Water

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Water may be analyzed for free available chlorine, chloramine, chlorine dioxide, and chlorite separately, and expressed as residual available chlorine in the order of fractional parts per million. The method consists of four amperometric titrations with sodium arsenite, from which the concentration of each constituent may be derived readily. Titration of a neutral solution, from which chlorine dioxide has been removed by hydrolysis at high pH, measures only free available chlorine in the absence of iodide, and measures both free available chlorine and chloramine in the presence of iodide. In neutral solution, iodide reduces chlorine dioxide to chlorite quantitatively, and, if not previously removed by hydrolysis, one fifth will be titrated by arsenite along with free available chlorine and chloramine. In acid solution, iodide reduces chlorite quantitatively to chloride, and subsequent titration in neutral solution measures not only the chlorite but also the chlorine dioxide already reduced to chlorite, plus the free available chlorine and chloramines reduced to chloride by iodide prior to acidification. Thus, the total oxidizing capacity of these compounds is measured. Temperatures as low as 0° C. do not affect results of titrations, but do retard the rate of hydrolysis of chlorine dioxide. Neither ferric nor chlorate ions interfere. Nitrites and manganese dioxide appear only as chlorite.

THE increasing use of chlorine dioxide in conjunction with chlorine in water treatment makes it possible for chlorine residuals to be present as free chlorine, hypochlorous acid, hypochlorites, the chloramines, and chlorine dioxide, along with chlorites and chlorates as secondary products. A method for the determination of each of these components individually is not to be found in the literature but would be of considerable importance in water treatment control and in studies of the changes that each component undergoes under various conditions.

Chloramine does not have the same bactericidal effectiveness as free available chlorine. Recent studies by Ridenour and Ingols (*5*) on the bactericidal properties of chlorine dioxide, indicate that the comparative effectiveness of chlorine and chlorine dioxide as bactericides varies with the type of contaminant and the alkalinity of the water, and show chlorine dioxide to be at least as effective as chlorine. Thus, with increasing interest in the use of chlorine dioxide as a bactericide, a rapid and simple method for distinguishing between these various chlorine compounds in the water system takes on added importance.

In choosing a method of analysis, care must be taken that the acidity imposed by the method does not inadvertently alter the constituents, but permits deliberate manipulation of pH for purposes of differentiation. For example, if the method required a high pH, chlorine dioxide would hydrolyze to chlorite and chlorate; if a low pH, chlorite would liberate chlorine dioxide. Reducing substances in the water, which are not attacked by chlorite under neutral or alkaline conditions, may reduce it in sufficiently acid solution. Reducing substances in the water, which are not attacked by free available chlorine, chloramine, or chlorine dioxide under the existing pH conditions, may reduce them to some extent in a sufficiently basic solution. In any case, if pH far from neutral were required, it is to be presumed that the constituents would be changed before measurement was possible. This restriction was recognized for the simple chlorine-chloramine system by Scott (*6*), Griffin (*1*), Hopkins (*2*), and others. It is even more imperative when the system is expanded to include chlorine dioxide and chlorite.

The amperometric titration of chlorine or iodine with standard arsenite solution as developed by Washburn (*8*), Treadwell (*7*), Kolthoff (*3*), and others offers a dependable method of measurement which may be performed at neutrality. It is particularly attractive because it is an absolute method, rather than dependent on uncertain standards. The electrical instrument, being merely an end-point indicator, introduces no variable and requires no calibration. As applied by Marks and Glass (*4*), elementary chlorine, plus hypochlorous acid and hypochlorites, is titrated by arsenite at pH 7 in the presence of chloramine without interference. Free iodine is also titrated under the same conditions, and, if the sample is first treated with potassium iodide, not only does the chlorine liberate an equivalent amount of iodine, but the chloramine also liberates iodine quantitatively and irreversibly. If the sample is now titrated, both free available chlorine and chloramine are titrated as equivalent iodine. With samples of water in which the available chlorine is known to be strictly limited to these two constituents, the method is excellent, but if chlorine dioxide is present, it cannot be distinguished from chloramine.

The system of analysis presented here measures each of four constituents: free available chlorine, to be understood as including chlorine, hypochlorous acid, and hypochlorites; chloramine, including any products of the reaction between chlorine and ammonia; chlorine dioxide; and chlorite, including chlorous acid as they exist in the sample of water when analyzed, and without interference from ferric or chlorate ions, turbidity, or color. Only in the case of the analysis for chlorites do nitrites and manganese interfere. All results are given in parts per million of chlorine equivalent to the total oxidizing capacity of the constituents, and are reproducible to 0.01 p.p.m. Complete analysis requires 20 minutes. The analytical system is here applied only to the low concentrations of chlorine compounds found in potable water, but with modifications is applicable to higher ranges and to the determination of nitrite and manganese. Work is under way in this laboratory to extend the method to higher concentrations for bleach liquors and similar applications and to analyses for other substances. Although chlorite in water is usually not of interest in water control, it is included in this system of analysis in order that the chemical changes which chlorine dioxide undergoes may be followed.

APPARATUS

The measuring element consisted of a porous clay tube 6 mm. in outside diameter, closed with fused glass at one end, and 30 mm. long, on the outside of which 65 cm. of No. 26 B. & S. plati-

num wire were wound to constitute the sensitive electrode. Within the porous tube a silver wire immersed in saturated sodium chloride solution constituted the reference electrode. The electrodes were directly connected to a microammeter, range 0 to 20 microamperes. A glass tubular extension fused to the open end of the porous tube provided a reservoir for the chloride solution and mechanical support for the measuring element. The level of chloride solution in the reservoir was kept always higher than that of the sample to reduce contamination by inward diffusion.

In many amperometric titrations it has been customary to supply a small polarization current from an external source and also to take advantage of such an arrangement to set the meter reading conveniently. In the present work the measuring element, as constructed, functioned adequately as a primary cell and required no external current supply. In most of the titrations, only the end point was of interest, and no provision for setting the meter reading was necessary. Elimination of the auxiliary source of current provided more constant sensitivity.

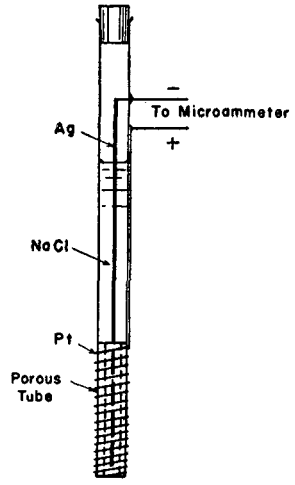

Figure 1. Electrode Assembly

Thorough stirring of the sample during titration is essential and it is advisable to prevent mixing in of air by cavitation. For these reasons the titration vessel was a square jar, made by cutting off a pint bottle, and fitted with a loose plastic cover. A small motor was mounted on the cover with an impeller shaft extending down into the liquid at the corner, and rotating so as to draw the liquid upward. The measuring element was mounted through the plastic cover in the corner opposite the stirrer. A hole in the cover permitted insertion of a delivery tube from a pipet, and the whole cover assembly could be readily transferred from one jar to another as required.

The measuring buret was made from a 1-ml. pipet graduated in 0.01 ml. and connected to a supply bottle and delivery tube by 2-mm. plastic tubing. In titrating, the tip of the delivery tube was placed in contact with the surface to permit smooth delivery, and the tip was drawn small to reduce diffusion. Glass pearls within the tubing served as satisfactory valves.

The amperometric titrator as supplied by Wallace & Tiernan may be used.

REAGENTS

The reagent solutions were made at sufficiently high concentrations to keep the amount of water introduced with the necessary quantity of reagent as small as possible, in order to minimize errors introduced by demand of the water used to dissolve the reagents.

As an added precaution against impure reagents, water which has been conditioned as described below may be used to prepare the reagent solutions excepting the arsenite. Then after standing several days they may be dechlorinated carefully to 0.01 p.p.m. of chlorine residual with sulfite, using the amperometric indicator. The neutral buffer should be dechlorinated first. Then the acid and acid buffer follow, using the neutral buffer on samples to adjust the pH. Finally the caustic solution is dechlorinated, and samples are neutralized with acid buffer.

Alternatively, dechlorination with sulfite may be followed by the aid of an outside indicator such as *o*-tolidine.

Neutral buffer, 100 grams of sodium dihydrogen phosphate monohydrate plus 400 grams of disodium hydrogen phosphate dodecahydrate, dissolved in distilled water and diluted to 1 liter.

Acid buffer, 750 grams of sodium dihydrogen phosphate monohydrate dissolved in distilled water and diluted to 1 liter.

Caustic solution, 10% sodium hydroxide in distilled water.

Acid solution, 6 *N* sulfuric acid.

Potassium iodide, U.S.P. crystalline.

Indicator paper for pH ranges at 2, 7, and 11.

Standard Arsenite Solution. C.P. sodium metaarsenite, $NaAsO_2$, Eimer and Amend, was dissolved in water to make a solution approximately 0.1 *N*, which was then standardized against standard iodine solution. The calculated amount was then diluted to 1 liter to produce a 0.00564 *N* arsenite titrating solution, each milliliter of which added to 200 ml. of sample is equivalent to 1 p.p.m. of available chlorine. A small amount of chloroform stabilized the arsenite solution.

The following test solutions were prepared and used in the experimental determinations.

Conditioned Water. To a large supply of distilled water which was known to have a small chlorine demand, a small amount of chlorine stock solution was added. The residual chlorine was titrated from time to time and held in slight excess by further addition of chlorine solution as required. After several days at room temperature, the small chlorine excess became virtually constant at a low value, and this water was used to make up the test solutions. This stabilized water could be used in comparative determinations and was less troublesome to prepare than chlorine-free and ammonia-free water.

Chlorine Stock Solution. Chlorine gas was led into distilled water to a concentration of about 6 grams per liter, 20 ml. of which were diluted to 2.5 liters for use.

Chloramine Stock Solution. A solution of ammonium hydroxide containing approximately 20 p.p.m. was prepared and then diluted tenfold. To the diluted solution sufficient chlorine stock solution was added to form the desired concentration of chloramine. Ammonia was always present in excess. These chloramine solutions were stored in the dark. They showed some instability, especially in the higher chloramine concentrations, and a loss of 0.01 p.p.m. was sometimes noticed at a concentration of 0.60 p.p.m. in 2 hours at 30° C.

Chlorine Dioxide Stock Solution. Chlorine dioxide gas, made by reduction of sodium chlorate by sulfur dioxide in strongly acid solution and containing less than 1% of the residual parts per million as free chlorine, was absorbed in water to a concentration of approximately 2 grams per liter, diluted tenfold to give a stock solution containing approximately 0.2 gram per liter, and stored in black bottles at 7° C. No deterioration was noticeable after standing 4 days. Chlorite solutions were made from sodium chlorite, analytical grade.

METHODS OF ANALYSIS

Separate amperometric titrations were made on each of four samples of water under test. The samples were prepared under different conditions, and were titrated with standard arsenite. As the active compounds were reduced, the meter reading decreased until the end point was marked by no further decrease. A standard sample of 200 ml. was found satisfactory for each titration.

In making a titration, arsenite was added from a buret if necessary to bring the meter needle on scale. Then a graph of meter reading against volume of arsenite added was drawn, which always resulted in a straight line, provided sufficient time was allowed between additions, particularly near the end point. With care, two points were sufficient to locate the line. Then excess arsenite was added to locate the end point on the line. An alternate method was to add equal small increments of arsenite until the last increment produced no further change, but this method was somewhat slower and the precision was limited to the value of the increment.

Titration I. Free Available Chlorine, Including Hypochlorite and Hypochlorous Acid. Sufficient 10% sodium hydroxide was added to a sample to bring it to at least pH 11, as tested by a pH meter or by Hydrion paper, if the sample was at 25° C., or pH 12 if at 0° C. The sample was allowed to stand for 5 minutes if at 25° and for 10 minutes if at 0° C. It was then brought to approximately pH 7 by the addition of a small amount of the acid buffer solution as checked by meter or paper, and was titrated by standard arsenite solution.

Under these conditions the content of free available chlorine is measured without interference from chloramine, chlorine dioxide, or chlorite which may have been originally present. The addition of alkali causes the conversion of any chlorine dioxide in the original sample to chlorite and chlorate according to the equation:

$$2ClO_2 + 2NaOH = NaClO_2 + NaClO_3 + H_2O$$

the products of which do not interfere when the pH is subsequently brought to 7 and titrated. Larger amounts of alkali than necessary to bring the pH to 11 may be added without harm, and serve to decrease the time required for hydrolysis especially at lower temperatures, but do increase the amount of acid buffer subsequently required to bring the pH to 7. Prepared solutions which contained known amounts of free available chlorine together with known additional amounts of chloramine, chlorine dioxide, and chlorite, when thus treated, showed on titration only the free available chlorine content.

Titration II. Free Available Chlorine Plus Chloramine. A second sample was made alkaline, allowed to stand about 5 minutes, and brought to pH 7 as in Titration I. Approximately 1.5 grams of crystalline potassium iodide were added, in large excess to ensure rapid completion of reaction, and the solution was then titrated. Under these conditions chlorine dioxide originally present was converted into chlorite and chlorate. Chloramine as well as chlorine liberated iodine, and on titration both were measured. Solutions containing known amounts of chloramine have been supplemented by the addition of chlorine, chlorine dioxide, and chlorite, and titrations of such solutions following the procedure described have consistently yielded accurate values for the content of free available chlorine plus chloramine. The difference between Titrations I and II measured the residual chlorine which was present as chloramine.

Titration III. Free Available Chlorine, Chloramine, and One-Fifth Chlorine Dioxide. With the third sample, the alkaline treatment was omitted, and the solution brought to pH 7 by neutral buffer. Potassium iodide was then added, and the solution was titrated. Iodine was liberated by the free available chlorine and chloramine and also by the chlorine dioxide. The latter was reduced to chlorite under these conditions with a valence change of 1, instead of being completely reduced to chloride with a valence change of 5, and only one fifth of the total oxidizing capacity was thus titrated. Five times the difference between Titrations II and III measured the chlorine dioxide.

Titration IV. Free Available Chlorine, Chloramine, and Chlorine Dioxide plus Chlorite. Potassium iodide was added to the fourth sample of water to be analyzed, mixed thoroughly, acidified with 6 *N* sulfuric acid to a pH of 2, and allowed to stand for 5 minutes. The pH of the solution was then brought to 7 by the addition of neutral buffer solution and titrated. Under these conditions the whole oxidizing capacity of free available chlorine, chloramine, chlorine dioxide, and chlorite was measured, all being reduced to chloride with the liberation of iodine.

ANALYTICAL PROCEDURE

For speed and efficiency the procedure has been reduced to the following steps. Reagents are added in the order given. None of the reagent quantities is critical. Changes in pH should be checked with indicator paper. The quantities are given for water at or near room temperature.

1. Measure four 200-ml. samples of water into jars marked I, II, III, and IV, respectively.
2. Add 10% sodium hydroxide to I and II to bring pH to 11 to 12 (about 3 drops).
3. Add neutral buffer to III to bring pH to 7 (1 ml.).
4. Add potassium iodide crystals to III and IV (1.5 grams).
5. Add acid solution to IV to bring pH to 2 (5 drops).
6. Titrate sample III. Result = C ml. of arsenite.
7. Add acid buffer to I and II to pH 7 (1 ml.).
8. Add 1.5 grams of potassium iodide crystals to II.
9. Titrate sample I. Result = A ml. of arsenite.
10. Titrate sample II. Result = B ml. of arsenite.
11. Add neutral buffer to IV to pH 7 (4 ml.).
12. Titrate sample IV. Result = D ml. of arsenite.
13. Cl_2 = A; chloramine = (B − A); chlorine dioxide = 5(C − B); chlorite = 4B − 5C + D.

EXPERIMENTAL

The following statements have been confirmed by experimental evidence. In only one case which unavoidably involved the gradual decomposition of chloramine, an error of 0.016 p.p.m. was observed. In the rest the error was less than 0.010 p.p.m.

1. Free available chlorine, including hypochlorites, is measured at pH 7 by titration with standard arsenite. Chlorine dioxide interferes with this titration but may be removed by preliminary basic treatment without altering the titration. Neither chlorites, chlorates, iodides, nor preliminary acid treatment alter the titration for chlorine. Excess ammonia converts the chlorine to chloramine and reduces this titration to zero.

2. After treatment with iodide, chloramine is measured at pH 7 by titration with arsenite. This titration is not altered by preliminary acid or basic treatment or the presence of chlorite or chlorates. Excess chlorine reacts with chloramine to give unstable solutions.

3. Chlorine dioxide is reduced by potassium iodide to chlorite at pH 7, and to chloride at pH 2. This is not altered by the presence of free available chlorine, chloramine, or chlorates.

4. If A, B, C, and D represent the titration quantities of standard arsenite required in Titrations I, II, III, and IV, respectively, the following relationships were experimentally confirmed with solutions containing known constituents:

Constituents Present	Relationships
Cl_2	A = B = C = D
NH_2Cl	A = 0; B = C = D
Cl_2 + NH_2Cl	A ≠ 0; B = C = D
NH_2Cl + ClO_2	A = 0; 5C − 4B = D
Cl_2 + NH_2Cl + ClO_2	A ≠ 0; 5C − 4B = D

5. The method of analysis yields additive results for each constituent independently. Three stock solutions consisting chiefly of chlorine, chlorine dioxide, and chlorite, respectively, were prepared. One milliliter of each was diluted to 250 ml. and analyzed as follows:

	P.p.m. of Available Chlorine		
	No. 1	No. 2	No. 3
Cl_2	0.265	0.000	0.000
NH_2Cl	0.093	0.012	0.000
ClO_2	0.000	0.220	0.000
$NaClO_2$	0.000	0.061	0.387

One milliliter of stock solution No. 1 plus 1 ml. of No. 2 were mixed together, diluted to 250 ml., and analyzed. The same procedure was followed also for the pairs Nos. 1 plus 3, and Nos. 2 plus 3. Finally a single dilution of 1 ml. of each of all three was mixed, diluted to 250 ml., and analyzed. If no changes occurred on mixing, and if each constituent were determined independently, the resulting analyses of the mixed solutions should have equaled the sum of the component analyses.

Mixture	1 and 2		1 and 3		2 and 3		1 and 2 and 3	
	Calcd.	Obs.	Calcd.	Obs.	Calcd.	Obs.	Calcd.	Obs.
Cl_2	0.265	0.274	0.265	0.270	0.000	0.000	0.265	0.280
$ClNH_2$	0.105	0.100	0.093	0.094	0.012	0.011	0.105	0.098
ClO_2	0.220	0.230	0.000	0.000	0.220	0.215	0.220	0.220
$NaClO_2$	0.061	0.058	0.387	0.383	0.448	0.446	0.448	0.454

6. Ferric ions do not interfere with the analysis. A chlorinated sample of water was divided into two parts. To one part ferric chloride solution was added to make 0.67 p.p.m. of iron. The two parts analyzed:

	Sample 1 without $FeCl_3$	Sample 2 with $FeCl_3$
	P.p.m.	*P.p.m.*
Cl_2	0.690	0.684
$ClNH_2$	0.046	0.048
ClO_2	0.010	0.007
$NaClO_2$	0.000	0.000

7. Sodium nitrite is titrated as if it were chlorite, and it does not otherwise interfere. A sample of water was divided into two parts: 2.0 p.p.m. of sodium nitrite were added to one part and both were analyzed. The part without nitrite gave zero for Titrations I, II, III, and IV. The part containing nitrite gave zero for Titrations I, II, and III. Titration IV showed 0.17 p.p.m. of residual chlorine apparently as chlorite.

8. Manganese dioxide is measured as if it were chlorite, and it does not otherwise interfere. A sample of water was divided into two parts. One part was treated with a colloidal solution of manganese dioxide so that it contained approximately 0.9 p.p.m. The colloidal manganese dioxide solution was prepared as follows: Potassium permanganate in 0.001 *N* solution was brought to pH 11 by the addition of 10% sodium hydroxide. Then it was just reduced by adding sodium metaarsenite solution, followed amperometrically. Both water samples were analyzed. Comparison showed no effect on Titrations I, II, or III, and that it was measured in Titration IV.

	Without MnO_2	With MnO_2
A	0.040	0.047
B	0.222	0.230
C	0.247	0.248
D	0.566	1.206

9. The temperature of the sample does not affect the result of the titration. A sample of chlorine solution buffered at pH 7 and containing potassium iodide was divided into two parts and titrated at 0° and 23° C., respectively. Both titrations showed 0.240 p.p.m. Both the actual microamperes at corresponding points and the gradient microamperes per milliliter of arsenite were much lower at the lower temperature, indicating lowered sensitivity, but the amount of arsenite required was the same in both cases.

10. The rate of hydrolysis of chlorine dioxide decreases with temperature. To reach completion of hydrolysis at 0° C. requires at least 10 minutes at pH 12. A solution which contained approximately 1.3 p.p.m. of chlorine dioxide and 0.34 p.p.m. of chloramine was prepared. One part was completely hydrolyzed at 25° C. pH 12.1, and on titration showed 0.337 p.p.m. of chloramine and no chlorine dioxide. A second and third sample were hydrolyzed at 0° C. and pH 12, 1 for 5 and 10 minutes, respectively, then neutralized and titrated for chloramine and residual chlorine dioxide. After hydrolyzing for 5 minutes the sample still contained 0.029 p.p.m. of chlorine dioxide. After hydrolyzing for 10 minutes 0.009 p.p.m. of chlorine dioxide remained, which is just within the accuracy of the method. For Titrations I and II at 0° C. at least 10 minutes and preferably longer should be allowed for hydrolysis.

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LITERATURE CITED

(1) Griffin, A. E., *J. Am. Water Works Assoc.*, **27**, 888 (1935).
(2) Hopkins, E. S., *Ind. Eng. Chem.*, **19**, 774 (1927).
(3) Kolthoff, I. M., and Pan, Yu-Djai, *J. Am. Chem. Soc.*, **61**, 3402 (1939).
(4) Marks, H. C., and Glass, J. R., *J. Am. Water Works Assoc.*, **34**, 1227 (1942).
(5) Ridenour, G. M., and Ingols, R. S., *Ibid.*, **39**, 561 (1947).
(6) Scott, R. D., *Ibid.*, **26**, 634 (1934).
(7) Treadwell, W. D., *Helv. Chim. Acta*, **4**, 396 (1921).
(8) Washburn, E. W., *J. Am. Chem. Soc.*, **30**, 31 (1908).

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Estimation of Hemicelluloses in Holocellulose from Nonwoody Plant Material

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A method is described for the determination of hemicelluloses from holocellulose of nonwoody plant material, based on quick extraction of hemicelluloses with an alkaline solution that is completely oxidized by an acid chromate solution. The reduced chromate is a measure of the organic matter, which is composed principally of hemicelluloses.

THE determination of hemicelluloses is made infrequently, despite the fact that they are probably present in all plants and in relatively large quantities in cereal straws. The hemicelluloses usually recognized are of two general types. The incrusting type consists of pentosans, hexosans, mixtures of these two, and polyuronides. The other type, referred to as cellulosans, are believed to be constituents of the cellulose pattern and do not contain uronic acid groups (*5*). The former group is the more readily soluble and probably corresponds most nearly to the β- and γ-cellulose of industry. The latter may be relatively insoluble. A portion of this fraction together with the cellulose corresponds approximately to the α-cellulose of industry.

The hemicelluloses determined by the present method are believed to be chiefly of the polyuronide type. Because some cellulosans as well as pentosans and hexosans may be extracted along with polyuronide hemicelluloses, the use of recognized terminology is abandoned and the whole fraction extracted is referred to as hemicelluloses, although the fraction extracted under the conditions prescribed is essentially similar to the β- and γ-cellulose fraction. It is felt that such a differentiation, while empirical, has certain advantages.

The methods ordinarily used for the estimation of hemicelluloses are based on the determination of the reducing values of the sugars formed during hydrolysis, the determination of furfural, and direct isolations (*3*, *7*, *9*). The older methods of isolations are reasonably specific but are rather laborious for routine procedures. A critical discussion of various procedures has been offered by Norman (*6*).

Essentially the present method is a modification of Launer's procedure for β- and γ-cellulose in pulps and papers (*4*). The variations are designed to shorten the time of operation and to include certain desirable features of other procedures (*1*). Holocellulose, freed of pectic compounds, is extracted with aqueous alkali and the resulting solution is oxidized completely with an acid chromate solution. The reduced chromate serves as an index of the organic matter present and is determined spectrophotometrically. Holocellulose from nonwoody plant tissue ordinarily contains some lignin, from 30 to 50% of the original percentage content of nitrogen, and substantially all the furfural-yielding substances (*2*).

PREPARATION OF HOLOCELLULOSE

The materials used in this work were beet pulp, citrus pulp, cranberry pulp, cornstalks, oat straw, and mixed hay. All samples were ground to pass a 25-mesh screen but were retained by a 50-mesh screen. Five holocellulose determinations were made on a 3-gram sample (*2*) of each type of tissue. Samples were first extracted with a solution of alcohol-benzene (1 to 2) for 6 to 8 hours, then twice with 0.5% ammonium citrate at 80° C., over a period of 24 hours; the first extraction period was for 4 to 6 hours. Samples were then subjected to the sodium chlorite–acetic acid treatment. The holocellulose produced was filtered through poplin in the usual manner and allowed to dry at room temperature before removal from the cloth. Hemicelluloses were then extracted from this material.

ESTIMATION OF HEMICELLULOSES

Preliminary extractions of holocellulose from cornstalks, using solutions of sodium hydroxide varying in concentration from 0 to 24%, indicated that a 12% solution, specific gravity 1.1309 at 20° C. or approximately 3.39 *N*, was most effective. The solution which contained the greatest amount of organic matter was considered the most effective.

In order to determine the extent of the removal of pentose

Table I. Hemicellulose as Anhydroglucose in Plant Tissues on an Ash- and Moisture-Free Basis

Trials	Beet Pulp %	Citrus Pulp %	Cranberry Pulp %	Corn-stalks %	Oat Hay %	Mixed Hay %
1	5.4	9.1	12.9	..	27.3	23.5
	5.8	9.1	12.9	26.6	26.3	23.2
	5.6	...	..	..	26.5	23.2
2	5.2	9.6	12.8	26.3	28.9	24.3
	5.2	8.9	13.4	26.6	28.0	23.4
	6.0	...	..	..	..	22.9
3	6.1	9.3	13.4	27.9	27.9	24.4
	5.4	9.3	13.4	27.9	..	..
4	6.2	8.8	13.1	25.7	26.4	22.4
	6.5	9.1	..	25.2	26.2	22.4
	...	8.7	..	..	26.4	..
5	6.8	9.3	13.2	26.7	28.7	22.5
	6.6	9.3	13.5	25.9	28.4	22.5
Av.[a]	5.9±0.8	9.2±0.5	13.1±0.4	26.5±1.4	27.5±1.4	23.1±1.0

[a] Weight basis.

units from the holocellulose by the above treatment, a furfural determination was made on an aliquot of the alkaline extract. In the case of cornstalks, over 85% of the furfural-yielding substances in the holocellulose were removed. More drastic means are usually necessary to remove so large a fraction (*5*).

A 0.2-gram sample of air-dried holocellulose was treated with 20 ml. of 12% sodium hydroxide in a 100-ml. volumetric flask and placed in a constant-temperature bath at 20° C. for 10 minutes. An equal volume of water at the same temperature was then added and the contents were agitated. The flask was left in the bath for a total of 90 minutes, the contents being agitated once more at about the half-way period. The contents were then diluted to volume, thoroughly mixed, and filtered by suction through a plug of glass wool jammed into the stem of a funnel just below the apex. Twenty-five milliliters of the filtrate were transferred to a 300-ml. Erlenmeyer flask together with 5 ml. of distilled water, 30 ml. of concentrated sulfuric acid, and 5 ml. of 1 *N* potassium dichromate. The solution was boiled gently for 2 minutes at 140° to 150° C. The passage of a slow current of air through the solution facilitated boiling considerably. After quick cooling under a tap, the transmittance of the reduced chromate was measured in a Coleman Universal spectrophotometer. This instrument operates on a spectral band of 35 millimicrons; the cuvettes were approximately 13 mm. thick. A wave length of 600 millimicrons and a PC-4 filter were used. Distilled water was used as the reference solution.

The yield of holocellulose must be recorded if the percentage content of hemicellulose is to be reported on the original basis. Results are reported on an ash-, moisture-, and nitrogen-free basis. The nitrogen is converted to protein by use of the factor 6.25. As the alkaline extraction procedure removed about 80 to 100% of the nitrogen of the holocellulose, it is believed to be in the form of protein. Samples bearing small percentages of nitrogen in the original material, such as wood, probably would not retain a sufficient amount of nitrogen to be significant. The extracts used in this work contained up to about 12 mg. as protein. Trial tests using casein to determine the oxidation values of proteinaceous materials indicated that 1 mg. is equivalent to about 0.94 mg. of glucose.

STANDARDIZATION OF REFERENCE CURVE

A curve was established which related the reduced chromate to glucose. Although pentose sugars are usually predominant in hemicelluloses, a reference curve can be made using the cheaper and more easily available glucose because the amount of reduced chromate produced by equal amounts of glucose and xylose under these conditions is not significantly different.

Data were obtained by taking the average of five or six determinations on each of eight solutions of glucose (expressed as anhydroglucose) that varied in concentration from 0 to 140 mg. per 100 ml., and oxidizing 25-ml. aliquots of these solutions in the manner described above. Percentage transmission values obtained were then plotted against milligrams of anhydroglucose per 100 ml. There is a slight tendency for the data for the last two concentrations to deviate from Beer's law, according to which one should obtain a straight-line relationship. The tendency, however, does not seem to be consistent or very significant; hence a straight line was drawn which satisfied all points. As the sugars in the hemicellulose are in the anhydro form, the concentrations of glucose were recorded as anhydroglucose.

Table I shows that, in general, agreement within any one set of determinations is better than that between sets of duplicates or triplicates. This is due to the fact that in each trial a new sample of holocellulose was prepared. The control of the latter procedure is not so good as the subsequent procedures that involve extractions with alkali and oxidation with chromic acid. The results indicate about as wide deviations as are likely to occur. It is essential that the holocellulose determinations be made under very definite conditions as regards time of digestion and temperature. More precise results can also be obtained if filtrations are made through crucibles, rather than cloth, although the latter facilitates filtering considerably.

DISCUSSION

In relatively small quantities starch was found to be removed from plant tissue by sodium chlorite. Starch treated with sodium chlorite as in the regular tests was not destroyed, as evidenced by the reaction with iodine. A recent patent covers the solubilizing action of sodium chlorite on starch (*8*).

Pectic materials are not completely removed by treatment with sodium chlorite. Treatment of commercial pectin with chlorite did not result in a destruction sufficiently great to prevent the formation of gels with calcium salts. In some instances incomplete removal of pectic substances may result in a slight enhancement of the percentage content of the hemicelluloses. This statement becomes more significant when one considers that citrus pulp yields about 9% of furfural, about 49% of which may be credited to pectic substances. The yield of furfural from cornstalks is practically unaffected by the use of ammonium citrate. The extensive removal of pectic substances from beet pulp and citrus pulp followed by a subsequent treatment with chlorite produces a residue which to a great extent has lost the characteristics of the particle and has acquired a somewhat sticky nature. The fibers from the cereal straws, being low in pectic materials, do not appear to have been altered.

It was found expedient to filter the residues from the hemicellulose extracts after dilution to volume. There was no significant difference in the reducing value of the filtrates whether filtered before or after dilution to volume. Solutions diluted to volume and allowed to stand before filtration did not change significantly in reducing value until after about 4 hours.

No correction in oxidizing values was made for the presence of uronic acids or for differences in uronic acid content of the hemicelluloses of different plants.

The manipulations described above are believed to make it possible to cut the time of operation down to about one fourth of the time used for the older gravimetric methods. If uniform conditions are maintained, the reproducibility of the method is good.

LITERATURE CITED

(1) Barton, C. J., and Prutton, A. J., IND. ENG. CHEM., ANAL. ED., **16**, 429 (1944).
(2) Bennett, Emmett, *Ibid.*, **19**, 215 (1947).
(3) Buston, H. W., *Biochem. J.*, **28**, 1028 (1934).
(4) Launer, H. F., *Bur. Standards J. Research*, **18**, 333 (1937).
(5) Norman, A. G., *Biochem. J.*, **30**, 2055 (1936).
(6) Norman, A. G., "Biochemistry of Cellulose Polyuronides, Lignin, etc.," London, Oxford University Press, 1937.
(7) Preece, I. A., *Biochem. J.*, **25**, 1304 (1931).
(8) Vincent, G. P., U. S. Patent 2,409,085 (1947).
(9) Weike, H. D., and Phillips, Max, *J. Agr. Research*, **74**, 77 (1947).

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Analysis of Liquid Phosgene

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A satisfactory and precise method is given for the analysis of high-purity liquid phosgene suitable for munitions and chemical manufacture. The cold liquid is taken up in a thin glass bulb and released by breaking the bulb in a stoppered flask containing specially treated anhydrous sodium iodide and anhydrous acetone. The released iodine is titrated with thiosulfate and the free acid is determined by titration with sodium hydroxide. Separate samples are broken similarly in cold water or aqueous potassium iodide for determination of free chlorine. Nonvolatile residue is determined by evaporation, and ferric chloride in the residue is determined iodometrically. Data are presented to show the precision of the determination, the effect of variations of the iodometric method, and the effect of high acidity on side reactions and accuracy.

NO METHOD appears to have been reported in the literature for the analysis of liquid phosgene. Methods (*1*, *2*, *3*) were developed for the determination of relatively low concentrations of gaseous phosgene rather than for the assay of high-purity liquid phosgene.

Current U. S. Army specifications for phosgene (*6*) cover two grades: Grade A, phosgene suitable for use in munitions, and Grade B, phosgene suitable for use in chemical manufacture. The minimum purity is 98%; acidity calculated as hydrochloric acid, 0.50% maximum; residue on evaporation, 0.50% maximum; free chlorine for Grade A, 1.0% maximum; for Grade B, 0.05% maximum.

It was not possible to obtain a correct assay of the liquid by sampling gas from the cylinder, as the vapor pressures of the main impurities, hydrochloric acid and chlorine, are much higher than phosgene.

In the first workable method that was used here (*5*), the cold liquid sample was placed in a bulb, sealed, weighed, and broken in a stoppered flask containing a small excess of 0.1 *N* sodium hydroxide. Excess barium chloride was added and the precipitated carbonate was titrated with 0.1 *N* hydrochloric acid added in excess and, back-titrated with 0.1 *N* sodium hydroxide. Chlorine and hydrochloric acid were determined by volatilizing a liquid sample and allowing it to pass through weighed absorption tubes containing antimony and Michler's ketone, respectively.

Although the method was useful, it was time-consuming and sometimes gave erratic results when used by different analysts. It was evident that a very careful and painstaking technique was necessary.

When large-scale manufacture of phosgene was undertaken during World War II, the available methods for the determination of phosgene were again studied. The iodometric method seemed most promising. The excellent article of Matuszak (*2*) allowed a better understanding of the side reactions occurring and gave ideas on how to proceed. Development of a satisfactory technique for drying and storage overcame objections to the use of sodium iodide, whereupon advantage was taken of the high solubility of this salt in acetone (molecularly about 15 times that of potassium iodide, *4*) to minimize the occurrence of undesired reactions.

In acetone solution sodium iodide reacts with phosgene to release iodine:

$$COCl_2 + 2NaI \longrightarrow CO + I_2 + 2NaCl$$

The released iodine is titrated with sodium thiosulfate. When water is present, some hydrolysis of phosgene occurs:

$$COCl_2 + H_2O \longrightarrow CO_2 + 2HCl$$

Treatment of the sodium iodide to remove alkali and moisture and the use of great excess in anhydrous acetone led to the procedure given below for high-purity liquid phosgene. The procedure was used by many laboratories throughout this country during the war, with highly satisfactory results. Directions should be followed rather meticulously, because variations in technique have usually resulted in the presence of moisture with attendant side reactions, low phosgene results, and high acidity.

APPARATUS

Erlenmeyer flasks, 500-ml. heavy-walled, glass-stoppered. Most flasks require regrinding with 300-mesh Carborundum powder. Test for leaks by placing 25 ml. of acetone in the flasks and heating to boiling, so that the vapors fill the flask. Grease the warm stopper with petroleum jelly. Stopper the flask and dip into ice water with the stopper above the water. A leak can be observed as a striation or bubbling in the grease.

Erlenmeyer flasks, 750-ml. heavy-walled, tested as above. Sampling apparatus as diagramed in Figure 1. Sampling bulbs, about 12 and 15 mm. in diameter.

REAGENTS

Anhydrous Acetone. Place 2 liters of reagent (A.C.S.) acetone in a brown glass-stoppered bottle. Add 200 grams of 20-mesh Drierite (anhydrous calcium sulfate), and allow to stand 24 hours with occasional shaking during the day. Store in a desiccator of suitable size. (A desiccator fitted with a low-form bell jar as a cover is satisfactory.)

Neutral Anhydrous Sodium Iodide. Place 500 grams of reagent grade sodium iodide in a porcelain evaporating dish, add about 20 ml. of dilute 1 to 2 hydrochloric acid, and stir thoroughly until the sodium iodide is uniformly moist. Place on a hot plate and heat slowly with constant stirring until the crystals are white and dry, but avoid decomposing the sodium iodide by too vigorous heating. Grind to a uniform powder with a mortar and pestle while the reagent is still hot, place in a Pyrex salt bottle, and store in an oven at 100° to 110° C. Test for acidity by adding about 5 grams of the reagent to 50 ml. of boiled distilled water. Add 2 to 3 drops of bromothymol blue–phenol red indicator. The reagent should be slightly acid, as indicated by a yellow-green color. One drop of 0.1 *N* sodium hydroxide should make the solution basic and violet in color. The sodium iodide is stable at 100° to 110° C. for at least several months.

Bromothymol Blue–Phenol Red Indicator. A 0.1% aqueous solution of the sodium salt of bromothymol blue added to an equal quantity of 0.1% aqueous solution of the sodium salt of phenol red.

Neutral, standardized 0.1 *N* and 0.05 *N* sodium thiosulfate. Standardized 0.1 *N* sodium hydroxide. Potassium iodide, reagent grade, iodate-free. Starch indicator. Solid carbon dioxide. Chloroform and carbon tetrachloride, equal parts by volume for the sample bath.

PHOSGENE DETERMINATION

The cylinder containing the phosgene should be previously cooled for at least 2 hours in a bath of ice, salt, and water, then removed and shaken. Place the cylinder, on a support, in an inclined position, so that the liquid phase occupies the lower end of the cylinder. The vapor in the cylinder should not be vented be-

fore withdrawal of the sample, and the temperature of the cylinder should not be allowed to rise above 0° C.

Dry the 50-ml. sampling bottle in an oven at 100° to 110° C. and cool in a desiccator. Place the bottle in the metal carrier and suspend the assembly in a Dewar cylinder previously charged with the chloroform–carbon tetrachloride mixture and cooled to −20° C. or below with dry ice. Discharge about 20 ml. of phosgene from the steel sample cylinder into the bottle.

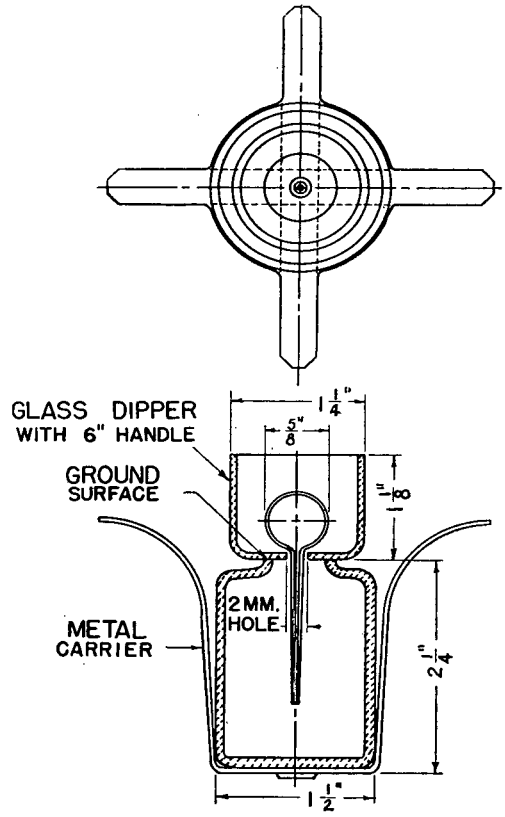

Figure 1. Sampling Apparatus

Swirl the phosgene to wash the inside and discard. Immediately replace the bottle in the bath and fill with phosgene to near the shoulder. Close with the dipper (Figure 1) holding a weighed glass bulb (dried in an oven and cooled in a desiccator before weighing). Place sufficient finely pulverized carbon dioxide ice in the dipper to cover most of the bulb, leaving sufficient surface uncovered to allow observation of the contents of the bulb. When about 0.4 gram of sample has been drawn in, remove the dipper, cover the bulb with more solid carbon dioxide, and hold at about a 45° angle with the stem pointing downward. Expel any visible liquid from the capillary stem by touching the stem with the fingers, or by bringing the stem near a gas flame to warm it slightly. When the liquid leaves the capillary, seal quickly in the flame with the bulb still in the dipper. Allow the bulb to come to room temperature in a desiccator and weigh to the nearest 0.1 mg.

Transfer approximately 5 grams of sodium iodide, still hot from the oven, into a 500-ml. flask that has been dried at 100° to 110° C. and is still warm after removal from the oven. Grease the stopper with pure petroleum jelly. Add approximately 30 ml. of acetone. Place the bulb containing the phosgene in the flask, stopper tightly, and cool in an ice bath to well below room temperature. Wrap the flask with a towel and holding the stopper down tightly shake to break the bulb. Continue to shake for 3 minutes, cool in the ice bath, remove the stopper, and wash down the neck of the flask with a small amount of boiled distilled water.

Titrate with 0.1 N sodium thiosulfate to the disappearance of the iodine color. Use no starch. When near the end point crush the fragments of the glass capillary with a glass rod to release the held iodine. Titrate a prepared blank containing the same amount of sodium iodide and acetone with 0.1 N thiosulfate if the solution is colored, or with 0.1 N iodine solution if the solution is clear. The blank should not exceed 0.1 ml. for properly prepared reagents. Reserve the solutions from the determination and blank for the acidity test.

$$\frac{(A \pm B) \times N \times 4.946}{W} - (C \times 1.396) - D \times 0.304 = \% \text{ phosgene}$$

where A = ml. of standard solution used for determination
B = ml. standard solution used for blank
C = % free chlorine
D = % ferric chloride
N = normality of standard solution
W = grams of sample

These symbols are used throughout subsequent calculations. The correction for ferric chloride is usually negligible.

Acidity. Add 3 to 4 drops of bromothymol blue–phenol red indicator solution to the flasks containing the sample and the blank from the phosgene determination. Titrate the contents of both flasks to the same blue-green color with the minimum amount of agitation. Stopper the flasks and shake. The color should hold for 20 seconds or more.

$$\frac{(A - B) \times N \times 3.65}{W} = \% \text{ acidity as HCl}$$

An alternative method is to add about 0.2 gram of potassium iodate and titrate the released iodine with sodium thiosulfate.

Free Chlorine. Grade A Phosgene. Place a 1.0-gram sample in the bulb as described above and place in a 750-ml. flask containing 100 ml. of cold 3% aqueous potassium iodide solution. Stopper the flask and break the bulb as above. Shake for 3 minutes or more. Cool the flask in the ice bath before removing the stopper and titrate immediately with 0.05 N sodium thiosulfate using starch as an indicator.

Grade B Phosgene. Determine as above, using 200 ml. of cold distilled water instead of the 3% potassium iodide. Add about 5 grams of solid potassium iodide after the stopper is removed and titrate immediately with 0.05 N sodium thiosulfate. (Water is used as the absorbent in order to prevent the slight side reaction of phosgene with potassium iodide, releasing iodine. In order to absorb the chlorine completely in a reasonable length of time, potassium iodide solution is used for Grade A phosgene. The possible alternative reaction of hydriodic acid with atmospheric oxygen releasing iodine was not found to be the cause of high results when potassium iodide solution was used. Using boiled distilled water and expelling the air from the flask with nitrogen resulted in released iodine with chlorine-free phosgene.)

$$\frac{A \times N \times 3.55}{W} = \% \text{ free } Cl_2$$

Nonvolatile Residue. Introduce into a tared Erlenmeyer flask of 50-ml. capacity, with a calibration mark at 35 ml., sufficient phosgene to fill the flask to the mark (50 grams). Allow the phosgene to evaporate in a vacuum desiccator at atmospheric pressure. When the liquid has evaporated apply a vacuum and leave at 200-mm. pressure or less for 0.5 hour. Then admit dried air. Repeat until the odor of phosgene cannot be detected in the flask, remove, and weigh.

$$\frac{R \times 100}{W} = \% \text{ residue}$$

where R = grams of residue
W = grams of sample (50)

Ferric Chloride. Add 20 ml. of water, 4 ml. of 1 to 3 hydrochloric acid, and about 1 gram of potassium iodide to the residue above. Titrate to starch end point with 0.05 N sodium thiosulfate.

$$\frac{A \times N \times 16.22}{W} = \% \text{ } FeCl_3$$

With careful weighing and titration the phosgene determination on specification grade material can be consistently checked with a precision of 5 parts in 10,000, as is shown in Table I.

Hydrochloric acid is present from chlorination of methane, ethane, and hydrogen, which are impurities found in small amounts in the carbon monoxide used, and also from any traces of moisture in the charcoal catalyst or carbon monoxide.

Chlorine is present from excess chlorine in the reaction mixture.

The residue has been examined and found to consist of hexachloroethane from chlorination of ethane, and ferric chloride from corrosion of iron or from iron carbonyls that may be present in the carbon monoxide.

The unaccounted for remainder probably consists of carbon tetrachloride from the methane present in carbon monoxide, and small amounts of dissolved carbon monoxide.

Method 1 is described in this article. Method 2 is essentially the Matuszak method (*2*) which corrected for side reactions that may give low phosgene results and high acidity. It was designed for acid-free phosgene and makes no provision for determining the acidity. In the modified method sodium iodide is used instead of potassium iodide and provision is made for the determination of hydrochloric acid. After the color of the released iodine has been discharged, potassium iodate is added to react with the free acid and the iodine color is discharged for the second time by titration with thiosulfate. This second titer is used in the modified procedure to calculate the acidity as hydrochloric acid after correction has been made for hydriodic acid resulting from the side reaction shown below. (The same amount of thiosulfate is used in the titration of iodine released by hydriodic acid as is consumed in the titration of the iodoacetone.) A measured excess of thiosulfate is then added and allowed to stand for 0.5 hour. The excess thiosulfate is then titrated back with 0.1 *N* iodine to distinct coloration and discharged with thiosulfate. The addition of excess thiosulfate is to correct for iodination of acetone (enol form) according to the equations:

$$CH_3COCH_3 \rightleftharpoons CH_3C(OH):CH_2$$

$$CH_3C(OH):CH_2 + I_2 \rightleftharpoons CH_3CI(OH)CH_2I$$

$$CH_3CI(OH)CH_2I \rightleftharpoons CH_3COCH_2I + HI$$

$$CH_3COCH_2I + Na_2S_2O_3 \longrightarrow CH_3COCH_2NaS_2O_3 + NaI$$

The thiosulfate consumed by the iodoacetone is doubled (phosgene releases 2 equivalents of iodine) and added to the initial thiosulfate titer to calculate phosgene.

$$\frac{(A + 2B) \times N \times 4.946}{W} = \%\ \text{phosgene (uncorrected for } Cl_2 \text{ and } FeCl_3)$$

$$\frac{(C - B) \times N \times 3.65}{W} = \%\ HCl$$

where A = ml. of thiosulfate used for initial titration of released iodine
B = ml. of thiosulfate consumed by iodoacetone
C = ml. of thiosulfate used to titrate I_2 released by KIO_3
N = normality of thiosulfate
W = grams of sample

The close agreement between methods 1 and 2 indicates that side reactions do not occur to any extent if moisture is absent and hydrochloric acid in the sample is within specification requirements. Further indications of the absence of side reactions are the negligible thiosulfate titrations shown in Table II and the fact that low acidic values ranging from 0.02 to 0.04% have been obtained by method 1 on a large number of plant samples containing small unknown amounts of hydrochloric acid which had not been removed.

Method 3 is an attempt to use acetone saturated with potassium iodide in the presence of excess solid potassium iodide rather than sodium iodide. The procedure of method 1 was used. The potassium iodide was thoroughly dried and the same anhydrous acetone used. The failure of potassium iodide as a reagent may be due to lower solubility in acetone. Probably the release of a large amount of phosgene uses up the iodide locally and the side reaction with acetone occurs:

$$COCl_2 + CH_3C(OH):CH_2 \longrightarrow CH_3C(OCOCl):CH_2 + HCl$$

Table III shows an experimental plant sample containing large amounts of hydrochloric acid, made from impure carbon monoxide. When the acidity as free hydrochloric acid approaches 1%, a value far outside the specification limits, the determinations become somewhat erratic, because of side reactions in acid solution, and thus confirm the findings of Matuszak. His modification of the iodometric method is helpful to correct for some of the side reaction products if abnormal phosgene must be analyzed. Reducing the size of the sample appears to be helpful, although the high precision obtainable with normal plant grade material appears to be lost, in any case, with all samples of high acidity. When less than 5 grams of sodium iodide are used, side reactions occur to a greater extent. Although sodium iodide is already present in great excess, the use of about 10 grams of sodium iodide in the usual amount of acetone appears to be helpful in such cases, further confirming previous findings (*2*, *3*) that large excess of iodide suppresses side reactions. Higher acidity than the values of Table III cause still lower phosgene values in all methods given.

Certain grades of phosgene produced abroad contain small amounts of sulfur compounds such as sulfur monochloride, thionyl chloride, and carbon oxysulfide from impure carbon monoxide. These compounds will affect the iodometric titration and other values, and consequently the determinations by the above method may be slightly in error. Phosgene containing large amounts of hydrochloric acid (1% or more) and impurities containing sulfur can best be determined by vaporizing the sample through a train containing absorbents for phosgene and the various impurities. This article does not deal with the analysis of gaseous phosgene.

Table I. Precision of Results Obtained on Specification Grade Phosgene

Sample	Sample Weight	Phosgene	HCl Acidity	Cl_2	Residue	Unaccounted for
	Gram	%	%	%	%	%
A	0.4595	99.08	0.48	nil	0.05	0.39
	0.3213	99.12	0.45	nil	0.04	0.39
	0.1521	99.10	0.47	nil	0.05	0.38
B	0.5040	99.54	0.05	0.29	0.04	0.08
	0.1215	99.59	0.04	0.27	0.05	0.05
	0.3028	99.57	0.05	0.26	0.04	0.08

Table II. Results by Variations of Method

Sample	Method	Sample Weight	Phosgene	HCl Acidity	0.1 *N* Thiosulfate to Correct for Side Reactions
		Gram	%	%	*Ml.*
C	1	0.4500	98.46	0.39	..
	2	0.4844	98.48	0.38	0.0
	2	0.3214	98.44	0.39	0.0
	3	0.4390	92.97	3.9	..
	3	0.2421	94.20	4.8	..
	3	0.1120	90.12	6.1	..

Table III. Effect of High Acidity on Determinations

Sample	Method	Sample Weight	Phosgene	HCl Acidity	0.1 *N* Thiosulfate to Correct for Side Reactions
		Gram	%	%	*Ml.*
D	1	0.4066	98.10	1.13	..
	1	0.3021	97.60	1.52	..
	1	0.5042	97.43	1.16	..
	2	0.5214	98.52	0.94	0.54
	2	0.4885	98.12	1.12	0.42
	1	0.1104	98.81	0.84	..
	1	0.1254	98.60	0.92	..
	2	0.1022	98.61	0.92	0.21
	2	0.1147	98.81	0.80	0.22

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Anon., *Berlin Jahresber. chem.-tech. Reichsanstalt*, 5, 11-20 (1926).
(2) Matuszak, IND. ENG. CHEM., ANAL. ED., 6, 457–9 (1934).
(3) Olsen, Ferguson, Sabetta, and Scheflan, *Ibid.*, 3, 189–91 (1931).
(4) Seidell, "Solubilities of Inorganic and Metal Organic Compounds," Vol. I, pp. 820, 1268, New York, D. Van Nostrand Co., 1940.
(5) U. S. Army Specification 96-21-3, "Phosgene" (Dec. 19, 1928).
(6) *Ibid.*, 96-21-3A (Sept. 20, 1944).

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Spectrophotometric Determination of Diacetyl

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With chromotropic acid in sulfuric acid diacetyl gives a purple dye which is nearly indistinguishable from that obtained with formaldehyde under the same conditions. The formation of this color, which is attributed to the production of a mole of formaldehyde by the sulfuric acid oxidation of a mole of diacetyl, has been utilized as the basis of a rapid spectrophotometric determination of diacetyl.

THE possibility of a spectrophotometric method of analysis for small concentrations of formaldehyde, based on the purple dye formed from this compound and chromotropic acid (4,5-dihydroxynaphthalene-2,7-disulfonic acid), was realized in the procedures developed by MacFayden (*6*) and Bricker (*2*). As shown in these investigations, and by the original qualitative observations of Eegriwe (*3*), this reaction of formaldehyde with chromotropic acid is highly specific. Its analytical application is also subject to few interferences.

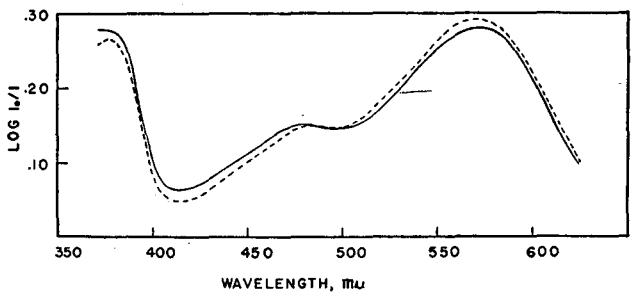

Figure 1. Absorption Characteristics

———— Diacetyl dye (from 1.16 × 10^{-6} *M* diacetyl solution)
— — — — Formaldehyde dye (from 1.13 × 10^{-6} *M* formaldehyde solution)

However, it has been observed in this laboratory that solutions of pure diacetyl give a color with chromotropic acid–sulfuric acid mixture, the absorption characteristics of which are nearly identical with those of the formaldehyde dye, as is shown in Figure 1. Moreover, the molar extinctions of the dyes, as based, respectively, upon moles of diacetyl and formaldehyde, are almost equivalent. These observations, other than implying a source of serious error in the chromotropic acid method applied to certain formaldehyde estimations, have indicated a basis for a rapid spectrophotometric estimation of diacetyl. Accordingly, the analytical possibilities of the diacetyl reaction have been investigated.

Most of the previous methods for determining diacetyl depend upon the formation of the dioxime and its precipitation as the nickelous complex, which is either weighed as such (*1, 5, 10, 13*) or measured colorimetrically in chloroform (*1*) or spectrophotometrically as the nickelic dimethylglyoxime complex (*12*). Diacetyl has also been determined colorimetrically by means of the ferric dimethylglyoxime complex (*9*) and as substituted quinoxalines formed by its reaction with *o*-phenylenediamines (*8*). A polarographic method has been developed by Fulmer *et al.* (*4*).

In a preliminary application of the chromotropic acid–diacetyl reaction to the quantitative estimation of diacetyl, it was found that the procedure described by Bricker for formaldehyde gave excellent results with little alteration, and that the relationship of extinction ($\log I_0/I$) to concentration was nearly linear over the range of 0.01 to 0.1 mg. of diacetyl per milliliter of solution analyzed. It was merely necessary to increase the time of heating in order to achieve maximum extinctions. The development procedure for the formaldehyde dye described by MacFayden, which employs a lower sulfuric acid concentration, gives lower extinction values.

PROCEDURE

To 1.0 ml. of solution containing 0.03 to 0.1 mg. of diacetyl per ml. in a test tube provided with a glass stopper is added 0.5 ml. of 10% chromotropic acid solution, and to this mixture are added gradually 5 ml. of 98% sulfuric acid. After mixing, the tube is heated in a water bath at 100° for 1 hour and cooled, and the contents are transferred to a 50-ml. volumetric flask and diluted to the mark with distilled water. The extinction is then determined at 570 mμ; a blank containing all the reagents and treated in the same manner is employed as the reference solution. The size of the test tube used in the development of the color is not important. However, the level of the liquid in the tube should be below that of the water in the bath throughout the period of heating.

EXPERIMENTAL

A Beckman spectrophotometer (model DU) and 1-cm. Corex cells were used in obtaining all extinction data.

The diacetyl used in these experiments was obtained from Eastman Kodak Co., Paragon Testing Laboratories, and Forest Products Chemical Co. The usual procedure for its purification involved two distillations through a 45-cm. (18-inch) Stedman column, the middle fraction boiling at 87.5–88° being taken each time.

Technical chromotropic acid was obtained from Eastman Kodak Co. and Paragon Testing Laboratories. It was not purified, but the solutions were merely filtered before use.

The reducing sugars which were examined as possible interfering substances were obtained from the Pfanstiehl Chemical Co. and were C.P. grade.

For comparison of the absorption characteristics of the formaldehyde dye with that resulting from diacetyl, a weighed amount of freshly resublimed hexamethylenetetramine was hydrolyzed with 2 *N* sulfuric acid, as recommended by MacFayden (*6*), and the color development carried out according to Bricker's procedure.

The effect of time of development of the color is shown in Table I. This was determined by maintaining the conditions of the above procedure constant with the exception of time of heating in the boiling water bath.

The marked effect of the sulfuric acid concentration, shown in Table II, was determined similarly.

Table I. Effect of Time of Development

Time of Development, Min.	Extinction ($\log I_0/I$) at 570 mμ
15	0.133
30	0.146
60	0.148

Table II. Effect of Sulfuric Acid Concentration

Concentration of Sulfuric Acid Added in Color Development, %	Extinction ($\log I_0/I$) at 570 mμ
98	0.292
80	0.242
60	0.006

For the comparison of this procedure with a standard method, diacetyl was precipitated from solution as the nickelous dimethylglyoxime complex; the precipitation conditions recommended by Wilson (*13*) were employed and the precipitates were weighed. These gravimetric analyses were carried out on 10.0-ml. aliquots from the original diacetyl solutions, which were approximately 2% diacetyl. The spectrophotometric analyses were carried out on solutions prepared by dilution of the original solutions either 1 to 25, 1 to 50, or 1 to 100. The values given in Table III correspond to the concentrations of the more dilute solutions. Those determinations designated by the subscript refer to analyses which were repeated after the original solutions had stood at room temperature for one week. Obviously, the present method gives much better agreement with the values obtained with freshly prepared solutions than does the precipitation procedure. This, probably, is due to a nonprecipitation of polymerized diacetyl as the nickelous complex, whereas some of the polymerized material is attacked by the chromotropic acid–sulfuric acid mixture. The proposed method should give high results, therefore, in cases where some polymerization of the diacetyl has occurred.

Although the data in Table III indicate that the precision of the method is somewhat outside the limits imposed by the spectrophotometer, the reproducibility of results obtained in the determination of the relationship of extinction to concentration was nearly that to be expected of the instrument over the range of extinctions covered. This precision is demonstrated in Table IV, in which are given the analyses of solutions of highly purified Forest Products' diacetyl by means of a standard curve obtained with highly purified Paragon Testing Laboratories' diacetyl.

Table III. Determination of Diacetyl

Determination	Found Gravimetrically *Mg./ml.*	Found Spectrophotometrically *Mg./ml.*
1	0.0752	0.071
2	0.0436	0.043
3	0.0212	0.022
4	0.0924	0.092
5	0.0464	0.048
6	0.0220	0.025
4a	0.0768	0.085
5a	0.0399	0.045
6a	0.0184	0.021

In order to obtain such reproducibility between separate lots of diacetyl, it was necessary to perform rectifications with great care and to protect the purified material against moisture as well as to use it within a short time after distillation. Otherwise, low results were always obtained.

The data shown in Table V, which were obtained with reducing hexoses in order to demonstrate their interference, are the extinctions found upon treating 0.01 *M* solutions of the sugars as described in the above procedure. The color obtained with galactose was brown; however, the glucose color was reddish purple, resembling very much the formaldehyde color.

DISCUSSION

It must be concluded from the near-congruency of their spectra that the dyes produced from diacetyl and formaldehyde are very probably of the same structure. Moreover, the most logical explanation for this structural identity is that, under these conditions, one mole of diacetyl is oxidized by the sulfuric acid with the production of one mole of formaldehyde, as is shown in Equation 1. This is borne out by the extinction data and by the fact that non-oxidizing acids produce relatively little effect. Although the "normal" oxidation of an aliphatic α-diketone (Equation 2) proceeds with great facility with most oxidizing

$$\begin{matrix} CH_2{=}C(OH)COCH_3 \end{matrix} + 2[O] \longrightarrow CH_2O + CH_3COCO_2H \qquad (1)$$

Table IV. Precision

Weighed, Mg./Ml.	Found Spectrophotometrically, Mg./Ml.
0.0192	0.020
0.0481	0.047
0.0871	0.087

Table V. Extinctions

Reducing Sugar	Extinction (log I_0/I) at 570 mμ
Galactose	0.266
Glucose	0.499

$$RCOCOR + [O] + H_2O \longrightarrow 2RCO_2H \qquad (2)$$

agents, it would hardly be expected to occur at a detectable rate in solutions in which the hydroxyl ion concentration is as low as that provided by concentrated sulfuric acid, as it is probable that this type of oxidation proceeds through the α-diketone hydrate.

Attempts to isolate pyruvic acid, the other product indicated in Equation 1, from the reaction of sulfuric acid with diacetyl have not been successful, possibly because at reasonable concentrations of diacetyl in sulfuric acid extensive polymerization and decomposition of this substance occur.

INTERFERENCES

All the substances that interfere in the formaldehyde determination by the chromotropic acid method interfere in this diacetyl estimation—for example, the presence of methyl ethyl ketone produces a marked diminution of the color developed with diacetyl. Formaldehyde is itself an interference.

Both hexoses and pentoses yield diacetyl as one of the products of acid degradation (*7*, *11*), and these substances give brown or purple colors with chromotropic acid–sulfuric acid mixture (*3*). Data indicating the extent of this reaction for two hexoses are given in Table V.

It is noteworthy that acetyl propionyl, the next higher homolog of diacetyl, gives no purple color with chromotropic acid.

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LITERATURE CITED

(1) Barnicoat, C. R., *Analyst*, 60, 653 (1934).
(2) Bricker, C. E., and Johnson, H. R., IND. ENG. CHEM., ANAL. ED., 17, 400 (1945).
(3) Eegriwe, E., *Z. anal. Chem.*, 110, 22 (1937).
(4) Fulmer, E., Kolfenbach, J. J., and Underkofler, L. A., IND. ENG. CHEM., ANAL. ED., 16, 469 (1944).
(5) Kunze, R., *Mikrochemie, Festschr. von Hans Molisch*, 1936, 279.
(6) MacFayden, D. M., *J. Biol. Chem.*, 158, 107 (1945).
(7) Nodzu, R., and Matsui, K., *Bull. Chem. Soc. Japan*, 10, 467 (1935).
(8) Pien, J., Baisse, J., and Martin, R., *Lait*, 16, 119, 243 (1936); 17, 675 (1937).
(9) Prill, E. A., and Hammer, B. W., *Iowa State Coll. J. Sci.*, 12, 385 (1938).
(10) Schmallfuss, H., and Rethorn, H., *Z. Untersuch. Lebensm.*, 70, 233 (1935).
(11) Speck, J. C., unpublished work.
(12) Stotz, E., and Raborg, J., *J. Biol. Chem.*, 150, 25 (1943).
(13) Wilson, J. B., *J. Assoc. Official Agr. Chem.*, 24, 655 (1941).

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Estimation of Ascorbic Acid in Food Preparations

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The dinitrophenylhydrazine and indophenol methods are equally valid for measuring the ascorbic acid content of garden-fresh vegetables. More than 90% of the ascorbic acid in garden-fresh plant materials studied was in the reduced form. Garden-fresh vegetables, slurried with 4 parts of 0.5% oxalic acid and stored for as long as 2 weeks at room temperature (70–77° F.), may be assayed by the indophenol method to determine the ascorbic acid and dehydroascorbic acid content at the time of assay, and by the dinitrophenylhydrazine method to determine the biologically active ascorbic acid at the time of harvesting. For longer storage times, lower temperatures of storage must be used. The ascorbic acid content of garden-fresh edible plants may be measured in a laboratory remote from the harvest area. Both methods may be employed to establish the freshness of vegetable foods.

OF THE various chemical methods that have been proposed for the assay of ascorbic acid, the indophenol method with modifications has been the most widely used. More recently, Roe and Oesterling (*10*) proposed a method based on the coupling of dehydroascorbic acid with 2,4-dinitrophenylhydrazine. The osazone dissolves in sulfuric acid to form a colored complex, the intensity of which serves as an indicator of the amount of ascorbic acid present.

However, these methods for the determination of vitamin C in foods must be applied with a clear understanding of interfering substances occurring naturally, or those derived from ascorbic acid itself. On the one hand, Tuba *et al.* (*11*), Harris and Olliver (*2*), and others have shown that the indophenol technique measures biologically active ascorbic acid in all foods tested, but that this reaction gives no indication of what the original ascorbic acid content of foods might have been in foods that are not fresh. Because it is only seldom that garden-fresh foods are taken for analysis, this latter point may be of extreme importance in judging data on foods of unknown history. On the other hand, Penney and Zilva (*6*) have confirmed the work of Herbert *et al.* (*3*), showing that ascorbic acid, in vitro, is easily oxidized to dehydroascorbic acid, which mutarotates to biologically inactive 2,3-diketogulonic acid. Similar products are formed in living plant materials by enzyme systems or by autoxidation. Penney and Zilva further showed that dinitrophenylhydrazine couples with *l*-ascorbic acid, and dehydroascorbic acid, and 2,3-diketogulonic acid, and (*7*) that the Roe and Oesterling method measures araboascorbic acid, glucoascorbic acid, and diketogulonic acid as well as *l*-ascorbic acid. Pijoan and Gerjowich (*8*) found that the Roe and Oesterling procedure would measure ascorbic acid together with other inactive compounds, one of which was believed to be 2,3-diketogulonic acid. Similar observations were made by Guild *et al.* (*1*).

The observations summarized above suggest that a concurrent use of these two methods might give not only information on the biological potency of a plant food at the time of analysis, but also an indication of the original potency of this food when taken from the soil. A series of experiments was designed to test the validity of these concepts.

These experiments were also planned to provide supplemental data on the proper method for the extraction and stabilization of ascorbic acid from plant materials, with particular reference to oxalic acid. In a study of thirteen acids, Ponting (*9*) found that 0.2% oxalic acid and 0.5% metaphosphoric acid were most suitable for stabilizing pure solutions of ascorbic acid. He recommended the use of twice this concentration of oxalic acid to stabilize ascorbic acid in food extracts. In the present study, 0.5% oxalic acid was used throughout.

EXPERIMENTAL

The dinitrophenylhydrazine and indophenol methods were used in this study. [In this paper dinitrophenylhydrazine method refers to the Roe and Oesterling procedure (*10*) and indophenol

Table I. Ascorbic Acid Content of Stored Food Slurries as Measured by Dinitrophenylhydrazine and Indophenol Reactions

[Garden-fresh foods were slurried with 0.5% oxalic acid (acid to food ratio, 7 to 1), filtered, and stored at room temperature (70° to 77° F.)]

	Dinitrophenylhydrazine Reaction, Total Ascorbic Acid, Mg. %					Indophenol Reaction, Mg. %										
						Total Ascorbic Acid					Reduced Ascorbic Acid					
Day	Cabbage	Chard	Spinach	Broccoli leaves	Broccoli blossoms	Cabbage	Chard	Spinach	Broccoli leaves	Broccoli blossoms	Cabbage	Chard	Spinach	Broccoli leaves	Broccoli blossoms	
0	78.8	63.7	112.5	146.0	181.1	85.9	66.0	114.1	164.0	192.0	77.1	50.0	79.9	138.5	176.8	
1	76.8	66.8	106.4	158.6	194.4	80.7	61.9	98.0	154.1	182.0	74.0	54.6	95.4	134.4	174.2	
2	76.8	65.6	106.2	154.8	184.0	50.0	25.3	56.6	103.2	115.5	44.7	17.3	53.3	94.4	105.4	
7	76.8	60.0	101.6	151.9	181.1	41.5	30.0	65.3	110.4	125.0	35.5	10.7	30.1	87.2	100.8	
	Radish roots	Lettuce	Turnip	Tomatoes	Radish greens	Lemon juice	Radish roots	Lettuce	Turnip	Tomatoes	Radish greens	Lemon juice	Radish roots	Lettuce	Turnip	Tomatoes	Radish greens	Lemon juice
0	20.5	6.6	57.6	11.2	64.0	34.5	18.9	5.3	55.4	16.5	66.9	37.6	16.0	2.3	51.2	11.7	59.7	33.7
2	20.4	6.2	58.6	13.1	68.0	36.6	17.8	3.6	57.0	14.5	64.0	39.3	14.8	2.3	47.5	11.2	55.3	34.3
6	20.3	5.8	59.2	14.1	69.5	38.8	15.0	3.2	47.3	18.0	61.3	45.2	9.2	[a]	39.0	8.2	40.3	24.0
13	18.7	6.6	60.8	13.9	64.0	37.6	11.5	4.1	44.3	14.7	49.4	38.0	2.2	[a]	27.6	5.0	23.4	29.7
24	16.5	4.4	47.2	13.3	62.3	32.8	3.9	1.6	24.4	6.0	19.4	30.6	[a]	[a]	15.0	[a]	3.3	7.4
31	13.3	1.2	47.3	10.4	54.2	29.2	2.2	0.7	13.3	3.0	9.2	12.3	[a]	[a]	2.4	[a]	3.7	2.7
0	14.4	..	..	17.6	26.1	..	17.2	..	..	16.7	22.4	..	12.8	..	..	14.0	18.7	..
2	13.9	..	..	16.4	24.0	..	13.5	..	..	13.7	20.6	..	10.3	..	..	11.0	15.3	..
4	..	..	..	..	..	..	17.0	..	..	16.0	..	..	9.0	..	..	11.0	11.7	..
7	13.9	..	..	16.1	23.2	..	12.0	..	..	15.0	19.0	..	7.8	..	..	8.5	10.0	..
12	14.1	..	..	14.4	20.6	..	6.6	..	..	7.7	6.7	..	3.2	..	..	6.1	2.7	..
14	14.9	..	..	16.5	24.8	..	5.9	..	..	9.5	8.0	..	3.0	..	..	5.6	1.7	..
20	13.9	..	..	16.3	22.4	..	3.5	..	..	5.3	4.1	..	0.7	..	..	2.1	1.9	..
22	14.4	..	..	14.6	25.9	..	3.8	..	..	5.0	4.7	..	0.8	..	..	2.4	[a]	..
27	13.9	..	..	12.0	23.6	..	..	..	..	..	..	..	..	..	..	..	..	..

[a] Too low to read.

method to the Hochberg, Melnick, and Oser (*4*) method for vitamin C assay.] As it had already been established that the indophenol method measures the biologically active ascorbic acid in a large number of garden-fresh foods (*2, 11*), the first part of this investigation was designed to determine whether the dinitrophenylhydrazine method also measures active ascorbic acid in garden-fresh foods.

Garden-fresh samples of cabbage, Swiss chard, spinach, broccoli, radish roots, tomatoes, lettuce, and turnip were obtained from a local grower and slurried with 7 parts of 0.5% aqueous oxalic acid in a Waring Blendor. The slurries were then filtered through a double layer of cheesecloth, distributed in a series of 6-ounce amber bottles, preserved with 2 ml. of chloroform, and stored at 70° to 77° F. At various time intervals, samples of each food were opened, the contents filtered, and aliquots assayed by the two methods mentioned above (Table I). New samples were used for successive analyses.

The efficiency of oxalic acid as an extractant and stabilizer was studied by slurrying samples of garden-fresh radish greens with 0.5, 1, 2, and 4 parts of 0.5% oxalic acid. Extracts of each sample were placed in six bottles, and 2 ml. of chloroform were added. As each sample was taken for analysis, it was filtered and assayed by both methods (Table IV).

The effect of storage temperature was determined on market samples of cucumbers, bell peppers, and radishes, treated as above and stored at 86°, 70° to 77°, 41°, and −10° F. (Table V).

Table II. Ascorbic Acid Values of Garden-Fresh Foods

Food	Dinitrophenylhydrazine Reaction	Indophenol Reaction
	Mg. %	*Mg. %*
Cabbage	78.8	85.9
Chard	63.7	66.0
Spinach	112.5	114.1
Broccoli leaves	146.0	164.0
Broccoli blossoms	181.1	192.0
Radishes	20.5	18.9
Radishes	14.4	17.2
Radish greens	64.0	66.0
Radish greens	26.1	22.4
Turnips	57.6	55.4
Tomatoes	17.6	16.7

DISCUSSION

The main purpose of this study was to determine whether values for ascorbic acid obtained by the dinitrophenylhydrazine method (*10*) on acid slurries of foods kept in storage for days or weeks could be used as indexes of the ascorbic acid content of the same food when it was fresh.

To facilitate a comparison of the results obtained by the dinitrophenylhydrazine method and the indophenol method on garden-fresh foods, Table II has been compiled from Table I. It appears that both the indophenol and dinitrophenylhydrazine methods measure the vitamin C potency of garden-fresh foods. It also follows that the garden-fresh foods used in this study contained only reduced ascorbic acid and dehydroascorbic acid, as the dinitrophenylhydrazine method values agree with those obtained by the indophenol method which others have shown to measure only ascorbic and dehydroascorbic acids (*2, 11*). The results on eight of the eleven samples fall within the limits of accuracy of the two methods. When the statistical "F" distribution test was applied, the results obtained by both methods showed complete homogeneity. The results with broccoli leaves, one sample of radish, and one sample of radish greens did not fall within this range. Possibly the results with radish and radish greens are due to technical errors, because other samples of this vegetable were found to be nearly the same by both methods.

Table III, calculated from the results in Table I (second and third sections), shows the relative amounts of ascorbic acid and dehydroascorbic acid present. It indicates that the ascorbic acid in oxalated slurries was oxidized almost completely to dehydroascorbic acid within 21 days. As measured by the indophenol technique, the ascorbic acid and dehydroascorbic acid present at the end of 21 days did not equal the total vitamin C in the food when garden-fresh, however. The values obtained by the dinitrophenylhydrazine method remained constant over a period of 14 days. In other words, the dinitrophenylhydrazine method under proper conditions will give, in stored foods, the ascorbic acid content of these foods when they were fresh. Consequently, accurate estimations of ascorbic acid in vegetables may be carried on in laboratories remote from the harvest area. These results also confirm the statement of Pijoan and Gerjowich (*8*) that the dinitrophenylhydrazine method measures compounds other than ascorbic acid, among which may be diketogulonic acid.

The ascorbic acid in different vegetables is not oxidized at the same rate. This may be attributed to the degree of maturity and to differences in the enzyme systems which operate at rates peculiar to each vegetable.

The data in Table IV show the effect of different ratios of 0.5% oxalic acid to food on the stabilit of the extracted ascorbic acid. With ratios of 2 to 1 and lower, there was a rapid loss in "ascorbic acid," as measured by the dinitrophenylhydrazine method. This loss increased with a decrease in oxalic acid content. The results obtained by the indophenol method indicate that insufficient 0.5% oxalic acid solution was used, for no reduced ascorbic acid could be detected when the lower ratios of acid to food were used. There is evidence that some oxidation to dehydroascorbic acid and beyond, probably to diketogulonic acid, occurred during slurrying.

As the ratio of 0.5% oxalic acid to food was increased above 2 to 1 the reduced ascorbic acid value increased, and the ratio of dehydroascorbic acid to total ascorbic acid decreased. Although Loeffler and Ponting (*5*) recommended a ratio of at least 7 to 1, the results in Table IV indicate that a 4 to 1 ratio is sufficient to prevent oxidation beyond diketogulonic acid during storage for at least 14 days at room temperature.

Table III. Ascorbic Acid Occurring as Dehydroascorbic Acid in Filtrates of Oxalic Acid Slurries of Foods Stored at Room Temperature (70° to 77° F.)

(Calculated from data in Table I)

Food	Total Ascorbic Acid, Per Cent, Day 0	2	6	13	24	31
Radish	15.1	16.8	38.9	80.0	91.6	100.0
Lettuce	57.2	29.2	95.9	100.0	100.0	100.0
Turnips	7.5	16.7	17.7	37.8	38.6	89.2
Tomatoes	29.2	23.0	54.7	66.0	83.3	100.0
Radish tops	10.8	13.5	34.2	52.6	82.8	70.7
Lemon juice	10.4	12.7	46.9	21.9	75.9	77.7

	Day 0	2	7	12	14	20	22
Radish	25.5	23.7	34.8	51.1	49.1	79.6	79.4
Tomatoes	16.2	19.7	43.3	21.5	41.2	60.8	51.2
Radish tops	16.5	25.8	47.4	60.2	79.2	53.8	100.0

Table IV. Ascorbic Acid Content of Stored Food Slurries

Garden-fresh radish greens slurried with varying ratios of 0.5% oxalic acid and stored at room temperature (70° to 77° F.)

	Total Ascorbic Acid, Mg. %: Dinitrophenylhydrazine Reaction, Ratio of Acid to Food				Indophenol Reaction, Ratio of Acid to Food				Reduced Ascorbic Acid, Indophenol Reaction, Mg. %			
Day	0.5:1	1:1	2:1	4:1	0.5:1	1:1	2:1	2:1	0.5:1	1:1	2:1	4:1
0	43.1	42.2	40.9	46.9	39.6	40.6	50.4	52.2	[a]	12.3	38.2	44.6
2	..	..	..	..	13.6	17.9	38.5	45.1	[a]	[a]	26.2	30.9
5	41.1	44.8	41.3	47.6	..	..	..	..	..	..	..	..
7	30.1	39.2	41.1	47.6	2.7	6.7	22.6	25.5	[a]	[a]	4.7	7.2
13	15.8	22.8	34.7	45.0	..	..	..	..	...	..	..	..
14	15.4	25.2	34.3	45.5	..	..	..	..	...	..	..	..

[a] Too low to read.

Table V. Effect of Temperature of Storage on Ascorbic Acid Content of Stored Food Slurries

Storage Temp., ° F.	Day	Ratio of Acid to Food 4:1 Cucumbers	7:1 Cucumbers	4:1 Peppers	7:1 Peppers	7:1 Radishes
		Mg. %	*Mg. %*	*Mg. %*	*Mg. %*	*Mg. %*
70–77	0	7.8	8.6	116.9	126.6	24.8
	7	9.5	9.6	102.8	136.0	24.4
	14	8.3	8.4	112.0	112.0	20.8
	45	1.5	4.4	50.7	...	8.6
86	0	7.8	8.6	116.9	126.6	24.8
	7	9.1	9.3	100.4	122.8	20.8
	14	6.4	6.7	91.0	108.0	18.4
41	0	7.8	8.6	116.9	126.6	24.8
	7	9.9	9.4	106.1	132.0	22.5
	14	9.5	9.6	134.1	129.4	24.0
	45	...	10.9	119.0	...	24.8
−10	0	7.8	8.6	116.9	126.6	24.8
	7	9.1	8.9	111.0	138.5	24.4
	14	9.0	9.3	107.1	131.0	23.4
	45	...	...	...	...	...
	77	8.9	9.0	111.5	128.0	24.6

The effect of storage at different temperatures upon the ascorbic acid values of radish greens as measured by the dinitrophenylhydrazine reaction is shown in Table V. When samples were stored at 86° F. for 14 days, there was an 18 to 26% loss of "ascorbic acid," as measured by the dinitrophenylhydrazine method. At room temperature, there was no loss during 14 days' storage, at 41° F. no loss during at least 6 weeks' storage, and at −10° F. no loss during at least 11 weeks' storage. If accurate estimations of ascorbic acid in food extracts are to be made, the influences of time and temperature of storage must be considered.

Inasmuch as the indophenol and dinitrophenylhydrazine methods agree when used to measure ascorbic acid in ground-fresh plant materials and disagree more and more during storage after the plant is taken from the ground, these methods should prove useful in checking the freshness of perishable vegetables.

LITERATURE CITED

(1) Guild, L. P., Lockhart, E. E., and Harris, R. S., *Science*, **107**, 226–7 (1948).
(2) Harris, L. J., and Olliver, M., *Biochem. J.*, 36, 155–82 (1942).
(3) Herbert, R. W., Hirst, E. L., Percival, E. G. V., Reynolds, R. J. W., and Smith, F. F., *J. Chem. Soc.*, 1933, 1270–90.
(4) Hochberg, M., Melnick, D., and Oser, B. L., IND. ENG. CHEM., ANAL. ED., **15**, 182–8 (1943).
(5) Loeffler, H. J., and Ponting, J. D., *Ibid.*, **14**, 846 (1942).
(6) Penney, J. R., and Zilva, S. S., *Biochem. J.*, 37, 403–17 (1943).
(7) *Ibid.*, 39, 392–7 (1945).
(8) Pijoan, M., and Gerjowich, H. J., *Science*, 103, 202 (1946).
(9) Ponting, J. D., IND. ENG. CHEM., ANAL. ED., 15, 389–91 (1943).
(10) Roe, J. H., and Oesterling, M. J., *J. Biol. Chem.*, 152, 511–17 (1944).
(11) Tuba, J., Hunter, G., and Steele, H. R., *Can. J. Research*, 24B, 37–45 (1946).

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Determination of Small Amounts of Ethyl Ether in Ethyl Alcohol

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A simple distillation procedure for the determination of 0.0 to 2.0% of ethyl ether in ethyl alcohol is described. Results found after the application of appropriate correction values are accurate within 0.05% of the weight of the sample. The presence of up to 0.5% of benzene in the alcohol does not affect the accuracy of the results.

IN THE manufacture of solvent types of smokeless powder during World War II, large quantities of ether-alcohol solutions had to be recovered and separated into their components by distillation. The recovered alcohol contained 5 to 7% water, 0 to 0.4% benzene, and 0 to 2% ether. For certain operations, it was necessary to use alcohol containing as little ether as possible; hence, an accurate method to determine small amounts of ether in recovered alcohol of the foregoing composition was needed.

Binary mixtures of alcohol and ether can be analyzed by the critical miscibility method of Kubias (*1*). The petroleum ether extraction procedure of Masson and McEwan (*2*) and the recently published density–refractive index method of Scott (*3*) are suitable for ternary mixtures of alcohol, ether, and water. None of the foregoing methods, however, can be used when benzene is present.

The distillation method for determining substantial amounts of ether in alcohol, previously described by the author (*4*), has been modified to make it suitable for the determination of small amounts of ether. Water and 0.5% or less of benzene do not interfere.

APPARATUS

The apparatus consists of a 1-liter round-bottomed flask into which a 3-bulb Snyder column is fitted tightly with a rubber stopper. The outlet of the column is connected to a condenser which is arranged vertically and attached to a suitable receiver. The manner of loosely attaching the receiver to a vacuum line, of cooling the lower bulb of the column with an air stream, and of arranging the thermometer to measure the vapor temperature at the top of the column has been described (*4*). The flask is heated by a hemispherical Glas-Col heating unit controlled by a 5-ampere Variac.

PROCEDURE

Place a weighed sample of about 630 ml. (about 500 grams) in the flask, and add about 250 ml. of water and a few particles of Carborundum.

In case it is estimated that less than 0.2% ether is present, add 2.00 ml. of ether to the sample. This may be readily accomplished by using a 2-ml. pipet which has just been chilled by rinsing with ether and then blowing air through it for a few seconds. A 2-ml. pipet cooled in this manner will deliver 1.38 grams of ether, which will increase the ether content of the standard sample by 0.28%. The addition of some of the substance being determined in an analytical procedure is always to be deplored, but in this case it is required to make the method function in the very low range of ether content.

Attach to the flask a 3-bulb Snyder column bearing a thermometer. Weigh the system consisting of flask, sample, added water, column, thermometer, and stoppers on a suitably identified cork ring to the nearest 0.1 gram, using a good torsion balance. Place a hemispherical Glas-Col heating mantle in position under the flask, direct a strong jet of air against the base of the column, and

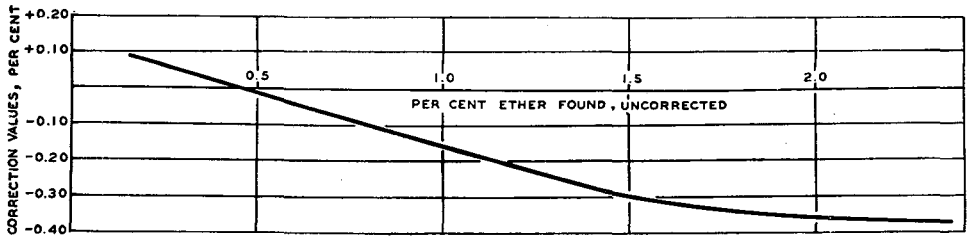

Figure 1. Curve for Correction Value

Table I. Analysis of Ether-Alcohol Solutions of Known Compositions by Proposed Method

Ether Present %	Ether Found, Uncorrected %	Correction Value Read from Curve in Figure 1 %	Ether Found, Corrected %	Difference %
1.94	2.34	−0.36	1.98	+0.04
2.00	2.34	−0.36	1.98	−0.02
1.03	1.21	−0.22	0.99	−0.04
0.49	0.58	−0.04	0.54	+0.05
0.31	0.27	+0.06	0.33	+0.02
0.23	0.16	+0.08	0.24	+0.01
0.43	0.36	+0.03	0.39	−0.04
0.78	0.92	−0.13	0.79	+0.01
1.44	1.82	−0.34	1.48	+0.04
0.68	0.81	−0.11	0.70	+0.02
1.29	1.57	−0.31	1.26	−0.03

heat the sample rapidly (approximately 10 minutes) to incipient boiling. Then lower the heating mantle and decrease its heat until it is just warm enough to permit gentle refluxing of condensed vapor from the lowest bulb of the column. Replace the heating mantle and allow the distillation to proceed slowly (approximately 0.5 to 5 minutes) until the vapor temperature reaches 52° C. Maintain the vapor temperature at 52° ±2° C. until the distillation rate falls below 3 drops per minute and then for an additional 10 minutes. If the initial rate of distillation never exceeds 3 drops per minute in the 50° to 54° C. vapor temperature range, continue the distillation within this range 20 minutes from the time the vapor temperature first reached 52° C.

Under the foregoing conditions, a small and reasonably constant amount of alcohol, for which a correction can be applied, will be distilled. The distillation temperature is controlled chiefly by adjusting either the Variac or the air jet. It can be raised slightly either by placing a towel around the upper half of the flask or by interposing a small watch glass between the end of the air outlet tube and the column for a few seconds.

Lower the heating mantle, disconnect the apparatus, and weigh the system to the nearest 0.1 gram.

CALCULATION

If no ether was added before distillation, the results may be calculated as follows:

$$\frac{\text{Loss in weight of system} \times 100}{\text{Weight sample of alcohol}} = \%\ \text{ether, uncorrected}$$

To the uncorrected per cent ether is applied the correction value read from a curve similar to Figure 1 which was constructed from data obtained by the distillation of 500-gram portions of alcohol to which known amounts of ether have been added. The known mixtures were prepared by adding ether from a Smith buret weighed to the nearest 0.01 gram and applying a correction in each case for the 0.02 gram of ether that is lost from such a weight buret when the reservoir is removed from its base.

If 2.00 ml. of ether were added to the sample prior to the distillation, the per cent ether is calculated as described above, a correction read from the curve in Figure 1 is applied, and from the result so obtained 0.28% is deducted to give the ether content of the original sample.

APPLICATION OF METHOD

The results found when the foregoing procedure was applied to known mixtures of ethyl ether in 2B ethyl alcohol, which contains 0.5% benzene, are shown in Table I.

LITERATURE CITED

(1) Kubias, J., *Chem. Obzor.*, 12, 5–8 (1937).
(2) Masson, I., and McEwan, T. L., *J. Soc. Chem. Ind.*, 40, 29–32T (1921).
(3) Scott, T. A., Jr., *J. Phys. Chem.*, 50, 406 (1946).
(4) Shaefer, W. E., IND. ENG. CHEM., ANAL. ED., 16, 432 (1944).

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1,2-CYCLOHEXANEDIONE DIOXIME

A Reagent for Palladium

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THE preparation of 1,2-cyclohexanedione dioxime, nioxime, and its use in the analytical chemistry of nickel have been reported (*1*, *4*). Nioxime also yields an insoluble yellow compound with palladium which can be used for the detection and determination of this metal. This reagent offers several advantages over dimethylglyoxime as a precipitant for palladium. Nioxime is soluble in water, in contrast to dimethylglyoxime which must be made up in alcohol or acetone, and the possibility of contamination of the palladium precipitate with excess reagent is eliminated. Palladium nioxime is very insoluble and may be filtered from a hot solution after a brief digestion period, whereas in many procedures it is recommended that the palladium dimethylglyoxime precipitate be allowed to cool and stand for long periods to ensure complete precipitation (*2*, *5*). Thus the nioxime procedure effects a great saving of time with no sacrifice of accuracy.

REAGENTS

An 0.8% aqueous solution of nioxime was used. A standard palladium solution was prepared from commercial palladium chloride. This salt was purified by first precipitating out platinum as ammonium chloroplatinate (*3*) and then precipitating the palladium as palladium dimethylglyoxime. This complex was destroyed with aqua regia and the solution evaporated to near dryness five times with concentrated hydrochloric acid to eliminate nitrate ions. The palladium content of the diluted palladium chloride solution was determined by precipitating the palladium in weighed amounts of this solution with dimethylglyoxime. These solutions were allowed to stand overnight to ensure complete precipitation of the palladium dimethylglyoxime.

The water-soluble 1,2-dioxime, 1,2-cyclohexanedione dioxime, called nioxime, yields a yellow precipitate with divalent palladium. This precipitant can be used as a qualitative and quantitative reagent for the palladium ion and offers several advantages over its analog, dimethylglyoxime. Palladium nioxime can be filtered from a hot solution after a brief digestion period, thus effecting a considerable saving of time. Qualitatively, nioxime is a more sensitive reagent for palladium than dimethylglyoxime.

The results of this standardization are given in Table I. A portion of this solution was evaporated to dryness and the residue spectrographically analyzed. Calcium, magnesium, aluminum, silicon, copper, chromium, barium, boron, and platinum were found to be present in less than 100 p.p.m. quantities, while only traces of manganese, silver, zinc, and nickel were found. Iridium, osmium, gold, rhodium, and ruthenium were not detected.

Table I. Standardization of Palladium Solution with Dimethylglyoxime

Trial	Palladium Solution Taken	Precipitate	Palladium	Palladium in Solution
	Grams	*Gram*	*Gram*	*G./g.*
1	20.30	0.1277	0.04044	0.001992
2	19.03	0.1197	0.03791	0.001992
3	21.70	0.1367	0.04329	0.001995
			Av.	0.001993

SENSITIVITY

Two series of five palladium solutions were made up in 100-ml. volumetric flasks with concentrations ranging from 1 to 10 parts in 10,000,000. To one series of solutions was added 1 ml. of nioxime solution, and to the other, 1 ml. of 1% dimethylglyoxime in alcohol followed by vigorous shaking. Observations made about 5 minutes after the addition of the reagent showed that the palladium solutions of 10, 7, and 5 parts in 10,000,000 which contained nioxime, exhibited a slight yellow coloration, while the corresponding concentrations of palladium with dimethylglyoxime did not. After standing several hours, precipitates were noticeable in the flasks containing the higher concentrations in each of the series. Thus, it can be concluded that nioxime is a more sensitive qualitative reagent for palladium ions than dimethylglyoxime. In making the test care should be exercised and the weakly colored precipitate allowed to coagulate before a definite conclusion is drawn.

GRAVIMETRIC DETERMINATION OF PALLADIUM

Although palladium nioxime, $Pd(C_6H_9O_2N_2)_2$, is formed by the union of one palladium ion with two molecules of nioxime with the liberation of two hydrogen ions, it is precipitated from a dilute mineral acid solution. Quantitative precipitation is obtained at pH values from 0.7 to 5 but is not assured at pH values below 0.7.

The effect of various anions upon the determination of palladium was investigated. In the cases of sulfate and nitrate, the chloride ions were expelled by evaporation with the respective acid before addition of the ammonium salts. A small amount of chloride was present in samples to which ammonium acetate, ammonium tartrate, and sulfosalicylic acid were added. Results obtained indicate that these anions do not affect the determination (Table II).

Table II. Effect of Various Anions upon Determination of Palladium

Anion Present	Anion	Palladium Taken	Weight of Precipitate	Palladium Found	Error
	Gram	*Gram*	*Gram*	*Gram*	*Mg.*
Chloride	0.5	0.0107	0.0391	0.0107	0.0
	0.5	0.0122	0.0445	0.0122	0.0
Sulfate	1.0	0.0127	0.0461	0.0127	0.0
	1.0	0.0119	0.0437	0.0120	+0.1
Nitrate	1.0	0.0117	0.0427	0.0117	0.0
	1.0	0.0126	0.0470	0.0129	+0.3
Acetate	1.0	0.0130	0.0483	0.0133	+0.3
Tartrate	1.0	0.0121	0.0439	0.0120	−0.1
Sulfosalicylate	1.0	0.0120	0.0438	0.0120	0.0

A series of determinations conducted in order to observe the effect of varying excesses of nioxime indicated that as much as 150% excess reagent could be added without affecting the accuracy of results obtained (Table III). The minimum excess of nioxime recommended is 30%, which is 0.43 ml. of reagent per milligram of palladium present.

Determinations conducted on a series of different sized samples indicated that palladium could be successfully determined over a range of 6 to 30 mg. However, filtration becomes somewhat difficult with the larger samples (Table IV).

RECOMMENDED PROCEDURE

Adjust the volume of the solution containing from 5 to 20 mg. of palladium to approximately 200 ml. The pH of the solution may vary from 1 to 5, depending upon other cations present. Heat the solution to about 60°. Add slowly from a pipet with stirring 0.43 ml. of 0.8% nioxime for each milligram of palladium present. Digest the solution with occasional stirring for 30 minutes at 60°, filter through a weighed filter crucible of medium porosity, and wash with five portions of hot water. Dry at 110° for 1 hour and weigh. The factor for palladium is 0.2743.

Table III. Effect of Excess Nioxime upon Determination of Palladium

Trial	Excess of Nioxime Added	Palladium Taken	Weight of Precipitate	Palladium Found	Error
	%	*Gram*	*Gram*	*Gram*	*Mg.*
1	30	0.0126	0.0462	0.0127	+0.1
2	60	0.0133	0.0481	0.0132	−0.1
3	100	0.0128	0.0467	0.0128	0.0
4	150	0.0125	0.0456	0.0125	0.0

Table IV. Determination of Palladium

Trial	Palladium Taken	Weight of Precipitate	Palladium Found	Error
	Gram	*Gram*	*Gram*	*Mg.*
1	0.0065	0.0240	0.0065	0.0
2	0.0121	0.0444	0.0122	+0.1
3	0.0185	0.0672	0.0184	−0.1
4	0.0268	0.0970	0.0266	−0.2

Table V. Determination of Palladium in Presence of Platinum

Trial	Platinum Present	Palladium Taken	Weight of Precipitate	Palladium Found	Error
	Gram	*Gram*	*Gram*	*Gram*	*Mg.*
			With Nioxime		
1	0.010	0.0112	0.0412	0.0113	+0.1
2	0.025	0.0118	0.0436	0.0120	+0.2
3	0.040	0.0130	0.0479	0.0131	+0.1
4	0.050	0.0132	0.0487	0.0134	+0.2
5	0.100	0.0114	0.0423	0.0116	+0.2
			With Dimethylglyoxime		
6	0.040	0.0133	0.0421	0.0133	0.0
7	0.100	0.0140	0.0446	0.0141	+0.1
8	0.100	0.0113	0.0355	0.0112	−0.1
9	0.050	0.0140	0.0447	0.0142	+0.2

Table VI. Determination of Palladium in Presence of Various Cations

Cation Present	Cation	Palladium Taken	Weight of Precipitate	Palladium Found	Error
	Gram	*Gram*	*Gram*	*Gram*	*Mg.*
Uranyl	0.030	0.0127	0.0468	0.0128	+0.1
	0.070	0.0117	0.0430	0.0118	+0.1
Ruthenium	0.010	0.0135	0.0501	0.0137	+0.2
	0.025	0.0125	0.0460	0.0126	+0.1
Beryllium	0.015	0.0117	0.0428	0.0117	0.0
	0.035	0.0130	0.0472	0.0130	0.0
Sodium, Potassium, Lithium, Barium, Strontium, Calcium	0.05 each	0.0135	0.0494	0.0136	+0.1
Aluminum, Lanthanum, Zinc, Cadmium	0.05 each	0.0119	0.0428	0.0121	+0.2

The determination of palladium in the presence of platinum was investigated. The separation was effected by simply using the procedure outlined above. The filtrates containing the platinum and excess nioxime yielded a blue precipitate only upon standing more than 24 hours after filtration (Table V). Determinations of palladium in the presence of platinum with dimethylglyoxime are also included in Table V. In these determinations the solutions were digested for 30 minutes at 60° and then allowed to cool for 1 hour before filtration. Spectrographic analyses of the decomposed precipitates from trials 5 and 7 revealed a stronger platinum line in the case of the dimethylglyoxime separation than when nioxime was used.

Palladium was determined in the presence of uranyl, beryllium, ruthenium, aluminum, lanthanum, zinc, cadmium, alkali, and alkaline earth ions (Table VI). Nioxime yields a precipitate with aurous ion at 60° which is contaminated with metallic gold.

LITERATURE CITED

(1) Rauh, E. G., Smith, G. F., Banks, C. V., and Diehl, H., *J. Org. Chem.*, **10**, 199 (1945).
(2) Scott, "Standard Methods of Chemical Analysis," Vol. I, p. 724, New York, D. Van Nostrand Co., 1939.
(3) Treadwell, F. P., and Hall, W. T., "Analytical Chemistry," Vol. II, p. 141, New York, John Wiley & Sons, 1942.
(4) Voter, R. C., Banks, C. V., and Diehl, H., ANAL. CHEM., **20**, 458 (1948).
(5) Zschiegner, H. E., *Ind. Eng. Chem.*, **17**, 294 (1925).

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Analysis and Composition of Lead Dioxide

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Complete analyses of four samples of reagent grade lead dioxide have been made, including total water and parallel assays by the standard Diehl-Topf method. The Kinch form of stating complete analyses is favored by the author.

IN SEVERAL complete analyses of lead dioxide and sulfate mixtures by the Diehl-Topf method (*9*), the summations were considerably less than 100% in spite of liberal allowances for common errors of analysis. The supposition that the figures for the main constituent, lead dioxide, were low was confirmed by checking against Kahlbaum's *Bleisuperoxyd*.

Three reported analyses of reagent grade lead dioxide by the Lux method (*18*) showed undetermined matter ranging from 4 to 8%. This seemed excessive. The author felt that neither the Diehl-Topf nor the Lux method had the order of accuracy achieved by volumetric procedures—for example, the permanganate methods for iron, manganese, and antimony. Consequently, the literature was carefully reviewed.

Kinch (*14*) was probably the first chemist to report an approximately complete gravimetric and volumetric analysis of mineralized lead dioxide, plattnerite. Similar but detached contributions have been made (*2, 10, 29*).

From 1880 (*18*) to 1937 at least 18 volumetric method assays of manufactured lead dioxide were reported, but Pamfilov and Ivančeva (*23*) and LeBlanc and Ebereus (*15*) found all unsatisfactory. Mrgudich and Clark (*20*), who checked the methods with Merck's reagent lead dioxide, obtained low results from all but the Diehl-Topf method (*9*)—average 96.5%—which they considered accurate. They also modified the Lux method (*18*) and obtained an average of 96.5%, but did not account for the undetermined matter that varied from 3.1 to 3.5%.

Summing up, volumetric assays of lead dioxide appear questionable. Evidently lead dioxide presents a real problem involving composition and structure.

The author concluded that complete gravimetric analyses offered the best means of obtaining a more truthful perspective of the composition and nature of lead dioxide, while parallel assays by the (Diehl-Topf) method and its active oxygen (Kinch) would serve as valuable adjuncts.

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EXPERIMENTAL

Materials Used. Four samples of reagent grade lead dioxide were selected. (1) Kahlbaum's *Bleisuperoxyd*, an anodic dioxide prepared according to the method of Dennstedt and Hassler (*8*). The color was dark chocolate brown and the particles were hard and dry. (2) An American product probably anodic and indistinguishable from (1) in physical properties. (3) A later American product (1925?), not anodic and advertised to contain 96.5% lead dioxide. The color was lighter than (1) and under the knife the sample was soft and plastic. (4) This sample (1937) was closely similar to (3) but differed in the stated analysis—i.e., "non H_2S substance—0.40, acid-insoluble—0.10, H_2S metals (not Pb)—0.02, Fe—0.005, Mn—0.003, SO_4—0.002, CO_3—0.003, NO_3—0.005, Cl—0.0015, and PbO_2—91.4%." Total assay was 91.94%.

All the samples were homogeneous under the microscope and the chemical properties were decidedly typical of lead dioxide.

Preparation of Material. The samples were carefully mixed in their containers, then ground portionwise in an agate mortar to 250- and 300-mesh and again thoroughly mixed.

Particular caution was used to avoid contamination with organic matter and dust. All subsequent weighings were made from the air-dried sample.

Assay of Free (Uncombined) Lead Monoxide. As there is no direct method for the assay of lead monoxide, hot extractions with a 10% lead nitrate solution were used, in general agreement with Löwe (*16*) for red lead but modified for small quantities (500.0 mg.) as described by West (*28*).

Löwe (*16*) found that 10 to 12% lead nitrate solutions dissolve free lead monoxide and metallic lead and decompose lead carbonate, but do not attack red lead. Lunge and Berl (*17*) confirmed these findings experimentally.

The author found that all the lead could not be removed by the modified procedure of West (*16, 28*); however, when the residues were boiled with an additional 25 ml. of lead nitrate solution and

washed with hot water (90° to 95° C.) before and after transfer to the Gooch crucible, no lead was found in the final wash water. The residues at 105° C. dried and extracted were then heated to constant weight at 225° C. Boiling water blanks were also made for the water-soluble and heat loss corrections at 225° C. of the air-dried samples. The net loss after adding the heat and water-soluble corrections was regarded as free lead monoxide. The improved procedure described was employed throughout the assays of the samples.

Sample 1 (500.0 mg.) was extracted several times, with a net weight after correction of 500.1 to 500.3 mg., well within the range of experimental error and indicating no free lead monoxide.

When the improved method was checked against two synthetic mixtures of sample 1 containing 4.13 and 12.13% pure lead monoxide, 99.5 and 99.8% of the monoxide was extracted, thus confirming the efficiency of the method.

As in sample 1, samples 2 and 3 after the assay corrections showed no appreciable net losses and consequently no free monoxide.

When 500.0 mg. of sample 4 were extracted, the weights of duplicate residues agreed closely. Heat and water corrections were 3.6 and 3.1 mg., and the net loss was 9.1 mg. or 1.82% of free lead monoxide.

The assays show that lead nitrate in 10% solution neither attacked lead dioxide nor the silicious and nonlead compounds contained. Evidently any net loss observed (sample 3) was due to free lead monoxide.

Assay of Red Lead. In mixtures with lead dioxide there appears to be no satisfactory method of assay. The nitric acid processing during manufacture probably eliminates most of the red lead (likewise the monoxide), a supposition that neither LeBlanc and Ebereus (*15*) nor Mrgudich and Clark (*20*) appear to question in their published analyses of lead dioxide.

Chemical Decomposition of the Samples for Analysis. The wetted samples were mixed with 5 to 6 ml. of nitric acid and 3 to 4 ml. of hydrochloric acid and concentrated to near dryness and the residues were taken up in 10 to 12 ml. of boiling water. If the samples proved refractory, the operations were repeated.

Assay of Total Lead. Reagent grade lead dioxide contains at least 84% of lead. Separate assays were therefore made by the standard sulfate, molybdate, and electrolytic methods (Table I). The results were averaged to obtain the highest accuracy for the relatively large amount of the lead contained.

Sulfate Method. The procedure of Hillebrand and Lundell (*12*) was extended to the recovery of filtrate lead by electrolysis.

Molybdate Method. Small quantities of calcium salts in lead dioxide suggested slight modifications of the Hillebrand and Lundell (*12*) procedure in agreement with Ibbotson and Aitchison (*13*) and Scott (*26*). Two precipitations of lead molybdate were made (increased ammonium salt) to obtain the normal lead molybdate (*27*). The collected molybdate on a close Munktell No. 00 filter after drying at 105° C. was ignited in a tared porcelain crucible.

When the method was checked against test lead and two synthetic test lead mixtures containing 0.38 and 0.77% of calcium oxide, 99.88, 99.98, and 99.88%, respectively, of the lead introduced was found, indicating no interference from the added calcium salt.

Electrolytic Method. Experiments indicated that the roughened laboratory platinum anodes would hold safely 85.0 mg. of lead dioxide. The empirical lead factor, 0.8620, was obtained by triplicate assays of 72.97 mg. of lead, calculated from Baker's analyzed test lead and in close agreement with the procedure of Hillebrand and Lundell (*12*). The assays of the samples were made in a closely similar manner.

Two to 8 assays were made per sample with a general average of 5. Deviations from the average value were greatest for the sulfate (±0.35%) and electrolytic (±0.25%) and least for the molybdate method (±0.15%). Sample 3 showed the least general deviation (±0.15) irrespective of the method used (Table I).

Table I. Assays of Total Lead

Method	Samples No. 1, %	No. 2, %	No. 3, %	No. 4[a], %
Sulfate	86.08	85.69	85.01	84.12
Molybdate	86.25	85.71	85.03	84.32
Electrolytic	85.90	85.83	84.96	84.17

[a] In sample 4, each result was corrected for 1.82% lead monoxide.

Accuracy Weights of Sulfate, Molybdate, and Electrolytic Methods. After over a year's intensive laboratory study of the methods, and in agreement with Mellor (*19*), the author concluded their relative weights of accuracy were in the ratio of 4 to 5 to 1, respectively.

The molybdate was favored over the sulfate method because of closer checking of assays, lower lead factor, no lengthy evaporations, and less possibility and significance of losses during analysis. The considerably lower weighted electrolytic method seemed justified on account of the high lead factor and low initial weight taken. In addition, at least 30% of the assays failed completely (Table II).

Table II. Final Averages of Total Lead from Weighted Assays

(Lead dioxide and lead monoxide calculated)

Constituent	Samples No. 1, %	No. 2, %	No. 3, %	No. 4, %
Total lead	86.14	85.71	85.02	84.25
Lead dioxide	99.44	98.95	98.15	97.27
Lead monoxide	92.79	92.32	91.58	90.75

Theoretical factors for lead dioxide and lead monoxide, 1.1544 and 1.0772.

Assay of Lead Dioxide by the Diehl-Topf Method. All standardizations and titrations were made with a 50.0-ml. buret graduated and calibrated twice at 30.0° C. The factor 0.1 *N* sodium thiosulfate solution was standardized against 0.1 *N* potassium permanganate solution in agreement with the procedure of Hillebrand and Lundell (*12*) (Table III).

Table III. Average Diehl-Topf Assays of Lead Dioxide and Active Oxygen (Kinch)

Constituent	Samples No. 1, %	No. 2, %	No. 3, %	No. 4, %
Lead dioxide	96.46	95.19	94.65	90.33
Active oxygen	6.43	6.38	6.40	6.05

Average deviation of duplicate and triplicate assays ±0.1%.

Assay of Acid-Insoluble and Nonlead Substance. The procedure was in general agreement with the A.C.S. specifications (*1*) but given sufficient latitude to include the practice of Hillebrand and Lundell (*12*) and Noyes and Bray (*22*) for increased accuracy of analysis.

Acid-insoluble included silica and silicates, nonlead substance, and metals of the III, IV, and V groups (in part as oxides) with smaller amounts of chloride, nitrate, carbonate, and sulfate. No assays were made of the nonlead hydrogen sulfide metals because of their minute quantity (Table IV). The deviations of the separate assays from the average results were usually less than ±0.02%.

Table IV. Averaged Triplicate Assays of Acid-Insoluble and Nonlead Substance

Constituent	Samples No. 1, %	No. 2, %	No. 3, %	No. 4, %
Acid insoluble	0.08	0.08	0.05	0.05
Nonlead substance	0.65	0.75	1.21	0.52
Total	0.73	0.83	1.26	0.57

Assay of Total Water. Water in lead dioxide was noted by several chemists (*4, 7, 8, 14, 21, 24, 25*) including Kinch (*14*), who probably first observed traces in plattnerite, and two others (*4, 7*) who proposed molecular water formulas, but obtained them by indirect methods. However, no quantitative methods of assay or analyses were reported.

Dennstedt and Hassler (*8*) found that lead dioxide parted with a stably bound water of hydration at 350° C., but the small part remaining parted only at the conversion temperature of red lead 450° to 500° C., an observation that suggested heating the samples to 500° C. and measuring accurately the water evolved.

IMPROVISED ABSORPTION METHOD. An available electric steel carbon combustion furnace was modified by replacing the first Ascarite bulb (air-drying train) with one containing Anhydrone and the second (afterbulb) with two Anhydrone U-tubes (stopcocks) in series followed by a sulfuric acid bubbler with guard tube.

The selected heating temperature, 500° ± 8° C. (middle of combustion tube) was easily maintained for 25 to 30 minutes by placing two rheostats in series with the circuit. According to the standard procedures, the apparatus was thoroughly dried out and freed from leaks.

Repeated assays indicated definitely that 2-gram samples (Silliman ware boat) when heated to 500° ± 8° C. for 20 minutes in a current of dry air (4 to 5 bubbles per second) evolved all the water (apparently) into the tared U-tubes. Obviously the additional weight of the tared U-tubes corrected for their average increase (blanks) represented the water evolved from the samples (Table V).

Samples 1 and 2 on heating developed a yellowish red color; samples 3 and 4 the typical red lead shade.

An experimental check of the results by the absorption method was made by employing the Penfield method (*3*) for water in rocks and highly recommended by Hillebrand and Lundell (*11*, *12*).

PENFIELD METHOD. The required tubes (one to two absorption bulbs) and capillary plugs were constructed of Pyrex (standard design), heated with the rubber connectors to 105° C., and dried in vacuo for 48 hours.

The ignition bulb containing the weighed sample (1 to 2 grams) was heated gently at first and then increased to the maximum temperature of the Tirrill flame, approximately 700° C. The remaining operations were essentially the same as those described in the standard procedure. Table V gives the average results of duplicate and triplicate assays.

Table V. Average Total Water Assays by Absorption and Penfield Methods

Method Used	Samples No. 1, %	No. 2, %	No. 3, %	No. 4, %
Absorption (500° ± 8° C.)	0.38	0.75	0.71	0.67
Penfield (700° C.)	0.37	0.76	1.02	0.67
Mean	0.38	0.76	0.86	0.68

Deviations of the separate assays from averages were approximately ±0.01%; sample 4 (absorption method) maximum ±0.02%; sample 3 (Penfield method) one assay, maximum, −0.06%.

In general agreement with (*8*) the total dioxide water was expelled at 500° ± 8° C. unless the nonlead substance exceeded 0.83% (sample 3) when the higher temperature 700° C. was required with possible loss of alkali metal salt.

Residual water at 225° C. by heating to constant weight was found to be 0.22% (average, 3 samples). Cumming and Kaye (*6*) reported 0.25% at 200° C.

Evidently the hygroscopic water in lead dioxide varies with the preparation, composition, and exposure to air, whereas that remaining is so firmly bound that its loss occasions the disruption of the dioxide molecule.

Assay of Combined Oxygen, Excluding That from Impurities. No planned assays could be made because of violent military activities in the Cavite area and the loss of most of the samples. Therefore, the combined oxygen was obtained indirectly from the difference of the accurately determined constituents and 100; or for No. 1, 12.75; No. 2, 12.70; No. 3, 12.86; and No. 4, 12.68%.

Oxygen Deficiency. The conclusion of Reinders and Hamburger (*24*) that the empirical formula of lead dioxide is always less than $PbO_{1.95}$ was confirmed by the recent findings of x-ray diffraction analysis that solid lead dioxide had an oxygen deficiency down to $PbO_{1.66}$ without structure change (*5*).

Oxygen deficiency in the samples is also definitely indicated by their increased lead-oxygen ratios—No. 1, 6.76; No. 2, 6.78; No. 3, 6.61; and No. 4, 6.64—over the theoretical dioxide ratio 6.47. It is more easily seen in the derived empirical formulas—No. 1, $PbO_{1.92}$; No. 2, $PbO_{1.92}$; No. 3, $PbO_{1.96}$; and No. 4, $PbO_{1.95}$. The formulas are approximate to a certain extent, as the combined oxygen carries the errors of analysis.

Summations of Complete Analyses. Table VI gives the gravimetric assays in detail. To avoid repetition the volumetric (Diehl-Topf) assays are summed up with the impurities. Table VII includes the assays of the impurities with lead monoxide calculated from gravimetric lead, and volumetric assays of active oxygen (Kinch).

DISCUSSION

Hillebrand and Lundell (*12*) set the lower and upper limits for satisfactory mineral analyses at 99.75 and 100.60%, providing no platinum vessels were used or corrections made for intruding foreign matter. If these criteria are extended to lead dioxide analysis, the gravimetric and Kinch (*14*) summations, but not those by Diehl-Topf, are satisfactory (Tables VI and VII).

Evidently the chief source of error lies in the determination of lead dioxide, in part due to the use of its theoretical gravimetric factor, 1.1544 and 0.1 *N* 119.6 gram equivalents per liter, both of which constants give high results because of the oxygen deficiency. This is easily shown by calculating the gravimetric lead dioxide by the tentative factor 1.1500 from the averaged empirical formula $PbO_{1.94}$. The results for the samples are No. 1, 99.06; No. 2, 98.57; No. 3, 97.77; and No. 4, 96.88% of lead dioxide. The volumetric assays (Diehl-Topf) and active oxygen (Kinch) are effected in a similar manner. However, the conventional theoretical values were used in the tables to avoid confusion.

The possible and serious errors when lead monoxide and red lead occur in lead dioxide have been considered.

In view of the abrupt ending of the experiments and the lack of additional data, the author favors the Kinch manner of stating analyses of lead dioxide.

SUMMARY

Complete analyses of four selected samples of reagent grade lead dioxide were made. The corrected percentages of lead

Table VI. Complete Gravimetric and Volumetric Analyses

Constituent	Samples No. 1, %	No. 2, %	No. 3, %	No. 4, %
Silicious and nonlead substance	0.73	0.83	1.26	0.57
Total water	0.38	0.76	0.86	0.68
Lead dioxide	99.44	98.95	98.15	1.82 PbO 97.27
Total, gravimetric	100.55	100.54	100.27	100.34
Total, volumetric[a]	97.57	96.84	96.77	93.40

[a] Totals include combined assays of impurities and those of lead dioxide by Diehl-Topf.

Table VII. Complete Analyses by Combining Gravimetric with Volumetric Methods (Kinch)

Constituent	Samples No. 1, %	No. 2, %	No. 3, %	No. 4, %
Silicious and nonlead substance	0.73	0.83	1.26	0.57
Total water	0.38	0.76	0.86	0.68
				1.82 (free PbO)
Lead monoxide[a]	92.79	92.32	91.58	90.75
Active oxygen	6.43	6.38	6.40	6.05
Total	100.33	100.29	100.10	99.87

[a] Calculated from total gravimetric lead by factor 1.0772.

dioxide in the samples by gravimetric assay (empirical factor 1.1500) were (1) 99.06, (2) 98.57, (3) 97.77, and (4) 96.68; by volumetric assay (Diehl-Topf, uncorrected) (1) 96.46, (2) 95.19, (3) 94.65, and (4) 90.33. Obviously, the Diehl-Topf assays were low. The corrected percentages of active oxygen in the samples (empirical oxygen equivalent, 0.78 gram per liter) were (1) 6.30, (2) 6.25, (3) 6.27, and (4) 5.92.

Free lead monoxide was assayed by an improved extraction method. The results were negative, except sample 4 which contained 1.82%.

Total water was assayed by an improvised absorption method and confirmed by the Penfield method. The averaged percentages obtained were (1) 0.38, (2) 0.76, (3) 0.86, and (4) 0.67.

Residual water in lead dioxide at 225° C. was found to be approximately 0.22% (average of samples 1, 2, and 3).

The percentages of gravimetric nonlead substance and silica in the samples were (1) 0.73, (2) 0.83, (3) 1.26, and (4) 0.57.

Oxygen deficiencies occurred in all the samples and are best shown approximately by the empirical formulas (1) $PbO_{1.92}$, (2) $PbO_{1.92}$, (3) $PbO_{1.96}$, and (4) $PbO_{1.95}$. From the averaged formula $PbO_{1.94}$ the tentative gravimetric factor (lead dioxide), 1.1500, and and the active oxygen decinormal equivalent 0.78 gram per liter were calculated.

The gravimetric and Kinch summations of analysis were within the limits, 99.75 and 100.60%, assigned for satisfactory mineral analyses.

LITERATURE CITED

(1) American Chemical Society, *Ind. Eng. Chem.*, **19**, 647 (1927).
(2) Breithaupt and Plattner, J., *J. prakt. Chem.*, **10**, 508 (1937).
(3) Brush, G. J., and Penfield, S. L., *Am. J. Science* (3), **48**, 31 (1894); *Z. anorg. Chem.*, **7**, 22 (1914).
(4) Carnelley, T., and Walker, T. J., *J. Chem. Soc.*, **53**, 59 (1888).
(5) Clark, G. L., "Applied X-Rays," 3rd ed., p. 362, New York, McGraw-Hill Book Co., 1940.
(6) Cumming and Kaye, "Quantitative Chemical Analysis," p. 241, 7th ed. rev., New York, D. Van Nostrand Co., 1939.
(7) Day, T. J., and Schrenk, W. T., School Mines Met. Univ. Missouri, *Bull. Tech. Ser.* **2**, No. 10, 31–6 (1935).
(8) Dennstedt, M., and Hassler, F., *Ber.*, **45**, 1197 (1910).
(9) Diehl-Topf, U. S. Bur. Standards, *Circ.* **90** (May 25, 1922).
(10) Hawkins and Hawkins, *Am. J. Science*, **38**, 165 (1889).
(11) Hillebrand, W. F., "Analysis of Silicate and Carbonate Rocks," U. S. Geol. Survey, *Bull.* **305** (1907).
(12) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," p. 181, 8th print., New York, John Wiley & Sons; London, Chapman & Hall, 1944.
(13) Ibbotson and Aitchison, "Analysis of Non-Ferrous Alloys," New York, Longmans, Green and Co., 1915.
(14) Kinch, E., *Mineralog. Mag.*, **7**, 63 (1886).
(15) LeBlanc, M., and Ebereus, E., *Z. anal. Chem.*, **89**, 81 (1932).
(16) Löwe, J., *Dinglers Polytech. J.*, **271**, 472 (1889).
(17) Lunge and Berl, "Tech. Chem. Research Methods," Vol. 8, 797 (1924), rewritten and revised by Ernst Berl, Berlin, J. Springer, 1921.
(18) Lux, A., *Z. anal. Chem.*, **19**, 153 (1880).
(19) Mellor J. W., "Higher Mathematics for Students of Chemistry and Physics," pp. 548–9, New York, Longmans, Green and Co., 1922.
(20) Mrgudich, J. N., and Clark, G. L., Ind. Eng. Chem., Anal. Ed., **9**, 256 (1937).
(21) Nissensen and Dennell, *Z. elekt. chem.*, **9**, 760 (1903).
(22) Noyes and Bray, *Tech. Quarterly*, **19**, 235 (1906).
(23) Pamfilov, A. V., and Ivančeva, E. G., *Z. anal. Chem.*, **88**, 23 (1932).
(24) Reinders, R., and Hamberger, L., *Z. anorg. Chem.*, **89**, 71 (1914).
(25) Sand, *Chem. News*, **100**, 264 (1909).
(26) Scott, W., "Standard Methods of Chemical Analysis," Vol. 1, p. 278a, New York, D. Van Nostrand Co., 1925.
(27) Wempe, *Z. anorg. Chem.*, **78**, 298 (1912).
(28) West, A. P., *Phil. J. Science*, **8**, No. 6, 429 (1913).
(29) Wheeler, *Am. J. Science*, **38**, 79 (1889).

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Index for X-Ray Powder Diffraction Patterns

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NOW that x-ray powder diffraction patterns for a comparatively large number of substances have been published, many users will find a real need for an appropriate over-all index to the patterns. The importance of such an index has been only too apparent in those laboratories that accumulated their own file of data cards from the literature before the collections of patterns were published by the joint committee of the A.S.T.M., the A.S.X.R.E.D., and the I.P. When additional supplements are issued, the need for a single index will become even more obvious.

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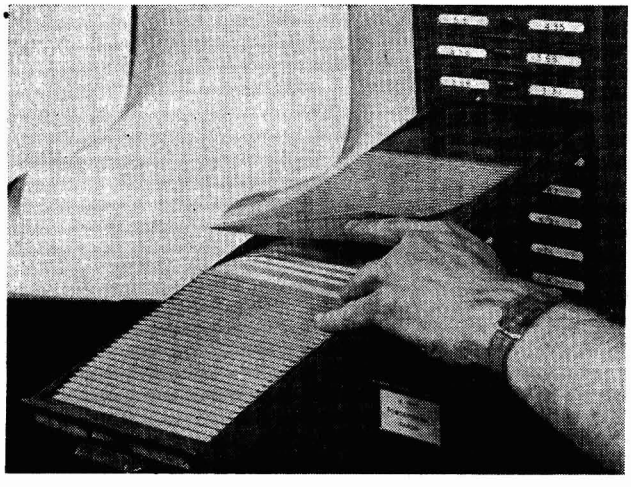

Figure 1. Kardex System

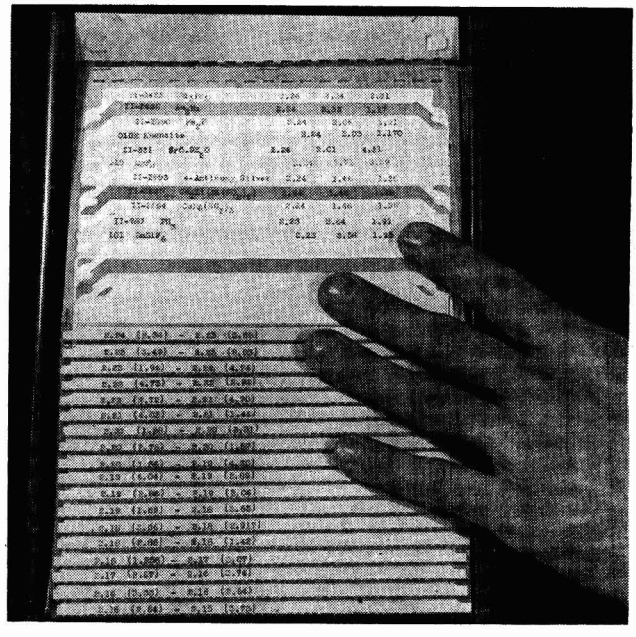

Figure 2. Chaindex Entries

As was pointed out by Bunn (*1*) the A.S.T.M. data cards are somewhat unwieldy for identifying an unknown substance. A large number of cards must be handled to allow for inaccuracies that exist in some of the lattice measurements, and there are three cards for each pattern. Bunn has set up an index consisting of single cards covering all the listings at a particular lattice spacing (to 0.01 Å.) with references to the complete pattern. He also mentions the usefulness of a card system listing innermost lines for all compounds.

During the past six years a Remington Rand Kardex system (Figure 1) has been found a most satisfactory answer to the problem of indexing data cards, and the authors' use of this system has been orally reported (*4*). Each one-line Chaindex entry (Figure 2) lists the key number of the complete pattern (*3*), the formula or name of the substance, and the three most prominent lines. This information is listed on each of three differently colored Chaindex cards, following the principle of the modified Hanawalt (*3*) scheme. The three strongest lines of a pattern are listed in the order of decreasing intensity on the white Chaindex entries (order—one, two, three). A red entry is used to list the lines in the order two, one, three, and a blue entry to list the lines in the order three, one, two. The system is flexible and the original Hanawalt scheme (*2*, page 245), by which the lines are listed only according to predetermined divisions of the scale in which they fall, may be used.

After more than five years' use in identification of many unknown compounds, the authors have found so little use for the blue cards (listing the third strongest line first) that there appears little justification for continuing to include them in the file. It is considered helpful, however, to retain the listing of three lines on the other two cards.

The advantages of this Kardex system are its speed and flexibility. As can be seen in Figure 2, some ten patterns are seen at a glance on one sheet, and sheets with compounds of the next ten larger or smaller, or any other lattice spacings, are immediately available. Each drawer holds 975 entries. By using the Chaindex entries of different colors, Bunn's system of filing the innermost lines could readily be incorporated in the file. When new patterns become available, the system can be rapidly expanded in any appropriate location, and all entries will appear in exact order.

LITERATURE CITED

(1) Bunn, C. W., *Nature*, **157**, 235 (1946).
(2) Hanawalt, J. D., and Rinn, H. W., IND. ENG. CHEM., ANAL. ED., **8**, 244 (1936).
(3) Hanawalt, J. D., Rinn, H. W., and Frevel, L. K., *Ibid.*, **10**, 457 (1938).
(4) Kronberg, M. L., reported at meeting of American Society for X-Ray and Electron Diffraction, Gibson Island, Md., 1944.

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Polarographic Determination of Free Monomer in Heteropolymerization Reaction Mixtures

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Free or uncombined monomers in heteropolymerization reaction mixtures were quantitatively determined by the polarographic method of analysis. By using standard polarographic linear curves as little as 0.01% of free monomer was determined within ±3 to 5%. Data for numerous samples of polymer showed the method to be rapid, accurate, and applicable in control work. The rate of reaction and ratio of monomers was conveniently studied with the polarograph. Photographs of typical Micromax recordings illustrate the type of polarogram obtained and the reproducibility of analyses.

THE need for a quick and accurate means of determining free or uncombined monomer in heteropolymerization reaction mixtures led to the work presented in this paper. It was necessary to find the amount of uncombined maleic anhydride in a styrene–maleic anhydride heteropolymer and the amount of uncombined vinyl acetate and uncombined maleic anhydride in a vinyl acetate–maleic anhydride reaction mixture.

Compounds that contain a conjugated double bond are known to be reducible at the dropping mercury electrode (*2*, *3*), and because analyses were to be made on this type of organic linkage the polarographic method appeared feasible.

SOLUTIONS AND APPARATUS

The research was conducted on a Leeds & Northrup "Electrochemograph, using a large constant-temperature bath controlled to ±0.05° C. Figure 1 shows the assembled apparatus. Standard Fisher 125-ml. lipless beakers were used for all polarographic base solutions and samples were run at 26.0° C. A Beckman pH meter was used to obtain pH readings.

Coleman and Bell C.P. redistilled mercury was used as an anode and as the dropping mercury electrode. The base solution used in styrene–maleic anhydride work was made up by dissolving 5 ml. of C.P. 85% phosphoric acid in 1500 ml. of distilled water and adjusting to pH 3.0 with a saturated solution of lithium hydroxide. Vinyl acetate, obtained from Shawinigan Chemical Company, was used in all experimental work on vinyl acetate–maleic anhydride analysis and was freshly distilled before using. The maleic anhydride was Monsanto solid grade and was reasonably free from maleic acid. The benzene used was obtained from Barrett Division of Allied Chemical Company at Cincinnati. A 50-50 mixture of 0.01 *N* hydrochloric acid and 0.25 *N* potassium chloride solution with ethanol, containing 2 grams per liter of a nonionic wetting agent known as Triton NE, was used as a base solution. As maxima inhibitor 2.5 ml. per liter of a 0.2% bromocresol green solution were added. The benzoyl peroxide was obtained from the Lucidol Corporation of Buffalo, N. Y., and the Triton NE was from Rohm and Haas Chemical Company, Philadelphia, Pa.

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EXPERIMENTAL

The data discussed are on two heteropolymerization reaction mixtures, styrene–maleic anhydride and vinyl acetate–maleic anhydride. The free maleic anhydride in the presence of water hydrolyzes to maleic acid and gives good reproducible polarographic diffusion currents, which were found to be proportional to the concentration. The linear polymer does not interfere. The maleic acid contains a conjugated double bond (*1*, *2*, *5*), which is reducible polarographically, while the polymer has no such linkage and hence is unaffected. Straight-line curves for maleic anhydride are obtained in both reaction mixture studies.

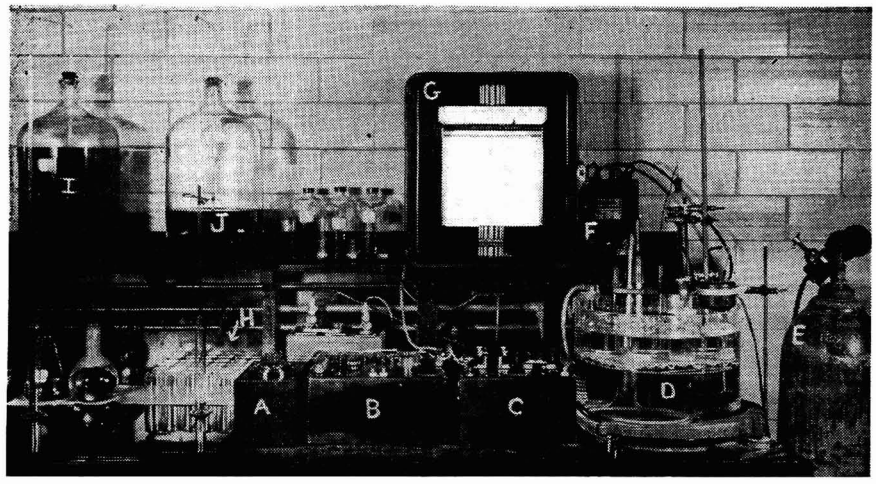

Figure 1. Assembled Electrochemograph

A. Shunt box
B. Polarizing unit
C. Thermionic amplifier
D. Constant-temperature bath
E. Tank nitrogen
F. Relay box (for constant-temperature bath)
G. Micromax recorder
H. Polarographic cells
I, J. 5-gallon bottles containing supporting electrolyte

Styrene–Maleic Anhydride. Several samples of the polymer were refluxed in the pH 3.0 buffer solution until complete solution or peptization of the polymer occurred. This appeared to vary in time according to the percentage of maleic anhydride present. These samples gave well defined polarographic waves and no better results were obtained with a pH of 4.0 or 5.0. When the cells were flushed with tank nitrogen, the dissolved oxygen reduction wave was lessened, so that it would not overlap that of maleic acid. Several standard curves were made by refluxing known amounts of pure maleic anhydride in the pH 3.0 base solution and polarographing the solution at different sensitivities of the instrument. From this, a sample was found, which, on analysis, showed less than 0.01% of free maleic anhydride, and this sample was then used to test the accuracy of the method. Samples containing from 0.2 to 1.0 gram of the (uncombined maleic anhydride free) polymer were prepared and various amounts of standard maleic anhydride were added. The samples were polarographed and gave results 8 to 12% low on using the standard maleic anhydride curves. This apparently showed that the presence of the sample in the base solution had a lowering effect on maleic anhydride wave. This lowering effect was thought to be due to an increase in viscosity, but samples using small amounts of polymer gave results as low as those with large amounts. Therefore, samples containing 0.5 gram of the blank polymer with aliquots of the standard maleic anhydride solution were refluxed for 3 hours and then polaro-

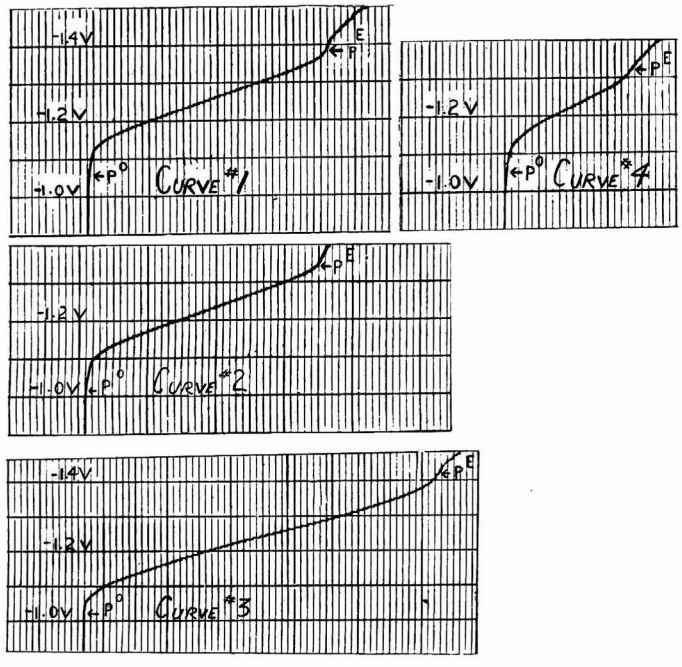

Figure 2. Current-Voltage Curves for Styrene–Maleic Anhydride Polymer

1, 2. Duplicate analyses on polymer containing approximately 0.5% of free maleic anhydride
3. Polymer containing approximately 3.0% of free maleic anhydride
4. Polymer containing approximately 0.1% of free maleic anhydride
Sensitivity. 50,000 ohms for curves 1, 2, and 3; 400,000 ohms for curve 4
Supporting electrolyte. pH 3.0 buffer solution
Temperature. 26.0° C.

Table I. Accuracy of Styrene–Maleic Anhydride Analysis

Sample		Maleic Anhydride Found	Maleic Anhydride Found	Maleic Anhydride Added
Gram	*Microamp.*	*Gram*	%	%
0.3128	44.1	0.00635	2.03	2.00
0.2491	33.9	0.00488	1.96	2.00
0.2606	35.8	0.00516	1.98	2.00
0.2573	34.6	0.00497	1.94	2.00

$m^{2/3}t^{1/6} = 1.66$ mg.$^{2/3}$sec.$^{-1/2}$

A sample of pure maleic anhydride flakes was used to make a 2.00% solution and aliquots of this (grams of sample) were added to pH 3.0 buffer solution containing 0.5 gram of R-56 dried (blank sample) per 100 ml. of solution.

Table II. Analysis of Laboratory Samples of Styrene-Maleic Anhydride

Sample No.	Sample		Maleic Anhydride Uncombined	
	Gram	*Microamp.*	*Gram*	%
R-8	0.2059	40.6	0.00585	2.85
R-8	0.2124	44.0	0.00633	2.98
R-65-A	0.5214	12.4	0.00180	0.34
R-65-A	0.5199	12.8	0.00185	0.35
R-64-A	0.5112	12.9	0.00186	0.36
R-64-A	0.5172	12.0	0.00174	0.34
R-62	0.2256	32.9	0.00126	0.56
R-62	0.2020	30.2	0.00116	0.57
R-62-C	0.5356	14.9	0.00057	0.10
2031-A	0.5109	19.2	0.000739	0.14
2031-A	0.5126	22.0	0.000846	0.16
R-60	0.5227	24.2	0.000988	0.19
R-56 (dried)[a]	0.5331	2.5	0.000100	0.01
2003	0.5147	9.8	0.000380	0.07
R-63	0.5072	15.4	0.00222	0.44
R-63	0.5037	18.6	0.00267	0.53
R-63-A	0.5088	12.7	0.00184	0.36
R-63-A	0.5168	13.2	0.00190	0.36
2515	0.2217	44.8	0.00645	2.91
2515	0.2738	56.1	0.00808	2.95
R-55	0.5151	9.7	0.00141	0.28
R-55	0.5427	10.8	0.00156	0.29
R-65-B	0.5119	10.0	0.00145	0.28
R-65-B	0.5291	11.5	0.00165	0.31
R-65	0.5125	15.6	0.00225	0.44
R-65	0.5309	16.0	0.00231	0.43
R-58-A	0.5012	18.9	0.00273	0.54
R-58-A	0.5104	20.4	0.00294	0.57
R-64	0.5045	9.5	0.00137	0.27
R-64	0.5046	9.6	0.00140	0.28
R-8A (dried)	0.5405	...	....	0.00 to 0.03[b]
R-67-B	0.4791	17.3	0.000663	0.14
R-67-B	0.5675	18.7	0.000718	0.13
R-61-A	0.5141	14.9	0.00215	0.42
R-61-A	0.5138	14.8	0.00214	0.42
R-67	0.5116	9.1	0.00132	0.26
R-67	0.5281	10.0	0.00145	0.27
R-67-A	0.5028	24.9	0.000952	0.19
R-67-A	0.5023	24.7	0.000948	0.19

[a] Sample used as blank for accuracy tests in Table I.
[b] Sample showed no measurable current, using greatest sensitivity.

graphed. Two standard curves were made up from these results and the accuracy of the method was tested as before (Table I). Four typical maleic anhydride waves found in samples of the polymer are shown in Figure 2. A number of styrene-maleic anhydride samples were then submitted for analysis (Table II). The value for the capillary constant $m^{2/3}t^{1/6} = 1.66$ mg.$^{2/3}$ sec.$^{-1/2}$.

Vinyl Acetate–Maleic Anhydride. An analytical method was needed which would determine both large and small amounts of free vinyl acetate and free maleic anhydride in solution of benzene, taken from the reaction mixture of vinyl acetate and maleic anhydride, and also in the presence of some heteropolymer formation. Because only a total value for both could be obtained by direct titration, and maleic anhydride has been determined with considerable success in other polarographic work, a combination of polarographic and volumetric analysis was decided upon.

Polarographic Analysis (for Maleic Anhydride). A pH 2.5 hydrochloric acid–potassium chloride buffer solution (*4*) was mixed 50-50 with laboratory ethanol and very good reproducible waves were obtained with as much as 2 ml. of benzene maleic anhydride solution. The reduction wave for maleic acid was proportional to concentration. Therefore, a pH 2.5 buffer solution, containing 0.01 *N* hydrochloric acid and 0.25 *N* potassium chloride with 2 grams per liter of Triton NE and 2.5 ml. per liter of a 0.2% bromocresol green solution, was used to obtain a standard curve for maleic anhydride. Standard maleic anhydride curves in the pH 2.5 buffer solution were made up, using both the 50,000-ohm shunt and 400,000-ohm shunt to govern the sensitivity of the instrument. The vinyl acetate had no effect on the maleic anhydride wave at a pH of 2.5 and good quantitative results were obtained with a standard maleic anhydride solution containing 0.00203 gram of maleic anhydride and 0.00220 gram of vinyl acetate per ml.

The benzene used contained thiophene; 2 ml. of the benzene were added to 48 ml. of the base solution and polarographed, using the 400,000-ohm shunt and then the 50,000-ohm shunt. No difference in residual current was noticed. Benzoyl peroxide (Bz_2O_2) was present in the reaction mixtures and was tested for interference. No deleterious effect was observed with double the amount expected in the reaction mixtures.

Several synthetic samples, comparable to those which were expected in the rate of reaction studies, were prepared and analyzed (Table III).

Volumetric Analysis (for Vinyl Acetate). Aliquots of reaction mixtures were shaken in 0.1 *N* sodium hydroxide, using phenolphthalein as indicator, and titrated for total acidity.

Maleic Anhydride–Vinyl Acetate Analysis (Combined). Several synthetic samples containing various amounts of vinyl acetate and maleic anhydride in benzene were made up and used for testing the accuracy of the method (Table IV). A number of laboratory samples, from the reaction of maleic anhydride and vinyl acetate in benzene solutions, were analyzed by this method (Table V). Reaction conditions such as temperature and catalyst were varied and account for the difference in data between percentage of free monomer and reaction time. The table, however, shows how one can obtain satisfactory data with the polarograph on rates of reaction of two monomers.

Table III. Accuracy of Maleic Anhydride Analysis

Sample No.	Maleic Anhydride Added	Maleic Anhydride Found	Error
	Gram	*Gram*	%
1	0.00112	0.00109	−2.6
2	0.00694	0.00715	+3.0
3	0.00347	0.00347	0.0
4	0.000562	0.00060	+6.7

Table IV. Accuracy of Combined Maleic Anhydride–Vinyl Acetate Analysis

Sample No.	Maleic Anhydride Added	Maleic Anhydride Found	Maleic Anhydride Error	Vinyl Acetate Added	Vinyl Acetate Found	Vinyl Acetate Error
	%	%	%	%	%	%
A	3.32	3.30	−0.6	5.23	5.29	+1.1
B	5.38	5.45	+1.3	4.73	4.80	+1.5
C	0.067	0.065	−3.0	0.106	0.103	−2.8
D	5.38	5.36	−0.4	4.73	4.94	+4.4

Table V. Analysis of Laboratory Samples

Sample No.	Hours	Min.	Maleic Anhydride %	Vinyl Acetate %
Starting material, 4.80% vinyl acetate and 5.45% maleic anhydride				
E-56-II	..	35	5.46	4.97
E-56-II	..	35	5.46	4.96
E-56-III	4	35	2.04	1.80
E-56-III	4	35	2.04	1.84
E-56-IV	5	30	1.45	1.42
E-56-IV	5	30	1.45	1.40
E-56-V	6	45	1.12	1.23
E-56-V	6	45	1.12	1.24
E-56-VI	8	45	0.94	1.14
E-56-VI	8	45	0.94	1.14
E-56-VII	12	45	0.82	0.92
E-56-VII	12	45	0.82	0.91
E-56-VIII	19	25	0.66	0.76
E-56-VIII	19	25	0.66	0.77
E-56-IX	24	..	0.53	0.84
E-56-IX	24	..	0.56	0.79
Starting material, 5.36% maleic anhydride and 4.94% vinyl acetate				
E-58-II	..	45	4.45	4.52
E-58-II	..	45	4.45	4.49
E-58-III	1	30	3.67	3.42
E-58-III	1	30	3.67	3.42
E-58-IV	2	32	2.88	2.60
E-58-IV	2	32	2.88	2.67
E-58-V	3	32	2.15	2.24
E-58-V	3	32	2.15	2.28
E-58-VI	4	35	1.75	1.81
E-58-VI	4	35	1.75	1.84
E-58-VII	5	35	1.62	1.42
E-58-VII	5	35	1.62	1.45
E-58-VIII	7	30	0.81	0.98
E-58-VIII	7	30	0.81	0.96
E-58-IX	10	40	0.36	0.57
E-58-IX	10	40	0.36	0.57
E-58-X	12	50	0.26	0.53
E-58-X	12	50	0.26	0.53
E-58-XI	18	40	0.25	0.49
E-58-XI	18	40	0.25	0.49
E-58-XII	24	..	0.21	0.50

RECOMMENDED ANALYTICAL PROCEDURE

Styrene–Maleic Anhydride. Weigh accurately 0.2 to 1.0 gram of the heteropolymer into a clean 125-ml. Erlenmeyer flask, add 50 ml. of pH 3.0 buffer solution, and reflux for approximately 3 hours or until the sample is in solution. (Some samples will dissolve in 30 minutes, while others may take 3 to 4 hours.) Wash the condenser down thoroughly with 20 ml. of pH 3.0 buffer solution and cool the flask to room temperature. Pour the contents into a 100-ml. graduated cylinder and wash flask out well with more pH 3.0 buffer solution, adding the washings to the graduate until diluted to 100 ml. Pour the solution from the graduate into a 125-ml. polarographic cell and flush for 10 minutes with tank nitrogen. Polarograph from −0.9 to −1.6 volts. Two polarograms are obtained for each cell and the average current rise is referred to a standard maleic anhydride curve. The temperature should be held constant within ±0.05° C. during the analysis.

Vinyl Acetate–Maleic Anhydride. Polarographic Method (for Maleic Anhydride). Weigh 5 ml. of sample into a volumetric flask and dilute to 100 ml. with benzene. Add 1-ml. or 2-ml. aliquot to hydrochloric acid–potassium chloride buffer, so that the final volume is 50 ml. Stir vigorously until slight foaming occurs. Now, flush with tank nitrogen for 10 minutes and polarograph from −0.70 to −1.35 volts, using 50,000-ohm resistance. If the wave is less than 15.0 microamperes use 400,000-ohm resistance and repolarograph. Calculate grams and per cent of maleic anhydride present from standard polarrogaphic curve. Convert grams of maleic anhydride into milliequivalents and use in obtaining per cent of vinyl acetate.

Volumetric Procedure (for Vinyl Acetate). Weigh 1 to 5 ml. of sample and add to 25 ml. of 0.1 *N* sodium hydroxide in a 125-ml. Erlenmeyer flask. Stopper and shake well for 2 minutes. Titrate excess sodium hydroxide with 0.05 *N* hydrochloric acid and add 2 to 5 ml. extra. Titrate excess acid with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator. Calculate total milliequivalents of acid present. Now, by subtracting milliequiva-

lents of maleic anhydride (obtained polarographically) from total milliequivalents of acid (obtained volumetrically), obtain the milliequivalents of vinyl acetate. The per cent of vinyl acetate and per cent of maleic anhydride are thus arrived at by a combination of two different types of chemical analyses.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Herasymenko, P., *Collection Czechoslav. Chem. Commun.*, 9, 104 (1937).
(2) Kolthoff, I. M., and Lingane, J. J., "Polarography," 1st ed., pp. 374–9, New York, Interscience Publishers, 1941.
(3) Semerano, G., and Bettinelli, G., *Gazz. chim. ital.*, 66, 744 (1936).
(4) Snell, F. D., and Biffen, F. M., "Commercial Methods of Analysis," pp. 117–18, New York, McGraw-Hill Book Co., 1944.
(5) Vopieko, E., *Collection Czechoslav. Chem. Commun.*, 8, 349 (1936).

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Lipides of the Cottonseed

Saponification Number

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The saponification number with crude cottonseed oils may be determined by carrying out the hydrolysis in an isopropyl alcoholic potassium hydroxide solution, adding ethylene or propylene glycol to give a 50-50 mixture of alcohol and glycol, and back-titrating with an alcohol-glycol solution of hydrochloric acid, using aniline blue as the indicator.

EVIDENCE from observations on yield, iodine number, and index of refraction of cottonseed extracts indicated that the extracted material obtained with a given seed specimen with any one solvent is not identical with the material extracted from that same seed specimen with another solvent (*3*). There is also a marked variation in the intensity and the quality of color of the extracts that are obtained. Those extracted with petroleum ether, for example, are fairly light in color, whereas oils extracted with dichloroethylene are extremely dark. The evidence from spectrophotometric studies with these oils indicates that a major portion of the pigments present are gossypol, gossypol tautomers, or derivatives of gossypol (*1*).

The determination of the saponification number of samples with crude cottonseed oil, in particular those that have been obtained on solvent extraction with the more polar solvents, where the determinations are carried out in the conventional manner on the back-titration of an alcoholic hydrolyzate with aqueous hydrochloric acid using phenolphthalein as an indicator, is difficult because of the difficulties involved in observing the end point of the titration. This difficulty occurs because of the presence of gossypol and gossypollike substances which are of various shades of red, and also have the property of behaving as indicators. Under these conditions it is impossible to perceive the fading of the red color of phenolphthalein.

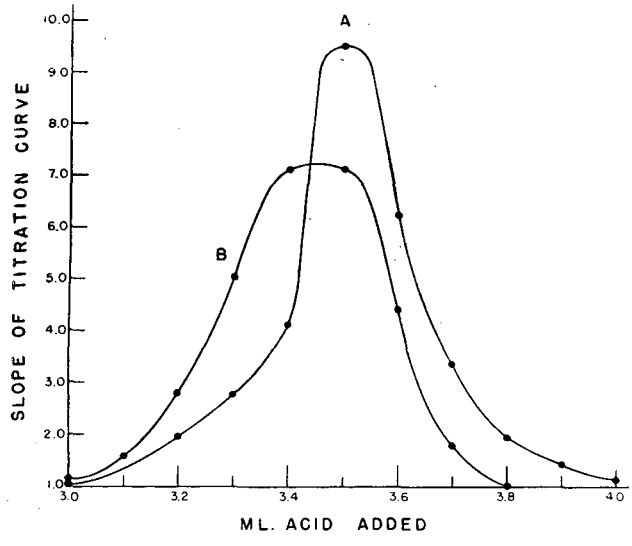

Figure 1. Slopes of Titration Curves at End Point Plotted against Acid Added

A. **Glycol-alcohol solution.** ***B.*** **Aqueous solution**

The observations reported by Palit (*4*) regarding the behavior of soap solutions prepared with mixtures of glycols and alcohols have given rise to the consideration of mixtures of these solvents in the hydrolysis and the back-titration of crude cottonseed extracts in the determination of the saponification number. This consideration is based also upon observations of the relative enhancement of the strengths of inorganic acids in organic solvents. Thus one might anticipate that the variation of the apparent pH with added hydrochloric acid at the end point corresponding to the neutralization of the excess base would be much greater in the organic than in the aqueous solution.

This prediction was verified in the experiments reported herewith. In Figure 1 the slopes of the titration curves at the end point are plotted against the volume of acid added. The end point is sharper with the ethylene glycol–alcohol solution than with the aqueous solution.

METHOD

In the determination of the saponification number the crude oil sample was weighed out in a glass weighing bucket (which weighed about 0.3 gram), equipped with a glass bale, which was lowered into a 100-ml. volumetric flask (Pyrex) with a hooked glass rod. Fifty milliliters of isopropyl alcoholic potassium hydroxide were added to the flask by pipet. The sample, together with the blank, was refluxed for 30 minutes in the volumetric flask. Adapters were prepared so that the flask could be connected directly to a condenser equipped with a $\overline{\S}$ ground-glass joint. It was established that the change in volume of the flask resulting from the refluxing was less than 0.1%.

After the contents of the flask were cooled, the flask was filled to the mark with ethylene glycol, and the contents were thoroughly mixed. Aliquots from the volumetric flask were then titrated potentiometrically with standard alcohol-glycol acid solution. A curve was drawn and the end points were thus determined. The same procedure was used with the control.

Table I. Saponification Number of Several Crude Cottonseed Oils

Solvent	Saponification No.	Solvent	Saponification No.
Chloroform	215.6	Petroleum ether	198.8
Diethyl ether	217.0	Petroleum ether[a]	200.1
Benzene	197.2	Petroleum ether[a] (seed pre-extracted with methyl alcohol)	190.2
Carbon tetrachloride	197.1		
Acetone	202.4		

[a] A different seed specimen.

EXPERIMENTAL

On titration in the ethylene glycol–alcohol solution of the hydrolyzates obtained on the extraction of cottonseed with the several solvents it was observed that the behavior of the indicators as listed by Palit was not the same as the behavior on the titration of sodium oleate. On the titration of the excess sodium hydroxide in a preparation of sodium oleate (in alcohol–glycol solution), the phenolphthalein end point occurs near the inflection point obtained corresponding to the neutralization of the excess alkali. The methyl orange and methyl red end points likewise were observed to be near the inflection points observed potentiometrically, corresponding to the replacement of the fatty acids with the hydrochloric acid. These indicators, however, do not yield satisfactory end points with the hydrolyzates of crude extracts from cottonseed. A typical titration curve, together with indications of indicator end points, is presented in Figure 2. This particular titration curve was obtained with the hydrolyzate of an isopropyl alcohol extract of cottonseed. The phenolphthalein end point, as may be seen from the curve, occurs before the true inflection point corresponding to the neutralization of the excess alkali, and the end points for methyl red and bromophenol blue occur before the end point corresponding to the replacement of the fatty acid with the hydrochloric acid. The methyl orange end point occurs after the second inflection point.

In the absence of an established principle that will permit one to translate the strength of acids as they occur in aqueous solutions to solutions of acids in organic solvents, it is not possible to predict which indicators may be useful in these titrations; the selection of a useful indicator is entirely empirical. The authors

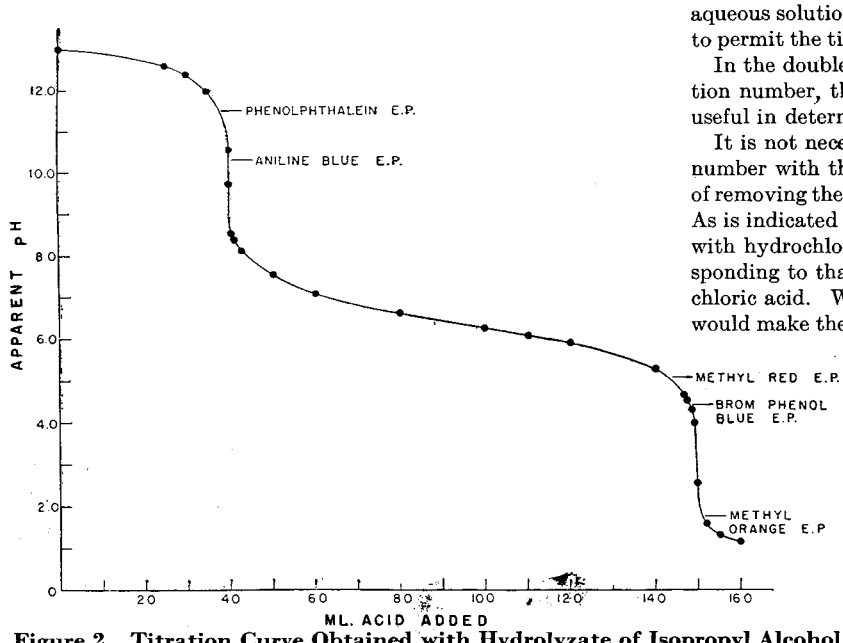

Figure 2. Titration Curve Obtained with Hydrolyzate of Isopropyl Alcohol Extract of Cottonseed

Apparent pH values where indicators change color in glycol-alcohol medium

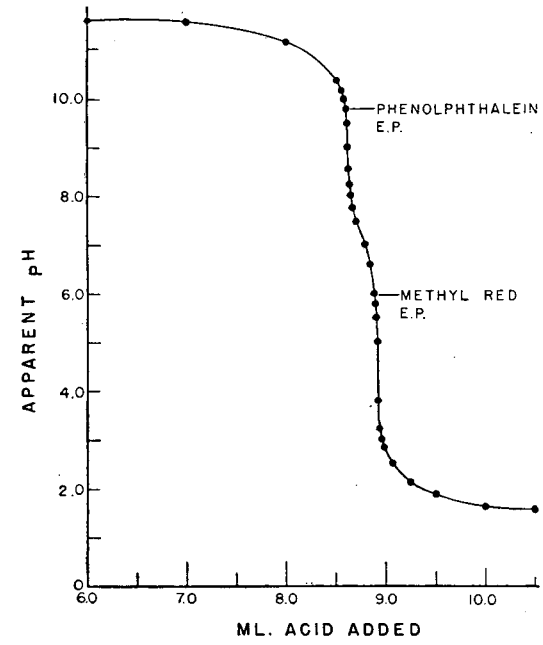

Figure 3. Titration in Glycol-Alcohol Medium of Control

Apparent pH value where carbonic acid is replaced by hydrochloric acid

have found that aniline blue is an excellent indicator for this purpose. The end point occurs at the inflection point and is extremely sharp. The procedure developed in this laboratory is capable of an accuracy comparable to that obtained in titrations of typically strong acids and bases in aqueous solution, if one takes the precaution to calibrate his pipets and is observant of the relatively viscous nature of the solvent. With purified esters saponification equivalents have been within 0.1% of the theoretical values.

Aniline blue, although it is an excellent indicator for these determinations, is not ideal in that the color occasionally fades, as, for example, with benzene extracts. There are other disadvantages with aniline blue; it does not behave as an indicator in aqueous solution and its solubility in isopropyl alcohol is too low to permit the titrations to be carried out in the absence of glycol.

In the double indicator method of determining the saponification number, the authors have not found an indicator which is useful in determining the second end point.

It is not necessary, in the determination of the saponification number with the alcohol–glycol mixture, to take the precaution of removing the carbonate from the potassium hydroxide solution. As is indicated in Figure 3, the replacement of the carbonic acid with hydrochloric acid occurs at the apparent pH value corresponding to that at which the fatty acids are replaced by hydrochloric acid. With the introduction of a second indicator which would make the double end point method applicable, it would be necessary to remove the carbonate from the potassium hydroxide solution, or to make suitable corrections.

Data were obtained for the saponification numbers of several cottonseed oils which were obtained with the Frampton-Giles low pressure extraction apparatus (*2*). These data are presented in Table I. The first six determinations were made with the oil from one seed specimen; the last two data were obtained with a second seed specimen.

The saponification numbers obtained with chloroform and diethyl ether are high. Those obtained with benzene, carbon tet-

rachloride, petroleum ether, and acetone are approximately the same as those recorded in the literature for refined cottonseed oil. On the extraction of the cottonseed with methyl alcohol before the extraction with petroleum ether, one obtains an extract with a saponification number considerably smaller than with the seed that has not been pre-extracted with methyl alcohol.

SUMMARY

A method for the determination of the saponification number of crude cottonseed extracts has been developed. The difficulties with colored cottonseed oil in observing the fading of the red color when the end point of phenolphthalein is reached in the conventional method are eliminated, and the difficulty because of the behavior of gossypol as an indicator has been circumvented by carrying the titrations out in a mixture of isopropyl alcohol and ethylene glycol with aniline blue as the indicator. The evidence is that the saponification number of oil obtained from a given seed specimen is a function of the solvent used in the extraction.

LITERATURE CITED

(1) Boatner, Charlotte, *et al.*, *J. Am. Oil Chemists Soc.*, **24**, 276 (1947); *Oil & Soap*, **21**, 10 (1944); *J. Am. Chem. Soc.*, **66**, 838 (1944); **69**, 668 (1947).
(2) Frampton, V. L., and Giles, F. K., IND. ENG. CHEM., ANAL. ED., **17**, 647 (1945).
(3) Frampton, V. L., Webber, H. H., and Giles, F. K., *Oil & Soap*, **23**, 318 (1946).
(4) Palit, S., *Ibid.*, **23**, 58 (1946), *J. Am. Oil Chemists Soc.*, **24**, 190 (1947); *J. Am. Chem. Soc.*, **69**, 3120 (1947); IND. ENG. CHEM., ANAL. ED., **18**, 246 (1946).

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Measurement of Heat of Combustion of Volatile Hydrocarbons

Liquid Sample Holder

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A sample holder, having advantages over existing liquid sample-handling techniques, for holding low boiling liquid fuels in determining the heat of combustion is described. Typical results are given for pure hydrocarbons and production grade motor and aviation fuels. Isopentane is securely held at 25° C., only 3° C. below its boiling point.

EVEN though the petroleum industry's largest volume product is gasoline, there is at present no standard procedure for handling samples of the material in the measurement of its heat of combustion (*4*). The usual procedure is to use the A.S.T.M. method for the thermal value of fuel oil (*2*) with either of two types of sample holders—gelatin capsules (*3*) or glass ampoules (*5*). The precautions necessary in the successful use of gelatin capsules are involved and lack of attention to any detail leads to erratic results. Two fundamental difficulties here are a large ratio of auxiliary material to fuel sample burned and permeability of the gelatin capsule which requires an evaporation correction during handling. In addition, the water content of the gelatin must be carefully controlled. The glass ampoule suggested by Prosen and Rossini (*5*) is very satisfactory where extreme accuracy is desired, but it is rather difficult to prepare these glass ampoules in routine work, where the glass blowing is not done by the calorimeter operator.

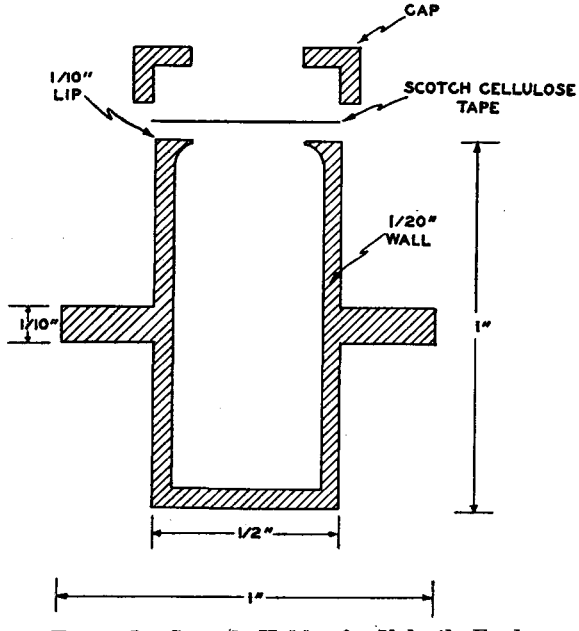

Figure 1. Sample Holder for Volatile Fuels

The sample holder described here is a simple corrosion-resistant cup with ordinary cellulose Scotch adhesive tape (Minnesota Mining and Manufacturing Company) cover. Others have suggested a similar scheme but have used an inferior covering material for the purpose. For example, Richter (*6*) described a steel crucible with collodion cap which he claimed is superior to the gelatin capsule; later he used a platinum crucible with the same covering scheme. Considerable difficulty has been experienced by the authors with both collodion and cellophane covers. They are either too brittle or of indeterminate moisture content and they are about as permeable as the gelatin capsule.

SAMPLE HOLDER

The sample holder, shown in Figure 1, is turned from a 2.5-cm. (1-inch) diameter bar of 52% nickel alloy steel to the dimensions shown, which is a convenient size for charging 1 ml. of liquid. (This high-nickel alloy is corrosion-resistant but not as good as the illium alloy customarily used for oxygen bombs. The Parr Instrument Company, Moline, Ill., will furnish this holder in illium.) The cap is turned from a separate piece of the same 52% nickel stock to the dimensions shown. The 0.5-inch diameter Scotch cellulose tape circle is conveniently cut with the appropriate cork borer.

Procedure for Handling Sample Holder. Before each use the lip of the sample holder is polished on crocus cloth. The sample holder with cap, but without Scotch tape, is weighed to the nearest 0.1 mg. on a suitable analytical balance. The Scotch tape is placed in the cap of the sample holder and the assembly is again weighed, with cap upside down on the balance pan beside the

Table I. Inspections on Fuels

Distillation D-86	Natural	Regular	Premium	91/96	100/130
Start, %	102	98	94	109	116
5	118	118	115	135	137
10	124	131	126	144	147
20	132	153	146	156	157
30	140	176	166	166	169
40	150	200	188	178	181
50	164	223	210	188	195
60	180	245	230	198	207
70	198	269	250	207	217
80	216	297	276	220	228
90	252	336	310	237	248
95	286	365	344	254	271
E.P.	346	402	377	291	338
Loss	2.0	1.2	1.2	1.2	1.2
A.P.I. gravity	70.1	59.2	62.6	63.9	67.3
Aniline point, ° F.	132.8	101.1	108.9	123.8	139.6

holder. The difference between these two weights gives the weight of Scotch tape used to seal the holder. This weight is in the order of 13 mg., which is equivalent to about 2% of the weight of gasoline. Exactly 1 ml. of fuel is pipetted into the holder with the tip of the pipet on the floor of the cup. The cap with Scotch tape is quickly placed on the cup and pressed into place with a slight twisting motion, and the assembled sample holder is again weighed to give the weight of sample enclosed. The sample holder is placed in the supporting ring attached to the bomb cap and the previously attached ignition wire is bent so that it lightly touches the Scotch tape cap without touching the metal retaining ring. No spiral or coil is necessary, just a simple hairpin bend. Just as the sample holder is being lowered into the bomb a tiny hole, which may vary in diameter from 0.05 to 0.10 mm., is pricked in the taut cover with a sharp needle. The bomb is closed and filled immediately but slowly with oxygen to a pressure of 35 atmospheres.

Calorimeter and other Accessories. In the tests described the Parr Model DD-11 adiabatic oxygen bomb calorimeter was used with a single-valve, illium bomb with self-sealing head. Solid-stem mercurial thermometers furnished with the calorimeter, graduated in units of 0.05° F., were used together with their calibrations. Although temperatures may be estimated to 0.005° F. with the aid of a telescopic reading lens, reproducibility in readings is not better than 0.01° F. and this is the limiting factor in accuracy so far attainable by this procedure. (Later work using a platinum resistance thermometer showed very satisfactory reproducibility.) The bomb used in these tests had a volume of approximately 360 ml. Iron firing wire supplied by the Parr Instrument Company was used. The water equivalent, evaluated with approximately 1-gram pellets of benzoic acid under the same conditions of test as with the liquid fuel samples, was 2450 grams for this particular experimental setup.

Table II. Accuracy

Hydrocarbon	Gross Heat of Combustion (Weight in Air) Observed	Lit. (*1*)
	B.t.u./pound	
2,2,4-Trimethylpentane	20,510	
	20,553	
	20,553	
	20,585	
	20,540	
Av.	20,548	20,556
Difference	8	
2-Methylbutane	20,852	
	20,927	
	20,873	
Av.	20,884	20,877
Difference	7	

Table III. Reproducibility of Gross Heat of Combustion

Regular Quality	Premium Quality	91/96 Grade Aviation	100/130 Grade Aviation
	B.t.u. per pound		
....	20,097	20,217	....
20,000	20,096	20,237	....
19,962	20,100	20,241	20,283
20,007	20,023	20,232	20,293
Av. 19,990	20,079	20,232	20,288
Largest deviation from average, %			
0.15	0.28	0.08	0.03

Calorimetric Procedure and Corrections. All of instructions of A.S.T.M. procedure D240–39 which applied in this setup were followed. No radiation correction was necessary because of adiabatic operation. Corrections for nitric acid formed were made by titration with standard alkali, and the length of burned iron wire was taken into account. A correction value of 10 B.t.u. per mg. weight of the cover was subtracted from the product of the water equivalent times ° F. rise for the weight of Scotch cellulose tape burned. This was an experimentally determined average value for several samples of Scotch cellulose tape.

FUEL SAMPLES

2,2,4-Trimethylpentane, primary fuel standard certified by the National Bureau of Standards, boiling point 99.23° C., refractive index n_D^{20} 1.39146, density d_4^{20} 0.69191, freezing point −107.40° C. Product of the Rohm and Haas Company.

2-Methylbutane, better than 99% purity, density d_4^{20} 0.6196, refractive index n_D^{20} 1.3575, boiling range 27.8–27.9° C.

A 10-pound Reid vapor pressure natural gasoline, inspections given in Table I.

Production fuels, regular and premium grade motor fuels, 91/96 grade and 100/130 grade aviation fuels, inspections given in Table I.

DISCUSSION OF RESULTS

The accuracy attainable with pure hydrocarbons is indicated by Table II.

The reproducibility on production grade engine fuels is shown by Table III.

The holder was developed especially to hold isopentane at about 25° C., only 3° C. below its boiling point. Scotch cellulose tape was found superior to other membranes for this purpose in several respects. The adhesive of the tape is made stickier by isopentane vapors and the seal is exceptionally tight, so tight in fact, that the holder may be turned upside down without any loss of sample once the seal is made. This same swelling of the adhesive in the hydrocarbon vapors "heals" the pricked hole required to prevent rupture of the taut membrane on addition of oxygen. This effect prevents evaporation of the sample into the bomb cavity before oxygen is added. It has been found easier to obtain ignition with this type of holder and liquid samples than with any other so far tried.

CONCLUSION

The technique described here has so greatly simplified the handling of gasoline samples that it is now the practice in these laboratories to determine heat of combustion experimentally where any question arises concerning the validity of a calorific value estimated from the physical properties.

ACKNOWLEDGMENTS

Experimental work by Paul E. Monson contributed greatly to the success of the investigation, and cooperation of the Laboratories Division of the Standard Oil Company of California helped to establish the general utility of the method. Their assistance is gratefully acknowledged.

LITERATURE CITED

(1) Am. Petroleum Inst., Project 44, Tables of Physical Properties of Hydrocarbons.
(2) Am. Soc. Testing Materials, Standard on Petroleum Products and Lubricants, A.S.T.M. Committee D-2, Designation D240–39 (December 1946).
(3) Dean, E. W., Williams, A. A., and Fisher, N. E., IND. ENG. CHEM., ANAL. ED., 16, 182 (1944).
(4) Federal Standard Stock Catalog, Section IV, part 5, Method 250.21, VV-L-791c-115 (May 1945).
(5) Prosen, E. J. R., and Rossini, F. D., *J. Research Natl. Bur. Standards*, 27, 297 (1941).
(6) Richter, M., *Deutsche Luftwissen*, 6, 128 (April 1939).

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Pore Size Distribution in Porous Materials

Interpretation of Small-Angle X-Ray Scattering Patterns

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The theory of small-angle x-ray scattering as developed by Guinier is applied simply and approximately to porous aggregates and the results are reduced to continuous distributions of pore size. A method is presented for the inversion of the Guinier integral under certain simplifying assumptions. A simple semi-empirical method of making the correction for a finite collimating system is developed and applied. Results are correlated with data from adsorption measurements and from the mercury porosimeter.

THE relation between the state of subdivision of a mass of matter and the angular distribution of x-ray intensity scattered by the mass has been discussed analytically by Guinier (*7*). Several methods of using this theory for interpreting the scattering patterns in terms of particle or pore size have been advanced.

Biscoe and Warren (*3*) evaluated the average particle size of carbon black on the assumption of only small departures from the average size. Jellinek and Fankuchen (*9*) did the same for certain inorganic gels. In a later paper, Jellinek, Solomon, and Fankuchen (*10*) amplified their method to the case where the size distribution may be considered as a collection of a small number of discrete constant sizes. Shull and Roess (*5, 13–15*) developed a workable method for determining a continuous distribution based on the determination of parameters in a postulated flexible distribution. More recently Bauer (*2*) and Roess (*12*) devised methods for the determination of continuous distributions wherein no *a priori* assumption concerning the kind of distribution is made. The latter two methods are tedious for numerical work and are perhaps too laborious to justify application to masses that obey only very approximately the assumptions made in the development of the Guinier theory.

This paper presents a method for analyzing small angle x-ray scattering data in terms of a continuous distribution of pore (or particle) sizes. Also presented are some typical results together with comparisons with the results of other physical methods of elucidating pore geometry.

In March 1947, before the present paper was submitted for publication, the work of Shull and Roess (*15*) appeared; this had been presented in substance before Gibson Island Conferences in 1944 and 1945. There is a strong similarity in the two treatments, and there is no doubt that Shull and Roess have established priority. The present paper is an independent work and places emphasis on the alternative pore size interpretation.

The experimental arrangement is familiar. Figure 1 shows a diagram of that used by the authors.

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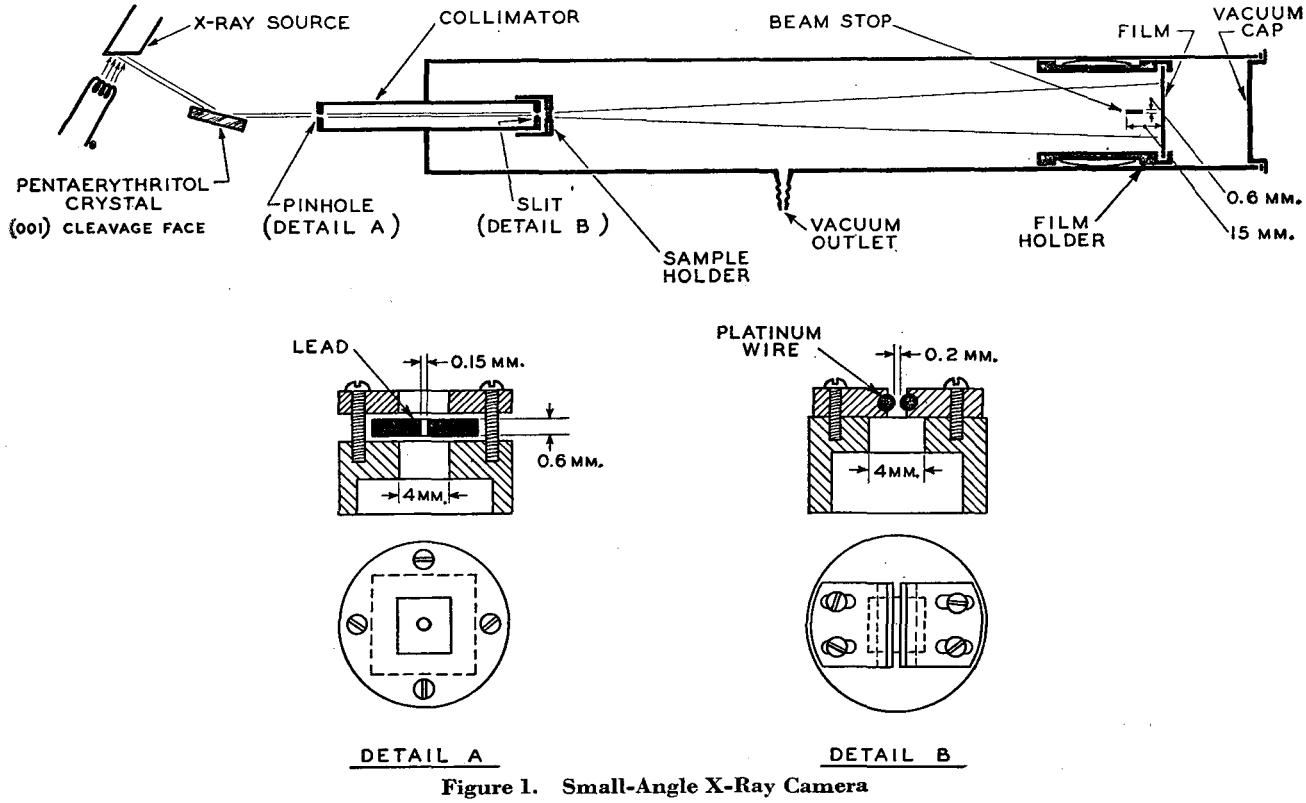

Figure 1. Small-Angle X-Ray Camera

The camera tube is a length of 10-cm. (4 inch) aluminum pipe, in one end of which is fitted a collimating tube made vacuum-tight by a piece of cellophane cemented over the entrance slit. The other end is capped with a gasketed disk. The film holder is attached to a piston which slides in the tube and may be placed at any desired position up to 60 cm. from the sample. Provision is made to evacuate the camera (to about 15 mm. of mercury) during exposure in order to eliminate air-scattering.

The main beam is caught by a beam-stop 0.6 mm. wide to prevent overexposure at the center of the film. Intensity readings are therefore possible to within about 0.3 mm. of zero scattering angle at sample-to-film distances up to about 60 cm. The collimator is 15 cm. long. Its entrance slit is a pinhole 0.15 mm. in diameter punched in a lead sheet 0.6 mm. thick. The exit slit is a narrow rectangle 4 mm. long and 0.1 mm. wide. The main beam is therefore collimated to a very thin and narrow fan shape. The edges of the exit slit are inlaid with 1-mm. platinum wire to prevent transmission through sharp slit edges, which affects the film in a manner indistinguishable from small-angle scattering.

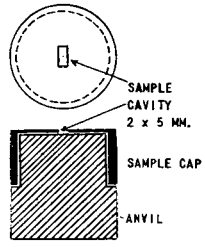

Figure 2. Sample Cap and Anvil

The x-ray beam from the target is monochromated by Bragg reflection from pentaerythritol (*6*). Although the life of this crystal under the x-ray beam is short (about 100 hours), the intensity of its 002 reflection is high enough to overweigh the bother of frequent renewal.

The sample is ground, mixed with very dilute collodion solution, and placed in the rectangular window of the sample holder by smearing it over a small anvil (Figure 2). The holder is then placed like a cap over the end of the collimating tube for the exposure.

Exposures ranged from 10 to 80 hours, depending on sample, sample-to-film distance, kind of radiation, and part of pattern required. Development was according to a standardized procedure on calibrated film, and intensities were determined by a Leeds & Northrup microphotometer. Intensities higher than density 1.1 were discarded, because the film began to depart from linearity above this density; and densities below 0.02 were discarded because of the relatively high "noise" shown by the microphotometer trace.

The experimental curve of observed intensity, $I_{obs.}$, versus the scattering chord, x, was obtained by averaging the microphotometer readings from the left and right sides of the theoretically symmetrical pattern and smoothing the data. Several exposures of one sample were normally made with long and short exposure times. This procedure permits piecing together several patterns to obtain a single intensity curve covering a range of intensities impossible to reach with one exposure because of the relatively narrow film latitude. Intensity ranges of at least 1000 to 1 were normally obtained, but the lower end of this range was frequently discarded as unreliable. The used intensity range was always at least several hundred to one. Fitting two patterns together at the region of overlap offers no difficulty, as only relative intensities are of interest here. Figure 3 shows a typical observed intensity curve (I, *A*) and the derived logarithmic curve (II, *A*). Use of the logarithmic curve greatly facilitates both the smoothing and the fitting processes.

Guinier (*7*) has shown that for a single spherical particle of radius r, immersed in a finely collimated beam of monochromatic x-rays of wave length λ and intensity I_0, the fraction of incident intensity scattered to a chordal distance z from the main beam is given approximately by

$$\frac{I}{I_0} \propto r^6 \exp(-a^2r^2z^2)$$

where

$$a^2 = \frac{4\pi^2}{5\lambda^2 s^2}$$

and

s = sample-to-film distance

The very fine beam required by this theory is not practicable because the scattered radiation is too weak. To increase the intensity of the main beam, and hence the scattered radiation, the beam is enlarged into a second dimension by opening up the pinholes to narrow rectangular slits. When the angular spread of the main beam is comparable to the scattering angle, however, it is necessary to employ a slit correction to reduce the experimentally obtained intensity back to what would have been obtained had pinholes been used. The complexity of this correction increases with departure of the collimating system (including the detector slit) from infinitesimal pinholes. The pinhole-slit combination described above makes the correction particularly simple, and is due to Shull. In both methods of correction described below, the detector slit is taken as small.

PORE GEOMETRY

The geometry involved in this system is diagramed in Figure 4. The plane of the fan-shaped beam is the plane of the paper, while the plane of the film is perpendicular to it. We seek the intensity distribution along the equator (x-axis) of the film.

Consider an element of the fan beam, dy, at Q on the film meridian, distant y from the center. The scattered intensity at P, distant z from Q, is given by Guinier's relation

$$d\left(\frac{I}{I_0}\right) \propto r^6 \exp(-a^2r^2z^2)\, dy$$

Inasmuch as evidently $z^2 = x^2 + y^2$

$$d\left(\frac{I}{I_0}\right) \propto r^6 \exp(-a^2r^2x^2) \exp(-a^2r^2y^2)\, dy$$

is the contribution to the intensity at Q due to the pinhole beam, OQ. The total for the entire fan beam is then

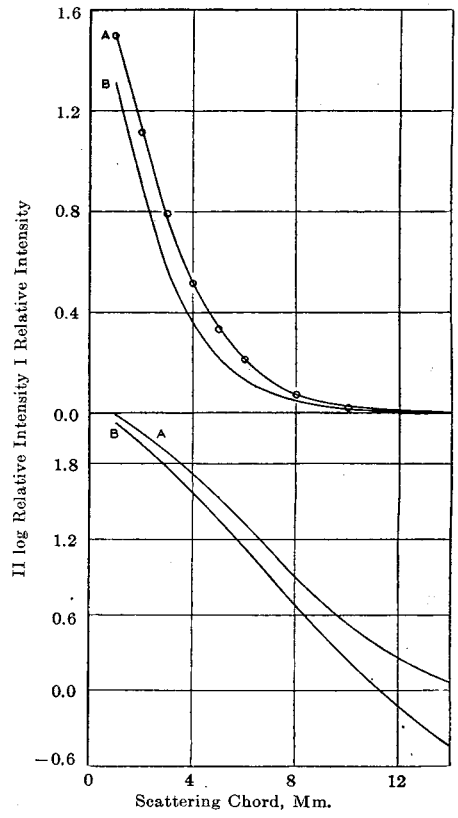

Figure 3. Observed and Corrected Intensity Curves

A. **Observed**
B. **Corrected**
O. **Observed computed**

$$I \propto \int_{-Y}^{Y} r^6 \exp(-a^2r^2x^2) \exp(-a^2r^2y^2)\, dy$$

The integral over y may be evaluated by means of the error function,

$$erf(T) = \frac{1}{\sqrt{2\pi}} \int_0^T e^{-t^2/2}\, dt$$

to give

$$I \propto r^5\, erf(\sqrt{2}\, aYr) \exp(-a^2r^2x^2)$$

as the scattered intensity at any point on the film equator due to a single spherical particle of radius r. The intensity due to dN such particles is just dN times this.

[The value of Y, half the length of the slit projected on the film, is a function of s and may be determined as follows: Short exposures are made, with no sample in place, with the film at a series of measured distances from the sample cap. After development the length of each slit image, $2Y$, is measured and a plot drawn of Y *vs.* s. The proper Y to use for any s is then either read directly from the graph or computed from the linear function, $Y(s)$.]

Let the volume distribution of pores be given by $D(r)$ where the total volume, dV, of all pores having radii between r and $r + dr$ is $dV = D(r)dr$. The number of pores within this range is

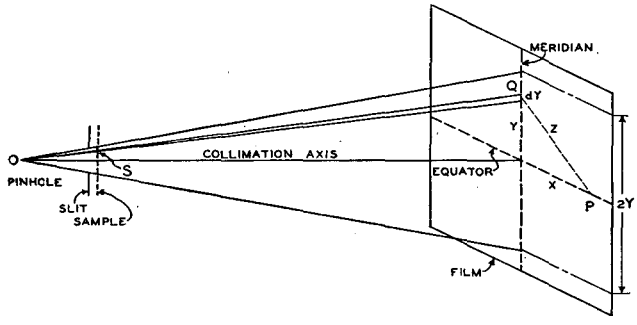

Figure 4. Geometry of Slit Correction

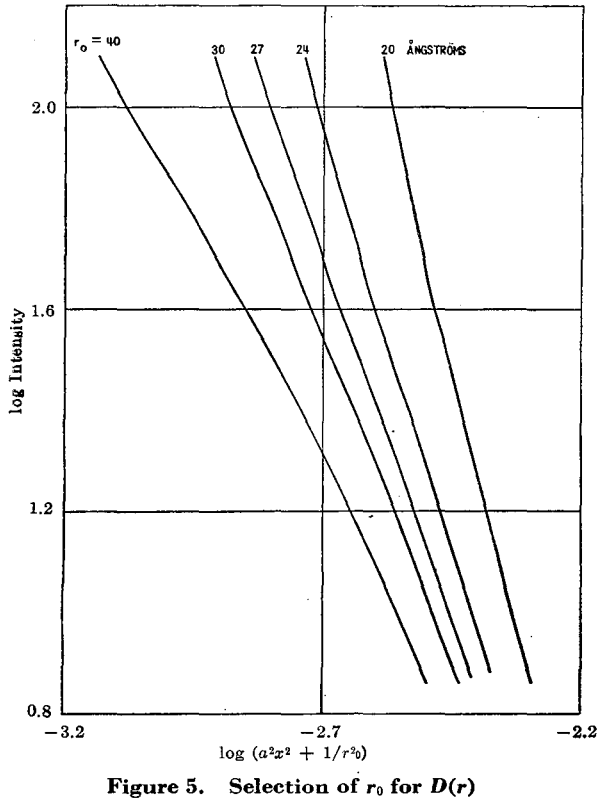

Figure 5. Selection of r_0 for $D(r)$

$$dN = \frac{dV}{kr^3} = k^{-1}r^{-3}\, D(r)dr$$

where kr^3 is the volume of one such pore. The scattering for these pores is then

$$dI \propto r^2\, D(r)\, erf(\sqrt{2}\, a\, Yr) \exp(-a^2x^2r^2)\, dr$$

and the total intensity at any point due to the entire distribution of sizes, providing the pores are randomly and widely separated spheres which produce no coherent interference, is

$$I(x) \propto \int_0^\infty r^2\, D(r)\, erf(\sqrt{2}\, a\, Yr) \exp(-a^2x^2r^2)\, dr \qquad (1)$$

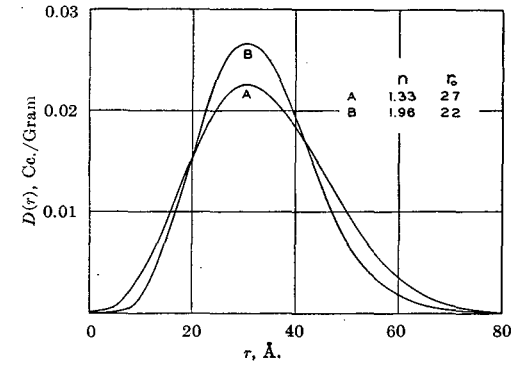

Figure 6. Distribution Function of Silica-Alumina Gel

Two methods of slit correction

This integral is not easily inverted—that is, there is no simple general method for computing $D(r)$, given $I(x)$. It happens, however, that the *erf* term is nearly constant for $r \gtrsim \frac{\lambda s}{Y}$. Using Cu-K$_\alpha$ radiation of $\lambda = 1.54$ Å., a sample-to-film distance of 15 cm. or less, and Y of about 5 mm., the *erf* term is practically constant for $r \gtrsim 40$ Å. and changes only slowly for $r \gtrsim 15$ Å. As a first approximation, then,

$$I(x) \propto \int_0^\infty r^2\, D(r) \exp(-a^2x^2r^2)dr \qquad (2)$$

We now assume that $D(r)$ is of a modified Maxwellian form (*8*), expressible analytically as

$$D(r) = A\left(\frac{r^2}{r_0^2}\right)^n \exp\left(-\frac{r^2}{r_0^2}\right) \qquad (3)$$

and attempt to find the values of n and r_0 which best fit the experimental data—that is, we seek that member of the double infinity of curves, Equation 3, which most closely approximates in x-ray scattering behavior the sample whose behavior is given by $I(x)$. The resulting expression for $I(x)$ can now be integrated in terms of the gamma function to give

$$I(x) = C\,\Gamma\left(n + \frac{3}{2}\right)\left[a^2x^2 + \frac{1}{r_0^2}\right]^{-\left(n+\frac{3}{2}\right)}$$

It is convenient to write this logarithmically, dropping the constant, to give

$$\log I(x) = \log\Gamma\left(n + \frac{3}{2}\right) - \left(n + \frac{3}{2}\right)\log\left(a^2x^2 + \frac{1}{r_0^2}\right) \qquad (4)$$

which equation is linear in $\log I$ and $\log\left(a^2x^2 + \frac{1}{r_0^2}\right)$.

In this last equation, I, a, and x are known and n and r_0 are to be evaluated. In application, one chooses various values of r_0 and plots $\log I$ against $\log\left(a^2x^2 + \frac{1}{r_0^2}\right)$. That value of r_0 which makes the plot most nearly linear is the best r_0; and from the slope of this line one may determine n.

Figure 5 shows this process for the material of Figure 3. Plots of log I *vs.* log $\left(a^2x^2 + \frac{1}{r_0^2}\right)$ are given for r_0 = 40, 30, 27, 24, and 20 Å. The definite curve to the right for r_0 too low and to the left for r_0 too high is evident, and r_0 = 27, giving the most nearly linear curve, was taken as the correct value. The slope of this line was found to be −2.83, which gives n = 1.33 according to Equation 4. $D(r)$ is therefore given by

$$D(r) = A\left(\frac{r}{27}\right)^{2.66} e^{-\left(\frac{r}{27}\right)^2}$$

Curve A of Figure 6 shows the form of this distribution. As plotted here the constant A in Equation 3 has been chosen to normalize the curve to make $\int dV$ from r = 0 to r = ∞ equal to V_0, the measured pore volume.

Having determined the parameters n and r_0 it is possible to evaluate $I(x)$ numerically, now including the *erf* term, according to Equation 1. If the computed I deviates too widely from the observed I, $D(r)$ may be distorted to give better agreement. If, however, there is a large contribution of r smaller than about 20 Å., it is usually more convenient to make the slit correction independently than to include the correction in the analysis—that is, it is simpler to reduce $I_{obs.}$ to $I_{cor.}$ before applying the Guinier theory. Further, it is necessary to make the slit correction before applying other methods of inverting the integral such as those of Bauer and of Roess. A method for making an independent slit correction is described below.

Referring again to Figure 4, and to the element, dy, of the fan beam which strikes the film at Q, the scattering due to this elementary beam alone is radially symmetrical about Q, depending only upon the distance from Q. In particular, the scattered intensity at the equatorial point, P, depends only on the distance, $QP = z$. This dependence is that given by the Guinier theory and is what we seek experimentally. Denote this relation between intensity I and radial distance, z, by $I = I(z)$. The intensity at P due to the beam, dy, is then $dI = I(\sqrt{x^2 + y^2})dy$. The intensity most conveniently measured is $I_e(x)$, the intensity along the equator, as a function of distance from the center. Along this line dI and dI_e are equal. Replacing dI by dI_e and forming the integral for all dy's,

$$I_e(x) = 2\int_0^Y I(\sqrt{x^2 + y^2})\, dy \qquad (5)$$

In this equation $I_e(x)$ is the experimentally determined equatorial intensity, and the required function is $I(z)$. Although there appears to exist no exact method for the inversion of this integral, the following approximate procedure has been found satisfactory:

Assume that it is possible to expand the unknown function $I(z)$ in a finite series of Gauss functions:

$$I(z) = \sum_i c_i e^{-\alpha_i z^2} \qquad (6)$$

Then the integral equation above becomes

$$I_e(x) = 2\int_0^Y \sum_i c_i e^{-\alpha_i (x^2+y^2)}\, dy = \sum_i \frac{c_i}{\sqrt{\alpha_i}} e^{-\alpha_i x^2}\, erf\,(\sqrt{2\alpha_i}\, Y),$$

where the error function has again been introduced and distributive constants dropped. Combining everything independent of x we may write

$$I_e(x) = \sum_i b_i e^{-\alpha_i x^2} \qquad (7)$$

where

$$b_i = \frac{c_i}{\sqrt{\alpha_i}}\, erf\,(\sqrt{2\alpha_i}\, Y)$$

Solving for c_i, one obtains finally

$$c_i = \frac{\sqrt{\alpha_i}\, b_i}{erf\,(\sqrt{2\alpha_i}\, Y)} \qquad (8)$$

Thus it appears that if the experimentally obtained equatorial intensity $I_e(x)$ be expanded empirically into the finite series of Gauss functions (Equation 7), the required slit-corrected intensity will be a new similar series of Gauss functions (Equation 6) differing only in that the new coefficients c_i have been changed from the old ones b_i according to Equation 8, and the argument, x, replaced by z. Experience has shown that from three to five (usually four or five) terms in the expansion will reproduce the experimental data well within the error of reading the microphotometer traces. Curves IB and IIB of Figure 3 show the slit-corrected values corresponding to the uncorrected curves, A. The necessity for applying the correction is obvious. Having obtained $I(z)$, the corrected intensity function, it is possible to check the accuracy of the approximation by placing $I(z)$ in Equation 5 and numerically evaluating the integral for $I_e(x)$ at selected values of x. The circled points in Figure 3 show the results of such a calculation at several values of the scattering chord. The negligible deviation from the observed intensity implies that the series (Equation 6) is an adequate approximation.

Using this new intensity function we may now employ Guinier's theory directly in the form

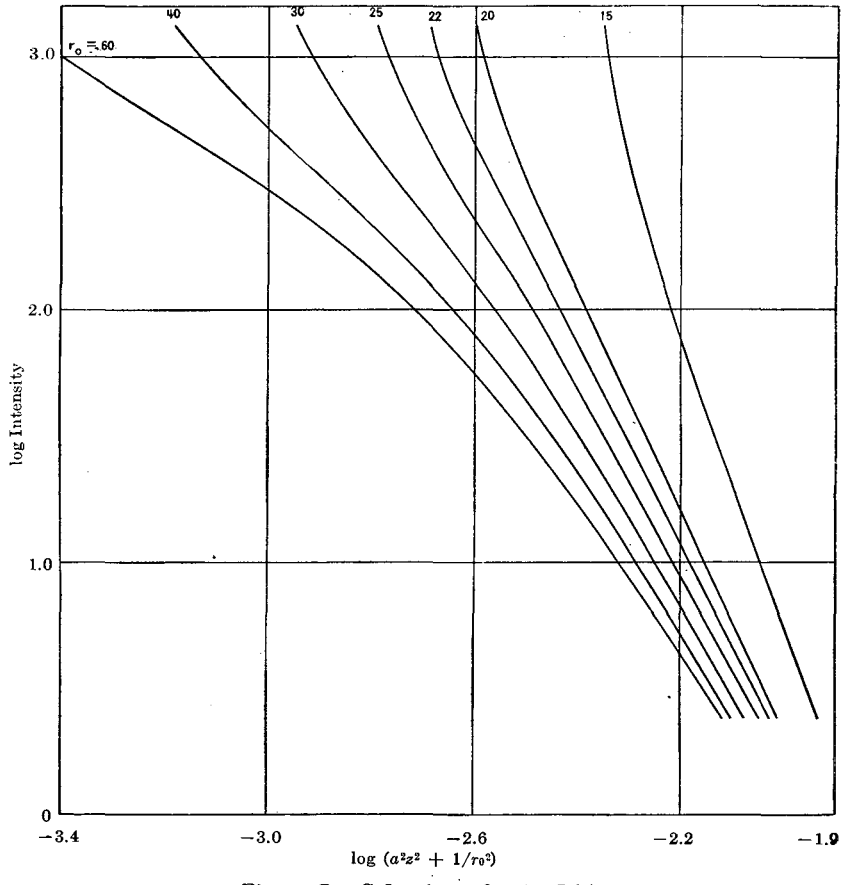

Figure 7. Selection of r_0 in $D(r)$

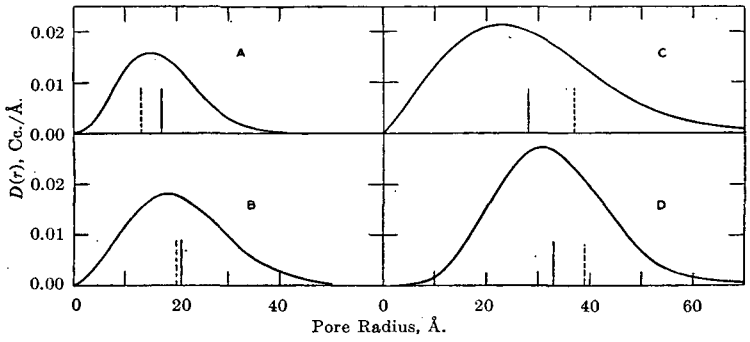

Figure 8. Pore Size Distributions for Some Silica-Alumina Gels

Other data given in Table I. Average pore radius indicated: r_c - - - - $\bar{r}$ ——

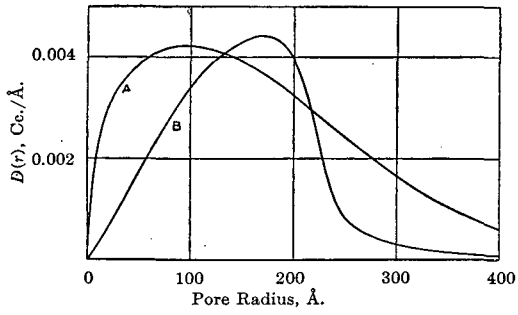

Figure 9. Pore Size Distribution for a Fuller's Earth

***A.* By small-angle x-ray scattering**
***B.* By mercury porosimeter**

$$d\left(\frac{I}{I_0}\right) \propto r^6 \exp(-a^2r^2z^2)dN$$

giving the contribution to the relative intensity at a scattering chord of Z due to dN pores of radius r. For $dN = k^{-1}r^{-3}D(r)\,dr$ such pores,

$$d\left(\frac{I}{I_0}\right) \propto r^3\,D(r)\exp(-a^2r^2z^2)dr$$

Thus for the whole range of r's from zero to infinity,

$$I(z) \propto \int_0^\infty r^3\,D(r)\exp(-a^2z^2r^2)\,dr$$

Again assuming $D(r)$ to be given analytically by Equation 3, the integration may be performed and the result written as

$$\log I(z) = \log\Gamma(n+2) - (n+2)\log\left(a^2z^2 + \frac{1}{r_0^2}\right) \qquad (9)$$

Thus a plot of $\log I$ against $\log\left(a^2z^2 + \frac{1}{r_0^2}\right)$ will be linear for the correct r_0 and the slope of this line affords a determination of n.

Figure 7 shows a set of these plots for the material of Figure 3, using r_0 = 60, 40, 30, 25, 22, 20, and 15 Å. Again there is a clear tendency to curve in opposite directions for r_0 too low and too high. The best r_0 was taken as $r_0 = 22$.

The slope of this line is -3.96, giving $n = 1.96$ according to Equation 8. The distribution function is then given by

$$D(r) = A\left(\frac{r}{22}\right)^{3.92} e^{-\left(\frac{r}{22}\right)^2}$$

where A may be chosen by normalizing to V_0. Curve B of Figure 6 compares the distribution obtained using this method of independent slit correction with that obtained with the earlier method. The fact that the two curves differ only slightly, even having their peaks at about the same value of r, shows that the less rigorous first method of slit correction is useful when, as here, there is a relatively small contribution of pores below about 15 Å.

Figure 8 shows the plotted distributions as determined by this second method for four silica-alumina gels of different characteristics. Each has been normalized to V_0, the pore volume, as measured by the usual density determinations. Figure 9 shows the distribution determined by the same method for a fuller's earth having a very much wider size range than that of the gels. Table I lists some other pertinent data for all five materials.

PORE VOLUME AND SURFACE AREA

In working with pore geometry there are two most useful measurements by well-established methods that provide data for correlation and orientation: the pore volume and the surface area as determined by adsoprtion methods. By comparing these, a value for "average pore size" may be selected. Thus, by considering the pores as uniform cylinders of constant cross-sectional radius, r_c, having a total volume = V_0 and a total inner surface area of S_0, the measured surface area [the authors have used the Brunauer-Emmett-Teller method (*4*) of computing surface area from nitrogen adsorption isotherms], it readily follows that $r_c = 2V_0/S_0$. Although not a true average, this quantity provides an excellent gage of reliability.

The comparison may be made by computing an average pore radius from $D(r)$. The conventional definition of the average $\bar{r}$ would be

$$\bar{r} = \frac{\int_0^\infty rD(r)dr}{\int_0^\infty D(r)dr}$$

By evaluating this expression for $D(r)$ given by Equation 3, it is found that

$$\bar{r} = \frac{\Gamma(n+1)}{\Gamma\left(n+\frac{1}{2}\right)}\,r_0 \qquad (10)$$

so that it is particularly simple to compute $\bar{r}$ when n and r_0 are known. The values, $\bar{r}$ from small-angle x-ray scattering, and r_c from density and adsorption data, are comparable values, and Table I shows some typical examples of the kind of agreement found. The values of $\bar{r}$ and r_c are also superposed on the plotted distributions of Figure 8.

Table I. Pore Data for Typical Porous Materials

		Adsorption Data		X-Ray Data		
Material	Pore volume, V_0, Cc./G.	Surface area, S_0, Cc./Å. G.	Av. pore radius, r_c, Å.	Parameters n	r_0	Av. pore radius, $\bar{r}$, Å.
Silica-alumina gel A	0.285	0.0440	13	1.00	15	17
B	0.459	0.0466	20	0.87	20	21
C	0.747	0.0409	37	0.58	30	28
D	0.733	0.0365	39	1.96	22	33
Fuller's earth	0.787	0.0129	122[a]	0.154	250	169[a]

[a] The average pore radius from porosimeter data is 142 Ångströms.

Although the average pore radii, $\bar{r}$ and r_c, are not strictly representations of the same quantity, it is not profitable to apply any of the obvious refinements. The unfortunate fact is that the whole theory of Guinier is based on the assumption that the scattering particles are spherical and sufficiently well and randomly separated to avoid coherent interference between particles. The circumstance of nonsphericity may be allowed for [as has been done by Guinier (*7*); see also Roess (*12*)]; but failure to meet the other essential requirement in most common samples

cannot be circumvented. The intensity of scattered radiation depends on a difference in scattering power between the two phases (solid and air in the case of porous solids), and the resultant size determination (neglecting interference) is a measure of the distance one can travel in the scattering volume without passing a phase boundary in either direction. In the case of a dispersed system of solid and air, when the particles are widely separated, the scattering due to their distances apart will be suppressed and one will obtain a particle size distribution. When the particles are squeezed so closely together that the interstices are small compared with particle size, the small-angle scattering will be indicative of pore size. There is no difference (except in over-all intensity) in scattering pattern between that obtained from a collection of particles with interstices of air, and that obtained from the reverse of this: a sponge in which the former particles are replaced spatially by air bubbles and the former interstices by a solid matrix.

The situation when pore and particle size are about equal is the worst case. Here one must necessarily obtain a combined distribution of both pore and particle sizes. In addition, the situation is complicated further by the fact that interparticle interference now enters importantly. The reason that results on pore size distributions in inorganic gels—which are typical of the worst case—seem to corroborate other measurements so nicely is probably that the pore and particle size distributions are about the same and therefore that the sum of the two is proportional to either one alone. A discussion of this whole problem by Bauer is in preparation (*1*).

The several assumptions made in applying geometrical significance to the quantities r_c and $\bar{r}$ vitiate from the start any rigorous comparison between the two. Thus r_c represents an "average pore size" only in the sense that a uniform open-ended cylinder of this radius has the same ratio of volume to surface as the porous sample. Similarly, $\bar{r}$ has significance as an average pore size only in so far as the Guinier theory is applicable—that is, only if the pores are actually spheres among which there is no diffractive interference. Inasmuch as the actual situation lies far from these assumed situations, correlation between r_c and $\bar{r}$ cannot be expected to be closer than merely qualitative. Thus Table I shows that all four silica-alumina gels have approximately the same order of pore size; and that both adsorption measurements and x-ray measurements arrange them in the same sequence.

It has been pointed out by Shull (*14*) that the ratio of pore size (from adsorption measurements) to discontinuity size (from x-ray scattering) shown by the materials of Table I shows not only the inconstancy expected from the argument above, but also a regular trend with the "porosity factor" for these materials. (Porosity factor is the ratio of empty volume to solid volume in any porous material.) Shull has found the same trend in a larger series of silica gels during the course of his own work and concludes that x-ray scattering for such materials is more indicative of particle than of pore size. Table II lists the solid volume, V_s, the porosity factor, V_0/V_s, the "average particle size," $r_s = 2V_s/S_0$, and the ratios $r_c/\bar{r}$ and $r_s/\bar{r}$ for the silica-alumina gels of Table I. Shull points out that the porosity factor should be proportional to the ratio of pore size to particle size; that such a proportionality, for gels A, B, C, and D, approximately exists between the porosity factor and the ratio $r_c/\bar{r}$, and that therefore $\bar{r}$ probably represents particle rather than pore size. On the other hand, the agreement between $\bar{r}$ and r_s is no better than that between $\bar{r}$ and r_c, and there is also an approximate proportionality between $\bar{r}/r_s$ and porosity factor. Additionally, the agreement among r_c, $\bar{r}$, and the porosimeter data for the fuller's earth is strikingly better than with r_s.

Clearly the exact geometrical significance of size determinations from x-ray scattering is still uncertain, and additional correlative data are urgently needed, particularly unambiguous methods of determining entire distributions of sizes.

Comparisons of entire distributions as obtained by x-ray scattering with entire distributions (rather than simply average sizes) as determined by other methods are difficult to make. The mercury porosimeter described in an earlier communication from this laboratory (*11*) affords one method of obtaining complete distributions but is limited to larger pore sizes. Figure 9 compares the porosimeter distribution for the fuller's earth (*16*) with the corresponding x-ray data. Considering the limitations of both methods the agreement is surprisingly good. If one proceeds to distributions having average radii of the order of a thousand times the wave length used, the method loses sensitivity. Thus, in an attempt to deduce by x-ray scattering the distribution found by the mercury porosimeter for Coors porous plate (*11*), the plot of log I *vs.* log $\left(a^2x^2 + \frac{1}{r_0^2}\right)$ was as nearly linear for $r_0 = 500$ as for $r_0 = \infty$, and on the criterion of linearity of this plot, any $r_0 > 500$ might have been chosen.

Table II. Comparison of Pore and Particle Size Data

Material	V_s, Cc./G.	V_0/V_s	$r_c/\bar{r}$	$\bar{r}/r_s$	r_s, Å.
Gel A	0.427	0.668	0.76	0.89	19
Gel B	0.421	1.108	0.95	1.19	18
Gel C	0.416	1.797	1.32	1.44	20
Gel D	0.421	1.748	1.77	1.47	23
Fuller's earth	0.376	2.09	0.72	2.9	58

For convenience the several steps in converting the observed intensity curve into a size distribution are summarized:

1. Expand empirically the observed equatorial intensity function, $I_e(x)$ into a closed series of Gauss functions, $I_e(x) = \Sigma b_i \exp(-\alpha_i x^2)$.
2. The slit-corrected intensity function will then be $I_{cor}(z) = \Sigma c_i \exp(-\alpha_i z^2)$, where $c_i = b_i\sqrt{\alpha_i}/erf(\sqrt{2\alpha_i}\,Y)$.
3. Plot log $I_{cor}(z)$ against log $(a^2z^2 + r_0^{-2})$ for a series of reasonable values of r_0. That curve that is most nearly linear gives the best value of r_0.
4. Determine the slope of this "best" curve, and find n from the relation, slope $= -(n + 2)$.
5. If desired, find A in the relation $D(r) = A\,\frac{r^{2n}}{r_0^{2n}}\exp\left(-\frac{r^2}{r_0^2}\right)$ by normalizing $\int dV = \int D(r)dr$ to the measured pore volume, V_0.

Obviously, the same method applies, with the same limitations, to a determination of particle size distribution.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Bauer, S. H., Cornell University, private communication.
(2) Bauer, S. H., *J. Chem. Phys.*, **13**, 450 (1945).
(3) Biscoe, J., and Warren, B. E., *J. Applied Phys.*, **13**, 364 (1942).
(4) Brunauer, S., Emmett, P. H., and Teller, E., *J. Am. Chem. Soc.*, **60**, 309 (1938).
(5) Elkin, P. B., Shull, C. G., and Roess, L. C., *Ind. Eng. Chem.*, **37**, 327 (1945).
(6) Fankuchen, I., *Nature*, **139**, 193 (1937).
(7) Guinier, A., *Ann. phys.*, **12**, 161 (1939).
(8) Hosemann, R., *Z. Physik.*, **113**, 751 (1939); **114**, 133 (1939).
(9) Jellinek, M. H., and Fankuchen, I., *Ind. Eng. Chem.*, 37, 159 (1945).
(10) Jellinek, M. H., Solomon, E., and Fankuchen, I., IND. ENG. CHEM., ANAL. ED., **18**, 172 (1946).
(11) Ritter, H. L., and Drake, L. C., *Ibid.*, **17**, 782 (1945).
(12) Roess, L. C., *J. Chem. Phys.*, **14**, 695 (1946).
(13) Shull, C. G., Gibson Island Conference, Aug. 23, 1944.
(14) Shull, C. G., private communication.
(15) Shull, C. G., and Roess, L. C., *J. Applied Phys.*, **18**, 295 (1947).
(16) Socony-Vacuum Laboratories, unpublished data.

RECEIVED July 25, 1947. Presented in part before the A.A.A.S. Gibson Island Conference on Catalysis, June 26, 1946. The first paper in this series appears in (*11*).

Microdetermination of Lead in Biological Material

With Dithizone Extraction at High pH

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The use of a procedure for the quantitative extraction of lead with dithizone at high pH has facilitated the determination of lead in biological material. The high pH extraction step has been incorporated in the final estimation step of the Bambach-Burkey method (*1*). The difficulties and advantages of the revised procedure are discussed and analytical data are given to prove the adequate nature of the revised procedure.

A PROCEDURE for the quantitative extraction of lead with dithizone at high pH, which was recently reported by Snyder (*4*), has facilitated the determination of lead in biological material. Following a personal communication early in 1945 (*5*), the procedure was incorporated in the Bambach and Burkey method (*1*) and has been used satisfactorily since then for the routine analysis of all types of samples. The revised procedure represents a significant advance in analytical technique, and is deemed worthy of presentation in order to point out the difficulties and advantages encountered in the application of the high pH extraction procedure to the analysis of biological material. For the convenience of analysts, the revised procedure is described stepwise, but only in so far as it differs from that given by Bambach and Burkey (*1*).

PROCEDURE

Samples are prepared without change, and only the following changes in reagents are required.

Ammonium Hydroxide–Potassium Cyanide Mixture. Dissolve 10 grams of potassium cyanide in 1000 ml. of ammonium hydroxide (specific gravity 0.900). Lead-free ammonium hydroxide of the proper specific gravity may be made by passing tank ammonia into distilled water immersed in an ice bath.

Dithizone Extraction Solution. Only one solution is needed—30 mg. of Eastman dithizone per liter of lead-free (redistilled and stabilized) chloroform.

Isolation of Lead and Removal of Bismuth Interference. The initial isolation of lead is carried out exactly as described by Bambach and Burkey (*1*). Because it is no longer necessary to observe each 5-ml. extraction in order to fit the samples into the three concentration ranges used by Bambach and Burkey (*1*), the extraction may be started with 10, 15, or 20 ml. of dithizone solution as the analyst prefers. The approximate total quantity of lead, however, must be known and this is determined from the total volume of extraction solution used. Each 5 ml. of dithizone extraction solution will extract approximately 40 micrograms of lead (*1*). If the quantity of the dithizone extraction solution used indicates the presence of more than 150 micrograms of lead, an aliquot is removed to bring the quantity of lead to or below 150 micrograms before adding the 50 ml. of the pH 3.4 buffer which is used to separate the lead from the bismuth.

Final Estimation of Lead. To the 50 ml. of pH 3.4 buffer (*1*) containing less than 150 micrograms, add 20 ml. of the ammonium hydroxide–potassium cyanide mixture. (The automatic pipet shown in Figure 1 has been found convenient for this purpose. It was designed and made in this laboratory by W. J. Younker.) Add 15 ml. of dithizone extraction solution and shake for 1 minute, releasing the pressure which develops through the stopper rather than through the stopcock. The colors in an entire series are developed in this way before proceeding to the photometry.

Photometry. Place a small pledget of cotton in the tip of the stem of each funnel, and just before filling the cells, discard 2 to 3 ml. of the chloroform phase by allowing it to pass through the cotton pledget. Fill the cell by filtering the chloroform solution through the pledget and read the density or the transmittancy of the solution at 510 mμ in any suitable photometer. The proper size of cell to use is determined as in Snyder's method (*4*), from the depth of color in the chloroform phase. It is usually possible to make measurements with only two cells, a cell of 50-mm. light path for quantities from 0 to 15 micrograms and a cell of 10-mm. light path for quantities from about 15 to 100 or 110 micrograms of lead. (A cell with a 5-mm. light path must be used when the amount of lead is between 110 and 150 micrograms.) The working curves are prepared from known amounts of lead added to the pH 3.4 buffer. A blank, starting with the pH 3.4 buffer, is run in order to obtain the daily zero points of the calibration curves. A blank of all of the reagents used in the complete extraction must also be determined and subtracted from the values read from the calibration curves.

RESULTS

In Table I are listed typical results chosen at random from approximately one hundred samples, duplicate aliquots of which were analyzed in parallel by the Bambach and Burkey (*1*) method and the modified procedure. In the case of the blood samples, these two sets of results are compared with the spectrographic findings.

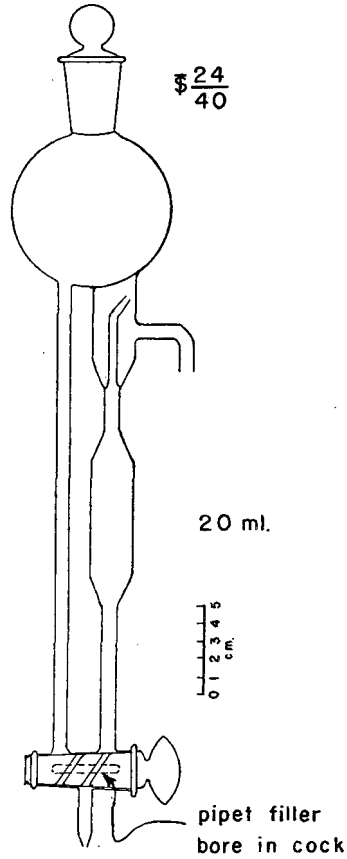

Figure 1. Automatic Dispensing Pipet

The comparative degrees of accuracy and reproducibility of results obtained on two series of samples by the old and new procedures are indicated in Table II. The ash of a series of samples of feces, in the one case, and of urine, in the other, which remained following certain other experimental observations, was composited and then divided into 40 equivalent samples, respectively, for the parallel analyses. The other parallel series consisted of two sets of samples of synthetic urine (*2*) to which known amounts of lead were added.

DISCUSSION

Inspection of Tables I and II shows that there is little difference in the findings obtained by the two methods. The differences between the respective mean values in Table II, however, are

Table I. Results Obtained on Duplicate Samples by Extracting with Dithizone at pH 9.5 and 11.5

Description		Found by Bambach and Burkey Method (*1*)	Found by Revised Procedure
		Mg.	*Mg.*
Urine 1185		0.325	0.320
Urine 1282		0.175	0.177
Urine 1299		0.160	0.160
Urine 1487		0.190	0.187
Feces 1216		0.430	0.430
Feces 1438		0.57	0.56
Feces 1442		0.52	0.51
Feces 1368		0.31	0.33
Food (mixed 24 hour total) 1369		0.19	0.19
Food (mixed 24 hour total) 1421		0.18	0.19
Food (mixed 24 hour total) 1576		0.16	0.16
Food (mixed 24 hour total) 1624		0.33	0.33
	Spectrographic (*2*)	*Mg./100 g.*	*Mg./100 g.*
Blood 2152	0.050	0.055	0.051
Blood 2153	0.060	0.066	0.066
Blood 2185	0.050	0.044	0.044
Blood 2192	0.055	0.061	0.061

statistically significant; slightly superior accuracy is shown by the revised method, particularly in the higher ranges of concentration. The reproducibility of the results, on the other hand, is somewhat superior in the case of the Bambach and Burkey method (*1*).

In the analysis of biological material, special precautions must be taken to prevent interference by bismuth. For this reason, it is necessary to make a preliminary extraction, which isolates lead and bismuth from the extraneous ash, and then to remove the bismuth before proceeding to the final lead step of extraction and photometry. The initial extraction may be made within the range of pH 8.5 and 11.5, but it is best to work at the lower pH (not exceeding 9) if the bismuth separation is to be made by washing the initial extract with the buffer solution at pH 3.4 (*1*). Changes in the buffer at this point will result in low recoveries of lead, and the losses are not reclaimed unless the initial chloroform extract is washed thoroughly with water to remove entrained alkali. When the initial extraction is made at pH 8.5, a single wash of the chloroform extract with 50 ml. of distilled water is sufficient to remove all the entrained alkali. On the other hand, at pH 11.5, interfering quantities of alkali remain even if the chloroform extract is washed with two 50-ml. portions of distilled water, and there may be significant losses of lead. Hence, pH 8.5 is chosen for the initial extraction.

Table II. Reproducibility and Accuracy of Two Analytical Procedures Applied in Parallel

Material	No. of Analyses	Method	Micrograms of Lead — Mean	Std. error	Std. dev.
Ashed feces, composited and subdivided	20	Bambach and Burkey	42.63	±0.064	±0.29
	20	Revised	44.00	±0.123	±0.55
Ashed urine, composited and subdivided	20	Bambach and Burkey	8.69	±0.056	±0.250
	20	Revised	8.90	±0.050	±0.224
Synthetic urine, 8 micrograms Pb added	10	Bambach and Burkey	7.9	±0.024	±0.077
	10	Revised	8.16	±0.073	±0.230
Synthetic urine, 40 micrograms Pb added	10	Bambach and Burkey	38.66	±0.209	±0.66
	10	Revised	39.8	±0.201	±0.64

The extraction at high pH possesses a distinct advantage when carried out in the presence of moderate amounts of phosphate ion. This fact is indicated in Table III, wherein are recorded the results obtained in the analysis of samples containing 50 micrograms of lead buffered at pH 3.4, to which various amounts of phosphate ion were added, following extractions at pH 9.5 and pH 11.5, respectively. Extractions with dithizone were made after the phosphate and lead had been allowed to remain in contact for various periods of time. The extraction of lead with dithizone at pH 11.5 is quantitative even in the presence of 5 mg. of phosphate ion. On the other hand, low recoveries are obtained at pH 9.5 in the presence of 1 mg. of phosphate or less. Actually, other experiments have shown that quantities of phosphate as low as 0.5 mg. in the final solution prevent the complete extraction of lead.

In the Bambach-Burkey method (*1*) solutions of lead dithizonate may be subjected to photometry without preliminary filtration. Although the same procedure may be followed in extracting at high pH, the results so obtained show a lesser degree of reproducibility than do those obtained after filtration. A convenient and rapid method of filtration is provided by the insertion of a small pledget of cotton into the stem of the funnel. No special purification of the cotton is required other than that accomplished by wasting 2 ml. of the chloroform phase through the pledget before filtering the remainder into the measuring cell.

The use of a specific type of cell (Style D, American Instrument Company, or equivalent) permits the employment of small volumes of solution and results in an increased sensitivity of detection. Only 15 ml. are used and only 10 ml. of this amount are required to fill the cell (having a light path of 50 mm.) which is used in the range 0 to 15 micrograms of lead.

Table III. Effect of Phosphate Ion on Extraction of Lead with Dithizone

(50 micrograms of Pb)

Added PO_4^-	pH of Final Extraction	Extracted Immediately	Extracted after 10 min.	20 min.	30 min.
Mg.		γ	γ	γ	γ
0	9.5	50	50	..	..
	11.5	49	49.9	..	..
1	9.5	33	42.5	..	..
	11.5	50	49.7	..	..
2	9.5	..	10.5	..	..
	11.5	48	49	..	..
3	9.5	..	8.5	..	..
	11.5	51	50	..	..
4	11.5	49.8	49	48	49
5	11.5	50	50	..	..

As shown by Snyder (*4*), it is no longer necessary to prepare separate standard solutions of dithizone for the three ranges of lead concentration, and the same extraction solution which is used for the initial extraction can be used in the final estimation of color. For the final extraction of lead, the proper quantity of dithizone also may be included in the ammonium hydroxide–potassium cyanide mixture. After adding the mixture, it is only necessary to add 15 ml. of clear chloroform to extract the lead. Such a mixture has been employed in a rapid screening test for lead in urine (*3*). Solutions of this type have been kept apparently unchanged for more than six months. The great convenience associated with the stability of such solutions is obvious, especially in laboratories in which only occasional lead determinations are carried out. Under these conditions, the buffer solution containing the dithizone can be stored in a refrigerator, and preserved further in an unchanged state. The initial extraction solution can then be made up as required and in sufficient amounts to take care of current samples.

LITERATURE CITED

(1) Bambach, K., and Burkey, R. E., IND. ENG. CHEM., ANAL. ED., 14, 904 (1942).
(2) Cholak, J., *Ibid.*, 7, 287 (1935).
(3) Cholak, J., Hubbard, D. M., and Burkey, R. E., *J. Ind. Hyg. Toxicol.*, 30, 59 (1948).
(4) Snyder, L. J., ANAL. CHEM., 19, 684 (1947).
(5) Snyder, L. J., Ethyl Corp., Baton Rouge, La., personal communication, 1945.

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Quantitative Microdetermination of Magnesium in Plant Tissue and Soil Extracts

A Rapid Colorimetric Method

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A rapid quantitative colorimetric method is proposed for the determination of magnesium in plant tissue and soil extracts. It is based on the use of thiazole yellow and a compensating starch solution to give a reproducible color that can be measured in a photoelectric colorimeter. It is not necessary to remove iron, aluminum, manganese, phosphorus, or calcium. Both the precision and accuracy are well within accepted standards for colorimetric methods.

A QUANTITATIVE procedure for determining small amounts of magnesium in plant material by using titan yellow has been described by Kidson (*2*). Gillam (*1*) described a titan yellow method for determining magnesium in fertilizers and soil extracts. In both methods iron, aluminum, phosphorus, and calcium have to be removed and ammonium salts destroyed by time-consuming processes. Peech and English (*4*) examined the titan yellow procedure carefully and developed a direct, rapid semiquantitative method for the determination of magnesium in soil extracts in the presence of calcium, iron, aluminum, and manganese. By using their procedure as a basis, and thiazole yellow as recommended by Mikkelsen and Toth (*3*) instead of titan yellow, a rapid quantitative colorimetric method has been developed for determining magnesium in tung leaves, and probably can be used for other plant tissue.

REAGENTS

Thiazole Yellow, 0.10%. Dissolve 0.10 gram of thiazole yellow (obtained from General Dyestuffs Corporation, New York, N. Y.) in 100 ml. of water and store in a dark bottle. This reagent will keep at least 2 months under ordinary conditions.

Hydroxylamine Hydrochloride, 5%. Dissolve 25 grams of hydroxylamine hydrochloride in water and dilute to 500 ml. Store in a dark-colored bottle.

Sodium Hydroxide, 2-5 *N*. Dissolve 100 grams of sodium hydroxide in water and dilute to 1 liter.

Starch Solution, 2%. To 2 grams of C.P. soluble starch add enough water to make a paste and add slowly, while stirring, the remainder of 100 ml. of hot water. Filter if necessary. This reagent should be prepared freshly as needed. Best results were obtained with Baker and Adamson's reagent grade.

Compensating Solution. Dissolve 3.7 grams of calcium chloride ($CaCl_2.2H_2O$), 0.74 gram of aluminum sulfate ($Al_2(SO_4)_3.18H_2O$), 0.36 gram of manganous chloride ($MnCl_2.4H_2O$), and 0.60 gram of sodium phosphate (Na_3PO_4) in about 500 ml. of water containing 10 ml. of concentrated hydrochloric acid. Dilute to 1 liter.

Starch Compensating Reagent. Mix equal volumes of the starch solution and compensating solution. Prepare daily as needed.

Standard Magnesium Solution (25 p.p.m. of magnesium). Dissolve 250 mg. of reagent grade magnesium metal in dilute hydrochloric acid solution (150 ml. of water plus 10 ml. of concentrated hydrochloric acid) and bring to volume in a 250-ml. volumetric flask. Dilute this solution 1 to 40 for the working standard of 25 p.p.m. of magnesium.

PROCEDURE

Take an aliquot of the hydrochloric acid solution of plant ash that should contain 0.025 to 0.15 mg. of magnesium. With tung leaves a convenient procedure has been to ash 2 grams of dry ground material, dissolve the ash in dilute hydrochloric acid, and make up the solution to 100 ml. A 1-ml. aliquot of this solution is satisfactory to cover the range 0.10 to 0.70% of magnesium in the leaves on a dry basis. For smaller or larger concentrations than this the aliquots should be adjusted accordingly.

Transfer the aliquot to a 50-ml. volumetric flask and add enough water to bring the volume to about 25 ml. Add 1 ml. of the hydroxylamine hydrochloride solution and 5 ml. of the starch-compensating solution and mix the solution. Add exactly 1 ml. of thiazole yellow solution and mix the solution, and then add 5 ml. of the sodium hydroxide solution. Bring the flask to volume with water, mix thoroughly the contents, and allow them to stand for 10 minutes before reading on a photoelectric colorimeter using a green filter. The color is stable for several hours. The concentration of magnesium is obtained from a standard curve as described below. A Cenco photelometer with the green filter 525 p was used in this work. The colorimeter is set to read 100% transmittance with a blank solution that is run by the same procedure used with the samples and standards.

STANDARD CURVES

When 0-, 1-, 2-, and 5-ml. aliquots of the standard solution of 25 p.p.m. of magnesium were analyzed exactly as described above and the readings plotted on semilog paper, a straight-line relationship was obtained. Because of temperature variations and possibly other factors, good reproducibility of the standard curve was not obtained. Therefore for the highest accuracy and precision it is necessary to run a 5-ml. aliquot of the standard solution in duplicate with each set of determinations. A curve for the set is then constructed by drawing a straight line from 0 concentration and 100% transmittance through the point obtained in the reading of the standard. It is convenient to prepare in advance a number of curves, one or two on each sheet of graph paper, and to use the appropriate curve for each set of determinations. The colorimeter reading for the 5-ml. aliquot of the standard usually is in the range 75.0 to 79.0 with the Cenco photelometer used in this laboratory.

ANALYTICAL RESULTS

The precision of the method was tested by analyzing 13 samples of tung leaves from widely different localities, ranging in magnesium content from 0.11 to 0.88% on an oven-dry basis. Each sample was ashed in triplicate, and triplicate determinations were made on each of the ashings. Thus nine determinations were made on each sample. A statistical analysis of the data obtained showed that the standard deviation of a single determination is 0.019%. Hence the standard deviation of the difference between two duplicate determinations is 0.027. This means that about two thirds of duplicate determinations should agree within 0.027% and 95% of them should agree within 0.054%. The standard error is 4% of the mean of all determinations, which is well within accepted standards for colorimetric methods.

The accuracy of the method was tested by comparison with the standard volumetric procedure of titrating the magnesium–ammonium phosphate precipitate with standardized 0.1 *N* hydrochloric acid. Ten samples of tung leaves from widely

Table I. Magnesium Determinations on Tung Leaf Samples

Sample	% Magnesium, Dry Weight Basis[a] Volumetric	Colorimetric
1	0.14	0.15
2	0.29	0.27
3	0.29	0.31
4	0.33	0.33
5	0.35	0.34
6	0.37	0.34
7	0.39	0.35
8	0.45	0.45
9	0.54	0.52
10	0.61	0.58
Mean	0.376	0.364

[a] Mean of triplicate determinations.

different localities and ranging in magnesium content from 0.14 to 0.61% by the standard method were used for the comparison. Triplicate determinations were run on each sample by the two methods. The data are given in Table I.

The values obtained by the two methods were in close agreement; the mean of the colorimetric determinations was lower than that of the volumetric determinations, but the difference was not statistically significant (Table I). It was noted that the differences between volumetric and colorimetric readings were greatest for high percentages of magnesium. The coefficient of correlation was found to be +0.992 and the coefficient of regression of volumetric on colorimetric readings was 1.069 ± 0.048 (*5*). As the regression coefficient does not differ significantly from unity, the trend observed in the ten samples of Table I is probably due to chance and one would not be justified in using values calculated from the regression equation.

The method was found applicable to the determination of exchangeable magnesium in soils, using a 0.1 *N* acid solution of the ammonium acetate extract. Eight soil samples from different parts of the tung belt were run for exchangeable magnesium by the method described above and by the standard gravimetric procedure, using 8-hydroxyquinoline. Duplicate determinations were made on each sample by the two methods (Table II).

The values obtained by the two methods were in good agreement. The coefficient of correlation was found to be + 0.9985; and the coefficient of regression of gravimetric on colorimetric values was 1.097 ± 0.025, which has high statistical significance.

DISCUSSION

In addition to the calcium and aluminum in the compensating solution suggested by Peech and English (*4*), it was found necessary to add manganese and phosphorus as well. Although hydroxylamine was used to prevent manganese interference as recommended by Peech and English, some samples high in manganese gave results that were too high. Addition of manganese chloride to the compensating solution corrected this error satisfactorily.

Because there was some intensification of color in the presence of phosphate ions, it is necessary to include phosphate in the compensating solution. The amount of phosphate as well as calcium, aluminum, and manganese added in the compensating solution is on the average about five times as much as is present in the aliquot of the ash solution being analyzed, so that the effects of these ions present in the sample are negligible.

Table II. Determinations of Exchangeable Magnesium in Soils

Location	Soil Type	Depth, Inches	Exchangeable Magnesium per 100 Grams of Soil[a] Colorimetric, thiazole yellow, m.e.	Gravimetric, 8-hydroxyquinoline, m.e.
Morriston, Fla.	Lakeland fine sand	7–14	0.05	0.05
		0–6	0.15	0.18
Hague, Fla.	Ft. Meade loamy fine sand	0–6	0.25	0.21
Fairhope, Ala.	Orangeburg sandy loam	0–6	0.29	0.27
Folsom, La.	Ora fine sandy loam	0–5	0.32	0.30
Sunnyhill, La.	Pheba fine sandy loam	0–6	0.64	0.62
Hague, Fla.	Gainesville fine sandy loam	0–6	1.04	1.22
Magnolia, Miss.	Ruston fine sandy loam	12–36	2.73	2.95

[a] Mean of duplicate determinations.

LITERATURE CITED

(1) Gillam, W. S., IND. ENG. CHEM., ANAL. ED., 13, 499–501 (1941).
(2) Kidson, E. B., *New Zealand J. Sci. Technol.*, 27, 411–13 (1946).
(3) Mikkelsen, D. S., and Toth, S. J., *J. Am. Soc. Agron.*, 39, 165–6 (1947).
(4) Peech, Michael, and English, Leah, *Soil Sci.*, 57, 167–95 (1944).
(5) Snedecor, G. W., "Statistical Methods," 4th ed., Ames, Iowa, Iowa State College Press, 1946.

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Microdetermination of Carbon Monoxide in Air

A Portable Instrument

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THE instrument described was developed in response to a request from the Office of Scientific Research and Development. Three models of the instrument were built; two were intended for laboratory use only, and the third was designed for field use by inclusion of an internal battery.

The instrument makes use of two chemical reactions, both of which go to completion at 180° C.

$$CO\ (gas) + HgO\ (s, red) = CO_2\ (gas) + Hg\ (gas) \quad (1)$$

$$3Hg\ (gas) + SeS_2\ (s) = 2HgS\ (s) + HgSe\ (s) \quad (2)$$

Although neither of these reactions is new, the conditions and method of application are radically different from those used by previous investigators. Both reactions have been extensively studied during the present investigation. In the case of Reaction 1 this study led to an accurate gravimetric method for the determination of carbon monoxide in air, based on the loss in weight of the mercuric oxide. The analytical procedure as well as the study of Reaction 1 has been described in an earlier communication (*4*). Nordlander (*5*) using Reaction 2 developed an instrument for the detection and semiquantitative determination of mercury vapor in air by use of a selenium sulfide paper prepared by coating the precipitated material onto white bond paper. The air stream being tested for mercury was heated to 70° C. and caused to impinge normally on the coated surface. The intensity of blackening as measured by photometry of the exposed paper gave an approximation of the mercury concentration in the air.

A portable instrument for the determination of carbon monoxide in air makes use of two reactions, one between carbon monoxide and red mercuric oxide to give mercury vapor and carbon dioxide, the other between mercury vapor and selenium sulfide. The mercury-containing air flows slowly along a selenium sulfide test strip, and produces a black coloration over a length which is a direct measure of the quantity of carbon monoxide in the original sample. Determinations can be made in a few minutes with an average error of less than 10%. The instrument may be used over wide ranges of carbon monoxide concentration from a few parts per million up to about 3%.

Improvements in the method employed both in making the selenium sulfide papers and in their use were developed during the present investigation. In the preparation of the papers a better result from every standpoint was obtained if the selenium sulfide was precipitated in the pores of the paper. This was accomplished by impregnating a good quality of smooth filter paper with aqueous selenious acid solution, followed by exposure to hydrogen sulfide gas. The improved method of using the papers is an adaptation of that described by Sanger and Black (*6*), who used mercuric chloride test papers in the microestimation of arsenic by the Gutzeit method.

Instead of impinging normally upon the test paper as in the Nordlander method, the gas stream flows lengthwise along a narrow strip of the test paper. The mercury vapor produces a black coloration which extends progressively along the strip in substantially linear relation with the mercury—i.e., carbon monoxide—concentration. The carbon monoxide concentration in the original gas sample is accordingly determined by the length of the blackened portion of the test strip, and the necessity for photometry is avoided.

REAGENTS AND MATERIALS

Red Mercuric Oxide. A granular (8- to 14-mesh) form of the oxide must be used. Its preparation from mercuric nitrate has been fully described (*1*).

Selenium Dioxide. Commercial selenium powder was purified as described by Campbell and McCullough (*2*). The pure selenium was then dissolved in C.P. nitric acid and the resulting solution evaporated to dryness over a sand bath or hot plate in a hood.

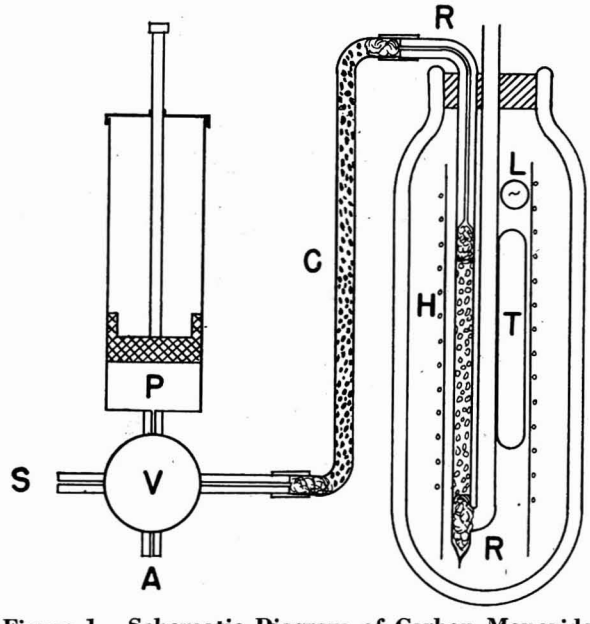

Figure 1. Schematic Diagram of Carbon Monoxide Instrument

A. Vent to atmosphere. *C*. charcoal trap. *H*. heater coil. *L*. pilot lamp. *P*. pump. *R*. reaction tube. *S*. sample intake. *T*. thermoswitch. *V*. four-way valve with positions *S* to *P*, *P* to *A*, *P* to *C*, and *S* to *C*

Final heating to about 140° to 150° C. for an hour or two yields a pure, white product.

PREPARATION OF SELENIUM SULFIDE PAPERS

Choice of Paper. Best results have been obtained with Whatman No. 1, Schleicher and Schuell (American) No. 602 Extra Dense and No. 598. A heavy paper such as S. and S. No. 598 takes a heavier deposit of selenium sulfide and is accordingly more useful in the higher concentrations.

Selenious Acid Solution. The areal concentration of selenium sulfide in the test papers is directly proportional to the concentration of selenious acid solution used. For carbon monoxide concentrations in the range 0 to 200 p.p.m. a solution 0.025 formal in selenious acid is satisfactory when Whatman No. 1 paper is used. In the range 10,000 to 30,000 p.p.m. of carbon monoxide, 1.0 *F* selenious acid on S. and S. No. 598 was used with success. The selenious acid concentration most satisfactory for any particular range may be estimated with sufficient accuracy from these figures; S. and S. No. 598 paper is about 2.5 times as thick as Whatman No. 1 and accordingly takes up proportionally more of the solution.

Impregnation of Papers with Selenium Sulfide. Large sheets of the paper are cut into pieces about 20 cm. (8 inches) square, and a strip about 0.5 inch wide is folded down on one edge and fastened with three staples. A stiff Chromel wire about 10 inches long is inserted under the folded part of each paper to give a suitable handle and also to keep the papers straight when wet.

In groups of eight the papers are immersed in the selenious acid solution for about 10 to 15 minutes. They are then hung up to drain for about 5 to 10 minutes, the time depending on the temperature and humidity of the air, until they are well beyond the dripping stage but not dry. After exposure to hydrogen sulfide gas for about 10 to 15 minutes in a large glass tank, the papers are hung up in a well ventilated place to dry. When completely dry they are stacked and pressed flat.

Baking Selenium Sulfide Papers. The stability of the selenium sulfide papers is increased by baking for about 30 minutes at 140° C. If forced air circulation is not provided in the oven, the papers must be rotated or turned frequently to prevent uneven sublimation of the active material During baking the color deepens from yellow-orange to red-orange and the colloidal particles of selenium sulfide fuse into larger glassy, amorphous beads. X-ray diffraction patterns of the material both before and after baking show no evidence of crystallinity.

Cutting of Test Strips. After about 1.5 inches from top and bottom and 0.5 inch from each side are trimmed away, the papers are cut into strips 0.125 by 4.5 inches. Because the accuracy of the analyses depends on the uniformity of width of the strips, the cutting must be done carefully. A metal spacing gage fastened to the cutting edge of a good quality photographic trimming board has been found satisfactory. The strips from a given square are fairly uniform, but there is often considerable variation from one square to another. The strips are therefore segregated according to the squares from which they are cut and stored in stoppered vials. They are stable if kept in the dark, especially if kept cold. Papers stored in a refrigerator for over a year have kept their original calibration. Under ordinary laboratory temperatures the papers retain their calibration values for several months if kept dark.

THE INSTRUMENT

A schematic diagram of the instrument is shown in Figure 1; Figures 2 and 3 are photographs of two models of the instrument.

The pump and valve mechanism are critical parts and required considerable experimental work in their development.

The pump body consists of a stainless-steel tube 1 inch in inside diameter by 4 inches long, the inner surface of which is

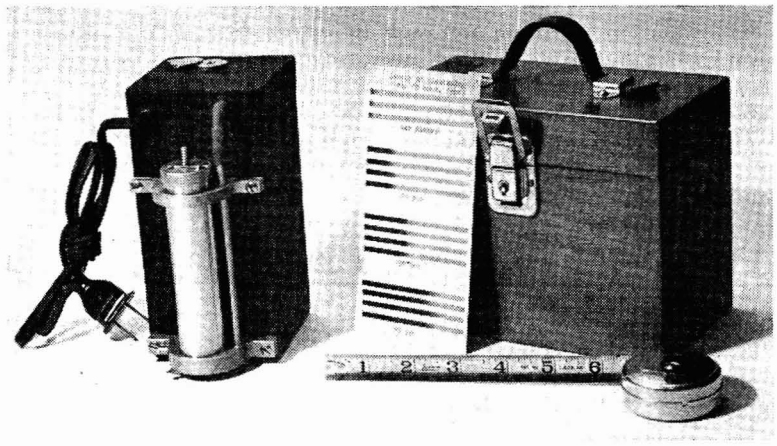

Figure 2. Carbon Monoxide Instrument, Model A

Table I. Calibration Data for Selenium Sulfide Papers

Paper No.	CO Concentration	Length of Blackening, Front	Length of Blackening, Back	Av.	Mean Deviation from Av.	Calibration
	P.p.m.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm./100 p.p.m.*
150	105	14.0	14.0			
		14.0	13.5			
		14.0	13.5			
		14.0	13.2			
		14.2	13.0			
				13.7	0.3	13.0
151	105	13.2	13.7			
		13.0	12.0			
		14.0	14.0			
		14.7	14.7			
		13.0	14.0			
				13.6	1.2	12.9
152	105	13.5	11.0			
		11.5	12.2			
		11.2	12.0			
		11.0	11.5			
		12.7	12.7			
				11.9	0.6	11.3
274	180	32.0	34.0			
		34.0	35.0			
		34.0	34.5			
		33.0	35.0			
				33.9	0.7	18.9
275	180	34.5	33.0			
		34.0	34.5			
		34.5	35.0			
		34.0	35.0			
				34.3	0.4	19.1

accurately ground and honed. An aluminum piston about 0.75 inch long is carefully turned to a diameter 0.001 inch less than the bore of the tube. It is operated without lubricant. As the gas pressure within the pump is only slightly above atmospheric pressure (being determined by the weight of the piston and rod), leakage past the piston is small and any variation in the rate of leakage is negligible. The piston and rod are made as light as possible and a slight constriction in the line suffices to slow the rate of gas flow through the instrument to the desired rate of 15 to 20 ml. per minute.

The four-way rotary valve consists of two flat, circular plates held tightly together by spring tension. Each plate has appropriately located grooves and ports for proper routing of the gas stream. The routes followed in each of the four positions are noted on Figure 1.

To remove unsaturated hydrocarbons and higher saturated hydrocarbons (butane and above) a charcoal trap is included between the valve and the reaction tube. This trap consists of a 5-inch length of 6-mm. glass tubing containing 8- to 14-mesh activated charcoal between Pyrex glass-wool plugs. The Pyrex reaction tube serves as the holder of both the mercuric oxide and the selenium sulfide test paper. The tubing into which the test strip is to be inserted is carefully chosen, so that its inside diameter is slightly larger than the width of the test strips.

The optimum operating temperature of the instrument is 175° to 180° C. At lower temperatures the reaction between carbon monoxide and red mercuric oxide is incomplete and at higher temperatures reaction of the oxide with hydrogen and hydrocarbons introduces errors when these substances are present. Proper reaction temperature is maintained by use of an electric heater controlled by an Edison thermoswitch. The reaction tube, heating coil, pilot light, and thermoswitch are contained in a half-pint Thermos bottle and are held in place by asbestos fiber insulation. The pilot light is a small neon lamp, which is placed next to the upper end of the exhaust tube, so that its glow may be seen through the tube.

OPERATION OF THE INSTRUMENT

Detailed instructions for use of the instrument are contained in the final report (*1*) by the authors to the office of Scientific Research and Development; hence only an outline is given here.

Testing Air Blank. Because mercuric oxide dissociates to a measurable extent, even at 175° C., the instrument will always show a small amount (1 to 2 mm.) of blackening with carbon monoxide–free air. As mercuric oxide tends to absorb free mercury at room temperature, the air blank should always be tested before making analyses.

This is done by allowing the instrument to heat to the reaction temperature, at which time the pilot light comes on. With the valve at the sample position, a sample of pure air is drawn in by slowly raising the pump to the top of its stroke. The valve is turned momentarily to the vent position, then on to the run position. A right-angle bend is made in a test strip about 0.5 inch from one end and the other end inserted in the reaction tube. The catch on the piston rod is released, permitting the piston to fall slowly. The piston is timed to fall in about 3 minutes. If the fall is too rapid, the boundary of the black coloration on the test strip is not sharp.

If the first air blank is more than 2 mm. (as it may be if the instrument has not been used for several days), two or three more determinations should be made. This will bring the blank down unless the reaction tube has been unused for a long period or is contaminated. In this case the tube should be conditioned by passing a slow stream of pure air through it for several hours at about 250° C.

Making an Analysis. The container holding the sample to be analyzed is connected to the sampling inlet and the same procedure is followed as outlined under "Testing Air Blank." For best results, test strips should be chosen so that the trace is between 20 and 40 mm. The first determination for each new sample should be discarded, because the reaction tube will contain some of the previous sample (or air). Five additional strips are usually

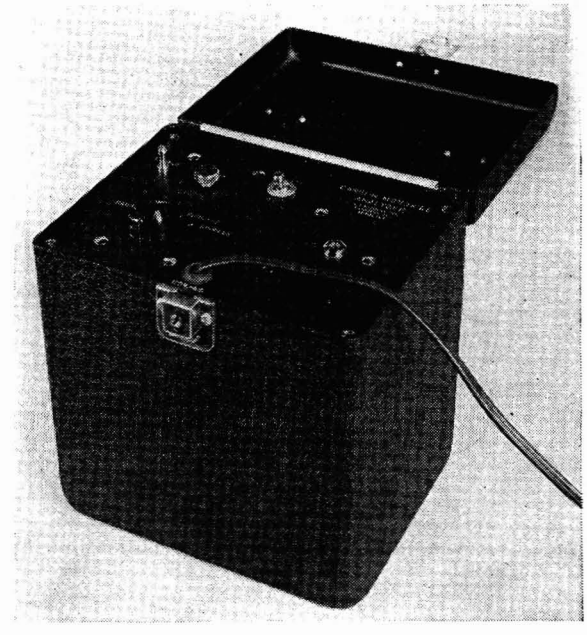

Figure 3. Carbon Monoxide Analyzer

Table II. Concentrations of Carbon Monoxide in Eight Carbon Monoxide–Air Samples

(Determined by Beckman-McCullough method in order of analysis compared with I_2O_5 reference method)

Analysis I_2O_5 Method	Carbon Monoxide, Parts per Million								
	90.5	184.6	44.8	320	244.7	25.8	66.2	137.8	137.8
	95	184	40	261	215	26	56	128	155
	96	202	54	279	214	23	55	119	142
	92	215	42	281	197	25	61	119	146
	102	204	49	278	216	25	57	123	149
Beckman-McCullough method	103	195	44	269	215	21	60	117	149
	93	201	42	264	208	22	57	122	146
	101	201	50	266	220	27	65	127	152
	92	169	47	261	208	27	55	121	159
	95	182	44	266	216	21	60	122	...
	90	192	43	276	214	25	61	119	...
Av.	95.9	194.5	45.5	270	212	24.2	58.7	121.7	148.5
Max.	103	204	54	279	220	27	65	128	155
Min.	92	169	40	261	197	21	55	119	142
	Per Cent Recovery of Carbon Monoxide								
Av.	106.0	105.4	101.6	84.4	86.6	93.8	88.7	88.7	107.6
Max.	113.8	110.5	120.5	87.2	90.0	104.7	98.2	92.9	112.5
Min.	101.7	82.9	89.3	81.6	80.5	81.4	83.1	86.4	103.0

prepared, the traces on both sides measured, and the ten resulting values averaged.

PERFORMANCE

The accuracy of the instrument may be judged from the data given in Tables I and II. Table I shows typical results obtained in the authors' laboratories in the calibration of five lots of selenium sulfide test papers. The data in Table II were obtained during comparative tests (*3*) of five methods for the determination of carbon monoxide in air at Naval Medical Research Institute.

Table II, which is taken directly from Consolazio's report, compares the results obtained with the mercuric oxide instrument with those obtained with an iodine pentoxide train. The mean deviation of the instrument values from those obtained by the iodine pentoxide method is 8.9%, which is good at these low concentrations. Some of the deviation is without doubt due to errors in the iodine pentoxide method, which was said by the author of the report to be perhaps as high as 5%. That the instrument error is almost entirely in the selenium sulfide step is shown by the high accuracy of the gravimetric method based on the mercuric oxide reaction (*1*). Instrument accuracy is high enough for many laboratory and most field requirements and the instrument has the distinct advantages of speed and simplicity.

In over five hundred determinations with one of these instruments in the writers' laboratory in the calibration of papers no servicing of the instrument was required.

LITERATURE CITED

(1) Beckman, A. O., McCullough, J. D., and Crane, R. A., *Report* **PB 5921**, Office of Publication Board, Department of Commerce, Washington 25, D. C.
(2) Campbell, T. W., and McCullough, J. D., *J. Am. Chem. Soc.*, **67**, 1965 (1945).
(3) Consolazio, W. V., and co-workers, *Report* 5, Project **X-417**, Naval Medical Research Institute, Naval Medical Center, Bethesda, Md., 1944.
(4) McCullough, J. D., Crane, R. A., and Beckman, A. O., ANAL. CHEM., **19**, 999 (1947).
(5) Nordlander, B. W., *Ind. Eng. Chem.*, **19**, 518 (1927).
(6) Sanger, C. R., and Black, O. F., *Proc. Am. Acad. Arts Sci.*, **43**, 295 (1907–8).

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Argentometric Microdetermination of Organic Chlorine

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A sample containing about 2 mg. of chlorine is digested with an excess of metallic sodium in butyl or amyl alcohol according to the method of Stepanow. The resulting chloride ion is titrated with 0.01 *N* silver nitrate solution and bromophenol blue is used as an adsorption indicator.

INTEREST in organic chlorine compounds has made necessary the establishment of a suitable method for the determination of chlorine. Most of the micromethods listed in textbooks—Pregl-Roth (*11*), Niederl-Niederl (*9*), and Ochiai-Tsuda (*10*)—as well as in the literature could not be performed because of the postwar shortage of equipment. Even the Carius micromethod, which is perhaps the most universal, could not be carried out because of the lack of suitable glass tubing and fuel gas for sealing the Carius tubes and heating the Carius oven. Other methods required special equipment or rare reagents or were too complicated or time-consuming.

In the authors' laboratory, carbon and hydrogen analyses are performed by specialists and the research chemist need only purify and submit the sample. Sulfur and halogen determinations must always be done by the research chemist himself.

According to the prewar literature available to the authors, no simple method of analysis exists. In order to determine the chlorine in organic combination, it must first be converted into the ionic form. For this purpose, chlorine compounds with the exception of hydrochlorides and alkyl chloride addition products of organic bases (*13*, *15*) must be decomposed by the Carius, oxidation, reduction, combustion, fusion, or other method. The Stepanow method (*14*), in which the compound is digested with metallic sodium to convert the chlorine to chloride ion, dispenses with special apparatus, is free from explosion, and is rapid and as in the Kjeldahl method for nitrogen, many analyses can be carried out in parallel at the same time. As the result of recent developments, the method is now improved in accuracy and reliability. Indeed, the analysis of a compound, for which even the Carius method failed to give a correct value, has been successfully carried out (*1*, *3*, *4*, *5*, *6*, *8*, *12*, *16*).

Chloride ion is best determined by means of the silver salt. Both volumetric and gravimetric procedures are used. Although Rauscher (*12*), in modifying the Stepanow method, employed gravimetric analysis on a micro scale, a volumetric modification is preferred because of its simplicity, even though it is less accurate than the gravimetric procedure. The errors are attributable to the buret and to the indicator. The back-titration (of the Volhard method), which doubles the largest error, must be avoided and the nature of the color change must be carefully studied. The original Stepanow method and many of its improvements, in which the Volhard back-titration is used, are unsatisfactory when applied to microanalysis. Another objection to the Volhard method is the fact that the reddish brown color of ferric thiocyanate often becomes indistinguishable from that of the solution titrated and is influenced by tempera-

ture, and that silver chloride reacts with ferric thiocyanate and thiocyanate ion.

When an adsorption indicator is used, only one standard solution, silver nitrate, is needed and the indicator undergoes not only a color change but also a change in fluorescence. The end point is therefore more clearly recognized. This kind of procedure must be applied in order to avoid the faults of the Volhard method. This modification has been described by Feldmann and Powell (*5*) in a macrodetermination of halogens, but is not applicable as it stands on a micro scale. Simple calculation shows that in order to obtain the usual analytical accuracy with milligram quantities, centinormal solutions must be used.

Table I. Results

Compound	Formula	Sample *Mg.*	0.01 *N* $AgNO_3$ *Ml.*	Factor	Found %	Calcd. %	Difference %
Hexachloroethane	C_2Cl_6	2.050	5.25	0.9900	89.90	89.87	+0.03
		2.290	5.89		90.29		+0.42
p-Dichlorobenzene hexachloride[a]	$C_6H_4Cl_8$	2.976	6.71	0.9887	79.04	78.89	+0.15
		3.165	7.10		78.64		−0.35
Benzene hexachloride[a]	$C_6H_6Cl_6$	2.925	6.33	0.9523	73.07	73.20	−0.13
		2.640	5.71		73.03		−0.17
DDT[a]	$C_{14}H_9Cl_5$	4.166	6.34	0.9451	51.00	51.23	−0.23
		4.383	6.70		51.22		−0.01
1,2-Dichloro-1,1,2,2-tetraphenylethane[a]	$C_{26}H_{20}Cl_2$	11.432	5.88	0.9433	17.20	17.63	−0.43
p-Dichlorobenzene	$C_6H_4Cl_2$	4.001	6.38	0.8590	48.57	48.29	+0.28
		4.574	7.29		48.54		+0.25

[a] Substances synthesized recently in authors' laboratory and analyzed by Miss Meizyô for their carbon and hydrogen contents to confirm the purity.

We need therefore to investigate anew the concentration of chloride that can be titrated and the kind of indicator that can be used. Bromophenol blue (*7*, *15*) has been used with success. Tetrabromophenolphthalein ester, according to Sakaguti (*13*) gives excellent results.

A sample containing about 2 mg. of chlorine is decomposed by reduction with metallic sodium in amyl or butyl alcohol. The sodium chloride produced is now dissolved in about 10 ml. of water and titrated with 0.01 *N* silver nitrate solution. The color change of the bromophenol blue is sharp even in the presence of alcohol and decomposition products.

Although the authors did not investigate compounds containing nitrogen, it is well known that the nitrogen is converted to ammonia by metallic sodium and escapes from the reaction mixture.

Kimura (*6*) and Bobranski (*2*) have shown that the decomposition with sodium and the titration with an adsorption indicator can be carried out on a semimicro scale using 20 mg. or more of sample weighed on an ordinary chemical balance and an ordinary buret with a 0.05 *N* silver nitrate solution. Ethyl alcohol can be used as solvent if the chlorine is not firmly attached to the nucleus or to an unsaturated carbon.

The time required for the analysis, including the weighing of the sample, digestion with sodium, and titration is less than an hour. It is much more rapid than the Carius and combustion methods. The errors are as large as in previous methods. The only apparatus necessary is a buret and digestion flask with ground-in reflux condenser. A test tube used as a cold finger can be used to replace the condenser. The indicator, also used as a pH indicator, is easily available.

This method is believed to be useful in microanalysis for research purposes as well as semimicroanalysis in industrial work.

APPARATUS

A 50- to 100-ml. flask with a ground-in reflux condenser—i.e., an acetylation flask. If a ground joint is not available, a flask fitted with a cold finger may work equally well.

A microburet with 0.05-ml. graduations.

REAGENTS

Sodium chloride, 0.001 *N*, used as a standard.

Silver nitrate, 0.001 *N*.

Bromophenol blue solution, 0.1%. Bromophenol blue is mixed with the equivalent amount of sodium hydroxide solution and diluted to 0.1%.

Acetic acid, 10%.

Metallic sodium. Although metallic sodium often contains sodium chloride as an impurity, this need not be considered, as the error produced can be eliminated by the blank test.

n-Butyl alcohol, isobutyl alcohol, or amyl alcohol. The alcohol must be digested with metallic sodium to remove aldehydes and distilled; otherwise the aldehydes polymerize by the action of sodium and give to the solution a brown coloration which hinders the easy distinction of the color change of the indicator.

PROCEDURE

A sample containing about 2 mg. of chlorine is weighed out in a manner analogous to the Dumas micromethod and transferred to the flask. A volume of 3 ml. of alcohol or less is added to dissolve the sample. If the sample is not easily soluble in alcohol it is dissolved in an appropriate solvent containing no halogen and then the alcohol is added.

A piece of clean sodium weighing 0.03 to 0.05 gram is added. The sodium need not be weighed every time, but the amount must be judged by the eye as constant as possible in order to keep the error produced by the impurities within the allowable limit.

After the condenser is attached, the flask is shaken at room temperature until the reaction ceases. Then the vessel is heated gradually during 10 minutes and finally the refluxing is continued with strong heating for 10 to 15 minutes.

The flask is allowed to cool and about 10 ml. of water are added to dissolve the sodium chloride. After addition of 1 to 2 drops of the bromophenol blue solution, the contents of the flask are neutralized with 10% acetic acid until the color of the indicator becomes yellow. The pH of the solution is therefore about 3.0. The solution is then titrated with a 0.01 *N* silver nitrate solution. Before arriving at the end point the color changes slowly, from yellow to blue-green, but this is not the true end point. The end point is sharply and clearly seen when one drop of the titrating solution changes the color from blue-green to violet. This point is also approximately distinguished by the mode of formation of the silver chloride mass.

The same procedure is repeated without the sample, but adding, after the digestion, 5.00 ml. of the 0.01 *N* sodium chloride solution. The factor of the silver nitrate solution is thus determined.

1.00 ml. of 0.01 *N* silver nitrate corresponds to 0.35457 mg. of chlorine.

ACKNOWLEDGMENT

The writers wish to thank Miss Yasuko Meizyô for her cooperation in this work.

LITERATURE CITED

(1) Bacon, *J. Am. Chem. Soc.*, **31**, 49 (1909).
(2) Bobranski, A., *Z. anal. Chem.*, **84**, 225 (1931).
(3) Cook and Cook, IND. ENG. CHEM., ANAL. ED., **5**, 186 (1933).
(4) Drogin and Rosanoff, *J. Am. Chem. Soc.*, **38**, 711 (1916).
(5) Feldmann and Powell, IND. ENG. CHEM., ANAL. ED., **11**, 89 (1939).
(6) Kimura, *J. Soc. Chem. Ind. Japan, Suppl. binding*, **37**, 590 (1934).
(7) Kolthoff, *Z. anal. Chem.*, **71**, 235 (1927).
(8) Landis and Wickmann, IND. ENG. CHEM., ANAL ED., **2**, 394 (1930).
(9) Niederl and Niederl, "Micromethods of Quantitative Organic Elementary Analysis," New York, John Wiley & Sons, 1938.
(10) Ochiai-Tsuda, "Yûki Biryô Syôryô Teiryô Bunsekihô," 3rd ed., 1942.
(11) Pregl-Roth, "Die quantitative organische Mikroanalyse," 4th ed., Berlin, Julius Springer, 1935.
(12) Rauscher, IND. ENG. CHEM., ANAL. ED., **9**, 296 (1937).
(13) Sakaguti, *J. Pharm. Soc. Japan*, **62**, 121 (1942).
(14) Stepanow, *Ber.*, **39**, 4056 (1906).
(15) Uyeo and Sakamoto, *J. Pharm. Soc. Japan*, **58**, 212 (1938).
(16) Walker and Rae, *J. Am. Chem. Soc.*, **33**, 598 (1911).

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NOTES ON ANALYTICAL PROCEDURES . . .

Determination of Iodine Numbers

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THE iodine number of oils and fats has long been used as a measure of their unsaturation. The Wijs method, commonly used, requires 30 minutes' absorption time. In 1939 the use of mercuric acetate to speed the reaction, introduced by Hoffman and Green (*2*), cut the absorption time to 3 minutes. Later Norris and Buswell (*3*) investigated the use of mercuric acetate with Hanus solution; they used a reaction time of 3 to 5 minutes.

In all the investigations of the use of mercuric acetate emphasis has been placed on the time factor. However, when hundreds of determinations have to be made, the volume of the solutions used also becomes a major factor. Norris and Buswell (*3*) have shown in the case of tung oil that when mercuric acetate is used the Wijs values vary only insignificantly in the reagent excess range of 30 to 225%. If the action of mercuric acetate is independent of the presence of a large excess of iodine, it should be possible to reduce the volume of Wijs solution from that normally used. Further, as the accelerated reaction leaves little time for the escape of halogens from the solution, stoppered flasks might not be necessary.

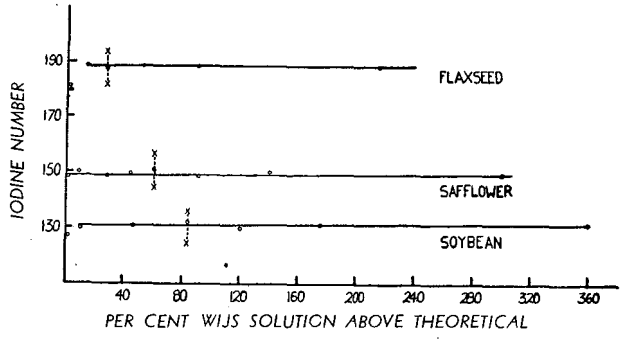

Figure 1. Effect of Volume of Wijs Solution on Iodine Numbers

With these points in mind the following method was developed:

Approximately 0.1 gram of the oil or fat was weighed and dissolved in 10 ml. of chloroform in a 250-ml. Erlenmeyer flask. Ten milliliters of Wijs were added, followed by 5 ml. of 2.5% mercuric acetate in glacial acetic acid solution. After 5 minutes (6 minutes for oil from flaxseed) 5 ml. of 15% potassium iodide and 25 ml. of water were added. The excess iodine was titrated immediately with 0.1 *N* sodium thiosulfate using starch as an indicator.

This method was compared with the official Wijs method of the A.O.A.C. (*1*) on a large number of samples. For this investigation Wijs solution was prepared in 18- to 20-liter quantities and kept in a stoppered bottle in the dark. Smaller volumes of 3 to 5 liters were transferred from this stock to a paper-wrapped bottle as required. It has been found in this laboratory over a period of 4 years that Wijs solution need not be prepared every 30 days as recommended by the A.O.A.C. Allowing for small variations due to temperature, the titer of blanks remained the same for 6 to 8 months. Norris and Buswell (*4*) found that Wijs solution could be kept well over a year.

Samples of oil-bearing seeds, chosen at random from groups grown across Canada, and commercial oils and fats were used in the investigation. The oil was pressed from the seeds on a Carver laboratory press and the iodine numbers were determined the same day. From a few samples the oil was extracted on the Goldfisch apparatus using petroleum ether as the solvent. Triplicate determinations were made on each sample by the two methods.

Because of the presence of ricinoleic acid, mercuric acetate cannot be used to determine the iodine number of castor oil (*3, 5*). The iodine number of a sample of this oil was 85.3 by the A.O.A.C. method and 91.4 by the new method. However, a sample of sulfonated castor oil 50% neutral had an iodine number of 30.1 by the A.O.A.C. and 29.5 by the new method.

Table I. Iodine Numbers of Oils, Fats, and Fatty Acids

(Each value is mean of three determinations)

Sample and Variety	Crop Year	Iodine Numbers A.O.A.C.	Iodine Numbers New method	Difference
Flaxseed				
Royal	1945	170.8	170.1	+0.7
Redwing	1945	182.7	183.4	−0.7
Dakota	1945	183.3	183.1	+0.2
Bison	1945	168.5	170.3	−1.8
Crystal	1945	187.1	187.3	−0.2
Dominion	1945	182.8	182.3	+0.5
Royal	1946	183.0	184.1	−1.1
Redwing	1946	191.2	192.3	−1.1
Dakota	1946	190.9	190.7	+0.2
Bison	1946	180.2	181.6	−1.4
Crystal	1946	195.0	195.5	−0.5
Dominion	1946	186.6	186.9	−0.3
Gossamer	1946	200.3	201.0	−0.7
Norfolk Queen	1946	193.0	192.3	+0.7
Custer[a]	1946	185.3	186.8	−1.5
Victory[a]	1946	190.4	189.5	+0.9
Viking[a]	1946	192.8	192.8	0.0
Novelty[a]	1946	180.7	181.3	−0.6
Soybeans				
Early Blackeye	1944	126.9	127.9	−1.0
Goldsoy	1944	134.5	134.9	−0.4
Manitoba Brown	1944	135.4	136.7	−1.3
Pagoda	1945	133.0	133.2	−0.2
Kabott	1945	131.7	132.6	−0.9
Manitoba Brown	1945	135.4	137.2	−1.8
Mandarin	1945	130.7	131.3	−0.6
La Pealon	1945	132.4	130.9	+1.5
M-229	1945	130.8	128.3	+2.5
Lincoln[a]	1946	137.1	137.5	−0.4
Mandarin[a]	1946	128.3	130.1	−1.8
Richland[a]	1946	126.1	127.9	−1.8
Harman[a]	1946	134.2	134.3	−0.1
Le Platou	1946	138.1	137.0	+1.1
Kabott	1946	141.2	139.8	+1.4
Pagoda	1946	141.2	141.3	−0.1
Sunflower				
Sunrise	1944	127.7	129.8	−2.1
3975	1944	134.5	132.4	+2.1
3977	1944	125.1	125.5	−0.4
3978	1944	130.3	128.8	+1.5
Sunrise[a]	1945	131.9	132.5	−0.6
Mennonite[a]	1945	136.0	135.7	+0.3
Sunrise	1946	136.5	138.2	−1.7
Safflower				
CD 3477[a]	1945	155.2	154.5	+0.7
Type 6[a]	1945	151.6	151.7	−0.1
Pusa 2[a]	1945	135.3	133.4	+1.9
Simla[a]	1945	147.9	150.3	−2.4
Karron[a]	1945	150.9	149.2	+1.7
Miscellaneous				
Peanut oil	Commercial	102.5	103.2	−0.7
Corn oil	Commercial	123.6	121.9	+1.7
Palm oil	Commercial	8.5	6.9	+1.6
Olive oil	Commercial	39.7	39.4	+0.3
Seal oil	Commercial	42.3	44.6	−2.3
Rapeseed oil	Commercial	93.6	92.1	+1.5
Croton oil	Commercial	112.5	109.1	+3.4
Cottonseed oil	Commercial	106.6	103.2	+3.4
Oleic acid	Commercial	52.3	53.1	−0.8
Palmitic acid	Commercial	54.6	53.1	+1.5
Stearic acid	Commercial	4.0	4.9	−0.9
Butter	Commercial	35.8	36.1	−0.3
Lard	Commercial	55.0	53.5	+1.5
Lamb fat	Commercial	42.8	42.6	+0.2
Total		8018.3	8017.9	+0.4
Mean		133.6	133.6	0

[a] Oil extracted on Goldfisch apparatus with petroleum ether as solvent.

The results of the iodine number determinations are given in Table I, which lists the means of the triplicate iodine numbers determined by the two methods and the differences between them. There is no difference between the means of the methods. In some individual samples the difference is relatively large but a statistical analysis of all the results showed that these differences are not significant. The reduction in the volume of Wijs solution did not affect the accuracy of the determination to any significant extent. The standard error of a single mean, based on triplicate determinations, was calculated for each type of oil. It was the same by both methods for soybean (±0.7), safflower (±0.7), and the miscellaneous group (±0.5). For flaxseed by the A.O.A.C. method it was ±0.9 and by the modified mercuric acetate method ±0.8, whereas for sunflower it was ±0.7 and ±0.3, respectively.

With the volume of Wijs solution reduced to 10 ml. it seemed advisable to determine how far this was above the theoretical requirements and how close to the theoretical the volume could be brought without affecting the iodine number. For this purpose composite samples of freshly pressed oil from soybeans, safflower, and flaxseed were used. The iodine number was determined in triplicate on 0.100-gram samples, using varying amounts of Wijs solution. Five-minute absorption periods were used except for oil from flaxseed, where 6 minutes were allowed. From the values obtained using 25 ml. of Wijs solution the theoretical volume required for 0.100 gram of each oil was calculated.

The results of this experiment are presented graphically in Figure 1. The equations for the lines were calculated from the first five values for flaxseed, six for soybeans, and all for the safflower. The lines have been extended in the graph only as far as these values. The position of 10 ml. of Wijs solution is indicated on each line by the vertical broken line marked *X*. The line extends well beyond this point in each case, so 10 ml. of Wijs are more than sufficient for the satisfactory determination of iodine numbers. The regression coefficient was calculated in each case and was insignificant. The lines, for all practical purposes, are parallel to the axis. Inspection of Figure 1 shows that for iodine numbers of 200 no excess of Wijs solution is present. Consequently when only 10 ml. are used, the weight of sample must be reduced if the iodine number is over 200.

In all but the safflower samples the value of the iodine number decreases when the volume of Wijs solution closely approaches the theoretical volume. This decrease may be partly compensated for by an increased time interval. When the smallest volume of Wijs solution was used in the experiment with flaxseed and the time was increased to 10 minutes, the value of the iodine number was increased from 181.5 to 183.7. With soybeans, the increase was from 127.1 to 128.2.

SUMMARY AND CONCLUSIONS

The mercuric acetate method of determining iodine numbers was modified by reducing the volume of Wijs solution and using unstoppered flasks. Comparison with the official Wijs method of the Association of Official Agricultural Chemists on a large number of samples showed no significant difference between the results obtained.

The effect of decreasing amounts of Wijs solution on the value of iodine numbers was investigated. With nonconjugated fats and oils 10 ml. of Wijs solution are sufficient for 0.1-gram samples, provided the iodine value is not more than 200.

ACKNOWLEDGMENT

Acknowledgment is made to the Adams Chemical Company for the supply of sulfonated castor oil.

LITERATURE CITED

(1) Assoc. Official Agr. Chem., Official and Tentative Methods of Analysis, p. 495 (1945).
(2) Hoffman, H. D., and Green, C. E., *Oil and Soap*, **16**, 236 (1939).
(3) Norris, F. A., and Buswell, R. J., IND. ENG. CHEM., ANAL. ED., **15**, 258 (1943).
(4) *Ibid.*, **16**, 417 (1944).
(5) Skell, P. S., and Radlove, S. B., *Ibid.*, **18**, 67 (1946).

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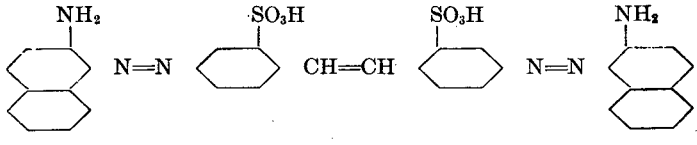
New pH Indicator for Titration of Sodium Carbonate

Disodium 4,4′-Bis(2-amino-1-naphthylazo)-2,2′-stilbenedisulfonate

MICHAEL TARAS, *Department of Water Supply, Detroit, Mich.*

THE dye resulting from the coupling of 1 mole of 4,4′-diaminostilbene-2,2′-disulfonic acid with 2 moles of β-naphthylamine is assigned the formula by Schultz (*4*). According to this



authority, the dye is known in the color trade as Hessian Purple N extra and Direct Purple.

A study of the dye's properties discloses that it may function profitably as a titrimetric indicator in the pH region near 4.0.

The defects of methyl orange indicator in the acid titration of carbonates have long been recognized. Up to the present, the remedy has consisted principally in modifying the indicator through the addition of inert dyes like cyanole xylene FF (*2*). Occasionally, bromophenol blue has been substituted for methyl orange in this titration (*6*). More recently, modified methyl yellow indicator (*1*) has been proposed for this titration. Each indicator possesses peculiar merits and, handled discriminatingly, provides advantages over the older methyl orange dye.

Inasmuch as the sharpest color change of disodium 4,4′-bis-(2-amino-1-naphthylazo)-2,2′-stilbenedisulfonate occurs at a pH of 3.8, the indicator lends itself advantageously to the titration of 0.2 *N* and 0.5 *N* sodium carbonate solutions on the one hand, or alternatively, to the direct titration of proportionate amounts of the solid salt.

INDICATOR PROPERTIES

The dye was prepared by the tetraazotization of 9.5 grams (0.025 *M*) of 4,4′-diaminostilbene-2,2′-disulfonic acid, Eastman Kodak T4614, and coupling with 8.0 grams (0.06 *M*) of β-naphthylamine, Eastman Kodak 174 (*5*). The amine was first dissolved in 50 ml. of glacial acetic acid and distilled water was added with stirring to a 100-ml. volume. Coupling was accomplished in this 50% acetic acid solution.

The disodium salt was formed by grinding a weighed quantity of the dye in a mortar with the calculated volume of 0.05 *N* sodium hydroxide and diluting to the proper concentration. Two concentrations of the dye were tested for indicator efficiency: 0.5 and 0.1%. Both solutions have a deep red color. Two or 3 drops of the 0.5% solution suffice for every 50-ml. volume titrated. In the case of the 0.1% solution about 10 drops are needed for the same volume.

The 0.5% solution possesses a sirupy consistency, presenting a minor problem of handling. On this account, most of the titrations reported in this paper were performed with the 0.1% solution.

The color characteristics of the indicator were tested using sodium dibasic phosphate–citric acid buffers (*3*). These buffer solutions showed the alkaline color of the indicator to be a delicate

Table I. Titration of Sodium Carbonate Solutions[a]

		Volume of H_2SO_4 Used					
Na_2CO_3 Taken	Na_2CO_3 in Solution	0.2 *N*	Av. deviation from mean	0.5 *N*	Av. deviation from mean	Na_2CO_3 Found	Deviation
Ml.	*Gram*	*Ml.*	*Ml.*	*Ml.*	*Ml.*	*Gram*	%
10.00	0.1060	10.02	0.01	...	..	0.1062	+0.19
25.00	0.2650	25.03	0.02	...	..	0.2653	+0.11
40.00	0.4240	40.00	0.02	...	..	0.4240	0
10.00	0.2650	...	..	10.00	0.01	0.2650	0
25.00	0.6625	...	..	25.03	0.02	0.6632	+0.11
40.00	1.0600	...	..	40.05	0.03	1.0612	+0.11

[a] All values represent average of five determinations.

red shade. The first transition occurs at a pH of 4.0 with the appearance of a faint mauve color. At a pH of 3.8, an emphatic and sharp change to purple takes place. This was the end point to which all titrations were conducted. The final conversion to a bluish purple takes place near a pH of 3.0.

EXPERIMENTAL DETAILS

In the experimental work standard 0.2 *N* and 0.5 *N* solutions of sodium carbonate were prepared by dissolving the reagent grade salt in carbon dioxide–free distilled water. Sulfuric acid solutions of the same concentrations were used as intermediate standards. Each acid solution was standardized by electrometric titration against the corresponding sodium carbonate solution. The acid solutions were then used as standards for the titration of the carbonate solutions, with the experimental indicator. Experimental conditions were equalized as far as possible by employing the same burets for the electrometric and the indicator titrations.

In the electrometric standardization of the sulfuric acid six determinations were made on each solution. Results of determinations on the 0.2 *N* acid showed an average deviation from the mean of 0.03%; those of determinations on the 0.5 *N* acid showed an average deviation from the mean of 0.014%.

The indicator titrations were conducted in Erlenmeyer flasks resting on a white background. Five determinations were made on each of 10.00-, 25.00-, and 40.00-ml. volumes of the sodium carbonate solutions. One drop of 0.1% indicator was used for each 10 ml. of solution at the end point. Titrations were conducted to the first definite purple color. No color standard was used; the color response is so good as to render such a standard superfluous, and artificial light or daylight can be used with equal effectiveness.

Results given in Table I indicate the precision and accuracy obtainable when the new indicator is used. Comparable results were obtained in a series of determinations in which samples of solid carbonate were titrated directly. One may conclude that the new indicator is satisfactory for ordinary titrations of sodium carbonate with sulfuric acid.

LITERATURE CITED

(1) Carmody, W. R., Ind. Eng. Chem., Anal. Ed., **17**, 141–2 (1945).
(2) Hickman, K. C., and Linstead, R. P., *J. Chem. Soc.*, **121**, 2502–6 (1922).
(3) McIlvaine, T. C., *J. Biol. Chem.*, **49**, 183–6 (1921).
(4) Schultz, Gustav, "Farbstofftabellen," p. 293, Berlin, Weidmannsche Buchhandlung, 1929.
(5) Taras, M., Anal. Chem., **19**, 339–41 (1947).
(6) Willard, H. H., and Furman, N. H., "Elementary Quantitative Analysis," p. 115, New York, D. Van Nostrand Co., 1935.

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Kinematic Viscometer Tube Cleaning Apparatus

JAMES McGLYNN, *Socony-Vacuum Laboratories, Technical Service Department, Brooklyn, N. Y.*

MANY petroleum laboratories, where large numbers of kinematic viscosity determinations are made, are confronted with the problem of cleaning the modified Fenske-Oswald kinematic viscometer tubes. This operation normally, in addition to being time-consuming, requires large volumes of solvent, necessitates a large stock of tubes, and is accompanied by high breakage.

This paper describes an apparatus by means of which a number of tubes may be attached to a manifold and solvent circulated through them until the mineral oil has been thoroughly flushed out. From one to eight viscometer tubes can be cleaned in approximately 5 minutes. In the author's laboratory this allows twice as many tubes to be washed in unit time. The use of this equipment results in a reduced consumption of solvent for cleaning and cuts down the amount of tube breakage by reducing the required handling.

A somewhat similar apparatus has previously been offered for sale by laboratory supply houses, including C. J. Tagliabue Company, Park & Nostrand Aves., Brooklyn, N. Y. The apparatus described in this article may be purchased from the Emil Greiner Co., 161 Sixth Ave., New York, N. Y.

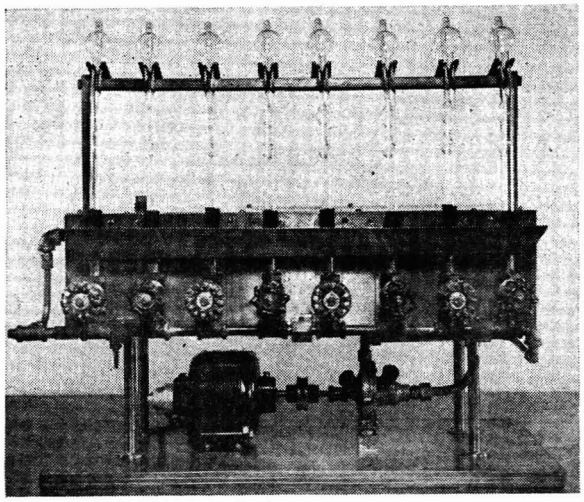


Figure 1. Kinematic Viscometer Tube Cleaning Apparatus

APPARATUS

The kinematic viscometer tube cleaning apparatus (shown in Figure 1) consists of the following parts: Solvent reservoir constructed of sheet brass having a capacity of approximately 3 gallons. G.E. explosionproof motor, 1/20 h.p., 110-volt, 60-cycle, 1-phase, G.E. catalog No. 5 KH 23 AC 15. Centrifugal pump, capacity 3 gallons per minute, M. L. Oberdorfer Brass Company, Syracuse, N. Y.

Valve manifold for connecting the modified Fenske-Ostwald kinematic viscometer tubes to the system. Pressure relief valve set at 1 pound pressure per square inch. Line filter, located on the discharge side of the pump. Rack for supporting the viscometer tubes in an inverted position.

An assembly drawing of the equipment is shown in Figure 2 (key cocks as indicated on this drawing are to be preferred to the needle valves shown in Figure 1).

PROCEDURE

The solvent reservoir is charged with a light petroleum solvent. The usual precautions relative to the use of inflammable solvents should be observed. The viscometer tubes, drained of excess oil, are connected to the valve manifold in inverted positions. The large-diameter side arm of the viscometer is connected to the manifold by means of a 2-inch length of thick-walled synthetic rubber tubing, 0.375 inch in inside diameter. The small-diameter side arm of the tube is placed in the opening in the top of the reservoir and supported in the rack provided. The valves are opened

and the solvent is forced into the large side arm of the tube, discharging through the small side arm into the reservoir. The circulation is continued until the tubes are washed free of oil. The valves are closed, and the tubes are removed from the apparatus, rinsed with acetone, and dried by drawing air through them. The entire cleaning operation for eight viscometers requires approximately 5 minutes.

When the oil concentration in the solvent is such that the viscometers are not thoroughly cleaned, the solvent is pumped out of the reservoir by attaching a length of synthetic rubber tubing to one of the valve outlets and is discharged to a suitable container for disposal. Three gallons of solvent will clean approximately three hundred tubes.

The author's laboratory has had occasion to test large numbers of competitive, used, and compounded oils containing many types of additives. Except for cases as mentioned below, no condition has been encountered where a light petroleum solvent will not satisfactorily clean the tubes when the procedure as outlined is followed. Viscometer tubes soiled with used oils, asphaltic materials, or wax products are rinsed with a suitable solvent before being mounted on the apparatus, in order to avoid excessive contamination of the solvent in the reservoir.

RECEIVED August 11, 1947.

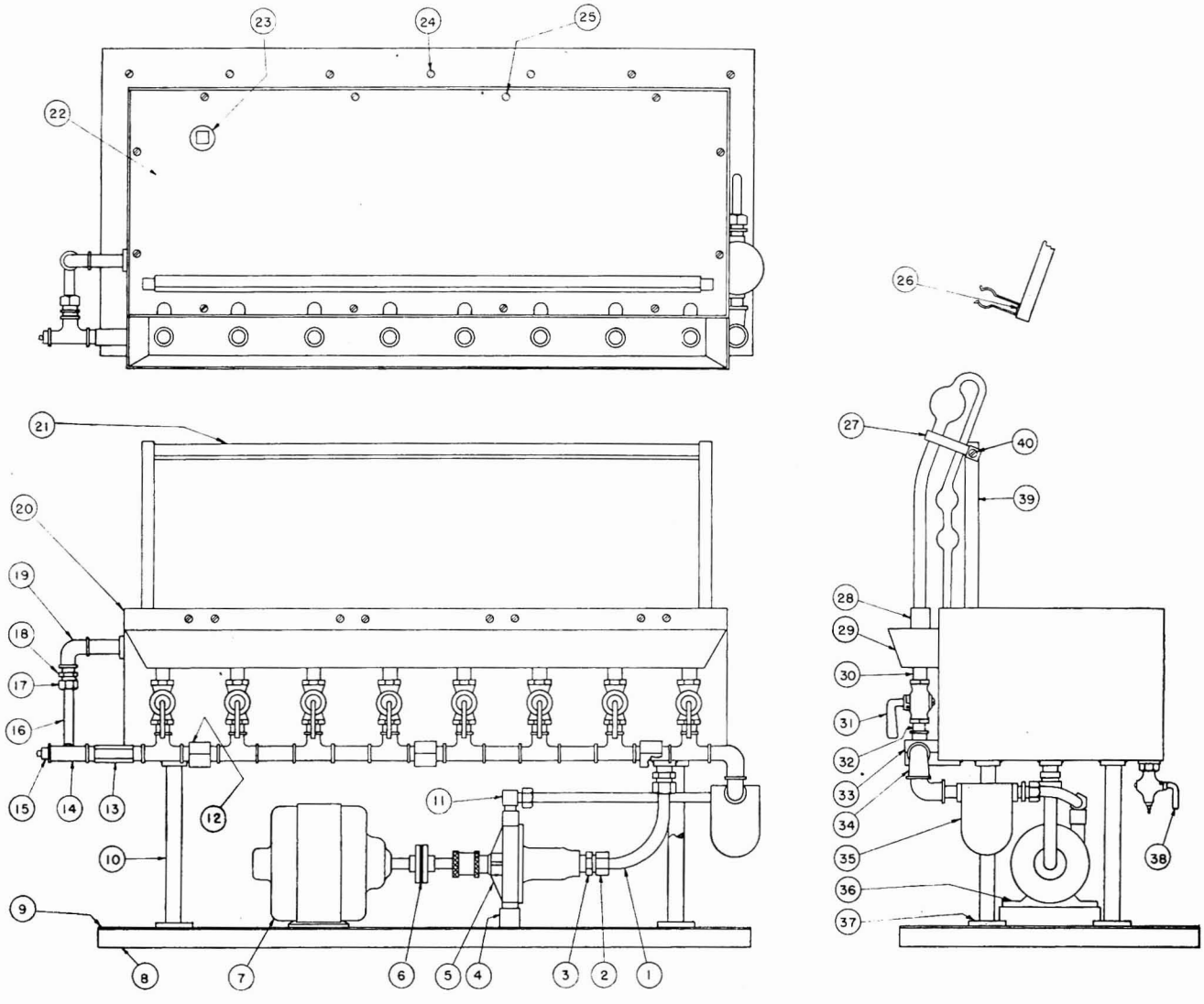


Figure 2. Diagram of Apparatus

1. 1/2 inch copper tubing, commercial
2. Two 1/2 inch coupling nuts, commercial
3. Two connector couplings, commercial
4. Wood pump base
5. Oberdorfer centrifugal pump
6. 3/8 × 5/16 inch flexible coupling, commercial
7. G.E. explosionproof motor, 1/29 h.p., 1725 r.p.m., 115-volt, alternating current
8. Wood mounting base
9. Brass mounting plate
10. 4 brass tank supports
11. Brass compression elbow
12. 12 brass No. 5–40 flat-head machine screws
13. 1-lb. check valve
14. 9 brass tees, 1/4 × 1/4 × 1/4 inch
15. 1/4 inch brass pipe plug
16. 3/8 inch copper tubing
17. 4 coupling nuts
18. 3 connector couplings
19. 1/4 inch brass elbow
20. Brass tank, 9 × 24 × 6 inches (No. 14 gage)
21. Brass tube strap bar
22. Brass tank cover
23. 3/8 inch brass pipe plug
24. 16 brass No. 4 flat-head wood screws
25. 31 brass No. 5–40 round-head machine screws
26. 8 commercial No. 4–40 flat-head machine screws
27. 8 steel spring clips
28. 8 rubber hose
29. Copper catch tray
30. 8 brass nipples
31. 8 brass ground key cocks (Lunkenheimer), 1/4 inch
32. 8 brass nipples, 1/4 inch
33. 3 brass nipple straps
34. 2 brass street elbows
35. Oil filter
36. 6 brass No. 12 round-head wood screws
37. 8 brass No. 5 round-head wood screws
38. Brass draincock
39. 2 brass tube strap bar supports
40. 2 brass 1/4 inch 20-thread round-head machine screws

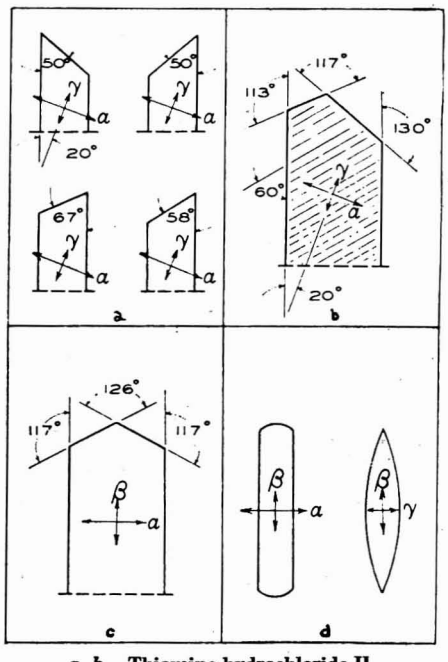
CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

AS THIS is the fifth in this monthly series of papers covering crystallographic data, the foundation feels justified in asking for critical comments and suggestions on this program. At the same time it wishes to thank those who have already sent in data for completion, checking, and publication; any suitable data or even compounds for study should be addressed to W. C. McCrone, Armour Research Foundation, 35 West 33rd St., Chicago 16, Ill.

8. Thiamine Hydrochloride (Vitamin B_1)

Thiamine hydrochloride is an important member of the vitamin B series and is interesting from a crystallographic point of view because it possesses at least three polymorphic forms, no one of which crystallizes under ordinary laboratory conditions as well-formed crystals. This compound is also unusual in that no one of the modifications can be easily obtained in a pure form and even more unusual in that modification II which is stable above about 182° C. is the common form, even in commercial preparations. For this reason this modification has been most completely studied. A complete study of all modifications of thiamine hydrochloride was considered impractical and unnecessary for this program.



a, b. Thiamine hydrochloride II
c, d. Thiamine hydrochloride I

Figure 1. Crystal Habit of Thiamine Hydrochloride

The most complete data on thiamine hydrochloride, vitamin B_1, were published by Bernal and Crowfoot (*1*) in which the crystals were shown to be monoclinic. They also state that the common habit is tabular with most of the crystals lying on well-developed clinopinacoid faces. Williams (*3*) has crystallized this compound after some difficulty, obtaining variations in habit from needles to plates. Keenan (*2*) proposes as a means of identification the change of habit from plates to spherites upon the addition of auric bromide.

The work on thiamine hydrochloride indicates that the crystals may exist in at least two crystalline modifications. Form I is orthorhombic (Figures 1 and 2) and exhibits low birefringence on the principal plate view. Form II is monoclinic (Figures 1, 3, and 4) and exhibits a much higher birefringence on the same view. Both polymorphs were obtained in varying amounts by recrystallization of the commercial product from common solvents slightly acidified with hydrochloric acid—e.g., acetone, alcohol, water, and dioxane.

Solution phase transformations were obtained at room temperature and at 80° C.; these indicated that I is the stable form over this range. Modification II dissolves in the saturated aqueous solution while I grows correspondingly. A typical solid-solid transformation of I to II is observed at about 182° C. on heating in the hot stage microscope. (The sample should be introduced at a temperature just below 182° C. in order to avoid extensive decomposition of the crystals before transformation.) The reverse transformation, II to I, which is observed in the solution phase, is not observed on cooling II in the absence of a solvent.

The transformation I to II can be defined positively as enantiotropic since the transition temperature (182° C.) is below the melting point. All of the crystals obtained in the various recrystallizations have been identified as I or II. There is then no direct evidence of a third polymorph III.

Another phase which appears when thiamine hydrochloride is recrystallized from aqueous acetone or alcohol is pseudomorphic. The optical properties agree with those of II although the ex-

Figure 2. Characteristic Crystals of Thiamine Hydrochloride I from Water on a Microscope Slide

Figure 3. Typical Sample of Commercial Thiamine Hydrochloride II

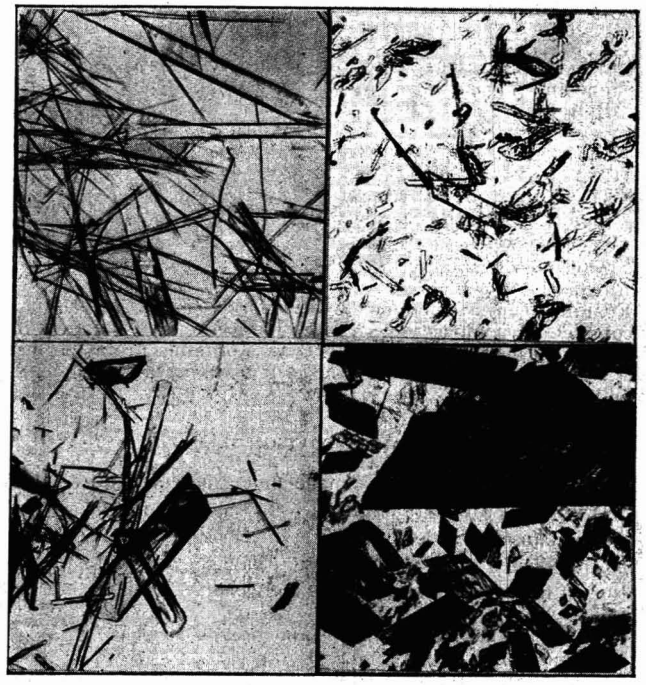


Figure 4. Characteristic Crystals of Thiamine Hydrochloride II from Water on a Microscope Slide

Figure 5. Typical Crystals of Pseudomorphic Thiamine Hydrochloride Obtained from Aqueous Alcohol Solutions

ternal shape is different (Figure 5). It is significant that these forms occur in products recrystallized from low boiling solvents in which they could not have undergone a transformation from I (transition temperature about 182° C.). In order to explain this form it is necessary to postulate a third polymorph which is less stable than I and II.

It proved to be very difficult to obtain massive crystals of thiamine hydrochloride which were suitable for microscopic examination. The best crystals were obtained by recrystallization from alcohol-water or acetone-water solutions containing enough hydrochloric acid to prevent dissociation of the amine hydrochloride.

MODIFICATION I

Crystals of thiamine hydrochloride I are, in general, obtained by slower crystallization than that used to yield II; they are invariably skeletal. Occasionally, in crystallization from water on a microscope slide, the terminal development is complete enough to allow measurement of the profile angles. Seldom, however, is there any further indication of specific crystal faces.

CRYSTAL MORPHOLOGY (determined and checked by W. C. McCrone and A. Smedal).

Crystal System. Orthorhombic.
Form and Habit. Elongated skeletal plates and tablets.
Profile Angles (True). 117°, 126°.

X-RAY DIFFRACTION DATA (determined and checked by J. F. Whitney and M. Tull).

Formula Weight. 36.8.
Density. 1.435; 1.43 (*1*).

d	I/I_1	*d*	I/I_1
11.002	0.43	3.192	1.00
8.543	0.37	3.131	0.28
5.894	0.47	2.941	0.52
5.356	0.65	2.846	0.15
5.099	0.30	2.725	0.23
4.866	0.17	2.614	0.05
4.634	0.27	2.565	0.20
4.522	0.22	2.498	0.13
4.272	0.13	2.441	..
4.101	0.13	2.388	..
3.829	0.45	2.333	..
3.722	0.33	2.248	..
3.632	0.35	2.134	..
3.508	0.03	2.086	..
3.398	0.33	2.060	..
3.304	0.43	2.002	..

OPTICAL PROPERTIES (determined and checked by W. C. McCrone and A. Smedal).

Refractive Indices (5893 A.; 25° C.). $\alpha = 1.618 \pm 0.002$. $\beta = 1.640 \pm 0.002$. $\gamma = 1.714 \pm 0.002$.
Optic Axial Angles (5893 Å.; 25° C.). $2V = 61°$. $2E = 112°$.
Dispersion. $r > v$ (strong).
Sign of Double Refraction. +.
Acute Bisectrix. γ.
Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.657$. R (calcd.) = 93.2. R (obsd.) = 86.4.

FUSION DATA. Thiamine hydrochloride melts with almost complete decomposition. Some fine crystalline sublimate is usually formed but no crystals can be obtained from the melt. A very characteristic odor reminiscent of baking bread is apparent on heating.

MODIFICATION II

Lath-shaped crystals of monoclinic thiamine hydrochloride (II) lying on 010 were obtained by recrystallization from water on a microscope slide. Modification II often occurs as pseudomorphs which show characteristic cleavages at 60° to the long edge of the crystal and ill-formed end faces.

CRYSTAL MORPHOLOGY (determined and checked by W. C. McCrone and A. Smedal).

Crystal System. Monoclinic.
Form and Habit. Plates and tablets lying on the clinodome, {010} with basal pinacoid, {001}, and positive hemiorthodome, {201}.
Axial Ratio. $a:b:c = 0.615:1:0.340$.
Interfacial Angles (polar). $100\Lambda001 = 67°$.
Beta Angle. 66° 5′ (x-ray) (*1*).

X-RAY DIFFRACTION DATA (determined by Bernal, *1*).

Space Group. $C_{2h}^5 - P_{1/a}^2$ (*1*).
Cell Dimensions. $a = 12.62$ Å.; $b = 20.53$ Å.; $c = 6.96$ Å. (*1*).
Formula Weights per Cell. 4 (*1*).
Density. 1.405 ± 0.003.

Principal Lines

Index	*d*	I/I_1
...	7.79	0.43
111	6.51	0.29
200	5.80	0.57
021	5.42	0.29
221	4.86	0.23
101, 111	4.683	0.86
121	4.363	1.00
...	4.056	1.00
300, 240	3.829	0.29
212	3.403	0.17
002	3.201	0.46
012, 312	3.122	0.69
400, 112	2.889	0.51
022, 420	2.787	0.20
222, 042	2.684	..
360	2.559	..
440	2.495	..
442	2.400	..
500, 222	2.333	..
090	2.259	..
242, 460	2.196	..
333, 462	2.156	..
003	2.106	..
023	2.062	..
550, 033	2.015	..

OPTICAL PROPERTIES (determined and checked by W. C. McCrone and A. Smedal).

Refractive Indices (5893 Å.; 25° C.). $\alpha = 1.600 \pm 0.002$. $\beta = 1.639 \pm 0.002$. $\gamma = 1.685 \pm 0.002$.
Optic Axial Angles (5893 Å.; 25° C.). $2V = 87°$.
Dispersion. Little or no dispersion.
Optic Axial Plane. 010.
Sign of Double Refraction. +.
Acute Bisectrix. γ inclined 20° to c in acute β.
Extinction. $\gamma\Lambda c = 20°$ in acute β.
Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.641$. R (calcd.) = 93.2. R (obsd.) = 86.6.

FUSION DATA. See under modification I.

LITERATURE CITED

(1) Bernal, J. D., and Crowfoot, D., *Nature*, **131**, 911–12 (1933).
(2) Keenan, Geo., *J. Assoc. Official Agr. Chem.*, **26**, 514–16 (1943).
(3) Williams, R. R., *J. Am. Chem. Soc.*, 57, 517–20 (1935).

Correspondence

Determination of Cobalt in Stainless Steel

SIR: I refer to the paper on "Colorimetric Method for the Determination of Cobalt in Stainless Steel" by Putsché and Malooly [ANAL. CHEM., **19**, 236 (1947)].

It is very likely because of war difficulties that the authors have failed to record the work on this subject by E. Stengel which appeared in *Stahl und Eisen* [**63**, No. 34, 621 (1943)]. This colorimetric method is now one of the usual tools in many industrial analytical Italian laboratories.

GAETANO GAVIOLI

Instituto Scientifico Tecnico E. Breda,
Milan, Italy

SIR: The authors regret that the Stengel 1943 publication in *Stahl und Eisen* was overlooked by them, and therefore not included among the nine literature references cited in their recent paper. This oversight, as surmised by Gavioli, was due to the exigencies of the recent war.

While Stengel described a photometric procedure utilizing the ammonium thiocyanate reaction in an acetone medium for the determination of cobalt in alloy steels, the article in question contains no reference to the important work of previous investigators, such as

Vogel, Kolthoff, and Tomula, nor does it contain any experimental or analytical data.

In certain respects the method of the authors differs from that of Stengel. In the first place, instead of dissolving a sample in dilute hydrochloric acid, oxidizing with nitric acid, and treating the dry residue with dilute hydrochloric acid, the authors employ aqua regia and hydrofluoric acid followed by treatment with perchloric acid, which effects the complete solution of all carbidic residues. Secondly, in the case of Stengel's procedure nickel interferes with the photometric reading for cobalt and must be separately determined. In the case of the authors' process the background color due to nickel, etc., is colorimetrically read before and after the development of the color due to the cobalt thiocyanate complex. In the third place, the method of the authors is far more rapid than that of Stengel. The entire procedure of the authors may be completed in the time required to take the Stengel solution to dryness on the steam bath at the outset of his procedure. Finally, the 24 different types of cobalt alloys shown in Table II of the authors' paper is an indication of the wide adaptability of the method in the presence of a large variety of other alloying elements.

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H. M. PUTSCHÉ

American Rolling Mill Company,
Baltimore 13, Md.

Book Reviews

Manual of Quantitative Analysis. *Donald R. Clippinger.* vi + 339 pages. Ginn and Co., Statler Building, Boston 17, Mass., 1947. Price, $3.50.

This text and laboratory manual was designed for a fundamental course in quantitative analysis for premedical or predental students or for students majoring in chemistry. Besides the conventional experiments in gravimetric and volumetric analysis, the book includes experiments in colorimetric, electrophotometric, potentiometric, and polarographic analyses. The reviewer believes that the latter experiments might well be left to a more advanced course, or to a course in instrumental analysis where the theory could be more completely and satisfactorily covered.

There are many errors in the text. On page 32 there is a misprint in the formula for the dichromate ion. On page 100 Equations 1 and 2 are in error. On page 213 both the ionic equation and the equation for the solubility product constant of Ag_3PO_4 are wrong.

In Table VIII, page 31, the author categorically gives the equivalent weight of H_3PO_4 as one third of the molecular weight. This is misleading to the student, who finds that one rarely titrates phosphoric acid to its third equivalent, and then only upon the addition of $CaCl_2$. The same is true in Table X, where the equivalent weight of Na_2HPO_4 is given as one third of its molecular weight on the basis of the total value of the positive ions, but in protolytic reactions it is not so used. On page 50, the titration curve for Na_2CO_3 *vs.* HCl shows only one break in the curve and the graph would seem to indicate that the equivalence point would occur at pH 6.

On page 141, in the discussion of the reaction $I_2 + SO_3^{--} + H_2O \rightarrow SO_4^{--} + 2H^+ + 2I^-$, the author refers to the table on page 101 for the oxidation-reduction potential of the sulfite-sulfate system, which is not found in the table. Furthermore, the author implies that the $^cH^+$ influences the reverse reaction between SO_4^{--} and I^-, which of course is not true, as the reaction is not reversible except with concentrated sulfuric acid. He fails to point out, however, that the chief source of error here is the air oxidation of I^-, which is greatly influenced by the pH of the solution. In Part III, Chapter XI, there is very little discussion of the proper selection of wash solutions for gravimetric analysis. In the procedure for gravimetric iron, 1*N* NH_4OH is used as a wash for ferric hydroxide instead of NH_4NO_3.

On the basis of the number of errors in the book, this reviewer does not feel that he can recommend this text for a fundamental course in quantitative analysis.

WILLIAM J. TOMSICEK

Standard Methods for Testing Petroleum and Its Products. *Institute of Petroleum,* 26 Portland Place, London W.1, England, 1948. 9th ed. xlvii + 616 pages. Available from American Society for Testing Materials, 1916 Race St., Philadelphia 3, Pa. Price, $6.75.

New methods include the determination of chlorine in unused and used lubricating oils, knock rating of aviation fuels (rich mixture), lead, copper, and iron in lubricating oils, oil separation on storage of grease, and a spectrographic method for the determination of inorganic constituents of petroleum ashes.

This new procedure is of particular interest. A flux containing ferric sulfate is added to the ash of the oil in a known proportion and the mixture is arced on copper electrodes using direct current. The large excess of added iron produces a uniform matrix and minimizes variations due to ashes of different compositions.

Revised methods include the copper strip corrosion, Diesel fuel diluent, cutback bitumen distillation, ignition quality of Diesel fuel, knock rating of aviation fuels (weak mixture), knock rating of motor fuel, grease penetration, saponifiable matter, smoke point, and unsaponifiable matter in drying oils. Two new thermometers are specified.

Close contact has been maintained with the work of the A.S.T.M. and it has been decided that the working year be altered so as to publish this volume simultaneously with the report of A.S.T.M. Committee D-2.

E. L. BALDESCHWIELER

Symposium on Electron and Light Microscopy

F. A. HAMM, ***General Aniline & Film Corp., Easton, Pa.***

THIS Symposium on Electron and Light Microscopy (sponsored by the Armour Research Foundation, Illinois Institute of Technology) was held in the Stevens Hotel, Chicago, Ill., on June 10, 11, and 12, 1948. It was the first meeting for the purpose of bringing together electron and light microscopists, who have much in common, not only in their technical background but also in the nature of their research problems. The program was well planned, and much credit must be given to W. C. McCrone and C. F. Tufts, co-chairmen, for their efforts in carrying out this excellent symposium idea. The opinion, expressed by many of the 300 attending, clearly indicates the need for making this symposium an annual affair.

The main feature of the program consisted of invited papers, presented by speakers well qualified to talk on their particular subject, and dispersed so as to command the attention of those in the audience who might be interested in either or both types of microscopy. Unfortunately, E. F. Burton of the University of Toronto and E. M. Chamot of Cornell University were not able to attend the symposium because of illness. These men might well be considered to be the "fathers" of electron and light microscopy, respectively.

The first two days consisted of formal presentations covering the corroborative nature of electron and light microscopy, as well as some specific developments in instruments (light microscopes) and some applications of both types of microscopy to research problems. Informal panel sessions on instrumentation, metal surfaces, and high speed microtomy were held on the third day. N. P. Allen and B. E. Hopkins of the National Physical Laboratory, Teddington, England, took an active part in the discussions on metallurgy.

New instruments such as phase contrast microscopes, an electron diffraction unit, high vacuum units, and high speed microtomes were displayed by several commercial organizations. A photographic exhibit of light and electron micrographs sponsored

by various research laboratories was displayed concurrent with the new instruments.

Mention should be made of an excellent color photomicrographic motion picture film presented by Henry N. Baumann of the Carborundum Company after the banquet on Friday night. The formation of various compounds of aluminum and silicon and their behavior on fusion and crystallization at extremely high temperatures were well illustrated. The quality of the color reproduction and the research information gained by this photographic record are noteworthy.

After the welcoming addresses by William A. Lewis, Illinois Institute of Technology, and Haldon A. Leedy, Armour Research Foundation, four general papers were presented illustrating the corroborative overlapping of electron and light microscopy. These papers were presented by W. G. Kinsinger, Hercules Powder Co., Wilmington, Del., Clyde W. Mason, Cornell University, Ithaca, N. Y., Robley C. Williams, University of Michigan, Ann Arbor, Mich., and Cecil E. Hall, Massachusetts Institute of Technology, Cambridge, Mass. The improvements in sample preparation, the usefulness of artifacts, and the analogy between dark-field methods in light and electron microscopy are just a few of the significant aspects discussed.

The papers abstracted below have been grouped in so far as possible according to techniques.

LIGHT MICROSCOPY

Ultraviolet, Visible, and Infrared Microscopy. K. J. Heinecke, Bausch & Lomb Optical Co., Rochester, N. Y.

Two new developments, the reflection microscope and the reflection-refraction microscope, were briefly discussed. The longer working distance with high numerical aperture objectives with no loss in resolution seems to be the chief advantage. It is relatively easy to make optics suitable for infrared microscopy, so that the utilization of absorption curves (thermister bolometers) should prove significant in the study of light microscopical samples.

The Microscope Objective and Its Function. L. V. Foster, Bausch & Lomb Optical Co., Rochester, N. Y.

The importance of using the central undiffracted light rays and the first order diffracted rays on both sides of the undiffracted rays for high resolution, aberration-free images was illustrated by means of light micrographs of diatoms. The effect of improper positioning of the various elements in an objective lens on the asymmetrical aberrations in the image was also illustrated.

The Principles of Phase Microscopy. F. Zernike, Johns Hopkins University, Baltimore, Md.

Special Experiments in Phase Microscopy. Charles P. Saylor, National Bureau of Standards, Washington, D. C.

Instrumentation in Phase Microscopy. Helen Jupnik, American Optical Co., Buffalo, N. Y.

These three papers demonstrated the theoretical and practical aspects of "phase-contrast" microscopy. It is now an established fact that greater contrast with no loss in resolution is possible with the proper use of diffraction plates in the substage condenser and in the back aperture of the objective. The degree of contrast may be varied by changing the amplitude ratios and phase differences in the deviated and undeviated light rays originating by diffraction in the specimen. The degree of contrast may be varied to the extreme so that a dark-field (negative contrast) image is realized. The radii and thickness of the dielectric material on the phase-contrast plates determine the degree of changes in amplitude and phase difference introduced in the undeviated and deviated rays. Consequently, the number of phase-contrast plates required is determined by the number of different specimens to be examined.

Crystal Optics on Microscopic Views. William A. O'Brien, Celanese Corporation of America, Summit, N. J., and J. D. H. Donnay, Johns Hopkins University, Baltimore, Md.

Crystal identification by light microscopical identification is difficult because the data measured on a certain view must be correlated with published goniometric data taken from all the principal orientations—that is, silhouette angles must be converted to true interfacial angles if the published data are to be used as a basis for identifying the unknown crystalline material.

The apparent interedge (silhouette) angles measured from microscopical views may be converted into interfacial angles. Or, if the morphological constants (axial elements, interfacial angles) are known, it is possible to calculate the apparent angle between any two edges that will be seen when the crystal lies on any given face.

The available data are plotted on a stereographic Wulff net for graphic constructions. For a detailed outline, reference should be made to Ind. Eng. Chem., Anal. Ed., **17**, 593 (1945).

Application of Microscopy to Polymorphism of Tristearin-Type Fats. Oscar T. Quimby, The Procter and Gamble Co., Ivorydale, Cincinnati, Ohio.

The mono-acid triglycerides exhibit monotropic trimorphism. All the solids are truly crystalline, and the various forms may be identified by x-ray diffraction. However, because of the polymorphic behavior, the x-ray diffraction pattern might not indicate the exact form initially isolated. Consequently, the polarizing microscope is useful for following the phase transformations, and furnishes the history of the sample without destroying it.

Resinography. T. G. Rochow, American Cyanamid Co., Stamford, Conn.

"Resinography" is proposed as the name for the graphic study of resins and their plastics. It is analogous to metallography. The filler, pigment, and matrix, and their relation to structure, strength, smoothness, opacity, etc., are being studied with both the light and electron microscopes. The author has a publication on this subject as applied to the paper industry [*Paper Trade Jl.*, **126**, No. 8, 80, 82, 84, 86, 88, 90 (1948)].

Effect of Particle Size on the Diffraction Image in Microscopy. Harold Osterberg, American Optical Co., Buffalo, N. Y.

Because the Airy disk increases rather rapidly when the radius of the particle falls in the range of 0.3 to 0.6 of the usual Airy limit of resolution, it follows that a good microscope objective reveals more about the size of small particles than is commonly believed. However, the shape and the particle size relative to the numerical aperture of the objective cause irregular disturbances in the diffraction image. Nevertheless, these regular and irregular growths in the Airy disk may be used to gain information about the physical properties of the particle.

Application of Microscopy to the Pigment Industry. Charles Maresh, American Cyanamid Co., Calco Chemical Division, Bound Brook, N. J.

The usual commercial organic pigments are often in the 0.2- to 0.4-micron range. Hence the electron microscope has been of greatest value in study of the physical properties of the pigment particles. Furthermore, the microstructural detail in texture, gloss, and weathering can best be evaluated by means of surface replicas. However, the light microscope has been used to evaluate the refractive index, pleochroism, and polymorphic behavior of these pigments. The data assembled by means of both instruments are obviously useful to the paint and pigment technologist.

The Study of Crystals, Oriented Aggregates, and Lyotropic Mesomorphs of Strongly Absorbing Substances with the Polarizing Microscope. Edwin E. Jelley, Eastman Kodak Co., Rochester, N. Y.

About three dozen color transparencies were shown illustrating the utilization of optical-crystallographic properties in the identification of organic crystals. Abnormal polarization colors, optical dispersion of the isogyres in interference figures, pleochroism, liquid crystal formation, etc., were used to identify various compounds. These compounds were sometimes relatively simple, or sometimes complex in the sense that various types of solvates might form. The various stages in the formation of the phenolate of 2,2′-cyanine chloride were illustrated.

ELECTRON MICROSCOPY

Electron Microscope Goniometry. Alan F. Kirkpatrick, American Cyanamid Co., Stamford, Conn.

The electron microscope image usually furnishes only a silhouette image of one (or at best only a few) morphological orientation of a crystal. These silhouette angles must be converted to interfacial angles if the data are to be compared with the literature for purposes of identification. The interfacial angles, as well as the axial ratios and interaxial angles, can be calculated from the silhouette angles by means of a stereographic projection on a Wulff net type of graph paper. The author presented the calculation for calcium carbonate.

Light and Electron Microscopical Studies of Cellulose Fibers. CHARLES W. HOCK, Hercules Powder Co., Wilmington, Del.

Natural cellulose fibers (cotton) have a laminated fabrillar structure. The relatively coarse fibrils observed by light microscopy are further subdivided into finer fibrils as seen in the electron microscope. A lengthwise periodicity has also been detected. This structure may play a significant role in the chemical reactivity of the fiber. Several of the synthetic cellulose fibers (rayons) have their own characteristic fibrillar pattern. However, this pattern is not so distinct as that in the natural fiber.

Electron Microscopy of Particle Aggregation in Carbon Black. JOHN H. L. WATSON, Medical Research Institute, Henry Ford Hospital, Detroit, Mich.

The need for evaluating the size and structure of small particle aggregates was demonstrated. Too often too much emphasis is placed on the fundamental particle size, which may not be so significant as the aggregate data. A critical discussion of the interpretation of the electron microscope image in terms of ellipticity and distance from the supporting membrane was presented. The accuracy of the technique and its relation to rubber compounding were mentioned.

Statistical Analysis of Electron Microscope Particle Size Determinations. PAUL L. COPELAND, Illinois Institute of Technology, Chicago, Ill.

This paper in part fills a strong need for a critical analysis of the accuracy of electron microscope particle size determinations. The practical results from a large number of measurements were compared with a theoretical (statistical) calculation. The measurements made on a large number of imaged particles do not agree with the calculated values, probably because of an inadequate representation of the whole sample due to the large number of very small particles.

Properties of Evaporated Gold. P. G. WILKINSON AND L. S. BIRKS, Naval Research Laboratory, Washington, D. C.

Evaporated gold blacks and thin films were examined in the electron microscope in order to relate continuity with thickness and electrical resistance. Gold films may be continuous or discontinuous, depending upon their thickness, as determined by electrical measurements. Gold blacks are discontinuous in nature.

Results of Electron Microscope Studies on Bacteriophage Action. THOMAS F. ANDERSON, Johnson Foundation, University of Pennsylvania, Philadelphia, Pa.

The electron microscope was shown to be a useful tool in examining viruses from the standpoint of their morphological identification, response to sonic vibrations, and the mechanism with which they adsorb on the host bacteria cell followed by proliferation within the infected cell. The sonic treatment breaks up only the larger tadpole-shaped viruses; the fragments continue to survive. Certain phages must be activated with amino acids before they will absorb on the host cell. Seven phages on strain B of *E. Coli* were cited as examples.

Electron Microscopy of the Tubercle Bacillus (BCG) by Metal Shadow Casting Technique. C. I. REED, S. R. ROSENTHAL, AND B. P. REED, University of Illinois, Urbana, Ill.

The advantages of metal-shadowing (chromium) the surface of bacteria were once again demonstrated. Surface (capsule) structure and also some internal details were made more recognizable by the increase in the contrast in the electron microscope image. The history (preparation) of the BCG vaccine and its relation to the morphology of the bacillus were presented.

Electron Metallography. ROBERT D. HEIDENREICH, Bell Telephone Laboratories, Inc., Murray Hill, N. J.

The combination of electron microscopy and electron diffraction in the study of surface metal structure might appropriately be called "electron metallography." Electron reflection diffraction, although subject to many qualifications, is useful for identifying the surface films. Slip planes, grain size and structure, height of surface elevations, etc., may be studied by several replica techniques. Three types (plastic, silica, and oxide film) of replicas were described with emphasis on their resolving power. The oxide film type is probably the only one that resolves better than 50 Å.

Electron Microscopical Study of Organic Pigments. F. A. HAMM, General Aniline & Film Corp., Easton, Pa.

A variety of organic pigments (dyes) have been analyzed for the sake of identification and evaluation of their microphysical properties (shape, size, porosity, aggregation, etc.). Three broad aspects of this long range research program were emphasized: (1) the use of sonic (audio, 9 kc., and ultra, 400 kc.) vibrations for dispersing severely aggregated pigments discussed from the standpoint of the quality of the electron microscope specimen; (2) the radiation-chemical effect of the electron beam and its relation to the polymorphism of several pigments; and (3) the utilization of the RCA Type EMU electron microscope in the "fingerprinting" of pigments by means of their electron diffraction patterns.

MISCELLANEOUS

Electron Diffraction—An Instrument and Some Applications. J. G. HUTTON, General Electric Co., Schenectady, N. Y.

The new G.E. electron diffraction instrument was described from its functional and applicability viewpoints.

High Vacuum Metallizing. GEORGE H. BANCROFT, Distillation Products, Inc., Rochester, N. Y.

A new 12-inch vacuum coater (D.P.I.) was described, including its required high vacuum technique.

Techniques of High Speed Microtomy. ERNEST F. FULLAM, General Electric Co., Schenectady, N. Y.

The need for a technique in the preparation of sections sufficiently thin for examination in the electron microscope has been partially filled. Animal tissue, fibers, plastics, etc., have been sectioned in thickness of 0.5 micron or less.

At the panel session on high speed microtomy, R. R. Allen of Custom Scientific Instruments, Inc., Arlington, N. J., and W. F. Fullam of Hemsdale International, Inc., 350 Fifth Ave., New York, N. Y., described their respective instruments and their new improvements. It is now possible to evacuate the chamber in which the sections are cut, cool (freeze) the sample before sectioning, and adapt the disk to serve as an ultracentrifuge.

The Analyst's Calendar

Second Symposium on Analytical Chemistry

Arrangements are now being made for holding the Second Annual Summer Symposium on Analytical Chemistry sponsored by the Division of Analytical and Micro Chemistry and ANALYTICAL CHEMISTRY, at Wesleyan University, Middletown, Conn., in June 1949. The conference will run two full days and will include four technical sessions and a get-together on the evening of the first day. M. G. Burford, Wesleyan University, will be chairman in charge of arrangements.

Symposium on Nucleonics and Analytical Chemistry. Division of Analytical and Micro Chemistry, Northwestern University, Evanston, Ill., Aug. 13 and 14.

Second Annual Summer Symposium on Analytical Chemistry. Wesleyan University, Middletown, Conn., June 1949.

Raman Spectra of Hydrocarbons—Correction

In the article on "Raman Spectra of Hydrocarbons" [ANAL. CHEM., 19, 700 (1947)], on page 710 the second paragraph under "Disubstituted Aromatics" should read: "The 1,4-disubstitution is characterized by"

Page 711, the dates for reference (7) should be 1939, 1940, and 1946.

Page 721, in spectrum table No. 162, the scattering coefficient value for the $\Delta\nu$ 651 cm.$^{-1}$ line should be 0.560.

M. R. FENSKE

AIDS FOR THE ANALYST . . . . .

Clamp and Pipet for Rapid Delivery of Approximate Volumes.
Frederic E. Holmes, 6515 Blueridge Ave., Cincinnati 13, Ohio.

THE apparatus shown in Figure 1 is suitable for rapid delivery of approximate volumes of reagent. As used for dispensing 5-ml. portions of Benedict's qualitative solution for determination of sugar in routine urinalyses, the variation in amount delivered is in the neighborhood of 0.3 ml. Considerably greater accuracy could be realized by use of a constant-level device delivering from the main reservoir into a small intermediate reservoir in the supply line. This device would also provide a fine adjustment of volume. However, when an approximate volume is adequate, the simplicity of the apparatus as shown outweighs the advantages of elaborate accessories.

The assembly embodies two essential features not commonly used in apparatus for similar purposes. The double-acting clamp, which controls the delivery of reagent and refilling of the pipet, operates against rubber tubing, thus eliminating glass or metal cocks which are subject to sticking and leaking due to corrosion or deposition of salts from concentrated solutions. The portion of the pipet above the bulb provides for an approximate zero: The inflow of reagent is checked at the level of that in the reservoir, and the small lumen minimizes change in volume with change in level. While this zero is less precise than those afforded by overflow or backflow tubes, manual operations or elaborate glass construction for return of reagent to the reservoir is avoided.

Further advantages are relative ruggedness, ease of construction from common materials with common tools and a minimum of glass-blowing, and simple operation. One of these pipets has been in operation in the clinical laboratory at Christ Hospital, Cincinnati, for several months. No breakage or other difficulties have occurred, even though several people have used it without prior experience or instruction.

The operation of the control clamp is shown in the top views at the right of Figure 1. When the movable arm of the control clamp, *MCA*, is depressed by pressure of the thumb against the outer (right) end, the rubber filling tube is closed before the delivery tube is opened. The contents of the pipet are delivered without leakage from the filling tube during delivery. This manner of operation is ensured by the free-floating action of the movable arm, which makes either tube the fulcrum of the lever when the other is being opened. Similarly, the delivery tube is closed first and then the filling tube opened when the pressure of the thumb is released.

SPECIFICATIONS

RB, rubber band or bands.
RL, retaining link, rectangular loop of wire resting loosely in notches in *FCA* and *MCA*.
DT, delivery tube, soft rubber, $^3/_{16} \times {}^1/_{16}$ inch (4.5 × 1.5 mm.).
FT, filling tube, from reservoir, conveniently same as *DT*.
Change of position of entire pipet upward or downward in relation to clamp increases or decreases volume of reagent delivered.
C, constriction to slow inflow and prevent trapping of bubble in delivery tube, approximately 2.5-mm. inside diameter.
SC, supporting clamp, wooden block with sloping ends and center notch to accommodate stem of pipet, rubber bands stretched over pipet stem to hold it in place.
S, main support, wooden board to which *FCA* is attached.
FCA, fixed arm of control clamp.
MCA, movable arm of control clamp.
PS, positioning studs, wire brads, fixed in *S* and *FCA* and extending through drilled holes in *MCA*, which have about three times the diameter of the studs to allow free movement.
F, funnel top of capillary stem of pipet to facilitate rinsing with water to redissolve any salt precipitated.
Pipet. The bulb accommodates the desired volume minus a small volume contained in the capillary stem and the amount required to fill the glass and rubber tubing below it to the point of cutoff in the clamp.
Small blocks are attached as shown to the fixed and movable arms between the positioning studs to allow wider amplitude of movement of *MCA*. A piece of wire, not shown, is laid over the positioning studs across both rubber tubes to concentrate the pressure against the tubes and make a sharper cutoff.

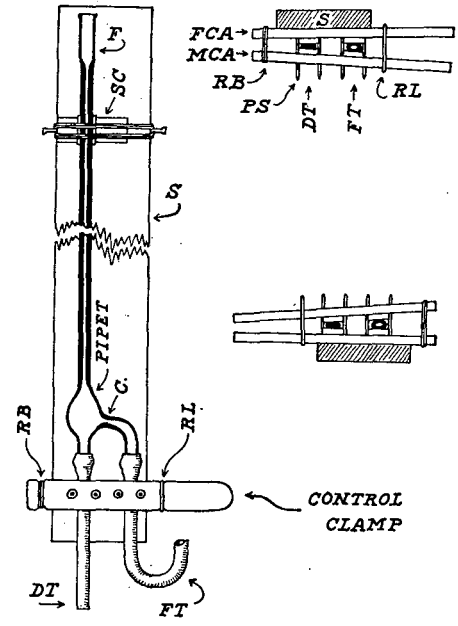

Figure 1. Assembly of Pipet and Clamp on Support

Right Above. Position during filling and when not in operation
Below. Position during delivery

The error due to change of level in the capillary stem of the pipet is absolute, therefore of smaller per cent in pipets of larger volume. The inside diameter should be as small as possible; approximately 2 mm. has been found satisfactory. The limiting minimum is that at which small amounts of precipitated solute will interfere with prompt drainage. The angle of *MCA* with *FCA* in the rest position is determined by *RL*, and should be just wide enough to open the filling tube without opening the delivery tube. The loose wire across the positioning studs helps to keep *DT* closed despite this angle. In larger pipets, the glass tubing below the bulb in the pipet and the rubber delivery tube could be made larger to facilitate more rapid refilling without danger of trapping bubbles. The entire assembly has been mounted on a ring stand by means of a carriage bolt attached near the upper end of *S*.

The pipet, control clamp, and support were built in a home basement shop; the only unusual equipment was an oxygen blast lamp, Pyrex tubing, and a graduated cylinder for rough calibration.