ANALYTICAL CHEMISTRY
Walter J. Murphy, Editor

ANNUAL REVIEWS

ANALYTICAL CHEMISTRY in 1949 will publish two comprehensive reviews. The fundamentals of analysis will be covered in the first review to be published in the January issue, and applied analysis in the second review to be published in February.

Emphasis will be placed in each review on a critical evaluation of the most outstanding developments in the field of analytical chemistry and rather extensive bibliographies will be provided. We hope the review numbers will become annual features of the publication.

We have requested each author in his initial review to cover all important developments of the past four or five years; thereafter the reviews will be on an annual basis.

The subjects selected for the January issue are:

Absorption Spectroscopy
Chromatographic Separations
Distillation
Electroanalysis
Electron Microscopy
Emission Spectroscopy
Extraction
Fluorescence
Indicators
Infrared Spectroscopy
Inorganic Gravimetric Analysis
Inorganic Microchemistry
Inorganic Volumetric Analysis
Instrumentation
Ion Exchange
Light Microscopy
Mass Spectroscopy
Nucleonics
Organic Gravimetric Analysis
Organic Microchemistry
Organic Volumetric Analysis
Polarography
Raman Spectra in Analysis
Statistics Applied to Analysis
Ultraviolet Spectroscopy
X-Ray Spectroscopy

The subjects selected to appear in February are:

Biochemistry
Coatings
Essential Oils and Aromatics
Fertilizers
Food
Fuel and Gas Analysis
Metallurgy, Ferrous
Metallurgy, Nonferrous
Petroleum
Pharmaceuticals and Natural Drugs
Rubber
Water

We were prompted to undertake this new venture after noting the success of such annual review features as "Unit Operations," and "Chemical Engineering Materials of Construction," appearing in *Industrial and Engineering Chemistry*. From time to time we send a questionnaire to a representative sample of readers of ANALYTICAL CHEMISTRY, *Industrial and Engineering Chemistry*, and *Chemical and Engineering News* and always find a keen desire for review articles. As the scientific literature expands in volume, it becomes increasingly difficult for those engaged in research to keep abreast of the developments reported in original manuscripts. We anticipate that the number of such papers published will increase materially as we expand research facilities in this country and research is resumed in the war devastated countries of Europe.

Over the past decade or two we have seen a high degree of specialization develop in the field of analytical chemistry. It is impossible for any one individual to be expert in every branch of the science, yet the professional analyst must possess a working knowledge of all if he is to utilize properly and to the fullest extent possible the advances made year by year in this fast-growing field.

We believe the time has arrived when it is necessary to provide periodic reviews of the scientific literature on analytical chemistry as an essential supplement to the publication of original research. We are grateful to the leading experts who have accepted publication assignments for the first two months of 1949. They will perform an essential and outstanding service for their co-workers and deserve our heartfelt thanks.

Mass Spectra of Some Organic Compounds

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Mass spectra on 53 organic compounds are presented as an aid to investigators in the field of analytical mass spectrometry.

BEFORE any known multicomponent mixture can be quantitatively analyzed by the mass spectrometer, it is imperative that the mass spectrum (or electronic dissociation pattern) of each of the pure components be accurately known. The spectrograms given here are presented with the intention of aiding other investigators in the field to predict the feasibility of the analysis of mixtures of any of the compounds whose spectra are tabulated. It is not to be inferred that these dissociation patterns are rigidly reproducible and fixed for a given molecule in any mass spectrometer, but rather that they are approximate descriptions of dissociation patterns which will vary somewhat from instrument to instrument. The salient features of all mass spectra are observed with nearly all analytical mass spectrometers when operated under the same conditions.

FACTORS AFFECTING DISSOCIATION PATTERNS

A great deal of work has been done in the field of ionization by electron impact with a view to understanding the processes and mechanisms giving rise to mass spectra. Smyth (*4*) has reviewed the field up to 1931. Hipple (*2*) has published a short bibliography covering the work up to 1941. This bibliography is not complete but provides a list of the substances that have been studied recently with the mass spectrometer, so that the earlier work can be traced. Washburn and co-workers (*5*) have discussed correlations between the structures of paraffinic hydrocarbons and their mass spectra and have shown that, in some cases, isomeric mixtures of the paraffins can be resolved because of sufficiently different dissociation patterns. They also discuss briefly some oxygen-containing compounds.

A great many factors affect the structure of the mass spectrum of a given pure compound in a mass spectrometer. The totality of these effects is generally not large enough to distort the major characteristics of any pattern but it is large enough to warrant a weekly (or sometimes daily) rerun of the spectrum during the course of accurate quantitative work. It is of primary importance that the ratio of any two ion currents (peaks) in any spectrum does not vary as a function of the individual peaks. If this is not the case, secondary effects are occurring in the ionization chamber or in the tube itself and quantitative analyses of mixtures cannot be easily made. All modern analytical mass spectrometers are designed to operate at such low pressures in the ion source and analyzer section that the probability of occurrence of secondary effects is negligibly small.

The temperature of the ion source affects the mass spectrum. Relative to the parent ion, some peaks increase, others decrease when the ion source temperature is raised. Fox and Hipple (*1*) have published some quantitative data on the variation of mass spectra with temperature. They studied *n*- and isobutane and 2,2,3-trimethylpentane. From their data one finds temperature effects varying from −3.3% per 1° C. for mass 114 of 2,2,3-trimethylpentane through 0.0 for some peaks in isobutane to positive values in some cases. This thermal effect is large enough to warrant operating the whole ion source at some constant, controlled, elevated temperature which is usually chosen a few degrees above the normal temperature attained by the ion source due to heat from the electron source filament.

The constancy of electron energy is a factor to be considered. However, this effect is usually small if the electron energy is maintained between 50 and 80 volts, as the ionization efficiency curves for nearly all ions studied so far are quite flat in this region.

The electron current density in the ion source used in this work was so low that probably no secondary effects from this cause were present.

There are transient effects such as field interactions in the ion source. These can perhaps be best illustrated by the fact that the dissociation pattern obtained at one value of H and V (magnetic field strength and ion accelerating potential, respectively) differs slightly from that obtained at other values of H and V. For analytical purposes this latter effect is of little or no consequence; the fields are varied in the same way for analysis as for calibration prior to the analysis. This variation of fields is important only in so far as comparison of mass spectra from different instruments is concerned—that is to say, the mass spectra of benzene obtained from two 90° sectored-field instruments will not be identical any more than that obtained from a 180° instrument compared to a sectored-field instrument. These differences are rarely, if ever, large enough to destroy the principal characteristics of any dissociation pattern.

RELATIVE IONIZATION EFFICIENCIES

The relative ionization efficiency (RIE) may be defined as the number of parent ions generated per molecule per electron for the molecule concerned, to that number for the reference compound under the same conditions. For example, the relative ionization efficiency of compound a relative to compound b may be calculated from the, so-called, pressure sensitivities of the parent ions of a and b.

A fundamental relation in mass spectrometry states that any peak in any mass spectrum must vary in strict linear proportion to the partial pressure of the compound at hand, so that

$$m_a^+ = Ap_a$$

where m_a^+ is the intensity of the ion current for the parent ion of molecule a, p_a is the partial pressure of a measured at any convenient point in the entering system, and A is a constant of proportionality usually called the "sensitivity factor." The same equation applies for the parent ion of the reference compound. Now, by dividing the first equation by the second, one finds,

$$\frac{m_a^+}{m_b^+} = \left(\frac{A}{B}\right)\frac{p_a}{p_b}$$

where A/B is now the relative ionization efficiency of a referred to b. The relative ionization efficiency is ambiguous unless it is explicitly stated which ions of the two molecules are being related. In the discussion to follow, only the parent ions are used in the relative ionization efficiency values. The reference compound was arbitrarily chosen to be *n*-butane (parent ion 58). The conditions under which these constants were obtained are described fully in a later section. All the relative ionization efficiency values given in Table I were obtained with the ion source and tube operating at 235° C. These constants are found to vary from day to day and are different from instrument to instrument; however, a series of relative ionization efficiency values from any instrument should be consistent among themselves.

On most mass spectrometers, these values rarely change more than 10% per month. It is difficult to place a figure of accuracy on the constants in Table I but it can be safely assumed that the data are valid to, at best, two significant figures.

Some interesting relations are apparent in the data of Table I. If one writes the relative ionization efficiency constants in order for a homologous series of compounds, it is seen that there is a steady decline in the values, from the lowest member of the series to higher members. Table I shows this up only through *n*-butane for the normal paraffin series. This same variation appears to hold for the mono-olefins. It appears to be true for the series benzene, toluene, ethylbenzene, *n*- or isopropylbenzene, etc.

Another obvious relation is that between an olefinic compound and its corresponding saturated molecule.

The relative ionization efficiency for the olefin is always the greater. This can be seen in the following pairs:

Ethane	1.8	Ethene	7.5
Propane	1.5	Propene	3.1
n-Butane	1.0	2-Butene	3.4
Ethylbenzene	5.4	Styrene	11.3
Isopropylbenzene	4.6	Isopropenylbenzene	6.8

When more data become available, it may be possible to find useful relations of the relative ionization efficiency among groups of isomers, thereby presenting a possibility of identifying a compound by means of its relative ionization efficiency. A future paper in this series will discuss this possibility further.

SOURCES AND PURITIES OF COMPOUNDS

The purities of the compounds used to obtain these spectra were only as high as the accuracy of the work warranted. Rather than discuss each compound in detail, the estimated purity is given at the top of each pattern table. In those cases in which isomers could exist, and no statement is made to the contrary, it is to be understood that the compound is a mixture of isomers. In these cases the purity figure indicates the quantity of materials not isomeric with the major component. In some cases, where the impurities were known, the mass spectrum was corrected by subtracting the contributions of the foreign materials, so that even though the impurity was as large as 10%, the spectrum represents the compound at a purity of very nearly 100%. Where no purity is given, it is understood that the purity was adequate for the problem in which the sample was used, which in most cases was better than 90%. As an example, *n*-hexane was purchased as "technical grade" and found to be too impure for use as received. A narrow boiling (0.5° C.) cut, taken during a 30-plate fractionation of this material, was further fractionated azeotropically through a 60-plate column to give the *n*-hexane that was used here. In all those cases where the purchased material was found to be inadequate, it was first purified by appropriate methods.

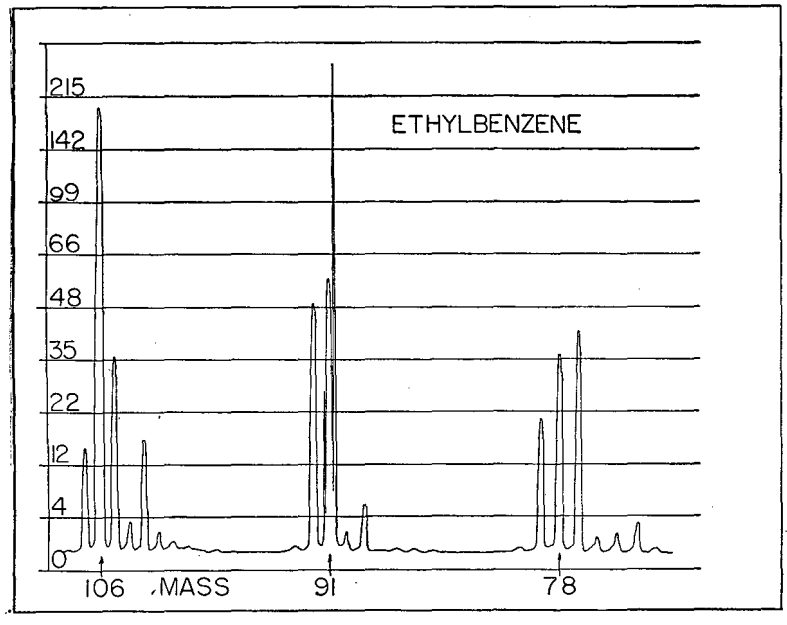

Figure 1. Mass Spectra of Ethylbenzene

Table I. Relative Ionization Efficiencies of Some Hydrocarbons Referred to *n*-Butane Parent Ion

Compound	RIE
Hydrogen	1.9
Methane	6.3
Ethane	1.8
Propane	1.5
n-Butane (reference)	1.00
Isobutane	0.24
2,2,4-Trimethylpentane	0.00
Ethene	7.5
Propene	3.1
Butene-2	3.4
1,3-Butadiene	3.9
2-Methyl-1,3-butadiene	2.3
1,4-Cyclopentadiene	4.1
Methylcyclopentane	1.4
Cyclohexane	5.6
Methylcyclohexane	2.5
Benzene	13.6
Toluene	6.9
Ethylbenzene	5.4
o-Xylene	6.7
Isopropylbenzene	4.6
Methylethylbenzenes	4.8
Diethylbenzenes	4.2
Styrene	11.3
Isopropenylbenzene	6.8

Table II. Instrument Conditions

Resolving power	142±3
Electron energy	75.0 volts
Electron current[a]	9.5 μamperes
Total electron current	21.0 μamperes
Ion accelerating voltage	600.0 volts (fixed)
Main magnet field	Variable
"Draw-out potential"	5.0 volts
Ion path deflection	90°
Analyzer radius	5.0 inches
Ion source temperature	235±1°C.
Over-all tube temperature	235±3°C.

[a] Only focused electrons responsible for positive ion formation.

TABLES OF MASS SPECTRA

All the patterns given were obtained under the same operating conditions. These conditions and some of the constants of the mass spectrometer used in this work are listed in Table II.

All the dissociation patterns presented here were originally obtained on a Leeds & Northrup Speedomax Type A strip chart recorder. The recorder and associated mass spectrometer were made by the Westinghouse Electric Corporation and have been described elsewhere (*2*, *3*). Although the recording mechanism has been discussed previously (*2*), the recorder operation is described in order to clarify the salient features of the mass spectra presented here.

As the recorder preamplifier receives a signal from the electrometer circuit due to positive ion current, the Speedomax pen moves up scale toward higher chart numbers. In order to record a

Table III. Mass Spectra Index

Compound	Parent Mass	Section of Table IV
Paraffins		
Methane	16	1
Ethane	30	2
Propane	44	3
n-Butane	58	4
Isobutane	58	5
n-Hexane	86	6
n-Heptane	100	7
2,2-Dimethylpentane	100	8
n-Octane	114	9
2,2,4-Trimethylpentane	114	10
n-Decane	142	11
Olefins		
Ethene	28	12
Propene	42	13
2-Butene	56	14
1-Pentene	56	15
2-Pentene	70	16
1-Octene	112	17
2-Octene	112	18
Diolefins		
1,3-Butadiene	54	19
1,3-Pentadiene	68	21
2-Methyl-1,3-butadiene	68	20
Cyclo-olefins		
1,4-Cyclopentadiene	66	22
Cyclohexene	82	23
4-Ethenylcyclohexene-1	108	24
d-Limonene	136	25
Naphthenes		
Cyclopentane	70	26
Methylcyclopentane	84	27
Cyclohexane	84	28
Methylcyclohexane	98	29
Ethylcyclohexane	110	30
Aromatics		
Benzene	78	31
Toluene	92	32
Ethynylbenzene	102	33
Styrene	104	34
Ethylbenzene	106	35
o-Xylene	106	36
Isopropenylbenzene	118	37
Isopropylbenzene	120	38
Methylethylbenzene	120	39
1,3,5-Trimethylbenzene	120	40
1-Ethyl-4-ethenylbenzene	132	41
o-Diethylbenzene	134	42
n-Butylbenzene	134	43
1-Methyl-4-isopropylbenzene	134	44
Oxygen compounds		
Methanol	32	45
Ethanol	46	46
Dimethyl ketone	58	47
Diethyl ether	74	48
Ethyl acetate	88	49
Sulfur compounds		
Carbon bisulfide	76	50
Thiophene	84	51
Nitrogen compounds		
Pyridine	79	52
Chlorine compounds		
Carbon tetrachloride	152	53

20,000-fold range of ion currents with the same percentage error for all values, the signal entering the recorder is attenuated logarithmically by the recording mechanism (pen drive gears). The attenuation is, of course, not strictly logarithmic but linear from the chart 0 to 10 divisions and approximately logarithmic above this. An additional shunting feature is also used. As the pen responds to a large ion current, it moves up scale until it trips a limit switch at about 96 divisions. This limit switch then immediately shunts the incoming signal by a factor of 10. At the same time that this switch shunts the signal, the whole mass scale is set back slightly to allow the pen to scan the peak top, which it would probably otherwise miss because of the time lost in going up scale and dropping back after shunting. After scanning the peak, the pen drops back toward the base line and when it crosses the tenth chart division, the shunting mechanism is cut out by a lower limit switch, after which the recorder proceeds at normal sensitivity.

The chart drive is linked mechanically to the main magnet control drive, so that in this case the mass scale is scanned by varying the magnetic field while maintaining the ion accelerating voltage fixed at 600 volts. The precise relation between chart reading and voltage developed across the electrometer grid resistor is easily obtained by inserting signals from a Type K potentiometer into the ground side of the grid resistor. When the recorder has once been calibrated in this way, all chart readings may be converted to millivolts by reference to the calibration table.

The chart given here, ethylbenzene, was redrawn exactly as the recorder presented it and is illustrated in Figure 1. The irregularities due to the factor-of-ten shunt can be seen at the 91 peak in the ethylbenzene spectrum.

Experience demonstrated in the course of this work that the dissociation patterns of *o*-, *m*-, and *p*-diethylbenzene and *o*-, *m*-, and *p*-xylene were so very much alike that no resolution of the isomers in either group could be made. For this reason only one isomer spectrum for each group is given here. As mentioned above, some analyses of isomeric mixtures of paraffins have been made, so it appears that larger differences in dissociation patterns of paraffin isomers are to be expected than for aromatics.

An index to Table IV is given in Table III.

The table of mass spectra is largely self-explanatory; however, some clarification may be necessary. All peak ratios given are expressed in terms of a parent ion peak height of 100 units. In those cases where the parent ion is absent (as in 2,2,4-trimethylpentane) or where the term "parent ion" can be ambiguous, some other ion is chosen as a reference. This is always noted clearly, to avoid confusion. Occasionally, base line irregularities, smeared peaks, and shoulders on peaks are observed. For want of a better name, these are all called metastable ions here, even though some may not actually be due to metastable ions. An attempt has been made in Table IV to report these peculiarities whenever they are observed. Three significant figures are always reported, as the data originally obtained generally warranted this accuracy. This is not meant to convey the impression that these same values can be exactly reproduced by other workers, for reasons outlined above.

ACKNOWLEDGMENT

The authors wish to thank J. A. Perry for his assistance in the preparation of the tables and to acknowledge gratefully the encouragement given by H. E. Morris during the course of this work.

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[Mass spectra data on 53 compounds are found in Table IV, pages 693 to 699.]

Table IV. Mass Spectra of Organic Compounds

No. 1. Methane

Source, Phillips. Purity, 95.0. RIE, 6.3

Mass	Peak Ratio
16	100.0
15	80.1
14	8.28
13	2.90
12	0.47

No. 2. Ethane

Source, Phillips. Purity, 99.0+ RIE, 1.8

Mass	Peak Ratio
30[a]	100.0
29	84.5
28	438.
27	11.6
26	87.0
25	1.18
24	0.77
15	0.85
14.5	1.12
14	5.99
13	0.37

No. 3. Propane

Source, Phillips. Purity, 99.9. RIE, 1.5

Mass	Peak Ratio
44[a]	100.0
43	115.
42	22.2
41	57.4
40	8.05
39	64.2
38	19.2
37	10.6
36	1.91
30	7.51
29	397.
28	232.
27	104.
26	22.7
25	0.60
20.5	0.33
20	1.86
19.5	0.79
19	1.68
18	0.79
15	5.41
14	0.70

No. 4. *n*-Butane

Source, Phillips. Purity, 99.78. RIE, 1.00

Mass	Peak Ratio
58[a]	100.0
57	18.2
56	2.56
55	3.62
54	0.41
53	2.23
52	0.66
51	3.72
50	5.53
49	1.10
44	26.7
43	782.
42	99.0
41	220.
40	6.25
39[b]	75.9
38	9.75
37	2.31
36	3.08
30	2.88
29	25.0
28	221.
26	25.5
25.5	0.84
25	0.66
18	2.15
15	3.89
14	0.43

No. 5. Isobutane

Source, Phillips. Purity, 99.88. RIE, 0.24

Mass	Peak Ratio
58[a]	100.0
57	129.
56	5.86
55	7.59
54	1.14
53	7.97
52	1.57
51	14.2
50	22.8
49	3.99
44	139.
43	4100.
42	1270.
41	1430.
40	78.1
39[b]	494.
38	81.7
37	34.2
36	6.66
30	1.34
29	194.
28	63.7
27	67.7
26	45.2
25.5	1.97
25	1.12
18	4.59
15	44.6
14	2.86

No. 6. *n*-Hexane

Source, Phillips. Purity, technical

Mass	Peak Ratio
86[a]	100.0
85	4.90
84	9.12
72	3.13
71	42.5
70	9.70
69	19.1
68	1.40
67	2.38
58	30.4
57	713.
56	392.
55	56.4
54	4.96
53	11.5
52	2.20
51	6.77
50	4.96
44	16.3
43	474.
42	237.
41	429.
40	16.6
39	94.5
38	7.45
37	5.00
36	1.52
30	7.25
29	282.
28	44.6
27	157.
26	12.2
18[c]	2.86
15	7.28

No. 7. *n*-Heptane

Source, Phillips. Purity, 99.0

Mass	Peak Ratio
100[a]	100.0
85	8.45
72	12.0
71	335.
70	130.
69	1.86
58	7.30
57	324.
56	179.
55	62.2
54	0.74
53	3.42
51	1.70
44	20.6
43	56.6
42	133.
41	281.
40	6.02
39	70.3
38	1.99
36	2.49
30	1.99
29	192.
28	24.7
27	116.
26	3.77

No. 8. 2,2-Dimethylpentane

Source, unknown. Purity, 95.0

Mass	Peak Ratio
100[a]	100.0
99	14.8
98	140.
97	6.06
88	3.84
87	111.
86	382.
85	1000.
84	278.
83	19.9
82	21.2
80	21.2
79	96.8
78	33.4
72	31.0
71	603.
70	928.
69	864.
68	81.3
67	82.1
66	8.20
65	23.3
63	13.3
58	72.3
57	1670.
56	3490.
55	1230.
54	137.
53	65.2
52	42.8
51	86.9
50	60.8
49	9.62
44	88.1
43	2450.
42	1230.
41	2390.
40	152.
39.5	12.1
39	693.
38	62.0
37	25.8
36	5 36
30	28.1
29	866.
28	275.
27	743.
26	64.3

No. 9. *n*-Octane

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
114[a]	100.0
86	35.7
85	544.
84	113.
83	5.03
72	21.0
71	398.
70	200.
69	22.7
67	3.59
65	2.00
64–62[d]	10.0
58	24.3
57	57.8
56	288.
55	146.
54	8.04
53	18.0
51	5.97
50	1.64
43	51.1
42	155.
41	199.
40	46.7
39	20.8
30	7.84
29	318.
28	57.5
27	205.
26	14.0

No. 10. 2,2,4-Trimethylpentane

Source, Phillips. Purity, 99 0. RIE, 0.00

Mass	Peak Ratio
100	26.5
83	2.90
80	0.26
72	0.18
71	4.44
70	1.07
69	2.01
68	0.35
67	1.73
66	0.22
65	0.58
58	24.1
57	553.
56	173.
55	16.3
54	0.91
53	4.25
52	0.63
51	1.92
50	0.92
49	0.44
42	4.02
41[a]	100.0
40	7.04
39.5	0.82
39	110.
38.5	1.27
38	6.19
37.5	0.44
37	26.5
36	1.65
35	1.41
33[d]	0.49
30	1.63
29	49.4
28	6.34
27	27.5
26	0.96

No. 11. *n*-Decane

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
142[a]	100.0
118	16.9
117	13.0
115	3.98
114	4.94
113	59.2
112	26.8
101	3.98
100	9.76
99	94.5
98	67.1
97	3.49
90	5.93
85	26.9
84	411.
83	132.
82	16.0
81	2.55
77	6.24
76	5.54
71	30.8
70	587.
69	213.
68	66.4
67	8.22
66	7.40
64	3.25
62	2.05
57	64.7
56	1470.
55	272.
54	194.
53	16.2
52	22.9
51	3.49
50	9.52
49	3.93
44	50.6
43	1540.
42	199.
41	521.
40	21.7
39	122.
38	4.31
36	2.00
32[d]	5.54
30	9.59
29	340.
28	56.1
27	216.
26	15.4

[a] Reference peak.
[b] Metastable ~39.2.
[c] May be H_2O^+.
[d] Metastable (?).

Table IV. Mass Spectra of Organic Compounds (*Continued*)

No. 12. Ethene

Source, Phillips. Purity, 99.8. RIE, 7.5

Mass	Peak Ratio
28[a]	100.0
27	57.2
26	49.9
25	7.79
24	1.97
15	0.06
14	1.28
13.5	0.09
13	0.27
12	0.14

No. 13. Propene

Source, Phillips. Purity, 99.2. RIE, 3.1

Mass	Peak Ratio
42[a]	100.0
41	164.
40	42.0
39	93.0
38	25.1
37	16.9
36	4.3
29	0.43
28	2.27
27	46.3
26	9.86
25	0.67
24	0.12
20.5	0.36
20	2.60
19.5	1.59
19	4.17
18	0.75
15	1.88
14	0.59
13	0.21
12	0.15

No. 14. 2-Butene

Source, Phillips. Purity, 96.2. RIE, 3.4

Mass	Peak Ratio
56[a]	100.0
55	41.1
54	7.40
53	15.9
52	2.91
51	10.8
50	12.7
49	3.50
48	0.26
44	0.11
43	0.21
42	6.75
41	199.
40	11.8
39	58.9
38	7.17
37	4.00
36	0.73
30	0.45
29	29.9
28	46.9
27.5	0.17
27	38.6
26.5	0.18
26	16.2
25.5	2.65
25	1.48
18	0.33
15	1.28
14	0.15
13	0.06
12	0.04

No. 15. 1-Pentene

Source, Phillips. Purity, 96.0

Mass	Peak Ratio
70[a]	100.0
69	5.36
68	1.37
67	4.60
65	1.45
64	1.30
63	1.15
56	5.05
55	8.49
54	173.
53	3.49
52	13.4
51	1.45
50	4.80
49	4.00
44	0.46
43	15.3
42	227.
41	108.
40	16.5
39	71.6
38	8.24
37	3.59
36	0.70
31	2.40
30	0.62
29	56.4
28	9.37
27	51.4
26	7.04

No. 16. 2-Pentene

Source, Phillips. Purity, 95.0+

Mass	Peak Ratio
70[a]	100.0
69	7.10
68	1.16
67	5.94
66	0.77
65	1.89
63	2.09
62	1.94
61	1.42
56	0.57
55	11.4
54	257.
53	6.46
52	19.8
51	2.41
50	6.42
49	5.25
48	0.96
42	7.10
41	102.
40	70.0
39	11.0
38	63.8
37	7.32
36	3.42
35	0.73
33	0.45
32.5	0.53
32	1.21
31.5	0.73
31	3.63
30	1.16
29	56.0
28	7.70
27	41.8
26	5.67
25	0.21

[a] Reference peak.
[b] Metastable ~39.2.
[c] May be H_2O^+.
[d] Metastable (?).
[e] Unresolved shoulder.

No. 17. 1-Octene

Source, Connecticut Hard Rubber. Purity, 95.0+

Mass	Peak Ratio
112[a]	100.0
97	20.5
85	10.5
84	130.
83	205.
82	32.7
81	4.01
79	3.29
77	4.26
72	3.29
71	77.5
70	553.
69	283.
68	33.3
67	34.4
66	3.53
65	6.62
64	3.74
57	3.04
56	119.
55	572.
54	615.
53	55.5
52	48.7
51	6.62
50	15.2
49	7.12
44	26.4
43	674.
42	467.
41	579.
40	34.9
39	216.
38	13.2
37	4.94
36	7.23
30	5.51
29	272.
28	54.9
27	211.
26	14.9

No. 18. 2-Octene

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
112[a]	100.0
97	5.38
93	1.22
92	3.15
86	2.10
85	28.5
84	72.4
83	9.13
82	3.15
80	2.09
78	2.61
72	1.56
71	17.4
70	172.
69	110.
68	15.3
67	18.0
66	1.98
65	4.00
63	1.67
58	4.68
57	69.0
56	203.
55	332.
54	27.8
53	26.1
52	3.69
51	8.75
50	4.78
44	3.29
43	68.6
42	142.
41	275.
40	13.9
39	85.0
38	4.77
37	2.85
36	1.33
30	3.38
29	140.
28	26.6
27	97.9
26	6.90

No. 19. 1,3-Butadiene

Source, Phillips. Purity, 99.8. RIE, 3.9

Mass	Peak Ratio
54[a]	100.0
53	70.0
52	11.6
51	24.3
50	26.2
49	8.26
48	1.05
41	0.08
40	3.16
39	101.
38	6.36
37	4.66
36	0.94
29	0.43
28[e]	32.9
27	49.1
26.5	0.19
26	17.1
25.5	3.77
25	2.54
24	0.10
18	0.17
15	0.07
14	0.15
13	0.07

No. 20. 2-Methyl-1,3-butadiene

Source, Phillips. Purity, 99.0. RIE, 2.3

Mass	Peak Ratio
68[a]	100.0
67	132.
66	11.2
65	12.2
64	1.00
63	5.01
62	4.07
61	2.95
60	0.61
54	1.17
53	4.01
52	88.9
51	5.66
50	12.1
49	9.29
48	2.15
47	0.19
42	0.55
41	19.6
40	33.9
39	35.9
38	53.3
37	9.21
36	4.59
35	0.49
33	0.42
32.5	0.42
32	0.61
31.5	0.48
31	3.06
29	2.19
28	3.23
27	24.0
26	3.16

No. 21. 1,3-Pentadiene

Source, Koppers. Purity, 90.0+

Mass	Peak Ratio
68[a]	100.0
67	133.
66	11.8
65	18.0
64	1.25
63	7.21
62	5.60
61	4.13
60	0.93
59	2.45
54	3.02
53	79.5
52	5.01
51	8.50
50	6.31
49	1.40
43	2.17
42	24.0
41	46.4
40	38.8
39	65.8
38	11.2
37	5.60
36	0.59
29	8.18
28	7.04
27	26.8
26	4.84
25	0.40

Table IV. Mass Spectra of Organic Compounds (*Continued*)

No. 22. 1,4-Cyclopentadiene

Source, Koppers. Purity, 99.5 RIE, 4.1

Mass	Peak Ratio
66[a]	100.0
65	47.9
64	4.75
63	8.78
62	6.33
61	4.70
60	1.11
51	2.59
50	1.81
49	0.79
48	0.13
41	1.07
40	28.3
39	31.8
38	8.82
37	4.70
36	0.68
33	1.82
32	1.80
31	5.02
28	0.31
27	2.48
26	1.18
25	0.23

No. 23. Cyclohexene

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
82[a]	100.0
81	28.1
80	2.74
79	18.5
78	4.87
77	13.5
75	1.05
74	1.97
73	0.68
69	1.01
68	13.9
67	245.
66	5.72
65	9.61
63	2.97
62	1.10
61	0.54
56	3.67
55	14.2
54	176.
53	26.1
52	8.10
51	17.2
50	11.2
49	1.54
43	0.91
42	6.35
41	82.7
40	10.2
39.5	1.64
39	72.6
38.5	0.44
38	6.97
37.5	0.66
37	2.85
36	0.40
29	5.40
28	11.1
27	37.8
26	5.76

[a] Reference peak.
[b] Metastable ~42.2.
[c] May be H_2O^+.
[d] Metastable (?).

No. 24. 4-Ethenylcyclohexene-1

Source, Dow. Purity, 95.0+

Mass	Peak Ratio
108[a]	100.0
107	15.0
106	2.05
105	11.3
104	4.58
103	7.04
94	16.7
93	229.
92	13.9
91	111.
81	33.6
80	340.
79	687.
78	157.
77	173.
76	3.86
75	7.35
74	6.63
68	15.0
67	276.
66	386.
65	76.1
64	3.86
63	20.5
62	6.46
61	1.57
55	57.8
54	1070.
53	119.
52.5	2.53
52	48.7
51	89.9
50	39.9
49	3.59
42	7.78
41	180.
40	31.5
39	333.
38	21.0
37	6.02
36	6 26
29	27.0
28	53.8
27	154.
26	16.0

No. 25. *d*-Limonene

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
136[a]	100.0
121	8.30
120	87.3
118	6.87
117	8.78
116	8.63
115	0.96
114	4.28
108	1.16
107	19.5
106	76.5
105	5.82
104	18.1
103	1.56
102	8.06
95	1.92
94	32.8
93	94.7
92	269.
91	81.4
90	57.8
89	1.70
88	2.37
80	9.18
79	50.4
78	49.7
77	105.
76	16.9
75	59.8
69	1.43
68	44.6
67	515.
66	234.
65	32.3
64	26.3
63	3.37
62	9.13
61	2.86
57	4.97
54	30.4
53	6.57
52	88.2
51	12.6
50	34.4
49	7.70
43	13.4
42	14.5
41	91.4
40	30.1
39	83.6
38	5.17
37	1.15
36	0.77
29	21.4
28	5.90
27	37.2
26	1.16

No. 26. Cyclopentane

Source, Phillips. Purity, 90.0+

Mass	Peak Ratio
70[a]	100.0
69	4.29
68	1.65
67	5.65
66	0.92
65	2.22
64	0.25
63	1.70
62	1.49
61	1.14
60	0.21
58	0.61
57	13.6
56	9.04
55	108.
54	3.22
53	5.72
52	0.78
51	2.67
50	2.16
49	0.40
44	0.85
43	25.5
42[b]	318.
41	86.0
40	18.3
39	51.7
38	6.55
37	2.84
36	0.36
30	0.21
29	16.0
28	6.68
27	28.8
26	4.72
25.5	0.50
25	0.43

No. 27. Methylcyclopentane.

Source, Phillips. Purity, Technical. RIE, 1.4

Mass	Peak Ratio
84[a]	100.0
83	5.46
82	1.10
81	1.74
79	4.25
78	22.7
77	6.50
76	1.10
75	0.66
74	1.74
71	1.26
70	11.8
69	229.
68	25.9
67	17.0
66	1.94
65	4.61
63	2.98
62	1.42
61	0.66
58	1.42
57	47.1
56	646.
55	153.
54	21.0
53	20.5
52	8.07
51	14.2
50	10.7
49	1.28
44	2.34
43	75.9
42	158.
41	335.
40	26.2
39.5	1.10
39	107.
38	11.8
37.5	4.26
37	5.35
36	0.86
30	2.01
29	51.9
28	38.3
27	79.3
26	8.92

No. 28. Cyclohexane

Source, Eastman. Purity, Eastman grade. RIE, 5.6

Mass	Peak Ratio
84[a]	100.0
83	6.06
82	0.24
81	0.39
79	0.63
78	0.39
77	0.93
70	1.92
69	34.6
68	2.50
67	3.75
66	0.33
65	0.79
57	6.45
56	133.
55	44.7
54	7.37
53	4.50
52	1.26
51	2.89
50	1.93
44	0.84
43	16.1
42	34.2
41	68.1
40	5.34
39	21.5
38	2.23
37	0.87
36	0.77
30	0.43
29	9.46
28	10.5
27	17.1
26	1.93

No. 29. Methylcyclohexane

Source, Dow. Purity, 95.0+. RIE, 2.5

Mass	Peak Ratio
98[a]	100.0
97	6.59
86	0.42
85	16.1
84	229.
83	33.9
82	3.18
80	1.62
79	0.52
78	1.78
72	2.69
71	50.1
70	51.5
69	20.4
68	10.2
67	0.87
66	1.97
64	0.87
63	0.41
57	10.4
56	66.1
55	177.
54	10.1
53	10.1
52	1.77
51	4.23
50	2.27
44	1.38
43	16.2
42	64.5
41	96.3
40	6.67
39	37.4
38	2.73
37	1.38
36.5	1.77
36	1.38
30	0.26
29	25.2
28	9.81
27	30.4
26	2.17

Table IV. Mass Spectra of Organic Compounds (*Continued*)

No. 30. Ethylcyclohexane

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
112[a]	100.0
111	2.25
85	0.57
84	39.1
83	523.
82	214.
81	9.64
80	0.63
79	4.30
78	1.41
77	4.40
71	1.08
70	15.9
69	43.6
68	11.3
67	56.9
66	1.81
65	4.11
63	1.38
61–59[d]	0.94
57	6.65
56	56.5
55	377.
54	24.0
53	18.3
52	3.28
51	7.08
50	2.71
44	1.29
43	35.6
42	43.0
41	182.
40	9.11
39	57.5
38	2.99
37–36[d]	5.05
30	0.71
29	42.6
28	14.3
27	51.1
26	3.01

No. 31. Benzene

Source, Baker. Purity, C.P. RIE, 13.6

Mass	Peak Ratio
78[a]	100.0
77	18.4
76	5.13
75	0.82
74	3.79
73	0.63
64	1.55
63	0.17
62	0.16
53	0.27
52	17.5
51	14.7
50	12.0
49	0.84
40	0.08
39.5	0.08
39	11.3
38.5	0.13
38	3.81
37.5	0.42
37	2.30
36	0.50
28	0.17
27	0.56
26	0.46

No. 32. Toluene

Source, Monsanto. Purity, nitration grade. RIE, 6.9

Mass	Peak Ratio
92[a]	100.0
91	153.
90	6.96
89	5.03
87	0.23
86	0.38
85	0.28
80	0.33
79	15.5
78	3.10
77	0.43
76	0.31
75	0.70
74	0.13
67	0.80
66	16.9
65	1.03
64	9.57
63	3.31
62	0.93
53	0.43
52	4.04
51	11.4
50	6.70
49	0.31
46.5	0.19
46	4.55
45.5	1.99
45	7.40
44.5	0.20
44	0.55
43.5	0.13
43	1.27
41	0.93
40	0.76
39	17.3
38	3.58
37	0.83
36	0.61
28	0.34
27	1.58
26	0.28

No. 33. Ethynylbenzene

Source, Farchan. Purity, 95.0+.

Mass	Peak Ratio
102[a]	100.0
101	2.48
100	0.41
99	0.66
98	1.84
97	0.41
87	1.04
86	0.39
85	0.13
79	0.25
78	2.62
77	2.97
76	6.89
75	6.48
74	8.46
73	1.31
64	0.28
63	5.43
62	2.58
61	1.59
60	0.31
57–55[d]	0.89
53	0.18
52	0.30
51	6.18
50.5	0.51
50	12.4
49.5	0.23
49	10.9
48.5	0.75
48	1.96
39	3.10
38	1.62
37	1.30
36	0.81
28	1.54
27	0.66
26	0.45

No. 34. Styrene

Source, Monsanto. Purity, 99.8. RIE, 11.3

Mass	Peak Ratio
104[a]	100.0
103	49.6
102	7.31
101	0.66
89	0.61
80	0.90
79	3.39
78	1.96
77	2.41
76	1.65
75	2.70
74	0.32
66	0.50
65	0.26
64	4.47
63	0.83
62	0.41
53	0.35
52	0.81
51.5	0.47
51	2.64
50	0.91
49	0.44
39	5.40
38	0.73
37	0.26
36	0.35
28	0.26
27	1.06
26	0.12

No. 35. Ethylbenzene

Source, Monsanto. Purity, 99.0+. RIE, 5.4

Mass	Peak Ratio
106[a]	100.0
105	17.8
104	1.55
103	7.67
102	0.98
101	0.54
92	24.6
91	288.
90	1.00
89	2.71
79	9.80
78	18.3
77	20.9
76	0.80
75	1.00
74	1.43
66	0.64
65	21.1
64	0.69
63	7.96
62	1.26
61	0.16
53	1.00
52.5	0.26
52	9.30
51.5	0.54
51	29.9
50	9.19
49	0.33
41	1.09
40	0.11
39	15.3
38	1.52
37	0.11
36	0.95
28	0.90
27	5.23
26	0.32

No. 36. *o*-Xylene

Source, Eastman. Purity, Eastman grade. RIE, 6.7

Mass	Peak Ratio
106[a]	100.0
105	42.4
104	5.50
103	10.4
102	2.30
101	0.79
99	0.26
98	0.60
97	0.76
96	0.26
95	0.19
93	0.52
92	13.4
91	170.
90	1.53
89	3.15
88	0.28
87	0.58
86	0.58
85	0.57
84	0.30
83	0.42
82	0.50
81	0.57
80	1.05
79	11.9
78	11.6
77	20.1
76	1.58
75	1 82
74	4.00
73	0.44
70	0.21
69	0.25
68	0.44
67	0.10
66	0.54
65	1.40
64	10.8
63	1.61
62	7.84
61	2.92
60	1.23
56	0.82
55	0.40
54	1.08
53	0.35
52	4.51
51.5	1.05
51	10.2
50.5	1.53
50	20.7
49.5	0.30
49	7.06
48.5	0.24
48	0.88
45	0.41
44	0.24
43	1.21
42	0.30
41	2.87
40	1.59
39	14.8
38	2.74
37	0.98
36	0.43
29	0.63
28	0.82
27	7.00
26	0.80

[a] Reference peak.
[b] Metastable ~39.2.
[c] May be H_2O^+.
[d] Metastable (?).

Table IV. Mass Spectra of Organic Compounds (*Continued*)

No. 37. Isopropenylbenzene

Source, Monsanto. Purity, 95.0+. RIE, 6.8

Mass	Peak Ratio
118[a]	100.0
117	71.0
116	9.10
115	21.1
114	1.43
113	1.08
107	1.58
106	4.00
105	45.6
104	4.43
103	0.59
102	0.16
101	0.18
100	0.33
91	1.60
90	17.9
89	1.47
88	3.56
87	0.41
86	0.89
85	0.68
84	0.36
78	2.40
77	28.9
76	19.9
75	2.93
74	2.60
73	3.07
72	0.48
67	0.07
66	0.15
65	0.47
64	5.01
63	0.97
62	6.53
61	2.67
60	0.91
58	1.47
57.5	1.35
57	11.8[b]
56.5	7.33
56	2.82
55	0.28
54	0.30
52	1.41
51	3.94
50.5	0.49
50	16.3
49	7.11
48	0.50
45	0.12
44	0.15
43	0.35
42	0.10
41	1.72
40	1.50
39	11.3
38	1.81
37	0.74
36	0.11
29	0.10
28	0.33
27	2.78
26	0.41

No. 38. Isopropylbenzene

Source, Fenske. Purity, 98.0. RIE, 4.6

Mass	Peak Ratio
120[a]	100.0
119	1.52
118	0.55
117	1.00
116	0.30
115	1.59
106	33.5
105	361.
104	13.4
103	28.2
102	2.95
101	1.08
91	17.6
89	1.18
81	1.03
80	39.1
79	17.9
78	45.4
77	1.49
76	1.49
75	1.62

No. 38. (*Contd.*)

Source, Fenske. Purity, 98.0. RIE, 4.6

Mass	Peak Ratio
66	4.73
65	0.45
64	5.94
63	1.06
62	0.40
60	2.50
58	1.56
57	1.18
53	1.78
52.5	2.45
52	8.07
51.5	1.11
51	36.7
50	9.13
41	8.78
40	0.50
39	16.4
38	1.11
37	0.29
36	0.80
28	1.19
27	10.1
26	0.24

No. 39. Methylethylbenzene

Source, Monsanto. Purity, 98.0+. RIE, 4.8

Mass	Peak Ratio
120[a]	100.0
119	11.5
118	2.07
117	7.41
116	1.99
115	7.10
107	1.29
106	30.8
105	330.
104	8.17
103	17.8
102	3.66
101	1.51
93	2.27
92	10.1
91	32.6
90	1.51
89	4.24
88	0.30
87	0.80
86	0.80
85	0.33
80	1.60
79	23.0
78	9.89
77	30.7
76	1.99
75	2.25
74	2.03
66	0.25
65	1.29
64	13.5
63	1.94
62	9.48
61	3.42
60	1.22
58.5	1.73
58	9.48
57.5	2.07
57	8.05
56.5	6.01
56	2.71
54	0.48
53	0.26
52	5.05
51.5	0.60
51	4.65
50.5	0.65
50	15.0
49	5.74
48	0.33
44	0.26
43	0.23
42	0.15
41	5.84
40	2.45
39	17.8
38	2.45
37	0.65
36	0.58
29	1.28
28	74.7
27	9.26
26	0.70

No. 40. 1,3,5-Trimethylbenzene

Source, Eastman. Purity, technical grade

Mass	Peak Ratio
120[a]	100.0
119	26.5
118	1.72
117	4.47
116	1.53
115	5.09
107	0.91
106	13.9
105	156.
104	5.36
103	9.36
102	2.31
101	0.97
97	0.52
96	0.59
95	0.61
94	1.15
93	1.98
92	3.60
91	12.1
90	0.64
89	1.40
87	0.36
86	0.41
85	0.20
83	0.36
82	0.21
81	0.50
80	0.76
79	8.24
78	4.89
77	14.2
76	0.91
75	1.20
74	1.23
70	0.39
69	0.45
67	0.66
66	0.71
65	4.88
64	0.93
63	3.86
62	1.42
61	0.52
60	0.82
59.5	0.78
59	4.12
58.5	1.05
58	3.74
57.5	2.55
57	2.03
55	0.88
54	0.25
53	3.65
52.5	0.30
52	2.65
51.5	0.25
51	7.65
50	2.85
43	1.17
42	0.25
41	5.29
40	1.14
39	10.1
38	1.19
37	0.31
29	0.78
28	0.63
27	5.72
26	0.34

No. 41. 1-Ethyl-4-ethenylbenzene

Source, University of Illinois. Purity, 90.0+

Mass	Peak Ratio
132[a]	100.0
131	9.38
130	1.97
129	3.60
128	6.10
127	3.06
126	0.70
120	0.94
119	2 65
118	24.1
117	23.8
116	13.8
115	38.1
106	1.37
105	11.9
104	5.00
103	6.81
102	5.39
101	1.64
92	2.18
91	28.1
90	1.08
89	4.56
88	0.38
87	0.87
86	0.57
79	3.09
78	5.16
77	15.2
76	2.52
75	3.09
74	2.75
65	4.04
64.5	0.81
64	12.1
63.5	1.79
63	7.52
62.5	1.79
62	10.2
61	2.78
60	0.74
58	2.61
57.5	1.83
57	6.51
53	0.28
52	3.49
51	3.37
50.5	0.34
50	15.7
49	5.77
48	0.34
41	2.02
40	0.92
39	12.9
38	1.66
37	0.34
36	0.64
29	0.73
28	2.20
27	5.74
26	0.66

[a] Reference peak.
[b] Metastable ~39.2.
[c] May be H_2O^+.
[d] Metastable (?).

Table IV. Mass Spectra of Organic Compounds (*Continued*)

No. 42. Diethylbenzene

Source, NACA. Purity, 99.0+. RIE, 4.2

Mass	Peak Ratio
134[a]	100.0
133	1.96
131	0.58
129	0.50
128	1.56
127	0.71
120	20.4
119	202.
118	6.43
117	32.0
116	6.93
115	19.9
106	19.0
105	201.
104	13.7
103	19.9
102	4.20
101	1.64
93	6.16
92	4.74
91	54.8
90	1.64
89	5.14
80	0.92
79	17.2
78	10.6
77	30.1
76	2.10
75	2.57
74	2.48
66	1.26
65	13.8
64	4.07
63	9.39
62	2.69
59	0.73
58.5	1.01
58	4.07
57.5	6.13
57	8.40
54	0.56
52	5.01
51	4.40
50	16.4
49	5.72
41	12.5
40	1.51
39	16.3
38	1.79
37	0.26
36	0.39
29	3.68
28	2.87
27	7.12
26	0.64

No. 43. *n*-Butylbenzene

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
134[a]	100.0
121	4.33
120	4.65
118	2.89
117	1.27
116	4.41
107	4.14
106	38.8
105	5.27
104	7.73
103	2.12
102	0.87
98	0.44
97	1.70
96	1.27
95	1.01
94	0.51
93	15.4
92	207.
91	378.
90	4.48
89	6.14
87	0.67
86	0.51
85	0.51
84	4.80
83	58.1
82	34.4
81	3.65
80	1.34
79	11.4
78	21.6
77	20.6
76	2.00
75	2.05

No. 43. (*Contd.*)

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
74	2.00
73	0.48
71	0.71
70	3.01
69	5.14
68	2.33
67	9.13
66	2.84
65	35.9
64	3.27
63	9.65
62	2.55
56	7.96
55	39.7
54	3.86
53	5.02
52	5.02
51	18.9
50	7.05
44	0.54
43	12.0
42	3.78
41	28.7
40	2.39
39	26.2
38	2.20
37	0.73
36	0.31
29	9.35
28	2.80
27	15.2
26	0.87

No. 44. 1-Methyl-4-isopropylbenzene

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
134[a]	100.0
133	3.34
132	0.73
131	0.44
121	2.07
120	40.1
119	396.
118	9.19
117	34.1
116	6.84
115	18.4
107	1.09
106	8.75
105	6.89
104	9.44
103	2.37
102	0.65
97	0.21
96	0.31
95	1.51
94	0.31
93	5.61
92	4.48
91	39.2
90	1.16
89	3.28
83	0.54
82	0.31
81	0.84
80	0.21
79	5.05
78	4.23
77	13.3
76	0.84
75	1.16
74	0.96
69	0.52
68	0.72
67	0.99
66	2.26
65	12.3
64	2.66
63	5.80
62	1.62
61	0.16
59.5	1.16
59	0.72
58.5	4.14
58	4.90
57.5	5.26
57	1.81
56	0.36
55	2.07
54	0.16
53	3.12
52	2.26
51	8.18
50	3.12

No. 44. (*Contd.*)

Source, Eastman. Purity, Eastman grade

Mass	Peak Ratio
43	1.37
42	0.66
41	14.4
40	1.33
39	13.4
38	1.24
37	0.17
36	0.24
29	0.76
28	1.33
27	6.46
26	0.37

No. 45. Methanol

Source, Carbide. Purity, C.P.

Mass	Peak Ratio
32[a]	100.0
31	138.
30	9.74
29	61.7
28	15.6
18	1.32
15	22.7
14	3.62
13	0.95
12	0.64

No. 46. Ethanol

Source, Carbide. Purity, C.P.

Mass	Peak Ratio
46[a]	100.0
45	230.
44	17.6
43	44.6
42	14.5
41	4.34
32	41.3
31	526.
30	29.0
29	111.
28	54.2
27	82.7
26	25.8
25	4.38
19	14.5
18	4.16
17	9.96
16	1.69
15	35.5
14	14.4
13	4 18
12	0.86

No. 47. Dimethylketone

Source, Carbide. Purity, C.P.

Mass	Peak Ratio
58[a]	100.0
57	2.81
55	0.86
53	1.08
44	0.72
43	6.35
42	275.
41	17.6
40	5.37
39	2.15
38	8.73
37	4.76
36	4.43
35	1.75
31	1.51
30	0.28
29	8.31
28	7.72
27	13.8
26	9.17
25	2.22
24	0.35
19	0.64
18	0.64
16	0.72
15	66.2
14	9.75
13	2.35
12	0.61

[a] Reference peak.
[b] Metastable ~39.2.
[c] May be H_2O^+.
[d] Metastable (?).
[e] Metastable ~ 28.8.

No. 48. Diethyl Ether

Source, Mallinckrodt. Purity, 95.0+

Mass	Peak Ratio
74[a]	100.0
73	10.2
72	1.13
59	5.21
58	159.
57	0.80
56	0.88
46	0.70
45	5.54
44	132.
43	6.06
42	23.7
41	4.63
40	16.2
39	0.38
35	0.34
33	0.48
32	4.07
31	359.
30	6.85
29	151.
28[e]	20.5
27	65.0
26	11.0
25	0.52
19	8.03
18	1.19
16	0.60
15	14.2
14	3.98
13	0.64

No. 49. Ethyl Acetate

Source, Baker. Purity, C.P.

Mass	Peak Ratio
88	1.76
87	31.1
86	1.66
73	0.95
72	29.4
70	2.46
69	59.7
61	2.43
60[a]	100.0
59	6.14
58	0.81
55[d]	2.43
45	2.72
44	104.
43	19.9
42	680.
41	35.1
40	3.06
35	0.85
31	8.54
30	4.25
29	107.
28	38.3
27	49.6
26	12.7
25	0.81
19	2.44
18	3.29
17	0.46
16	1.31
15	54.6
14	13.3
13	2.37
12	0.36

No. 50. Carbon Bisulfide

Source, Baker. Purity, C.P.

Mass	Peak Ratio
80	2.51
79	1.97
78[a]	100.0
77	29.7
76	1120.
66	1.00
64	11.4
59	2.08
46	4.65
45	1.94
44	106.
39	6.72
38.5	1.93
38	76.2
36	0.53
34	3.94
33	0.75
32	77.9
28	9.97
27	0.33
25	0.64

Table IV. Mass Spectra of Organic Compounds (*Continued*)

Mass	Peak Ratio	Mass	Peak Ratio	Mass	Peak Ratio	Mass	Peak Ratio
No. 51. Thiophene		No. 51. (*Contd.*)		No. 52. Pyridine		No. 53. Carbon Tetrachloride[b]	
Source, Socony-Vacuum. Purity, C.P.		Source, Socony-Vacuum. Purity, C.P.		Source, Eastman. Purity, Eastman grade		Source, Sharples. Purity, redistilled commercial	
87	0.16	46	1.83				
86	4.47	45	1.02	79[a]	100.0	123	10.9
85	5.47	44	41.8	78	1720.	122	1.29
84[a]	100.0	43	1.57	77	218.	121[a]	100.0
83	6.33	42	0.22	76	8.83	120	3.98
82	2.54	41.5	0.22	75	23.3	119	306.
81	3.81	41	3.86	74	29.2	118	4.03
80	0.56	40.5	0.22	73	5.71	117	314.
77	0.16	40	1.83	62	4.74	86	7.29
75	1.76	39.5	1.18	61	2.16	85	1.40
70	0.32	39	1.83	60	2.16	84	43.3
69	0.32	38	19.5	59	1.41	83	2.06
68	6.63	37	4.96	53	3.53	82	66.6
67	0.53	36	3.56	52	119.	76	1.87
66	0.53	35	2.28	51	990.	71	1.57
59	2.76	34	0.19	50	416.	69	2.60
58	2.26	33	0.27	49	260.	60	3.59
57	60.6	32	0.36	48	55.2	59	10.6
56	9.91	31	1.60	47	9.82	58	10.8
55	1.10	28	0.58	39	7.82	48	16.4
51	0.14	27	0.76	38.5	10.1	47	0.75
50	2.75	26	2.07	38	106.	46	50.8
49	4.15	25	0.68	37.5	15.1	43	2.79
48	1.88	24	0.17	37	43.1	42	0.44
47	0.46			36	27.8	41	1.12
				35	7.94	40	1.93
				28	28.5	38	4.37
				27	28.2	37	9.84
				26	86.2	36	11.9
				25	9.07	35	28.8
				24	1.24	28	21.8

[a] Reference peak.
[b] No ionization at parent mass.

Depolymerization of Butylene Polymers

Analysis of Isomeric Octenes by the Mass Spectrometer

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In a study of the catalytic depolymerization of butylene polymers over Attapulgus clay, several analyses of octene feed and products of the experiments were needed in order to determine the extent and the manner in which some of the original compounds were isomerized over the catalyst. The method employed consisted of hydrogenation to the corresponding paraffins, fractional distillation, and analysis of the fractions by means of the mass spectrometer. The analytical data are presented, and the method of arriving at the various carbon structure isomerizations occurring in two typical experiments employing cold acid polymer and a codimer made over U. O. P. phosphoric acid catalyst, respectively, is described in detail. Summaries of the over-all reactions that occurred with these two stocks gave mole balances of 99.4 and 95.3% of the compounds charged and accounted for as undergoing depolymerization or as being isomerized into other octenes.

IN A study of the catalytic depolymerization of butylene polymers in the presence of Attapulgus clay (*3*), leading to the development of a means of manufacturing pure isobutylene from "cold acid polymer," certain chain isomerizations of the octenes were suspected as accompanying or preceding depolymerization. To determine what changes took place and to what extent, exhaustive analyses of both feed and liquid products of a few typical experiments were needed. This paper describes the method of analysis used and the results obtained on analyzing these complex mixtures of hydrocarbons by means of the mass spectrometer.

Direct analysis of these mixtures as olefins was not possible, as the necessary calibrating compounds were not available. If these pure materials had been available, there is also considerable doubt as to whether the unknowns could have been analyzed with the desired degree of accuracy in view of the difficulties experienced in mass spectrometric analysis of olefins of lower molecular weight. By hydrogenating the olefins to the corresponding paraffins the octanes can be analyzed with good accuracy (*2*), providing the number of isomers in any particular mixture analyzed is not overly large. This method does not show the composition of the mixture as far as position of the double bond in the various isomers is concerned, but it does indicate the carbon structure of the isomers, which was the main issue of this investigation.

EXPERIMENTAL

The depolymerization procedure, apparatus, Attapulgus clay catalyst, and hydrocarbon feeds are described elsewhere (*3*). The specific hydrocarbons used for the experiments reported here were a fraction of cold acid polymer obtained from the Neches Butane Products Company, Port Neches, Tex., boiling between 101° and 103° C., which is essentially diisobutylene, and a 95° to 119° C. fraction of a polymer formed in the presence of a Universal Oil Products Company phosphoric acid catalyst. In the preparation of this polymer the ratio of *n*-butylene to isobutylene consumed in forming the polymer was 0.7.

Gas Analysis. The gaseous products were analyzed in a Consolidated Engineering Corporation mass spectrometer. Because the isobutylene content of the gas was very high the modified technique reported by Melpolder and Brown (*4*) was used. At the concentration of isobutylene found in the present work the accuracy of its determination by this technique is within ±0.3%.

Liquid Analysis. A complete analysis of the feed stocks and the liquid products was made by hydrogenating the olefins to the corresponding paraffins, carefully fractionating the product, and analyzing the fractions by means of the mass spectrometer.

The olefins were hydrogenated at atmospheric pressure and at 230° to 260° C. over a catalyst composed of 10% nickel on Harshaw alumina. The hydrocarbon recovery and hydrogen consumption showed the absence of depolymerization during the hydrogenation. A question might be raised as to the possibility of chain isomerization's taking place during hydrogenation. No proof has been obtained that it does not take place over the above catalyst. However, inasmuch as such isomerization was not observed in the hydrogenation of pure diisobutylene to 2,2,4-trimethylpentane over Universal Oil Products Company nickel hydrogenation catalyst under similar conditions, proof was not considered necessary.

The fractionation of the hydropolymers was carried out in an 8-mm. Podbielniak Hyper-Cal column (*5*). The manufacturer reported that at a back pressure of 25 mm. of water this particular column tested better than 92 theoretical plates at total reflux when a *n*-heptane–methylcyclohexane text mixture was used. The distillations were performed at a 100 to 1 reflux ratio with a take-off rate of 0.5 ml. per hour. Approximately 45 to 50 ml. of hydropolymer were charged to the distillation and 25 ml. of pure *n*-octane added to act as a chaser.

After a study was made of the graph of temperature *vs.* volume distilled, those fractions were combined for mass spectrometer analysis which would give relatively few compounds in an individual combination. It was also necessary to keep in mind that the C_7 paraffins should not contain C_8's above 2,2,4-trimethylpentane in boiling point, and compounds boiling higher than the octanes could not be tolerated in mixture with the C_8's if the mass spectrograms were to be successfully resolved. These combined fractions were then analyzed in the mass spectrometer, using American Petroleum Institute–National Bureau of Standards pure hydrocarbons for calibration purposes.

Before analysis of the hydropolymer fractions was attempted, a number of known mixtures of pure C_7 and C_8 hydrocarbons were analyzed (*2*). It was found that in the C_8's the two pairs of compounds, 2,2-dimethylhexane and 2,2,4-trimethylpentane, 3,3-dimethylhexane and 2,3,3-trimethylpentane, could not be readily resolved. This necessitated analyzing these compounds in groups. In the C_7's, 2,2-dimethylpentane and 2,2,3-trimethylbutane also could not be resolved. The presence of triptane in any of the hydropolymers analyzed in this work was considered so doubtful that this combination has been evaluated as only 2,2-dimethylpentane.

It was also found that in the presence of 4-methylheptane, 2,3-dimethylhexane and 2,3,4-trimethylpentane could not be accurately resolved. In the analyses of cold acid polymer feed and product the assumption was made that no 4-methylheptane was present. It was felt that this assumption was justified, in that the other methylheptanes were present in quantities of 0.1% or less. A similar amount of 4-methylheptane could cause no appreciable error in the resolution of 2,3-dimethylhexane and 2,3-4-trimethylpentane. The analyses of the codimer feed and products, however, showed appreciable amounts of 2- and 3-methylheptane, so that the absence of 4-methylheptane could not be assumed. Fortunately, the boiling point of 4-methylheptane (117.7° C.) is significantly higher than that of 2,3,4-trimethylpentane (113.5° C.) and 2,3-dimethylhexane (115.6° C.) boils just halfway between. Hence, the assumption was made that no 4-methylheptane occurred in fractions in which 2,3,4-trimethylpentane was present, and that the latter was not present where the former would be expected. In the analyses of codimer depolymerization products this split could be made largely on the basis of the boiling range of the fractions but in the case of the codimer feed, definite evidence was obtained of 2,3,4-trimethylpentane in fractions boiling higher than the boiling point of 4-methylheptane, so that the latter was assumed to be present in only the two highest boiling fractions.

The possible error of the analyses reported in this work varies with the particular compound under consideration. In general, the mass spectrometer analyses of known octane mixtures indicate that for most of these compounds the error in the spectrometric analysis may amount to ±1.5%, which extends over a large range of concentrations but decreases as the concentration of a component approaches 100%. The error may be larger, ±2 or 3%, for the C_8 paraffins boiling higher than 2,3-dimethylhexane—i.e., 3,4-dimethylhexane and the methylheptanes. These spectrometer errors when calculated back to the original liquid charged to distillation become smaller, depending on the volume of fraction analyzed. In the analyses reported here the possible error, if the distillation loss is assumed to be evenly distributed over the entire distillation, has been calculated to be as low as ±0.1% for some compounds and as high as ±1.0% for others.

RESULTS

Experimental data for cold acid polymer (run 118) are shown in Table I. The feed for this run was prepared by distilling a commercial sample of cold acid polymer from the Neches Butane Products Company through a 10-ball Snyder column, and collecting a fraction boiling from 101° to 103° C. The depolymerization, carried out under the stated conditions, gave 59% conversion to gas (C_5's and lighter). Mass spectrometer analyses show small concentrations of C_3's and C_5's in the gas which were not found by the low temperature distillation analysis used in the earlier work (*3*). The C_4 fraction is essentially isobutylene (99.3%); the remainder is butanes and *n*-butylenes in about equal amounts. Other analyses carried out in this laboratory on pilot plant samples have indicated that the *n*-butylenes are essentially 1-butene.

The liquid portion of the product was hydrogenated and carefully distilled through a Podbielniak Hyper-Cal column as described in the section on experimental procedure. Similar hydrogenation and fractional distillation of the feed were also carried out for comparison. The results of these distillations are shown in Figure 1, wherein the refractive indices of the fractions, determined with Bausch & Lomb precision refractometer, are

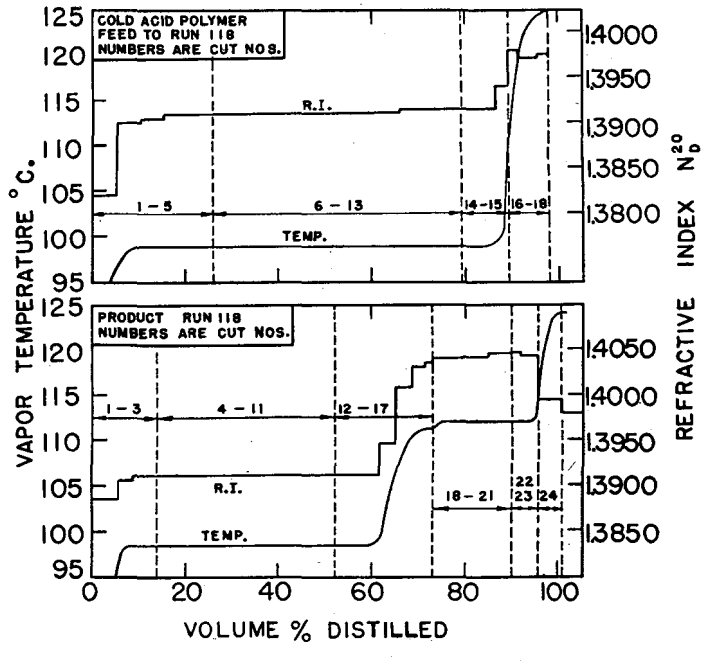

Figure 1. Catalytic Depolymerization of Cold Acid Polymer over Clay

Fractional distillation of hydrogenated cold acid polymer feed and hydrogenated liquid product

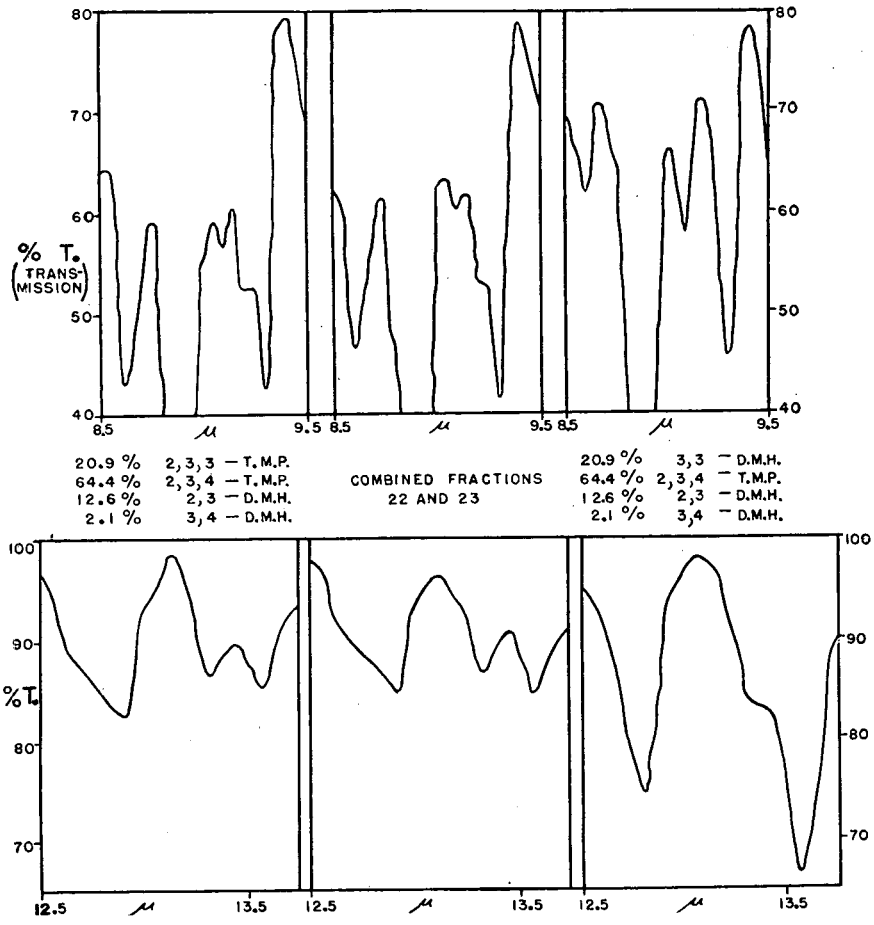

Figure 2. Infrared Comparison of Combined Fractions 22 and 23 from Run 118 with Synthetic Mixtures

plotted along with their boiling points and those fractions combined for mass spectrometer analysis are indicated by vertical dashed lines. The vapor temperatures reported in this figure should not be regarded as true boiling points, inasmuch as previous experience on distilling pure compounds through this column has shown that the vapor temperature recorded by a calibrated thermocouple gave readings that were low by about 0.6° C.

Tables II and III show the mass spectrometer analyses of the various combinations of distillation fractions from the hydrogenated cold acid polymer feed and liquid product, respectively. The analysis of the combined fractions 22 and 23 shows the presence of 20.9 volume % of either 2,3,3-trimethylpentane or 3,3-dimethylhexane or both, which the mass spectrometer cannot resolve. Inasmuch as proof of the presence or absence of 2,3,3-trimethylpentane was desired because of its bearing on the course of the reaction, the infrared absorption patterns of the combined fractions 22 and 23 were compared with those of two synthetic mixtures. These synthetic mixtures were made to conform to the mass spectrometer analysis, but in one case 20.9 volume % of pure 2,3,3-trimethylpentane and in the other the same amount of pure 3,3-dimethylhexane (A.P.I.-N.B.S. spectrometer calibration compounds) was included. Figure 2 shows two portions of the infrared absorption curves that are critical in differentiating 2,3,3-trimethylpentane and 3,3-dimethylhexane; the remainder of the curves are for the most part so similar that reproduction is unnecessary. It is obvious that the unknown conforms closely with the synthetic mixture containing 2,3,3-trimethylpentane. If there is any 3,3-dimethylhexane in the product it is there in only very small amounts. These infrared absorption curves are also of interest in that they substantiate the mass spectrometer analysis of the other components of this particular combination of fractions.

Table IV summarizes the analytical results calculated on the basis of hydrogenated material charged to the distillation. The volume per cent figures given in this table are indicated on a basis of no loss during the distillation. Correction for loss was made on the assumption that the loss was evenly distributed over the boiling range.

Table I. Depolymerization of Cold Acid Polymer over Clay Catalyst

(Experimental data for run 118)

Feed stock	
Source	Cold acid polymer
Total polymer, vol. %	69.4
Boiling range, ° C.	101–103
Density, g./ml., 20° C.	0.718
Bromine No.	133
Test conditions	
Catalyst temp., ° C.	264
Space velocity, ml./ml./hour	1.1
Hours on test	5.0
Products, weight % of feed	
Gas, C$_5$'s and lighter	58.9
Liquid, C$_5$'s and heavier	41.1
Gas composition, mole %	
Propylene	0.2
Propane	0.2
Isobutylene	98.5
Isobutane	0.2
n-Butylene	0.3
n-Butane	0.2
Pentanes and pentenes	0.4
Yields, weight % of feed	
Propylene	0.1
Propane	0.1
Isobutylene	58.0
Isobutane	0.1
n-Butylene	0.2
n-Butane	0.1
Pentanes and pentenes	0.3
C$_5$'s and heavier	41.1
Liquid product inspections	
Boiling range, ° C.	95–115
Density, g./ml., 20° C.	0.725
Bromine No.	130

Table II. Analysis of Hydrogenated Cold Acid Polymer Feed to Run 118

Fraction No.	1–5	6–13	14–15	16–18
Boiling point, ° C.	98.8	98.8–98.8	98.8–108.6	108.6–124.8
Volume, ml.	13.12	26.68	4.94	4.26
Hydrocarbon, vol. %				
2,2-Dimethylpentane	16.9	...	...	...
2,3-Dimethylpentane	2.7	...	...	...
2,2,4-Trimethylpentane / 2,2-Dimethylhexane	80.4	100.0	94.7	...
2,5-Dimethylhexane	...	...	0.8	...
2,4-Dimethylhexane	...	...	0.5	...
2,2,3-Trimethylpentane	...	...	4.0	20.5
2,3,4-Trimethylpentane	...	...	...	0.7
2,3-Dimethylhexane	...	...	...	7.9
n-Octane	...	...	...	70.9

Table III. Analysis of Hydrogenated Liquid Product of Run 118

Fraction No.	1-3	4-11	12-17	18-21	22-23	24
Boiling point, ° C.	... 98.4	98.4- 98.4	98.4- 111.1	111.1- 112.0	112.0- 116.2	116.0- 124.0
Volume, ml.	7.01	19.08	10.27	8.53	2.99	2.42
Hydrocarbon, vol. %						
2,2-Dimethylpentane	11.1	...	...	...	...	...
2,3-Dimethylpentane	9.9	...	...	...	...	...
2,2,4-Trimethylpentane / 2,2-Dimethylhexane	79.0	100.0	63.0	...	...	...
2,5-Dimethylhexane	...	...	2.0	...	...	...
2,4-Dimethylhexane	...	...	1.5	...	...	...
2,2,3-Trimethylpentane	...	...	16.5	2.4	...	...
2,3,3-Trimethylpentane / 3,3-Dimethylhexane	...	...	0.2	1.6	20.9	2.9
2,3,4-Trimethylpentane	...	...	16.8	93.9	64.4	17.1
2,3-Dimethylhexane	...	...	...	2.1	12.6	3.6
3,4-Dimethylhexane	...	...	...	...	2.1	1.4
2-Methylheptane	...	...	...	...	...	1.9
3-Methylheptane	...	...	...	...	...	0.2
n-Octane	...	...	...	...	...	72.9

Table IV. Analytical Summary of Hydrogenated Cold Acid Polymer Feed and of Liquid Product from Run 118

Hydrocarbon	Fresh Feed, 50.00 Ml. Vol., ml.	Fresh Feed, 50.00 Ml. Vol. %[a]	Product, 50.00 Ml. Vol., ml.	Product, 50.00 Ml. Vol. %[a]
2,2-Dimethylpentane	2.22	4.82	0.78	1.61
2,3-Dimethylpentane	0.35	0.76	0.69	1.42
2,2,4-Trimethylpentane / 2,2-Dimethylpentane	41.90	91.12	31.09	64.04
2,5-Dimethylhexane	0.04	0.09	0.21	0.43
2,4-Dimethylhexane	0.03	0.07	0.15	0.31
2,2,3-Trimethylpentane	1.07	2.33	1.90	3.92
2,3,3-Trimethylpentane / 3,3-Dimethylhexane	...	....	0.86	1.77
2,3,4-Trimethylpentane	0.03	0.07	12.06	24.85
2,3-Dimethylhexane	0.34	0.74	0.65	1.34
3,4-Dimethylhexane	...	....	0.09	0.19
2-Methylheptane	...	....	0.05	0.10
3-Methylheptane	...	....	0.01	0.02
Total	45.98	100.00	48.54	100.00
Recovery, vol. %	92.1		97.1	

[a] On no loss basis in distillation.

Table V. Depolymerization of Codimer over Clay Catalyst

(Experimental data for run 119)

Feed Stock	1st Pass	2nd Pass	Over-all
Boiling range, ° C.	95-119	...	..
Density, g./ml., 20° C.	0.736	0.732	..
Bromine No.	126	125	..
Vol. % of total polymer	70.5	...	..
Test conditions			
Catalyst temp., ° C.	374	378	..
Space velocity, ml./ml./hour	1.85	1.93	..
Hours on test	7.0	5.0	..
Products, weight % of feed			
Gas, C_5's, and lighter	41.0	31.1	59.3
Liquid, C_6's, and heavier	59.0	68.9	40.7
Gas composition, mole %			
Propylene	2.3	8.8	3.6
Propane	0.1	1.8	0.5
Isobutylene	82.9	66.7	79.7
Isobutane	0.5	1.4	0.6
n-Butylene	7.1	9.9	7.7
n-Butane	0.8	0.8	0.8
Pentenes	5.6	10.3	6.5
Pentanes	0.7	0.3	0.6
Yields, weight % of feed			
Propylene	0.7	2.1	1.9
Propane	0.1	0.5	0.4
Isobutylene	33.4	20.7	45.6
Isobutane	0.2	0.4	0.4
n-Butylene	3.0	3.1	4.8
n-Butane	0.3	0.2	0.4
Pentenes	2.9	4.0	5.3
Pentanes	0.4	0.1	0.5
C_6's and heavier	59.0	68.9	40.7
Liquid product inspections			
Density, g./ml., 20° C.	0.732	0.735	..
Bromine No.	125	125	..

From consideration of the gas analysis, it was calculated on the basis of 100 moles of feed that 57.8 moles of octenes, undoubtedly 2,4,4-trimethyl-1-pentene and -2-pentene, have depolymerized in a manner yielding 2 moles of isobutylene for every mole of octene disappearing, with an assumed 0.4 mole of isobutylene coming from the dissociation of an octene into isobutylene and *n*-butylene. From the liquid analysis, however, it was apparent that 70.6 moles of the original 100 moles of octenes were converted to other products. The difference in these values is accounted for by the

The over-all change in composition of cold acid polymer (*3*, Table III), depolymerized in run 118 was calculated from the data of Table IV, using density values for the individual octanes obtained from American Petroleum Institute tables (*1*), and the gas analysis and conversions reported in Table I. In calculating this change in composition, the heptanes were eliminated from the liquid analysis because of a primary interest in changes occurring in the octanes and the fact that the heptane resolution in the first combination of fractions was poor. The column used for the distillation of hydropolymer had a slight imperfection in the take-off valve which allowed a small leakage to the receiver during periods of total reflux. Hence at the start of a distillation before equilibrium was established within the column, a small amount of poorly fractionated material collected in the receiver and was included in the first distillate fraction. This caused difficulty in resolution of the mass spectrometer patterns of the heptanes, as compounds boiling higher than iso-octane interfere in their analysis. In making this calculation it was assumed that the analysis of the feed was correct, as the remaining material is mainly iso-octane which does not interfere with the heptane resolution, and that the C_7's were not depolymerized by the catalyst. The small amount of propylene in the gaseous product justifies the latter assumption.

These results substantiate the qualitative indications of isomerization of the octanes previously obtained (*3*).

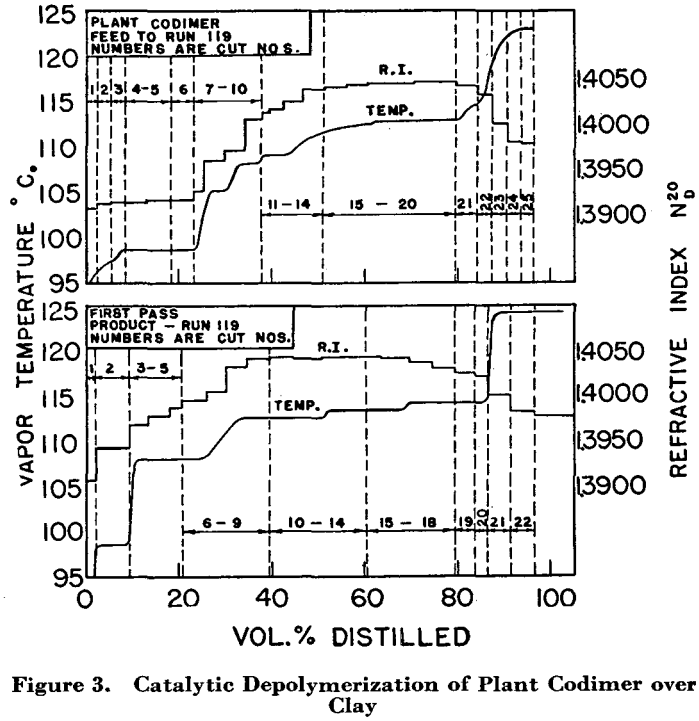

Figure 3. Catalytic Depolymerization of Plant Codimer over Clay

Fractional distillation of hydrogenated codimer feed and hydrogenated first pass liquid product

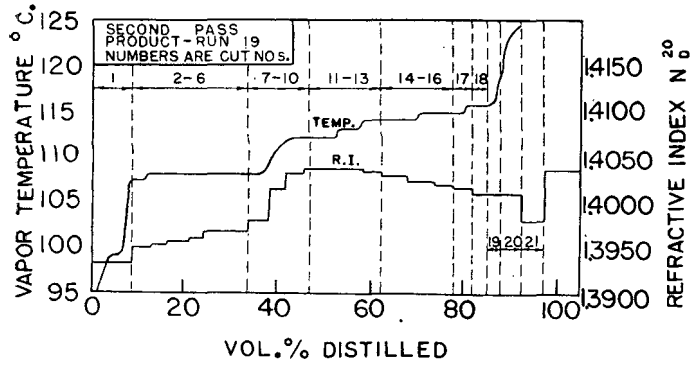

Figure 4. Catalytic Depolymerization of Plant Codimer over Clay

Fractional distillation of hydrogenated second pass liquid product

formation of a considerable amount of 2,3,4-trimethylpentenes, a smaller but definite amount of 2,3,3-trimethylpentenes, and possibly small amounts of 2,5- and 3,4-dimethylhexenes through isomerization of the original octenes.

Table V shows the experimental data for the depolymerization of a commercial codimer sample (ratio of *n*-butylene to isobutylene approximately 0.7) when two passes over the catalyst were employed. A fraction of the total plant polymer boiling between 95° and 119° C. was passed over the clay at 374° C. This temperature, 110° C. higher than that employed on the cold acid polymer, was used to obtain a total conversion similar to that realized in run 118. A portion of the liquid product from the first pass was set aside for analysis in the manner previously described and the remainder used as a charge for the second pass over the catalyst. Figure 3 shows the distillation of the hydrogenated original feed and hydrogenated first pass liquid product or feed to the second pass. Figure 4 shows the distillation of the hydrogenated second pass or final product. Tables VI, VII, and VIII show the mass spectrometer analyses of the various combined distillation fractions of the hydrogenated feed, first pass product, and second pass product, respectively. Table IX summarizes the results of the liquid analyses calculating the volume per cent figure on a "no-loss" basis during the distillation. The hydrocarbon marked "heavier" in the table is that material which boiled higher than the *n*-octane used as a distillation chaser, the amount of which was determined from the increase in volume of the *n*-octane recovered.

The composition and changes in composition accompanying the depolymerization of codimer over clay (*3*, Table IV) were calculated from the data of Tables V and IX, using 100 moles of octenes charged to the initial depolymerization as a basis for comparison. Elimination of the hydrocarbons boiling higher than the octanes was believed justified, as calculations, assuming that they are nonanes, showed that no depolymerization of these heavier hydrocarbons occurred. The resolution of the C_7's was again poor for the reasons previously described, but the sum of the heptanes in

Table VI. Analysis of Hydrogenated Feed, Run 119

Fraction No.	1	2	3	4–5	6	7–10	11–14	15–20	21	22	23	24	25
Boiling point, ° C.	... 96.4	96.4–97.2	97.2–98.6	98.6–98.6	98.6–98.6	98.6–108.8	108.8–110.7	110.7–112.8	112.8–114.6	114.6–119.5	119.5–123.2	123.2–124.0	124.0–124.0
Volume, ml.	1.32	1.32	1.67	4.74	2.52	7.38	6.68	14.26	2.24	1.60	1.63	1.56	1.31
Hydrocarbon, volume %													
2,2-Dimethylpentane	8.0	2.2	1.4	0.2	0.4	..	..	..	..	..	..	..	..
2,4-Dimethylpentane	3.0	..	..	0.6	..	..	..	..	..	..	..	..	..
2,3-Dimethylpentane	21.9	4.6	3.6	1.2	0.9	..	..	..	..	..	..	..	..
2-Methylheptane	..	..	0.4	0.1	0.1	..	..	..	..	..	..	..	..
3-Methylheptane	4.0	1.9	0.7	0.2	0.3	..	..	..	..	..	..	..	..
2,2,4-Trimethylpentane / 2,2-Dimethylhexane	52.5	91.3	93.9	97.7	98.3	47.4	..	..	..	..	..	..	..
2,5-Dimethylhexane	1.6	..	..	..	..	4.7	3.2	..	..	..	..	..	..
2,4-Dimethylhexane	0.8	..	..	..	..	6.5	8.4	..	..	..	..	..	..
2,2,3-Trimethylpentane	8.2	..	..	..	..	38.1	53.3	3.1	..	..	..	..	..
3,3-Dimethylhexane / 2,3,3-Trimethylpentane	..	..	..	..	..	2.9	2.3	9.1	23.5	17.8	3.7	..	..
2,3,4-Trimethylpentane	..	..	..	..	..	0.4	32.8	82.1	35.9	19.6	7.7	..	..
2,3-Dimethylhexane	..	..	..	..	..	..	..	5.7	30.7	30.4	10.6	0.1	..
3,4-Dimethylhexane	..	..	..	..	..	..	..	..	8.0	16.2	10.7	3.8	2.0
2-Methylheptane	..	..	..	..	..	..	..	..	0.2	1.4	1.2	..	1.2
3-Methylheptane	..	..	..	..	..	..	..	..	1.4	3.3	1.4	..	..
4-Methylheptane	..	..	..	..	..	..	..	..	..	..	..	3.3	1.2
n-Octane	..	..	..	..	..	..	..	..	0.3	11.3	64.7	92.8	96.8

Table VII. Analysis of Hydrogenated First Pass Product, Run 119

Fraction No.	1	2	3–5	6–9	10–14	15–18	19	20	21	22
Boiling point, ° C.	... 95.3	95.3–104.7	104.7–108.0	108.0–112.8	112.8–113.2	113.2–114.2	114.2–114.2	114.2–115.2	115.2–124.2	124.2–124.2
Volume, ml.	0.97	3.40	5.03	8.42	9.30	8.83	1.97	1.07	1.97	2.17
Hydrocarbon, volume %										
2,4-Dimethylpentane	0.6	..	..	..	..	..	..	..	..	..
3,3-Dimethylpentane	..	1.3	..	..	..	..	..	..	..	..
2,3-Dimethylpentane	57.4	..	..	..	..	..	..	..	..	..
2-Methylhexane	..	6.1	..	..	..	..	..	..	..	..
3-Methylhexane	..	2.1	..	..	..	..	..	..	..	..
2,2,4-Trimethylpentane / 2,2-Dimethylhexane	31.0	48.8	8.2	..	..	..	..	..	..	..
2,5-Dimethylhexane	6.0	15.2	19.7	5.5	..	..	..	..	..	..
2,4-Dimethylhexane	5.0	7.6	36.0	15.4	..	..	..	..	..	..
2,2,3-Trimethylpentane	..	18.9	33.6	30.5	0.3	..	..	..	..	..
3,3-Dimethylhexane / 2,3,3-Trimethylpentane	..	..	..	5.2	6.2	23.1	..	4.2	0.1	1.4
2,3,4-Trimethylpentane	..	..	2.5	43.4	90.4	35.3	8.0	..	..	..
2,3-Dimethylhexane	..	..	..	..	3.1	39.4	56.0	33.2	9.5	..
3,4-Dimethylhexane	..	..	..	..	..	1.7	21.3	38.2	21.8	5.7
2-Methylheptane	..	..	..	..	..	..	8.3	8.5	4.1	0.4
3-Methylheptane	..	..	..	..	..	0.5	6.4	13.9	9.8	0.8
4-Methylheptane	..	..	..	..	..	..	..	0.5	6.3	5.8
n-Octane	..	..	..	..	..	..	..	1.5	48.4	85.9

Table VIII. Analysis of Hydrogenated Second Pass Product, Run 119

Fraction No.	1	2–6	7–10	11–13	14–16	17	18	19	20	21
Boiling point, ° C.	... 107.2	107.2– 108.0	108.0– 112.0	112.0– 114.0	114.0– 114.8	114.8– 115.6	115.6– 115.7	115.7– 119.4	119.4– 124.6	124.6– 124.6
Volume, ml.	4.52	12.04	6.69	7.61	7.81	2.09	1.68	1.28	2.38	2.48
Hydrocarbon, volume %										
2,2-Dimethylpentane	29.0	..	..	..	..	..	..	..	..	..
2,4-Dimethylpentane	2.0	..	..	..	..	..	..	..	..	..
2,3-Dimethylpentane	15.0	..	..	..	..	..	..	..	..	..
3-Methylhexane	15.0	..	..	..	..	..	..	..	..	..
2,2,4-Trimethylpentane / 2,2-Dimethylhexane	9.0	5.7	..	..	..	..	..	..	..	..
2,5-Dimethylhexane	9.0	25.5	5.8	..	..	..	..	..	..	..
2,4-Dimethylhexane	12.0	46.1	14.0	..	..	..	..	..	..	..
2,2,3-Trimethylpentane	9.0	14.7	13.3	..	..	..	..	..	..	..
3,3-Dimethylhexane / 2,3,3-Trimethylpentane	...	2.2	14.2	11.3	10.5	..	..	..	..	0.9
2,3,4-Trimethylpentane	...	5.8	37.3	46.8	6.5	2.2	...	...	...	...
2,3-Dimethylhexane	...	..	15.4	39.2	60.7	30.4	18.1	10.6	3.8	..
3,4-Dimethylhexane	...	..	..	2.7	13.7	27.4	35.2	30.3	13.5	3.8
2-Methylheptane	..	..	..	..	4.8	13.5	14.4	11.4	3.4	..
3-Methylheptane	..	..	..	..	3.8	26.5	28.3	37.1	17.0	..
4-Methylheptane	..	..	..	..	..	..	4.0	5.3	3.6	2.2
n-Octane	..	..	..	..	..	..	..	5.3	58.7	93.1

Table IX. Analytical Summary of Hydrogenated Codimer Feed and Liquid Products, Run 119

	Fresh Feed, 50.00 Ml.		1st Pass, 45.00 Ml.		2nd Pass, 50.00 Ml.	
Hydrocarbon	Vol., ml.	Vol. %[a]	Vol., ml.	Vol. %[a]	Vol., ml.	Vol. %[a]
2,2-Dimethylpentane	0.18	0.39	...	....	1.31	2.67
2,4-Dimethylpentane	0.07	0.15	0.01	0.02	0.09	0.18
3,3-Dimethylpentane	...	....	0.05	0.12	...	....
2,3-Dimethylpentane	0.49	1.07	0.56	1.31	0.68	1.39
2-Methylhexane	0.02	0.04	0.21	0.49	...	....
3-Methylhexane	0.10	0.22	0.07	0.16	0.68	1.39
2,2,4-Trimethylpentane / 2,2-Dimethylhexane	14.07	30.6	2.37	5.52	1.09	2.22
2,5-Dimethylhexane	0.59	1.28	2.02	4.71	3.86	7.88
2,4-Dimethylhexane	1.05	2.28	3.42	7.97	7.03	14.4
2,2,3-Trimethylpentane	6.92	15.1	4.92	11.5	3.07	6.27
3,3-Dimethylhexane / 2,3,3-Trimethylpentane	2.54	5.52	3.13	7.29	2.91	5.94
2,3,4-Trimethylpentane	15.16	32.9	15.46	36.0	7.30	14.9
2,3-Dimethylhexane	2.16	4.68	5.41	12.6	9.91	20.2
3,4-Dimethylhexane	0.70	1.51	1.53	3.57	3.23	6.59
2-Methylheptane	0.06	0.13	0.34	0.78	1.13	2.30
3-Methylheptane	0.10	0.22	0.53	1.23	2.20	4.49
4-Methylheptane	0.07	0.15	0.23	0.53	0.28	0.57
Heavier	1.73	3.76	2.66	6.20	4.22	8.61
Total	46.01	100.00	42.92	100.00	48.99	100.00
Recovery, vol. %	92.2		95.4		98.1	

[a] On no loss basis in distillation.

the feed and in the final product indicates no appreciable depolymerization. The method of eliminating the C_7's involved using the heptane analysis as obtained, inasmuch as none could be considered more reliable than the other.

These calculations reveal that of the octenes, the trimethylpentenes with the possible exception of 2,2-dimethylhexenes are practically the only components of the butylene codimer that undergo reaction in the presence of Attapulgus clay under the conditions studied. Except for the small conversion to C_3, C_4, and C_5 paraffins, these trimethylpentenes were converted to olefins of lower molecular weight by depolymerization and to less highly branched octenes by isomerization. The octenes formed by isomerization appear to be mainly dimethylhexenes, although the formation of the methylheptenes cannot be entirely ignored.

DISCUSSION

The accuracy of the analyses reported here, in general, is believed to be good. In interpreting the course of the depolymerization reactions (*3*), the molecular balance of compounds formed and accounted for amounted to 99.4 and 95.3%, respectively. These figures, however, cannot be used as a true indication of analytical accuracy; they simply mean that the proposed reactions account for the observed results.

The most likely source of errors in the analysis of the individual components in this work may be attributed to the fractional distillation step. Distillation loss was assumed to be distributed evenly over the whole boiling range but may have taken place while only one fraction was being collected. Poor rectification of the first fraction collected in each distillation caused difficulty in resolution of the mass spectrometer patterns. The latter difficulty may be peculiar to the specific distillation column used in this work but the former is probably a general problem encountered whenever a small amount of material is subjected to thorough rectification. Distillation of a larger amount of material would minimize the loss but increase the time required for this step of the analysis.

Aside from the discrepancies introduced by distillation, the results of this work show that olefin mixtures can be analyzed quantitatively by hydrogenating to the corresponding saturated compounds followed by mass spectrometer analysis. Although this method of analysis provides no information on the various olefin isomers which differ only in the position of the double bond, it does offer a procedure for the analysis of complex olefin mixtures, such as those employed in this investigation, in which the number of olefin isomers may be so great as to defy attempts at complete analysis as the original olefins.

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Determination of Functionality in Methyl Silicone Systems

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Oil viscosity and gel test methods are described for determining small amounts of mono- and trifunctional methylsiloxane units in primarily difunctional methylsiloxane systems. Such information is important in the preparation of silicone oils and silicone rubber. The methods are based on the fact that methylpolysiloxane systems equilibrated with sulfuric acid form gels when the functionality is greater than 2, oils when the functionality is less than 2. The viscosity of the oils prepared is related to the mono-, di-, and trifunctional unit concentration. The addition of a known amount of monofunctional material to an oil of unknown di- and trifunctional unit composition permits upon equilibration and viscosity determination definition of the system by reference to a standard viscosity curve. In the gel test method the amount of monofunctional units required for just preventing gelation is directly related to the trifunctional unit concentration of the original oil.

IN THE preparation of polymeric systems it is important that the functionality be carefully controlled. This is especially true in the preparation of methyl silicone oil and rubber where small amounts of cross-linking and chain ending groups markedly affect the properties of the final product. It is therefore essential that methylchlorosilanes used as starting materials in the preparation of silicone oil and rubber be of high purity. This necessitates careful fractionation, particularly in the separation of methyltrichlorosilane and dimethyldichlorosilane which boil at 66° and 70° C., respectively.

Although the hydrolyzable chlorine and density determinations used by this laboratory (*1*, *2*) for general control measurements are satisfactory for most binary methylchlorosilane mixtures, they are neither positive nor sufficiently sensitive for determining small amounts of methyltrichlorosilane (0 to 0.5%) in dimethyldichlorosilane. The oil viscosity and gel test methods described in this paper permit the most accurate and precise measurement of the small amounts of mono- and trifunctional material that suffice to modify the properties of difunctional silicone systems.

THEORY

When the methylchlorosilanes are hydrolyzed and the resulting methylpolysiloxanes, or silicone oils, are shaken with sulfuric acid, catalytic rearrangement takes place and equilibrium is established, providing the functionality of the system is sufficiently low to allow adequate mobility of the molecules. The general technique of equilibrating methylpolysiloxanes with sulfuric acid has been described by Patnode and Wilcock (*3*). The equilibria involved have been studied and discussed by Wilcock (*7*) and Scott (*6*).

Table I shows the polymeric units obtained upon hydrolysis of the methylchlorosilanes with which this paper is concerned.

Table I. Polymeric Units Obtained

Methylchlorosilane	Polymeric Hydrolysis Unit	Functionality
$(CH_3)_3SiCl$	$(CH_3)_3SiO_{1/2}$	1
$(CH_3)_2SiCl_2$	$(CH_3)_2SiO$	2
CH_3SiCl_3	$CH_3SiO_{11/2}$	3

The oil viscosity and gel test methods are both based on the fact that methylpolysiloxane systems with a functionality of less than 2 form oils upon treatment with sulfuric acid, whereas those with a functionality greater than 2 form gels with sulfuric acid.

In the oil viscosity method advantage is taken of the proportionality that exists between the viscosity of an equilibrated silicone oil and its mono-, di-, and trifunctional unit concentration. If a constant number of monofunctional units are added to a series of silicone oils composed solely of di- and trifunctional units in varying but known amounts, the viscosity of the oil after sulfuric acid treatment can be plotted as a function of the original trifunctional unit concentration. The trifunctional unit content of an oil of unknown di- and trifunctional unit composition can then be readily ascertained by equilibration with the same quantity of monofunctional material and reference to the standard curve.

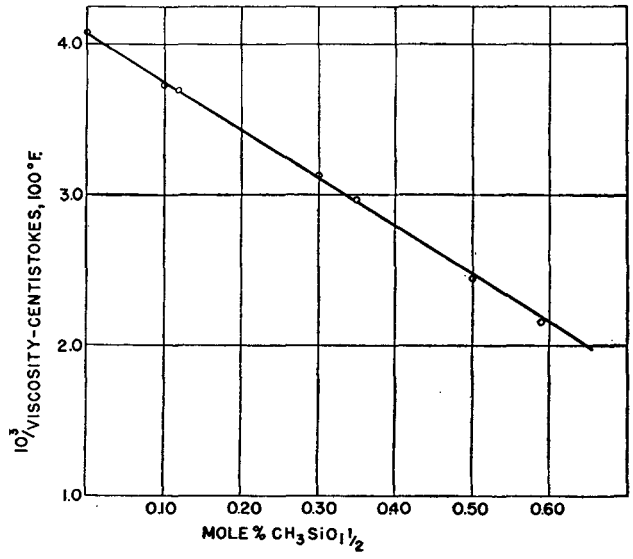

Figure 1. Viscosity *vs.* Mole Per Cent of Trifunctional Units

In the gel test method graduated amounts of monofunctional units are added to an oil composed of di- and trifunctional units to cover the transition range of oil to gel. The amount of monofunctional units required to just prevent gelation is a function of the trifunctional unit concentration of the original oil. Both methods can also be used to determine small amounts of monofunctional units in an oil composed of mono- and difunctional units by the addition of a known amount of trifunctional units.

OIL VISCOSITY METHOD

Standard Curve. The compounds which were used as the source of pure mono-, di-, and trifunctional units are listed in Table II. The systems composed of known di- and trifunctional unit concentration were prepared by dissolving varying amounts of crystalline compounds containing trifunctional units—the methylpolycyclopolysiloxanes $(CH_3)_{10}Si_6O_7$ and $(CH_3)_{10}Si_8O_{11}$ of Table II—in octamethylcyclotetrasiloxane. To these stock solu-

Table II. Source of Pure Mono-, Di-, and Trifunctional Units

Siloxane	Formula	Polymeric Unit Composition
Hexamethyldisiloxane (*4*)	$(CH_3)_3SiOSi(CH_3)_3$	$2(CH_3)_3SiO_{1/2}$
Octamethylcyclotetrasiloxane (*3*)	$[(CH_3)_2SiO]_4$	$4(CH_3)_2SiO$
A methylpolycyclopolysiloxane (*5*)[a]	$(CH_3)_{16}Si_6O_7$	$4(CH_3)_2SiO$; $2CH_3SiO_{11/2}$
A methylpolycyclopolysiloxane (*5*)[b]	$(CH_3)_{10}Si_5O_{11}$	$2(CH_3)_2SiO$; $6CH_3SiO_{11/2}$

[a] Compound B of Scott's Table I.
[b] Compound E of Scott's Table I.

Table III. Viscosity of Oils Composed of Di- and Trifunctional Units upon Equilibration with 1.10 Mole % of $(CH_3)_3SiO_{1/2}$

Sample	Mole % $CH_3SiO_{11/2}$	Viscosity[a], Centistokes at 100° F
1	0[b]	243
2	0[b]	248
3	0[b]	243
4	0[b]	245
5	0.10[c]	268
6	0.12[d]	271
7	0.30[c]	321
8	0.35[d]	338
9	0.50[c]	410
10	0.59[d]	465

[a] Ostwald-Cannon-Fenske viscometer.
[b] $[(CH_3)_2SiO]_4$.
[c] Source of trifunctional units, the methylpolycyclopolysiloxane $(CH_3)_{10}Si_5O_{11}$.
[d] Source of trifunctional units, the methylpolycyclopolysiloxane $(CH_3)_{16}Si_6O_7$.

tions containing 0.0 to 0.59 mole % of $CH_3SiO_{11/2}$ were added 1.10 mole % of $(CH_3)_3SiO_{1/2}$ in the form of hexamethyldisiloxane. The mixtures were equilibrated with concentrated sulfuric acid. The series in which the methylpolycyclopolysiloxane $(CH_3)_{16}Si_6O_7$ was employed as the source of trifunctional units was shaken for 40 hours. With the methylpolycyclopolysiloxane $(CH_3)_{10}Si_5O_{11}$ as the source of trifunctional units, equilibration was continued for 70 hours. Upon dilution with water the oils were shaken further for 2 hours, centrifuged, and filtered.

The viscosities obtained for these oils containing known amounts of trifunctional units are listed in Table III. When the mole per cent of trifunctional units is plotted against fluidity a straight-line relationship exists, as is shown by Figure 1. The equation for the straight line is:

Mole % $CH_3SiO_{11/2}$ = 1.26−309/viscosity (centistokes at 100° F.)

Procedure. Weigh 30.0 grams of hydrolyzed oil into a 2-ounce (60-ml.) screw-cap bottle. Add 363 mg. of hexamethyldisiloxane [1.10 mole % of $(CH_3)_3SiO_{1/2}$] to the oil on the balance. Addition of the hexamethyldisiloxane should be rapid, as the oil slowly loses weight when permitted to stand uncapped. It is also desirable to cap the bottle and shake the hexamethyldisiloxane into the oil before making the final weighing, as the disiloxane has an appreciable vapor pressure at room temperature. Add 1.2 ml. of concentrated sulfuric acid and shake the mixture vigorously by hand to disperse the acid. Place on a reciprocating mechanical shaker and shake at room temperature. The time required for equilibration depends on the type of mixing obtained and should be determined for the equipment to be used. With a common mechanical shaker an equilibration period of 24 hours has been found adequate for hydrolyzed oils. When the source of trifunctional units is concentrated in compounds such as the methylpolycyclopolysiloxanes of Table II, longer periods of equilibration have been found necessary. Upon equilibration add 3 ml. of water and shake for 2 hours. Centrifuge and filter the oil layer. In some cases opalescence persists after centrifuging. Shaking with a little anhydrous sodium carbonate before filtering will give clear oils. Determine the viscosity of the oil in centistokes at 100° F.

GEL TEST METHOD

Procedure. Weigh 10.0-gram samples of hydrolyzed oil into 1-ounce screw-cap bottles. Add graduated amounts of hexamethyldisiloxane to the weighed samples [11 mg. of hexamethyldisiloxane represents 0.1 mole % of $(CH_3)_3SiO_{1/2}$]. The same precautions in adding the hexamethyldisiloxane described under the oil viscosity method should be observed here. Pipet 0.4 ml. of concentrated sulfuric acid into each bottle. Shake vigorously by hand to disperse the acid, place on a reciprocating mechanical shaker, and equilibrate for at least 3 hours at room temperature. Originally the period of shaking was at least 15 hours but subsequent tests showed that with ordinary hydrolyzed oils 3 hours sufficed. When the source of trifunctional units is compounds such as the methylpolycyclopolysiloxanes of Table II more time is required. Add 10 ml. of 65% sulfuric acid and shake vigorously. Place on shaker for about 16 hours. Check solubility of solid polymers by shaking 1-gram portions with 20 ml. of toluene in the presence of an equal volume of water. The water prevents possible depolymerization. Record formation of gel, soluble solid or semisolid polymer, or oil. The amount of monofunctional units added to the sample which is just soluble [the addition of a little less $(CH_3)_3SiO_{1/2}$ gives a highly insoluble gel] represents the mole per cent of $(CH_3)_3SiO_{1/2}$ at incipient gelation. Read the mole per cent of $CH_3SiO_{11/2}$ of the original sample from Figure 2.

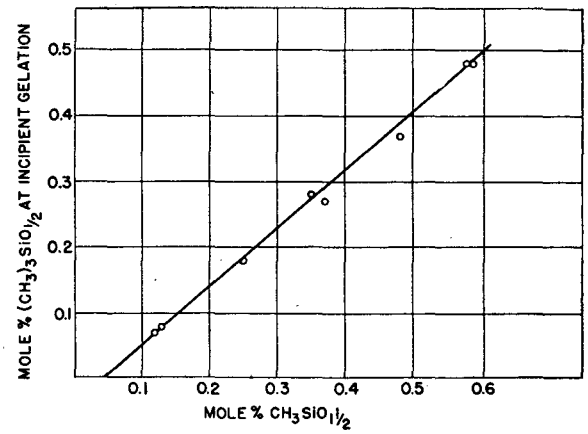

Figure 2. Relationship of Mono- and Trifunctional Units at Incipient Gelation

Discussion. To obtain an insoluble gel using the sulfuric acid polymerization technique, a slight excess of tri- over monofunctional units is required. This is illustrated in Table IV and in Figure 2 constructed from the data in the table. The amount of $CH_3SiO_{11/2}$ in a silicone system composed of di- and trifunctional units necessary to produce a gel lies between 0.04 and 0.08 mole % of $CH_3SiO_{11/2}$. As the trifunctional unit content of the oil increases the tri-/monofunctional unit ratio at incipient gelation increases.

DISCUSSION OF RESULTS

These methods can be used to give high accuracy with well defined silicone systems and are capable of extension beyond the

Table IV. Relationship of Mono- and Trifunctional Units at Incipient Gelation

Sample	Mole % $CH_3SiO_{11/2}$	Mole % $(CH_3)_3SiO_{1/2}$ at Incipient Gelation
1	0.04[a]	0 (no gel)
2	0.08[a]	0 (gel)
3	0.12[b]	0.07
4	0.13[c]	0.08
5	0.25[c]	0.18
6	0.35[b]	0.28
7	0.37[c]	0.27
8	0.48[c]	0.37
9	0.58[c]	0.48
10	0.59[b]	0.48

[a] Source of trifunctional units, $(CH_3)_8Si_5O_6$ (Compound A of Scott's Table I) (*5*).
[b] Source of trifunctional units, the methylpolycyclopolysiloxane $(CH_3)_{16}Si_6O_7$ of Table II.
[c] Determined by oil viscosity method on mixture of $[(CH_3)_2SiO]_4$ and a high $CH_3SiO_{11/2}$ containing oil of di- and trifunctional unit composition.

range discussed in this paper. The precision is such that with care the mole per cent trifunctional unit values can be determined to ±0.01 mole % of $CH_3SiO_{1\,1/2}$.

When both the oil viscosity and gel test methods are applied to the same system of di- and trifunctional units obtained by hydrolysis of a mixture of methyltrichlorosilane and dimethyldichlorosilane, the results may vary somewhat. This indicates the presence of other than di- and trifunctional units in the hydrolyzed oil. A hydrocarbon impurity, for example, will produce a greater discrepancy in the oil viscosity result than in the gel test result.

In general, it is desirable to use the oil viscosity method when the silicone is to be used for making silicone oils, the gel test when silicone rubber is the intended end product.

ACKNOWLEDGMENT

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REDUCTION OF NOISE

Perkin-Elmer Infrared Spectrophotometer Recording System

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Sources of noise in the amplification and recording system were located in magnetic fields that induce currents in the amplifier input circuit, in the voltage stabilizers and power supplies for the General Motors amplifier and the source of radiation, in the low screen grid potential of the second stage pentode of the amplifier, and in the fiducial marker circuit. The filter system was redesigned to provide a better match between filter and recorder. The over-all time constant of the system is 2.2 seconds. The calculated Johnson noise level is ±0.33 × 10^{-9} volt. The measured r.m.s. noise voltage is ±0.57 × 10^{-9} × volt. The probable maximum of peak noise voltage is ±1.3 + 10^{-9} volt.

IN THE course of installing and testing a Perkin-Elmer infrared spectrometer and recording system in the Gas Chemistry Section of the Chemistry Division, National Bureau of Standards, it was noted that the spectral resolution obtainable was limited not by the optical system but by the electrical system required for the amplification and recording of the thermocouple potential. An attempt has been made to reduce the electrical noise that limits the amount of amplification that may be used, and, because wider slits are required to produce a given deflection, results in lowered resolution. (For the purposes of this discussion any disturbing electrical effects that cannot be attributed to the quantity to be measured are referred to as noise.)

At this writing the optical system has not been thoroughly studied, but it probably is of sufficiently high quality to warrant reduction of the noise to the lowest possible level. Although this paper deals with a single installation of a model 12B spectrometer (serial No. 113) and a type 12-071 amplifying and recording system (installed in the spring of 1946), it is believed that an account of the troubles encountered in its installation in this laboratory may prove of value to others installing infrared equipment for the first time.

In a system of this kind where electrical and optical components have been combined, variability in the record which may be classed as noise may be grouped into four categories:

1. Uncertainties arising from variations in the temperature of the source of radiation. Such variations are caused by irregularities in the power used to heat the source and in its rate of cooling.
2. Noise introduced in the amplifier. Included in this group are such irregularities as variations in the power supplied to the amplifier, tube noises such as microphonic and shot effects, unusual operating potentials on tube elements, and other effects.
3. Drift. Variations having a long period and caused primarily by thermal voltages developed in the input to the amplifier exclusive of that developed by the thermocouple proper.
4. Johnson noise (*1*, *4*), caused by thermal agitation of the charges in the conductors constituting the input circuit to the amplifier.

Johnson gives the equation

$$V = (4k\,TR\,[f_2 - f_1])^{1/2}$$

where V = root mean square voltage developed across the resistor
k = Boltzmann constant, 1.38×10^{-16} erg per ° K. = 1.38×10^{-23} joule per ° K.
T = absolute temperature
R = resistance of the conductor across which the voltage is developed.
$(f_2 - f_1)$ = width of the band of frequencies passed by the amplifier

For a temperature of 25 ° C. the above reduces to

$$V = 12.8 \times 10^{-11}\,(R\,[f_2 - f_1])^{1/2}$$

At room temperature and with a given thermocouple resistance the only parameter that may be varied is the width of the frequency band passed by the frequency-discriminating network. Furthermore, the thermocouple has a fairly long time constant and develops signals containing low frequency components. This requires that the amplifier with its filter network pass low frequencies. If the Johnson noise is to be reduced, the upper limit of the frequency band passed by the filter must be lowered. This results in slower response of the electrical system and reqiures a longer time for scanning the spectrum, which in turn results in more trouble from drift. Opposed to this disadvantage, the higher amplification permitted by the lower Johnson noise level permits narrower slit settings and correspondingly higher resolution and less deviation from Beer's law, which is based on

monochromatic radiation. When wider slit settings are permitted by the nature of the project, the ratio of signal to noise is raised proportionately as the amplifier gain is decreased.

Johnson's equation is useful only for determining when the limit of amplification has been reached. The Johnson noise level, when expressed as a root mean square voltage, is appreciably smaller than the maximum noise peak which will probably be encountered in a typical series of observations. It is the latter quantity which is of interest to the analyst, as it is the limit of error in measurements of absorption.

THE INSTALLATION

The installation is shown schematically in Figure 1.

The source of radiation is a $^3/_{16}$ inch globar heated by an alternating current of 5 to 6 amperes at 40 to 50 volts. After passing through the optical system the radiation is received on the target of the thermocouple. Characteristics of the thermocouple (*5*) are: total resistance 12 ohms, response time 1 second (approximately), sensitivity 0.3 microvolt per erg per second.

The voltage developed by the thermocouple is amplified by a General Motors direct current amplifier. This amplifier, which is of the carrier-current type, is described elsewhere (*3*). The amplified voltage of the thermocouple is filtered and used to operate a Brown Electronik strip chart recorder, which itself contains a direct current amplifier. The recorder requires an input of 10 mv. to produce full-scale deflection of the pen and 4 seconds to reach a final position for full-scale deflection; 2 seconds to reach a final position for 10% of full-scale deflection, and 1.5 seconds to reach a final position for 1% of full-scale deflection.

The power circuits shown in Figure 1 are in some respects unconventional. The explanation is given in the discussion of the various circuit components.

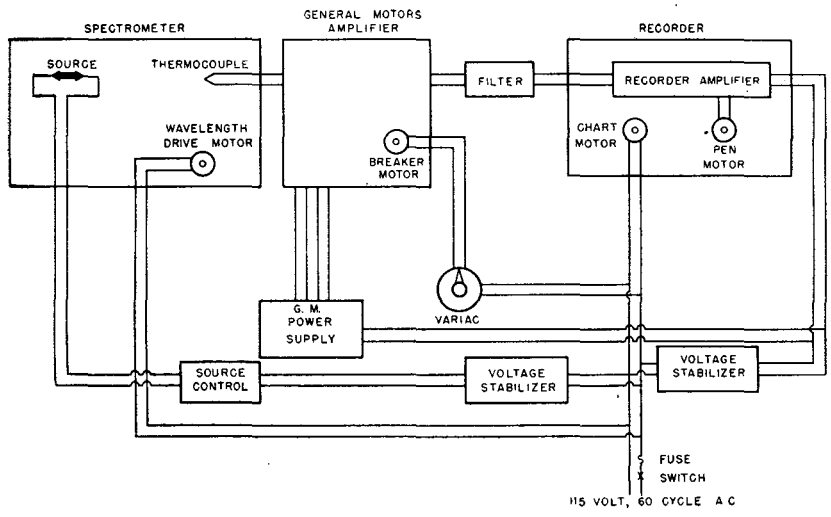

Figure 1. Schematic Diagram of Globar Control, Amplifier, and Recorder System

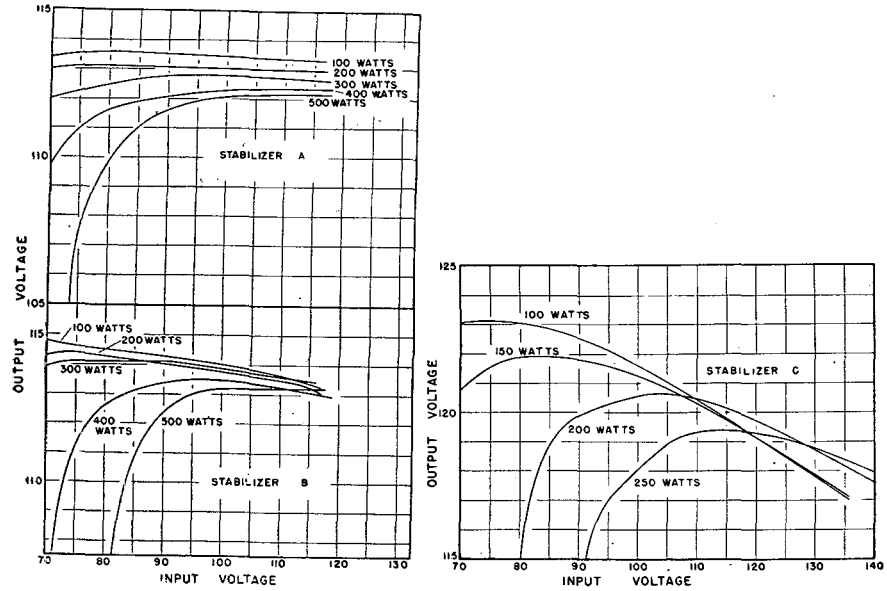

Figure 2. Stabilization Characteristics of Three Voltage Stabilizers Taken at Different Loads

CIRCUIT COMPONENTS

Voltage Stabilizers. Because variations in the voltage of the 110-volt 60-cycle supply are always a potential source of noise in amplifiers a study was made of the regulation characteristics of the voltage stabilizer supplied with the spectrometer. Its characteristics are shown in Figure 2,*C*. Data for somewhat larger stabilizer already in the laboratory are shown in Figure 2,*A*. Because of its excellent regulation it was decided to operate the General Motors amplifier and the recorder amplifier which together present a load of between 100 and 200 watts from this stabilizer. A duplicate of this stabilizer was obtained and found to have its best regulation at full load (see Figure 2,*B*). The source and its control equipment draw less than 500 watts, so additional load was provided as shown at *L* in Figure 3.

Source Control. The conventional circuit for operation of the source places the Variac brush in series with relatively high current at relatively low voltage. Under these conditions the brush deteriorates, and changes in its contact resistance can produce undesirable changes in the source current. A bucking circuit is used in this laboratory to put the Variac brush in a part of the circuit where it carries only a small current.

The source control circuit is shown at the left in Figure 3. The ratio of primary to secondary turns of transformer *A* is 2 to 1. Transformer *B* has a ratio of approximately 3.5 to 1 between primary and secondary and is connected so that its secondary is 180° out of phase with the primary of transformer *A*.

The ends of the Variac coil are connected across the stabilized voltage as shown. A double-pole double-throw switch is used to connect a dummy load in place of the globar. This permits leaving the circuit on continuously without reducing the life of the globar. The stabilizers should be left on continuously for best operation, for they tend to drift as they warm up (*6*).

The control operates as follows:

When the Variac brush is to the left as shown in the diagram, no potential is supplied to the primary of transformer *B*. Transformer *A* receives approximately full voltage on its primary and delivers approximately 55 volts to the globar. When the Variac brush is set to the right in the diagram,

full voltage is applied to the primary of transformer B and because of the out-of-phase relationship between the two transformers, the potential across the primary of transformer A is reduced by approximately 32 volts. Under these conditions approximately 33 volts are applied to the globar.

The output voltage of a Variac always varies stepwise. In some cases, because of mechanical considerations, the output voltage may fluctuate between two values, depending on the turn of wire with which the brush makes contact. In the system described above, the current through the Variac brush is reduced to 0.6 to 0.7 ampere, and brush life is correspondingly lengthened. Brush contact resistance is of smaller consequence. Transformer A serves as an isolation transformer to prevent possible short-circuiting in case a broken globar touches the water jacket.

Power Supply for General Motors Amplifier. The circuit of this unit as supplied is shown in Figure 4.

The high voltage supply to the plates of the amplifier tubes is conventional. Voltage regulation is provided by two OD3/VR150 tubes. Some filtering is provided by the 4-μf. condenser and the 5000-ohm resistor. Final smoothing occurs in the amplifier proper. Direct current is supplied to the heaters of the amplifier tubes by means of the bridge type dry rectifier, Re, and its associated circuit. Regulation of the heater current is provided by the barretter tube. For best regulation the barretter should operate with slightly less than half of its filament at a red heat. Resistor R_3 is provided to make this adjustment possible. Difficulty was encountered in one power supply received at this laboratory in that the dry rectifier had so much resistance that even with R_3 set to zero resistance there was insufficient voltage to produce a red spot on the barretter filament. With this power supply the noise level was unusually high, and major noise fluctuations could be correlated with variations in the line voltage.

The cable carrying power to the amplifier consists of three lines carrying direct current and three lines carrying alternating current. Connections 5 and 6 on the Jones plug run to a switch on the amplifier panel. Connections 3 and 5 run to the alternating current motor used to operate the breaker. It was thought desirable to eliminate the alternating current lines in this cable because of possible leakage and feedback into the direct current leads. The switch was eliminated by removing the leads from connections 5 and 6, soldering the leads together, and taping them. The lead was also unsoldered from connection 3 and taped.

The negative plate return and the positive heater lead are connected together and grounded to the power supply chassis. Care should be taken that the chassis is not inadvertently grounded by contact with other grounded metal objects.

The breaker motor is a 110-volt, alternating current motor which, in the instrument as received, was supplied from an 85-volt tap on the primary of the power transformer. It was reconnected to operate from a Variac as shown in Figure 1, through a shielded cable connected directly to the terminal strip in the back of the amplifier chassis. An Amphenol cable connector was later installed to permit easy removal of the cable. Connected in this way it no longer operates from the stabilized alternating current, but directly from the line. Changes in the load on the motor as reconnected do not cause voltages across the motor to be reflected by the stabilizer into some other part of the system. Operation of the breaker motor in this fashion eliminated from the record a small but persistent fluctuation of about 20-second period. It was observed that, as the voltage supplied by the Variac to the motor was changed, rather large noise voltages were recorded at some settings. It is therefore necessary to select a voltage that gives minimum noise.

General Motors Amplifier. The wiring diagram of the General Motors amplifier as given in the instruction manual accompanying the instrument is shown in Figure 5. Connections to the terminal strip as shown in this diagram are for operation from a 6-volt storage battery. Connections for operation from the power supply are shown in Figure 4.

In checking the amplifier circuit against the diagram, several differences were found, but most of them had only minor effects on the operation of the amplifier.

A mica condenser of 0.001-μf. capacity was connected between the control grid and cathode of the first 6SJ7 tube. The stepwise sensitivity control, which operated by varying the screen grid potential of the second 6SJ7 tube, had been eliminated. The sensitivity had been set to a permanent value by removing the connection between the connector, P_4, and the amplifier control panel and connecting P_4 to ground through a 35,000-ohm resistor. The right-hand tap switch on the diagram of the control panel (Figure 6) had been replaced by a variable resistor, which, with the one shown, gives continuous fine and coarse adjustments of the voltage used to balance spurious thermal voltages out of the input system. The circuit found on the amplifier control panel is shown in Figure 6.

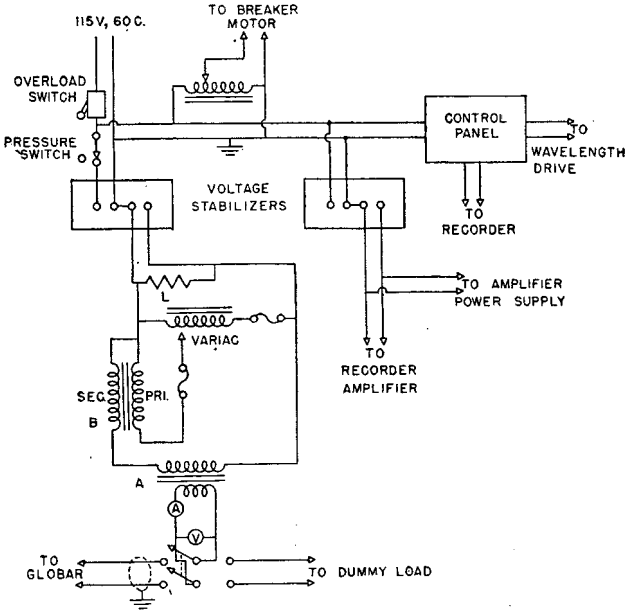

Figure 3. Schematic Diagram

Connections of breaker motor, wave-length drive, recorder, and General Motors amplifier to 60-cycle a.c. mains and detailed circuit of radiation source control

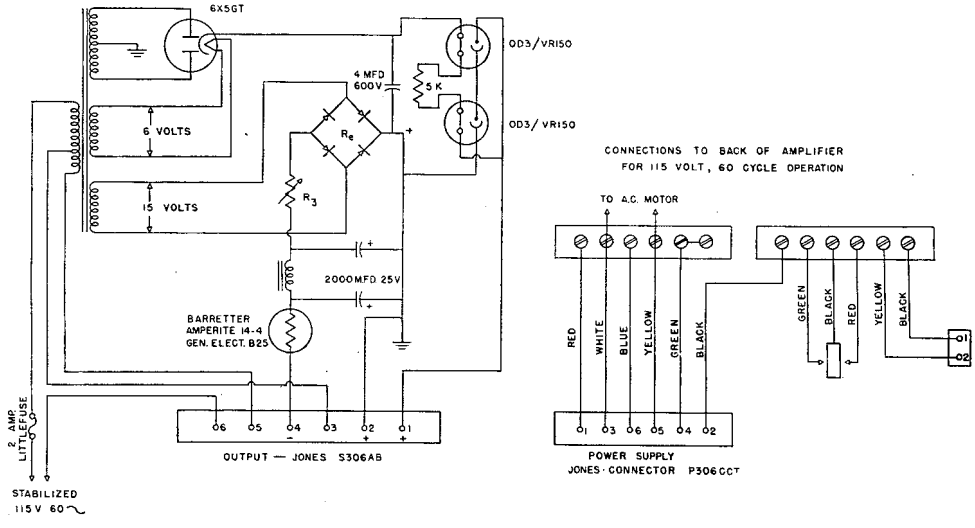

Figure 4. Circuit Diagram of Power Supply for General Motors Amplifier

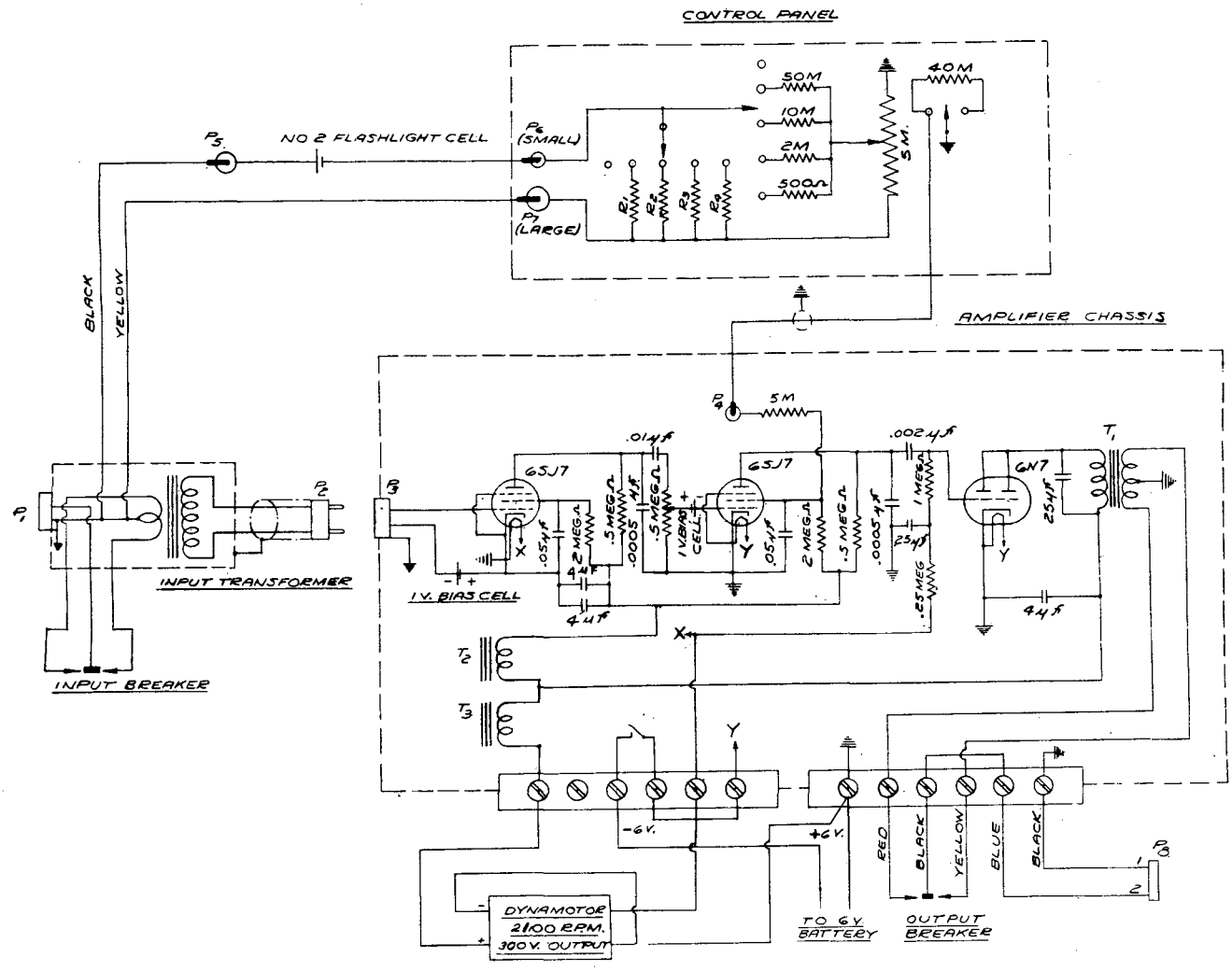

Figure 5. Circuit Diagram of General Motors Amplifier

Microphonic tubes are a frequent source of noise in an amplifier of this type. Noise from this source is reduced by careful selection of tubes. After nonmicrophonic tubes have been acquired, the noise is further reduced if the tubes are aged under normal heater voltage and plate current for 200 hours.

Examination of the amplifier circuit diagram indicates that the second stage is a pentode operating with 6-volt current supplied to screen grid. Because of this condition of operation the gain of the second stage is reduced and the over-all gain of the amplifier depends to a large extent on the individual tube used in this stage. In order to reduce the change in amplifier characteristics with change of tube, a triode (6J5) was substituted for the pentode (6SJ7) and negative current feedback used to reduce the gain of the stage to a usable value. An unbypassed 50,000-ohm cathode resistor provided the requisite amount of feedback.

An alternative method of stabilizing the amplifier is as follows (a system somewhat similar to this has been recommended by the Perkin-Elmer Corp.):

The 35,000-ohm resistor is removed, leaving the 2-megohm resistor as a series screen resistor. This results in extremely high gain in the amplifier. The gain is then reduced by introducing negative feedback across the whole amplifier. A connection is made between the positive output terminal and the yellow lead (P_7) to the amplifier control panel (see Figure 6) through a resistor which may have any value between 500 and 2500 ohms. The lower the resistance the greater the feedback and the lower the over-all gain. However, too low a resistance upsets the balance controls.

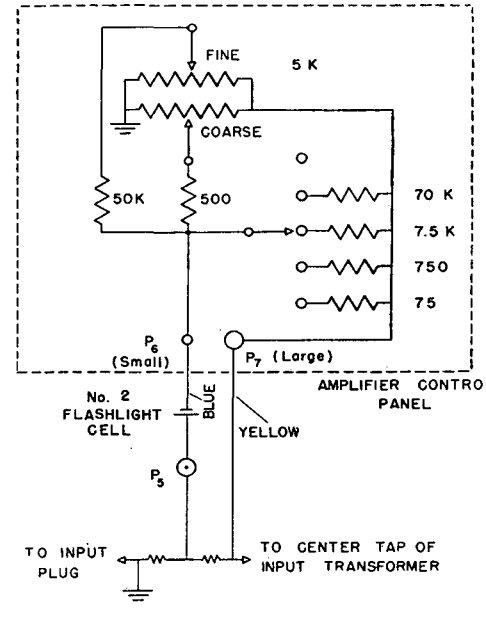

Figure 6. Balance Control and Standardizing Potential Circuits and Part of Input Circuit of General Motors Amplifier

There seemed to be little difference in the amplifier performance whether the triode second stage or the over-all negative feedback was used. Those who contemplate using the Perkin-Elmer 5-cycle chopped beam system should not rebuild the amplifier, as the negative feed-back system is used in the new equipment.

A second source of noise, which is as troublesome as noisy tubes, is in the 1-volt bias cells. Replacement with other cells, selected to produce the lowest noise, is the only satisfactory remedy for noisy cells. The No. 2 flashlight cell supplying voltage to the control panel may also be a source of noise.

The input circuit of the amplifier proper, including the input transformer and the input breakers, is shielded against magnetic effects (except as has been noted previously). The input cable supplied with the amplifier was tested by soldering the far ends together and comparing the noise picked up by the cable with that found when the shorting cap was in place on the input. This cable was found to pick up very little noise. The same cannot be said, however, about the cable furnished with the spectrometer and connected to the thermocouple. The noise picked up by this cable was very large and the cable could not be effectively shielded against the magnetic disturbances causing the trouble. The most noticeable difference between the two cables was that the General Motors cable was tightly twisted and the wires were held tightly together by the electrostatic shielding which was shrunk onto the twisted pair. The Perkin-Elmer cable was twisted about 1 turn per foot and was loose inside the braided shield.

The extreme sensitivity of the low impedance thermocouple and amplifier input circuit to 60-cycle magnetic fields prompted a search for the sources of such fields. The laboratory was found to be full of them. Fields were found at every 90° bend in electrical conduit, around the electric clock, and particularly near the voltage stabilizers and the wave-length drive motor. The summation of all fields, measured in the vicinity of the thermocouple, had peak valves of about 0.02 to 0.05 oersted (about 0.1 to 0.2 the horizontal component of the earth's field in this vicinity). In spite of their low magnitude it was felt that pickup of magnetic fields was the largest source of noise.

In reducing noise from the above sources the cable between the amplifier and the thermocouple was shielded by means of a soft iron conduit; the cable was brought out underneath the spectrometer in such a way as to keep it as far as possible from the wave-length drive motor; the drive motor was removed and another motor which did not produce strong fields was substituted for it (a capacity starting motor is recommended); and, although it did little good, iron shielding was placed around the voltage stabilizers. It would have been preferable to move them to another part of the building.

Filter Circuit. The time constants of the filter and recorder are interdependent and must be considered together. The circuit of the filter and the input of the recorder as originally received are shown in Figure 7,*A*. The impedance into which a filter operates determines, to a large extent, the rapidity with which the filter responds.

In Figure 7,*A*, this impedance is essentially 100 ohms shunted by 250 μf., assuming that the recorder is at all times able to maintain equilibrium between its potentiometer and the signal from the filter, so that its impedance, as seen from the filter, is infinitely high. Looking back into the filter, the impedance presented to the recorder is determined approximately by 50 ohms' resistance, shunted by 50 ohms' resistance in series with 250-μf. capacitance. The time constant of this combination is too short to provide adequate damping for the recorder. As received, underdamping had been corrected by building into the input of the recorder a condenser-resistor combination as shown in Figure 7,*A*.

Sensitivity may be increased by changing the recorder input connection from point *X* to point *Y* of Figure 7,*A*. However, both signal and noise voltages will be increased by the same amount. If this connection is made and the two 50-ohm resistors are replaced by resistors having higher values, the signal is increased while the noise level is decreased. When higher resistance is used another effect is noted; the time constant of the filter, as seen from the recorder, will have been increased, and will result in overdamping the recorder. This difficulty was solved by removal of the damping impedance in the recorder.

The final value chosen for the resistor (1000 ohms) was arrived at by trial and error. There is relatively small effect beyond about 700 ohms. Damping of the pen motion seems to be about right, using any value of resistor between 700 and 1000 ohms. Each change of resistance required readjustment of the gain setting of the recorder amplifier for optimum sensitivity of the recorder. If values greater than about 1000 ohms are used, advancing the gain control of the recorder amplifier to its full "on" position does not provide enough sensitivity to eliminate dead spots from the record. Overall sensitivity is controlled by means of the gain control on the General Motors amplifier. It is preferable, because the noise of the first stage of amplification limits the ultimate sensitivity of an amplifier, to control the gain as near the first stage as possible rather than at the output of the filter.

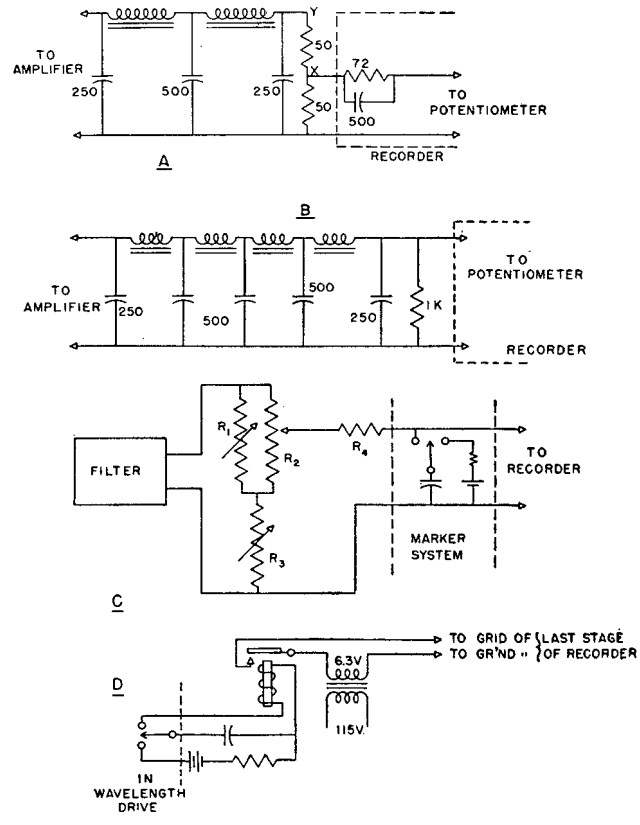

Figure 7. Circuits

A. **Diagram of filter circuit as received**
B. **As modified**
C. **Diagram of attenuator and marker circuit as received**
D. **Marker circuit as modified**

It was found that additional filtering action could be had at the expense of only slight loss in gain by doubling the number of elements in the filter. The initial response of the recorder to a suddenly applied signal is limited by the speed at which the pen is driven (3.8 seconds for full-scale deflection). The final part of the response is limited by the filter circuit between the amplifier and the recorder. The filter shown (Figure 9,*B*) gives 95% response in 5.5 seconds when a signal is applied to the input of the filter. The time constant (time required to attain $1 - 1/e$ or 63% of full response) of filter and recorder together is 2.2 seconds.

In Figure 7,*C*, are shown the circuit of the filter and some additional components that were received with the microdrive after the above changes were made. Component values were changed as follows:

	As Received	As Modified
R_1	110-ohm decade	2000-ohm decade
R_2	100-ohm gear driven	2000-ohm gear driven
R_3	20-ohm fixed	1000-ohm decade
R_4	100-ohm fixed	Eliminated

With this set of resistances it is possible to vary the correction required to produce a horizontal background curve and at the same time maintain a constant impedance across the output of the filter. Although this system of producing a horizontal background curve has never been used in this laboratory, it was felt that under certain conditions it might be useful and has therefore not been completely discarded.

A network for putting fiducial marks on the record at regular intervals of rotation of the wave-length drum is also shown in Figure 7,*C*. The single-pole, double-throw microswitch operates from a notched wheel on the wave-length shaft. Two faults were found in the instrument as supplied.

If the filter is to have a sufficiently long time constant to produce good smoothing action it will also tend to smooth out pulses introduced for marker purposes. The resulting marks on the record will show a steep side as the condenser discharges into the filter and a sloping side as the filter recovers its equilibrium value. This may take several seconds, during which time the record is useless as a measure of thermocouple potential. The second objection is the extremely practical one that leads from the microswitch were cabled with leads carrying 110-volt alternating current from which they picked up noise voltage. Undesirable alternating current voltages of sufficient magnitude to overload and block the recorder amplifier were thus introduced directly into the recorder, fortunately without benefit of amplification by the General Motors amplifier.

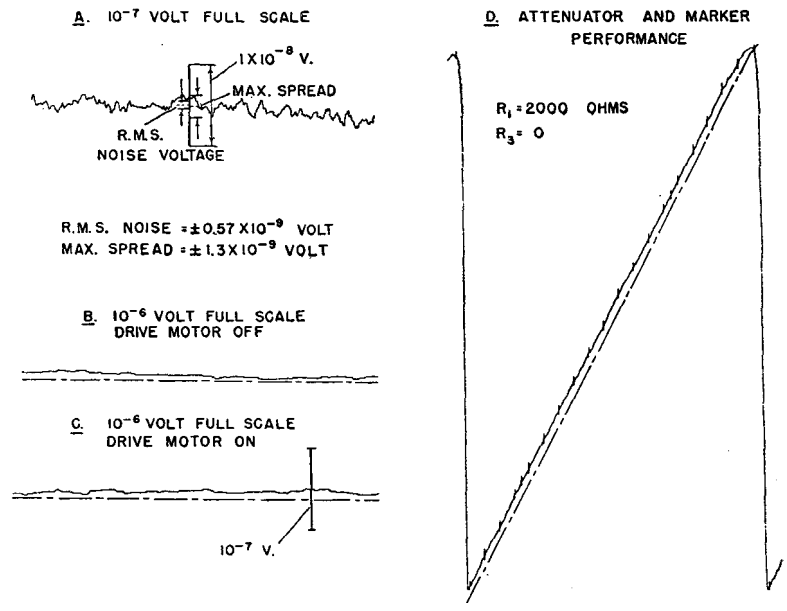

Figure 8. Photographs of Recordings

A. **Made at 0.1 microvolt for full scale, showing relation between r.m.s. noise voltage and maximum error**
B. **Made at 1 microvolt for full scale, wave-length drive motor turned off**
C. **Made at 1 microvolt for full scale, wave-length drive motor turned on**
D. **Made at low gain to show performance of attenuator and marker systems**

Both these deficiencies were corrected simultaneously by the circuit shown in Figure 7,*D*.

As before, a condenser is charged through a current-limiting resistor to full battery potential. When the microswitch trips, the condenser is discharged through the coil of a relay. The relay introduces momentarily an alternating current voltage across the grid resistor of the last stage of the recorder-amplifier. The pen can be made to deflect in either direction, depending on the phase relation between the transformer voltage and the signal on the grid. The condenser capacity was chosen to produce an appropriate pen deflection with the use of a standard filament transformer (6.3 volts) and a relay which was already on hand. The circuit is, of course, subject to wide variation in the choice of components. As the microswitch operates a relay in this circuit, it makes no difference if a small amount of alternating current is picked up by the cabled leads.

The Recorder. Aside from the changes in the input circuit of the recorder already outlined, little was done to the recorder.

The recorder wiring was rearranged so that the motor driving the chart could be operated from the unregulated 115-volt, alternating current line. The recorder amplifier, which drives the pen motor, was connected to the stabilizer that supplies power to the General Motors amplifier. Unstabilized voltage is supplied to the other parts of the recorder through the control box.

On several occasions, following some circuit change, it was noted that the recorder suddenly lost sensitivity. Turning up the recorder-amplifier gain control seemed to do no good. The pen motor lacked power and the dead space, when the instrument was allowed to come to balance from either direction, was excessive. Measurement of the signal voltage (alternating current) at the grid of the output stage of the recorder amplifier indicated that the cause of this condition was simple overload. Removal of the overload voltage returned the recorder to its original sensitivity. The source of the overload voltage sometimes was difficult to locate because when present at the input of the recorder amplifier either direct current voltages or alternating current voltages of the right phase can produce these results.

Ground Connections. Extending from the thermocouple through the General Motors amplifier, its power supply, the filter, the recorder, and the voltage stabilizer to the grounded side of the alternating current supply line is a common (grounded) lead. Any voltage developed in this lead is in series with the signal from the thermocouple either before or after amplification. It is therefore imperative to avoid any connections that permit closed circuits in the ground leads. The job is made more difficult because the cases of both the G.M. amplifier and its power supply are connected to the common (grounded) lead. The particular voltage stabilizers used in this installation have a common connection and provide no isolation from the alternating current lines. The alternating current line was therefore grounded to a water pipe in the laboratory.

Drift. Because of the excellent construction of both thermocouple and amplifier it is believed that drift is caused principally by thermal e.m.f.'s at the connections between the thermocouple leads and the amplifier cable. Drift was measured by measuring the total variation in output of the electrical system as a function of temperature. The air temperature in the neighborhood of the spectrometer was varied $\pm 0.2°$ C. in cycles of 14 to 16 minutes and produced variations corresponding to $\pm 0.8 \times 10^{-8}$ volt at the input to the amplifier ($\pm 4 \times 10^{-8}$ volt per ° C. change in room temperature).

RESULTS

In Figure 8 are shown records made under various conditions of operation. Record *A* was made for the purpose of showing the relation between r.m.s. and maximum noise voltages. The r.m.s. noise voltage was measured on a section of the same recording as is shown in *A*. The figure for maximum noise voltage appearing during a normal period of recording is that for an envelope which would enclose all points of the record (*6*). The Johnson noise level was computed, assuming $R = 13$ ohms and $f = 0.5$ cycle per second to be $\pm 0.33 \times 10^{-9}$ volt. For comparison, values for Johnson noise, measured r.m.s. noise, and maximum noise voltages are tabulated below.

The figure given in the table below is that determined on a 15-minute record, part of which is shown in Figure 8,*A*. It is obvious

that the larger the noise burst the less likely is its occurrence, and that the longer the period of recording the more likely is the recording of a large burst. While the below figure is taken from a single record, it represents fairly closely the author's experience with many records. The ratio of $\frac{\text{max. peak}}{\text{r.m.s.}} = 2.3$ is slightly lower than that reported by Landon (3.4) (*2*).

Johnson noise (calculated)	$\pm 0.33 \times 10^{-9}$ volt
R.m.s. noise (measured)	$\pm 0.57 \times 10^{-9}$ volt
Maximum peak noise (measured)	$\pm 1.3 \times 10^{-9}$ volt

In making record *A* the wave-length drive motor was turned off and the gain was set to give 0.1 microvolt for full-scale deflection.

Record *B* was made with the gain reduced to that required to give full-scale deflection for 1.0 microvolt, and the wave-length drive motor was turned off. The maximum noise was about $\pm 0.2\%$ of full scale (2.0×10^{-9} volt). The percentage error in this case appears to exceed that for record *A* because the potentiometer in the recorder is incapable of interpolating between voltages smaller than 0.1% of full scale. The potentiometer slide wire is helically wound and has about 1000 turns for full scale. The sliding contact must therefore operate in a stepwise fashion in units of about 0.1%. In view of this fact it is believed that the maximum noise voltage recorded at 1 microvolt for full scale is essentially the same as that for recording at 0.1 microvolt for full scale.

Record *C* was made under the same conditions as record *B* except that the wave-length drive motor was turned on. Pickup from the drive motor approximately doubles the noise voltage. Reference lines (broken) are ruled beside the recorded lines in records *B*, *C*, and *D* to aid in estimating noise voltage and in the case of record *D* to show linearity.

Record *D* was made to show the action of the attenuator and fiducial marker. The record is very nearly linear and shows no stepwise variations attributable to the attenuator. For this record R_1 and R_3 (see Figure 9,*C*) were 2000 and 0, respectively.

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Instrument for Measuring Stress Relaxation of High Polymer Materials

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A compact instrument is described for measuring stress relaxation of high polymer materials. It contains a minimum of moving parts, is essentially free from draft and vibration effects, and measures the relaxation characteristics of a substance under constant strain (constant sample deflection).

STANDARD test methods in the field of stress relaxation of high polymer materials have been neglected partly because satisfactory instrumentation has not been available. One method of measurement recommended by the A.S.T.M. (*1*) has been described as a compression set test, under which a sample of standard size and shape is distorted by clamping between metal plates, heated for a prescribed time in an oven, then unclamped and allowed to resume its shape for a period of one hour. The ratio of the thickness of the sample before clamping to that after release is used as a figure of merit of the material tested.

This A.S.T.M. method does not, of course, give a dynamic measurement of stress relaxation under strain, and although the measurements provide an index by which materials may be graded, it is often desirable to obtain more accurate and detailed information and to be able to predict by extrapolation the expected future behavior of the material. Furthermore, as the sample is clamped before being heated, it is difficult to separate the effects of temperature change from those caused by the fundamental decay of the material.

The authors were desirous of making rapid measurements that would allow the classification of materials to a higher degree of accuracy than could be obtained through the use of the compression set method. It was hoped that enough information would be obtained to enable predictions to be made of relaxation values as a function of time following the application of a given load to the material. It was expected that this might be accomplished by extrapolation of data taken in a short period of time with a suitable instrument.

Theories of elasticity have been treated mathematically (*4*), but the behavior of an elastic body over a period of time under constant strain has not been represented by an equation involving but one constant of proportionality. The apparatus developed by the authors to solve this problem consists basically of a measuring jig that applies a predetermined initial stress to the sample; the strain thus built up in the sample is then maintained essentially constant, and the stress is allowed to relax. The stress as a function of time is recorded automatically for the entire period of the test.

Through experimentation with this instrument it was found that the data obtained by plotting stress relaxation against time at constant temperature yield logarithmic curves. Therefore, plotting on semilog paper gives straight lines, the slopes of which can be used as indexes of the stress relaxation characteristic of the materials. This is in agreement with work done on creep tests of textiles (*3*). The straight lines obtained in tests of a few hours' duration apparently may be extrapolated to periods of a month or more; actual tests carried to over 1800 hours have indicated very close adherence to the straight-line plot. Tests are currently being extended for several months to provide additional experimental verification of the logarithmic stress relaxation behavior.

REQUIREMENTS

The requirements for a suitable measuring jig are as follows:

It should be able to apply in less than a second a predetermined initial stress of several hundred pounds per square inch to a sample 1 square inch in cross section and 0.5 inch thick.

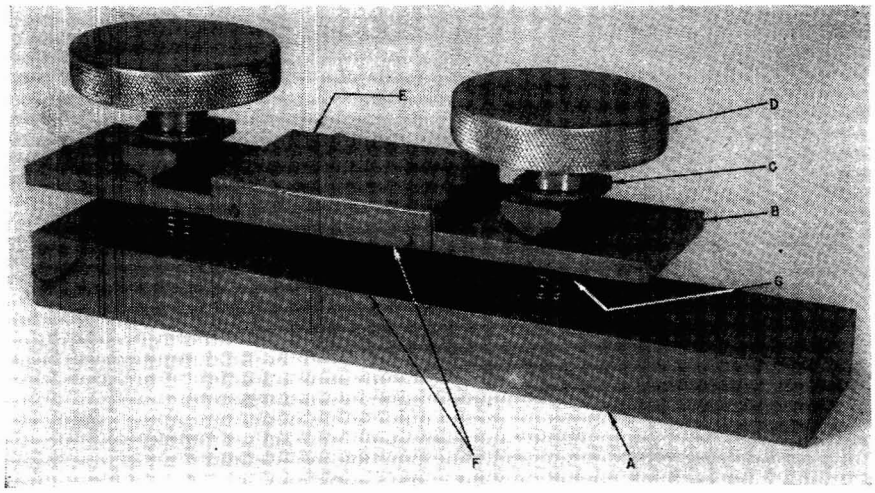

Figure 1. Early Model of Jig

The measuring spring deflection should be small (less than 5% of the sample compression under load) in order that the spring may be affected only by relaxation, and that the sample may remain under nearly constant strain.

The anvil upon which the sample to be measured is clamped should deflect less than 1% of the spring deflection under load.

The measuring element should be temperature-compensated, so that measurements can be taken at any desired temperature without instrument sensitivity or zero shifting.

It should be possible to measure samples in compression, tension, and shear.

Provision should be made for keeping the surfaces of the samples from slipping in the clamps.

The recorder should be able to record the output of the measuring jig over periods of several days with an error of less than 0.2% of full instrument scale.

APPARATUS

The unit described meets the above requirements. It consists of two components: a measuring jig that applies a desired initial stress to the sample and measures its relaxation on a continuous basis after the test starts, and a suitable recorder.

In accordance with the requirements that the allowable spring travel be small, a flat leaf spring is used as the measuring element. The stress in the sample causes a proportional deflection of the leaf spring which is measured by means of an electrical resistance strain gage bridge cemented to the spring. This bridge can be accurately balanced and calibrated; and as all four arms are cemented directly to the spring's surface, the bridge can easily be temperature-compensated.

An early model of jig, shown in Figure 1, was made to check the method and to obtain information for further development.

The base or anvil plate, *A*, was made of 1-inch cold rolled steel; the spring, *B*, of 0.25-inch cold rolled steel 2 inches wide and 5 inches between knife edges. The knife edges, *C*, were driven by clamping bolts, *D*. The strain gage bridge was cemented to the upper surface of *B* at *E*, and the two clamping surfaces, *F*, were knurled to prevent sample slippage. Springs, *G*, held the measuring spring plate, *B*, from contact with base plate *A* before the clamping bolts were tightened.

This jig proved the usefulness of the instrument but lack of means of applying quickly a known load to the test sample made it difficult to duplicate readings for a given material.

To overcome this, the jig illustrated in Figure 2 was designed.

The fixed anvil of the previous model is replaced by a movable, variable-length plunger driven by a lever-operated cam, *A*. The spring plate, *B*, is the anvil upon which the sample under test is clamped. The adjustable plunger allows for the use of test samples of varying thicknesses, and for predetermining the strain (and initial stress) to be applied to the sample. The cam drive always moves the plunger through a fixed distance, and assures positive clamping in a minimum amount of time. The strain gage bridge, after being cemented to the lower spring surface, *B*, is balanced and temperature-corrected so that it holds its zero reading within 0.2% from 70° to 180° F. under no-load conditions. Subsequently, there is run a calibration curve of load in pounds against millivolts per volt applied to the bridge.

A modified Foxboro Dynalog, *C*, is used for recording the output of the electrical strain-gage bridge. This recorder was designed specifically for use with strain gages and incorporates the voltage supply for the bridge in addition to performing its main function of recording output voltage. The modification consists of adding a range-extending switch to suppress the zero of the instrument in three steps, thus changing the normal 4-inch deflection range of the instrument to 12 inches.

The final version of the apparatus is illustrated in Figure 3. The measuring jig, Figure 2, a temperature-regulated oven, and a Foxboro recorder have been integrated into a compact unit.

PROCEDURE

The specimen size selected for tests is the standard pellet of the American Society for Testing Materials, normally used for measuring rubber compression set. This pellet measures 0.5 inch thick by 1.129 inches in diameter (1 square inch in area). The standard 2-inch high plastic sample, however, may be used.

The testing procedure consists of subjecting the specimen to a predetermined initial stress and recording the decay continuously as ordinate against time as abscissa. A pellet is centered on the

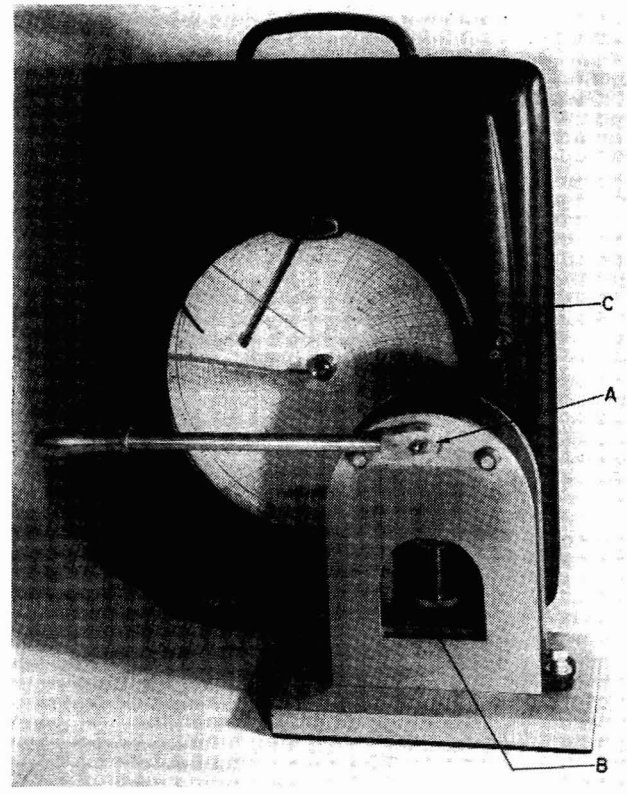

Figure 2. Model of Revised Jig

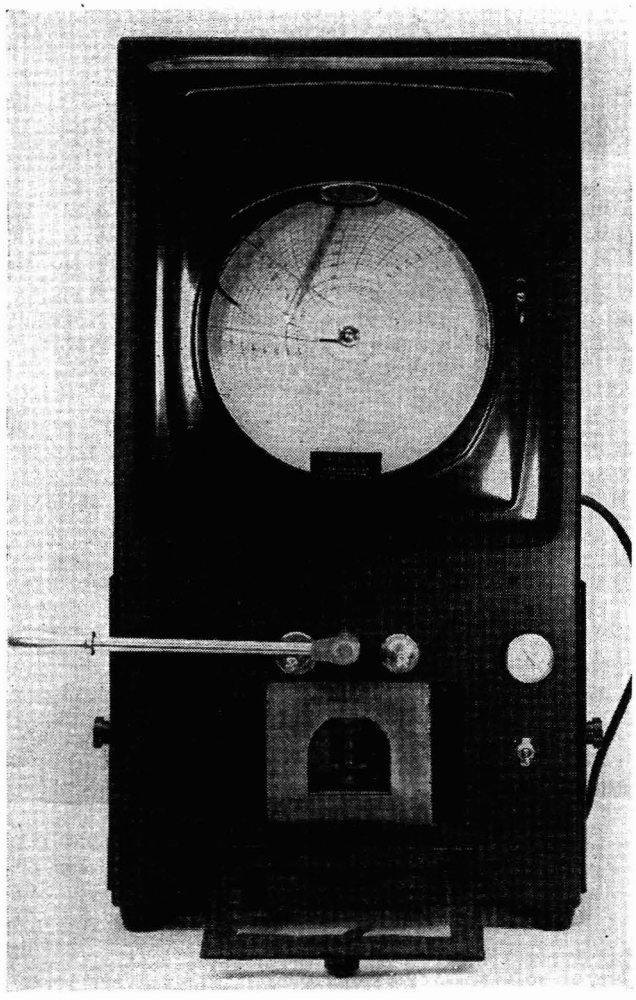

Figure 3. Final Apparatus

spring and the plunger adjusted by trial until the pressure exerted by the spring on the specimen reaches the desired value. The expended specimen is then removed in each case and a new one of the same material substituted before the recorder is started.

It has been found desirable to hold the temperature of the testing jig constant during tests; otherwise the volume expansion of the material produces an error in reading. Although the expansion error might be corrected, it is more straightforward to control the measuring jig temperature. Individual tests have covered periods ranging from 1 hour to 3 months. At the end of each test a check reading of the instrument zero makes certain that no drifts have occurred during the analysis.

The test data are analyzed by plotting the ratio between the stress at each point on the record curve and that at a common reference point as ordinate against a logarithmic time scale as abscissa.

Because the rate of decay of stress is at first in all instances very rapid, it has been found impractical to use the peak recorded stress as the common denominator for the interpretation of results. Accordingly there is selected the earliest point for which accurate measurements can be made after the relaxation of the specimen has stabilized. In the work covered by this report this time was established as 0.01 hour after the stress had been applied to the specimen.

After the above-defined stress ratios have been plotted against a logarithmic time scale for each test run, the negative slopes of the curves are determined graphically. Figures 4, 5, 6, and 7 are characteristic of the plots obtained. Figure 4 shows a comparison between the curves for certain samples of natural rubber, neoprene, and Buna S. Error in choosing the starting time at 0.01 hour has displaced the neoprene curve downward but has not affected the slope which is the important parameter. Figure 5 shows the effect of ambient temperature on specimens of Buna S. Figure 6 shows the effect of humidity variation at constant temperature; 100% humidity was maintained after the sample had been soaked in water. Figure 7 shows the curves of other materials and suggests that the test is useful for a wide variety of plastics.

All these measurements were made with an initial stress of approximately 300 pounds per square inch. Additional measurements with initial stress up to 600 pounds per square inch in-

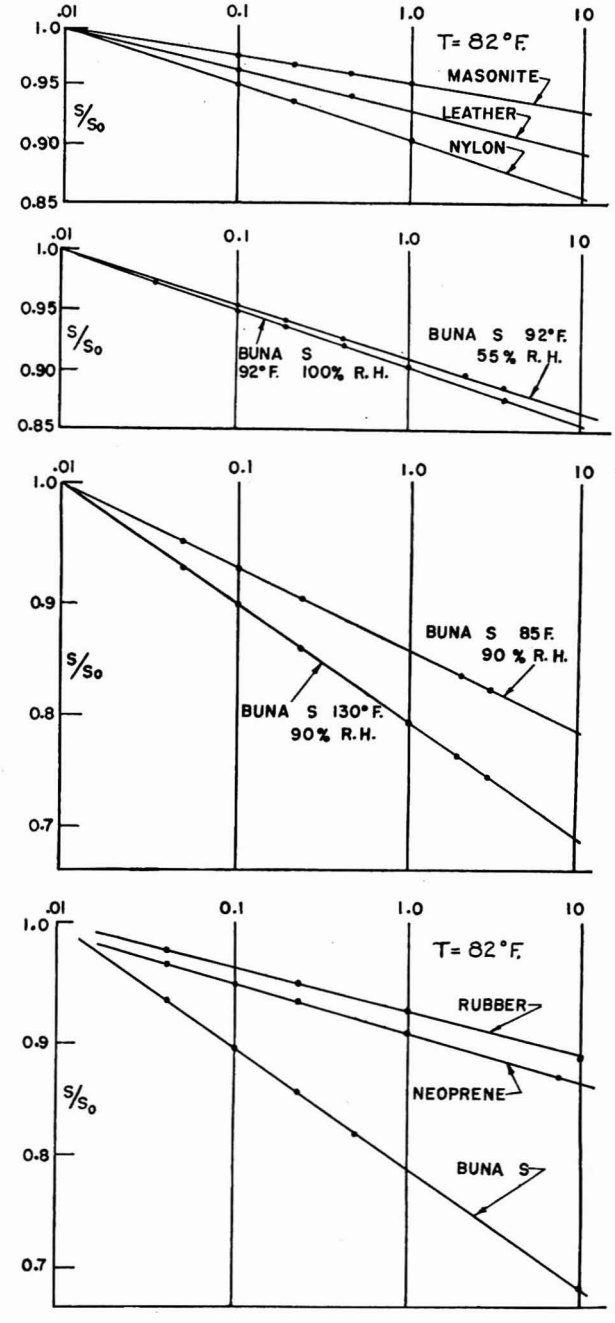

Figure 4 (lower). Comparison of Natural Rubber, Neoprene, and Buna S. Figure 5 (second from bottom). Effect of Temperature. Figure 6 (second from top). Effect of Humidity. Figure 7 (upper). Masonite, Leather, and Nylon

Table I. Material Stock Numbers and Code Letters for Samples Illustrated in Figures 4 through 8

Figure	Material	Code	Du Pont Stock No.
4	Rubber	UE	846-14
4	Neoprene	UH	846-N-1592
4	Buna S	UG	846B-27
5	Buna S	CB	1333B-2A
6	Buna S	CB	1333B-2A
7	Buna S	UG	846B-27
8	Rubber	UE	846-14

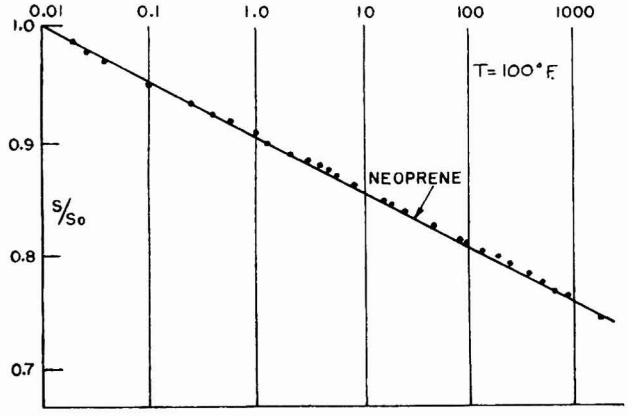

Figure 8. Extended Period Test

dicated variations in the slopes smaller than inherent variations among individual sample pellets of any one material.

Although cylindrical samples were used, the shape factor is not important as long as comparisons are made between specimens of identical geometry.

The nature of the stress-time decay curves as illustrated in Figures 4 to 8 (see Table I) is apparently that of a logarithmic relaxation in stress with respect to time. Figure 8 shows an extended period test with measurements up to 145 hours, in which

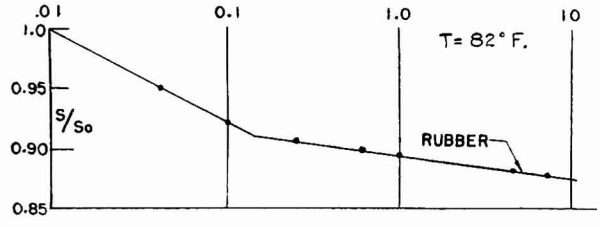

Figure 9. Effect of Slippage

the logarithmic stress relaxation is very closely maintained. On the basis of these data the pressure exerted on relatively stiff clamping members by a plastic sample at times greater than 0.01 hour may be calculated from the equation:

$$(S/S_0) = 1 - K(\log t/t_0)$$

where S = clamping pressure at time t
S_0 = clamping pressure at time t_0
t_0 = 0.01 hour
K = slope of time decay curves

The case for which t_0 is less than 0.01 hour is excluded, because the instrument does not lend itself to analysis over such short periods.

At the start of each test a slight slippage of the material along the surface of the clamping plates is apparent. This effect lasts for varying lengths of time, depending on the material under test. Because it is also logarithmic in character, it appears in the final plot of the test results as an increase in the slope of the curve. The slippage, when it is noticeable, seldom lasts for more than 0.1 hour and is easily separated from the stress relaxation of the specimen, by the appearance of a discontinuity in the slope of the curve. This may be seen clearly in Figure 9. The clamping plates are roughened to reduce the slippage effect and avoid the necessity of cementing or vulcanizing the specimen to them.

To give further experimental support to the method of extrapolating the data, several long-term tests were conducted on Buna S and neoprene covering periods in excess of 60 days. These showed satisfactory linearity when plotted on semilog paper, and, therefore, agreed closely with extrapolation from the early data of the respective tests In connection with these and all other tests it was also found that the linearity of the data improved with the care taken in performing the experiment. Such items as stabilizing the oven and sample temperatures before test have relatively large influence upon the appearance of the resulting data.

Specimens have also been tested in tension and shear by simple adaptation of the clamping device. The resulting curves have been indistinguishable from the ones taken in compression.

APPLICATIONS

A stress relaxation measurement, making possible the extrapolation to a period of months of a curve requiring but a short test to establish, lends itself admirably to industrial problems involving gaskets, shock mounts, retaining bands, supports, etc. In an effectively rigid system the high polymer member may be adjusted to ensure proper pressures or positions in the future. As the shape of the sample is not a limiting factor, jigs can be adapted to an unlimited number of articles to be tested. Thus, the guesswork of choosing a material best suited to a given set of conditions is reduced once a set of these tests has been performed.

Curves may be run on materials that are in process of curing, aging, and dehydrating. Extrapolations of purely theoretical interest may also be carried out on samples whose properties are not stable over a long period. Successive curves taken on a series of supposedly identical samples will indicate the effect of a process, whether it be natural or the result of a predetermined set of imposed conditions. Controlled atmospheres containing moisture, ozone, or solvents may be introduced.

The instrument can be used for quality control. Samples from production lines may be tested to be sure that the effects of plasticizer, accelerator, or solvent remain constant. Variations in

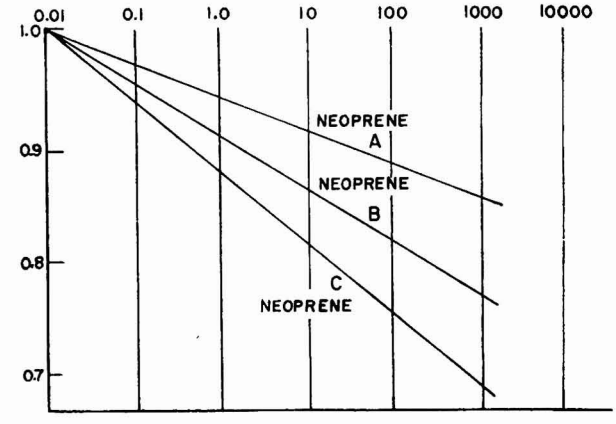

Figure 10. Example of Stress Relaxation of Gasket

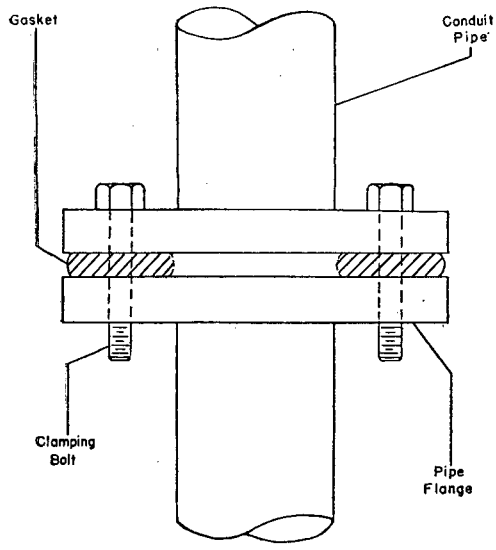

Figure 11. System for Testing Gasket

performance beyond those permissible for the process would be quickly recognized and call for corrective action.

EXAMPLE

A problem of the stress relaxation of a gasket will serve as an example of the use of information obtained in the manner described above. Assume that a neoprene gasket must be compressed to 500 pounds per square inch in order to prevent leakage in a pipe joint. Three arbitrary formulations are available: A, B, and C (Figure 10). The mechanics of the system (Figure 11) limit the maximum gasket stress to 700 pounds per square inch. Which composition may be used to prevent leakage over a period of a thousand hours?

If all three gaskets are originally tightened to 700 pounds per square inch, gaskets A, B, and C will in 10 hours relax to 640, 610, and 570 pounds per square inch, respectively. At the end of 100 hours they will have relaxed to 625, 580, and 525 pounds per square inch, respectively. Gasket C will fail at 400 hours, but at 1000 hours gaskets A and B will still be exerting safe loads of 600 and 545 pounds per square inch.

CONCLUSIONS

Up to now little use has been made of the strain gage as a means of measuring stress, although the idea is not original and has been used recently in textile and building materials laboratories. Chain balances (*5*), sliding balance weights actuated by wires (*6*), and servo-mechanisms (*2*) have been incorporated in most constant strain tests which have necessitated cumbersome equipment.

The instrument used in the experiments described above is a compact unit, measuring 2 feet 8 inches × 1 foot 4.5 inches × 1 foot, containing a minimum of moving parts, essentially free from draft and vibration effects, and with the advantage that it measures the relaxation characteristics of a substance under constant strain (constant sample deflection).

As identical curves have resulted from samples in tension and from those under compression, the compression test is generally used because of its relative simplicity.

The relaxation characteristics of a number of materials may be specified by one constant when tested under given temperature and humidity conditions and for a specified sample shape. Because all characteristic curves are straight lines on semilog paper, the suggested ideal parameter is the slope K of the characteristic curve as indicated by the equation

$$(S/S_0) = 1 - K \log (t/t_0)$$

The unit requires a 110-volt alternating current supply but is voltage-stabilized to withstand normal industrial line voltage variations.

ACKNOWLEDGMENT

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A commercial model of the apparatus described has recently been made available by the Baird Associates, Inc., Cambridge, Mass., under the commercial name Hi-Po-Log.

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Determination of Free Carbon in Compounded Rubber and Synthetic Elastomers

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THERE appears to be no record in the chemical literature of a general method for the estimation of carbon black in both natural rubber and all types of synthetic elastomer compounds.

The A.S.T.M. method for the chemical analysis of synthetic elastomers (*1*) states that the carbon black in elastomers containing polyisobutylene cannot be determined by the method described, but it may be determined in the decantate from the polyisobutylene estimation, provided the polyisobutylene content is not over 25%. This method, thus, could not be used for straight Butyl rubber compounds.

McCready and Thompson (*6*) have modified the A.S.T.M. method for rubber compounds to include the estimation of free carbon in Butyl rubber reclaim. The essential change is the substitution of a hot digestion in mineral-seal oil in place of the hot nitric acid digestion of the sample. These authors, however, recommend further checking of this method before applying it to

In a procedure for the quantitative estimation of free carbon in compounded rubber and synthetic elastomers the usual nitric acid decomposition of the polymer is preceded by a softening period in boiling tetrachloroethane. The use of tetrachloroethane, under the conditions described, enables subsequent rapid decomposition of the sample and separation of the carbon black even in cured Butyl rubber, an elastomer that is very difficult to decompose. The mixture resulting from the decomposition is extracted with diethyl ether, and the carbon black remains in the raffinate. This residue can be filtered rapidly by dispersing it with acetone and pouring the dispersion into a Gooch crucible containing sufficient ether to cause the free carbon to coagulate and settle quickly. The usual acid and alkali washes appear to be unnecessary. The carbon black is washed thoroughly with ether, dried at 250° to 300° C., and weighed. The free carbon content is calculated from loss in weight upon ignition at 900° C. The method has been successfully applied to cured compounds of natural and synthetic rubbers.

vulcanized Butyl rubber stock, inasmuch as trial determinations of the black in two samples of cured Butyl rubber tube stock were stated as being only "fairly accurate."

Galloway and Wake have reported a method for the estimation of both the polymer and the free carbon in a sample of vulcanized Butyl rubber (*4*), in which, they claim, the elastomer is degraded to polyisobutene by nitric acid. The polyisobutene is then quantitatively extracted with light petroleum (boiling range 40° to 60° C.). The free carbon and fillers remain as a residue, and the carbon is determined from the loss in weight on ignition of this previously dried and weighed residue. The time required for removal of the polymer makes this method undesirable as a procedure when only carbon black is wanted, as is very often the case.

The use of cresol for decomposing rubber samples, previous to free carbon estimation, has been recommended instead of the nitric acid decomposition (*8*). This method requires a long digestion period, a large volume of diluent, and a long settling time. From the standpoint of rapidity, these are undesirable features. No data concerning the application of this method to synthetic elastomers were presented.

Scott and Willott (*9*) state that neoprene compounds are not dissolved by nitric acid alone. They used nitrobenzene to swell the sample, followed by heating with 1 to 3 nitric acid to disintegrate the polymer. They stated that the method could be applied to the determination of free carbon in Buna S, but was not applicable to Perbunan or to soft rubber compounds.

Earlier papers deal chiefly with the nitric acid method (*10*) and its modification (*7*), which do not work with Butyl rubber (*1*) or with neoprene (*8*).

The distillation method for determining carbon black has been applied to vulcanized rubber mixtures by Dekker (*3*), and more recently has been revised for GR-S (*11*). No data could be obtained, however, as to the general applicability of this method to all types of elastomers.

The method proposed in this paper has been successfully used, not only for natural rubber compounds, but also for all types of synthetic elastomers encountered in present day compounding. The use of 1,1,2,2-tetrachloroethane and nitric acid under the conditions described will decompose all the types of cured rubber and elastomer compounds tested. The often encountered difficulty in filtering out the carbon black has been overcome in this method by the use of ether. Ether had been used previously by Ging (*5*) to coagulate the black and prevent it from running through the filtering crucible.

APPARATUS AND REAGENTS

Standard stock apparatus and reagents were employed in this procedure. The electric hot plate used for heating was modified, by inclusion of a variable transformer, to maintain a surface temperature of 170° to 180° C. The temperature was checked by means of a mercury thermometer enclosed in a brass block.

Redistilled technical grade 1,1,2,2-tetrachloroethane (acetylene tetrachloride) was used for all determinations. The diethyl ether, concentrated nitric acid, and acetone used were reagent grade.

Gooch crucibles were employed in filtration. The mats for the crucibles were prepared of two tightly packed layers of reagent grade asbestos, each layer approximately 0.16 cm. ($^1/_{16}$ inch) thick. For the bottom layer an aqueous suspension of medium fiber asbestos was used. A second aqueous suspension of finely divided asbestos was prepared for forming the top layer. This fine division of the asbestos was accomplished by using a high speed mixer to agitate the suspension. The prepared Gooches were then dried and finally ignited for several hours in a muffle furnace at 900° C.

PROCEDURE

The recommended standard methods of preparing rubber samples for chemical analysis should be used (*2*). After the milling operation the sample should be sheeted as thinly as possible.

Set the electric hot plate under a hood, and adjust it so that its surface temperature remains between 170° and 180° C. Measure out a slight excess of the required quantity of 1.42 specific gravity nitric acid (15 ml. for each sample) and heat so that the acid will have reached its boiling point and will be simmering by the time it is needed. (Because the vapors of the halogenated hydrocarbons are toxic, the heating with tetrachloroethane should be conducted in a well ventilated place. As the solvent may also cause dermatitis if left in contact with the skin, the hands should be thoroughly washed with soap and water after handling the tetrachloroethane. Any spilled on the skin should be rinsed off with alcohol and then the skin thoroughly soaped and rinsed.)

Accurately weigh 0.1000 to 0.1500 gram of sample and place in a 150-ml. beaker. Add 10 ml. of tetrachloroethane, insert a glass stirring rod, and cover the beaker with a 125-ml. Erlenmeyer flask filled with cold water. (A glass rod is inserted in the beaker at this point as an aid to prevent bumping; it is retained in the beaker and later can be used for a stirring rod. The Erlenmeyer flask filled with cold water serves as a condenser to prevent excessive loss of tetrachloroethane vapor, and after the addition of nitric acid the stream of condensing vapors running down the sides of the beaker will aid in keeping the dispersed black from creeping up toward the top of the beaker.)

Set the covered beaker on the hot plate and heat until the sample is well softened. Measure out 15 ml. of the boiling nitric acid and add it to the contents of the beaker, moving the Erlenmeyer flask just enough to allow addition of the hot acid. Keep the mixture simmering until the rubber or elastomer is completely decomposed and the black residue is thoroughly dispersed. (Most compounds of rubber and some of the elastomers will soften after 20 to 30 minutes' heating in the boiling tetrachloroethane. Butyl stock and other heavily reinforced stocks usually require heating for 30 to 40 minutes in the solvent to become sufficiently softened. A sample that is properly softened will decompose quickly after the addition of the boiling nitric acid. A 20- to 40-minute simmering period following the addition of acid is usually sufficient to ensure the complete decomposition of the rubber or elastomer and to allow dispersion of the carbon black.)

At the end of the digestion period, remove the beaker from the hot plate, keeping it covered with the Erlenmeyer flask, and place it in a shallow pan containing sufficient ice to cool the contents of the beaker until its temperature drops slightly below 20° C.

Remove beaker from bath and uncover. To the beaker add approximately 25 ml. of diethyl ether, stirring quickly and thoroughly. Allow the beaker to stand only until two layers form; then immediately decant the top layer through the prepared Gooch crucible under suction. Retain the lower layer, which contains the carbon black, in the beaker. Repeat, adding 25-ml. portions of ether; stirring, settling, and decanting as before until

the top layer becomes colorless. (The dilution and decantation with ether should be carried out quickly to prevent possible reaction between the nitric acid that remains in the beaker and the ether. Keeping the beaker cold will also aid in preventing a reaction. Usually only three 25-ml. portions of ether are required, and the third portion of ether is clear of color.)

Immediately following the final decantation with ether, wash down the sides of the beaker with about 5 ml. of acetone, delivered from an acetone wash bottle, and stir thoroughly to dilute and disperse the black residue.

Wash the crucible with a small quantity of ether and reduce the suction to the point where ether will just slowly drain through. Fill the Gooch about three-quarters full with ether, then slowly pour the remaining contents of the beaker into the crucible, keeping the suction reduced until the black has coagulated and settled down. (To prevent the black from redispersing and running through the crucible, a ratio of at least 2 parts of ether to each part of acetone added should be maintained within the Gooch. If the level of the liquid in the crucible falls below the three-quarters full mark before all of the acetone dispersion can be transferred, the transfer should be stopped and the proper level restored by the addition of ether. A reservoir of ether, connected to a capillary tube that just dips into the Gooch, may be used to permit a continuous flow of ether into the crucible during transfer of the acetone dispersion. However, proper transfer technique will make this latter procedure unnecessary.)

Gradually increase the suction, draw the liquid through the Gooch, and wash the residue once with ether. Scrub out the beaker with a 5-ml. portion of acetone. Reduce suction and add ether to the crucible as before, then pour the acetone wash into the Gooch. Wash the residue in the crucible thoroughly with ether and suck dry. If necessary, wash off outside of Gooch with acetone. Dry Gooch in oven at 250° to 300° C. for 30 minutes, cool in desiccator, and weigh. Place crucible in muffle furnace at 900° C. to burn off the carbon black and again cool and weigh. Calculate loss in weight to per cent free carbon.

$$\% \text{ free carbon} = \frac{(A - B) \times 100}{\text{weight of sample}}$$

A = weight of crucible and carbon
B = weight of crucible after ignition

EXPERIMENTAL DATA

Smith and Epstein (*10*) found that carbon black heated with nitric acid gained in weight. They obtained recoveries of 100 to 108% on the black alone, dependent upon the time of heating. To compensate for this increase in weight, they divided the per cent carbon black found by the factor 1.05. The A.S.T.M. method (*1*) also uses this correction factor. Later work has shown that the use of the 1.05 correction factor could be eliminated simply by drying the free carbon residue at 200° C. before igniting it (*11*). When samples of furnace and channel black were treated by the procedure described in this paper, average percentages of black recovered varied from 98.2 to 99.2. However, when these blacks were treated in the presence of approximately 70% rubber or other elastomer, the average amount of black recovered was nearly 100%. It is thus evident that no correction factor is necessary in this method. The effect of the reagents upon the recovery of carbon black is shown in Table I. Results obtained on certified stocks are shown in Table II. The accuracy of these results is further evidence that a correction factor is unnecessary.

The stocks designated as certified, which were used in the trial determinations of free carbon, were carefully compounded under conditions designed to prevent any loss of ingredients. A checker was assigned to verify all weighings and to follow the stocks through the milling operations.

The average deviation of results on certified stocks, even in the hands of different analysts not acquainted with the method, was never larger than 0.30%. The deviation of the average from the calculated black content was never more than 0.5%. The certified stocks were compounded with percentages of black varying from 15 to 30. All stocks were cured before sampling.

Table I. Effect of Treatment on Recovery of Black

Black	Elastomer Present	No. of Detns.	Av. % Black Recovered	Average Deviation, %
		Carbon Black Alone		
Furnace 1	...	4	99.2	±0.6
Furnace 2	...	4	98.3	±0.8
Channel 1	...	4	98.2	±0.3
Channel 2	...	4	98.5	±0.7
		Carbon Black Recovered in Presence of Approximately 70% Elastomer		
Furnace 1	Natural rubber	3	100.3	±0.9
Furnace 2	Natural rubber	3	100.4	±0.2
Channel 1	Natural rubber	3	100.2	±0.9
Channel 2	Natural rubber	3	99.6	±0.5
Channel 2	GR-S	3	99.8	±0.4
Channel 2	Butyl	3	99.9	±0.6
Av. % black recovered in presence of elastomers			100.03	

Table II. Determination of Free Carbon in Cured Stocks

Stock	Analyst	No. of Detns.	% Free Carbon Found (Av.)	Av. Deviation, %	Theoretical % Free Carbon
		A. Certified Stocks			
Butyl	A	12	30.95	±0.20	30.6
	B	2	30.45	0.25	30.6
	C	1	30.60	...	30.6
Natural rubber 1	A	2	27.90	0.10	28.0
	C	2	27.50	0.10	28.0
Natural rubber 2	A	2	31.80	0.20	32.0
	B	2	32.00	0.30	32.0
GR-S 3	A	2	30.65	0.25	31.0
GR-S 4	A	2	27.90	0.10	28.0
GR-S 5	A	6	27.90	0.30	28.25
Neoprene GN 1	A	6	23.70	0.15	23.55
Neoprene GN 2	A	6	23.70	0.25	23.55
Butyl	A	6	29.10	0.15	28.85
Butyl[a]	A	4	27.70	0.25	27.9
		B. Uncertified Stocks			
Natural rubber 1	A	6	26.0	±0.20	25.9
Natural rubber 2	A	6	25.8	0.15	26.0
GR-S 1	A	6	28.2	0.25	28.3
GR-S 2	A	6	14.8	0.25	14.9
Butaprene N	A	6	29.6	0.20	29.8

[a] Highly loaded stock.

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Determination of Carotene in Plant Materials

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A rapid method for the routine determination of carotene in plant materials has been worked out by which the green tissues are triturated with a mixture of lime and anhydrous sodium sulfate. This results in their disintegration as well as adsorption of chlorophyll and other noncarotenoid pigments; the carotene itself is rendered readily extractable by petroleum ether or benzene and can be determined either as gross carotene or as its isomerides after chromatographic resolution. The method is particularly suitable for the analysis of green, leafy materials, feeds, and fodders and gives excellent agreement with standard procedures, but it can be applied with equal success to fresh fruits and vegetables.

IN THE determination of carotene in plant tissues, the two general methods of removal of noncarotenoids are phasic and chromatographic separations (*4, 30, 34*). In view of the recognized variations in the extent of digestion and absorption of carotene and its interdependence on the presence of varying amounts of antioxidants (*1, 7, 14, 15, 16, 39, 40*), complete chromatographic analysis, involving the separation of various carotene isomers, is neither practical nor necessary for routine analytical purposes, particularly in the nutritional evaluation of foods, feeds, and fodders (*29*). The phase separation method, on the other hand, is not always quantitative (*11, 23, 24, 25*); certain biologically inert degradation products are also taken up in the epiphasic layer (*36, 50*). In addition, a small percentage of the xanthophylls remains in the petroleum phase and appreciable amounts of carotene are taken up in the hypophasic methanol layer (*21, 46*). The saponification step with hot alcoholic alkali which precedes phase separation (*35*) is inconvenient and causes inaccuracies due to isomerization and, occasionally, loss of carotene (*5, 12, 19, 51*).

The possibility of a more direct method for obtaining carotene was indicated in the observations of Fremy (*13*) that barium hydroxide could be used to remove chlorophyll from alcoholic extracts of green plants. Tswett (*45*) had shown that, if a mixed solution of chlorophyll and carotene is shaken with an excess of finely divided calcium carbonate, the chlorophyll is completely adsorbed, leaving the carotene in solution. Kernohan (*18*) and Cooley *et al.* (*6*) suggested the use of soda ash for the selective adsorption of noncarotenoids, while Petering *et al.* (*26, 32, 33*) used baryta for removal of chlorophyll and saponifiable lipides from acetone extracts of alfalfa meal. More recently, technical hydrated lime has been employed for the adsorption of noncarotenoid pigments from petroleum ether extracts of vegetable leaf wastes (*47*) and directly from fresh plant tissues (*28*).

Another adsorbent which is found efficient in preferentially adsorbing chlorophyll and other noncarotenoid plant pigments is dicalcium phosphate, originally recommended by Moore (*23, 24, 25*) and since extensively used. A column of fat-free bone meal has been suggested as equally effective (*20*).

In this communication is described the development of a method for the determination of carotene in which saponification and phasic separation are eliminated. A combination of freshly ignited lime and anhydrous sodium sulfate in one operation dehydrates green tissues and strongly adsorbs chlorophyll and noncarotene pigments, after which carotene can be quantitatively extracted with petroleum ether for estimation as such or, where required, after chromatography.

MATERIALS AND METHODS

All preliminary trials were carried out with drumstick (*Moringa oleifera*) or lucerne (*Medicago sativa*) leaves, two rich natural sources of carotene (*38, 43, 44*). The method, as finally worked out, has been tested on a large variety of fresh materials, listed in Table I. Carotene, referred to in this paper, includes both α- and β-isomerides although, in most cases, the α-isomer formed only a very small, if variable, fraction of the total carotene. Carotene determinations, for comparison, were made by an extraction procedure using a Waring Blendor and a solvent mixture of alcohol and petroleum ether similar to that of Moore and Ely (*25*), followed by adsorption and elution from a magnesia column according to the method of Wall and Kelley (*46*). This method, like the proposed method, does not involve saponification or phasic separation. Color measurements were made using a Klett-Summerson photoelectric colorimeter with a No. 42 filter in place. A calibration curve with a commercial source of carotene (90% β- and 10% α-, kindly supplied by Barnett Laboratories, 6256 Cherry Ave., Long Beach 5, Calif.), purified according to Fraps and Kemmerer (*11*), and standardized at 450 mμ with a Billingham and Stanley visual spectrophotometer, was used to read off carotene contents.

PRELIMINARY OBSERVATIONS

Most plant materials, when fresh, contain about 80% of moisture; hence, any method of direct extraction of carotene should be preceded by drying, for which purpose preliminary blanching, and use of alcohol, acetone, and water-binding agents like anhydrous sodium sulfate, sodium carbonate, and calcium sulfate have been recommended (*3, 8, 17, 26, 41, 42, 48*). As freshly ignited lime is a good desiccant as well as adsorbent for chlorophyll and xanthophylls, a number of experiments were carried out in which known amounts of leaf were macerated in a mortar and further triturated with varying proportions of freshly prepared lime, chemically pure and ignited for a few minutes at a temperature of 250° to 300° C. before use. When drumstick or lucerne leaves were used, it was found that a proportion of leaf to lime of 1 to 3 gave a loose, fluffy mass, apparently dry and readily extractable with petroleum ether or benzene. However, contrary to the observations of Murri (*28*), the percentage recovery of carotene from this meal was very low; partial heating for a few minutes inside a steam oven or, for longer periods, under vacuum did not result in much improvement. But practically all the carotene could be quantitatively recovered after refluxing with 10% aqueous sodium carbonate, extraction with acetone, and phasic separation between petroleum ether and methanol.

It would therefore follow that treatment with lime in the cold did not result in destruction of carotene but that, in the proportion used for efficient absorption of all the moisture in the leaf tissue, the plant pigments, inclusive of carotene, were adsorbed by the lime. Further trials with reduced quantities of lime together with varying amounts of anhydrous sodium sulfate showed that about equal weights of leaf, lime, and sodium sulfate gave, on grinding, a fluffy meal in which all the chlorophyll and practically all the xanthophylls were strongly adsorbed and where the carotene was so weakly adsorbed that it could be quantitatively eluted with petroleum ether. It was found best first to grind the leaf to a paste in a mortar and then add the lime–sodium sulfate

Table I. Carotene Values

Sample	Moisture, %	Proposed Method	Wall and Kelley Method
		γ/g. fresh basis	
Agathi leaf (*Sesbania grandiflora*)	74.4	143	146
Amaranth (*Amaranthus gangeticus*)	75.2	110	106
Pan leaf (*Piper beetle*)	81.6	130	132
Coriander leaf (*Coriandrum sativum*)	75.9	300	300
Drumstick leaf (*Moringa oleifera*)	71.0	320	330
Dhub grass (*Cynodan dactylon*)	80.0	111	108
Lucerne (*Medicago sativa*)	71.4	296	304
Curry leaf (*Murraya koenigii*)	68.3	250	264
Neem leaf (*Azedirachta indica*)	71.7	143	139
Carrot tops (*Daucus carota*)	76.5	174	170
Turnip tops (*Brassica rapa*)	74.9	136	142
Carrot root (*Daucus carota*)	79.0	162	158
Bengal gram, tender (*Cicer arietinum*)	60.3	81	84
Tomato, green (*Lycopersicum esculentum*)	84.0	9	9
Chilies, green (*Capsicum annum*)	84.6	7	7
Bitter gourd (*Momordica charantia*)	90.2	50	53
French bean (*Phaseolus vulgaris*)	84.3	105	110
Plantain, ripe (*Musa paradisica*)	73.0	7	8

mixture, when further grinding gave a loose, powdery material ready for extraction. As, however, during the initial grinding, small quantities of carotene, varying at times up to 2% of that originally present, were destroyed, evidently through enzymic degradation (*22*), incorporation of about 5 mg. % of sodium cyanide during this stage was found effective in inhibiting this enzymic destruction (*2, 31, 41*).

Among the solvents tried for extraction of carotene, petroleum ether and benzene were found to be best; acetone and chloroform eluted some of the adsorbed chlorophyll and were therefore unsuitable. It was convenient and did not affect the accuracy of the results obtained (Table II) if the leaf meal, after trituration with lime and sodium sulfate, was given a short period of drying for about 15 to 20 minutes in the steam oven before extraction of the carotene.

Occasionally, with some of the plant materials examined, small quantities of xanthophylls were extracted along with the carotene by the petroleum ether solvent. In only one instance—viz., bitter gourd (Table I)—a small amount of chlorophyll remained unadsorbed by the lime and could not be removed even by increasing the proportion of lime. Evidently, this chlorophyll and the xanthophylls from the other sources are not completely adsorbed by lime. They could, however, be readily freed by passing the extracts through a column of dicalcium phosphate as recommended by Moore (*23*). Removal of such traces of chlorophyll or xanthophyll, where present, could be effected equally well when the adsorbent was added along with the lime to the ground meal or when, as was preferred, the petroleum ether extract was just shaken with a small quantity of it.

PROCEDURE

A weighed amount of the material (2 to 5 grams, corresponding to approximately 100 to 500 micrograms of carotene) is transferred to a glass or porcelain mortar and ground intimately with 1 to 2 grams of quartz sand and 1 or 2 drops of a 2% solution of sodium cyanide. A quantity of anhydrous sodium sulfate, equal to the weight of the material taken, is now added and the mixture triturated again. This is followed by the addition of an equal amount of freshly ignited lime and further maceration when a loose, fluffy meal with a light greenish yellow tint results. This is transferred to a beaker and dried in the steam oven for 15 to 20 minutes.

The mixture is next extracted in the cold or, preferably, over a water bath maintained at 50° to −60° C. with successive small quantities of low-boiling petroleum ether till no more carotene is extracted. Three or four extractions are usually sufficient. The extract, after decantation each time, is passed through a filter paper. For routine purposes, some type of continuous extractor may be used, if preferred. The combined petroleum ether extract is shaken with a small quantity (0.5 to 1 gram) of dicalcium phosphate to remove any chlorophyll and xanthophylls that may be present in solution. The extract, if made turbid by suspended materials, is filtered and made up to volume, when it is ready for estimation of carotene. This may be done either tintometrically (*9, 10*) or spectrophotometrically (*27*), chromatographic resolution of the isomerides being effected, if required, by any suitable procedure.

RESULTS AND DISCUSSION

In Table I, the values for carotene, yielded by the above procedure and using a few natural sources are compared with those obtained according to the method of Wall and Kelley (*46*).

Data on carotene recovery, by the proposed as well as comparison methods, are given in Table II. For these experiments, 1-gram lots of fresh tissues, either as such or after preliminary blanching by immersion in boiling water for 5 to 10 minutes as described by Zscheile and Whitmore (*52*), were treated with aliquots of a standard carotene solution in petroleum ether and the solvent was evaporated off by a very short period of drying in a steam oven before trituration or blending. As observed by Wall and Kelley (*47*), carotene is not affected by evaporation on a steam bath under these conditions.

The results for carotene obtained by the proposed method compare favorably with those given by the standard procedure (Table I). Quantitative recovery of carotene is also obtained with carotene added to plant samples (Table II); in the latter case, the slightly better recovery resulting from blanched tissues, particularly with drumstick leaf and lucerne, is evidently due to inactivation of carotene-destroying systems in these sources (cf. *22*). The differences, however, are small (*52*).

Table II. Recovery of Carotene Added to Plant Samples

Sample	Carotene in Sample	Carotene Added	Proposed Method		Wall and Kelley Method	
			Carotene found	Recovery	Carotene found	Recovery
	γ	γ	γ	%	γ	%
Fresh						
Drumstick leaf	304	96	386	96.5	388	97.6
Drumstick leaf	304	48	345	98.0	345	98.0
Lucerne	316	48	358	97.7	362	99.5
Amaranth	118	96	214	100.0	210	98.1
Blanched						
Drumstick leaf	297	96	402	102.3	396	100.8
Lucerne	308	48	350	98.4	350	98.4
Dhub grass	89	96	182	98.4	185	100.0
Turnip tops	124	96	209	95.0	212	96.4

The method outlined here is especially suited for the determination of carotene in green and leafy materials; it may also be used with success for fresh fruits and vegetables except those like ripe tomatoes, chilies, and papaya which may contain noncarotene chromogens (*37*), not likely to be removed under the conditions of these experiments. Such instances are, however, rare, as most fresh vegetable tissues have practically all the carotene in the normal all-*trans* configuration.

The method may not lend itself for use with dehydrated products, as there is evidence to suggest that, with petroleum ether alone, long periods of extraction are necessary (*49*) and that quantitative extraction of carotene is usually best accomplished with a 30% solution of acetone in petroleum ether (*42, 49, 52*); besides, dehydrated materials may have considerable quantities of carotene isomers with much less biological activity than β-carotene.

Because leafy materials contain mostly β-carotene (*27*), estimation as gross carotene is sufficient for most practical purposes. The determination of carotene as individual isomerides is not always necessary in the nutritional evaluation of feeds and fodders, in view of the varying degrees of their assimilation. The steps for chromatography and spectrophotometry may be omitted in these cases. By employing colorimetric or tintometric procedure for estimation, the whole operation, could be completed in less than 1.5 hours and, in a 6-hour period, as many as twenty-five to thirty-

samples could be dealt with. A food blender, cutter, or chopper and the refluxing procedure with solvents are unnecessary.

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Colorimetric Determination of Copper with Carbon Disulfide and Diethanolamine

An Improved Dithiocarbamate Reagent

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A reagent prepared from carbon disulfide and diethanolamine reacts with the cupric ion to form a brownish-yellow salt of bis(2-hydroxyethyl)dithiocarbamic acid. The reaction has been made the basis of a colorimetric method for the determination of copper. As the colored compound is water-soluble, the use of a stabilizing colloid or extraction with an organic solvent is unnecessary. Of the metals whose compounds are soluble under the conditions used, bismuth, chromium, cobalt, iron, mercury, nickel, silver, and uranium interfere seriously. Procedures are described for eliminating the interference of appreciable amounts of bismuth, chromium, ferric iron, and uranium. Among the anions studied, only cyanide, dichromate, nitrite, and sulfite interfered appreciably.

ALTHOUGH sodium diethyldithiocarbamate has been used extensively for the colorimetric determination of copper, the reagent possesses the disadvantage that it forms an insoluble copper salt and requires the use of gum arabic for stabilization of the colloidal suspension (*6*) or extraction with a suitable organic solvent (*5*) before colorimetric comparison can be made.

Geiger and Müller (*4*) have shown that the bis(2-hydroxyethyl)-dithiocarbamate of diethanolamine reacts with the cupric ion to form a brownish-yellow salt which is soluble in water. They prepared the reagent by dissolving carbon disulfide and diethanolamine in alcohol. The present paper describes the application of this reagent in slightly modified form to the colorimetric determination of copper. It is suggested that the trivial name, cuprethol, be applied to the new chromogenic agent.

REAGENTS

Hydrochloric acid, 1.2 *N* and 8 *N*.

Nitric acid, 1.2 *N* and 8 *N*.

Sodium Pyrophosphate Solution. Dissolve 30 grams of sodium pyrophosphate decahydrate, ($Na_4P_2O_7 \cdot 10H_2O$) in water and dilute to 1 liter.

Sodium Acetate Solution. Dissolve 200 grams of sodium acetate trihydrate in water and dilute to 1 liter.

Acetate Buffer solution, pH 5. Dissolve 96 grams of sodium acetate trihydrate and 17 ml. of glacial acetic acid in water, and dilute to 1 liter.

Ammonium hydroxide, 15 *N*.

Standard Copper Solution. Dissolve 0.1000 gram of pure copper in 5 ml. of 8 *N* nitric acid, add 1 ml. of concentrated sulfuric acid, and fume to dryness. Dissolve the residue in water and dilute to 1 liter; 1 ml. contains 0.100 mg. of copper. Dilute 50 ml. of this solution to 1 liter; 1 ml. of the solution contains 0.005 mg. of copper.

Diethanolammonium Bis(2 - hydroxyethyl)dithiocarbamate (Cuprethol) Reagent. Solution A. Dissolve 4.0 grams of diethanolamine in 200 ml. of methanol. Solution B. Dissolve 1.00 ml. of carbon disulfide in 200 ml. of methanol. Prepare sufficient reagent for one day's use by mixing equal volumes of Solutions A and B.

Standard solutions of various cations and anions containing either 1 or 10 mg. of the ion per ml.

All chemicals were reagent grade. No difference in results was noted when diethanolamine, Eastman Kodak Company No. 1598, was replaced by a commercial grade.

APPARATUS

Klett-Summerson filter photometer, Model 900-3. The 12.5-mm. tubes were employed in conjunction with the No. 42 Klett violet filter.

EXPERIMENTAL

Composition and Stability of Cuprethol Reagent. The amounts of diethanolamine and carbon disulfide used in preparing the cuprethol are such that a 15% excess of the amine is present. One milliliter of the reagent provides over six times the quantity of dithiocarbamate theoretically required to react with 0.2 mg. of copper. Although the cuprethol is stable for at least a week, it is preferable to prepare the reagent as needed by mixing equal volumes of the component solutions. The latter are perfectly stable; and as the mixing requires but a few minutes' time, any ultimate instability of the reagent is of minor importance.

Color Development. Quantities of standard copper solution to give final concentrations up to 4 p.p.m. were diluted to approximately 60 ml. with water, 1 ml. of cuprethol reagent was added, and the solutions were diluted to 100-ml. volume. The curve prepared from the photometer readings showed that Beer's law was valid for concentrations up to 2 p.p.m. of copper, but deviated thereafter, so that with 4 p.p.m. the dial reading was 92% of the value expected if the curve had remained linear. Increasing the amount of cuprethol to 2 ml. gave no improvement in the results. For this reason the upper limit of copper concentration was set at 2 p.p.m.

A curve prepared from photometer readings made with the No. 54 Klett green filter was linear up to 10 p.p.m. of copper when 3 ml. of cuprethol reagent were used. The violet filter, however, permits more accurate determination of very small amounts of copper, and was used exclusively in subsequent work.

A Lumetron filter photometer, Model 402-E, became available after the experimental work described in this paper had been completed. By using the set of narrow-band filters supplied with the instrument, it was found that the colored solution showed minimum transmittancy with the M 440 filter. A plot of copper concentration versus optical density was perfectly linear up to at least 4 p.p.m. of copper when the above filter, together with a rectangular absorption cell of 10-mm. light path, was used. The behavior of higher copper concentrations was not studied.

The effect of pH on the color intensity and stability is shown in Table I.

Each solution contained 0.100 mg. of copper, 2 ml. of 1.2 *N* nitric acid, sufficient sodium acetate solution to give the final pH desired, and 1 ml. of cuprethol reagent, in a total volume of 100 ml. Measurements of pH were made with a Precision-Shell dual titrometer, on which the glass electrodes were calibrated against a standard phthalate buffer. Photometer readings were corrected for traces of copper in the reagents by running appropriate blank determinations.

At pH 2 the color tended to increase gradually with time, whereas at pH 4 the color decreased under the same conditions. The two effects appeared to be balanced at pH 3, resulting in a constant color reading. At pH 5 the color was constant for at least 90 minutes, and was not changed by increasing the pH to 6. Essentially the same results were obtained when hydrochloric acid was used instead of nitric acid.

Effect of Cations. It is often desirable to determine copper without preliminary removal of small amounts of iron which may be present. Cuprethol, like sodium diethyldithiocarbamate, gives a dark brown color with ferric salts. In the presence of sufficient pyrophosphate the brown color may appear on adding cuprethol, but fades out completely in 5 to 10 minutes, and the copper determination can be made thereafter without interference from iron. Although 5 ml. of the pyrophosphate solution were found to eliminate the interference of iron in concentrations up to 100 p.p.m., certain other cations may be precipitated. By using only 1 ml. of pyrophosphate solution, interference of 20 p.p.m. of iron could be prevented, and at the same time appreciable amounts of other metal ions could be tolerated. Large amounts of iron must be removed by careful precipitation with ammonia or by extraction with isopropyl ether (*2*).

The extent of interference for various cations in the presence of 1 p.p.m. of copper was studied by two procedures.

METHOD I. A measured volume of solution containing the ion in question was added to 20 ml. of the copper sulfate solution containing 0.005 mg. of copper per ml., followed by the addition of 2 ml. of 1.2 *N* nitric or hydrochloric acid. The solution was diluted to approximately 50 ml. with water, 1 ml. of sodium pyrophosphate solution was added, and the solution was brought to a pH of approximately 5 with sodium acetate solution. One milliliter of cuprethol reagent was added, the solution was diluted to 100 ml., and the dial readings were obtained at intervals of 10, 30, and 60 minutes after color development. If interference was pronounced, smaller quantities of the ion were used successively until the limiting permissible concentration could be estimated, 2% error being set arbitrarily as a reasonable tolerance.

METHOD II. The solution to be tested contained 0.100 mg. of copper, a measured volume of solution containing the ion in question, and 5 ml. of 8 *N* nitric or hydrochloric acid. After dilution to approximately 50 ml. with water, the solution was

Table I. Effect of pH on Color Intensity and Stability with 1 P.P.M. of Copper

Time after Adding Cuprethol, Min.	Photometer Readings				
	pH 2	pH 3	pH 4	pH 5	pH 6
10	91	93	94	94	94
30	93	93	94	94	95
60	95	94	91	94	94
90	97	93	89	94	94
210	96	93	83	91	94

Table II. Effect of Cations on Determination of Copper by Method I

Ion	Added as	Maximum Permissible Concentration, P.p.m.	Cause of Interference above Permissible Concentration
Aluminum	$AlCl_3$	20	A
Ammonium	NH_4NO_3	1000	..
Antimony	Sb_2O_3	1000	..
Arsenic	As_2O_3	1000	..
Arsenic	Na_2HAsO_4	1000	..
Barium	$BaCl_2$	200	A
Bismuth	$Bi(NO_3)_3$	0	B
Cadmium	$Cd(NO_3)_2$	20	A
Calcium	$Ca(NO_3)_2$	400	A
Chromium	$Cr_2(C_2H_3O_2)_6$	5	C
Cobalt	$CoCl_2$	0	B
Iron	$FeSO_4$	50	B
Iron	$FeCl_3$	20	A, B
Lead	$Pb(NO_3)_2$	10	A
Lithium	LiCl	1000	..
Magnesium	$MgSO_4$	1000	..
Manganese	$MnSO_4$	10	A
Mercury	$Hg_2(NO_3)_2$	1	D
Mercury	$Hg(NO_3)_2$	20	D
Molybdenum	$(NH_4)_6Mo_7O_{24}$	1000	..
Nickel	$NiSO_4$	0	B
Potassium	KCl	1000	..
Silver	$AgNO_3$	0	A
Sodium	NaCl	1000	..
Strontium	$SrCl_2$	1000	..
Tin	$SnCl_2$	10	D
Tin	$SnCl_4$	20	A
Tungsten	Na_2WO_4	1000	..
Uranium (as UO_2)	$UO_2(C_2H_3O_2)_2$	10	B
Zinc	$ZnSO_4$	20	A

A. Turbidity or precipitate formed.
B. Ion gives color with cuprethol.
C. Colored ion.
D. Ion inhibits color development.

Table III. Effect of Cations on Determination of Copper by Method II

Ion	Added as	Maximum Permissible Concentration, P.p.m.	Cause of Interference above Permissible Concentration
Aluminum	$AlCl_3$	100	A
Barium	$BaCl_2$	1000	..
Cadmium	$Cd(NO_3)_2$	1000	..
Calcium	$Ca(NO_3)_2$	1000	..
Iron	$FeCl_3$	0	B
Lead	$Pb(NO_3)_2$	1000	..
Manganese	$MnSO_4$	1000	..
Silver	$AgNO_3$	0	A
Tin	$SnCl_4$	20	A
Zinc	$ZnSO_4$	1000	..

A. Turbidity or precipitate formed. B. Ion gives color with cuprethol.

Table IV. Determination of Copper in Lubricating Oil

Oil	Elements Present	Copper Present, %	Copper Found, %	No. of Detns.
A	Cu, Fe, Pb, S	0.029	0.029	4
B	Ca, Cu, P, Pb, S, Zn	0.052	0.050	4
C	Ba, Ca, Cl, Cu, Fe, P, Pb, S, Zn	0.011	0.011	2
D	Al, Ba, Ca, Cd, Cl, Cu, Fe, K, Mg, Na, P, Pb, S, Si, Sn, Zn	0.005	0.006	2

neutralized with 15 *N* ammonium hydroxide, then made just acid to litmus by the dropwise addition of 1.2 *N* acid. After addition of 10 ml. of acetate buffer solution and 1 ml. of cuprethol, the solution was diluted to 100 ml. and the readings were obtained as in Method I.

Table II summarizes the results obtained with various cations by Method I. In those cases where interference was due to precipitation of the metal pyrophosphate, the effect of the ion in the absence of pyrophosphate was studied by Method II.

The interference of ferric salts and its elimination by pyrophosphate have been discussed. Bismuth, cobalt, ferrous iron, nickel, and uranium also interfered by forming colored salts with the cuprethol reagent. Drabkin (*3*) has pointed out that the coloration given by bismuth in the diethyldithiocarbamate method is not destroyed by cyanide, whereas that of copper is. Following his suggestion it was found that at least 10 p.p.m. of bismuth or 300 p.p.m. of uranyl ion did not interfere in the present method if photometer readings were obtained on the solution before and after addition of a few milligrams of potassium cyanide; copper was thus found by difference.

Stannous, mercurous, and mercuric ions inhibited the color development, the effect being especially marked with the latter ion. The addition of a few milligrams of a mercuric salt to a solution containing copper and cuprethol instantly destroyed the color.

Silver must be absent, as it produced a brown turbidity even in very small amounts.

When cuprethol was added to a copper solution containing the tungstate ion, a greenish hue was at first noticed, but this disappeared in a few minutes and caused no interference in the copper determination. The color may be due to reaction of the cuprethol with a pyrophosphate-tungstate complex, as the green color was not observed in the absence of pyrophosphate.

More than 5 p.p.m. of chromic ion interfered because of its green color; however, the interference of at least 100 p.p.m. of chromium could be avoided by obtaining dial readings on two aliquots of the solution, with and without the addition of cuprethol, or by decolorizing with cyanide or mercuric ion.

Table III shows the results of determinations made by Method II. In the absence of pyrophosphate no interference was shown by barium, cadmium, calcium, lead, manganese, and zinc.

When copper was determined in a slightly ammoniacal solution the results were in agreement with those obtained at pH 5, but low values were obtained in the presence of cadmium, and the interference became greater with increasing concentrations of ammonium hydroxide. Addition of more cuprethol did not improve the results. The effect of other ions under these conditions was not investigated.

Effect of Anions. The possible interference of various anions was studied by Method II. For solutions containing 1 p.p.m. of copper, the following ions may be present in concentrations of at least 1000 p.p.m. without causing an error greater than 2%: acetate, bromide, carbonate, chlorate, chloride, citrate, fluoride, iodide, nitrate, perchlorate, phosphate, pyrophosphate, silicate, sulfate, tartrate, tetraborate, and thiocyanate. Oxalate did not interfere if readings were obtained within 10 minutes after color development, but thereafter the color faded slowly. Thiosulfate was decomposed by the nitric acid; and with more than 200 p.p.m. the turbidity due to sulfur interfered. When the nitric acid was eliminated and the determination made only in the presence of acetate buffer, 1000 p.p.m. of thiosulfate caused no difficulty if the reading was obtained within 10 minutes. The color faded rather rapidly thereafter.

Serious interference was encountered only with cyanide, dichromate, nitrite, and sulfite. No more than 20 p.p.m. of cyanide was permissible. Dichromate oxidized the cuprethol reagent, giving large negative errors in concentrations above 2 p.p.m. Twenty parts per million of nitrite and 100 p.p.m. of sulfite could be tolerated if the readings were obtained within 10 minutes.

DISCUSSION

The proposed method possesses several distinct advantages over the diethyldithiocarbamate procedure. The solubility of the colored copper salt eliminates the need for a stabilizing colloid or extraction with an organic solvent. The chromogenic agent is conveniently prepared by mixing two stable solutions, whereas a solution of sodium diethyldithiocarbamate slowly deteriorates. In the absence of pyrophosphate large amounts of cadmium, lead, manganese, and zinc produce no interference. These ions often cause difficulty in the diethyldithiocarbamate procedure.

RECOMMENDED GENERAL PROCEDURE

Low Iron Content. The solution should contain from 2 to 10 milliequivalents of mineral acid, and no more than 10 mg. of ferric iron and 0.2 mg. of copper. Dilute to 50 ml., add 1 ml. of sodium pyrophosphate solution for each 2 mg. of ferric iron present, and adjust the acidity to a pH of 5 to 6 with sodium acetate solution. If no turbidity develops in 5 minutes add 1 ml. of cuprethol reagent, dilute to 100-ml. volume, and obtain the photometer reading within 1 hour.

High Iron Content. If the solution contains large amounts of iron or other metals precipitated by pyrophosphate, remove the iron by careful precipitation with ammonia. Dilute an aliquot of the ammoniacal filtrate containing no more than 0.2 mg. of copper to approximately 50 ml. and make just acid to litmus with 1.2 *N* nitric or hydrochloric acid. Add 10 ml. of acetate buffer solution and 1 ml. of cuprethol reagent. Dilute to 100-ml. volume and obtain the photometer reading within 1 hour.

DETERMINATION OF COPPER IN LUBRICATING OILS

Oil samples are wet-ashed and lead is removed as sulfate by A.S.T.M. Method D-810 (*1*). An aliquot of the filtrate is used for the copper determination. The small amounts of other metals present will usually cause no difficulty when pyrophosphate is added to prevent interference of iron; however, if a turbidity develops when the solution is adjusted to pH 5 it is best to start with another aliquot and remove iron with ammonia before proceeding with the copper determination.

Some typical results on lubricating oils are shown in Table IV.

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Determination of Aromatic Compounds in Petroleum Products

Chromatographic Method

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A rapid chromatographic method for the determination of aromatic compounds in hydrocarbon mixtures in the gasoline and kerosene range utilizes a combination of silica gel separation and ultraviolet-stimulated fluorescence. The method is reproducible to within ±2% and requires a maximum of 45 minutes per determination. Olefins and other hydrocarbons do not interfere.

A RAPID method was needed for determining the percentage of aromatic compounds in numerous samples taken from various fractions of petroleum.

Present methods for determining aromatic content of petroleum compounds are either time-consuming or inaccurate on account of interfering hydrocarbons. The use of silica gel percolation as recommended by Mair (*2, 5*) requires from 4 to 8 hours per determination. The present A.S.T.M. acid absorption method (*1*) and the Kurtz controlled acid absorption method (*4*) depend upon accurate determinations of olefinic content as well as molecular weight, both of which are subject to numerous potential errors. Specific dispersion methods (*3*) have been found inaccurate on samples boiling over a wide temperature range and on cracked stocks containing olefins.

The method described here is based upon the procedure developed by Mair (*2, 5*) for separating aromatic hydrocarbons from hydrocarbon mixtures. While employing the same limitations for the type of silica gel, ratio of silica gel to sample, and rate of percolation, the author has developed a rapid procedure for estimating the aromatic content, which employs a much smaller sample and a smaller column.

Instead of eluting the sample from the column, the determination was completed through the use of fluorescing chromatograms while the sample remained adsorbed on the silica gel. The fluorescence of the entire adsorption band of the sample on the column was stimulated by the addition of a trace of a hydrocarbon-soluble fluorescing impurity. With ultraviolet radiation, chromatographic bands became visible demarking the area covered by the highly fluorescent aromatic compounds and that covered by the other weekly fluorescing hydrocarbons. Sharp lines of demarcation became visible when the column was carefully packed and the separation was complete. The ratio of the length of the aromatic band to that of the whole sample was found to be equal to the percentage of aromatic compounds in the sample. These ratios remained constant as the sample percolated through a specific portion of the tube.

EQUIPMENT

Ultraviolet illumination. Mercury arc lamp with the filter encased in a black box surrounding the column. A slit is made in the box to permit observation at an angle of 90°.

Glass tubing, 9 mm. in inside diameter and approximately 1 meter long.

Silica gel, 28- to 200-mesh, dried at oven heat of 120° C. for 1 hour and cooled in desiccator (Davison Chemical Corporation, Baltimore, Md., No. 659,528–2000).

Parasheen (Enjay Company, Paraflow Sales Department, Chemical Products, 26 Broadway, New York 4, N. Y.)

Ethyl or isopropyl alcohol.

PROCEDURE

Clean and dry glass tubing.

Dry silicate gel in oven at 120° C. for 1 hour and cool in desiccator. Connect bottle of silica gel covered with glass wool to a vacuum pump and pump for 10 minutes.

Place plug of glass wool in bottom of tubing.

Fill tube to within 15 cm. (6 inches) of top with dry silica gel and pack tube by tapping firmly for 10 minutes while rotating tube. Particles of silica gel should show no downward movement at the end of the tapping period.

Insert cotton plug into top of tube.

To a 2- to 5-ml. sample add 2 drops of Parasheen and place in top of the column.

When the sample has completely entered the column, measure the total length of its adsorption band.

Add 5 mm. of silica gel and tap gently.

Add ethyl or isopropyl alcohol equal to approximately twice the volume of the sample.

When the sample has moved down the column a distance equal to twice the length of its original adsorption band, direct ultraviolet rays on it.

Mark and measure accurately the limits of purple fluorescent layer and the limits of the entire sample.

Repeat measurements at 5-minute intervals as the bands move down the column until their ratios are constant.

Calculation

$$\frac{\text{Length of purple aromatic band}}{\text{Length of entire sample}} \times 100 = \%\ \text{aromatics}$$

DISCUSSION OF METHOD

Considerable care was necessary to obtain uniformity in packing the column to secure straight-line separations between the aromatic and the nonaromatic bands. Whenever a determination was attempted on a poorly packed column, irregular rates of percolation produced wavy cross-section lines of separation, clearly visible under ultraviolet light. As such irregularities rarely became rectified by prolonged percolation, the determination was discarded and another column prepared.

Ordinarily gasoline samples entered the silica gel and could be moved down the column at a satisfactory rate. Samples boiling over a high temperature range, however, usually moved too slowly down the column—i.e., less than 4 ml. per minute. In this case, a medium of lower viscosity and higher rate of percolation was secured by proper dilution. A 50-50 mixture of sample plus *n*-heptane was generally used. The *n*-heptane was purified by percolation through a silica gel column.

When a sample boiling over a high temperature range required such dilution, it was frequently noted that the percentage of aromatics in the dilution mixture was too low to be linearly measured with satisfactory accuracy. In this case the blending procedure was modified to include both *n*-heptane and benzene, also purified in a silica gel column. By this means the optimum conditions of aromatic concentration and rate of percolation were secured. Petroleum samples of the gasoline and kerosene range have been satisfactorily analyzed by correct choice of blending ratios and it is believed that this will work for higher

Table I. Analysis of Known Mixtures of Hydrocarbons for Per Cent Aromatics

	Theoretical	Experimental	
		Mair method	Fluorescence
	%	%	%
Toluene	100	100	100
Toluene and heptane	50	50	50
Toluene, heptane, 1-octene	30	29.5	29.2
Toluene, cyclohexane, 1-octene	30	29.0	28.9

Table II. Analysis of Typical Samples

	Per Cent Aromatics	
	Mair method	Fluorescence
Mississippi crude		
250–300° F.	4	3.8
300–350° F.	7	6.3
300–560° F.	16	15.1
400–430° F.	13	14.0
Cracked gasolines		
I.B.P. to 250° F.	4	4
250° to E. P.	45	48
250° to E. P.	16	16
Hydroforming products, I.B.P. to 400° F.	41.0	40.0
	29.0	29.5
	28.0	29.0
	23.0	22.7
Average time per analysis	6 hours	45 minutes

boiling mixtures. Corrections for such dilutions were then included in the calculation.

Because many aromatic compounds do not fluoresce in the visible range, it became necessary to produce conditions that would stimulate visible fluorescence for all types of aromatic compounds. Adsorption on silica gel was found to move the range of fluorescence of toluene from the invisible to the visible. Additional measures were necessary to secure visible fluorescence of benzene. Traces of fluorescing impurities were found capable of producing this effect. Parasheen was considered very satisfactory, as its addition to a hydrocarbon mixture produces visible fluorescence of the entire silica gel column covered by the hydrocarbons. With Parasheen the aromatic band fluoresces in a brilliant purple while the paraffin and olefin bands fluoresce in a light blue color.

Some standardization was necessary to determine where on the column the aromatic separation had become complete and linear measurements could be made. Mixtures of hydrocarbons were blended quantitatively and samples previously analyzed by the method of Mair were used as standards. Measurements were repeated at 5-minute intervals while the samples percolated through the entire column length. In all cases it was found that under proper conditions complete separations occurred when the sample had percolated through a length of column equal to twice the length of its original adsorption. Thus a sample covering a 10-cm. length as it first entered the column could be measured when its lower limit had reached 20 cm. below the top of the silica gel. In every case, the ratios of the length of the aromatic bands to the length of the sample band did not vary over a period required to make three or four 5-minute readings. Samples that required blending occasionally evidenced a fanning out of the front ends of the sample after this period. No measurements were taken after the lower limit of the sample had wetted the bottom of the silica gel, as sample packing then usually occurred.

Results obtained on mixtures of known composition and on typical samples are shown in the tables, compared to those obtained by analysis following the Mair method. Reproducibility is shown to be within ±2%.

CONCLUSION

A chromatographic method is described for the determination of aromatic compounds in hydrocarbon mixtures such as are present in low boiling petroleum products. A fluorescent tracer and ultraviolet light are employed for the observations. A linear ratio between the two zones of differing fluorescence is shown to be directly proportional to the aromatic compound content. Advantages of this method include the need of only a small amount of sample (2 ml.), the consumption of less time per analysis than most methods of similar accuracy (45 minutes), and the adaptability of procedures to cover a wide range of sample types. The reproducibility has been shown to be ± 2%.

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Chromatographic Separation of Aliphatic 2,4-Dinitrophenylhydrazones

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Chromatographic adsorption on bentonite from ether and/or hexane has been used to separate many aliphatic dinitrophenylhydrazone mixtures. Twenty-two pairs of derivatives representing twelve aliphatic aldehydes and ketones were examined. Eighteen were separated satisfactorily; four pairs were incompletely separated.

IN 1935 Strain (*8*) proposed chromatographic adsorption for the separation of 2,4-dinitrophenylhydrazones. He described the separation of the β-ionone and camphor derivatives and of the geronic acid and levulinic acid derivatives. He used talc as the adsorbent, and suggested alumina, aluminum phosphate, magnesium phosphate, and fuller's earth as other adsorbents. Lucas, Prater, and Morris (*6*) separated acetaldehyde dinitrophenylhydrazone from propionaldehyde dinitrophenylhydrazone on alumina; later Buchman, Schlatter, and Reims (*3*) used chromatographic separation on alumina to separate two cyclobutylcyclobutanone derivatives. Roberts and Green (*7*) have reported the chromatographic separation on silicic acid–Super Cel of any mixtures of the 2,4-dinitrophenylhydrazones of acetaldehyde, acetone, propionaldehyde, and methyl ethyl ketone except

acetone-propionaldehyde. Johnston (*5*) has recently described the separation of the dinitrophenylhydrazones of certain androgens on an alumina column.

During a study of the volatile components of apple juice, it became necessary to separate and identify small quantities of aliphatic carbonyl compounds. It was found that 325-mesh Volclay bentonite strongly adsorbed and gave excellent separation of many dinitrophenylhydrazones. Results of adsorption of 22 pairs of derivatives involving 12 aldehydes and ketones are reported here. (Three other bentonites were found unsatisfactory. Magnesium oxide and silicic acid did not appear to be as useful as the adsorbent selected. The first decomposed the derivatives and the last showed considerably less strength of adsorption with more diffuse bands than did the Volclay bentonite.)

EXPERIMENTAL

Materials. The adsorbent mixture consisted of three parts by weight of the bentonite and one part of a diatomaceous filter aid, well mixed. No special activation was necessary with the batch used unless the mixture had been exposed to the atmosphere for several weeks. Heating for 2 hours in a vacuum at 70° C. was sufficient to restore the adsorbent. Columns were packed dry, with vacuum applied to the lower end of the column. Solvents were not specially purified. (The ether was a chemically pure diethyl ether. The label carried the statement "Ether conforming to this specification normally contains about 2% of alcohol and about 0.5% of water." It was not further purified before use. The hexane was a petroleum ether boiling at 63° to 70° C.)

Procedure. Samples of 10 to 20 mg. of each derivative were mixed, dissolved in 5 to 20 ml. of the appropriate solvent (Table I), and adsorbed on a 13 × 200 mm. column. It was developed by washing with 30 to 200 ml. of the appropriate solvent, which was chosen to give maximum spread of the adsorbed zones. Improper choice of developing solvent resulted in either failure of the bands to move down the column or very weak adsorption without separation. Air pressure (30 cm. of mercury) was used to increase filtration rates. Development was continued until complete separation of the zones was noted, or if this was not attained, until more than one zone showed. In some cases two adsorptions were necessary.

When convenient, the lower zone was washed through the column. Otherwise both zones were removed and eluted with methanol, ethanol, or ether-ethanol (in order of decreasing strength of adsorption of the 2,4-dinitrophenylhydrazones). The solutions were filtered through Selas crucibles to remove traces of adsorbent, evaporated dry on a steam bath, and weighed. Melting points (uncorrected) were determined on the evaporated residues rather than after recrystallization, in order to avoid any fractionating effect of crystallization. When derivatives melting at the same point were separated, mixed melting points with known compounds were used to identify them.

Example. Eighteen milligrams of formaldehyde 2,4-dinitrophenylhydrazone (melting point 165° C.) and 20 mg. of acetone 2,4-dinitrophenylhydrazone (melting point 124° C.) were adsorbed from ether and developed with 5% acetone in ether until the following zones appeared:

40 mm.	Colorless
55 mm.	Orange
15 mm.	Colorless
18 mm.	Bright yellow

The zones were removed, eluted with methanol-ether, and evaporated to dryness. The upper zone yielded 19 mg. of acetone 2,4-dinitrophenylhydrazone, melting point 124° C., and the lower zone yielded 17 mg. of formaldehyde 2,4-dinitrophenylhydrazone, melting point 166° C.

Application. As an application of the separation procedure, a crude preparation of dinitrophenylhydrazones of volatile carbonyl compounds from the chromic acid oxidation of technical 2-ethylbutanol was adsorbed from hexane and developed with 75% hexane in ether. Six bands were observed. The two predominating zones were removed. The upper band yielded diethyl ketone 2,4-dinitrophenylhydrazone, melting point 153.5–154.5° C.; the lower band, after readsorption to remove traces of unidentified compounds, yielded 2-ethylbutyraldehyde 2,4-dinitrophenylhydrazone, melting point 136–136.5° C. [94.5–95° (*2*); 129–130° (*4*)]

Table I. Chromatographic Separation of the 2,4-Dinitrophenylhydrazones of Twelve Aldehydes and Ketones

Upper Zone	Lower Zone	Developing Solvent
A. Completely Separated		
2,4-Dinitrophenylhydrazine	Acetone	1% acetone in ether
Acetone	Formaldehyde	5% acetone in ether
Acetone[a]	Acetaldehyde	Ether
Acetone	Propionaldehyde	Ether
Acetone	Methyl ethyl ketone	Ether
Acetaldehyde	Methyl ethyl ketone	Ether
Acetaldehyde	Propionaldehyde	Ether
Methyl ethyl ketone	Methyl *n*-propyl ketone	25% hexane in ether
Methyl ethyl ketone[a]	*n*-Butyraldehyde	25% hexane in ether
Propionaldehyde[a]	*n*-Butyraldehyde	50% hexane in ether
Methyl *n*-propyl ketone	*n*-Butyraldehyde	67% hexane in ether
Methyl *n*-propyl ketone	*n*-Valeraldehyde	50% hexane in ether
n-Butyraldehyde	Isobutyraldehyde	50% hexane in ether
n-Valeraldehyde[a]	Isovaleraldehyde	50% hexane in ether
Methyl *n*-butyl ketone	*n*-Valeraldehyde	67% hexane in ether
n-Butyraldehyde[a]	*n*-Valeraldehyde	67% hexane in ether
Methyl *n*-propyl ketone[a]	Methyl *n*-butyl ketone	67% hexane in ether
n-Valeraldehyde	Isobutyraldehyde	67% hexane in ether
B. Incompletely Separated[b]		
Formaldehyde	Acetaldehyde	
Propionaldehyde	Methyl ethyl ketone	
Methyl *n*-butyl ketone	*n*-Butyraldehyde	
n-Valeraldehyde	*n*-Hexaldehyde	

[a] Two adsorptions necessary.
[b] In all cases, sections of zone showed different melting points.

Diethyl ketone dinitrophenylhydrazone $C_{11}H_{14}O_4N_4$ requires 21.05% N; found 21.01% N.

2-Ethylbutyraldehyde dinitrophenylhydrazone $C_{12}H_{16}O_4N_4$ requires 19.99% N; found 20.18% N.

RESULTS AND DISCUSSION

Table I shows the 2,4-dinitrophenylhydrazone pairs studied and the developing solvent. The first named of each pair was the more strongly adsorbed. In some cases two adsorptions were necessary for satisfactory separation. In all pairs listed as incompletely separated only one zone was observed. Material from the upper and lower parts of the zone showed different melting points, indicating that a mixture was present, inasmuch as a single compound shows the same melting point throughout the zone. These pairs were not completely separated by any mixture of ether and hexane, the mixtures being chosen to cover the entire range from strong adsorption to virtual elution.

The pairs were chosen to include all those that might be expected to be difficult to separate. By examination of Table I, the separability of other pairs or of mixtures of more than two dinitrophenylhydrazones can be ascertained. For example, as the dinitrophenylhydrazones of acetone and of methyl ethyl ketone are separated and those of methyl ethyl ketone and methyl *n*-propyl ketone are separated (in each case the first-named being more strongly adsorbed), it is obvious that acetone dinitrophenylhydrazones can be separated from methyl *n*-propyl ketone dinitrophenylhydrazones. Similarly, a mixture of the first four methyl ketone dinitrophenylhydrazones can be separated by proper development. In making such a separation, the mixture should be adsorbed from hexane and washed successively with 67% hexane–ether, 25% hexane–ether, and ether. These separations have been made.

When an unknown mixture is being examined, it is often of value to adsorb from ether and develop with ether, thus dividing the mixture into two fractions, those that remain adsorbed and can be separated by ether development, and those that are eluted with ether and must be readsorbed from hexane and developed with hexane-ether solution.

Use of the correct solvent mixture for development results in clear, definite zones with sharp lower boundaries, which permit observation of faint zones near the more prominent bands. Occasionally a single derivative will give rise to two adjacent bands that cannot be separated by continued washing. Elution and evaporation yield apparently identical compounds. In all such

cases, the upper of the two bands is an orange color and the lower may be buff or yellow. To avoid confusion, a mixture or unknown should be developed until the bands actually separate.

Variability of Adsorbent. The work reported herein was done with a single lot of bentonite which had been in open storage in the laboratory for approximately 4 years. No activation or drying was necessary for good results. A sample from current production was obtained which also gave satisfactory results; however, it was necessary to heat the material at 110° in a vacuum oven for 6 hours before mixing with the filter aid. A new Volclay product, BC dust Volclay bentonite, also gave excellent separation but had a slower filtration rate than the 325-mesh Volclay bentonite. The former should show greater uniformity from lot to lot than the other product. The addition of more filter aid (to a 1 to 1 ratio) increased the flow rate satisfactorily. Some indication was noted that the BC dust might require drying of the solvents for best results.

Mixed Crystals. Brandstätter (*1*), in a study of mixed crystal formation with the 2,4-dinitrophenylhydrazones has stated that certain errors may be made in the estimation of their purity or identity. In some cases the presence of a homologous impurity may raise the melting point of a dinitrophenylhydrazone. She also observed that certain mixed crystal systems may be mistaken for pure compounds. She found eight dinitrophenylhydrazones which showed thirteen complete series of mixed crystals. Of these thirteen pairs, six were studied by the author's procedure in this laboratory, and all were chromatographically separable.

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Accumulation of Traces of Arsenate by Coprecipitation with Magnesium Ammonium Phosphate

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A procedure is given for the quantitative coprecipitation of traces of arsenate with magnesium ammonium phosphate. In this way 0.075 mg. of arsenic dissolved in 500 ml. of solution can be determined with an accuracy of 2%. Metal ions that are precipitated in ammoniacal medium are made harmless by the addition of an excess of tartrate. In the presence of much antimony a reprecipitation is necessary. The method can be applied to the determination of arsenic in steel that contains more than 0.01% of arsenic.

GENERALLY, coprecipitation is a great nuisance in quantitative gravimetric analysis. In rare cases, however, it can be used to advantage in the quantitative separation of traces of constituents from solutions and their separation from other substances. In order to accomplish this, a precipitate is produced in the solution which carries the microconstituent down quantitatively. Quantitative coprecipitation can be expected when the microcompound forms mixed crystals with the macroprecipitate, when the distribution coefficient is favorable, and when the proper conditions of precipitation are chosen. For example, lead sulfate forms mixed crystals with barium and strontium sulfates. Traces of lead can be coprecipitated quantitatively, if a barium or strontium salt and then an excess of sulfate are added to the lead-containing solution. The principle can also be used in the coprecipitation of traces of arsenate with magnesium ammonium phosphate. The salts $MgNH_4PO_4.6H_2O$ and $MgNH_4AsO_4.6H_2O$ are isomorphous (orthorhombic), and their axial ratios are almost identical (*5*):

$MgNH_4PO_4.6H_2O$	$a:b:c = 0.5667:1:0.9122$
$MgNH_4AsO_4.6H_2O$	$a:b:c = 0.5675:1:0.9122$

Moreover, the solubilities of the two compounds are of the same order of magnitude. Use of the coprecipitation of traces of arsenate with magnesium ammonium phosphate has been made in the literature (*1*, *2*). However, there have been no systematic studies of the best conditions for quantitative coprecipitation of arsenate and application of the method to the determination of small amounts of arsenic in the presence of other constituents in the solution. Such an investigation is described in the present paper.

PROCEDURE AND ANALYSIS

In general, a definite amount of monopotassium phosphate was added to the solution containing a known volume of arsenate solution. After the solution had been made distinctly acid with hydrochloric acid an excess of magnesia mixture (50 grams of magnesium chloride hexahydrate and 100 grams of water) was added. (A slight excess of ammonia was added and the solution allowed to stand overnight. If a precipitate was formed, the solution was filtered, slightly acidified with hydrochloric acid, and diluted to 1 liter.) Concentrated ammonia was then added until the solution became just alkaline to methyl red. The concentration of reagents used was the same as given by Hillebrand and Lundell (*3*) in the procedure for the determination of phosphate. After most of the precipitate had formed, 5 ml. of ammonia were added in excess. The solution was allowed to stand for 4 hours unless otherwise stated, and the precipitate was filtered and washed with dilute ammonia (1 to 20).

As a great number of arsenate determinations had to be made, the simple procedure described by Kolthoff (*4*) was used.

The precipitate was dissolved in 20 ml. of 3 *N* hydrochloric acid and the arsenate reduced on the steam bath in a closed vessel after addition of 0.5 gram of potassium iodide. After being heated for 2 to 3 minutes, the flask was cooled quickly, the liberated iodine was removed with sodium thiosulfate, the solution was care-

Table I. Coprecipitation of Arsenic Pentoxide with Phosphate

(10 ml. of arsenate required 10.04 ml. of 0.01 *N* iodine)

P_2O_5, Grams per 100 Ml. of KH_2PO_4 Solution		
1	0.100	0.025
	0.01 *N* Iodine Used, Ml.	
10.05	10.04	8.56
10.04	10.00	8.78
10.08	10.05	8.25

Table II. Effect of Time of Standing upon Coprecipitation

(10 ml. of 0.01 *N* As_2O_5 in 500 ml.; blank, 10.11 ml. of 0.01 *N* I_2)

Time of standing after precipitation, min.	0.5	30	60	120	240
0.01 *N* iodine, ml.	8.65, 9.10	9.50, 9.75	9.35, 9.60	9.55, 10.00	10.09, 10.10

fully neutralized with sodium bicarbonate, and an excess of bicarbonate was added. The arsenic trioxide was then titrated with standard 0.01 *N* iodine solution in the presence of an excess of bicarbonate, using starch solution as indicator. Blank experiments with 5- to 10-ml. portions of 0.01 *N* arsenate yielded theoretical results within 0.3%.

COPRECIPITATION OF ARSENATE WITH MAGNESIUM AMMONIUM PHOSPHATE

Effect of Amount of Phosphate Used. Ten milliliters of 0.01 *N* arsenate were added to 500 ml. of monopotassium phosphate solution of varying strength; hence the original concentration of arsenate was only 0.0002 *N*. After precipitation according to the procedure described, the results given in Table I were obtained. An amount of 500 mg. of phosphorus pentoxide per 500 ml. of solution to be precipitated was sufficient to give a quantitative coprecipitation of the arsenate. Therefore, this amount of phosphate was used in the following experiments.

Effect of Time of Standing before Filtration of Precipitate. In the general procedure the precipitate was allowed to stand for 4 hours before filtration. In the experiments reported in Table II the precipitate was filtered after various periods of standing following addition of the excess of magnesia mixture. After 30 minutes of standing 96 to 97% of the arsenate was found in the precipitate. As the results depend somewhat upon the exact manner of precipitation, which is hard to reproduce, waiting at least 4 hours before filtration is recommended.

Effect of Shaking. Shaking the suspensions after addition of the excess of magnesia mixture promotes precipitation of the arsenate, but the effect is not large enough to make shaking imperative in the general procedure. Thus, in the coprecipitation of the arsenate in 500 ml. of 0.0001 *N* solution with 100 mg. of phosphorus pentoxide the results given in Table III were obtained.

Excess of Magnesia Mixture Added. The speed of precipitation of arsenate increases with increasing excess of magnesia mixture added. When 500 ml. of 0.00008 *N* arsenate and 5 ml. of magnesia mixture were used—i.e., 2 ml. in excess—90% was found coprecipitated if the precipitate was filtered after 30 minutes, 95% after 1 hour, and 99.5% after 2 hours. When 10 ml. of magnesia mixture—i.e., 7 ml. in excess—were used the figures were 98, 99, and 100% after 0.5, 1, and 2 hours of shaking. Thus, with an amount of phosphate corresponding to 500 mg. of phosphorus pentoxide in 500 ml. the use of 10 ml. of magnesia mixture is recommended.

Recommended Procedure. To the solution containing the arsenic are added enough bromine water to give a yellow color (oxidation of arsenic trioxide to arsenate), an amount of phosphate corresponding to 500 mg. of phosphorus pentoxide per 500 ml. of solution, 1 ml. of hydrochloric acid, and 10 ml. of magnesia mixture. The solution is neutralized with ammonia and after most of the precipitate has been formed 5 ml. of concentrated ammonia are added in excess. It is filtered after 2 to 4 hours of standing, washed with dilute ammonia, and arsenate in the precipitate is determined by the procedure mentioned or by any other suitable procedure. When only traces of arsenic have to be determined, a blank is run with the phosphate solution and reagents and the amount of standard iodine solution used in the blank is subtracted from that used in the actual determination. The iodine solution is standardized under the same conditions as prevail during the titration of the unknown.

The procedure gave satisfactory results even with very dilute arsenic solutions. As little as 0.075 mg. of arsenic in 500 ml. of solution could be determined with an accuracy of 2% (six determinations). In this case the iodine was standardized by taking 2 ml. of 0.001 *N* arsenic trioxide which was oxidized with bromine water and reduced by the procedure given. A blank determination was run to determine the amount of 0.001 *N* iodine, added from a microburet, which was necessary to give a perceptible color change of the starch. This blank was subtracted from the amounts of iodine used in the standardization and in the actual titrations.

Determination of Small Amounts of Arsenic in the Presence of Iron, Antimony, Tin, Aluminum, and Zinc. The precipitation of antimony, tin, aluminum, and iron in ammoniacal medium was prevented by the addition of tartaric acid. These metals in the highest state of oxidation form complex compounds with tartrate which are not precipitated with ammonia.

Procedure. To the solution containing 5 ml. of 0.01 *N* arsenate (1.9 mg. of arsenic) and 100 mg. of one of the above metals in the form of its chloride, bromine water was added until an excess was present. Two hundred milliliters of water, 1 gram of monopotassium phosphate, 3 grams of tartaric acid, and 25 ml. of magnesia mixture were added and then slowly 15 ml. of concentrated ammonia. After 2 hours of standing the precipitate was filtered and washed with dilute ammonia (1 to 20). The arsenate was determined in the precipitate by the method described previously. In the presence of antimony a reprecipitation was necessary, as some of the antimony was coprecipitated and interfered in the determination of arsenate. After the precipitate was dissolved in hydrochloric acid, 0.5 gram of tartaric acid, 5 ml. of magnesia mixture, and ammonia were added, and filtration was made after 2 hours of standing. As evidenced from the data in Table IV, good results were obtained by the procedure.

The method was also used in the determination of arsenic in samples of steel that contained more than 0.01% of arsenic. With such unfavorable ratios of arsenic to iron a reprecipitation is necessary.

PROCEDURE. A sample of steel containing at least 0.1 mg. of arsenic is weighed into a 250-ml. Erlenmeyer flask and dissolved in 25 ml. of 6 *N* nitric acid. In order to ensure complete oxidation of arsenic to arsenate a few milliliters of bromine water are added. The solution is boiled for a few minutes and cooled. Ten grams of tartaric acid for each gram of steel are added with 0.2 gram of monopotassium phosphate and 50 ml. of magnesia mixture (tenfold excess). The solution is transferred to a 250-ml. bottle, and concentrated ammonia is added until there are at least 10 ml. in excess. The mixture is shaken for 4 hours, and the

Table III. Effect of Shaking and Time of Standing

(Blank 5.04 ml. of 0.01 *N* iodine)

Time of Standing after Precipitation, Minutes	0.01 *N* Iodine, Ml.	
	Shaken	Not shaken
30	4.55	4.33
60	4.98	4.85
120	5.04	4.53
240	5.03	5.06

Table IV. Determination of Arsenic in Presence of Other Ions

(1.9 mg. of arsenic. 100 mg. of other ions. Blank, 4.51 ml. of 0.01 *N* iodine)

Element added	Iron	Tin	Antimony[a]	Aluminum	Zinc
0.01 *N* iodine, ml.	4.48, 4.49, 4.51	4.49, 4.50, 4.52	4.48, 4.50	4.47, 4.49	4.48, 4.50

[a] Reprecipitation: after one precipitation 7.05 ml. of iodine were required.

precipitate is filtered off and dissolved in 3 *N* hydrochloric acid. One gram of tartaric acid, 10 ml. of magnesia mixture, and an excess of ammonia are added to the solution. After shaking for 2 hours the precipitate is filtered and washed with dilute ammonia (1 to 20). The precipitate is dissolved in 3 *N* hydrochloric acid and the arsenate determined as described previously. When the amount of arsenic is small (of the order of 0.1 mg.) the titration is carried out with 0.005 *N* iodine added from a microburet. A blank is run following the same procedure, except that no steel is added. The amount of iodine required in the blank is subtracted from that used in the determination.

The method was checked with a Bureau of Standards sample of ingot iron, No. 55, containing 0.012% of arsenic. The results in eight determinations varied between 0.011 and 0.14%, with an average of 0.012%.

When the amount of arsenic in the steel was less than 0.01%, the blank was so large compared to the amount of iodine used in the determination, that the results were not satisfactory.

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Determination of Diphenyl Carbonate

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Three analytical methods are described for estimating diphenyl carbonate in cloth: bromination of the phenol produced by alkaline decomposition, estimation of the blue indophenol color formed by the phenol decomposition product with 2,6-dibromoquinone chloroimide, and ultraviolet absorption measurements. A modification of the colorimetric indophenol method markedly stabilizes the color and is applicable to determinations of small amounts of phenol as well as diphenyl carbonate.

DIPHENYL carbonate, one of the new industrial chemicals available in commercial quantities, is being used for pharmaceutical manufacturing purposes. It has been found, by the U. S. Department of Agriculture, Bureau of Entomology, Orlando, Fla., to be a promising miticide and larvacide, and is permitted for restricted use on the skin by the Division of Pharmacology of the Food and Drug Administration. In connection with the study of miticides for impregnation in clothing being conducted at the Army Chemical Center, it became necessary to devise methods of analysis for this compound applicable to clothing impregnated with the compound. A diversity of methods is desirable during development stages.

The methods described in this paper involve brominating the phenol produced by alkaline decomposition of the compound; estimating the blue color formed by phenol with 2,6-dibromoquinone chloroimide by transmittance measurements at 565 to 630 millimicrons, using a photoelectric colorimeter; and measuring ultraviolet absorption. They were found satisfactory for estimating various amounts of the compound in cloth and with minor changes may be of value in other applications of diphenyl carbonate.

APPARATUS

Soxhlet type extraction apparatus with standard-taper Erlenmeyer flask.

Beckman quartz spectrophotometer, Model CUV, range 220 to 1000 millimicrons, with interchangeable hydrogen discharge lamp in housing, and a pair of fused silica absorption cells with Pyrex covers.

Klett-Summerson photoelectric colorimeter, Model 900-3, with brown color filter No. 59 with maximum transmittance at 565 to 630 millimicrons, and calibrated test tubes.

REAGENTS AND MATERIALS

Undyed pure-finish cotton herringbone twill, undyed, sized cotton herringbone twill, and olive drab cotton herringbone twill cloth.

Diphenyl carbonate, prepared by Chemical Division, Army Chemical Center, and recrystallized three times from ethanol, melting point 79°–80° C.

Reagents for Bromination Method. C.P. ethyl ether; potassium hydroxide solution approximately 0.2 *N*; C.P. hydrochloric acid, concentrated; potassium bromate–potassium bromide solution of 3.5 grams of C.P. potassium bromate, and 13.0 grams of C.P. potassium bromide, made up to 1 liter with water; potassium iodide solution, 10%; sodium thiosulfate solution, 0.1 *N*; and starch indicator solution, 1%.

Reagents for Colorimetric Indophenol Method. Buffer solution of 28.4 grams of C.P. sodium tetraborate ($Na_2B_4O_7 . 10H_2O$), and 5.9 grams of C.P. sodium hydroxide, made up to 1 liter with boiled and cooled distilled water; 2,6-dibromoquinone chloroimide (Eastman Kodak No. 2304) solution prepared by dissolving 0.08 gram of the compound in 50 ml. of 95% ethanol and filtering into a dark brown bottle; and 0.3 *N* sodium hydroxide solution. The 2,6-dibromoquinone chloroimide solution should be kept in a cool, dark place and made up fresh at least every second day. The buffer solution should be adjusted to a pH of 10 with boric acid or sodium hydroxide, so that a solution of 0.20 ml. of the buffer and 10 ml. of distilled water will have a pH of 9.8.

DEVELOPMENT OF METHODS

Ultraviolet Absorption Measurement. Some preliminary work was done to make ultraviolet absorption measurements the basis of an analytical method. The procedure applied was essentially the same as described by Gibb (*5*) and Klotz (*9*). In order to obtain the optimum wave length, which is regarded as the wave length of maximum absorption, the absorption spectra of diphenyl carbonate in concentrations of 0.01, 0.10, and 0.80 mg. per ml. in 95% ethanol were determined. It was indicated that the most pronounced absorption of diphenyl carbonate in 95% ethanol solution occurs in a wave band somewhere below 210 to 220 millimicrons, which is the lower limit of the Beckman quartz spectrophotometer used for this work. Another lesser absorption peak for this compound occurs at about 256 millimicrons, and it was found that concentrations of diphenyl carbonate in the range of 0.05 to 0.8 gram per liter of 95% ethanol may be estimated by

absorption measurements at 256 millimicrons in the absence of interfering material. Much greater sensitivity was obtained by breaking the compound down to phenol and carbon dioxide by alkali and applying ultraviolet absorption measurements to the estimation of the phenol. Phenol in 95% ethanol has a pronounced absorption band at about 274 millimicrons (*8*) and can be estimated by a method indicated by Klotz (*9*).

Both methods based on ultraviolet absorption measurements were rapid and accurate for pure-finish cloth samples impregnated with diphenyl carbonate, but marked interference was experienced in estimating small percentages of the compound in cloth containing large amounts of ultraviolet-absorbing material such as sizing preservatives. Slight interferences may be compensated by use of suitable blanks, if they are available, or by a method such as the one used for determination of some inhibitors in rubber by ultraviolet absorption (*1*).

Bromination Method. Attempts to brominate diphenyl carbonate directly in alcohol, carbon tetrachloride, or acetic acid solutions (*14*) were unsuccessful; the carbonate group evidently inhibited the bromination of the phenyl groups in this case. However, no difficulty was experienced when the compound was decomposed by alkali to phenol, which is readily brominated in aqueous solution to 2,4,6-tribromophenol by methods described in the literature (*4, 10, 12*). Recoveries of 99.8, 99.6, and 99.8% were obtained when weighed amounts of diphenyl carbonate were decomposed with 0.2 *N* potassium hydroxide, followed by bromination with a solution of potassium bromide and potassium bromate.

When the method was applied to cloth impregnated with diphenyl carbonate, the first trials were run by extracting the cloth with ethyl or methyl alcohol, decomposing the extracted compound in alcohol solution by addition of 0.1 *N* potassium hydroxide solution, and refluxing, followed by bromination. In the presence of alcohol, unless the bromination was carried out under carefully controlled conditions of temperature and time, side reactions took place between the bromine and alcohol, giving variable results. But when the cloth was extracted with ethyl ether, followed by evaporation of the ether, decomposition with 0.1 *N* potassium hydroxide solution, and bromination, the results were uniform and recoveries of 99.8, 99.8, and 100.0% were obtained with samples containing as little as 5 mg. of the compound. Several trials also indicated that there was no loss of the compound when the ether was evaporated from the extract. When blanks were run using pure-finish cloth and chlorinated paraffin, no interference was found. When blanks were run using sized cloth, a very slight blank titration was obtained. This indicated that, for greater accuracy, a blank correction should be made.

It was also found that the determination can be made by heating a weighed sample of cloth impregnated with diphenyl carbonate directly with 0.1 *N* potassium hydroxide solution, followed by filtration of the resultant phenol solution and bromination. In this case, higher blanks were obtained, but in all cases the blank titrations were relatively low compared to the titration of the sample.

Detailed Bromination Procedure. Cut the cloth sample into 1-cm. squares and mix to make the sample more homogeneous. Extract a weighed sample of the cloth containing about 50 mg. of diphenyl carbonate for 1.5 hours with ethyl ether in a Soxhlet type extractor with a paper thimble. (If Soxhlet equipment is not available, the cloth may be heated directly with the potassium hydroxide solution and the extract separated by filtration. Blanks will be slightly higher if this is done.) Evaporate the ether carefully from the extract in the flask of the extractor and add 25 ml. of aqueous 0.2 *N* potassium hydroxide solution. (If a sample containing about 200 mg. or more of diphenyl carbonate is used, 0.5 *N* potassium hydroxide solution should be used in place of 0.2 *N* potassium hydroxide and an aliquot portion should be used in place of the entire extract.) Attach a reflux condenser to the flask and heat to boiling until the compound is saponified and in solution (requires from 20 to 30 minutes). Cool, transfer to a 500-ml. iodine flask, add a measured amount of potassium bromate–bromide solution (25 to 35 ml., depending on the amount needed to have at least 2 to 5 ml. excess over that required for bromination), and add 4 ml. of concentrated hydrochloric acid (if the volumes of the potassium hydroxide and potassium bromate–bromide solutions are changed, use an amount of hydrochloric acid to have an acidity between 0.7 and 0.8 *N*).

Insert the stopper quickly and shake intermittently for 5 minutes. Add 10 ml. of 10% potassium iodide solution, taking care to avoid loss of bromine while the stopper is being lifted. Wash down the stopper and sides of the flask with water and titrate the liberated iodine with 0.1 *N* sodium thiosulfate solution, using starch indicator at the end of the titration. Run a cloth blank under the same conditions. The difference between the titration volume of the blank and that of the sample after bromination is the net titration. Then:

$$\% \text{ diphenyl carbonate} = \frac{(\text{net titration of } Na_2S_2O_3 \text{ solution}) \times (\text{normality of } Na_2S_2O_3)(1.7851)}{\text{sample weight}}$$

Results obtained on pilot-plant-impregnated batches of cloth, some of which had been aged outdoors and some aged in simulated tropical storage for varying lengths of time, are given in Table I. The cloth used was either pure-finish or sized undyed cotton herringbone twill and some of the samples were impregnated with chlorinated paraffin as a fixative in addition to the diphenyl carbonate.

Colorimetric Indophenol Method. This method was selected for estimating smaller amounts of the compound, as Snell and Biffin (*11*) reported it to be very satisfactory and the best available. It involves decomposition of diphenyl carbonate by alkali to phenol, formation of a blue color by treating a buffered solution (pH 9.4 to 9.8) of the phenol with a solution of 2,6-dibromoquinone chloroimide, and estimation of the amounts of phenol by light transmittance measurements at 610 millimicrons (*6, 13*).

When the method was applied as described by Snell (*11*) and transmittancies were read on a photoelectric colorimeter, satisfactory results could be obtained under carefully controlled conditions of time and pH, but some inaccuracy was found because the sample and blank transmittancy readings increased in a variable manner with time. Gibbs (*6, 7*) and others (*2, 3*) reported that the 2,6-dibromoquinone chloroimide decomposes slowly in alkaline buffers, giving rise to discoloration of the solutions, and it is thought that the variable transmittancy readings are due chiefly to the decomposition of the excess 2,6-dibromoquinone chloroimide over that required to form the colored indophenol compound. Because an excess of the chloroimide over phenol must be used (*6*), some means of removing or destroying this excess after formation of the color was indicated as a possibility in increasing the stability of the color. Beshgetoor, Greene, and Stenger (*2*), in order to increase the sensitivity in de-

Table I. Comparison of Bromination and Colorimetric Indophenol Methods for Determination of Diphenyl Carbonate in Cloth Impregnated in a Pilot-Plant Impregnator

	Diphenyl Carbonate, Per Cent		
	Bromination Procedure		Colorimetric indophenol method
Sample No.	Individual determinations	Av.	
Samples with chlorinated paraffin as fixative			
1	4.4 , 4.6	4.5	4.57
2	3.9 , 3.8	3.9	3.85
3	0.06, 0.04	0.05	0.044
4	.....	<0.05	0.010
5	.....	<0.05	0.002
6	.....	<0.05	0.002
7	.....	<0.05	0.005
Samples without fixative			
8	5.4 , 5.5	5.5	5.47
9	2.8 , 2.9	2.9	2.75
10	0.9 , 0.9	0.9	0.78
11	0.04, 0.06	0.05	0.055

termining mere traces of phenol, concentrated the colored compound by acidifying the colored solution with hydrochloric acid, extracting with chloroform, separating the chloroform extract, adding alcohol to the extract, and finally making alkaline with sodium hydroxide. Although this procedure (*2*) is also reported to increase the stability of the color, it was desired to avoid the increased number of steps in the determination and the amount of handling of the colored compound for the authors' application which involved much larger amounts of phenol.

Table II. Effect of Addition of Sodium Hydroxide on Stability of Indophenol Color

Sample	Readings of Klett-Summerson Colorimeter at Various Time Intervals								
	15 min.	20 min.	25 min.	30 min.	45 min.	60 min.	90 min.	120 min.	150 min.
0.01 mg. of diphenyl carbonate	142	146	147	150	153	153	156	156	157
Blank	36	41	43	45	48	49	52	51	54
Net reading	106	105	104	105	105	104	104	105	103
0.01 mg. of diphenyl carbonate + 0.1 ml. of 0.3 *N* NaOH after 10 min.	144	144	143	143	143	143	143	142	140
Blank + 0.1 ml. of 0.3 *N* NaOH after 10 min.	37	36	36	37	37	37	37	37	37
Net reading	107	108	107	106	106	106	106	105	103

Some efforts were made to find a suitable reagent which could be added to the solution to destroy the excess of 2,6-dibromoquinone chloroimide after formation of the blue indophenol color. Reducing agents such as sodium sulfite, hydrazine, and hydroxylamine destroyed the blue color. Ammonium hydroxide, which reacts with chloramides, and triethanolamine did not decolorize the blank solution, but had a pronounced stabilizing effect on the colors of the sample and blank solutions; reagents such as ammonium acetate, ammonium carbonate, ammonium oxalate, and urea did not. This indicated that increasing the pH after development of the color had a stabilizing action, and sodium hydroxide, which was tried next, gave good results (Table II).

It may also be observed in Table II that the net readings for the untreated sample are fairly constant although the blank and sample readings increase with time. But if the times of the blank and sample readings were not carefully controlled (which is inconvenient when many samples are run), large variations in the net readings occurred.

Trials with 0.1 ml. of 0.2 *N*, 0.3 *N*, and 0.6 *N* sodium hydroxide solutions gave like results, and it was decided to use the 0.3 *N* strength. The addition of 0.1 ml. of 3 *N* sodium hydroxide changes the pH from 9.8 to 10.9. Using 0.3 *N* sodium hydroxide as described in the procedure below, a curve was prepared relating Klett-Summerson colorimeter readings (logarithmic scale), using filter No. 59, with diphenyl carbonate concentrations. A straight line was obtained up to a concentration of 0.004 gram per liter with a reading of about 400; this concentration also applies to phenol. In applying the method to cloth samples containing known amounts of diphenyl carbonate, no interferences were experienced in analyzing both pure-finish and sized cloth with and without chlorinated paraffin as a fixative. The method was found to be satisfactory for small amounts of compound down to 0.01 mg., but large amounts involved the error of large dilution. Some results in comparison with the bromination method are given in Table I.

DETAILED INDOPHENOL COLORIMETRIC PROCEDURE. Cut the cloth sample into 1-cm. squares and mix. Extract a weighed sample and saponify the extract as directed in the bromination procedure. Neutralize the resultant alkaline solution with dilute hydrochloric acid to a pH of about 7 and make up to a known volume with distilled water. Dilute portions of this solution to such a volume that not more than 0.0025 mg. per ml. of the diphenyl carbonate will be present. If the amount of diphenyl carbonate is completely unknown, prepare several dilutions until the right range is obtained. In each case, place 10 ml. of the diluted solution in a calibrated Klett test tube, add 0.20 ml. of the borate buffer solution, and mix well. Add 0.20 ml. of the 2,6-dibromoquinone chloroimide solution, mix well, and allow to stand for 10 minutes. After 10 minutes, add 0.10 ml. of 0.3 *N* sodium hydroxide solution and allow to stand for another 10 minutes.

Prepare a blank solution under the same conditions, starting with 10 ml. of distilled water. Using the blank solution to adjust the instrument to 100% transmittancy, read the transmittancy of the solution in a spectrophotometer at a wave length of 610 millimicrons or in a photoelectric colorimeter such as the Klett-Summerson with brown filter No. 59 (maximum transmission 565 to 630 millimicrons). The reading should be taken within 2 hours, as the color fades gradually with time after this period. Prepare a curve relating colorimeter readings with diphenyl carbonate concentration, using known solutions treated as described above, and make the quantitative determination of the unknown by applying the reading obtained to the curve.

The curve readings should be checked with known solutions each time a new buffer solution is prepared, as a slight change in pH may make it necessary to prepare a new curve.

DISCUSSION

The best methods evolved for diphenyl carbonate determination are the bromination method for larger amounts (down to 20 mg.) and the colorimetric indophenol method for small amounts (20 down to 0.01 mg.). The method based on ultraviolet absorption measurements of diphenyl carbonate solution in 95% ethanol or of phenol solutions is offered only as an alternate or check method.

In the bromination and colorimetric indophenol methods for diphenyl carbonate, the phenol liberated on alkaline decomposition of the compound is estimated. Any free phenol present as an impurity or from a prior breakdown of the compound would give high results. However, in the many samples of impregnated cloth handled which had been stored outdoors, or in tropical storage, or had undergone launderings, the odor of free phenol was not detectable. If phenol is found to be present in any samples; it may be determined separately by the bromination procedure, alkaline decomposition of the diphenyl carbonate is omitted, as diphenyl carbonate will not brominate without prior alkaline decomposition. The free phenol can also be removed by extraction with water, in which the diphenyl carbonate is insoluble.

Although no appreciable interferences were encountered in determining diphenyl carbonate in cloth by either the bromination or colorimetric indophenol methods, the phenol could be separated from nonvolatile interfering materials by distillation (*2*).

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Determination of Boiling Range of Methyl Bromide

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The boiling range of methyl bromide is determined by distillation under a reflux condenser; the distillation flask and receiver are enclosed in a dry ice box to permit measurement of the vapor temperature. The heater consists of a coil of resistance wire wound around a Pyrex finger sealed to the bottom of the flask. By use of a thermometer divided into 0.1° C. divisions, distillation temperatures may be measured with a precision of ±0.05° C. during the first 95% of the distillation, during which time superheating is essentially absent. Distillation temperatures may be quantitatively correlated with the concentration of known impurities that do not form constant-boiling impurities.

THE boiling range is commonly used as an index of the purity of liquids. The determination of the boiling range of methyl bromide offers unique problems as a result of the fact that its boiling point is below room temperature. When conventional distillation equipment is used, a thermometer in the vapor indicates a temperature close to that of the laboratory. One method of measuring the distillation temperature in applications of this sort is to immerse the thermometer bulb in the liquid. When this procedure was used, methyl bromide invariably superheated, the amount of superheating being as much as 6 ° C. Another method involves carrying out the distillation in a flask, the neck of which is jacketed. A refrigerant is circulated through or simply introduced into the jacket and the vapor temperature is measured. By this procedure wide and erratic boiling ranges were obtained.

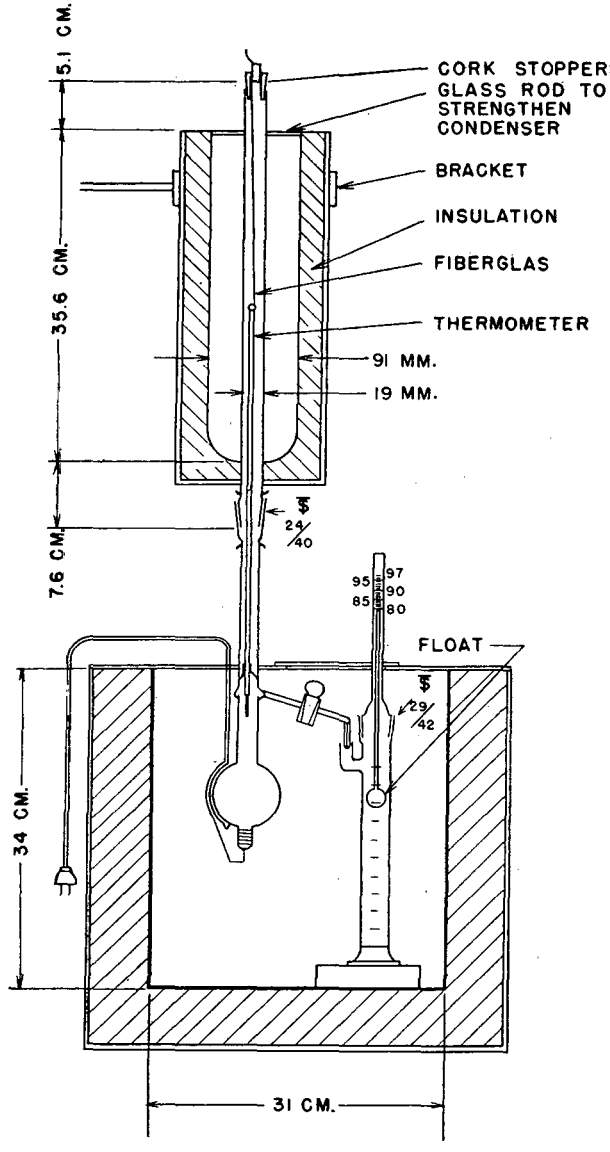

Figure 1. Methyl Bromide Distillation Apparatus

The method that was finally chosen involved distillation in a refrigerated cabinet which reproduced on a small scale the conditions that obtain during the distillation of higher boiling liquids. An adaptation of the boiling-point flask described by Quiggle, Tongberg, and Fenske (*2*) provided a convenient and efficient heater for carrying out the distillation inside the cabinet.

Normally the methyl bromide used for determination of the boiling range is measured at a temperature much below its boiling point and the temperature of the sample before distillation may be substantially different from that of the distillate. Because any change in temperature will result in a change of volume, and the temperature of the distillate, and hence its volume, may be greater than that of the distilland, it is impractical to take a 100-ml. sample for distillation. Accordingly, a 95-ml. sample is taken and the data are reported in per cent of distillate, calculated from the total volume of distillate.

Although this method has been applied only to the determination of the boiling range of methyl bromide, it should be applicable to any liquid that has a boiling point between room temperature and the sublimation point of dry ice.

MATERIALS

Commercial methyl bromide was used unless otherwise specified. The methyl bromide used for testing the feasibility of the method and apparatus was purified by scrubbing with sulfuric acid, followed by rectification. The scrubbing was carried out by passing vaporized methyl bromide through a 180-cm. (6-foot) tower packed with 6-mm. glass rings and filled with 95% sulfuric acid. Boiling range data indicated that this method of purification removed most of the high boiling impurities but was ineffective in removing low boiling impurities and that the subsequent rectification through a 6-foot column packed with 0.125-inch (0.3-cm.) helixes removed essentially all the low boiling impurities. The rectified product was used in the tests described below where a purified product is indicated.

APPARATUS

The distillation apparatus is illustrated in Figure 1.

The distillation flask is made by modifying a 100-ml. Engler distillation flask. The condenser-flask assembly is supported by a suitable clamp attached to the condenser. A tight fit is made at the standard-taper joint at the top of the flask by means of two small springs.

The dry ice box is divided into two compartments (front and rear) with a substantial wire partition between. One compartment is for dry ice and the other for the flask and receiver. The box is covered with a two-piece Plexiglas cover through which ap-

propriate holes are drilled. One large hole over the receiver is covered with an auxiliary piece of Plexiglas, so that access is provided to the stopcock on the take-off tube of the distillation flask.

The receiver consists of a 100-ml. graduated cylinder, provided with a side arm through which the distillate enters. A 29/42 standard-taper joint is sealed to the top of the cylinder and is used to attach a narrow Pyrex tube which extends through the cover of the cabinet. A glass float rides on the surface of the methyl bromide and is provided with a long stem that extends into this tube. The tube is calibrated to permit easy reading of the volume of distillate. This calibration is accomplished by introducing methyl bromide into the cylinder, noting the volume, then putting the float and tube in place and marking the height on the stem of the float on the tube. Three or four points are obtained by direct measurement and other points by interpolation.

During the experimental work volume measurements were made by means of a mirror located at the level of the liquid in the receiver and tilted 45° from vertical. This permitted measurement of the volume at any point during the distillation by moving the mirror upward as the distillation progressed but is less convenient for routine use than the float.

The heating coil consists of 9 feet of No. 30 varnished chromel C wire, resistance 6.9 ohms per foot. The wire is closely wound around a finger which is sealed to the bottom of the distillation flask. The insulation is stripped from the ends of the wire and they are soldered to short lengths of No. 20 copper wire which are twisted around two glass knobs, one sealed to the bottom of the finger and the other to the bottom side of the bulb. These heavier copper wires serve as convenient terminals for the ends of the windings and for the permanent electrical connection which plugs into a Variac. Several glass prongs are sealed to the bottom of the finger and three or four turns of the resistance wire are held in place by these prongs to ensure adequate heating at the tip of the finger and complete volatilization of the sample. The heating coil is covered with several turns of asbestos wicking which serves as heat insulation.

A thermometer guide placed inside the neck of the flask above the take-off tube keeps the thermometer in the center of the neck. This guide consists of a short length of 9-mm. Pyrex tubing, held in place in the center of the neck by three glass rods inclined downward and sealed to the flask. The diameter of the inside of the collecting ring must be approximately the same as that of the inside of the neck of the flask.

A Precision Thermometer and Instrument Co. thermometer (Catalog No. 72-B, range −10° to +50° C. in 0.1° C. intervals) is used. It should be calibrated to the nearest 0.01° C. at the ice point by immersion in crushed ice. The thermometer is supported by means of a 24-inch length of 1/8-inch Fiberglas sleeving threaded through a hole in a notched cork that fits the top of the condenser. A small cork is fitted into the hole in the larger cork and is used to grip the sleeving and support the thermometer at the proper level.

A thermometer reader (Precision Scientific Co., Catalog No. 8968) is used for accurate temperature readings. It is held in place on the neck of the distilling flask by several small springs, obtainable from Ace Glass, Inc., Vineland, N. J.

A 100-ml. Pyrex cylinder is used for measuring the sample.

An accessory dry ice box is required for cooling the graduate and samples.

All ground-glass joints and stopcocks are lubricated with Dow-Corning silicone stopcock lubricant.

PRECAUTIONS

Methyl bromide vapors are poisonous, and great care should be exercised to avoid breathing them. All boiling ranges should be carried out in a well-ventilated hood, and the analyst should be provided with a canister-type respirator which should be used whenever methyl bromide is transferred or there is any possibility of high concentrations of methyl bromide in the atmosphere.

PROCEDURE

Place the sample and the 100-ml. graduate, closed with a cork stopper, in an accessory dry ice box to cool. Assemble the apparatus as shown in Figure 1, but do not insert thermometer, and close the top of the condenser tube with a cork stopper to prevent condensation of moisture from the atmosphere. Fill the dry ice compartment with dry ice. Fill the condenser reservoir with small pieces of dry ice, then cautiously pour in trichloroethylene to a point about 2.5 cm. (1 inch) from the top. The trichloroethylene evaporates and must be replenished from time to time. Add dry ice as required during the course of the distillation.

Measure the temperature of the methyl bromide sample to make sure that it is below −50° C. When this condition is met, measure 95 ml. of sample into the precooled cylinder, *transfer to* the distillation flask through the top of the condenser, and immediately replace the cork in the condenser. Plug the flask heating coil into a Variac and adjust the voltage to give a distillation rate of 5 to 6 ml. per minute (about 60 volts). Carefully lower the thermometer through the top of the condenser until the bottom of the mercury capillary is opposite the bottom of the take-off tube. Secure it in this position by wedging the corks at the top of the condenser against the Fiberglas sleeving to which the thermometer is attached.

Observe the distillation temperature at 1-minute intervals. When two successive readings agree within 0.01° C., record the temperature as the starting temperature. Now open the stopcock in the take-off tube and record the time. Read the distillation temperature when the volume reaches 80, 85, and 88 ml. and every milliliter thereafter until the dry point is reached.

The temperature readings must be taken every 10 to 12 seconds during the latter part of the distillation. Obviously, the analyst must be extremely alert in order to make readings with this rapidity. If one reading is missed, proceed to the next without delay. The dry point cannot be determined in the usual manner because of lack of visibility. After the liquid level disappears inside the finger, carefully watch the intermittent flow of liquid through the take-off tube. When this flow becomes inappreciable, the dry point has been reached. Immediately turn off the current to prevent burning out the heating coil. Record the volume of distillate, the distillation temperature, and the time at this point. Immediately remove the thermometer to prevent breakage due to freezing the mercury. Restopper the condenser. When the flask is removed, stopper the bottom of the condenser and replace the Plexiglas cover on the dry ice box.

Divide the total volume of distillate by the time to obtain the rate of distillation, which should be 5 to 6 ml. per minute. Read the barometer and the temperature alongside during the course of the distillation. Correct the pressure to 0° C. as follows:

$$P_0 = P_t - (t \times 0.122)$$

where P_0 and P_t are the barometric pressure in millimeters of mercury at 0° and t° C., respectively, and t is the temperature of the barometer. Correct the distillation temperature to 760-mm. pressure as follows:

$$T_{760} = T_{obs.} + dT/dP\ (760 - P_0)$$

where T_{760} and $T_{obs.}$ are the corrected and observed distillation temperatures, respectively. A value of 0.035, calculated from the data of Egan and Kemp (*1*), is used for dT/dP for normal variations in atmospheric pressure. Add the thermometer ice-point correction, if any. Calculate the per cent of distillate collected at each point at which the temperature is read by dividing this volume by the total volume of distillate collected and multiplying this ratio by 100. It is convenient to compare lots of methyl bromide by calculating the temperature range over which the first 95% of the distillate is collected. The temperature of the 95% point is obtained by plotting the per cent distillate against the temperature and reading the 95% point from the curve.

Table I. Comparison of Flasks

Distillate, %	Commercial CH_3Br		Purified CH_3Br	
	QTF flask[a]	Simplified flask[b]	QTF flask[a]	Simplified flask[c]
Start	3.70	3.60	3.66	3.61
5	3.71	3.62	3.66	3.61
50	3.70	3.66	3.65	3.62
90	3.75	3.74	3.63	3.64
95	3.84	3.81	3.65	3.63
97	3.92	3.90	3.66	3.64
Dry	4.61	4.88	3.84	3.83

[a] Average of 2 boiling ranges.
[b] Average of 4 boiling ranges.
[c] Average of 3 boiling ranges.

EXPERIMENTAL

To adapt the Quiggle-Tongberg-Fenske flask for routine boiling range determinations the inner tubes that served to pump the liquid-vapor mixture over the temperature-measuring element were omitted. The heating finger was shortened as much as possible and the stopcock at the bottom of the heating finger was omitted. It was considered desirable to measure the temperature of the vapor, but low and erratic temperature measure-

Table II. Effect of Thermometer Position

Distillate, %	Temperature, ° C.			
	Position 1	Position 2	Position 3	Position 4
Start	4.39	3.72	3.69	3.64
5	4.39	3.71	3.71	3.66
25	4.31	3.74	3.72	3.72
50	4.26	3.78	3.74	3.74
80	4.04	3.83	3.80	3.78
90	4.09	3.89	3.86	3.84
95	4.09	3.93	3.92	3.92
96	4.09	3.94	3.94	3.95
97	4.19	3.96	3.97	4.02
98	4.3	4.1	4.1	4.2
100	8.7	6.1	4.7	5.3

ments were obtained during the initial tests due to cold condensate running down the thermometer. This was eliminated by means of the thermometer guide, which centers the thermometer in the neck of the flask, and by removing the bulge from the outer member of the standard-taper joint of the top of the flask.

A comparison of the performance of the Quiggle-Tongberg-Fenske flask with that of the simplified flask when both commercial and rectified methyl bromide are used is shown in Table I. In general, slightly lower temperatures are obtained with the simplified flask. This flask would be expected to have a tendency toward fractionation of a sample and this may account for part of the temperature difference, especially at the beginning of the distillation, when commercial methyl bromide is used. The data for purified methyl bromide in the Quiggle-Tongberg-Fenske flask indicate that no superheating occurs during 97% of the distillation. As a matter of fact the temperature falls appreciably near the end of the distillation. This effect is real, as it occurred consistently, but no explanation is known. The dry point temperatures are, of course, of only qualitative value because of lack of visibility. The data for purified methyl bromide in the simplified flask indicate only a slight tendency of 0.02° C. superheating during the first 95% of the distillation.

Because the Quiggle-Tongberg-Fenske flask is a boiling point apparatus measuring the temperature of the liquid and vapor in equilibrium, it should give reliable boiling point data. The data in Table I show that the boiling point of methyl bromide, when this flask is used, is 3.65° C. if the 50% distilled temperature is taken. When the simplified flask is used, the boiling point is 3.62° C. Table III indicates that this value is precise to ±0.04°C. The boiling point of methyl bromide is variously reported in the literature. The most reliable value is probably that of Egan and Kemp (*1*), 3.56° ± 0.05° C. Although the authors' value is slightly higher than theirs, the two ranges overlap, indicating satisfactory agreement.

The effect of using the thermometer at the following positions was determined:

1. Thermometer bulb inside heating finger.
2. Thermometer bulb in liquid just above heating finger.
3. Thermometer bulb just above the surface of liquid at start of distillation (6 cm. below normal position).
4. Thermometer bulb just below take-off tube (normal position).

The first position gave definite superheating throughout the distillation with the minimum superheating after about 90% of the sample had distilled. While the other three positions gave somewhat different temperature indications, the differences between them are small and it appears that the temperature could be measured at any one of these positions. The highest position was adopted because it gave the greatest temperature spread over the significant portion of the range (0 to 95%), which seemed advantageous from the standpoint of estimating purity (see Table II).

Distillation rates between 3.8 and 8.4 ml. per minute gave distillation temperatures that agreed well within the precision of the method. However, it is considered desirable to maintain a uniform rate even though no effect of variation in rate could be observed. Because of the low lag of the heater it is possible to control the rate precisely by setting the Variac at any predetermined point. A rate of 5 to 6 ml. per minute was chosen arbitrarily as being sufficiently rapid to complete the distillation in a reasonable length of time.

The precision was determined by distilling ten portions of the same lot of methyl bromide in succession. The limit of uncertainty (LU_1) of several points between the start and the 91% point was ±0.04° C. or better, but increased sharply at 97%. The poorest precision (±0.57° C.) was obtained at 99%. The rapid rise in the temperature during the last 4% of the distillation, due to superheating as well as to the presence of small amounts of high boiling impurities and the effect of slight errors in the measurement of the volume, are sufficient to account for poor precision in this region. Representative precision data are shown in Table III.

One of the impurities to be expected in methyl bromide is dimethyl ether. A plot of distillation temperatures against the composition of methyl bromide-dimethyl ether mixtures was shown to be linear up to a concentration of 0.84% by weight of dimethyl ether. The starting temperature was lowered 1.09° C. per weight per cent of dimethyl ether and the 5-ml. temperature was lowered 0.81° C. per per cent. Judged by the precision of the temperature measurements, the precision of measuring the dimethyl ether content at either the first drop or the 5-ml. point is ±0.037%. The following equation applies to the first drop temperature:

$$C = 0.92\,(3.62 - t)$$

where C is the concentration of dimethyl ether in weight per cent and t is the starting temperature in degrees Centigrade.

Table III. Precision of Boiling Range

Test No.	Start	91%	96%	97%	99%	100%
1	3.64	3.85	3.93	4.00	4.23	5.63
2	3.65	3.85	3.94	3.99	4.07	5.33
3	3.63	3.83	3.94	3.97	4.23	5.33
4	3.65	3.86	3.97	4.02	4.55	5.35
5	3.65	3.87	3.95	3.97	4.27	5.32
6	3.65	3.86	3.95	4.00	4.70	5.53
7	3.64	3.88	3.97	4.06	4.53	5.41
8	3.63	3.87	3.98	4.09	4.41	5.31
9	3.62	3.86	3.95	3.99	4.33	5.32
10	3.62	3.85	3.95	4.02	4.42	5.42
Average	3.64	3.86	3.95	4.01	4.37	5.39
LU_1 of method	±0.04	±0.04	±0.05	±0.12	±0.57	±0.33

Methanol might also be expected in methyl bromide. The addition of 0.55% by weight of methanol lowered the entire boiling range by approximately 0.1° C. Larger amounts, 0.73 and 0.97%, tended to raise the distillation temperature, although the starting temperature of even these mixtures was below that of the original. These data show that methanol forms a constant boiling mixture with methyl bromide. The exact composition of the constant boiling mixture cannot be determined from these data but it must be near 0.55% by weight of methanol, as this mixture gave uniform lowering of the distillation temperatures throughout the entire range. These data indicate that the boiling range is of little value in detecting the presence of small amounts of methanol in methyl bromide.

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Determination of Phosphorus in Hexaethyl Tetraphosphate and Tetraethyl Pyrophosphate

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From the many published methods for determining phosphorus, a rapid and reliable procedure has been chosen that is especially applicable to technical grades of tetraethyl pyrophosphate, including so-called hexaethyl tetraphosphate. Conversion of the organic phosphorus by alkali-nitrate fusion and dilute nitric acid digestion is followed by a colorimetric determination in which the yellow molybdivanadophosphoric acid method of Misson is used, as modified by Kitson and Mellon.

INCREASED interest in the use of hexaethyl tetraphosphate and tetraethyl pyrophosphate as agricultural insecticides and the variety of procedures used in their commercial manufacture have made rapid and reliable analytical methods for their determination highly desirable. This paper describes a method for determining the phosphorus content of these materials.

Of the known methods for the conversion of organic phosphorus compounds to orthophosphates, the alkali-fusion method described by Clark (*1*), with certain modifications, has been found by the authors most suitable from the standpoint of ease and rapidity. In order to avoid time-consuming operations to attain constant weight, gravimetric determination of the phosphate was discarded in favor of a colorimetric procedure. The blue-color method of Dickman and Bray (*2*) was inapplicable because of interference of the nitrate ion from the nitric acid used in conversion of pyrophosphate to orthophosphate. However, the yellow molybdivanadophosphoric acid method of Misson (*5*), as modified by Kitson and Mellon (*4*), proved both rapid and reliable. The entire procedure, from the weighing of the sample to reading of the color, can be run in about an hour.

REAGENTS

A. Sodium Hydroxide–Potassium Nitrate Fusion Mixture. Four parts of powdered sodium hydroxide and one part of powdered potassium nitrate were intimately mixed in a mortar. Potassium hydroxide may be used in place of sodium hydroxide.

B. Nitric acid, C.P. grade, 70%.

C. Nitric acid, dilute (1 to 2).

D. Ammonium hydroxide, C.P. grade, 28 to 29%.

E. Ammonium vanadate (0.25%). In 500 ml. of warm water 2.5 grams of ammonium vanadate were dissolved, the solution was cooled, 20 ml. of concentrated nitric acid were added, and the mixture was diluted to 1 liter.

F. Ammonium molybdate (5%). Fifty grams of ammonium molybdate were dissolved in 1 liter of warm water.

PROCEDURE

Preparation of Standard Graph. Twice-recrystallized potassium dihydrogen phosphate (43.9 mg.) was weighed out on a small piece of cigaret paper and transferred to a 100-ml. volumetric flask, and the flask was made up to the mark with water and shaken. This solution therefore contained 0.1 mg. of phosphorus per milliliter. Aliquots of 1 to 10 ml. were measured into a 50-ml. volumetric flask from a 10-ml. buret that could be estimated to 0.01 ml. Five milliliters each of the 1 to 2 nitric acid, ammonium vanadate, and ammonium molybdate were added in that order, and the flask was made up to the mark with water and shaken. The solution was allowed to stand for at least 10 minutes, and then a portion was poured into a clean, dry photometer test tube and the color was measured in a photometer. The color was measured in this laboratory in a photoelectric photometer, employing a No. 46 blue filter (optical centroid at about 460 millimicrons) and a solution of reagents C, E, and F as a blank to balance at 100% transmittance. The results were plotted on semilogarithmic paper as per cent transmittance against concentration of phosphorus and the straight line obtained provided a standard graph for reference of all analyses made on the same instrument with the same reagents.

Fusion of Unknown Sample. From 40 to 50 mg. of hexaethyl tetraphosphate or tetraethyl pyrophosphate were weighed by means of a weighing pipet (specially designed to handle hygroscopic liquids) (Figure 1) into a small platinum crucible of about 7-ml. capacity, and approximately 0.9 gram of the alkali-nitrate fusion mixture was added. A small glass scoop holding the required amount was found very convenient for adding the fusion mixture. The crucible was covered and gently heated over a small burner until foaming ceased, and then more heat was applied until the mass became a clear liquid. The progress of the fusion, which usually requires 5 or 6 minutes, was followed by gently removing the crucible cover at about 2-minute intervals. Upon cooling, the mass was dissolved from the crucible and cover with 75 ml. of hot distilled water, transferred to a 125-ml. Erlenmeyer flask, acidified to Congo red with concentrated nitric acid, and slowly simmered until the volume was 50 ml. (about 25 minutes). After cooling, the solution was made just acid to litmus by careful addition of ammonium hydroxide, transferred quantitatively to a 100-ml. volumetric flask, made up to the mark with water, and shaken. A 5-ml. aliquot of this solution was transferred to a 50-ml. volumetric flask, 5 ml. each of 1 to 2 nitric acid, ammonium vanadate, and ammonium molybdate were added, and the solution was made up to the mark and allowed to stand.

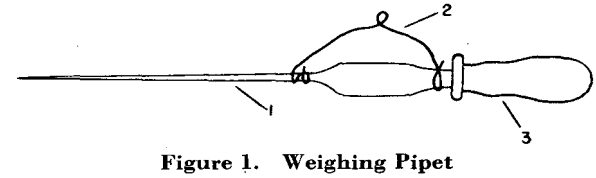

Figure 1. Weighing Pipet

1. Glass tube with fine capillary tip
2. Wire holder
3. Rubber bulb

The percentage of light transmittance was then measured in the photometer and referred to the standard graph. The concentration of phosphorus per milliliter of solution was read from the graph, and the percentage of phosphorus in the unknown sample was calculated as follows:

$$\% \text{ of P} = \frac{\text{amount of P} \times 2000}{\text{mg. of unknown sample}}$$

Table I. Percentage of Phosphorus in Samples Tested

Product	Calcd.	Found
Triethyl orthophosphate, $(C_2H_5)_3PO_4$ (purified)	17.01	17.06
Hexaethyl tetraphosphate, $(C_2H_5)_6P_4O_{13}$		
Schrader[a]	24.47	25.20
Woodstock[b]	24.47	26.28
Tetraethyl pyrophosphate, $(C_2H_5)_4P_2O_7$		
Schrader[c]	21.35	23.96
Woodstock[d]	21.35	22.25
Purified	21.35	21.86

[a] $POCl_3 + 3(C_2H_5)_3PO_4 \longrightarrow 3C_2H_5Cl + (C_2H_5)_6P_4O_{13}$
[b] $P_2O_5 + 2(C_2H_5)_3PO_4 \longrightarrow (C_2H_5)_6P_4O_{13}$
[c] $POCl_3 + 5(C_2H_5)_3PO_4 \longrightarrow 3C_2H_5Cl + 3(C_2H_5)_4P_2O_7$
[d] $P_2O_5 + 4(C_2H_5)_3PO_4 \longrightarrow 3(C_2H_5)_4P_2O_7$

Fused mixtures of alkali hydroxides and nitrates attack platinum and prolonged fusion in platinum, especially at higher temperatures, is very undesirable. In the authors' experience, however, the brief fusion of the small quantity of fusion mixture resulted in a loss in weight of the crucible of only a few milligrams after 35 separate fusions. Gold is much less attacked than platinum by fused alkali hydroxides and nitrates. If the fusion is to be repeated many times, the use of a gold crucible might be preferable.

DISCUSSION OF RESULTS

Triethyl orthophosphate, purified by fractionation, was used to check the accuracy of the procedure before any unknown samples were run. The percentage of phosphorus found for all materials tested is shown in Table I. In each case the average value found was the result of at least three determinations, and excellent checks were obtained in all cases. Theoretical per cent phosphorus is on the basis that the reactions shown in the table were carried out quantitatively, except in the cases of the true compounds triethyl orthophosphate and tetraethyl pyrophosphate (purified). The samples of hexaethyl tetraphosphate and tetraethyl pyrophosphate tested were high in phosphorus content, a fact that is further borne out by the values for refractive index and ethoxyl determination reported previously (*3*) for these samples.

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Analysis of 1,2,3,4,5,6-Hexachlorocyclohexane for Gamma Isomer

A Polarographic Method

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A polarographic method is described for the determination of the gamma isomer content of the new insecticide—1,2,3,4,5,6-hexachlorocyclohexane. Under the conditions employed, the gamma isomer is the only one of the five isomers (alpha, beta, gamma, delta, and epsilon) that is reduced at the dropping mercury cathode. Comparative results are given for the bioassay method and for the method described in this paper.

RECENTLY 1,2,3,4,5,6-hexachlorocyclohexane (benzene hexachloride) has been announced as an insecticide that promises to become of considerable importance to agriculture. The present technical material is a mixture of at least five of the sixteen possible stereoisomers, of which the so-called gamma isomer is by far the most biologically active. The activity of the gamma isomer is so marked and superior to that of the other isomers that the evaluation of hexachlorocyclohexane is usually expressed in terms of the per cent gamma content, and the latter is regarded as an index of the biological activity (*5*).

Development work in both the manufacturing and the biological fields has been hampered by lack of rapid analytical methods. Analysis by biological assay has been employed and should probably be considered as the final test of any insecticidal material. However, this method does not lend itself to high accuracy and is further limited by the requirements of a rather specialized technique and the fact that it is time-consuming. Daasch (*1*) has recently described an infrared method which should prove valuable, particularly as it permits the determination of all five isomers.

The present paper describes a polarographic procedure for the determination of gamma isomer. It is based upon the observation that the gamma isomer is the only one of the five isomers (alpha, beta, gamma, delta, and epsilon) that is reduced at the dropping mercury electrode under the conditions employed. Apart from a brief reference by Keller *et al.* (*2*), no description of this approach has appeared in the literature.

REAGENTS AND MATERIALS

All reagents were of C.P. quality.

The hexachlorocyclohexane isomer samples used in this work possessed the following melting points: alpha isomer 157–158° C.; beta isomer 180–196° C. (sublimes); gamma isomer 112.8–114° C.; delta isomer 138–139° C.; epsilon isomer 217–219° C. (obtained through the courtesy of the Dow Chemical Co., Midland, Mich.). These values are in good agreement with those obtained by other workers (*4*, *5*). The values for alpha, gamma, and delta isomers are corrected capillary melting point values. The sublimation range value for the beta isomer was obtained by using a Fisher-Johns melting point block. These isomers were separated from the commercial material by the recrystallization process described by Slade (*5*). A sample of one of the heptachlorocyclohexane isomers (melting point 85–86° C.) was also available for study (obtained through the courtesy of U. S. Department of Agriculture, Beltsville, Md.).

The potassium chloride–sodium acetate buffer solution was prepared by dissolving 2 grams of potassium chloride and 20 grams of sodium acetate trihydrate in 50 ml. of water. A solution of 0.10 gram of carpenter's glue in 20 ml. of hot water was prepared separately, then added to the sodium acetate solution and diluted to 100-ml. volume. This solution was prepared fresh, daily.

Stock solutions of the beta, delta, and epsilon isomers were prepared by dissolving 0.4000 gram of the isomer in 80 ml. of acetone and diluting to 100-ml. volume with distilled water. A gamma isomer stock solution was prepared by transferring 0.060 gram of gamma isomer to a 100-ml. volumetric flask, adding 60 ml. of acetone, and diluting to the mark with distilled water.

The isomer-mix stock solution was prepared by weighing out 0.280 gram of alpha isomer, 0.040 gram of beta isomer, and 0.040 gram of delta isomer, dissolving in 80 ml. of acetone, and diluting to 100 ml. with distilled water. This solution together with varying amounts of the gamma stock solution was intended to approximate the composition of commercial hexachlorocyclohexane. The addition of the epsilon isomer was omitted because of the lack of a suitable amount of this material.

The potassium chloride–alcohol-water mixture was prepared by dissolving 0.010 gram of carpenter's glue in 20 ml. of hot water, diluting to 110 ml. with distilled water, adding 2 grams of

potassium chloride, and diluting to 250-ml. total volume with ethyl alcohol (95% grade).

APPARATUS

A Leeds & Northrup Electro-Chemograph was used to obtain polarographic data. The dropping mercury cathode possessed an m value of 1.73 mg. per second. The drop time was 4.5 seconds at −0.5 volt in the electrolyte that was employed. Data were obtained using a 22,222-ohm shunt across the galvanometer circuit. When this shunt is used, the 100-unit scale of the chart corresponds to 20 microamperes.

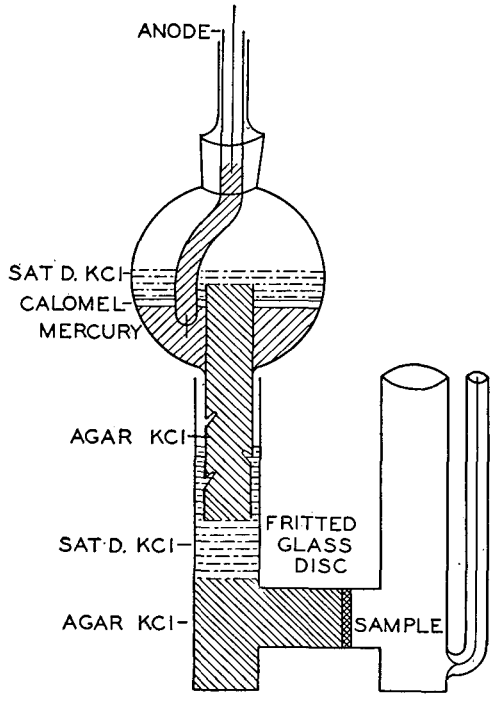

Figure 1. H-Cell and Calomel Reference Assembly

An H-cell of the type described by Lingane and Laitinen (*3*) was employed with an auxiliary saturated calomel cell as a separate unit. Electrical contact between the left and right arms of the H-cell and the calomel cell was maintained through a potassium chloride–saturated agar plug (see Figure 1).

A bath with temperature maintained at 25° ± 0.5° C. was used in obtaining all polarographic data.

Oxygen interference was removed by the use of oil-pumped nitrogen. Because of the volatile nature of the solvent, two bubblers were used in the nitrogen train to ensure proper saturation of acetone vapor. The first of these was a large 25-cm. (10-inch) gas-washing bottle containing acetone, which was placed alongside of the bath at room temperature; the second was a small bubbler of the absorption bulb type and was partially immersed in the bath. A solution similar in composition to the sample solution was introduced into this bubbler to prevent either enrichment or dilution of the test solution contained in the H-cell.

EXPERIMENTAL DATA

Calibration Using Pure Isomers. Preliminary polarographic runs indicated that the alpha, beta, delta, and epsilon isomers show no appreciable reduction at the dropping mercury cathode. Thus Figure 2 (curves 1, 2, 3, and 4) shows the data obtained on the epsilon, beta, delta, and isomer-mix (alpha, beta, and delta) stock solutions when 10-ml. aliquots were treated with 20 ml. of acetone-water mixture (60-40 ratio) and 5 ml. of sodium acetate buffer and diluted to 50-ml. volumes with potassium chloride–alcohol-water mixture. The beta, delta, and epsilon isomers showed no reduction beyond that which was typical of the condenser current. The isomer-mix stock solution showed a slight reduction somewhat in excess of the condenser current. A blank correction for this reduction was made in the calculation of wave height.

The effect of the presence of the gamma isomer is shown in Figure 2 (curves 5 and 6). These data were obtained by transferring known volumes of gamma stock solutions to volumetric flasks of 50-ml. capacity, adding 10 ml. of isomer-mix stock solution, diluting to 30-ml. volume with acetone-water mixture (60-40 ratio), and making up to 50-ml. volume with sodium acetate buffer and potassium chloride–alcohol-water mixture as above.

The standard curves were determined in an isomer mixture which approximates the mixture found in commercial hexachlorocyclohexane. Under these conditions the maximum in the polarographic wave was obtained at a voltage of −1.70 volts against the saturated calomel electrode. It was noted that the polarographic wave was very much more drawn out than the waves normally encountered in inorganic work. For this reason the half-wave potential was not calculated.

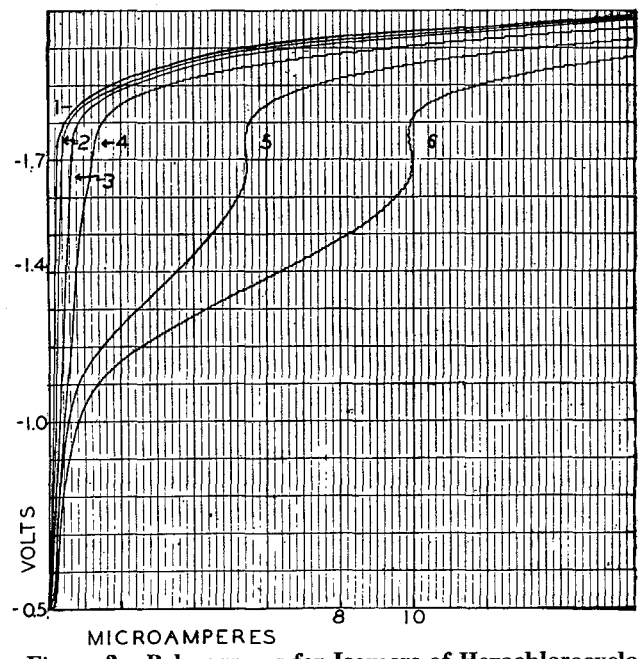

Figure 2. Polarograms for Isomers of Hexachlorocyclohexane

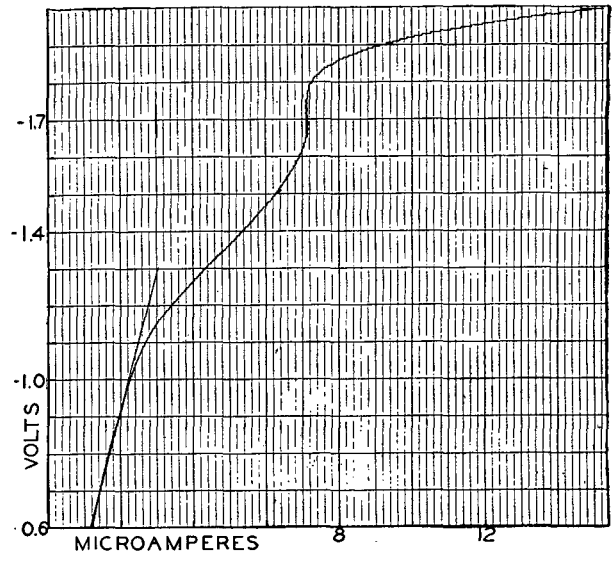

Figure 3. Gamma Isomer Polarogram for Technical Hexachlorocyclohexane

An empirical method was developed for determination of the wave height. Using this method for wave height determination, a straight-line relationship was obtained between galvanometer deflections and gamma isomer content throughout the range 0 to 12 mg. of gamma. On the basis of the aliquoted sample, this corresponds to a concentration range of 0 to 30%. Beyond this point the curve deviates from a straight line because of the shift in the base line slope of the polarogram. These data were used as a calibration curve.

Use on Technical Grade Samples. Effect of Heptachlorocyclohexane. The application of the above technique to technical grade hexachlorocyclohexane indicated the presence of an impurity not present in a simple mixture of hexachlorocyclohexane isomers. The presence of this impurity showed itself by an initial displacement from the galvanometer zero at −0.5 volt and a marked change in the base line slope (see Figure 3). Its presence made the interpretation of the polarogram difficult, as the drawing of the base line to determine the wave height was clouded with uncertainty.

Experimentation indicated that the same effect was produced when a solution of the four isomers was modified by adding a small amount of material produced in laboratory chlorination experiments and analyzing as heptachlorocyclohexane. This material showed a chloride content of 75.9% (theoretical for $C_6H_5Cl_7$ 76.3%). It is believed to be a mixture of the isomeric heptachlorocyclohexanes. The wave obtained by the polarization of a solution containing 4 and 8 mg. per 50 ml., respectively, of this heptachlorocyclohexane is indicated in Figure 4. A similar polarographic wave was obtained on a pure sample of one of the heptachlorocyclohexane isomers (melting point 85–86° C.), which was separated from the technical hexachlorocyclohexane by chromatographic means (*4*).

A study of the heptachlorocyclohexane polarographic wave indicated that the additive effect produced upon the normal gamma isomer wave can be eliminated, if the base line slope drawn along the lower portion of the curve (using the −1.0 volt locus as the point of departure) is extended to −1.30 volts and then drawn parallel to the voltage axis. Using this method of calculation, satisfactory agreement for gamma isomer content was obtained on synthetic mixtures of isomers modified by the addition of various amounts of heptachlorocyclohexane. These data are summarized in Table I and Figure 5.

This study indicates that the polarographic approach can serve as an absolute method for the determination of gamma isomer.

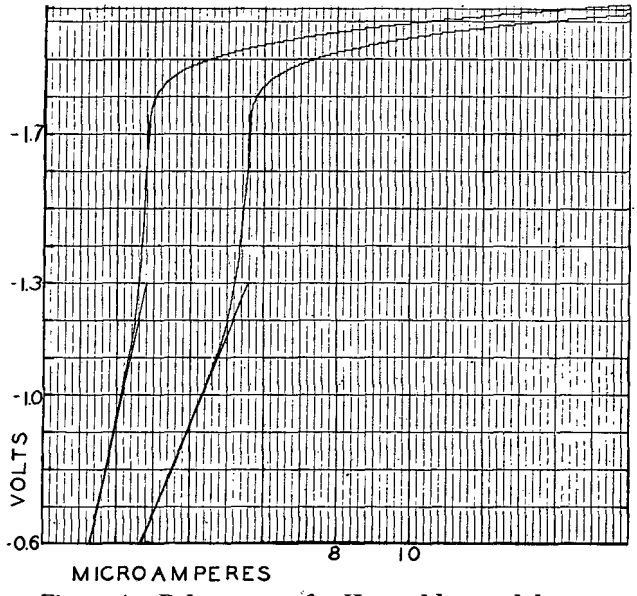

Figure 4. Polarograms for Heptachlorocyclohexane

Table I. Effect of Heptachlorocyclohexane

Heptachlorocyclohexane Added, Mg.	Wave Height		Gamma Found, %	Gamma Present, %
	From curve	Corrected		
0.0	21.2	18.2	12.3	12.0
4.0	20.5	17.5	11.8	12.0
8.0	20.0	17.0	11.5	12.0

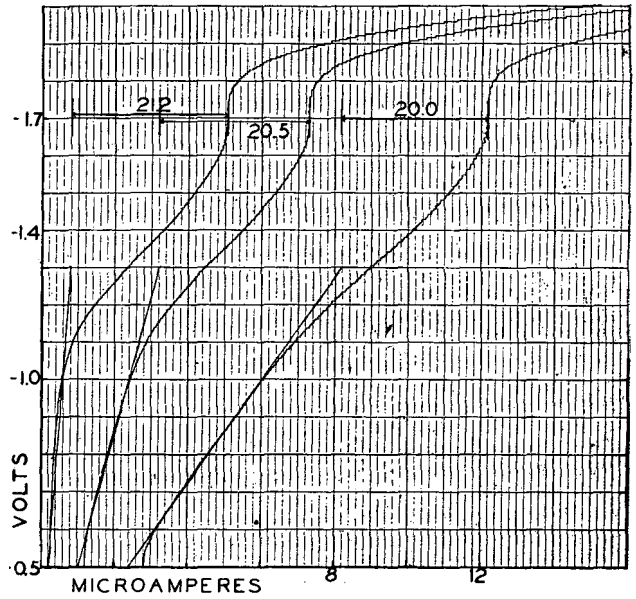

Figure 5. Gamma Polarograms, Showing Effect of Heptachlorocyclohexane

Results Obtained for Gamma Isomer Content. Using this method it was possible to interpret the curves obtained on commercial samples. A typical curve for technical grade material is shown in Figure 3.

The method as finally adopted consisted of the solution of a 0.4-gram portion of sample in 80 ml. of acetone and dilution to a 100-ml. volume with water. A 10-ml. aliquot of this solution was transferred to a 50-ml. volumetric flask, and 20 ml. of 60-40 acetone-water mixture were added, followed by potassium chloride–sodium acetate buffer solution and potassium chloride–alcohol-water mixture in the amount described above. Approximately 15 ml. of this solution were transferred to the H-cell, and the dropping electrode was put in place. The solution was then thoroughly deoxygenated by a 15-minute gassing with nitrogen and the polarogram was obtained through the range −0.5 to −2.0 volts. The wave height was obtained by extending the base line slope to −1.3 volts and then drawing a line parallel to the zero current line. The perpendicular distance between this line and one drawn through the wave maximum at −1.7 volts (see Figure 3) was taken as the wave height. This value, after correction for blank, when multiplied by the milligrams of gamma per scale unit as determined in the standardization and divided by the sample weight, gave the percentage gamma present.

Results obtained are shown in Table II. Repeated analyses of the same samples indicated that the method possesses a precision of ±0.5% gamma isomer. Bioassay values as determined against house flies, also shown, possess a precision of ±1 to 2% gamma isomer.

These results are representative of a total of approximately fifty comparisons. Approximately 25% of the results agree within 1%, 75% agree within 2.0%, and some 25% show a discrepancy of 2 to 3%. That the polarographic values are invariably low indicates the possibility that the bioassay technique is measuring the toxicity of other ingredients (apart from the isomer) present in the technical material. This is further corroborated by the observation that the best agreement between

Table II. Comparison of Polarographic with Bioassay Results

Sample	Per Cent Gamma Isomer		
	Polarograph	Bioassay	Difference
1	10.9	12.1	−1.2
2	11.6	13.5	−1.9
3	11.6	13.2	−1.6
4	10.2	12.2	−2.0
5	10.2	12.5	−2.3
6	11.7	12.0	−0.3
7	11.9	14.0	−2.1
8	10.4	11.8	−1.4
9	11.0	11.8	−0.8
10	10.9	13.5	−2.6
11	10.8	12.7	−1.9
12	11.2	12.1	−0.9
13	9.8	10.4	−0.6
14	9.3	9.6	−0.3
15	10.2	10.7	−0.5
16	11.5	12.8	−1.3
17	12.9	13.0	−0.1

Table III. Effect of Heptachlorocyclohexane on Comparisons between Bioassay and Polarographic Values for Gamma Isomer

Base-Line Slope	Av. Difference between Bioassay and Polarograph, %	No. of Samples Included in Av
2 or below	1.8	10
2–3	1.6	21
3 or above	1.1	8

polarographic and bioassay results was obtained in those cases where the heptachlorocyclohexane impurity (as evidenced by the slope of the polarographic wave) was low. The data in Table III indicate the evidence obtained on this point.

The evidence is not clear-cut and there were exceptions in each group; however, the data definitely show that best agreement is obtained in cases where the heptachlorocyclohexane impurity is low. This would indicate that the heptachlorocyclohexane possesses a degree of biological activity. This has been experimentally verified, although the degree of activity was not sufficient to account for the discrepancy. Possibly we are dealing here with a synergistic effect.

The method is primarily intended for the analysis of technical samples in the 13% gamma range. With proper calibration and choice of sample size it can be extended to the analysis of samples containing larger amounts of gamma or to dust preparations of lower gamma content. In the latter case a polarographic examination of all the components of the dust mixture is desirable in order to determine their effect upon the polarographic wave.

SUMMARY

A polarographic method is described for the determination of gamma isomer in hexachlorocyclohexane. The accuracy and precision of the results are ±0.5% on 13% gamma isomer samples and the method is believed to be more reliable and specific than the bioassay technique.

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Rapid Evaluation of Solvent Extraction Processes

New Liquid-Liquid Extractor

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A convenient continuous liquid-liquid extractor for evaluation of solvent extraction processes and for routine analytical procedures may be used with solvents heavier or lighter than the extracted liquid. A new value, the half extraction volume, for the comparison of the efficiency of continuous extractions is described. The half extraction volume is related to the distribution factor and is easily and rapidly determined by use of the new extractor. The characteristics of the extraction during the entire course of the operation may be followed. Examples of the use of the extractor and the half extraction volume are given.

THE value of solvent extraction processes in analytical chemistry has been recognized for many years and large numbers of separations accomplished by means of batch or continuous extractions have been reported. Certain limitations have restricted their use in cases where the inorganic, organic, or metal-organic materials under consideration were only slightly soluble in the given solvent.

Where the distribution factor is great, batch extractions may be used to advantage, for a few batches are sufficient to effect the desired separation. These extractions are carried out with some type of separatory funnel and each batch extraction may usually be made in a reasonably short time. However, in many cases the distribution factor is not great and the batch process is undesirable, for the required large number of extractions makes the method slow and tedious and experimental error is increased as an increasing number of extractions is made. Quantitative batch extractions are also difficult to accomplish if one of the components is viscous. Under such conditions as these, continuous extractions are desirable. Most of the continuous extractors described in the literature require long periods for the separation of the desired constituents. Continuous extractors such as those of Ashley and Murray (*1*), McNair (*8*), and Quick (*9*) permit the extracting solvent to rise or fall through the solution undisturbed. The apparatus described by Hossfeld (*6*) incorporates a stirrer.

CONTINUOUS, LIQUID-LIQUID EXTRACTOR

The apparatus illustrated in Figure 1, designed for the study of continuous liquid-liquid extractions of inorganic and metal-organic compounds with various immiscible solvents, is convenient also for routine use in standard analytical separations. It

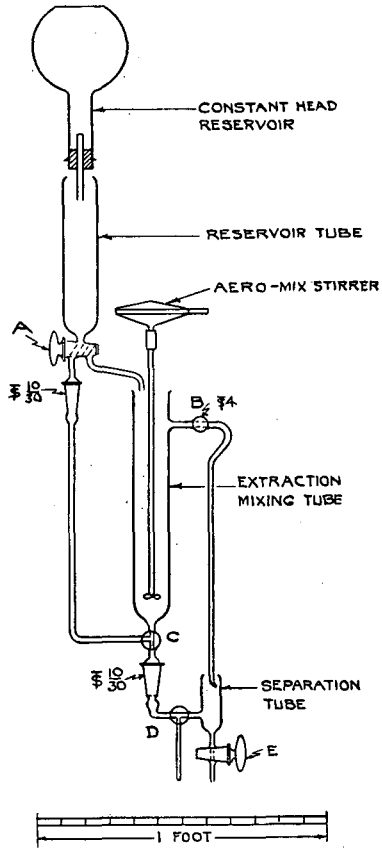

Figure 1. Extraction Apparatus

C and *D* are ₹, 3-way T stopcocks

permits speedy separations with excellent precision in cases where the distribution factor is low. It is possible to continue the extraction until the desired degree of separation has been effected, and the characteristics of the continuous extraction during the complete procedure may be determined. Any change in the distribution factor with concentration may be easily discovered. The efficiencies of various solvents for the extraction of a given material may be readily determined.

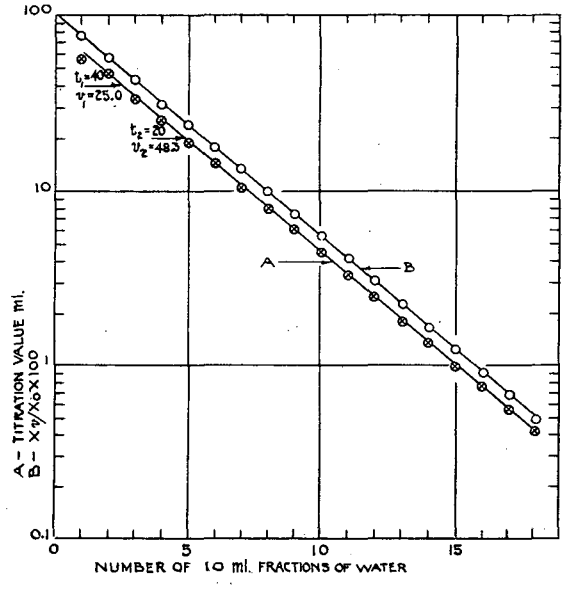

Figure 2. Water–Boric Acid–Isoamyl Alcohol

$V = v_2 - v_1 = 23.3$ ml.

The apparatus was constructed from Pyrex with standard-taper stopcocks and ground-glass joints. The parts were made interchangeable, so that only one portion, the extraction mixing tube, need be replaced in order to convert from the extraction of a large to a small volume of liquid. It is convenient to have several mixing tubes of various capacities. The apparatus may be used with solvents either heavier or lighter than the liquid being extracted. It may be set in operation simply, and after initial adjustments have been made, further changes are seldom required. An inexpensive Aero-Mix stirring motor was used to operate the glass stirrer. This increased the effective liquid-liquid surface and permitted more rapid extraction than simple gravity flow. The apparatus may be dismantled readily when cleaning is necessary.

When used with light extracting solvents the vertical delivery and reservoir tubes were filled with the solvent. In order to have a constant head of solvent in the reservoir tube, a 500-ml. flask fitted with a one-hole stopper and a short length of tubing was held inverted over this tube. This may also be constructed entirely of glass if desired. The solution to be extracted was measured into the extraction mixing tube to about 3.75 cm. (1.5 inches) below the outlet tube at *B*. Stirring was begun, tap *C* was opened partially into the extraction tube, and the solvent flow rate was regulated by tap *A*. The extracting solvent passed through tap *B* and the removal from the settling tube was controlled by tap *E*.

When the apparatus was used with heavy extracting solvents, taps *A*, *B*, *C* and *E* were closed and the solution to be extracted was measured into the extraction tube. Tap *D* was opened horizontally. The stirrer blades were placed about 11.25 cm. (4.5 inches) above tap *C* for the extraction tube illustrated. The stirrer was started and the extracting solvent permitted to drop into the top of the extraction tube. When about a 2.5-cm. (1-inch) layer of clear solvent had collected at the bottom of the extraction tube, tap *C* was partially opened and the liquid drawn off at the desired rate. The rate of flow of solvent was regulated by *A*. Tap *E* was used to measure the extract into the desired container.

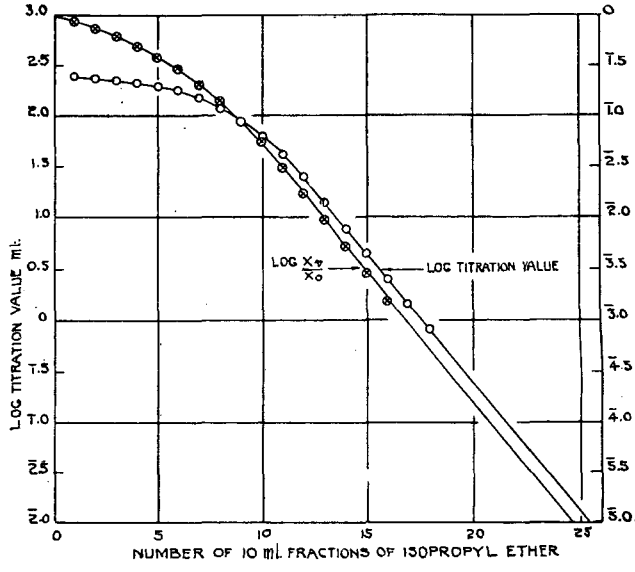

Figure 3. Isopropyl Ether–Ferric Chloride

If any of the original solution passed over with the extracting solvent, a separation of the two phases occurred in the separating tube, the lighter phase being removed by tap *D* and the original solution being returned to the extraction tube. However the extraction was usually easily regulated, so that this phenomenon seldom occurred after the operator had become experienced in carrying out the extractions. If dark colored solutions were used, the interface between the phases in the extraction tube was easily made visible by the use of a spot lamp.

Half Extraction Volume. The efficiency of continuous extraction processes may be conveniently evaluated by comparisons of

a simple practical value. This value, the "half extraction volume," V, is the volume in milliliters of extracting solvent required to decrease the amount of extracted constituent to one half its former value. It can be obtained as follows:

A series of aliquots of the extract is drawn off from the separation tube and the amount of extracted constituent in each is estimated by titration. The logarithm of these titration values is plotted against the number of aliquots (or the volume in milliliters) of the extracting solvent; this is done conveniently on semilogarithmic paper. Examples of this plot are shown in Figures 2, 3, and 4; usually a straight line is obtained (the upper part of Figure 3 is an exception). Two points on the rectilinear part of this plot are now chosen, such that the titrating value corresponding to one of them is equal to half the titration value corresponding to the other; the difference of the corresponding abscissas (converted to milliliters of solvent) gives the value of the half extraction volume, V. V makes possible a numerical comparison of changing conditions in continuous extractions, and the distribution of the solute between the two liquid phases during a continuous extraction may be estimated from it.

Distribution Factor. The distribution factor, k, may be obtained directly from the relation:

$$k = \frac{0.693W}{V}$$

where k is the concentration of solute in the extracting solvent divided by the concentration of solute in the original solution, W is the volume in millliters of original solution, and V is the half extraction volume.

The difference between the distribution factor, k, and the distribution constant, K, should be emphasized. The distribution factor is the number characteristic of the easily reproducible conditions of the continuous extraction, whereas the distribution constant is the value obtained for equilibrium with respect to the passage of the dissolved constituent between the two phases under specific conditions. The distribution factor for a particular system closely approaches the distribution constant if the value of the latter is low.

There is an unfortunate lack of agreement in the literature concerning the definition of the term distribution constant or coefficient, K, obtained for equilibrium conditions. The values of K are generally and more conveniently reported as numbers greater than 1 in favorable extraction processes and this custom is followed in the report. However, many published articles and texts give formulas for the derivation of a K which is the reciprocal of the distribution constant (*2, 3, 4, 7*).

Development of Distribution Factor Equation. In many cases the amount of the unextracted solute in the original solution during a continuous extraction was found to be proportional to the amount of extracted constituent of successive fractions of solvent; and the plot of the logarithm of X_v/X_0 versus v gave a straight line, as shown in Figure 2, where

X_v is the weight in grams of unextracted solute after passage of v ml. of solvent
X_0 is the weight in grams of solute present in the original solution before extraction
v is the total volume in ml. of extracting solvent
The equation of this straight line may be expressed as

$$\log \frac{X_v}{X_0} = -Cv + a$$

When $\frac{X_v}{X_0} = 1$, $v = 0$ and hence $a = 0$.

Therefore, $\log \frac{X_v}{X_0} = -Cv$, where C is a constant, or

$$\frac{X_v}{X_0} = e^{-Cv} \qquad (1)$$

For constant, but not necessarily equilibrium, conditions the weight of solute in the original solvent, X_n, after n batch extractions may be expresssed as

$$X_n = X_0\left[\frac{W}{\frac{vk}{n} + W}\right]^n \qquad (2)$$

where X_0 is the weight in grams of solute in the original solution before extraction.

Equation 2 is similar to that given by Griffin (*3*), except that k is defined as above to give a number greater than 1 for most practical extractions.

Equation 2 becomes $\frac{X_n}{X_0} = e^{\left(\frac{-kv}{W}\right)}$ (3)

and X_n approaches X_v when $n \xrightarrow{\text{in limit}} \infty$ as shown by Griffin (*4*).

By comparing Equations 1 and 3 it may be seen that

$$Cv = \frac{kv}{W}$$

and Equation 1 becomes $\frac{X_v}{X_0} = e^{\left(\frac{-kv}{W}\right)}$

When the amount of solute in the original solution is reduced to one half its original amount, then

$$\frac{X_v}{X_0} = 0.5 = e^{\left(\frac{-kV}{W}\right)}$$

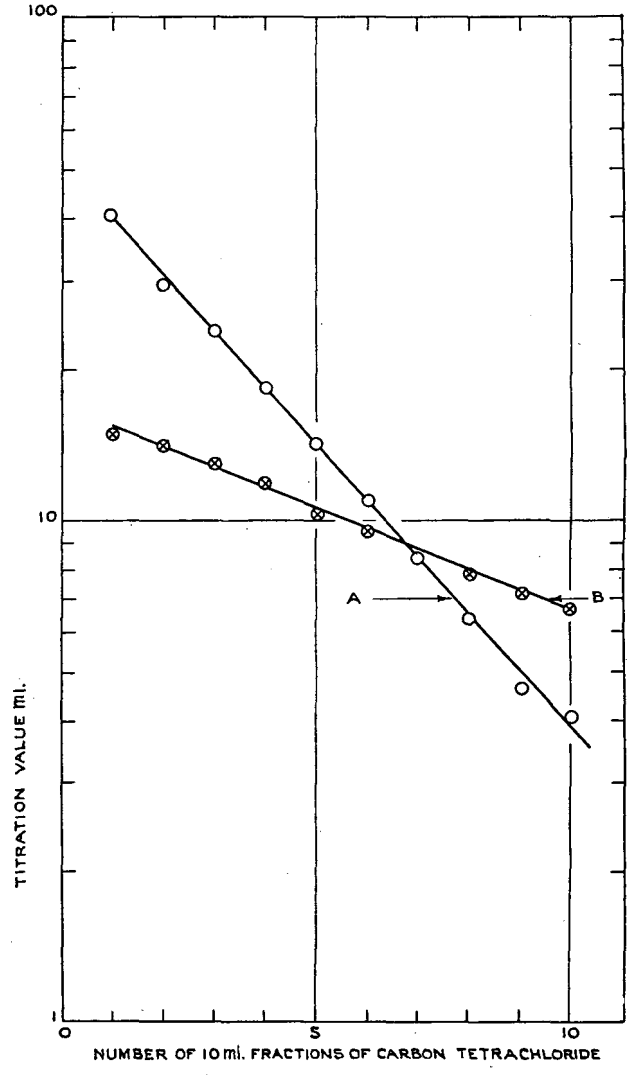

Figure 4. Carbon Tetrachloride–Aqueous Iodine

A. 8 grams of KI per liter
B. 24 grams of KI per liter

where V is the half extraction volume.

Also $\log_e 0.5 = \frac{-kV}{W}$ and $k = \frac{0.693W}{V}$

EXPERIMENTS AND RESULTS

Various standard extractions have been made with the apparatus described above, and the characteristics of three of these are given below.

Water Extraction of Boric Acid from Isoamyl Alcohol. This is an example of a system having a low distribution constant in which the solvent is denser than the extracted solution. Griffin and von Saaf (*4*) have reported the results of batch water extractions of isoamyl alcohol containing boric acid.

In the authors' experiments 90 ml. of isoamyl alcohol solution containing 1.36 grams of boric acid were extracted with water. The heavier water extract was measured into 10-ml.volumetric flasks. The amount of boric acid in the alcohol and the water extracts was determined by titration with standard 0.1 N sodium hydroxide, mannitol, and phenolphthalein, as described by Hollander and Rieman (*5*).

The extractions were carried out at a rate of 2 ml. of water per minute. After passage of 100 ml. of water, 94.5% of the boric acid was extracted.

Results are given in Figure 2. The titration values of the successive extraction aliquots and the calculated values of X_v/X_0 (multiplied by 100 for convenience) are plotted against the corresponding numbers of the fractions of extracting solvent. From these curves the value of 23.3 ml. may be derived for V and $k = \frac{0.693 \times 90.0}{23.3} = 2.68$. Griffin and von Saaf (*4*), using the value 1/3.15 which is their experimentally determined distribution constant, showed that the amount of boric acid remaining unextracted in 100 ml. of solution after treatment with 100 ml. of water divided into an infinite number of infinitesimally small portions of water is 4.29%. With this latter number it may be shown graphically that an ideal continuous extraction would have a value of 22.0 ml. for V with the conditions they described.

Isopropyl Ether Extraction of Ferric Chloride. These experiments illustrate the results obtained with a system in which the solvent is less dense than the extracted solution.

Ninety milliliters of an aqueous solution containing 10.0 grams of iron (III) and 8.2 N in hydrochloric acid were treated in the extraction tube with redistilled isopropyl ether. During the initial stage the solution was stirred slowly, so that small droplets of the aqueous layer were not carried over with the isopropyl ether. After about 60 ml. of ether had been collected it was possible to increase the speed of the stirrer. Occasionally two ether layers formed after about 90 ml. of ether had been collected. This condition sometimes persisted while 40 to 50 ml. more were collected. This phenomenon has been observed by other authors (*10*). Both solvent layers were collected. The rate of extraction in various experiments was 2 to 10 ml. per minute. The 10-ml. ether fractions measured in volumetric flasks were transferred with water to conical flasks and the ether was evaporated on a steam bath. Any reduced iron was oxidized with 10 ml. of 3% hydrogen peroxide on the steam bath. The excess peroxide was destroyed by boiling for 4 minutes. The solutions were transferred to 100-ml. volumetric flasks and diluted to volume, and aliquots were taken for analysis. These aliquots (10 or 25 ml.) were treated with 4 ml. of 6 N hydrochloric acid and 3 grams of potassium iodide and allowed to stand in stoppered flasks for 5 minutes. After dilution with 75 to 100 ml. of water the iodine released was titrated with 0.1 N sodium thiosulfate. Starch solution was added near the end point. A blank of 0.15 ml. was determined and subtracted from each titration.

The results given in Figure 3 show that the values for V and hence for k vary during the first part of the extraction, but then become constant. Extrapolation of the curve predicts that approximately 75 micrograms of iron (less than 0.001% of the original amount) would remain in the aqueous layer after extraction by 250 ml. of ether. This was confirmed by colorimetric determination of the aqueous solution by the thiocyanate method.

Carbon Tetrachloride Extraction of Iodine. Carbon tetrachloride extractions of two 0.02 N aqueous iodine solutions containing different concentrations of potassium iodide were carried out. Results plotted in Figure 4 illustrate the manner in which the changes in V and k are made readily evident under varied conditions of extraction. The values for k for the two iodine extractions were:

(1) $k = \frac{0.693 \times 90}{26.6} = 2.34$ (8 grams of KI per liter)

(2) $k = \frac{0.693 \times 90}{75} = 0.83$ (24 grams of KI per liter)

During these experiments it was found that the experimental data for the various extractions could be duplicated with excellent precision.

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Determination of Specific Gravity of Pigments

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A method is proposed for the determination of the specific gravity of pigments. It must be used with reservations when applied to very finely divided pigments.

IN CONNECTION with research on the specific surface of pigments the authors required a convenient and accurate method for determining the specific gravities of a variety of pigment which has a diameter of less than 1 micron. Determination of the specific gravities of such solids is complicated by the difficulty of removing gases that are held on the very large surfaces by adsorptive forces and are mechanically entrapped among the particles. Gooden (*2*) developed a method of removing the entrapped air by outgassing while applying mechanical vibration to the liquid-pigment mixture in the pycnometer in which the specific gravity determination was made. Baker and Martin (*1*) used Archimedes' method, suspending the pigment in kerosene.

Table I. Specific Gravity of Pigments

Pigment	Specific Gravity	% Variation from Average	Pigment	Specific Gravity	% Variation from Average
$BaSO_4$[a]	4.505	0.07	TiO_2, anatase	3.856	0.05
	4.501	0.02	(0.428 μ)[b]	3.854	0.00
	4.500	0.04		3.848	0.16
	4.499	0.07		3.853	0.03
	4.505	0.07		3.856	0.05
Av.	4.502	0.05		3.854	0.06
Irox	5.246	0.04	ZnO, U.S.P. 12	5.652	0.05
(0.806 μ)[b]	5.250	0.04	(1.035 μ)[b]	5.654	0.02
	5.244	0.08		5.657	0.04
	5.251	0.06		5.654	0.02
	5.248	0.00		5.660	0.09
Av.	5.248	0.04		5.655	0.04
Lithopone	4.191	0.02	ZnS	4.001	0.00
(0.471 μ)[b]	4.191	0.02	(0.479 μ)[b]	4.006	0.12
	4.195	0.07		4.000	0.02
	4.191	0.02		3.996	0.12
	4.195	0.04		4.001	0.00
Av.	4.192	0.05		4.001	0.05
SiO_2[a]	2.371	0.04	Zinc tetroxychromate[a]	3.999	0.03
	2.373	0.04		4.001	0.03
	2.372	0.00		4.003	0.08
	2.372	0.00		4.000	0.00
	2.370	0.08		3.999	0.03
Av.	2.372	0.03		4.000	0.03

[a] Diameter of pigments not included because it was impossible to interpret and measure photomicrographs.
[b] Average diameter $\bar{d}_i$ of pigments.

Their procedure required a preliminary drying of the pigment for 2 hours and subsequent removal of gases by heating and stirring the kerosene-pigment mixture for 0.5 hour.

The method reported here is a modification of that of Baker and Martin. A dilute water solution of a wetting agent was used, instead of kerosene, in order to eliminate the incompatibility effect between adsorbed water and kerosene. The preferential wetting action of the wetting agent eliminated the necessity of heating the liquid-pigment mixture. This modified method consists of weighing the pigment in air, dispersing it in a suitable water solution, centrifuging the slurry, and finally suspending the centrifuged sample in the same water solution and weighing the immersed system. An exhaustive investigation of the dispersing and flocculating effect of 100 wetting agents on the pigments used in this study showed marked specific effects between pigment and wetting agent. Inspection of the data indicated that Hornkem No. 1 (Horn Research Laboratories, Inc., Long Island, N. Y.) was among the more universally effective dispersing agents for these pigments. This was tried first and it was not necessary to try other agents. The pigments listed in Table I were studied. Approximate diameters, where it was possible to interpret photomicrographs, are given. Of these pigments the silicon dioxide had the smallest specific surface and the zinc tetroxychromate the largest. These pigments are all hydrophilic. The method has not been tested with hydrophobic pigments such as carbon black, Prussian blue, etc. It might well be that hydrophobic pigments would require other wetting agents of a more specific nature, or that it would be necessary to use oil-soluble wetting agents dissolved in a suitable oil.

PROCEDURE

The immersion liquid was a 0.2% solution of Hornkem No. 1 in water. The absolute density of this solution as determined in a pycnometer was 0.9973 gram per ml. at 25° C. and compares with a density of 0.9971 gram per ml. for water at this temperature. This difference in density would give an error of 0.02% in the specific gravity of a solid if it were assumed that the density of the solution was the same as the density of water. The actual density of the solution was used in calculating the specific gravities presented below.

All weighings were made on an analytical balance, the weights being corrected for the buoyancy of air. The immersion liquid was contained in a 500-ml. Dewar tube which was placed above one pan of the balance. This Dewar tube was fitted with a cork stopper which had openings for the suspension wire of the specific gravity tube and thermometer. This maintained the temperature to within 0.01° of 25.00° C. for 0.5 hour. The specific gravity tube, illustrated in Figure 1, consisted of a glass tube having a glass handle that was fitted into small bulges in the walls of the tube. It was suspended by means of a platinum wire (No. 24) and contained a glass stirring rod which remained in the tube during all weighings. The weights of this apparatus in air and in the liquid were constants and were subtracted from the corresponding weights for the apparatus containing the pigments, in obtaining weight data for the pigments.

Ten grams of dry pigments were placed in the specific gravity tube, enough liquid was added to cover the powder, and the mixture was stirred to give a uniform slurry. The apparatus was centrifuged for a few minutes at a maximum relative centrifugal force of 1350 times gravity (2000 r.p.m. on the authors' centrifuge) until the pigment was well packed in the bottom of the tube. It was then immersed in the liquid in the Dewar tube and weighed after coming to temperature. The specific gravity was calculated from the formula

$$S.G. = \frac{W_1}{W_1 - W_2} s.g. \qquad (1)$$

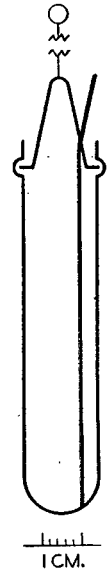

Figure 1. Apparatus

where W_1 = weight of pigment in air, W_2 = weight of pigment immersed in the solution, and $s.g.$ = density of the solution.

DISCUSSION

The data obtained are tabulated in Table I. These pigments were commercial pigments and the data are not to be considered as accurate densities for pure substances. The method gives results with a limit of uncertainty of the average of $LU_{av.} = \pm 0.0024$ and a limit of uncertainty under the best conditions of $LU_1 = \pm 0.0078$ (3).

An error inherent in the application of Archimedes' principle to the determination of the specific gravity of solids having very large surfaces, as is the case with pigments, is due to the weight and volume of the adsorbed layer on the surface of the solid. This condition exists when the solid is suspended in pure liquids as well as when it is suspended in solutions. That even pure liquids are adsorbed is evidenced by the large heat of wetting when the pigment is immersed in the liquid. This error is some function of the difference in density of the adsorbed layer and the surrounding solution. Present information does not suffice to evaluate the density of the adsorbed layer when it is composed of either adsorbed pure liquid or adsorbed solute from a solution.

In order to illustrate a possible magnitude of the error involved due to the adsorbed layer of Hornkem on the pigments, the following approximate calculations have been made.

The weight of Hornkem adsorbed on the pigments in Table I has been determined. The data indicate that for these pigments the silica has the smallest specific surface and the zinc tetroxychromate has the largest. On the silica 0.01 gram of Hornkem was adsorbed per 10 grams of pigment and on the zinc tetroxychromate, 0.08 gram per 10 grams. It was assumed that the density of the adsorbed Hornkem film was 0.8 gram $ml.^{-1}$. The formula for calculating the specific gravity of the pigment can be written

$$S.G. = \frac{\text{weight of solid}}{\text{weight of liquid displaced}} = \frac{\text{weight of solid}}{\text{vol.} \times s.g. \text{ of solution displaced}} \qquad (2)$$

Assuming that the density of the solution is approximately 1 gram $ml.^{-1}$, the formula simplifies to

$$S.G. = \frac{\text{weight of solid}}{\text{vol. of solution displaced}} \qquad (3)$$

The volume of the solution displaced is the volume of the pigment plus the volume of the adsorbed Hornkem. The volume of the solution displaced by 10 grams of silica, as experimentally determined, is 10/2.372 = 4.2158 ml. This is actually the volume of 10 grams of silica plus 0.01 gram of adsorbed Hornkem. The assumed volume of the adsorbed Hornkem is 0.01/0.8 = 0.0125 ml. The difference, 4.2158 − 0.0125, gives 4.2033 ml. as the volume of the silica. Its density then becomes 10/4.2033 = 2.379 gram ml.$^{-1}$, which corresponds to the value 2.372 in Table I.

A similar calculation for the zinc tetroxychromate pigment gives the true density as 4.167, corresponding to 4.000 in the table.

The corrections are based on a guess as to the density of the adsorbed layer, and the nearer the density of the adsorbed layer is to the density of the surrounding solution, the smaller will be the correction. It follows that the values for the specific gravities in Table I are based on the assumption that the densities of the adsorbed layers are the same as the densities of the surrounding solutions and that the method must be applied to very finely divided pigments with reservations. The same reservations must be considered when the pigment is immersed in a pure liquid.

The pigments considered in this report are all essentially hydrophilic. With hydrophobic pigments other wetting agents might have to be employed. If water-soluble wetting agents having a specific affinity for hydrophobic pigments are not available, it might be necessary to use oil-soluble wetting agents of the required characteristics dissolved in some organic solvent.

No solution is suitable for use with pigments that have appreciable solubilities in the solvent.

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Colorimetric Determination of Certain Dinitro Aromatics

In the Presence of the Corresponding Mononitro Compounds

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Procedures are presented for the estimation of small amounts of dinitrobenzene, dinitrochlorobenzene, dinitronaphthalene, dinitrotoluene, and dinitroxylene in their mononitro derivatives and for dinitrocresol in mononitrotoluene. The first five depend upon the color formed when an acetone solution of the sample is treated with dilute alcoholic sodium hydroxide; the last, upon the color of the sodium salt of dinitrocresol in aqueous solution. In all cases, the color intensity is read with a photoelectric colorimeter. A single determination can be run in 10 to 15 minutes and results are reproducible within about ±3% of the amount of dinitro compound present.

THE well-known color reactions of certain dinitro aromatic compounds when treated in acetone solution with alcoholic sodium hydroxide were intended, as originally published (*3*), for qualitative purposes only. Preliminary experiments with these reactions showed that in general the hue, intensity, and stability of the color formed are largely influenced by the concentration of sodium hydroxide and, to a lesser extent, by the amount of water present. To attain quantitative reproducibility it is therefore necessary to exercise proper care in the preparation of the caustic reagent.

Even with accurately prepared solutions of sodium hydroxide in ethanol (denatured, 2B formula) somewhat erratic results were obtained and in many cases the colors were too fugitive for quantitative purposes. The substitution of ammonia for sodium hydroxide was ineffective; the colors formed, if any, were of too low tinctorial power, while potassium hydroxide yielded colors of such a low order of stability as to be entirely hopeless from the analytical standpoint. It was found, however, that if the caustic reagent is prepared from sodium monoxide (Na_2O) (obtainable from E. I. du Pont de Nemours & Co., Inc., R & H Chemical Division, Niagara Falls, N. Y.) instead of the hydroxide, the resulting colors are sufficiently stable for a colorimeter reading, presumably because of dehydration of the alcohol. Solutions of sodium hydroxide in methanol give feeble colors, if any. The different dinitro compounds studied require somewhat different concentrations of sodium hydroxide to produce maximum color.

The colorimeter used in this work was a Cenco-Sheard-Sanford Photelometer (Catalog No. 12,335) provided with 1-cm. fused glass cells and the Cenco light filters normally supplied with the instrument. A calibration curve for each determination was prepared. Because all the methods are identical in principle and manipulation (except for dinitrocresol which is treated separately), differing only in the amounts of sample, reagent, etc., used, the following procedure is expressed in general terms with a subjoined table giving the required quantity of sample, the aliquot used, and reagent composition for each specific case.

SODIUM HYDROXIDE REAGENT

Using a 25-ml. graduated cylinder, measure into a dry 8-ounce bottle the desired amounts of 95% ethanol and acetone, add the sodium monoxide (*not* peroxide), swirl gently for 2 minutes, cool to 20° to 25° C., add sufficient acetone (also at 20° to 25°) to bring the volume to 200 ml., shake vigorously for about 10 seconds, and filter through a folded paper into a dry bottle. The filtrate must be perfectly clear and only slightly yellowish. This reagent is not stable for longer than about one hour.

PROCEDURE

Pipet (or weigh, in the case of dinitronaphthalene) the desired amount of sample into a 100-ml. volumetric flask, allowing the pipet to drain 30 seconds with its tip touching the flask neck. Dilute to 100 ml. with acetone. Into a 50-ml. beaker pipet 25 ml. of the sodium hydroxide reagent, add the proper aliquot of the

sample solution, stir briefly, and immediately read the color intensity on the colorimeter, using the appropriate light filter and some of the sodium hydroxide reagent in the comparison cell. Obtain the corresponding percentage of dinitro compound from the calibration curve.

Table I. Sample and Reagent Quantities

Determination	Sample	Aliquot	Sodium Hydroxide Reagent: Acetone	Sodium Hydroxide Reagent: Alcohol	Sodium Hydroxide Reagent: Na_2O	Light Filter
	Ml.	*Ml.*	*Ml.*	*Ml.*	*G.*	
DNB	1	1	10	10	2	Green[a]
DNCB	2	1	10	10	2	Green
DNT	1	1	10	10	2	Red
DNX (meta and 3°)	2	1	15	15	3	Red
DNX (ortho-para)	4	1	15	15	3	Red
DNN	5 grams	1	15	15	3	Blue

[a] Maximum transmittances of these filters were 410, 525, and 610 millimicrons, respectively, for the blue, green, and red.

NOTES

The preparation of the sodium hydroxide reagent is fairly critical. The initial volumes of acetone and alcohol should be accurate within 0.5 ml. and the sodium monoxide weighed on a rough balance within 0.1 gram. Sodium peroxide should not be used because of its violent reaction with alcohol.

These procedures will easily detect 0.05% of the compound sought and cover the range up to about 5%. However, the sensitivity and scope can be changed within wide limits by suitable variation in sample size and aliquot taken.

The colorimeter readings should be taken as soon as possible (preferably within 0.5 minute) after the sample aliquot is added to the reagent because the colors change fairly rapidly.

In the early experimental work, it was found that the different isomers of a given dinitro compound yield more or less different colors in both hue and intensity when treated by the above procedure. For this reason, it is essential that the calibration curve be prepared on the basis of standard samples containing the same isomers and in substantially the same proportions as will be present in the samples to be analyzed. To accomplish this, the following general procedure, illustrated in the case of nitroxylene was adopted.

Table II. Calibration Data for Determination of Dinitroxylene in Nitrated 3°, *m*- and *o,p*-Xylenes

% DNX Present	Photelometer Reading: 3°	Meta	Ortho-para
0.0	99.5	100.0	99.4
1.0	85.0	84.0	82.0
2.0	71.7	70.0	67.8
4.0	51.0	49.5	46.8
8.0	25.8	25.6	22.8
12.0	13.1	13.4	12.0

PROCEDURE

The xylene was nitrated with 115% (theory) of nitric acid, in the form of a mixed acid containing 26% nitric and 61% sulfuric acid, at 45° to 50° C. The product, containing about 15% dinitro, was fractionally distilled through a 30-cm. (12-inch) packed column at 1- to 5-mm. pressure until most of the mononitro had passed over. The residue was then transferred to a small flask and distilled practically to dryness at 1-mm. pressure without fractionation. The dinitro content of this distillate was calculated from its total nitrogen value, determined by titanous sulfate titration, on the assumption that it contained only mono- and dinitroxylene. The mononitro distillate was then refractionated, a small foreshot and about 20% residue were discarded, and the dinitro-free middle cut was combined with the distilled dinitro to produce a series of standards of known dinitro content. Three such preparations were made, starting, respectively, with 3° distillation range commercial xylene containing all the isomers, purified *m*-xylene, and the mixture of ortho and para remaining after removal of the meta from 3° xylene by selective sulfonation. These three series of standard samples were analyzed by the colorimetric method previously described (Table II).

The 3° and meta figures are so nearly the same that their average may be taken to serve for both without significant error, but a separate curve is required for the ortho-para.

A series of known composition mixtures of mono- and dinitrotoluene was prepared in similar manner, and three analyses were made of each. The results are given in Table III to illustrate the degree of concordance that can be expected in multiple determinations upon a given sample.

Table III. Calibration Data for Determination of Dinitrotoluene

% DNT Present	Photelometer Reading: A	B	C
0.0	99.3	99.6	99.5
1.0	66.0	66.2	66.0
2.0	44.0	44.3	44.5
3.0	30.0	30.0	30.0
4.0	20.2	20.5	20.8
6.0	10.1	9.9	9.6

It is thus evident that in spite of the rather poor color stability, consistent results are readily possible. This conclusion is further confirmed by the fact that in the hands of routine analysts these methods have produced entirely satisfactory results for more than 3 years.

The 99.5 reading instead of 100 for 0% dinitrotoluene is due to a slight color developed by mononitro and not to accidental contamination by dinitrotoluene, for exhaustively purified *o*- and *p*-nitrotoluene both gave the same reading separately. In some cases, this effect is much more pronounced, pure mononitronaphthalene (approximately 94% alpha and 6% beta isomer) giving a reading of 76.0.

Incident to the mononitration of toluene, a small amount of dinitrocresol is formed (*1*, *2*, *4*) which must be removed by an alkaline wash to avoid explosion hazard in the subsequent fractional distillation. The following procedure was devised to estimate traces of dinitrocresol in washed nitrotoluene and thus check the efficiency of the washing operation.

Procedure for Dinitrocresol. Pipet 10 ml. of the clear oil into a 100-ml. volumetric flask, add 75 ml. of 0.5% aqueous sodium hydroxide solution, shake vigorously for 1 minute, dilute to the mark with more of the sodium hydroxide solution, mix thoroughly, let stand about 5 minutes to settle, and filter a portion of the aqueous layer. The filtrate must be perfectly clear. Read its color intensity against the 0.5% sodium hydroxide solution using the blue filter. The sample quantity specified is such as to make the analysis cover the range up to about 0.1%, 0.001% being readily detectable.

To prepare the standard samples for calibration, wash 1 liter of crude mononitrotoluene direct from the nitrator several times with water to remove the mineral acids, then with successive 50-ml. portions of 1% sodium hydroxide until the extracting solution is only slightly colored. Combine the caustic extracts, acidify with hydrochloric acid, filter off the precipitated material, which will consist of all the nitrated phenol derivatives normally present in crude mononitrotoluene, and dry in a vacuum desiccator. Distill the washed mononitro oil through a short fractionating column at 10- to 20-mm. pressure, discarding a 10% foreshot and 30% residue. Prepare a series of standard samples covering the desired range by dissolving weighed portions of the crude dinitrocresol in the distilled oil.

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Storage and Titration with Oxygen-Sensitive Solutions

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A convenient apparatus for the storing and quantitative dispensing of highly oxygen-sensitive solutions of chromous ion involves storage of the active solution under a pressure of nitrogen or other inert gas. Sufficient volume of the inert gas is provided above the stored solution and the buret to serve as a compensator when the reagent is introduced or removed from the storage or buret systems. This method is of particular advantage to workers who use oxygen-sensitive reagents only occasionally and must maintain such a reagent ready for use at all times. Two forms of the apparatus are described and the relative merits of each are pointed out. The effectiveness of the system is demonstrated by data which show that a highly sensitive chromous chloride–hydrochloric acid solution did not change in titer during more than 10 months.

A METHOD of protecting oxygen-sensitive reagents from the atmosphere during storage and use, by means of nitrogen gas under slight pressure, is described. The process, which is particularly advantageous to those who titrate with such a solution only intermittently, has been indicated in principle by Crowell and Baumbach (*2*) of this laboratory. These authors, however, did not realize the full possibilities of the principle, and workers are still publishing descriptions of the older, less satisfactory techniques, when confronted by the problem of excluding the atmosphere in analytical procedures.

The system most commonly reported in the literature for titration with oxygen-sensitive reagents involves the use of the Kipp generator for maintaining an oxygen-free atmosphere through the application of a slight pressure of hydrogen. Thornton and Wood (*7*) and Zintl and Rienacker (*8*) offer illustrations of this method. In more recent years cylinders of inert gases, with pressure-reducing valves, such as the carbon dioxide described by Stone and Beeson (*5*), have been used to replace the Kipp generator system. Particularly with nitrogen and to a lesser extent with hydrogen and carbon dioxide it is necessary to wash the inert gas with an oxygen absorber to prevent loss of the active reagent. The pressure-storage system, discussed in this paper, eliminates the necessity both of maintaining the pressure by Kipp or cylinder and of maintaining an oxygen removing system for the inert gas over long periods of time.

APPARATUS

The accompanying drawings illustrate two modifications of the apparatus which have been used with this inert gas pressure storage-dispensing system. Figure 1 indicates a type in which the gas pressure-compensating systems for the storage and the buret are separate.

The storage reservoir may be made from a round-bottomed flask. The buret, stopcocks, and gas and liquid lines are most conveniently made from the same kind of glass. The stopcocks should be one of the commercially available types for pressure work, though ordinary stopcocks can be converted into pressure stopcocks as described by Hamilton (*3*) and Connelly (*1*). The author has found the less elegant method, of simply winding No. 28 B. and S. gage copper wire in the groove of the stopcock plug in such a way as to produce a pressure against the shell of the stopcock, to be satisfactory.

In this type of apparatus (Figure 1) it is necessary to adjust the nitrogen pressure in the reservoir to slightly above that in the buret, so that the solution will rise to the proper height in the buret during the process of filling. The nitrogen pressure in the buret must be not only less than that in the reservoir but slightly above the atmospheric pressure in order to expel the solution in the process of titrating.

A second type of apparatus, used with the oxygen-sensitive reagents, is shown in Figure 2. The top of the buret is connected to the top of the reservoir. In addition, this modification has a single three-way pressure stopcock in place of the two stopcocks used in the type shown in Figure 1. This type also has the neck of the flask, used to make the storage bulb, located on the under side in a much better position for attaching the supporting clamp.

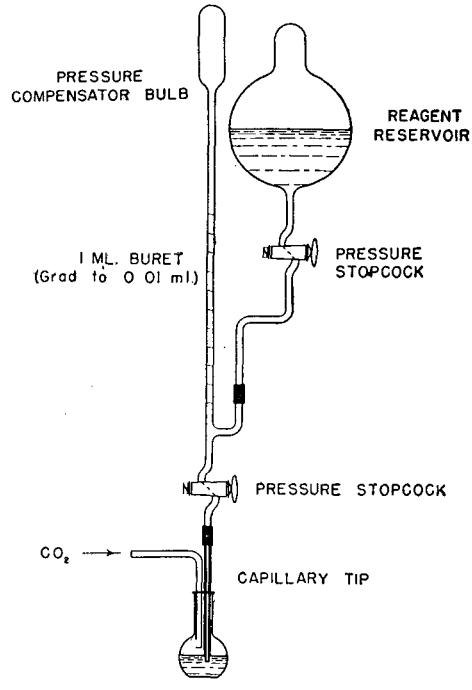

Figure 1. Pressure Differential Apparatus

The second type requires that the bottom of the reservoir be above the top of the graduations on the buret as the buret is filled by a gravity flow of the reagent. This gravity system has the advantage over the pressure differential system in that only one pressure adjustment is required in preparing the apparatus for use. It has the disadvantage that a careless operator may leave the buret cock open, allowing the liquid to empty completely, followed by the loss of the nitrogen pressure and subsequent diffusion of oxygen from the air into the storage bulb. With the other type, such a mistake requires only the removal of the oxygen from the buret, as the storage part of the system is unaffected by such an error.

Table I. Stability of Chromous Chloride–Hydrochloric Acid Solution under Pressure Type Storage

	Ml. of $CrCl_2$ Required to Titrate 0.900 Ml. of KIO_3		
	Oct. 10, 1946	May 19, 1947	Aug. 19, 1947
	0.302	0.303	0.303
	0.305	0.303	0.303
	0.304	0.303	0.295
	0.302	0.303	0.300
	0.302	0.303	0.300
			0.314
			0.315
			0.301
			0.300
Av.	0.303	0.303	0.303
Normality	0.0297	0.0297	0.0297

INTRODUCTION OF OXYGEN-SENSITIVE REAGENT

The technique of removing oxygen from the air-filled apparatus and introducing the oxygen-sensitive reagent under proper pressure of nitrogen is illustrated in Figure 3.

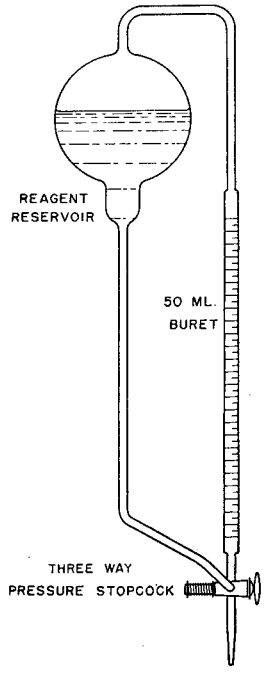

Figure 2. Gravity Type of Apparatus

The buret stopcock at the top of the figure corresponds to the one on the apparatus of Figure 2. At the start, the screw clamp, *D*, is closed while those at *B*, *C*, and *E* are open. Air, at approximately 1 atmosphere above the pressure of the room, is provided and vented through *B*. Now the screw clamp at *B* is gradually closed until the pressure in the bottle is sufficient to cause the chromic chloride–hydrochloric acid solution to flow up through the zinc amalgam reductor. The resulting chromous solution passes from *E* through *C*, clearing the line of air. [Details of the preparation and properties of chromous solutions for oxygen-sensitive work are discussed in (*4*, *5*, *6*).] Next the buret stopcock is opened and *C* is closed, causing the chromous solution to flow into the reservoir. The rate of flow is regulated by manipulation of the screw clamp at *B*. When sufficient chromous solution has been introduced into the reservoir and buret to absorb all the oxygen, both clamp *E* and the buret stopcock are closed. The apparatus is then shaken to complete the removal of the oxygen.

The absorption of oxygen has reduced the pressure in the reservoir, so that it is necessary to introduce additional nitrogen to make up for the loss in pressure. This is accomplished by opening the buret stopcock and the clamp at *D* and allowing nitrogen to enter the storage flask. A pressure-reducing valve on the nitrogen cylinder supplying this gas is used to regulate the pressure in the system to slightly above atmospheric pressure. The buret stopcock is closed again and the apparatus is shaken once more to ensure the absorption of any oxygen entering as an impurity in the nitrogen.

To clear the storage flask of the partially spent chromous solution and the excess nitrogen pressure it is only necessary to close *D* and open the buret stopcock and *C*. When this is done the system is adjusted to approximately 1 atmosphere pressure of nitrogen by venting the solution and gas through *C*. The apparatus is now ready to receive the oxygen-sensitive reagent. This is accomplished by closing *C*, opening *E*, and forcing the chromous reagent into the storage by turning down screw clamp *B*.

It is customary to discontinue filling the storage when it becomes half full of liquid because of the hazards resulting from the greater pressure if more of the solution is introduced. At this stage the nitrogen pressure in the reservoir is approximately 1 atmosphere above the room pressure and the reagent is ready for use.

A different technique has been used for filling such a system in certain cases, particularly one in which the apparatus was designed for dispensing water free from gaseous oxygen.

A 5-liter flask is equipped with a 100-ml. buret. Oxygen is removed from the system by alternately pumping out with an oil pump and filling with oxygen-free nitrogen until the desired reduction in the partial pressure of the oxygen has been achieved. Then distilled water, which has been boiled and cooled in a stream of nitrogen free from oxygen, is forced into the reservoir by the method described above for the chromous solution. It is apparent that the chromous reagent could be used in place of the vacuum technique to remove the oxygen if its use were followed by rinsing the portions of the oxygen-free water.

Other gases such as hydrogen may be used in place of the nitrogen, provided the solubility does not change too rapidly with pressure or temperature. Carbon dioxide is unsatisfactory for this reason. When it was used, bubbles appeared in the buret tip during titrations.

Rubber connections, such as the one shown in Figure 1, cause no difficulty due to the diffusion of oxygen through them, provided they do not lie between the stopcock and the reservoir. The custom of replacing the reagent in the lines with fresh solution before the first titration of any working period is effective in eliminating trouble from this source.

In titrating with chromous chloride–hydrochloric acid reagent the author has been accustomed to attach a capillary tip to the buret with a rubber connection. Such a tip is removed for the operation of introducing reagent into the reservoir; otherwise the solution flow is restricted by the capillary and the time required for filling is much increased. During titration, the capillary tip dips below the surface of the liquid in the titration flask and here the small diameter of the capillary is an advantage, as it restricts the diffusion between the reagent in the buret tip and the liquid of the titration flask. A stream of an inert gas is delivered over the liquid in the titration flask at a

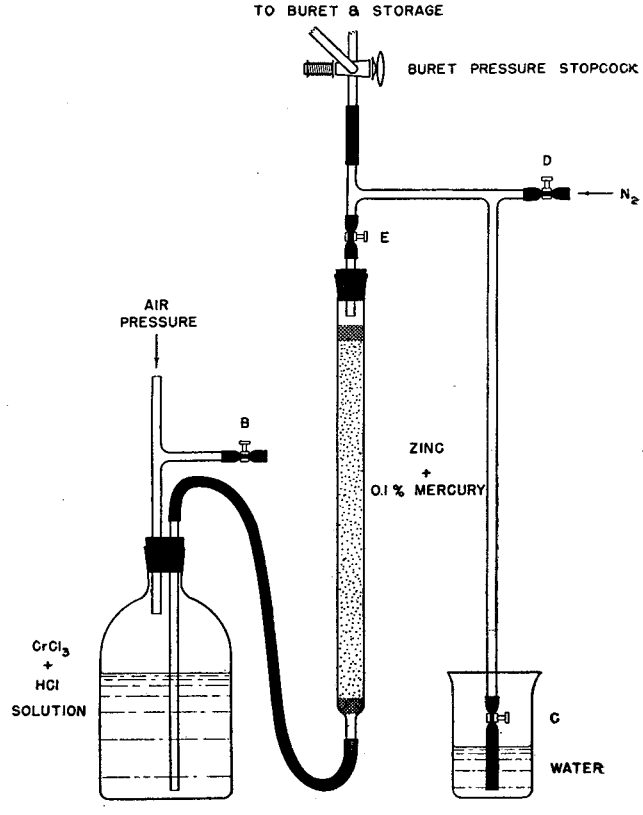

Figure 3. Buret-Filling Apparatus

rate of 200 to 300 ml. per minute to ensure exclusion of air during the titration. Commercial carbon dioxide is most satisfactory for this purpose, as because of the nature of its preparation it has a partial pressure of oxygen too low to interfere with ordinary quantitative work and does not need to be passed through a wash system for removing the oxygen. Commercial nitrogen may show oxygen up to a few tenths of a per cent and needs to be treated for the removal of this gas. Hydrogen is not so suitable for this purpose because of its greater diffusibility. The time required to eliminate the air from the titration flask at 200 to 300 ml. per minute rate of carbon dioxide flow should be determined by each worker. The author found that a 5-minute flow of carbon dioxide at the rate given above was sufficient for this work once the oxygen had been eliminated from the line leading from the carbon dioxide cylinder. However, a much longer period of flow was required to clear this line of oxygen at the beginning of any working period.

The effectiveness of this pressure storage system in protecting oxygen-sensitive solutions from the atmosphere is demonstrated by the data of Table I.

The reactions used in obtaining the data are indicated by the following equations.

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

$$3I_2 + 6Cr^{++} \longrightarrow 6Cr^{+++} + 6I^-$$

For this work the apparatus of Figure 1 was used. The buret was made from a serological pipet of 1-ml. capacity in which 0.01 ml. is represented by about 2 mm. in length. Thus the volumes could be estimated to approximately 0.002 ml. A sample of a 0.0100 *N* solution of potassium iodate was measured from a buret similar to the one described into a 15-cm. (6-inch) Pyrex test tube and brought to a boil over a gas flame. After the iodate solution had been cooled under a stream of carbon dioxide, chromous chloride–hydrochloric acid solution was added with the capillary tip delivering the chromous solution, below the surface of the iodate solution. Excess solid iodide and a drop of starch solution were added just before the end point was reached. The latter was exceedingly sharp, and the additions of reagent near the end point were less than a drop. Such small additions were possible because of the location of the capillary tip below the surface of the iodate solution. No iodine color appeared during the titration until the potassium iodide was added near the end point. This addition of the iodide near the end point kept the losses due to the volatility of the iodine at a minimum.

A standard solution of this reagent which does not change titer in more than 10 months is unusual. Even with this method of storage, solutions of higher concentrations in acid and chromous reagent might well deteriorate, owing to the reduction of the acid hydrogen by the chromous ion. This system requires no attention while not in use and yet is ready for use at a moment's notice. The facts that no inert gas supply has to be maintained outside the system, and that no oxygen-absorbing unit is required, represent important improvements.

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Spectrophotometric Determination of Phosphorus as Molybdiphosphoric Acid

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A spectrophotometric study of the molybdiphosphoric acid method for phosphorus was made using a Beckman spectrophotometer. The effect of various variables on the color development was determined and optimum conditions were ascertained. Conformity to Beer's law was found for concentrations from 0 to 25 p.p.m. of phosphorus, using 1.000-cm. absorption cells. The main disadvantage of this method is the interference caused by iron and silica. The recommended general procedure is rapid, convenient, and sensitive.

A YELLOW hue develops when excess molybdate solution is added to an acidified solution containing orthophosphate ions. The color may be attributed to the formation of molybdiphosphoric acid, $H_3[P(Mo_3O_{10})_4]$.

The colorimetric determination of phosphorus utilizing the molybdiphosphoric acid complex was first suggested by West-Knights (*10*) in 1880. As silicic acid complexes similarly, a procedure eliminating the deleterious effect by dehydration of the silica was developed by Lepierre (*4*). Several modified procedures (*2, 5, 7, 9, 11, 12*) have been developed, principally for the determination of the phosphate content of natural waters. Estes (*1*) studied the effect of certain salts on the color of molybdiphosphoric acid. Krumholz (*3*) made a colorimetric study of certain heteropoly complexes, including molybdiphosphoric acid, with a Pulfrich photometer (*6*). He studied the interrelationship of temperatures and the ratio of central ion (P) concentration to that of the coordinating group (MoO_3) on the extinction coefficient. Acidity also affected the extinction coefficient.

Although extensive use has been made of the formation of molybdiphosphoric acid for subsequent reduction to give the so-called molybdenum blue, and of the formation of molybdivanadophosphoric acid, insufficient attention seems to have been given to the molybdiphosphoric acid method itself. Therefore, a spectrophotometric study of the reaction involving the formation of this complex was made in connection with a study of the heteropoly blue reaction. The information presented in this article pertains only to the applicability of the molybdiphosphoric acid method for the determination of orthophosphate ions in solution.

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Table I. Interfering Diverse Ions

Ion	Added as	Amount Added *P.p.m.*	Error %	Permissible Amount *P.p.m.*
NH_4^+	NH_4NO_3	1000	−2.5	750
Ba^{++}	$Ba(NO_3)_2$	1000	−2.4	750
Bi^{+++}	$Bi(NO_3)_3$	40	Hue changes	0
Cu^{++}	$Cu(NO_3)_2$	200	−2.7	100
Co^{++}	$Co(NO_3)_2$	200	−1.0	200
Fe^{+++}	$Fe_2(NH_4)_2(SO_4)_4$	20	16.7	0
Pb^{++}	$Pb(NO_3)_2$	200	−0.5	200
Ni^{++}	$NiSO_4$	200	4.0	40
AsO_4^{---}	Na_2HAsO_4	40	17.8	0
BO_3^{---}	H_3BO_3	1000	2.7	750
Cl^-	KCl	1000	−3.2	500
F^-	NaF	40	−2.7	20
SiO_3^{--}	Na_2SiO_3	20	Over 100	0
WO_4^{--}	Na_2WO_4	1000	−35.0	0
VO_3^-	KVO_3	40	67.0	0

GENERAL EXPERIMENTAL WORK

Apparatus. All transmittancy measurements were made with a Beckman spectrophotometer and 1.000-cm. absorption cells. A blank solution (containing the reagents added) was used in the reference cell unless otherwise designated. The pH values were determined with a Beckman pH meter.

Solutions. A standard phosphate solution containing 0.1 mg. of phosphorus per ml. was prepared by dissolving 0.4395 gram of potassium dihydrogen phosphate in redistilled water and diluting to 1 liter.

A 10% sodium molybdate solution was prepared by dissolving 25 grams of sodium molybdate ($Na_2MoO_4.2H_2O$) in redistilled water, filtering, and diluting to 250 ml. The acids used were C.P. grade reagents.

Color Reaction. In acidic solution the orthophosphate ion condenses with molybdate ions to give molybdiphosphoric acid, $H_3[P(Mo_3O_{10})_4]$. This heteropoly complex has a yellow hue.

In order to study the effect of certain variables on the color development, the following procedure was used.

A definite volume of the standard phosphate solution was transferred by means of a microburet to a 100-ml. beaker, and the desired amount of acid was added. In the study of diverse ions a definite amount of solution containing each ion was added, followed by sufficient water to give a total volume of 50 ml. To this were then added 5 ml. of the solution of sodium molybdate, the solution was mixed, and the transmittancy readings were taken. The concentration of the desired constituent is expressed in terms of parts per million of phosphorus in solution prior to the addition of the molybdate reagent.

Molybdate Concentration. A series of solutions was prepared containing 25, 50, 75, 100, 125, 250, 500, and 1250 mg. of sodium molybdate. To each were added 2.5 ml. of the standard phosphate solution (0.25 mg. of phosphorus), 5.5 ml. of 2 *N* sulfuric acid, and enough water to bring the final volume of each solution to 55 ml. The transmittancy curves were obtained with water in the blank cell. These data indicate that absorption increases appreciably with concentration until there are 250 mg. of sodium molybdate. Thus, it is evident that a large excess of molybdate is necessary. In order to ensure sufficient molybdate for higher concentrations of phosphate ion, 500 mg. of sodium molybdate were selected as the most suitable amount to have present. Five milliliters of a solution containing 10 grams of sodium molybdate dihydrate in 100 ml. of solution fulfill this requirement when added to a 50-ml. sample.

Acid Concentration. The effect of various concentrations of sulfuric acid was determined with 5 p.p.m. of phosphorus and 5 ml. of the molybdate solution, containing 10 grams of sodium molybdate dihydrate per 1000 ml. of solution, in a final volume of 55 ml. A final acidity of 0.2 to 0.35 *N* (pH 0.9 to 1.25) gives maximum color development. Precise duplication of acidity is necessary for reliable results.

When the experiment was repeated with hydrochloric acid, it was found that a final acidity of 0.2 to 0.3 *N* gave maximum color development, but that beyond 0.3 *N* fading resulted. With nitric acid, a final acidity of 0.2 to 0.3 *N* resulted in maximum color development. This acidity corresponded to a pH of 0.5 to 0.8. A slight variation (±0.05 *N*) in nitric acid does not cause appreciable error. A final acidity corresponding to 0.25 *N* nitric acid was used in further applications of this method. This acidity was obtained by adding 5 ml. of a 2.8 *N* nitric acid solution in the procedure previously given.

Phosphorus Concentration. Figure 1 shows the effect of 1, 5, 10, 15, 20, and 25 p.p.m. of phosphorus. Conformity to Beer's law was found for transmittancy measured at 380, 400, or 420 mμ.

Temperature. A slight increase in color was detected when the sample was heated to 60° C., either before or after addition of the molybdate. Heating is not recommended because of increased possibility of introducing silica, and of decomposition of the complex in presence of high concentrations of certain salts. Development of color at room temperature is recommended.

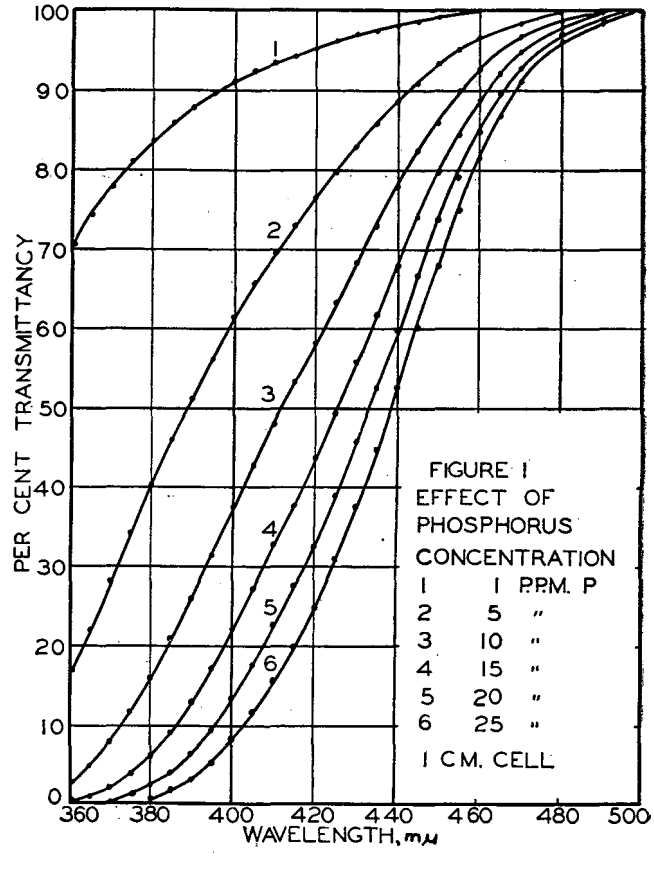
Stability of Color. Maximum color development is obtained almost immediately and the color is stable for at least 1 hour. Contamination with a minute amount of silica will cause a slow and gradual increase in intensity of color. The ease of silica contamination precludes the use of these solutions as permanent standards.

Diverse Ions. The effect of various diverse ions was studied with 10 p.p.m. of phosphorus. Transmittancy readings were taken at 380, 400, and 420 mμ in order to detect any changes in hue. Errors of less than 2% of phosphorus present were considered negligible. A negligible error was obtained with 1000 p.p.m. of the following ions: aluminum, beryllium, cadmium, calcium, magnesium, manganese (II), potassium, sodium, zinc, bromide, chlorate, sulfate, and nitrate.

Table I lists the permissible amounts of ions that were found to interfere. Because of the easy reducibility of molybdiphosphoric acid, any reductants such as iodide, tin (II), sulfite, and iron (II) ions should be absent.

Discussion. The amount of molybdate present is important. A large excess over the stoichiometrical amount results in an intensification of the color. This suggests the probability that the law of mass action applies to the formation of molybdiphosphoric acid.

In general, a pH of 0.5 to 1.2, depending on the acid used, is required for maximum development of color. Solutions 0.25 *N*



in nitric acid are recommended in determining phosphorus as molybdiphosphoric acid.

The procedure developed is more rapid and convenient, and conforms to Beer's law for a greater range (0 to 25 p.p.m. of phosphorus) of phosphorus concentration than the "molybdenum blue" method. It is not so sensitive as the latter method. Its main disadvantage is the interference caused by iron and traces of silica. Silica error can be eliminated by dehydration with nitric acid; but the error due to Fe^{+++} color, which presumably forms ferric molybdate, needs to be eliminated either through removal or complexation of the interfering ion.

RECOMMENDED GENERAL PROCEDURE

Sample. A representative sample is obtained containing 0 to 1 mg. of orthophosphate ions. Silica, if present, should be removed by dehydration with nitric acid (*8*). Any interfering ions present must be reduced to concentrations within limits specified in Table I. The solution is made neutral to litmus, 5 ml. of 2.8 *N* nitric acid are added, and the solution is diluted to 50 ml.

Desired Constituent. To 50 ml. of the above-mentioned solution 5 ml. of the 10% molybdate solution are added. The transmittancy is measured at 380, 400, or 420 mμ, the wave length depending upon the amount of phosphorus present and the sensitivity desired. Use of distilled water in the reference cell is recommended, provided it was also used as a reference in standardization.

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Determination of Unsaturation in Dehydrogenated Diethylbenzene

By Use of Mercuric Acetate

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A new method for the determination of the unsaturation in certain types of olefinic compounds is described, in which use is made of the well-known addition reaction of mercuric salts, especially mercuric acetate, to double bonds. The mercury in the addition compounds thus formed is titrated with standard ammonium thiocyanate solution. This method has proved satisfactory as an assay procedure for styrene and styrene derivatives such as ethylvinyl and divinylbenzenes, vinyltoluene, and α-methylstyrene.

DURING recent years divinylbenzene has seen an increasing demand for use in the synthetic rubber and plastic industries. It is produced by the dehydrogenation of diethylbenzene over suitable catalysts at elevated temperatures, which results in mixtures containing principally divinylbenzene, ethylvinylbenzene, and uncracked diethylbenzene.

These mixtures were usually susceptible to substitution when the unsaturation was determined by procedures involving the use of the halogens.

The well-known bromate-bromide procedure of Koppeschaar (*4*) gives erratic results owing to the substitution caused by the nascent bromine unless very carefully controlled conditions are maintained. The brominating procedure of McIlhiney (*5*), using bromine in carbon tetrachloride, did not offer much improvement over the bromate-bromide titration, even though it was possible to correct for substitution. The method of Wijs (*14*), using iodine chloride, and the method of Hanus (*1*), using iodine bromide, gave good results when the correct amount of excess halogen was used, but substitution occurred with a large excess of reagent. The Hübl method (*2*), using a solution of mercuric chloride and iodine in methanol (active agent, iodine chloride), gave reasonable results in most cases. A procedure for analytical bromination by the Kaufman method (*3*), using a solution of bromine and sodium bromide in methanol, is reported in the literature to give good results for styrene. However, because about one half of the bromine used undergoes the substitution reaction, it was not tried in the analysis of mixtures of divinylbenzene, ethylvinylbenzene, and diethylbenzene.

Because of the ease of substitution of the halogens in mixtures of divinylbenzene, ethylvinylbenzene, and diethylbenzene, it was decided to investigate the use of the well-known addition reaction of mercuric acetate with olefinic linkages as a measure of the unsaturation.

OUTLINE

Whitmore (*10*) states that aqueous mercuric salts add the groups —HgX and —OH to the double bond of olefinic compounds. A general equation for this reaction may be shown as follows:

$$RCH{=}CH_2 + HgX_2 + H_2O \longrightarrow RCHOHCH_2HgX + HX$$

the addition in general following Markownikoff's rule (*11*), and mercury going to the carbon having the most hydrogen atoms.

In 1919 Tausz and Peter (*9*) prepared the mercury compound of styrene by the use of an aqueous mercuric acetate solution.

Manchot (*6*) found that styrene reacted with aqueous mercuric acetate to give a mercury-containing product, which was perhaps a basic compound, in which the mercury was not firmly held.

In 1928, Priewe (*8*) reported that styrene reacted with mercuric acetate in acetic acid solution to form β-phenyl-β-acetoxymercuriethyl acetate.

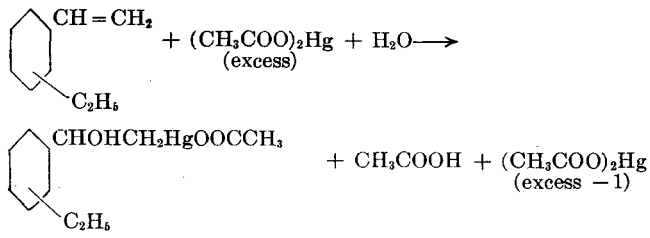
Wright (*15*) obtained α-acetoxymercuri-β-methoxy-β-phenylethane when he treated styrene with a methanol solution of mercuric acetate.

Nesmeyanov and Freidlina (*7*) made β-acetoxymercuri-α-hydroxyethylbenzene by reacting styrene with aqueous mercuric acetate.

Whitmore states that nearly all the compounds formed from unsaturated substances and mercuric salts are soluble and stable

in sodium hydroxide solution (*13*) but that they react readily with halogen acids to give back the original unsaturated compound (*12*).

In the procedure given below, a weighed sample of dehydrogenated diethylbenzene reacts with an excess of mercuric acetate dissolved in water containing 40% (by volume) 1,4-dioxane; the dioxane is used to increase the solubility of the addition compound:



The reaction is similar for divinylbenzene, the mercuric acetate adding to both vinyl groups.

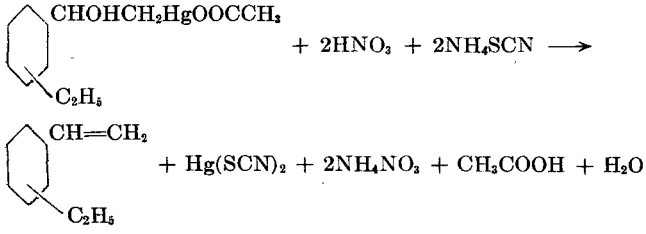
The excess mercuric ion is then precipitated as mercuric oxide by the addition of sodium hydroxide; the mercuric acetate addition compounds of ethylvinylbenzene and divinylbenzene are stable in the basic solution:

$$(CH_3COO)_2Hg + 2NaOH \longrightarrow HgO + 2CH_3COONa + H_2O$$

The mercury in the mercuric oxide is reduced to metallic mercury by adding 30% hydrogen peroxide and warming:

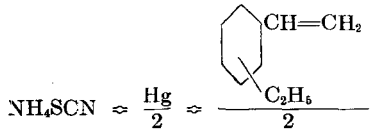
$$HgO + H_2O_2 \xrightarrow{NaOH} Hg + H_2O + O_2$$

The solution is then acidified with nitric acid and titrated with standard ammonium thiocyanate, using ferric alum indicator:



The titration reaction is similar for the addition compound of divinylbenzene.

As the total unsaturation owing to both ethylvinylbenzene and divinylbenzene is calculated as per cent ethylvinylbenzene:



Thus, pure divinylbenzene is equivalent to

$$\frac{\text{Mol. wt. of ethylvinylbenzene} \times 100}{\frac{\text{mol. wt. of divinylbenzene}}{2}} = \frac{132.196 \times 100}{\frac{130.18}{2}} = \frac{132.196 \times 100}{65.09} = 203.1\%$$

as ethylvinylbenzene

REAGENTS

Mercuric Acetate, approximately 0.27 *N*. Dissolve 44.0 grams of A.R. grade mercuric acetate in about 400 ml. of water and add 5.0 ml. of glacial acetic acid and 400 ml. of 1,4-dioxane. Dilute with water to exactly 1 liter.

1,4-Dioxane, 40% by volume aqueous solution made by dissolving 200 ml. of 1,4-dioxane in 300 ml. of water.

Sodium Hydroxide, approximately 4 *N*. Dissolve 82 grams of A.R. grade sodium hydroxide pellets in water and dilute to 500 ml.

Hydrogen Peroxide, 30%, or Superoxol.

Nitric Acid. Mix equal volumes of concentrated nitric acid (42° to 44° Bé.) and distilled water.

Ferric Alum Indicator. Dissolve 100 grams of ferric alum in water, add 12 ml. of concentrated nitric acid, and dilute with water to 1 liter.

Ammonium Thiocyanate, standard 0.1 *N*.

Care must be taken that all reagents are halide-free.

APPARATUS

Any shaker suitable for shaking 125-ml. glass-stoppered Erlenmeyer flasks.

PROCEDURE

For unsaturation above 50% as ethylvinylbenzene pipet 25.0 ml. of mercuric acetate solution into a 125-ml. glass-stoppered Erlenmeyer flask and add a sample that will titrate between 35 and 50 ml. of 0.1 *N* ammonium thiocyanate. Place the stopper and secure it with Okonite or other suitable tape. Shake the flask in a shaker for 20 minutes.

Unstopper the flask and add 5.0 ml. of 4 *N* sodium hydroxide and 1 ml. of 30% hydrogen peroxide. Add Carborundum chips and slowly heat to boiling with occasional swirling to reduce all the excess mercuric oxide to mercury. If some mercuric oxide remains, cool, cautiously add more hydrogen peroxide, and heat to complete the reduction. Boil gently for 1 minute to decompose the excess hydrogen peroxide. Cool in a cold water bath.

Add 5 ml. of diluted nitric acid and 2.0 ml. of ferric alum indicator and titrate immediately to the ferric thiocyanate end point; shake the contents of the flask well to minimize ion adsorption.

For correspondingly smaller amounts of unsaturation, the amounts of reagent shown in Table I are the most suitable for the titration ranges indicated, the balance of the procedure remaining the same. In each case the indicated amount of 40% aqueous 1,4-dioxane solution is placed in the 125-ml. glass-stoppered Erlenmeyer flask to make a total of 25 ml. of solution.

Table I. Reagent Requirements

Range of Unsaturation, % as Ethylvinylbenzene	40% 1,4-Dioxane, Ml.	Hg Acetate Soln., Ml.	Titration Range Regulated by Sample Size, Ml. 0.1 N NH_4SCN
50–203.1	..	25	35–50
5–50	15	10	10–20
3–15	20	5.0	4–10
0–3	23	2.0	<4

CALCULATION

Calculate the unsaturation as follows:

$$\frac{A \times 0.00661 \times 100}{\text{Wt. of sample}} = \%\ \text{unsaturation as}$$

% ethylvinylbenzene (by weight)

where A = ml. of 0.1 *N* ammonium thiocyanate used to titrate the mercury

ANALYTICAL DATA

A sample of divinylbenzene boiling at 32° C. at 1 mm. was used as the basis for the experimental work. Determining the purity of this compound was extremely difficult, as an established analytical method for divinylbenzene was not available with which to check the described procedure; the cryoscopic method, for instance, was not satisfactory because various isomers of divinylbenzene, ethylvinylbenzene, and diethylbenzene possibly were present. Infrared analysis indicated the presence of only a small amount of ethyl group which was attributed to ethylvinylbenzene. Owing to the nonavailability of pure standards, a liberal maximum of 3% ethylvinylbenzene in the sample was ascertained, with a lower percentage very probable. The 201.4% unsaturation as ethylvinylbenzene obtained by the method described in this paper gave calculations of 1.6% ethylvinylbenzene and 98.4% divinylbenzene, assuming no diethylbenzene present. All this information indicated that the procedure gave good analytical results for determining the total unsaturation in this sample of high purity divinylbenzene.

The ethylvinylbenzene sample was a material boiling at 50° C.

Table II. Unsaturation of Mixtures

Known	Calcd. % EVB as % EVB	Calcd. % DVB as % EVB	Total Unsatn. as % EVB	Unsatn. as % EVB Found	Av. % as EVB
Divinylbenzene	....	...	....	201.1, 201.8, 201.3	201.4
Ethylvinylbenzene	....	...	....	94.1, 94.3, 93.8	94.1
4	163.23	17.83	181.1	181.4, 181.0, 181.0	181.1
3	140.66	25.08	165.7	166.0, 165.5, 165.4	165.6
1	136.03	24.49	160.5	160.5, 159.5, 160.3	160.1
2	127.78	23.13	150.9	151.1, 150.4, 150.8	150.8
5	89.07	40.41	129.5	129.6, 130.1, 129.8	129.8
6	41.96	37.61	79.6	79.3, 79.8, 80.0	79.7
7	30.22	27.17	57.4	56.8, 57.6, 57.5	57.3
8	23.47	21.09	44.6	44.6, 44.3, 44.2	44.4
9	11.77	10.05	21.8	21.7, 21.7, 21.5	21.6
10	7.14	6.61	13.8	14.4, 14.2, 13.9	14.2
11	2.55	2.39	4.94	5.21, 4.98, 4.89	5.03
12	0.77	0.35	1.12	1.29, 1.15, 1.08	1.17

at 54 mm. It contained only traces of divinylbenzene as analyzed by ultraviolet. The major impurity in this material was diethylbenzene.

These two samples were analyzed by this procedure and the unsaturations calculated as per cent ethylvinylbenzene. Portions of these samples were then mixed in known proportions with diethylbenzene and the unsaturation was calculated for each mixture. Each mixture was then analyzed by this method. The values for per cent unsaturation as ethylvinylbenzene obtained by analysis of the samples compared with the calculated values are given in Table II.

To show that the procedure as given was quantitative for the vinyl group attached to the benzene ring, a sample of 99.6% styrene (as determined by the freezing point method, an entirely independent established procedure) was analyzed together with known mixtures of styrene and ethylbenzene (Table III).

DISCUSSION

All the reagents must be halide-free. The mercuric halides are very slightly ionized and thus interfere with the thiocyanate titration of mercuric ion.

If the sample contains polymer, reduction of some of the precipitated mercuric oxide may be retarded by polymer on the side of the flask. After most of the mercuric oxide is reduced but before the excess hydrogen peroxide is decomposed, the polymer with mercuric oxide should be policed off the side of the flask to aid complete reduction.

The 1,4-dioxane is used to increase the solubility of the mercuric acetate addition compounds. Commercial 1,4-dioxane often contains reducing substances such as ethylene acetal and acetaldehyde which slowly reduce mercuric ions and cause the precipitation of mercurous acetate. In order to eliminate this reduction, the commercial product should be purified; usually a good distillation is sufficient.

Table III. Analytical Results for Styrene

Known	Calculated % Styrene	% Styrene Found	Average % Styrene
99.6% styrene	...	99.5, 99.6, 99.4	99.5
3	40.8	41.0, 40.4, 40.5	40.6
1	25.1	25.4, 25.1	25.3
2	0.91	1.00, 1.03	1.02
4	0.40	0.51, 0.41, 0.41	0.44

Besides ethylvinylbenzene, divinylbenzene, and styrene, this method is suitable for various styrene derivatives such as the α-methylstyrenes and the vinyltoluenes.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of the Infrared and Ultraviolet Departments of the Dow Spectroscopic Laboratory in establishing the purity of ethylvinylbenzene and divinylbenzene used in this work.

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Estimation of Small Amounts of O,O-Diethyl O,*p*-Nitrophenyl Thiophosphate

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THE new synthetic insecticide, O,O-diethyl O,*p*-nitrophenyl thiophosphate, has acquired in this country the generic name "parathion" and the commercial name of Thiophos 3422 insecticide. The compound has been discussed by Thurston (*4*) and by Martin and Shaw (*3*), and its applications by Gleissner, Wilcoxon, and Glass (*2*).

Research on the application of a new insecticide to fruits and vegetables makes necessary a method for the determination of minute amounts such as would occur in spray or dust residues on the plant material. In the case of parathion, it seemed that the nitro group might offer the best approach to a more or less specific method for colorimetric determination, particularly if it is first reduced to the amino group. Bratton and Marshall (*1*) describe a method for the determination of sulfanilamide in blood and urine, in which the sulfanilamide is diazotized and coupled with *N*-(1-naphthyl)-ethylenediamine. This procedure, when applied to parathion after reduction to the corresponding amino compound, showed such promise that further possibilities were not investigated.

This paper deals mainly with the actual procedure for the de-

A colorimetric procedure is described for the estimation of small amounts of O,O-diethyl O,*p*-nitrophenyl thiophosphate (parathion, Thiophos 3422 insecticide). Reduction with zinc to the amino compound, diazotization, and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride produces an intense magenta color. Amounts of the insecticide of 20 to 200 micrograms in the final 50-ml. aliquot are readily determined using a photocolorimeter or spectrophotometer. This method may be applied to the determination of parathion in spray and dust residues on fruit, vegetables, and foliage.

termination of small quantities of parathion. The extraction of spray and dust residues from fruit and vegetables is treated briefly.

EXPERIMENTAL

The basic steps in the determination of small amounts of parathion are: (1) reduction with zinc dust in acid solution to the amino compound, diethyl *p*-aminophenyl thiophosphate; (2) diazotization of the amino compound with sodium nitrite, removal of the excess nitrite with ammonium sulfamate, and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to produce an intense magenta color; (3) evaluation of the developed color, using a photocolorimeter or spectrophotometer and a standard transmittance-concentration curve prepared from data on known amounts of the insecticide.

Transmittance–wave-length curves of the colored solutions were run on the G.E. recording spectrophotometer. A typical curve, shown in Figure 1, has an absorption maximum at about 555 mμ. The Cenco-Sheard-Sanford photelometer was used for routine determinations of parathion throughout this investigation. The green filter supplied with this instrument has a transmittance peak at about 520 mμ, which is sufficiently near the desired value to give a satisfactory transmittance gradient.

Preparation of Standard Curve. A sample of the best parathion available was used in constructing a standard curve. A total nitrogen determination indicated an apparent purity of 98%. A stock solution in benzene was made up, aliquots of which were carried through the procedure outlined below. Each aliquot was diluted to 10 ml. with benzene. The transmittances of the colored solutions were determined in a Cenco-Sheard-Sanford photelometer, using the green filter; the transmittances of the different solutions containing from 20 to 200 micrograms of parathion were plotted against concentrations to make a standard curve (Figure 2).

Effect of Evaporation of Benzene from Solution Aliquots. The choice of a solvent for the extraction of insecticide spray or dust residues from plant material depends on the following considerations: (1) The insecticide must be easily soluble in the solvent; (2) the solvent must be fairly low boiling for easy removal; (3) the loss of insecticide during evaporation of the solvent must be low; and (4) there must be a minimum of extraction of vegetable coloring materials such as chlorophyll and carotene or other interfering substances.

Both alcohol and acetone proved unsatisfactory because (1) they cause rapid breakdown of cellular structure, producing highly colored extracts; (2) colored compounds in solution in these solvents are not removed by common adsorbents; and (3) evaporation of solutions of parathion in alcohol or acetone is accompanied by excessive loss of the insecticide. Carbon tetrachloride is better in these respects, but is not so good a solvent for extracting parathion from its inert carrier. Benzene was found satisfactory in respect to extraction of color from plant materials and decolorization of the extracts by adsorbents. In order to test the loss of insecticide on evaporation, stock solutions of parathion in benzene were made up, and aliquots containing 20 to 200 micrograms of parathion were measured into beakers and diluted to 100 or 250 ml. with benzene. The benzene was evaporated down to 10 ml. on the steam bath with a jet of air passing over the liquid surface, then down to dryness at room temperature by means of the air jet. The residue was taken up in 10 ml. of alcohol and analyzed by the colorimetric procedure using the standard curve. The recoveries of parathion are shown in Table I.

The data indicate a slow steady loss of parathion in proportion to the volume of benzene evaporated. When 250 ml. of benzene are evaporated, the recovery averages about 93%; when 100 ml. are evaporated, the recovery averages about 98%. The loss of parathion is therefore roughly 2 to 3% for each 100 ml. of benzene evaporated. If desired, a correction may be applied to compensate for this loss.

Removal of Vegetable Colors from Benzene Extracts. The principal color compounds found in plant material, particularly leafy vegetables or leaves of any kind, are chlorophyll and carotene. The removal of these colors from benzene extracts is essential because of interference in the colorimetric determination. Several of the more common chromatographic adsorbents were tried with indifferent success. Attapulgus clay (may be obtained from the Attapulgus Clay Company, Philadelphia, Pa.), however, was found to be a very satisfactory adsorbent for chlorophyll and carotene.

Accordingly, aliquots of a stock solution of parathion in benzene, diluted to 175 ml., were passed through a mixture of Attapulgus clay and Hyflo Super-cel (may be obtained from Johns-Manville Company, New York, N. Y.); the latter serves only as a filter aid. The "decolorized" extracts plus washings, a total

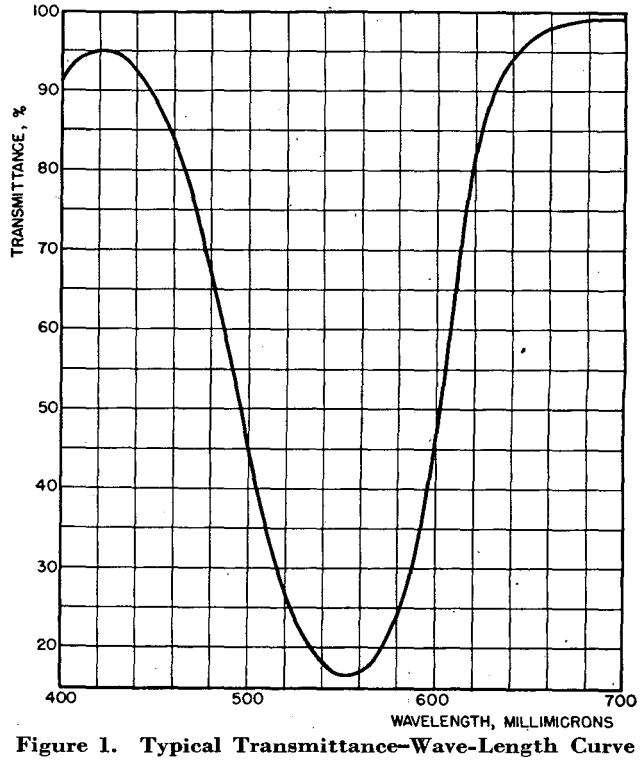

Figure 1. Typical Transmittance–Wave-Length Curve

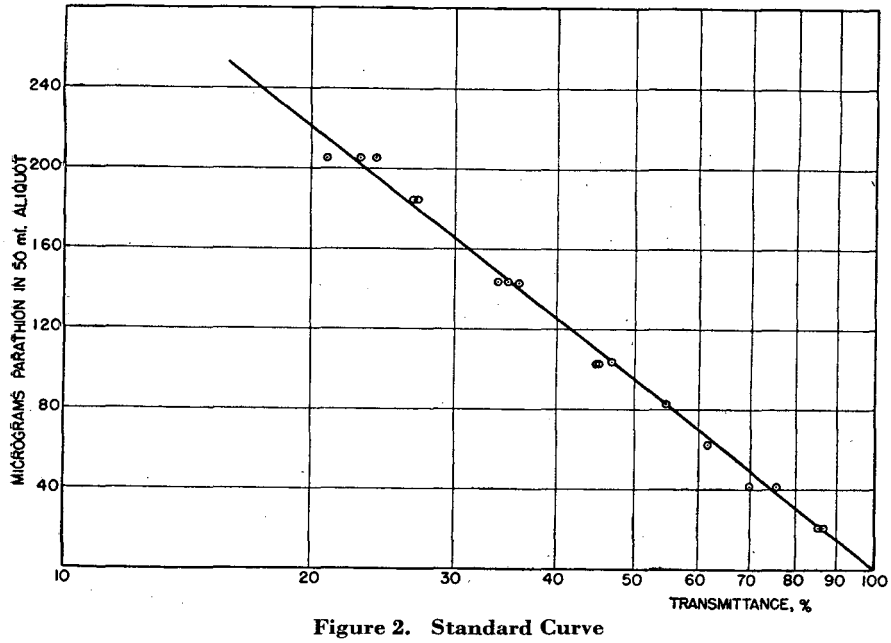

Figure 2. Standard Curve

Table I. Recovery of Parathion after Evaporation of Benzene

Benzene Evaporated, Ml.	Micrograms of Parathion Added	Micrograms of Parathion Recovered	Recovery, %
250	21	20	95
	41	40	98
	62	60	97
	82	76	93
	103	98	95
	103	98	95
	143	123	86
	164	148	90
	164	152	93
	205	180	88
	205	186	91
100	41	42	102
	41	40	98
	103	101	98
	103	101	98
	205	196	96
	205	196	96

Table II. Recovery of Known Amounts of Parathion from Benzene Solution after Passage through Attapulgus Clay

Micrograms of Parathion Added	Micrograms of Parathion Found	Recovery, %
41	36	88
	36	88
103	98	95
	93	90
	91	88
205	180	88
	190	93
	180	88

volume of 250 ml., were evaporated and analyzed as before. The recoveries shown in Table II average about 90%, or slightly less than when the extract is not passed through clay.

The recovery of parathion by the method described here may thus be low by as much as 10% as a result of losses during passage of the benzene extract through clay and evaporation of benzene. In the determination of insecticide residues in plant material, however, 90% recovery is satisfactory for the purposes served.

Interference by *p*-Nitrophenol. *p*-Nitrophenol is reduced, diazotized, and coupled with naphthylethylenediamine to produce a blue color whose absorption maximum is at 585 mμ as compared to 555 mμ for parathion. The color develops hardly at all in the 10-minute reaction period recommended for parathion, and only very slowly on longer standing. The possible presence of small amounts of free *p*-nitrophenol as a result of hydrolytic decomposition does not, therefore, cause any measurable error in the determination of parathion by this method.

ANALYTICAL PROCEDURE

The procedures described below were used in obtaining the results given in Tables I and II.

Preparation of Standard Curve. REAGENTS. Parathion. A sample of Thiophos 3422 insecticide may be obtained from American Cyanamid Company, 30 Rockefeller Plaza, New York 20, N. Y. Determine approximate purity by total nitrogen (theory = 4.81%).

Standard solution 1, 0.1000 gram of parathion (allowing for purity) in benzene to make 500 ml. 1 ml. = 200 μg. of parathion.

Standard solution 2, 25 ml. of standard solution 1, diluted to 250 ml. with benzene. 1 ml. = 20 μg. of parathion.

Sodium nitrite, 0.25 gram of reagent grade sodium nitrite in water to make 100 ml. Make solution up fresh weekly.

Ammonium sulfamate, 2.5 grams of technical ammonium sulfamate in water to make 100 ml. Make solution up fresh weekly.

N-(1-naphthyl)-ethylenediamine dihydrochloride, from Eastman Kodak Company, Chemical Division, Rochester 4, N. Y.; 1 gram in water to make 100 ml. Make solution up fresh weekly.

Zinc dust. Ethyl alcohol, 95%. Benzene, C.P. or technical.

APPARATUS. Colorimeter. Cenco-Sheard-Sanford Photelometer, using tubular cells of 1.5-cm. thickness, and green filter having transmittance peak at 520 mμ.

Beakers. Volumetric flasks, 50 ml.

PROCEDURE. Aliquots of standard solution 2 are run into beakers from a 10-ml. microburet: 1, 2, 3, 4, 5, 7, 9, and 10 ml., equivalent to 20, 40, 60, 80, 100, 140, 180, and 200 μg. of parathion. Each aliquot is diluted to 10 ml. with benzene, and a blank of 10 ml. of benzene is placed in another beaker. The benzene is evaporated off completely at room temperature by passing a gentle stream of air over the liquid surface, and the evaporation is stopped as soon as the residue is just dry.

The residue is dissolved in 10 ml. of ethyl alcohol, and then 10 ml. of water, 2 ml. of 5 *N* hydrochloric acid, and 0.2 gram of zinc dust are added. Each beaker is covered with a watch glass and the solution is heated to boiling on a hot plate and boiled gently for 5 minutes. The watch glass and sides of the beaker are washed down with water, and the contents are allowed to cool and are filtered through No. 42 Whatman filter paper into a 50-ml. volumetric flask. Beaker, residue, and paper are washed with small portions of water until the total volume of filtrate is about 40 ml. One milliliter of 0.25% sodium nitrite is added to each flask, and the solutions are mixed well and let stand 10 minutes. One milliliter of 2.5% ammonium sulfamate is added to each solution, which is again mixed well and let stand 10 minutes more. Finally, 2 ml. of 1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution are added to each solution, then water to 50 ml., and the solutions are mixed well and let stand 10 minutes. The blank solution is used to set the photometer scale at 100% transmittance, and the transmittance of each solution is taken. The standard curve is prepared by plotting transmittance against concentration (micrograms per 50 ml.).

Determination of Parathion in Benzene Solution. REAGENTS AND APPARATUS. Essentially the same as for preparation of the standard curve.

PROCEDURE. The benzene solution of parathion is concentrated to 10 ml. in a 300-ml. tall-form beaker on a steam bath; a gentle stream of air is passed over the liquid surface to hasten evaporation. The last 10 ml. of benzene are removed at room temperature by passing air over the surface, and the evaporation is stopped as soon as the residue is just dry.

The residue is taken up in 10 ml. of ethyl alcohol, and the analysis continued as in the preparation of the standard curve. A blank is run, starting with the same volume of benzene as in the

test solution. The final blank solution may then be used to set the photometer scale at 100% transmittance, or, if the same benzene and reagents are used throughout the day, one blank may be run for all the determinations of that day. In the latter case, the scale is set at 100% against distilled water, and the transmittances are corrected as follows:

$$\log 100/T \text{ (corr.)} = \log 1/T \text{ (sample)} - \log 1/T \text{ (blank)}$$

or

$$T \text{ (corr.)} = 100 \times T \text{ (sample)}/T \text{ (blank)}$$

where transmittances are expressed as per cent. When the corrected transmittance has been obtained, the number of micrograms of parathion in the final solution is read off the standard curve.

Notes on Procedure. The loss of parathion from filtration of the benzene extract through clay and by evaporation of the benzene may be as much as 10% of the total present. Although this amount has a negligible effect on the conclusions to be drawn, it should be borne in mind.

The pH of the solution in the coupling step should be in the range 0.6 to 1.0. If the conditions outlined in the procedure are adhered to, the acidity will be in this range.

In the reduction step, a 5-minute reaction period is sufficient. Fifteen minutes do not change the recovery in either direction.

The developed color was found to be stable on standing up to at least 4 hours. In the analysis of plant extracts, however, interfering colors may develop on standing. The transmittance reading should, therefore, be taken 10 minutes after the coupling reagent is added.

The transmittance of the reagent blank will be observed to decrease with the age of the reagents, particularly the naphthylethylenediamine. The reagents should, therefore, be made up fresh weekly.

DISCUSSION

The method of analysis described was developed for the purpose of determining very minute amounts of parathion such as might be expected in residues on fruit, vegetables, or foliage after spraying or dusting. For the analysis of parathion itself, or for the determination of parathion in commercial dusts or wettable powders, other methods are more suitable but are outside the scope of this paper.

Parathion is applicable as an insecticide to a wide variety of plant materials. It would be difficult, therefore, to set forth a concise universal procedure for the extraction of residues and preparation of the extract for analysis for such diverse materials as apples, grapes, cabbage, tobacco, etc., as well as soil. The basic elements of such a procedure are outlined below, with some mention of vegetable materials treated; the basic procedure may then be modified as necessary for use on specific plant materials.

Extractant. Benzene has been found the most satisfactory of the common solvents for use as an extractant of parathion from plant materials.

Apparatus. The extraction apparatus consists essentially of liquid-tight jars and a mechanical means of rolling or tumbling the jars. The jars may be ordinary preserve jars of 2- or 4-quart capacity, or larger jars up to 3-gallon capacity. The closures of the jars should have tight gaskets protected from contact with the benzene by heavy tin foil. The agitation machine may be built for the purpose, or ordinary motor-driven rollers, geared down to slow speeds, may be used. For end-over-end tumbling, a large can or fiber container, in which the extraction jars may be placed sideways, may be used.

Extraction Time. A minimum of 30 minutes' extraction time has been employed, and 1 hour is recommended. The extra time does no harm in the extraction of most materials, with the exception of soft-fleshed fruits or vegetables; special effort must be made toward gentler treatment in the determination of surface residues on such material.

Sample Size. The amount of material taken should be large enough to be representative; in general, the larger the sample, the greater the precision. In some cases, such as pea vines or wax beans, where there is a high content of natural waxes, too large a sample may mean an excessive amount of wax in the extract, which occasionally appears in the final colored solution as a turbidity. A guiding principle to be kept in mind is that, under the conditions outlined in the procedure, the aliquot of benzene extract taken for analysis should contain 20 to 200 μg of parathion, so that the transmittance of the colored solution will be in the right range; for different instruments, filters, or cell depths, the proportions may differ.

Decolorization of Extracts. Benzene extracts of leafy material are usually green or yellow-green with chlorophyll or carotene; other materials as well show a considerable content of carotene. This color may be removed satisfactorily by filtration through Attapulgus clay.

A small plug of cotton is placed in the bottom of a 150-ml. separatory funnel, and 10 grams or more of Attapulgus clay adsorbent (2 parts of Attapulgus clay, 1 part of Hyflo-super-cel, well mixed) are added; 50 ml. of benzene are added, and the mixture is stirred until completely wetted and free of air bubbles; the benzene is drawn through by suction almost to the surface of the adsorbent. The benzene extract of the plant material is poured into the separatory funnel and drawn through by suction at about 25 ml. per minute into a beaker or volumetric flask. Container and column are washed with at least three separate 25-ml. portions of fresh benzene. The column must not be allowed to suck dry until the last wash is passed through, or some color will pass into the filtrate.

If there is no color in the extract, or only very light tints, the decolorization step may be omitted. Such light tints are usually destroyed during the reduction step.

Control Analyses. In the analysis of most materials investigated in this laboratory, control analyses on untreated materials showed an apparent parathion content of less than 0.1 p.p.m. A few materials, however, showed an appreciable blank. With some samples of tobacco, for instance, a yellowish color was present just before the addition of the coupling agent (naphthylethylenediamine). If the observed transmittance after adding the coupling agent was corrected for the reagent blank, there was still an appreciable control blank. However, if the transmittance of the solution was taken before addition of the coupling agent, and applied as a correction to the transmittance taken after addition of the coupling agent the resultant blank was negligible. Brussels sprouts and cabbage show a pinkish color in the solution before the coupling agent is added; this color may or may not precipitate out on standing and is not corrected for as in the case of tobacco. Some varieties of grapes have shown an appreciable control blank. It is, therefore, important when working on materials not previously investigated, that control analyses be run, at least at the outset, to determine whether or not interfering colors are introduced by the material itself.

Plant Material Analyzed. The following materials have been analyzed in this laboratory for residues of parathion: apples, pears, grapes, tobacco, cabbage, Brussels sprouts, corn, tomatoes, cherries, pea vines, wax beans, grapefruit, and soil. Other materials have been investigated by collaborating experimental laboratories.

ACKNOWLEDGMENT

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Determination of Vitamin A Ester in Fortified Poultry Mashes

With Activated Glycerol Dichlorohydrin

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A study has been made of the application of the vitamin A–activated glycerol dichlorohydrin reaction to the estimation of vitamin A in fortified poultry mash. Certain constituents present in both unsaponified and saponified petroleum ether feed extracts repress the vitamin A–glycerol dichlorohydrin reaction. Adsorption on a mixture of 3.1 Hyflo Super-cel–activated magnesia No. 2641 removed most of the interfering substances. By carefully regulating the length of the adsorption column, volume of eluant, and quantity of sample adsorbed, it was possible to elute vitamin A ester while retaining carotene and most unknown interfering compounds on the column. With most feeds, vitamin A concentrations of 3000 or more I.U. per pound could be accurately determined within ±5%, and concentrations as low as 1500 I.U. per pound could be determined with accuracy sufficient for routine purposes, except when the mash contained a large proportion of fish meal. Quantities as low as 500 I.U. per pound could not be accurately determined. Chief disadvantages: It is necessary to use an empirical correction formula and the method is not applicable to free vitamin A alcohol.

SOBEL and Werbin have recently published papers (*6*, *7*) on a new method for the colorimetric determination of vitamin A in which activated glycerol dichlorohydrin is used as the color-producing agent. Because the color formed is relatively stable and with fish liver oils the glycerol dichlorohydrin reaction gives results in close agreement with the antimony trichloride and ultraviolet absorption methods, these workers propose the adoption of glycerol dichlorohydrin in preference to antimony trichloride as the color-producing agent (*7*).

The authors attempted to apply the glycerol dichlorohydrin reaction to petroleum ether (boiling point, 63° to 70° C.) extracts of fortified poultry mash. (All the petroleum ether used in this investigation had a boiling point range of 63° to 70° C. and received no special purification treatment.) In preliminary experiments with both unsaponified and saponified samples, inability to recover more than a small fraction of added vitamin A ester indicated that certain constituents in both the unsaponified and saponified extracts suppressed or interfered with the glycerol dichlorohydrin reaction with vitamin A. Brew and Scott (*1*) and Cooley *et al.* (*2*) have shown that constituents of certain feed extracts give false vitamin A color reactions with antimony trichloride. The problem with the glycerol dichlorohydrin reaction is somewhat different in that the chief effect of interfering substances seems to be a suppression of the glycerol dichlorohydrin–vitamin A reaction.

A chromatographic study was made with a number of adsorbents in an attempt to find one that would not only remove the substances that interfered with the glycerol dichlorohydrin–vitamin A reaction but would also separate vitamin A ester from any carotenoids present, so that a correction for the presence of carotenoids could be avoided. It was found that under carefully controlled conditions an adsorbent consisting of 3 parts of Hyflo Super-cel and 1 part of activated magnesia No. 2641 would meet most of the requirements. It was impossible to remove all the substances in various feed extracts which repress the glycerol dichlorohydrin–vitamin A reaction. An empirical correction formula was developed that permitted reasonably good estimation of vitamin A ester in a variety of poultry mashes and at concentrations as low as 1500 I.U. per pound.

METHOD

Extraction and Purification. All manipulations are carried out in a semidarkened room shielded from direct sunlight, with all-glass apparatus. Ten to 20 grams of mash are extracted with petroleum ether for 3 hours in a Soxhlet extractor. For best results the sample should have a minimum of 6 to 8 I.U. per gram, although feeds containing half this quantity can be analyzed with reasonable accuracy.

The extract is concentrated under vacuum and mild heating, transferred to a 50-ml. volumetric flask, and made to volume with the petroleum ether. Aliquots equivalent to 4 to 5 grams of original sample are taken for purification by adsorption. The adsorbent is a mixture of 3 parts of Hyflo Super-cel (Johns-Manville Company) and 1 part of activated magnesia No. 2641 (Westvaco Chemical Corp.). The components are mixed in a ball mill (stones are omitted). A 7 × 2.5 cm. column of adsorbent is prepared by firmly tamping the dry adsorbent in a suitable tube under full vacuum. Details of this technique have been reported (*9*).

The column is wet with petroleum ether followed immediately by the aliquot. The vitamin A ester is eluted with 25 ml. of 5% acetone in petroleum ether. The flow of solvent through the column is regulated so that a rapid drop rate is maintained.

In order to remove fine adsorbent particles, the eluate is filtered on a small pad of Hyflo Super-cel in a sintered Hirsch funnel, which is then washed several times with petroleum ether. The filtrate is concentrated, and transferred to a 50-ml. standard taper Erlenmeyer flask, and the solvent is evaporated to dryness with vacuum and mild heating. The residue is dissolved in 1 ml. of chloroform.

To a duplicate aliquot is added a suitable quantity of standard vitamin A ester. Twenty International Units were used in this investigation, and the fortified sample was adsorbed and eluted in the manner described.

Colorimetric Determination. A calibration curve of International Units of vitamin A against density in the range 0 to 50 I.U., is prepared according to the method of Sobel and Werbin (*6*); a standard concentrate of vitamin A ester, capsules PC-3 from Distillation Products Company, is used as the source of vitamin A. To the vitamin A in 1 ml. of chloroform are added 4 ml. of activated glycerol dichlorohydrin (Shohan Laboratories), the mixture is shaken vigorously, and the density is determined 2 minutes after mixing. The authors used a Fisher electrophotometer provided with microtubes and a 550-mμ filter. A blank consisting of 1 ml. of chloroform and 4 ml. of glycerol dichlorohydrin is used for the zero setting of the instrument. The calibration curve secured with the Fisher electrophotometer was not linear, and it was necessary to make use of the standard reference curve in all calculations.

The density of the purified mash solutions is determined in the same manner as that of the standards. In calculating the results it is necessary to allow for the repression of the vitamin A–glycerol dichlorohydrin reaction which occurs to some extent even in extracts purified by adsorption. Vitamin A in the aliquot taken can be calculated by the following formula:

$$\text{I.U. of vitamin A} = \left(\frac{X + Y}{Z}\right) \times X$$

in which X = I.U. of vitamin A found in unfortified aliquot, Y = I.U. of standard vitamin A added to fortified aliquot, and Z = I.U. of vitamin A found in fortified aliquot.

The expression $\left(\frac{X + Y}{Z}\right)$ represents an empirical correction factor which usually had a value of 1.05 to 1.15 when an aliquot equivalent to 4 to 5 grams was taken for analysis. The reason for using this factor instead of the repression formula $\left(\frac{Y}{Z - X}\right)$ developed by Oser, Melnick, and Poder (*4*) is discussed below.

Table I. Effect of Size of Sample on Repression Factor

(40 units vitamin A ester added to sample in all cases)

Sample, Grams	Density	Repression Factor
0	0.265	1.0
4.0	0.241	1.1
7.0	0.204	1.3
8.0	0.189	1.4
10.0	0.177	1.5
16.0	Impossible to read because of turbidity	...

DISCUSSION AND RESULTS

In order to determine vitamin A in poultry mashes with the glycerol dichlorohydrin reaction, it is necessary to separate it from many interfering substances present in the original extract. This separation is based on a critical relationship among the quantity of adsorbent, volume of eluant, and amount of sample taken for analysis. Standard solutions of vitamin A ester equivalent to 10 to 100 I.U. could be recovered within ±5% when a firmly tamped magnesia adsorbent, 7 × 2.5 cm., and 25 ml. 5% acetone in petroleum ether eluant were used. When longer columns were tested, considerable vitamin A was lost even when much more eluant was used. These results show that it is necessary to use a minimum quantity of magnesia adsorbent in order to avoid loss of vitamin A.

The use of 25 ml. of 5% acetone in petroleum ether as the eluant is based on the fact that this volume of liquid will not only elute vitamin A ester from a 7 × 2.5 cm. column, but will also separate the vitamin A ester from carotene and most of the substances that repress the glycerol dichlorohydrin reaction. Under the experimental conditions, carotene is washed almost to the end of the column, but so little passes into the eluate that no correction for the reaction of glycerol dichlorohydrin with carotene need be made. Larger volumes of eluant will quantitatively elute the carotene, along with many substances which repress the glycerol dichlorohydrin reaction.

The third critical factor is the quantity of sample adsorbed. Petroleum ether extracts 2 to 6% of the solids in poultry mashes depending on the nature of the sample. From this it is evident that vitamin A in quantities usually present in mashes constitutes a small proportion of the extract. If too large a sample is used, the adsorbent will be saturated by the other components of the extract, with the result that many substances will not be retained on the column and will be eluted along with vitamin A ester. The effect of increasing the quantity of sample adsorbed on the repression of the vitamin A–glycerol dichlorohydrin reaction is shown in Table I. Forty units of standard vitamin A are added to various aliquots of a mash extract containing no vitamin A, and the samples are adsorbed and eluted in the usual manner. The data show that as the quantity of sample increases the density decreases, and indicate that the larger the sample the less the removal of substances which repress the vitamin A–glycerol dichlorohydrin. The quantity of sample adsorbed should therefore be restricted to the equivalent of 4 to 5 grams of mash. The repression factor found with a number of feeds of different composition ranged from 1.05 to 1.15 with 4- to 5-gram samples.

The procedure presented here applies only to vitamin A ester. That the free vitamin A would be strongly adsorbed (*10*) is due to the well-known effect of a free OH group on adsorption affinity (*8*). Ordinarily vitamin A is added to feeds in unsaponified fish liver oils. Because almost all the vitamin A in such oils occurs in the ester form (*3, 5*), the method should be applicable to many fortified feedstuffs.

A number of poultry mashes were obtained with considerable variations in composition, as shown in Table II. None contained a measurable quantity of vitamin A. In order to test the recovery of vitamin A, a standard 3000A-400D feeding oil was mixed with the various feeds to give final concentrations of 500, 1500, and 3000 I.U. per pound (1.1, 3.3, and 6.6 I.U. per gram). A distilled vitamin A ester concentrate was used to give concentrations as high as 300,000 I.U. per pound. As shown in Table III, the vitamin A in most mashes can be determined within ±5% at the 3000 level and within +5 to +9% at the 1500 level.

In mash 5, the recovery of vitamin A was 108% at the 3000 level and 130% at the 1500 level. This mash was unusual, in that it contained high proportions of fish meal and distiller's solubles. The extract from this feed contained pigments that could not be completely removed, which gave a false vitamin A reaction with glycerol dichlorohydrin. Cooley *et al.* have reported similar findings, using the antimony trichloride reaction on a mash containing 20% fish meal (*2*).

The results with mashes containing 500 I.U. per pound were unsatisfactory, as the recoveries were invariably at least 20% too high. On the other hand, feeds containing more than 3000 I.U. per pound could be analyzed with an accuracy as good as or better than that found with the 3000 level. The glycerol dichlorohydrin reagent is less corrosive than the conventional antimony trichloride reagent and produces a more stable colored reaction product with vitamin A. On the other hand its use for determination of vitamin A in poultry mashes is associated with some inherent difficulties. These can be traced to the

Table II. Composition of Poultry Mashes Used in Recovery Experiments

Ingredient	Mash 1 %	Mash 2 %	Mash 3 %	Mash 4 %	Mash 5 %
White corn	17	..	...	....	30
Yellow corn	...	20	30	30	...
Ground wheat	23	20	10	12	10
Wheat bran	15	7.5	10	15	...
Ground oats	15	7.5	15	15	20
Ground barley	...	10	...	....	...
Gluten meal	...	2.5	...	5	...
Soybean meal	20	7.5	20	15	...
Alfalfa leaf meal	...	..	8	....	...
Broccoli leaf meal	...	2.5	...	1	...
Fish meal	...	10	...	5	20
Meat scraps	5	5	5	....	...
Steam bone meal	1	..	1	1	1
Whey	...	..	...	....	13
Dried skim milk	2	..	...	....	...
Molasses	...	2.5	...	....	...
Distiller's solubles	...	..	...	....	5
Delsterol	0.05	..	0.05	0.075	0.05
Salt mix	0.5	1.0	0.5	0.5	...
Oyster shell flour	1.5	3.0	1.5	1.5	0.5

Table III. Recovery of Vitamin A Added to Poultry Mashes

	500 I.U./Lb. Added		1500 I.U./Lb. Added		3000 I.U./Lb. Added		300,000 I.U./Lb. Added	
Mash	I.U./lb.	%	I.U./lb.	%	I.U./lb.	%	I.U./lb.	%
1	603	120	1590	106	2920	97	298,000	99
2	611	122	1640	109	2880	96	290,000	95.5
3	650	130	1580	105	2820	95	302,000	100.5
4	632	126	1620	108	3120	104	285,000	95.0
5	787	157	1950	130	3240	108	320,000	107

fact that some constituents of poultry mash (apparently unsaponifiable) suppress or interfere with the reaction of glycerol dichlorohydrin with vitamin A. Most of the interfering substances, but not all, could be removed by adsorption on activated magnesia Super-Cel. It was therefore necessary to rely on an increment procedure somewhat similar to that described by Oser *et al.* (*4*). However, when the true repression factor $\left(\frac{Y}{Z-X}\right)$ was applied to the data, many of the results obtained were too high. The use of an empirical factor $\left(\frac{X+Y}{Z}\right)$ resulted in fairly good recovery of vitamin A with a variety of mashes and with vitamin A concentrations as low as 1500 I.U. per pound. However, the necessity for using an empirical factor must be regarded as an undesirable feature.

Because the method is restricted to the analysis of products containing only vitamin A ester, its general application is limited. For routine or control purposes, the present method offers advantages in simplicity and ease of operation. It should not be used for samples of unknown origin.

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Spectrophotometric Determination of Serum Calcium

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A precise method (standard deviation ±2.2%) for the determination of calcium in blood serum is based on the oxidation of the oxalate equivalent with excess ceric sulfate and the spectrophotometric determination of the residual ceric ion. The residual ceric concentrations have been measured with the Beckman spectrophotometer at 315 mμ (absorption maximum) and with the Coleman Universal spectrophotometer at 390 mμ with equal precision. As the optical densities can be read more rapidly with the Coleman spectrophotometer, this would be the instrument of choice for routine analyses. For a single calcium determination by the ceric method, 15% of the total error variance is ascribable to the trichloroacetic filtration, 79% to the oxalate precipitation, and 6% to the ceric oxidation.

MOST existing methods for determining serum calcium depend on the precipitation of calcium as calcium oxalate and the determination of the oxalate equivalent by titration with permanganate. In the more recent methods, however, cerium salts are gradually replacing permanganate for the oxidation of the oxalate. In certain methods (*1*, *2*, *5*) the calcium oxalate, dissolved in acid, is titrated with standard ceric solutions; in others (*3*, *4*), an excess of the ceric solution is added and the excess is titrated with standard ferrous ammonium sulfate. In a modification by Sendroy (*7*), the excess ceric sulfate is reduced with potassium iodide and the liberated iodine is then determined photoelectrically. The present paper reports a method in which the excess ceric sulfate is measured directly on the spectrophotometer.

REAGENTS

Cerous Sulfate, 0.2% in approximately 1 *N* sulfuric acid. Dissolve 4 grams of cerous sulfate (G. Frederick Smith Chemical Co., Columbus, Ohio) in approximately 300 ml. of distilled water and 55.8 ml. of concentrated sulfuric acid with heating. Cool and dilute to 2 liters.

Ceric Sulfate Stock Solution. Weigh 3.2072 grams of oven-dried ceric sulfate (G. Frederick Smith Chemical Co., Columbus, Ohio). Dissolve in and dilute to 1 liter with the 0.2% cerous sulfate–sulfuric acid solution. This solution is approximately 0.0081 *N* as standardized against sodium oxalate and is stable for at least 10 months.

Ceric Sulfate Working Solution. Dilute 1 volume of the ceric sulfate stock solution to 10 volumes with the 0.2% cerous sulfate solution. In the procedure below, the ceric sulfate solutions prepared in this manner give a working range of between 6 and 14 mg. of calcium per 100 ml. of serum. This range is adequate for the analysis of normal serums from most mammals.

Primary Standard Sodium Oxalate. Weigh exactly 234.0 mg. of oven-dried Bureau of Standards sodium oxalate and dissolve in 1 liter of approximately 1 *N* sulfuric acid. Two milliliters of this solution are equivalent to 0.14 mg. of calcium (14 mg. of calcium per 100 ml. of serum). For the preparation of calibration curves, 6-, 8-, 10-, and 12-ml. portions of the primary standard are diluted to 14 ml. with 1 *N* sulfuric acid. Two-milliliter portions of these dilutions are equivalent to 6, 8, 10, and 12 mg. of calcium per 100 ml. of serum, respectively. Two milliliters of the undiluted standard furnish the fifth datum for the calibration.

ANALYTICAL PROCEDURE

The calcium from a 5-ml. aliquot of a trichloroacetic acid filtrate, representing 1 ml. of serum, is separated as the oxalate according to the procedure described by Wang (*9*).

The washed and dried calcium oxalate is dissolved in exactly 2 ml. of 1 *N* sulfuric acid. Solution of the precipitate may be facilitated by immersing the tubes in a boiling water bath for 5 minutes. Exactly 10 ml. of the ceric sulfate working solution are added, and the contents are thoroughly mixed by stoppering the tube and inverting repeatedly. The presence of the cerous ion catalyzes the reaction so that oxidation is rapid and complete at room temperature. After 5 minutes the residual ceric concentration is determined spectrophotometrically. These solutions are stable for at least 3 to 4 hours.

Simultaneously, 2-ml. portions of the sodium oxalate standards are oxidized with 10-ml. volumes of the ceric solution. The calibration curve is plotted from their residual optical densities. Typical calibration curves appear in Figure 1. The calcium content of the serum samples may be read directly from the curve or, preferably, may be computed from the linear regression equation representing the calibration data.

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In the studies reported here, the optical densities of the unreduced ceric ion were measured both with the Beckman spectrophotometer at 315 mμ (tungsten lamp and either Corex glass or fused silica cells, 1-cm. path length) and with the Coleman Universal spectrophotometer, Model 11, at 390 mμ (PC-6 filter and cylindrical cells, inside diameter approximately 17.5 mm.). Distilled water was used as the reference solution.

ABSORPTION SPECTRA

Partial absorption spectra of ceric-cerous solutions, as measured on the Beckman spectrophotometer using the tungsten lamp, and fused silica cells (1-cm. path length), and read against distilled water are shown in Figure 2. The ceric ion exhibits a distinct absorption maximum at 315 mμ. This maximum is accentuated if the total measured absorption of a ceric-cerous system (curve I) is corrected for absorption by the cerous ion (curve III). The absorption properties of these solutions have been examined at shorter wave lengths (down to 280 mμ) using the hydrogen discharge lamp. Below 315 mμ, absorption by these solutions decreases regularly.

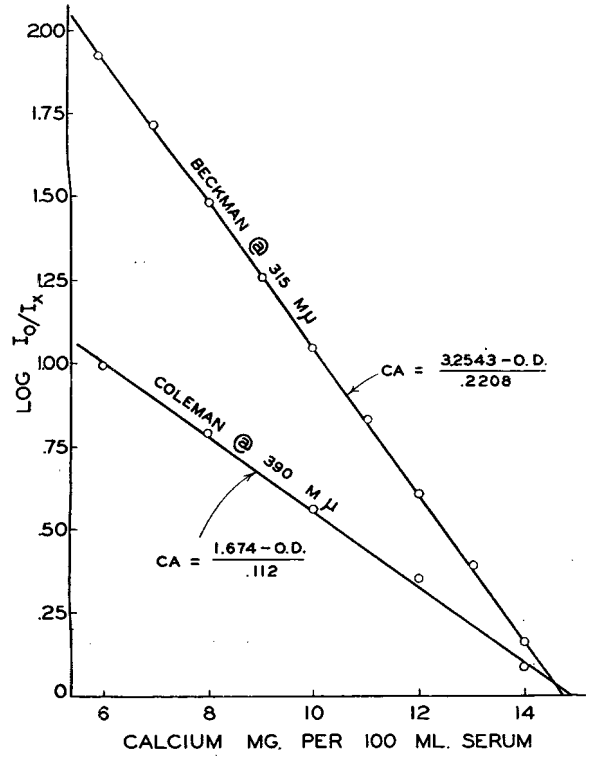

Figure 1. Typical Calibration Curves

Calcium equivalents of sodium oxalate standards *vs.* optical density

The similarity in the character of the absorption curves of diluted ceric solution (curve I, Figure 2) and the partially reduced ceric solution (curve II, Figure 2) is evidence that the measured optical properties of the analytical solutions are due solely to the unreduced ceric ion.

COMPARATIVE ANALYSES

Serum Analysis. In an experiment designed to permit subsequent statistical treatment, calcium analyses by the proposed procedure were compared with analyses by the permanganate titration method. The serums from the bloods of ten sows of the swine breeding herd were used in this study.

Trichloroacetic acid filtrates were prepared in triplicate from each serum sample, and quadruplicate calcium precipitates were made from each filtrate. During the preparation of the calcium oxalate precipitates, all samples and subsamples were completely randomized and treated uniformly by one operator. The four oxalates from any one filtrate were randomly assigned to either the ceric or the permanganate procedure, two to each method. After the oxalate precipitates had been distributed to the two methods, all tubes in each complete series were assigned a coded randomized sequence number for the final oxidation of the oxalate.

In order that the experiment be restricted to a practical size, the same series of tubes was used for measuring the residual ceric concentrations with the two spectrophotometers independently. Different individuals measured the oxalate equivalents by the different methods. Table I contains the results of this experiment.

Oxidation of Sodium Oxalate Standards. This experiment was designed to permit an evaluation of the precision of the oxidative step in the calcium procedure, free of other major sources of variation, by the ceric and permanganate methods. Accordingly, in this study, quintuplicate aliquots of sodium oxalate standard solutions, representing five randomly selected levels of calcium equivalence within the range of mammalian serums, were oxidized by each method.

As in the previous experiment, all tubes within each series were given a random sequence number to obscure the identity of the replicates. In addition, a fourth nonrandom series of samples, in which the replicates were consecutive and clearly identifiable, was titrated with permanganate. These data are given in Table II.

DISCUSSION

Values for the calcium content of swine serums, as determined by the three methods, are in excellent agreement. On the aver-

Table I. Calcium Content of Swine Serums

Sample No.	T.C.A. Filtrate	(Oxalate equivalents) Spectrophotometric Method: Beckman at 315 mμ *Mg./100 ml.*		Coleman at 390 mμ *Mg./100 ml.*		$KMnO_4$ Titration *Mg./100 ml.*	
1	1	11.13	11.13	11.35	11.21	10.96	10.48
	2	10.60	10.47	10.79	10.62	10.72	10.48
	3	10.21	10.54	10.22	10.56	10.96	10.24
Mean and $s_{\bar{x}}$[a]		10.68 ± 0.15		10.79 ± 0.17		10.64 ± 0.12	
2	1	11.12	10.93	11.29	10.83	11.43	11.43
	2	...	11.05	...	10.88	11.67	11.20
	3	11.26	10.74	11.21	10.61	10.48	11.20
Mean and $s_{\bar{x}}$		11.02 ± 0.13		10.96 ± 0.13		11.24 ± 0.17	
3	1	9.69	9.84	10.01	10.04	9.53	9.29
	2	9.46	9.80	9.51	9.79	9.77	10.00
	3	10.26	9.55	10.30	9.67	10.00	11.91
Mean and $s_{\bar{x}}$		9.77 ± 0.12		9.89 ± 0.12		10.08 ± 0.38	
4	1	10.63	11.15	10.92	11.13	10.48	10.48
	2	11.06	10.84	11.10	11.02	11.43	11.20
	3	11.22	11.06	11.39	11.02	11.67	10.96
Mean and $s_{\bar{x}}$		10.99 ± 0.09		11.10 ± 0.07		11.04 ± 0.20	
5	1	10.40	10.18	10.56	10.29	10.24	10.48
	2	10.66	10.39	10.57	10.49	10.00	11.20
	3	10.31	10.65	10.30	10.53	9.53	9.77
Mean and $s_{\bar{x}}$		10.43 ± 0.08		10.46 ± 0.05		10.20 ± 0.24	
6	1	10.97	10.89	10.91	11.01	11.43	11.43
	2	11.09	10.89	11.12	10.88	11.43	10.72
	3	11.01	10.99	11.10	11.03	10.48	9.53
Mean and $s_{\bar{x}}$		10.97 ± 0.03		11.01 ± 0.04		10.84 ± 0.31	
7	1	10.65	10.71	10.62	10.70	9.77	10.00
	2	10.53	10.34	10.57	10.41	10.72	10.48
	3	11.12	10.84	11.11	10.87	10.48	10.72
Mean and $s_{\bar{x}}$		10.70 ± 0.11		10.71 ± 0.10		10.36 ± 0.16	
8	1	10.60	10.49	10.62	10.71	11.20	10.96
	2	10.60	10.45	10.62	10.46	10.72	11.20
	3	10.49	10.19	10.64	10.21	10.96	11.20
Mean and $s_{\bar{x}}$		10.47 ± 0.06		10.54 ± 0.08		11.04 ± 0.08	
9	1	9.84	10.11	10.12	10.13	10.48	10.24
	2	10.18	10.48	10.38	10.56	10.24	10.24
	3	10.30	10.19	10.53	10.29	9.77	10.72
Mean and $s_{\bar{x}}$		10.18 ± 0.09		10.34 ± 0.08		10.28 ± 0.13	
10	1	9.44	9.80	9.69	9.96	9.77	9.05
	2	10.18	9.47	10.16	9.63	9.77	10.00
	3	9.72	9.59	9.79	9.55	10.00	10.00
Mean and $s_{\bar{x}}$		9.70 ± 0.11		9.80 ± 0.09		9.77 ± 0.15	

[a] $s_{\bar{x}}$ = standard deviation of mean = $\sqrt{\frac{S(x - \bar{x})^2}{n(n-1)}}$

age, the ceric values are, as determined on the Beckman, 99.4%, and on the Coleman spectrophotometer, 100.1%, as compared with the values obtained by permanganate titration. Excellent agreement between the two ceric methods was expected in this experiment, because the determinations were made on the same set of solutions. The average standard deviation for a single determination of calcium in serum was computed for the three methods after correction for animal-to-animal variation. These deviations were ±2.2, 2.2, and 4.9%, respectively, for the ceric-Beckman, ceric-Coleman, and permanganate titration determinations.

In the precision experiment in which the calcium equivalents of sodium oxalate standards were determined by the three methods, the standard errors of the means for the ceric methods, except for the 8.96-mg. level, approach the respective theoretical sensitivities (0.005 optical density unit times the slope of the calibration curve). Although the reproducibility of the analyses by the ceric-Beckman method is good in this experiment, the determinations were biased. On the average, the recoveries were 3.0% too low. Furthermore, the regression of the bias on concentration was negative and highly significant ($P < 0.01$)—that is, the departure of the mean from the true value became less as the concentration of the sample increased. Apparently the slope of the calibration curve for this particular experiment was too steep.

The design of these experiments permitted the statistical isolation of the contributory components of variance [for a discussion of components of variance see Snedecor (*8*)] in the calcium method and the estimation of their associated pure variances. These estimated variances are given in Table III. The estimated animal-to-animal variances are large compared with errors from other sources in the calcium procedure. These estimates of animal variability, however, do not necessarily express the variability to be expected for all swine populations, since they are based on a limited number of observations.

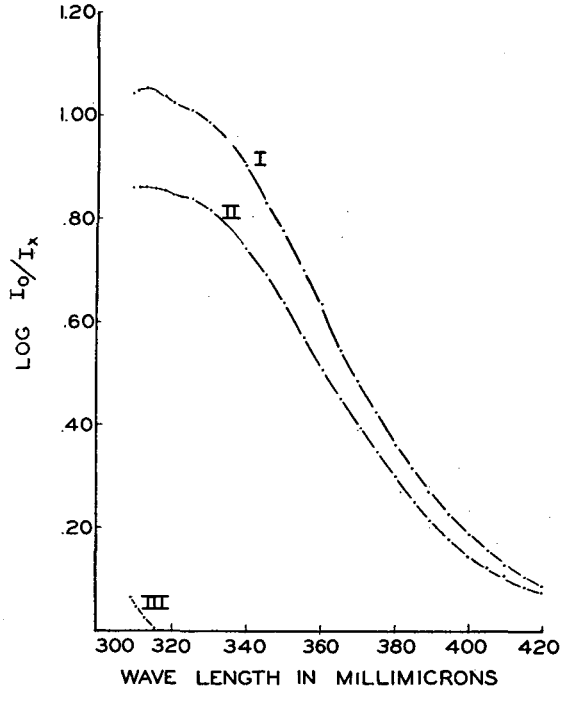

Figure 2. Absorption Spectra of Ceric-Cerous Systems in 1 *N* Sulfuric Acid

I. Ceric sulfate stock solution diluted 1/30 with 0.2% cerous sulfate in 1 *N* sulfuric acid
II. Ceric sulfate working solution partially reduced with sodium oxalate
III. 0.2% cerous sulfate in 1 *N* sulfuric acid

Table II. Calcium Equivalents of Primary Standard Sodium Oxalate

Sodium Oxalate Added	Spectrophotometric Method: Beckman at 315 mμ	Spectrophotometric Method: Coleman at 390 mμ	$KMnO_4$ Titration Method: Randomized series	$KMnO_4$ Titration Method: Nonrandom series
	Mg. per 100 ml. of serum			
10.08	9.78	10.03	10.24	11.20
	9.84	9.96	9.53	9.77
	9.78	10.04	9.77	9.77
	9.80	10.08	9.77	10.48
	9.87	10.12	10.00	10.24
Mean and $s_{\bar{x}}$[a]	9.81 ± 0.02	10.05 ± 0.03	9.86 ± 0.12	10.29 ± 0.27
11.48	11.31	11.55	11.43	11.91
	11.26	11.32	11.43	11.43
	11.21	11.39	10.96	11.20
	11.25	11.58	10.72	11.67
	11.27	11.39	11.67	12.15
Mean and $s_{\bar{x}}$	11.26 ± 0.02	11.45 ± 0.05	11.24 ± 0.17	11.66 ± 0.17
8.96	8.58	9.04	9.05	9.53
	8.94	8.96	9.29	9.53
	8.20	8.36	8.58	9.05
	8.61	9.04	8.81	9.05
	8.63	8.62	8.34	9.53
Mean and $s_{\bar{x}}$	8.59 ± 0.12	8.80 ± 0.14	8.81 ± 0.17	9.34 ± 0.12
7.84	7.36	7.96	8.58	8.10
	7.49	7.85	8.10	7.86
	7.46	8.07	7.38	8.34
	7.45	8.07	8.34	8.58
	7.44	8.03	8.10	7.86
Mean and $s_{\bar{x}}$	7.44 ± 0.02	8.00 ± 0.04	8.10 ± 0.20	8.15 ± 0.14
10.92	10.72	10.94	11.20	11.43
	10.72	10.88	11.20	11.91
	10.57	10.71	11.20	12.39
	10.72	10.96	10.96	11.20
	10.71	10.93	10.24	10.72
Mean and $s_{\bar{x}}$	10.69 ± 0.03	10.88 ± 0.05	10.96 ± 0.19	11.53 ± 0.29
Coefficient of variation, %	1.3	1.6	3.9	4.6
Mean bias, %	−3.0[b]	−0.2	−0.6	+3.4[b]

[a] $s_{\bar{x}}$ = standard deviation of mean = $\sqrt{\frac{S(x - \bar{x})^2}{n(n-1)}}$

[b] Highly significant, $P < 0.01$.

The nature of titrimetric oxidations precludes more than a single determination on each oxalate precipitate; hence, the errors associated with oxalate precipitations and permanganate oxidations are always confounded. Comparing the combined variances of filtrates, precipitates, and oxidations for the two methods, it may be noted that the error variance of a calcium determination by the ceric method is less than one half of that by permanganate titration.

A single determination of serum calcium by the ceric method involves the preparation of one trichloroacetic filtrate, one oxalate precipitation, and one measurement of the oxalate equivalence. When the variances associated with each of these procedural steps are compared with their sum, it may be shown that, of the total error variance accompanying such a determination, 15% is ascribable to the trichloroacetic filtration, 79% to the oxalate precipitation, and 6% to ceric oxidation. Thus, refinement in the preparation of the oxalate precipitates offers the most promising way for further increasing precision of calcium determinations.

Table III. Estimates of Components of Variance in Calcium Method

Component of Variance	Ceric Method[a]: Estimate	Ceric Method[a]: D.F.[c]	$KMnO_4$ Titration[b]: Estimate	$KMnO_4$ Titration[b]: D.F.[c]
Animal	0.272	24	0.240	24
Filtrates within series	0.013	2	...	..
Precipitates within groups	0.070	62	...	..
Reading error (mean for Beckman and Coleman)	0.006	135	...	..
Sum of analytical	0.089	48	0.268	4
Beckman	0.002	...	...	..
Coleman	0.011	...	...	..

[a] Weighted average of two experiments using combined data for Coleman and Beckman determinations.
[b] Weighted average of two experiments.
[c] Degrees of freedom were computed by method of Satterthwaite (*6*).

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Iodometric Determination of Vinyl Alkyl Ethers

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A rapid method for determining vinyl alkyl ethers involves the consumption of iodine by the vinyl ether and the back-titration of the excess iodine. The average deviation of the method was 0.2% with a maximum deviation of 0.5%.

THE previous methods for determining vinyl alkyl ethers involve hydrolysis of the vinyl ether to acetaldehyde and the corresponding alcohol, and the determination of the acetaldehyde.

$$R{-}OCH{=}CH_2 + H_2O \xrightarrow{H^+} ROH + CH_3CHO$$

The formation of the oxime of acetaldehyde with hydroxylamine hydrochloride was used by German industrial laboratories to analyze vinyl ethers. This method involved reaction times up to 6 hours and the final end point depended upon color comparisons for accurate results; any vinyl ether sample that was somewhat colored by impurities gave very poor results or could not be analyzed at all because of difficulty in observing the end point. Recent modifications of the German method decrease the reaction time; however, the end-point difficulties remain, especially with colored samples.

A method involving hydrolysis of the ether and determination of the acetaldehyde by the bisulfite addition reaction has been developed (*1*). The reaction time for this method is only about 15 minutes but a potentiometric titration is necessary to obtain accurate results. The apparatus provides for carrying out the hydrolysis in one section of the apparatus, then transferring the contents of the hydrolysis vessel to the second section which contains the sodium sulfite for the aldehyde reaction. The bisulfite method yields results accurate to better than 0.4% with no equilibrium or end-point difficulties.

The iodometric method described is by far the simplest method yet devised which will still yield results of a high degree of precision and accuracy. The reaction time is 10 minutes, and the entire analysis is carried out in one vessel. The end point is very sharp to the naked eye and no color comparisons or electrical indicating devices are necessary.

The reaction between the iodine and the vinyl ether requires the presence of an alcohol, as the end product of the reaction is the corresponding iodoacetal.

$$R{-}OCH{=}CH_2 + I_2 + CH_3OH \longrightarrow \underset{\displaystyle OCH_3}{\overset{\displaystyle |}{ROCH{-}CH_2I}} + HI$$

The mechanism of the reaction was established in several ways. On omission of the alcohol, no quantitative reaction between the ether and the iodine occurred. The pH of the solution was measured before and after the sample was introduced; the pH dropped considerably on introduction of the ether, indicating the formation of hydriodic acid. To substantiate these qualitative observations, several reactions were run using *n*-butyl vinyl ether. The final reaction mixtures were combined and extracted with carbon tetrachloride. The carbon tetrachloride extracts were distilled and a product was obtained whose carbon, hydrogen, and iodine analysis corresponded to the methyl butyl iodoacetal.

The impurities usually associated with vinyl alkyl ethers—namely, alcohols, acetaldehyde, acetals, acetylene, and water—do not interfere under the conditions of the iodometric analysis. This procedure is an excellent method for determining vinyl alkyl ethers in the presence of acetals and acetaldehyde. Because all previous methods for vinyl alkyl ethers depended on acid hydrolysis and determination of the resulting acetaldehyde, they would react with the acetal along with the ether. As the ultimate analysis was for acetaldehyde, samples containing acetaldehyde presented difficulty.

Acid impurities in the sample will not interfere unless they lower the pH of the analysis solution below 2. In such an acid solution the hydriodic acid is sensitive to atmospheric oxygen, and oxidation to free iodine results. Because the sample is titrated to the disappearance of the free iodine color, the oxidation of the hydriodic acid obscures the end point by causing the color to reappear very rapidly.

Alkaline impurities interfere in the analysis by causing the reaction to take a different course. In alkaline solution the ethers react with the iodine, as does acetaldehyde, to form iodoform. Samples containing free alkali or too much free acid should be neutralized before analysis.

Acetaldehyde shows no interference whatsoever so long as the sample contains no free alkali. When a sample containing approximately 0.060 gram of ether and 2.0 grams of acetaldehyde was analyzed, there was no interference, even in the presence of such a large amount of acetaldehyde.

PROCEDURE

Vinyl ether samples are accurately weighed by sealing 0.001 to 0.002 mole in small glass ampoules. Bulbs of soft glass are blown to about 1.25-cm. (0.5 inch) diameter, having a capillary stem of about 10 cm. (4 inches). An ampoule is weighed and is then filled by dipping the capillary below the surface of the vinyl ether, and cooling the bulb with a dry ice–acetone cooling mixture until the desired amount of ether has entered the bulb. The ampoule is sealed by touching the end of the capillary to a small flame while keeping the bulb in the cooling mixture. The ampoule is then left to dry and come to room temperature before reweighing.

The reaction vessel used is a 500-ml. wide-mouthed glass-stoppered bottle. About 25 ml. of glass beads are placed in the bottle, and 50 ml. of standard iodine (0.1 *N*) are added by pipet. To this are added 50 ml. of C.P. methanol and the vinyl ether sample. The stopper is well greased to prevent any leaks, and the bottle is shaken violently to enable the glass beads to crush the sample ampoule thoroughly. After the ampoule is crushed, the bottle is placed on a mechanical shaker for 10 minutes. Shaking is very

Table I. Analysis of Vinyl Alkyl Ethers

	Sample	Analysis by Hydrolysis and Bisulfite Addition, %	Iodometric Analysis, %
1.	Methyl vinyl ether	96.7	96.51
			96.48
			97.02
2.	Methyl vinyl ether	[a]	99.61
			99.01
3.	Ethyl vinyl ether	[a]	99.03
			99.75
			99.51
			99.72
4.	*n*-Butyl vinyl ether	98.7	98.45
			98.85
			98.81
5.	Isobutyl vinyl ether	[a]	99.34
			99.35
			99.25

[a] Ether was purified by washing five times with alkaline water (pH = 8) to remove acetaldehyde and alcohol; cooled to −50° C. and filtered from any ice which had separated; and finally distilled from sodium.

important to complete the reaction with methyl vinyl ether which, because of its low boiling point, will be present largely in the vapor phase. As the higher boiling ethers will be in solution at room temperature, complete reaction may take place without continuous shaking for 10 minutes.

After shaking, the stopper and side walls of the bottle are rinsed, and the contents of the bottle are titrated with standard 0.1 *N* thiosulfate. Inasmuch as starch indicator in the presence of methanol is not satisfactory, the end point is taken as the disappearance of the last trace of iodine color. On standing a few minutes after titrating, a slight iodine color may return to the solution, but in all cases the first disappearance of iodine is the correct end point. If the sample size is large, the excess iodine titration will be small and the iodine color will return more rapidly. If a smaller sample is used there will be a large excess of iodine left to titrate and the iodine color will return much more slowly. The iodine color returns so slowly that it does not noticeably affect the determination of the end point.

It is very important to use C.P. methanol in the above reaction. As a check, it has been the authors' custom to run a blank titration of 50 ml. of standard iodine in 50 ml. of C.P. methanol. If the iodine titration in the presence of the methanol differs from the calculated titer, a correction for the blank must be applied. From the titration the excess iodine present in the reaction mixture is determined. Subtracting this excess from the original 50 ml. iodine added yields the iodine used in the reaction. Per cent vinyl ether is then calculated as follows:

$$\frac{\text{Ml. of } I_2 \text{ used in reaction}}{1000} \times \text{normality of } I_2 \times \frac{\text{M.W. of ether}}{2} \times \frac{100}{\text{wt. of sample}} = \% \text{ vinyl ether}$$

DISCUSSION

The method was found to work for practically all the vinyl ethers tried. Lauryl vinyl ether and octadecyl vinyl ether could not be determined by this method because of their limited solubility in the reagents used. Attempts to analyze these samples by eliminating water from the system entirely, using alcoholic solutions of iodine and solutions of iodine in carbon tetrachloride, gave very unsatisfactory results. Apparently water is necessary to obtain a clean-cut reaction. The use of the standard aqueous solution of iodine provides sufficient water to obtain a good reaction, and the aqueous iodine is a very stable reagent.

The end point in this analysis is very sharp. Starch is not used because of its inactivity in an alcoholic medium; however, the disappearance of the iodine color can be very easily detected (within one drop).

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Composition of Vapors from Boiling Solutions

Improved Equilibrium Still

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Scores of systems of binary and ternary volatile liquids have been studied at atmospheric pressure, high vacuum, and high pressures by numerous investigators in modifications of a simple unit first developed 20 years ago. Subsequent studies on models incorporating various improvements suggested by some of these workers have shown that a newer form has some advantages in securing readily more precise data for engineering design. A carefully scaled drawing of this newer unit is presented with some discussion of its advantages and the technique of such determinations.

ALTHOUGH Rosanoff and co-workers (*17*, *18*, *19*) used various methods in their early attempts at precise determination of vapor-liquid equilibria, their last system (*17*) depended on an analysis of successive fractions distilled, and extrapolation back to the point where distillation started. This has seldom been used recently; and practically all workers have attempted to utilize the apparent advantages of the recycling type of equilibrium still. Most of the numerous types used have been similar to those discussed in previous articles of this series (*4*, *7–10*, *13*, *14*, *15*).

Chilton (*1*) suggested an additional feature, the interposition of what amounts to one bubble plate between the still and the condenser. The vapor leaving the liquid in this plate was presumed to be more nearly in equilibrium therewith than vapor leaving the liquid in the still itself. Scatchard, Raymond, and Gilmann (*20*) modified the unit in an elaborate pattern for use at constant temperatures rather than the constant pressure conditions required for engineering work. As much as 2.5 hours were required for the temperature adjustment. Jones, Schoenborn, and Colburn (*6*) greatly simplified the system; others working with this modification found that considerable attention was necessary in the control of the flash boiler used, and that trouble was experienced with entrainment. Gillespie (*2*) questioned the results obtained in this still, because of the return of the condensate as a more or less superheated vapor. A still further modification (*11*) also gave indication of values of vapor composition below the true values, owing to entrainment. Entrainment should be watched for in the use of an added "bubble plate" in an equilibrium still because of the small volume and disengaging surface area compared to a usual boiler, as well as the relatively very high velocity of vapor bubbling through the liquid.

Gillespie (*2*) attempted to improve the recycling still by the use of a Cottrell boiling tube, his theory being that vapor and liquid leaving its separator would be more nearly in equilibrium with each other than would vapor that had risen in fine

droplets through a liquid owing to a regular boiling action, and then disengaged relatively quietly from a relatively very large surface. No data were presented to demonstrate the advantages claimed. Because the volume in the boiler has to be almost fixed (and may have to be adjusted after the condensate has been partially collected) a somewhat less flexible procedure has to be followed, which may require a longer time. The only available data taken with this modification (*16*) have indicated that results differing from those obtained by every other method and apparatus are given by this technique and apparatus, even in using a time of from 3 hours to as much as 41.5 hours of boiling for reaching each equilibrium point. (The average time of boiling used was 7.5 hours for each run.)

If it is necessary to use a Cottrell pump because of vapors in the boiler not being in equilibrium with the liquid there, and this results in an interchange between the liquid and vapor after leaving the boiler (the use of a Cottrell pump presupposes this), then the equilibrium liquid leaving the vapor at the disengagement chamber is not of the same composition as that in the pot, because its composition has been changed in this tube. The vapor also is of different composition than would be in equilibrium with the liquid in the boiler from which the sample is taken. Furthermore, heat losses in the system above the boiler (and usually uninsulated) will result in a slight condensation of vapors with a resulting change of composition in both phases. The extent of both effects is directly dependent upon the recirculation rate.

In the development of the simple recycling still without either the bubble plate or Cottrell boiling tube features, a compact, unitary apparatus is desired, which would be readily set up, would be operable with the minimum of time, skill, and attention, and would give results of sufficient precision for the design of engineering equipment, principally distillation columns.

Methods have been developed (*12*) for correlating data taken at several pressures or temperatures by the use of a logarithmic straight-line plot of vapor compositions, relative volatilities, or activities against pressure. (These methods also allow the ready transposition of data from conditions of constant pressure to constant temperature, or vice versa.)

A review of the desirable considerations in design and operation of apparatus for determination of vapor-liquid equilibrium has been given (*8*), together with a detailed drawing of an apparatus for securing these results. More recent work has shown that modifications will secure the desired results with greater precision and with the automatic elimination of possible errors. The apparatus shown in Figure 1 incorporates many features of the previous model, and several such units have been built and operated in various academic and industrial laboratories. The results obtained indicate that its use has some advantages. (The unit shown has been constructed by E. Machlett and Son and Emil Greiner, New York, N. Y., and by Scientific Glass Apparatus Co., Bloomfield, N. J.)

BOILING FLASK

The boiling flask desirably is large, as there should be a large volume of boiling liquid compared to volume of condensate (*8*). An 800-ml. standard Kjeldahl flask is indicated but a standard round-bottomed flask may be used or a balloon about 11.25 cm. (4.5 inches) in diameter may be blown. A much shorter neck minimizes the chance of radiation losses, condensation, and corresponding fractionation in the central tube.

The still may be heated directly by a small Bunsen flame, an external electric heater, or an internal electric heater of uncovered resistance wire. The small bubbles formed in large numbers probably represent nearly an equilibrium boiling condition. Electrical connections are made through openings (drawn in the front view, at right angles to their true position). (An alternative method for attaching leads uses spherical ground joints rather than ⅁ connections. In a stainless steel, bronze, or mild steel ball of corresponding size is drilled and tapped a hole for a binding post and wire connections on each side. The standard clamp for joining the spherical joint to its socket then gives direct electrical contact through the ball itself.) Brass plugs (ground to the ⅁ taper) previously suggested (*8*) have been found unsatisfactory, as they seat tightly; if they expand (or the flask contracts), the flask is broken.

A vapor outlet tube of relatively large diameter allows a minimum of pressure drop for vacuum work, down to a few millimeters of mercury. If even lower absolute pressures are to be used, the cross section for vapor travel may be increased correspondingly; up to about 2.5 cm. (1 inch) in diameter is satisfactory for most determinations even under 1-mm. pressure. A correspondingly larger inlet of the condenser is necessary.

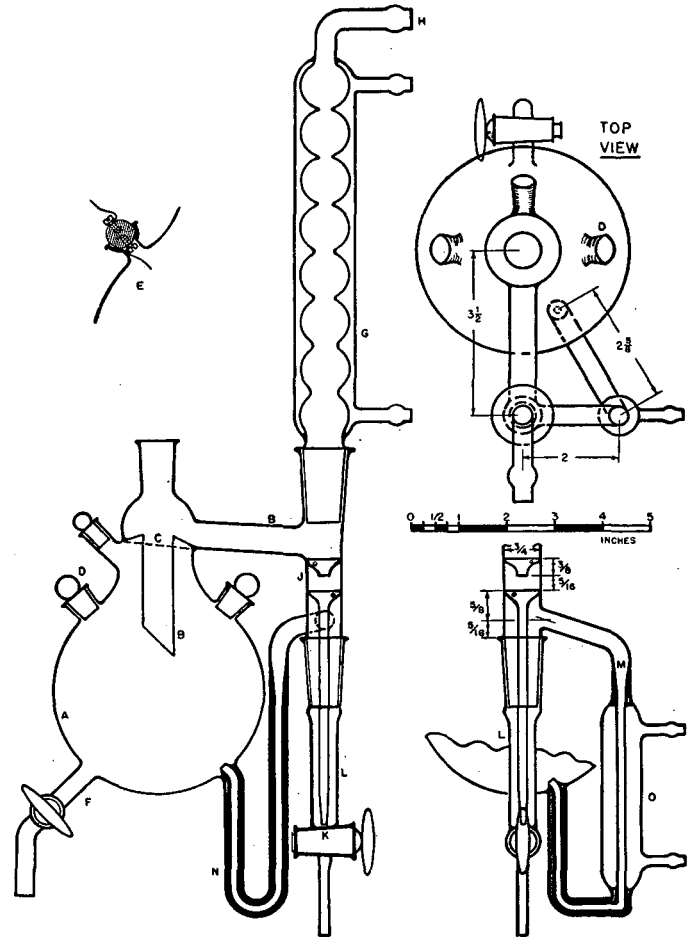

Figure 1. Equilibrium Still One Quarter Size. All Dimensions in Inches, ⅁ in Millimeters. Left, Front View. Right, Alternate Side View, Showing Condensate Cooler

Still *A*. Body, 800-ml. Kjeldahl flask, or 4.5-inch (approx.) diameter balloon. Top vapor jacket purge, ⅁ 9. Thermometer opening, ⅁ 22
B. Central and outlet vapor tubes 5/8 inch i.d.; may be enlarged for vacuum work. Tip of central tube is 3 inches above bottom of flask and its length is 2.5 inches from tip to top
C. Sloped gutter and vapor neck for draining to receiver
D. Openings for electrical connections are in front and back as shown in top view, instead of on sides as in front view (⅁ 13)
E. Alternate connection for electrical wires to internal heater through binding posts in metal ball fitting standard spherical joint and held together with standard clamp
F. Liquid sample cock, as close as practical to still
Condenser. *G*. Approximate dimensions of condenser shell, 8.5 × 1.25 inches with 1 inch diameter balls, constrictions varying from maximum opening at bottom to 0.25 inch at top. Bottom connection ⅁ 24/40
H. Connection to atmosphere or vacuum
Receiver and Connections. *J*. Drop counter as close to vapor inlet as possible, with 3/16 inch outlet. Vent holes on drop counter and receiver funnel 1/16 inch diameter
K. Bottom of receiver funnel not more than 1/8 inch from stopcock
L. Interchangeable receivers for samples of different volumes. Length to cock, 4 inches. Connection ⅁ 19/38
M. Heavy-walled tubing 1/16 to 1/8 inch i.d. connecting overflow from this point to still
N. Return trap may be shortened if no cooler is used. This connects in back of receiver and is back of plane of cross section shown (as in alternate side view and top view)
O. Cooler approx. 4 inches in length, with 1/8 inch light wall inner tube

Experiments have been conducted to determine the amount of entrained liquid carried out of the still. This is practically zero even at high rates of distillation.

CONDENSER

Various workers have objected to the cold condensate obtained from the bottom of a usual condenser. Cold condensate contacts the air or other gas present and may dissolve some. It is not believed that this dissolved gas would displace equilibrium conditions; however, such noncondensable gas will be liberated in the boiling flask and may tend to accumulate in its neck. Thus the desired jacketing of the central vapor tube with vapors will be reduced, with increase of possible heat transfer, reflux, and rectification. A hot condensate, however, is obtained from the base of the reflux condenser shown in Figure 1, wherein the vapors entering and the liquid leaving are in contact. Besides the elimination of the effects of gas solution, a further advantage is that with a countercurrent condenser no additional vent condenser (*8*) is needed.

Larger openings to the balls of the condenser near the bottom minimize pressure drop of entering vapors and allow excellent drainage, while smaller openings near the top minimize possibility of vapors diffusing out without condensing. Straight tube condensers have also been used in somewhat longer length where the making of the ball type shown presented a problem. The outlet may be connected to a pressure-regulating system (*3*, *5*).

The condenser may be made an integral part of the unit, or it may be fitted as shown, with a $\overline{\$}$ connection to allow ready demounting for more convenient storage. For determinations at pressures below about 10 mm., a cup at the rim of this joint may be made to hold mercury and prevent leakage. Similar mercury-sealed joints may be used for other connections.

CONDENSATE RECEIVER

The drop counter below the condenser discharges directly into a funnel (both provided with a vent hole) which conducts the liquid to the bottom of the receiver in order to ensure positive displacement therein. This funnel is shown as an integral part, but it may also be made as a separate funnel, in which case it can rest on the plug of the cock. This allows somewhat more flexibility and less danger of breaking if condensate receivers of different sizes are used. The top of the funnel should then fit closely to the inside of the tube; and the $\overline{\$}$ ground-glass connection to the receiver is enlarged to accommodate.

To minimize time required for taking samples, it is highly desirable (*8*) to use a condensate receiver no larger than actually needed to hold sufficient volume for the particular method of analysis.

In the drawing a separable receiver is shown (although it may be integral with the still as in previous models). It has a capacity of about 18 ml. to the overflow. An even smaller volume of sample may suffice. If the analyses are to be made by refractive index, where only a few drops are necessary, the receiver may be partly or completely filled with glass beads to minimize the effective volume, or the body may have a smaller diameter.

Where larger samples are necessary, they are taken in a larger condensate receiver which has the same standard taper connection and exactly the same length to the cock, but a larger diameter (or even a spherical body). For general analytical methods, a condensate reservoir somewhat larger than that shown, and with an outside diameter of about 1.8 cm. (0.75 inch), is recommended, which will give a total capacity of about 28 ml. of sample. Several interchangeable receivers of different sizes are useful, even with the same system, wherein a larger sample may be necessary near one end of the curve. (Thus in the water–acetic acid system, where acid titration is used, a much larger sample would be required to give the same precision at 99% water as at 1% water.)

The front view of Figure 1 is idealized somewhat to show the overflow from the reservoir in the plane through the axes of the still, condenser, and receiver. This is too close, particularly when a larger reservoir is used. To avoid increasing the width of the unit, the overflow may be taken from the back of the unit as shown by the dotted circle. A better representation of this construction is that of the side and top views at the right side.

Usually it is desired to return cold condensate to the still in order to minimize its tendency to flash, particularly when the boiling points of the two components are far apart, and thus allow a better chance of mixing it with the bulk of the boiling liquid. The overflow from the condensate reservoir in the side and top views passes through a smaller cooler, in the form of the usual Liebig condenser, thence through a re-entrant 0.3-cm. (0.125-inch) nozzle into the boiling liquid (*8*). In order to minimize holdup and hence the time required to obtain an equilibrium sample, this tube is approximately 0.3 to 0.15 cm. ($^1/_8$ to $^3/_{16}$-inch) inside diameter. For strength, it should be thick-walled, as shown in the side view of Figure 1, with a thin-walled tube through the cooler. To prevent the entrainment of vapor, the outlet from the reservoir should be maintained full size as shown in the side view, funneling down just above the cooler.

PREVENTION OF HEAT LOSSES

It is necessary to minimize any condensation in the central vapor outlet leaving the distilling flask, so that reflux and consequent fractionation may be prevented. In the present unit the length of neck and hence the heat losses, the condensation, and length of travel for fractionation have all been greatly reduced; and the jacketing of the central tube with vapors is much more effective than previously, as the hot condensate prevents solution of gases which, after re-evolution in the still, collect around the long central tube of the former model. When boiling temperatures are below about 100° C., merely insulating the upper part of the still pot and its neck is effective in reducing the heat losses to a negligible amount. This may be done readily by applying a heavy coat of a paste of asbestos and water and allowing to dry. Where considerably higher temperatures are encountered, heat may be applied to the outside of the upper part of the still pot and the neck, in an amount equal to that radiated.

Electrical resistance wires are wound around a thin layer of asbestos applied to the glass. The wires are covered with more asbestos; and a rheostat or transformer controls the heat input. The correct amount may be determined and controlled by embedding a thermometer or thermocouple immediately adjacent to the flask and underneath the insulation. (Alternatively, the resistance of the heating wires themselves may be measured by a bridge or other electrical circuit and calibrated against temperature.) The outside temperature is controlled to approximately that of the boiling liquid by adjustment of electrical input.

Attention has been called (*8*) to the danger of superheating the flask immediately adjacent to the boiling surface, and the disadvantages attendant on the use of agitators or a high rate of ebullition which splashes liquid on the superheated glass surface. Such splashings vaporize completely; and the percentage composition of more volatile constituent in the vapors is unduly lowered.

The vapors may be superheated above the boiling surface to prevent condensation; but the thermometer bulb in a stream of superheated vapor will give erroneous temperatures. (A droplet of liquid should remain on the thermometer bulb.) Alternatively, the boiling temperature is determined by having the bulb or thermocouple in the body of the boiling liquid.

ACKNOWLEDGMENT

Appreciation is expressed to the many who have used this and previous models of the equilibrium still and have offered suggestions based on their experience. Thanks are especially due to

Salvatore Silvis for his considerable help and for drafting the figure.

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Modified Glycerol Dichlorohydrin Reaction for Vitamin D_3

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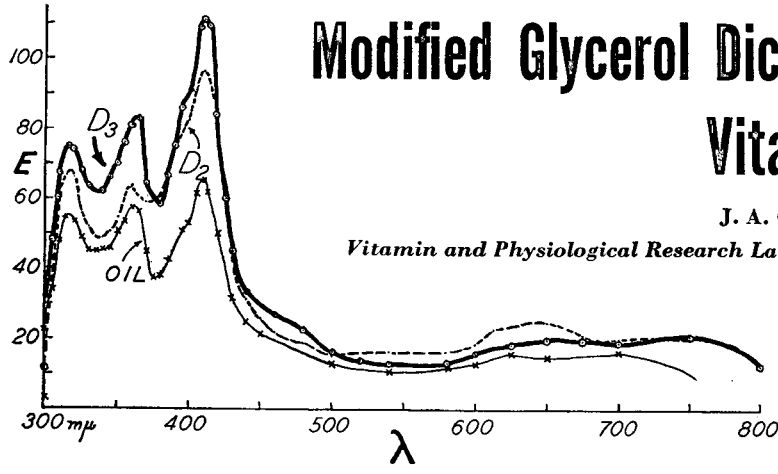

Figure 1. Absorption Curves for Vitamins D_2 and D_3 and a High Potency Oil

Readings begun at 300 mμ, 20 minutes after adding solvent to reagent at ratio of 3:2

MANY reactions have been proposed for the colorimetric estimation of vitamins D but at the present time the antimony trichloride procedure (*1*, *2*) is probably more widely used than any other method. Although this reaction is relatively sensitive, it has several disadvantages. As an alternative method Sobel, Mayer, and Kramer (*3*) proposed the reaction with activated glycerol dichlorohydrin and pointed out some of its advantages. The glycerol dichlorohydrin reaction is influenced by several factors but may be criticized chiefly for its relatively low sensitivity. This paper presents the results of studies of various factors affecting the sensitivity of the method particularly as applied to vitamin D_3. A modified procedure has been developed which is more than 20 times as sensitive as the original method.

EXPERIMENTAL RESULTS

The Beckman spectrophotometer was used for all studies reported in this paper. For the estimation of calciferol Sobel *et al.* (*3*) recommended, on the basis of less interference from the provitamins, a solvent-reagent ratio of 3 to 2 and a reagent containing 1% acetyl chloride. They also showed, however, that the reaction was somewhat more sensitive to calciferol at other wave lengths and with a ratio of 1 to 4. They determined the density of the color at 625 mμ in the Coleman spectrophotometer 15 minutes after mixing reagent and solvent.

Using Sobèl's procedure and beginning readings 20 minutes after adding the reagent, the author determined the absorption curve

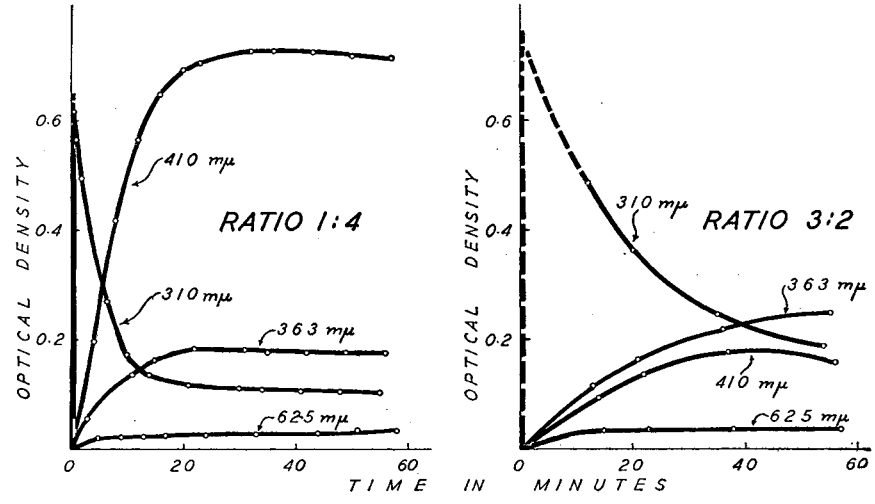

Figure 2. Variation with Time in Optical Densities of Solutions at Two Ratios of Solvent to Reagent

200 micrograms of vitamin D_3 in 10 ml. of colored solution

A modified glycerol dichlorohydrin reaction for vitamin D_3 is proposed, which is more than 20 times as sensitive as the original reaction. Under the conditions outlined the effects of certain interfering materials are relatively at a minimum. The method is simple and offers possibilities of adaptation to filter colorimeters. There is a linear relation between optical density at 410 mμ and concentrations of 2 to 25 micrograms of vitamin D_3 per ml. of colored solution. Comparisons of the results of the reaction with chick tests indicate that the method may be useful for estimating vitamin D_3 content of high potency oils.

of the color formed with vitamin D_3 with the Beckman spectrophotometer as shown in Figure 1. There are three points of maximum absorption at approximately 310, 363, and 410 mμ. At 625 mμ the maximum was not so well defined so at the other wave lengths. The curves for vitamin D_2 and a high potency oil determined under similar conditions are in general similar, although vitamin D_2 has a relatively lower absorption than vitamin D_3 at 410 mμ and a somewhat higher absorption at 625 mμ, as mentioned by Sobel *et al.* When the reaction time was increased to 30 minutes, an entirely different type of curve was obtained although the maxima were in the same place. In view of this evident variability in the reaction over a period of time it was decided to limit the study to four maxima—310, 363, 410 and 625 mμ.

The effect of time on the reaction of glycerol dichlorohydrin with vitamin D_3 was studied at these four wave lengths, using solvent-reagent ratios of 1 to 4 and 3 to 2 as shown in Figure 2. At 310 mμ the intensity of color reaches a maximum in less than 1 minute, then fades rapidly. It is not suitable for a routine reaction, as the maximum density is difficult to determine accurately in the short time. With a 1 to 4 solvent-reagent ratio the reaction, measured at 410 mμ, was more sensitive than at the other wave lengths studied and also offered possibilities of adaptation to filter colorimeters. At 625 mμ the absorption was very low with both ratios. It is evident that the measurement of the optical density of the solution at 410 mμ with a 1 to 4 solvent-reagent ratio yields a method about 25 times as sensitive as the original procedure.

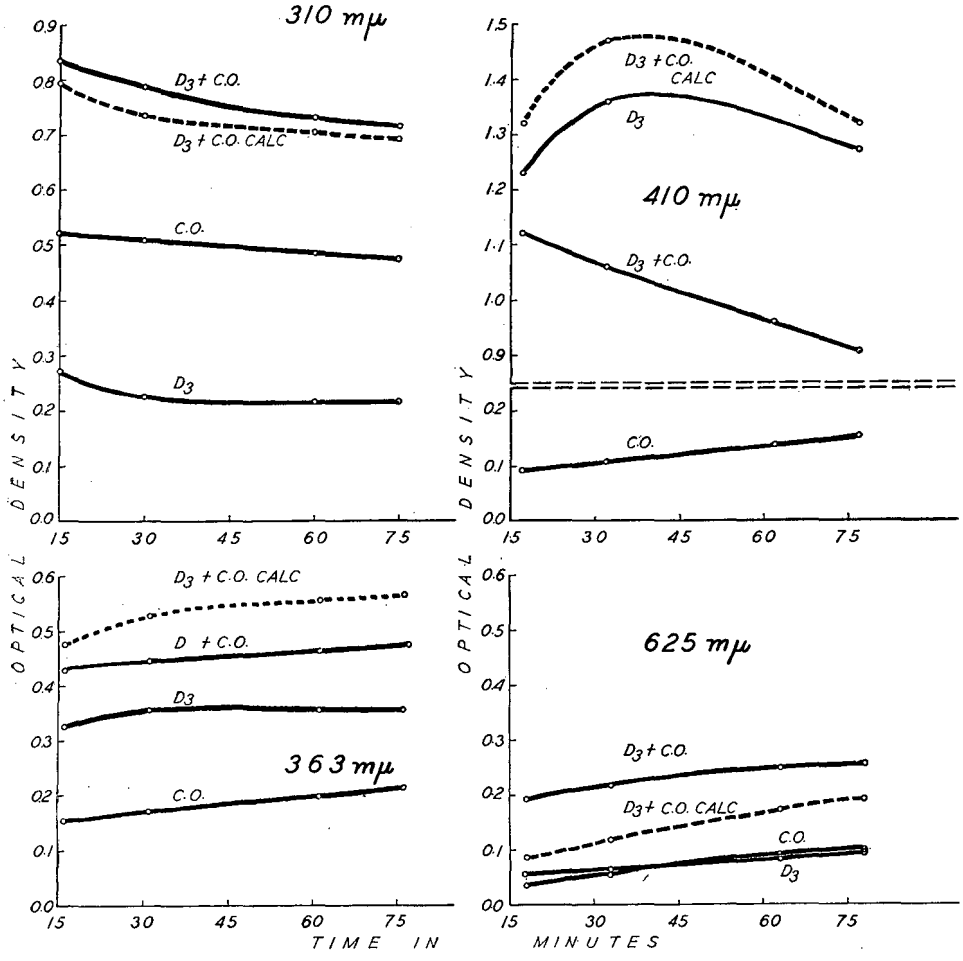

Figure 3. Effect of Corn Oil on Glycerol Dichlorohydrin Reaction Measured at Four Wave Lengths

D_3 = 500 micrograms (approx.). C.O. = 0.2 gram. Dotted lines indicate sum of densities of D_3 and C.O. when reacted separately

To test the general applicability of the reaction the effect of the addition of small amounts of corn oil and fish oil was studied by applying the reaction to these materials alone and to the same materials in a solution containing vitamin D_3. Four tubes were prepared, using 2 ml. of chloroform as solvent:

1. Blank.
2. 500 micrograms (approximately) of vitamin D_3.
3. 0.2 gram of oil.
4. 500 micrograms of D_3 plus 0.2 gram of oil.

Eight milliliters of glycerol dichlorohydrin were added to each tube and well mixed. The light absorption was measured at 310, 363, 410, and 625 mμ at four times over a period of about 75 minutes.

The optical densities for the different wave lengths and times are thus strictly comparable, for they were determined on the same solutions. The data for corn oil have been plotted in Figure 3. The dotted line in each graph was obtained by simply

Table I. Optical Densities

(Color produced 40 to 45 minutes after adding reagent containing varying amounts of acetyl chloride to 200 micrograms of vitamin D_3)

Acetyl Chloride in Reagent, Vol. %	Wave Length, mμ			
	310	363	410	625
1	0.108	0.179	0.726	0.030
2	0.104	0.213	0.795	0.020
4	0.067	0.184	0.677	0.013

summing the optical densities for vitamin D_3 and for the oil. This line illustrates the density that would be expected if the two substances when put together had reacted with the glycerol dichlorohydrin in the same way as when separate and had not interacted with each other.

It is evident from Figure 3 that some constituent of corn oil reacts with glycerol dichlorohydrin to yield a substance that absorbs light to a certain extent at all four wave lengths. At 410 mμ the reaction to corn oil is proportionately much less than at the other three maxima studied. Furthermore, the effect of corn oil on the reaction in the presence of vitamin D_3 cannot be predicted from the reaction of glycerol dichlorohydrin with corn oil alone. For example, at 363 mμ the optical density of the mixture is less than would be expected from adding the two densities, at 410 mμ it is less than for vitamin D_3 alone, and at 625 mμ it is greater than would be expected. When a solvent-reagent ratio of 3 to 2 was employed, these relations were somewhat changed. If the difference between the curve for vitamin D_3 plus corn oil and the curve for vitamin D_3 alone is considered as the effect of corn oil and expressed as percentage of the density of the solution of vitamin alone, it is obvious that at 310 and 625 mμ the amount of corn oil employed introduces an error of about 100%, while at 363 and 410 mμ it amounts to approximately 10 to 15%.

To determine what substance might be responsible for this interference the nonsaponifiable fraction was isolated from a similar aliquot of corn oil and the experiment was repeated using this fraction (Figure 4). At all wave lengths the interference was reduced by removing the saponifiable fraction. However, the fact that all the curves are in the same relative position indicates that the compound causing a reduction in the apparent vitamin content at 410 mμ and an increase at the other wave lengths is present in the nonsaponifiable fraction of the oil.

There is no explanation available for the reduction in optical density at 410 mμ with corn oil. Other experiments have shown that the causative substance is present in that part of the nonsaponifiable fraction of corn oil which is not precipitated by freezing in methanol—i.e., the nonsterol fraction. The substance is present in samples of corn oil from several sources.

Similar experiments were carried out with fish oils that contained 200 to 400 A.O.A.C. units of vitamin D and 2000 to 3000 I.U. of vitamin A per gram. The effect of various types of oils differs greatly. The curves for one oil that gave an intense purplish color with the reagent and showed relatively great interference in the vitamin readings are presented in Figure 5. Only at 410 mμ is the interference of the fish oil sufficiently low to permit an estimate of the vitamin D content. At the other wave lengths the response is greater to the fish oil than to the vitamin. It is doubtful if the purplish color was caused by vitamin A in the oil, for other oils containing the same amount of the vitamin did not show this color. Many oils, on the other hand, show relatively little interference (Figure 6). Measurement of the light absorption at 410 mμ in this case also is more satisfactory than at any other region of the spectrum. It is further evident that the substance that is present in corn oil and causes a reduction of apparent vitamin D content is not present in the fish oils studied.

In preparations containing synthetic vitamin D the presence of small amounts of ergosterol and 7-dehydrocholesterol could cause difficulty. With ten times the concentration of these substances as for the vitamins, reaction curves were determined with solvent-reagent ratios of 1 to 4 and 3 to 2 (Figure 7). The color formed under these conditions with 7-dehydrocholesterol is relatively negligible. With ergosterol a denser color is obtained, but in relation to that formed with vitamin D_3 the density is proportionately less at 410 mμ than at the other wave lengths. The reaction with ergosterol seems variable and may be influenced by other factors. The optical density of the

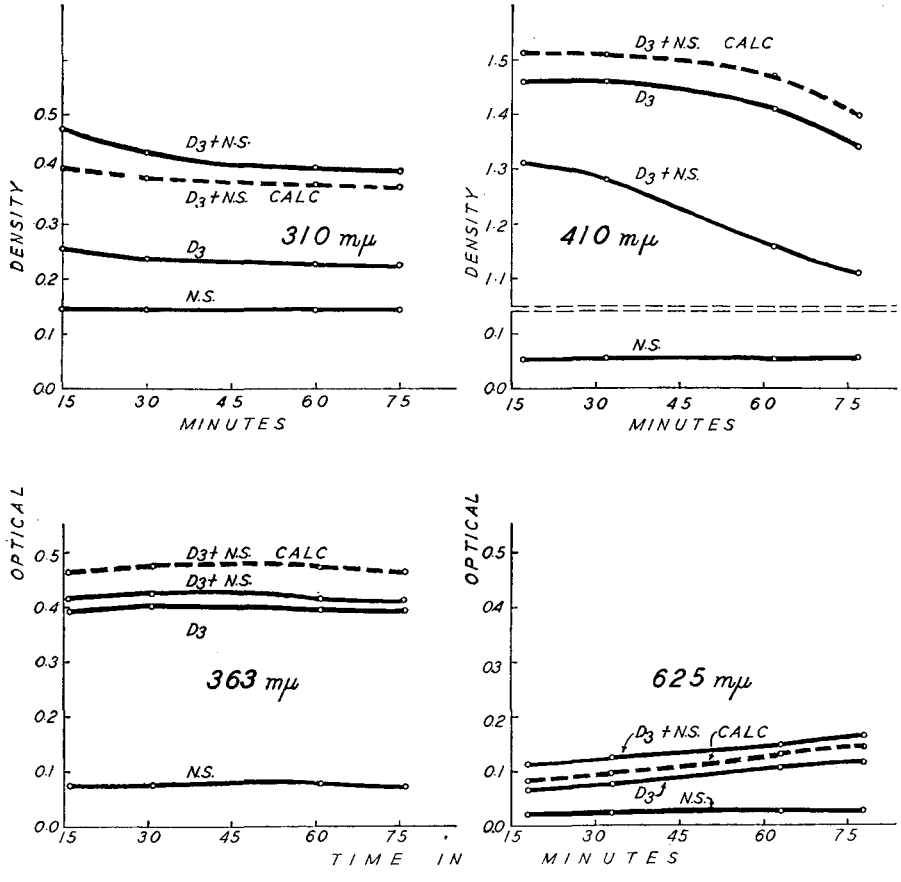

Figure 4. Effect of Nonsaponifiable Fraction of Corn Oil on Glycerol Dichlorohydrin Reaction

D_3 = 500 micrograms (approx.). N.S. = nonsaponifiable fraction from 0.2 gram of oil. Dotted lines indicate sum of densities of D_3 and N.S. when reacted separately

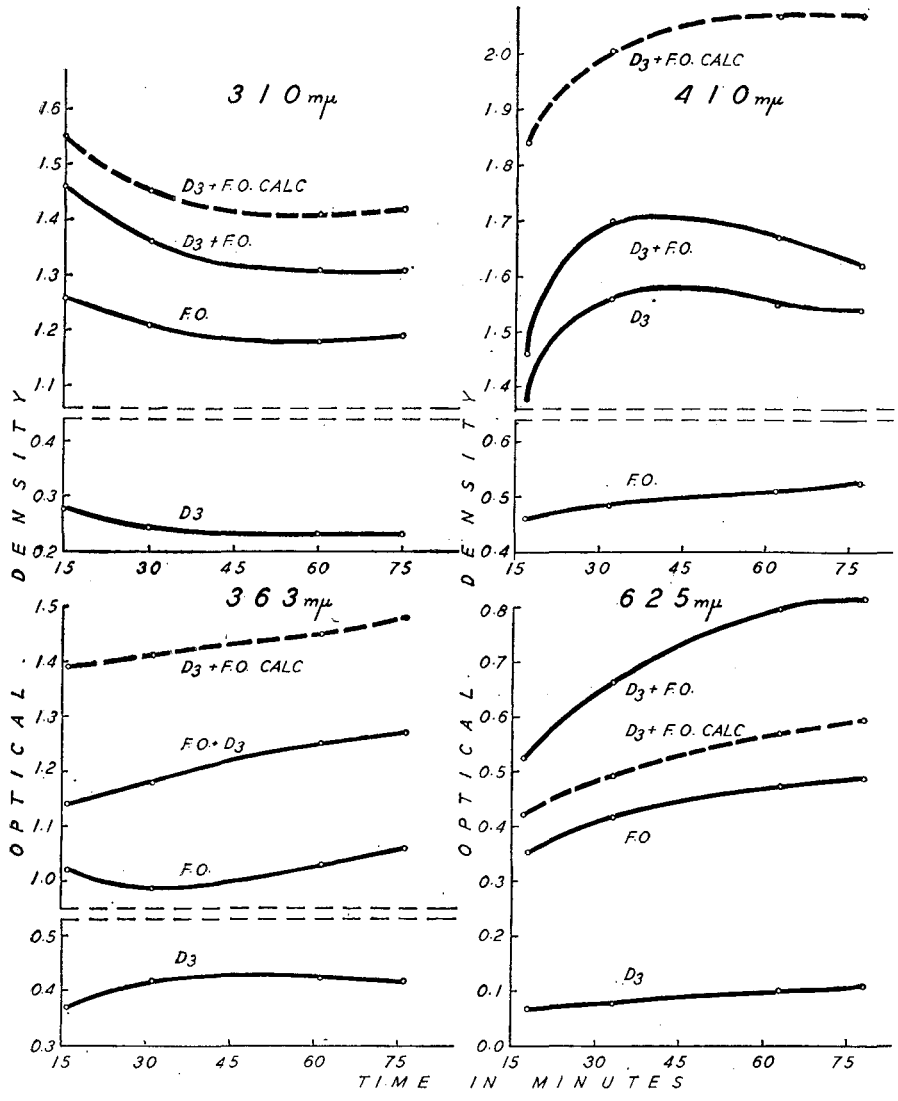

Figure 5. Effect of a Fish Oil Exhibiting Relatively Great Interference with Glycerol Dichlorohydrin Reaction

D_3 = 500 micrograms (approx.). F.O. = 0.2 gram of fish oil. Dotted lines indicate sum of densities of D_3 and F.O. when reacted separately

color formed with cholesterol and glycerol dichlorohydrin using a solvent-reagent ratio of 1 to 4 is approximately one half that with 7-dehydrocholesterol. In view of the relatively lower interference at 410 mμ the proposed modification would seem particularly suitable for the determination of vitamin D_3. Sobel and Werbin (*4*), using the Beckman spectrophotometer, have shown that the color formed on the addition of glycerol dichlorohydrin to a solution of vitamin A or carotene is as low, or lower, in absorption at 410 mμ as at any other band in the visible range.

The effects of other factors on the color reaction have also been investigated. Variations from 1 to 4% in the amount of acetyl chloride in the reagent as shown in Table I had relatively little effect on the density of color produced when readings were taken at 0 to 45 minutes. At 410 mμ the reagent containing 2% acetyl chloride gave slightly more color and that containing 4% slightly less than the one containing 1% acetyl chloride. The effect was not the same at all wave lengths. Little change could be detected in the intensity of color when the ratio of solvent to reagent was further widened to 1 to 9 and 0.5 to 9.5. Investigation of the width of the maximum at 410 mμ indicated that the absorption is approximately the same from 410 to 412 mμ. At the other three wave lengths the width of the maxima was somewhat greater.

At 410 mμ the time required to reach maximum density varied somewhat from day to day (Figures 3, 4, and 5). Temperature was a major factor in determining the rate of development and disappearance of color. The effect of variations in temperature from 20° to 40° C. is shown in Figure 8. It is evident that to ensure reproducible results the temperature of the reaction must be controlled and that 25° C. is probably the best temperature to employ.

Another factor causing variation from time to time is the lot of reagent used. Almost all the work in this paper was carried out using the reagent prepared by Shohan Laboratories. Occasionally some lots of activated glycerol dichlorohydrin darkened over a period of time and required redistilling to remove the yellowish color. These lots were slightly slower in producing color with vitamin D_3. Similar observations were made on lots of Eastman glycerol dichlorohydrin which was activated by the author by distilling from antimony trichloride. It is possible that the conditions of distillation may influence activation of the reagent. However, this difficulty is not serious and differences in reagents can be readily detected if each lot is checked against previous calibration curves.

The calibration curve shown in Figure 9 was drawn up using a solvent-reagent ratio of 1 to 4 and a reagent containing 1% acetyl chloride. Density readings were made 35 minutes after addition of reagent. The line was drawn through points calculated from the mean optical density per microgram of vitamin D. The observed points show good agreement with the calculated ones. There is a linear relation between the optical density and concentration within the limits of 2 and 25 micrograms of vitamin

Table II. Potencies of Oils Determined by Modified Glycerol Dichlorohydrin Reaction and by Chick Assay

Oil No.	Potencies Millions of A.O.A.C. Units per Gram: G D H reaction[a]	Chick assay[b]	Difference, %
1	3.13	3.06	+ 2
2	1.35	1.30	+ 4
3	1.30	1.35	− 4
4	1.38	1.45	− 5
5	1.40	1.30	+ 8
6	3.10	2.86	+ 8
7	2.43	2.16	+13
8	2.49	2.91	−14
9	2.54	3.38	−25
10	3.27	2.25	+45

[a] Conversion factor = 55,000,000 units per gram of crystalline vitamin D_3 (*5*).

[b] Data and oils supplied by A. D. Grieve, Ayerst, McKenna & Harrison, Ltd., and G. H. Kennedy, E. I. du Pont de Nemours & Co., Inc.

D_3 per ml. of colored solution. The effect of the addition of corn oil is also shown in Figure 9. As would be expected, this alters the position of the curve.

The usefulness of any colorimetric reaction lies in its ability to reflect biological activity of unknown oils. To determine the validity of the procedure outlined here the reaction was applied to ten oils of high potency which had been accurately assayed by several chick tests. A factor of 55 units per microgram of vitamin D_3 was used to convert the vitamin content as determined directly by reference to the standard curve, to a unit basis. This factor is an approximate average of the values reported by Waddell and Kennedy (*5*). The comparison (Table II), shows that the reaction gives a fair indication of biological potency, considering the error of the chick assays. The last two oils show a higher percentage difference between the chemical and biological tests than the others. Antimony trichloride tests of these oils agree more closely with the glycerol dichlorohydrin results than with the bioassay values. Although it seems more likely that the chick tests are at fault, it must not be overlooked that the chemical tests may not reflect the true biological potency of these particular oils.

The increment procedure gave results similar to the direct method and offered no advantage in the estimation of the vitamin content of the oils themselves but, as might be expected from Figure 9, was useful when the oils were diluted with corn oil. However, the variability of results indicated that further refinements of procedure are necessary for the separation of interfering materials in these lower potency oils. This was considered a problem of sufficient importance to merit separate investigation.

In view of the number of factors affecting the reaction it is possible that other modifications may make the reaction still more sensitive. The difference between the results reported here and those reported by Sobel *et al.* (*3*) cannot be ascribed to the different instruments employed, as the ratio of absorption at 410 to that at 625 mμ using a solvent-reagent ratio of 1 to 4 has been found to be approximately the same with the Coleman Universal spectrophotometer as with the Beckman. The modifications proposed here have been developed with particular reference to vitamin D_3, whereas Sobel worked chiefly with calciferol; nevertheless, preliminary investigations suggest that the two vitamins react similarly to activated glycerol dichlorohydrin. The modification has increased the extinction coefficient ($E^{1\%}_{1\ cm.}$) of the colored solution to approximately 360. The $E^{1\%}_{1\ cm.}$ for the antimony trichloride reaction has been reported as approximately 1800 to 2200, depending on the solvent used (*1*). The modified method is thus about one fifth as sensitive as the antimony trichloride procedure. For oils of high potency this difference in sensitivity is not critical. For oils of lower potency, however, only experience will determine whether the lower sensitivity of the glycerol dichlorohydrin reaction will be sufficiently balanced by greater ease of manipulation and by less interference from provitamins and from other similar substances present in the oils.

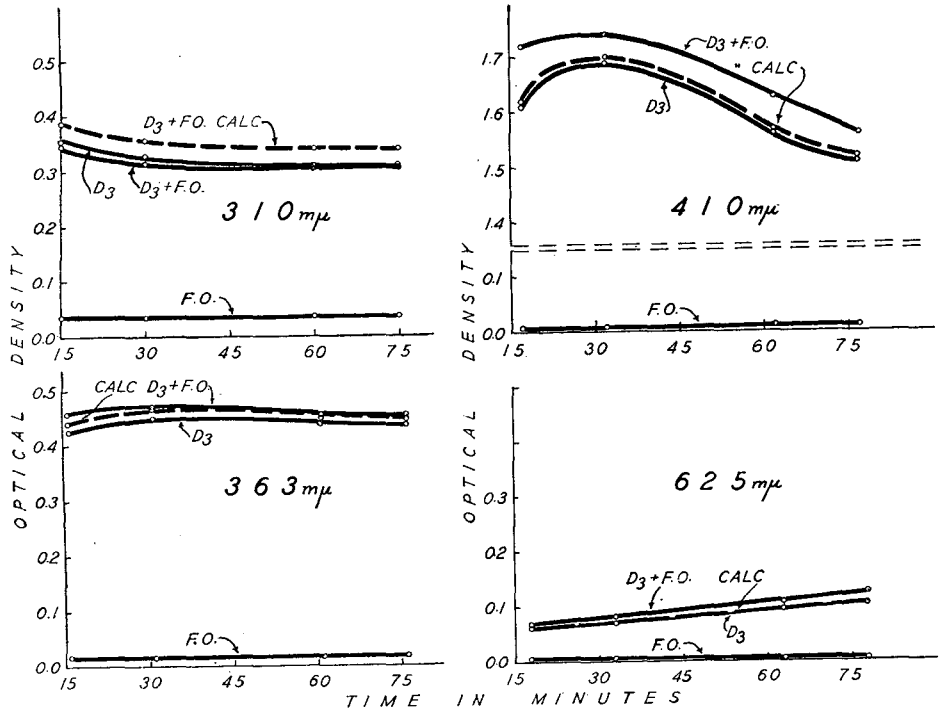

Figure 6. Effect of a Fish Oil Exhibiting Relatively Little Interference with Glycerol Dichlorohydrin Reaction

D_3 = 500 micrograms (approx.). F.O. = 0.2 gram of fish oil. Dotted lines indicate sum of densities of D_3 and F.O. when reacted separately

MODIFIED PROCEDURE

Apparatus. The Beckman quartz spectrophotometer model DU with Corex cells is recommended. Sensitivity knob is kept at 1 to 3 turns from clockwise limit.

Reagents. Activated glycerol dichlorohydrin (obtainable from Shohan Laboratories, 78 Wheeler Point Road, Newark 5, N. J.). One milliliter of reagent grade acetyl chloride is added to 99 ml. of glycerol dichlorohydrin.

Chloroform, commercial U.S.P. grade, is used without purification.

Method. Two milliliters of chloroform containing 25 to 250 micrograms of vitamin D_3 are pipetted into a test tube or glass-stoppered cylinder, 8 ml. of activated glycerol dichlorohydrin are added, and the solution is thoroughly mixed. The cylinders are placed in a water bath at 25° C. out of strong light and the color is allowed to develop. The density of color is measured at 410 mμ in the Beckman spectrophotometer during the period of maximum intensity—i.e., in 30 to 40 minutes.

A calibration curve is made up using crystalline vitamin D_3 and should be checked with each new lot of reagent. The optical density obtained from the spectrophotometer is used to determine the concentration in micrograms of vitamin D_3. A factor of 55 A.O.A.C. units per microgram of vitamin D_3 (*5*) is used to transfer to the basis of A.O.A.C. units.

SUMMARY

The glycerol dichlorohydrin reaction has been studied in detail with the aid of the Beckman spectrophotometer. Particular attention has been paid to factors influencing the sensitivity of the reaction as applied to vitamin D_3.

The sensitivity may be increased over 20 times by using a solvent-reagent ratio of 1 to 4 and determining the density of the color so formed at 410 mμ. Under these conditions interference offered by constituents of corn oil, fish oils, ergosterol, and 7-dehydrocholesterol is relatively slight compared with that at other wave lengths. The temperature of the reaction is important but the amount of acetyl chloride in the reagent and wider ratios of solvent to reagent have comparatively little effect on the color density. The density-concentration

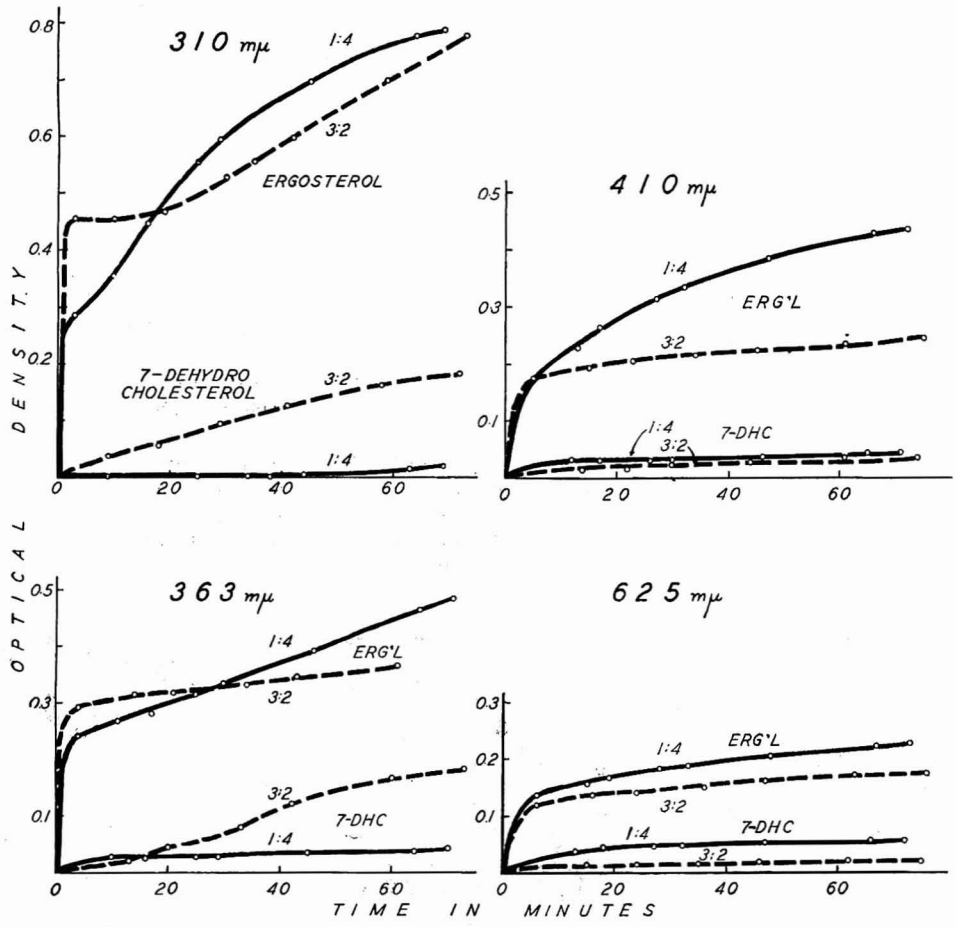

Figure 7. Time Reaction Curves for 5 Mg. of Ergosterol and 5 Mg. of 7-Dehydrocholesterol

Solvent to reagent ratios, 1:4 and 3:2

curve is a straight line between 2 and 25 micrograms of vitamin D_3 per ml. of colored solution.

Limited comparisons with chick assays indicate that the method may be useful for estimation of the vitamin D content of high potency oils, but further refinement in the separation of interfering substances seems necessary before it can be applied to low potency oils.

ACKNOWLEDGMENT

The author is indebted to G. A. Grant of Ayerst, McKenna & Harrison, Ltd., Montreal, and to J. Waddell and H. R. Rosenberg of E. I. du Pont de Nemours & Co., Inc., New Brunswick, N. J., for generous supplies of crystalline vitamin D_3, 7-dehydrocholesterol, cholesterol, and high potency oils used in this study.

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RECEIVED January 9, 1948. Part of the data presented here was taken from a thesis submitted by the author in partial fulfillment of the requirements for the degree of doctor of philosophy at McGill University, Montreal, 1947. The results were summarized at the 11th Annual Meeting of the Canadian Physiological Society, London, October 23 to 24, 1947. Contribution No. 147 of the Division of Chemistry, Science Service.

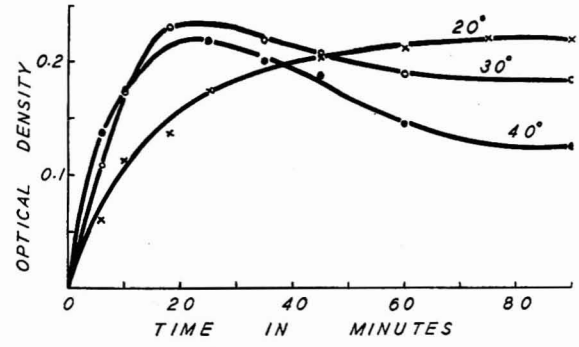

Figure 8. Effect of Temperature on Rate of Formation and Disappearance of Color at 410 mμ

60 micrograms of vitamin D_3 in 10 ml. of colored solution

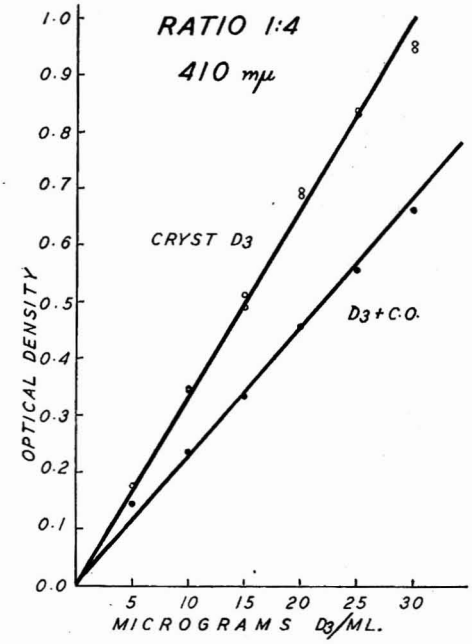

Figure 9. Density Concentration Relation as Measured at 410 mμ for Vitamin D_3 Alone and in Presence of 0.2 Gram of Corn Oil

Solvent to reagent ratio, 1:4

Determination of Lead in Air

Rapid Micromethod for Field Use

L. J. SNYDER, W. R. BARNES, AND J. V. TOKOS, *Ethyl Corporation, Baton Rouge, La.*

Rapid analytical methods are essential not only to production control but also to industrial health control. In the lead-handling industries as elsewhere where toxic materials are processed, the health control problem is paramount. This is particularly true in plants handling tetraethyllead. In this form the lead is relatively volatile, and the problem of controlling atmospheric concentrations is rather difficult. To aid in rapidly detecting and avoiding dangerous concentrations of tetraethyllead in air, a field micromethod was developed, which consists of collecting an air sample through a specially constructed scrubber containing an iodine–potassium iodide solution. The solution containing the lead is drained from the scrubber into a comparator tube containing an alkaline reducing solution. The aqueous mixture is shaken with a dithizone solution and the resulting color is compared with permanent glass color standards in a Hellige comparator. All the equipment is contained in two portable cases, so that an air sample may be collected and analyzed on the spot. The method requires approximately 10 minutes and is accurate to better than 1 microgram of lead per cubic foot of air.

TETRAETHYLLEAD vapor is not successfully sampled by electrostatic precipitator, impingers, and filter paper devices, which are the typical means of sampling inorganic lead in air.

The methods described in the literature for determining tetraethyllead in air are long and tedious (*2*, *5*), as a large air sample must be collected and analyzed by a day-long laboratory procedure. This laborious procedure is undesirable from both a laboratory and physiological viewpoint. [Recently a new method for determining tetraethyllead in air was introduced (*1*), which depends upon sampling tetraethyllead in air with silica gel impregnated with silver nitrate. To date a copy of this paper has not been available.]

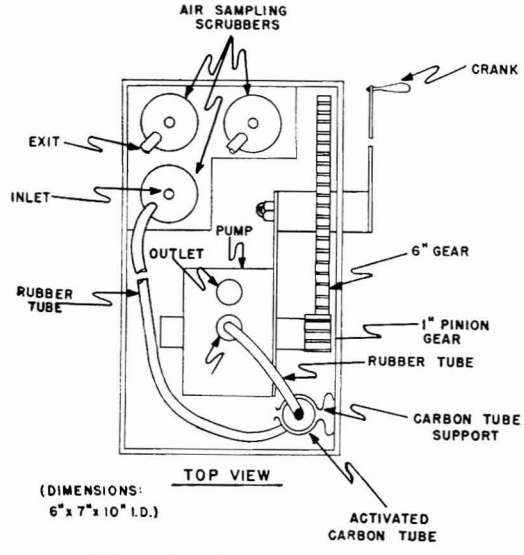

Figure 1. Sampling Apparatus

This paper describes a rapid method for sampling and determining tetraethyllead in air. The method, applicable also to inorganic lead samples, is based upon an adaptation of the high pH dithizone method previously developed in this laboratory (*7*). This dithizone method is essentially a single-color method which differs from the conventional single-color methods (*3*, *6*, *8*) in that repeated extraction with an ammoniacal solution is not necessary to remove the excess dithizone from lead dithizonate. A single extraction contains all the lead dithizonate and an insignificant amount of free dithizone. The pH of extraction is approximately 11.0.

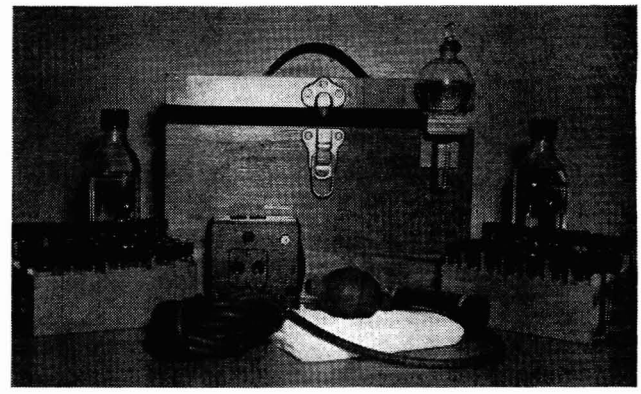

Figure 2. Reagent Box and Contents

REAGENTS

Iodine Solution (1.0 *N*). Dissolve 250 grams of potassium iodide in 750 ml. of water in a separatory funnel. Delead this solution as follows:

First add thymol blue indicator and make alkaline with ammonium hydroxide. Shake with 25-ml. portions of dithizone solution until the last portion added retains its original color. Wash the aqueous solution twice with 25 ml. of clear chloroform and filter the aqueous solution through a fluted filter paper (previously wetted with water) to remove the chloroform. Add dilute nitric acid with constant stirring until the solution is neutral to the thymol blue indicator previously added. In this solution dissolve 125 grams of resublimed iodine and dilute to 1 liter with lead-free water. Pipet 10-ml. portions into 4-dram screw-cap bottles, close with paraffin-gasketed caps, and seal with gel caps. The iodine solution is stable indefinitely. However, it solidifies at approximately 24° C.; therefore, to collect samples below this temperature, a small amount of alcohol may be added to lower the freezing point.

Solution A. Dissolve 10 grams of potassium cyanide, 100 grams of sodium sulfite (anhydrous), and 20 grams of ammonium citrate in 550 ml. of distilled water. Delead this solution as described above, but do not acidify after deleading. Dilute the deleaded solution with 1950 ml. of lead-free ammonium hydroxide (specific gravity 0.900). Pipet 30-ml. portions into 8-dram bottles, close, and seal as described for the iodine solution. Kimble glassware is satisfactory for the storage of Solution A. In a 9-month storage test a batch of Solution A stored in Kimble glassware showed no appreciable lead blank.

Chloroform. Baker's C.P. chloroform was found satisfactory

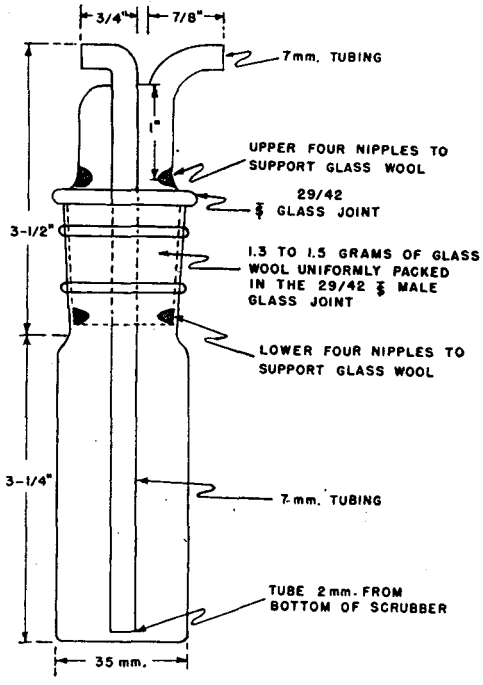

Figure 3. Air-Sampling Scrubber

for use without further treatment. Pipet 10-ml. portions into 4-dram bottles, close with silver-gasketed caps, and seal with gel caps.

Dithizone. Dissolve 40 mg. of dithizone in 20 ml. of chloroform. Pipet 0.20-ml. portions of this solution (from a micropipet) into 4-dram screw-cap brown bottles. Place the bottles in a vacuum desiccator and evaporate the chloroform by reducing the pressure to 15 ml. of mercury for about 25 minutes. Close with screw caps; no special gasket or gel cap is necessary. At time of use the dithizone is redissolved in chloroform to make the dithizone solution referred to.

To ensure the use of a stable dithizone-chloroform solution after several months' standing, it was necessary to dispense the dithizone and chloroform separately. All attempts to preserve the stored mixture proved unsuccessful. Use of an all-glass ampoule sealed with an oxygen-gas torch proved unsatisfactory because the heat from the torch pyrolyzed some of the chloroform vapor, which in turn gradually destroyed the dithizone. Use of screw-cap bottles utilizing various gaskets such as aluminum, copper, cellophane, chemically treated paper, Tygon, saran, silver, and glass also proved unsatisfactory. Mixtures stored in contact with the best of these gaskets decomposed in 2 months. Because of these failures it was necessary to dispense the dithizone and chloroform separately. Dithizone stored in this manner has been found satisfactory after a year's storage.

APPARATUS

The field kit consists of two parts, the air-sampling apparatus (Figure 1) and the reagent box (Figure 2).

Air-Sampling Apparatus. The box housing the sampling apparatus is constructed from plywood, 0.94 cm. (0.375 inch) for the base and 0.3 cm. (0.125 inch) for the sides and hinged top. The box contains an air-sampling pump, three air-sampling scrubbers (Figure 3), an activated carbon tube (Figure 4), and a small oil can for the pump. The over-all dimensions of the box are $15 \times 17.5 \times 25$ cm. ($6 \times 7 \times 10$ inches).

Weighing a total of 4.5 kg. (10 pounds), the box is equipped with a 150-cm. (6-foot) adjustable strap to facilitate carrying while samples are being collected.

Air-Sampling Pump. The air-sampling pump is basically a No. 26-1 1/2 Leiman pump or its equivalent. The purchased model is adapted by adding a crank and gear assembly (6 to 1 ratio) and by rebuilding the heavy iron side plates and housing of aluminum metal to reduce weight. Thus modified, the pump has a capacity of 28.3 liters (1.0 cubic foot) of air per 90 crank revolutions. S.A.E. No. 10 oil is used to lubricate the pump periodically.

The pump is calibrated by connecting the vacuum side to a gas meter or a flowmeter and counting the number of revolutions necessary to collect a 113.3-liter (4-cubic foot) air sample. If the pump is operated at approximately the same rate at which it was calibrated, the sample may be collected with an accuracy of ±2%. The pump calibration should be periodically checked to ensure accurate results.

Air-Sampling Scrubbers. The air-sampling units are modified impinger scrubbers specially developed for this application (Figure 3). These can now be purchased from the Ace Glass Company.

Activated Carbon Tube. The activated carbon tube (Figure 4) is an adsorption unit to remove corrosive iodine vapor from the air stream entering the pump. It is made from a glass tube 12.5 cm. (5 inches) long and 3.44 cm. (1.375 inches) in diameter. It contains 20 grams of 4 × 10 D granular activated carbon, such as that supplied by the Darco Corporation, and it should be replaced after 50 samples have been collected. The efficiency of the activated carbon tube was determined by passing through it air from a saturated iodine solution (0.15 *N* potassium iodide) and using starch solution to detect iodine in the exhaust air. The starch solution turned a light blue color after 504 cubic feet of air passed through the system at a rate of 40 cubic feet per hour. This test showed that a carbon tube can be used for collecting 100 samples and led to the conservative recommendation that the tube be replaced after 50 samples.

The activated carbon tube also serves as a reservoir for moisture carried over during sampling. The water collecting beneath the carbon should be periodically drained after the tube is removed from the sampling box.

Reagent Box. The reagent box is constructed from 0.25-inch plywood. The over-all dimensions are $6.5 \times 8 \times 12$ inches. This box contains reagents for 14 determinations and the accessory equipment needed in the analysis. When thus filled, it weighs 11.5 pounds. A leather hand grip is provided.

Reagents. The reagents consist of 14 bottles each of iodine solution, Solution A, chloroform, and dithizone as previously described.

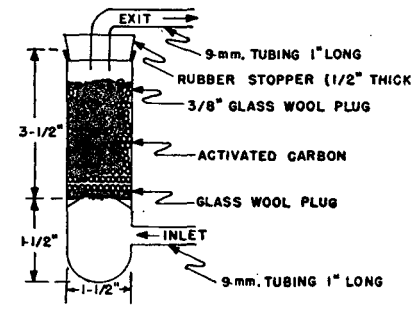

Figure 4. Activated Carbon Tube

Color Comparator. With the cooperation of the Hellige Company a special lead dithizonate disk No. 351-D was prepared for use with the standard Hellige comparator No. 605. Known lead nitrate solutions were prepared and analyzed by the procedure previously described. The colors obtained in these known lead dithizonate solutions were used by the Hellige Company for the preparation of the permanent glass color standards.

The lead dithizonate disk is calibrated in micrograms of lead per cubic foot of air based on a sample of 4 cubic feet of air. The disk readings are 1, 2, 3, 4, 6, 8, 10, 15, and 20. The discrimination between the colors in the 0- to 4-microgram range is good enough that units of 0.5 microgram of lead per cubic foot are easily distinguished. The comparator and lead dithizonate disk can be purchased from the Hellige Company.

Color Comparator Tube. The color comparator tube (Figure 5) is a specially designed vessel in which the analyst mixes the reagents, develops the color, and views the density of the lead dithizonate complex directly in the Hellige comparator. This equipment was constructed to eliminate the lead contamination error normally experienced in transferring the chloroform solution from one container to another.

The comparator tube is made by sealing a 3-inch piece of square Pyrex tubing (13 × 13 mm.) to a 125-ml. glass-stoppered bulb. This tube fits into the Hellige comparator for directly estimating the amount of lead present in a sample. This tube can now be purchased from the Fischer & Porter Company, Hatboro, Pa.

Comparator Tube Support. This unit is a support for the

comparator tube during transfer of reagents (Figure 2). It fits on the side of the reagent box.

RUBBER BULB. A small rubber bulb attached to a 12-inch piece of rubber tubing is used to force the iodine and wash solution from the sampling scrubber. It also serves to force rinse water out of the glass wool between uses. Normally, the water will drain from the scrubber by gravity, but the glass wool will remain saturated with water. The collection of an air sample through glass wool saturated with water will cause flooding and possibly lead losses by entrainment. It is not necessary, however, to dry the glass wool completely.

PROCEDURE

Pour the iodine solution (10 ml.) from the screw-cap bottle into an air-sampling scrubber and draw a sample of 4 cubic feet of air through the iodine solution at the rate of 40 to 60 cubic feet per hour. (As the sampling apparatus contains three scrubbers, three samples may be collected in succession and analyzed later.) After collecting the sample, pour Solution A (30 ml.) from the screw-cap bottle into the comparator tube. Drain the iodine solution from the scrubber into the comparator tube and rinse the scrubber with two successive 20-ml. portions of distilled water. Use a small rubber bulb to force the iodine and wash solutions from the air scrubber into the comparator tube.

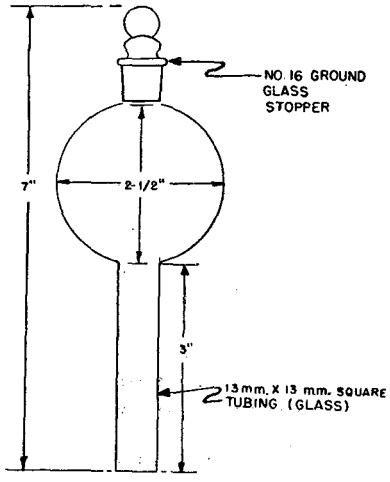

Figure 5. Color Comparator Tube

Stopper the comparator tube, shake it for 3 seconds to ensure complete reduction of the iodine solution, and add 10 ml. of dithizone solution. (Prepare the dithizone solution on the spot by pouring 10 ml. of chloroform into the bottle containing the dry dithizone, which dissolves on contact.) Shake the comparator tube vigorously for 30 seconds and observe the color of the dithizone solution which constitutes the lower layer. This will be colorless, red, or orange. To distinguish between orange and red, a pilot plate containing these colors is provided in the rear of the comparator.

If the solution is colorless, the air sample is free from lead. If the solution is cherry red, insert the comparator tube in the Hellige comparator. Match the unknown with a standard and read the number in the upper right-hand corner of the comparator. For the specified 4 cubic foot air sample, this number represents the micrograms of lead per cubic foot of air.

If the solution is orange, a low reading will be obtained in the comparator. If the temperature is below 60° F., the orange color probably indicates incomplete tetraethyllead decomposition. In this case another sample must be collected and heated before analysis. While it is satisfactory to heat to 80° F. for 10 minutes, it may be more convenient to heat above 150° F. for 2 minutes. After heating the sample, treat the warm iodine solution as previously described, and read the red color directly on the Hellige comparator.

TETRAETHYLLEAD-IODINE REACTION

Tetraethyllead reacts with iodine to form a series of ethyllead iodides, depending upon temperature, contact time, and concentration of iodine. Triethyllead iodide, diethyllead diiodide, and ethyllead triiodide are formed before the reaction proceeds to completion (lead iodide). Even at low temperature (32° F.) the reaction of tetraethyllead with 1.0 *N* iodine proceeds immediately to the first stage (triethyllead iodide). An increase in the contact time and/or in the temperature will drive the reaction toward completion.

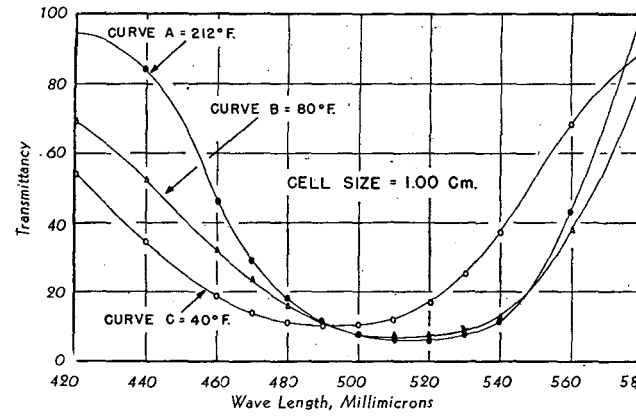

Figure 6. Absorption Curves for Lead Dithizonate and Ethyllead Dithizonate

As in the case of lead iodide, the ethyllead iodides form colored complexes with dithizone. The partitioning of the ethyllead dithizonates between an aqueous and an organic (chloroform) solvent depends upon the pH of the solution. If the aqueous solution is alkaline, the ethyllead dithizonate will concentrate in the chloroform phase. If the aqueous solution is acid (1% nitric), the ethyllead dithizonates will decompose; the lead portion will dissolve in the aqueous solution and the liberated dithizone will remain in the chloroform layer. If the aqeuous portion is again alkalized and shaken with dithizone in chloroform, the ethyllead dithizonates will reappear and again dissolve in the chloroform.

The ethyllead dithizonates possess a different maximum absorption point and also a different extinction coefficient from lead dithizonate. Figure 6 shows the absorption curves for 100 micrograms of lead obtained from tetraethyllead decomposed in 1.0 *N* iodine for 10 minutes at 40°, 80°, and 210° F.

Curve A (Figure 6) shows the absorption spectrum for lead dithizonate obtained by completely decomposing tetraethyllead to lead iodide. The transmittancy of this curve, at 510 millimicrons, agrees with that previously published for 100 micrograms of lead obtained from the reaction of lead nitrate with dithizone (7). At 40° F., curve C shows that tetraethyllead does not completely react with 1.0 *N* iodine in 10 minutes. A considerable amount of ethyllead iodides are produced and these in turn react with dithizone to give the corresponding dithizonates.

Because temperature is the most important variable affecting tetraethyllead decomposition in the iodine scrubber, the effect of temperature has been studied. The reaction is practically complete at temperatures above 80° F. For temperatures below 80° F., the reaction is progressively less complete. If a

Table I. Efficiency of Scrubber on Samples of Tetraethyllead in Air

Sampling Rate, Cu. Ft./Hr.	Volume of Air, Cu. Ft.	TEL, Micrograms Added	TEL, Micrograms Found	% Efficiency
10	1	350.0	350.0	100.0
40	4	150.5	150.0	99.7
41	8	104.4	104.0	99.6
60	4	110.2	109.0	98.9
60	4	83.3	81.0	97.2
60	4	43.5	42.0	96.6
68	4	139.0	138.0	99.3
			Av.	98.8

sample were collected at 60° F. and the color developed immediately, the quantity of tetraethyllead indicated by the analysis would be only about 80% of the actual amount trapped in the scrubber. At 50° F., it would be about 60% and at 32°F., about 45%. The low result is due to the formation of ethyllead dithizonates which possess a different extinction coefficient and therefore appear less dense than the corresponding lead dithizonate. The tetraethyllead is quantitatively trapped in the scrubber, but it is not completely decomposed to lead iodide. It is for this reason that samples of tetraethyllead in air collected on a cold day (below 60° F.) must be heated before the color is developed.

Table II. Efficiency of Scrubber on Samples of Inorganic Lead in Air

Total Lead Found in Three Scrubbers	Found, Lead Per Cent		
	Scrubber 1	Scrubber 2	Scrubber 3
8.5	100.0	0.0	0.0
27.3	67.0	27.5	5.5
84.8	99.1	0.9	0.0
90.7	99.8	0.0	0.2
100.4	95.1	4.5	0.4
125.8	85.1	12.7	2.2
145.7	94.0	2.7	3.3
269.2	95.1	3.5	1.4
1,038.0	97.4	1.1	1.5
Mean	92.5	5.9	1.6

AIR-SAMPLING SCRUBBER

From earlier work in this laboratory and elsewhere, it was known that tetraethyllead can be determined in air by: (1) passing the air sample through a glass tube containing activated carbon, decomposing the carbon, and determining the amount of lead remaining in the ash (*2, 5*), and (2) by passing the sample through a dispersion type of scrubber containing a large amount of 0.1 *N* iodine, boiling off the excess iodine, and determining the lead in the remaining salts. Both methods are time-consuming. The latter procedure is objectionable further because it is difficult to remove lead compounds from the dispersion disk; the large scrubber required for a satisfactory sampling rate is difficult to transport and handle without excessive breakage; the fine porosity of the dispersion disk causes excessive foaming and entrainment of the iodine solution; and the large pressure drop across the fine dispersion disk requires the use of a large air-sampling pump to collect samples.

To collect large air samples conveniently in a short period of time, it was necessary to develop a small sampling scrubber capable of removing microquantities of tetraethyllead from air at high sampling rates. A scrubber of this type was developed (Figure 3) and tested on known samples.

The scrubber (Figure 3) is a modified impinger. The air inlet tube (impinger) extending to within 2 mm. of the bottom has a wide opening (approximately 5 mm.). A tube with a smaller opening (2.3 mm.), as described by Greenburg and Smith (*4*), was found to give a much higher back pressure. For sampling tetraethyllead in air with a hand-operated pump, the larger opening is preferable.

The glass wool in the upper portion of the scrubber is necessary to assure complete removal of tetraethyllead from air. The amount of glass wool is important; the use of too small an amount will result in lead losses. In efficiency tests at the rate of 60 cubic feet per hour, it was found that at least 1.5 grams of glass wool must be used, if lead removal efficiency is to be satisfactory. The glass wool must be carefully placed in position, firmly contacting the walls, if channeling and consequent efficiency losses are to be avoided.

The scrubber (Figure 3) is easily cleaned by flushing with tap water and then rinsing with distilled water. (Rinsing the glass wool with hot acid or alkali during analyses will dissolve some of the glass to give high results.) All excess water must be removed from the glass wool. The clean scrubber is sealed by connecting a 6-inch piece of rubber tubing between the openings.

The efficiency of the standard scrubber (Figure 3) was determined by evaporating known amounts of tetraethyllead dissolved in methyl alcohol. The vapors diluted with air were passed through the standard scrubber and into a large glass fritted scrubber (2 inches in diameter) which had previously been proved to be 100% effective for collecting samples of tetraethyllead in air. The amount of lead remaining in the evaporator and the amount collected in each scrubber were measured with a Beckman DU spectrophotometer at a wave length of 510 millimicrons. The efficiency of the standard scrubber was calculated from the amount of lead collected in the standard scrubber as compared with the amount evaporated into the scrubbing system. The results (Table I) show an average tetraethyllead recovery of 98.8%.

The efficiency of the scrubber was also determined for inorganic lead (fume and dust) in air. Samples were taken by passing 4 cubic foot air samples through three scrubbers connected in series. The amount of lead collected in each scrubber was then accurately measured with a Beckman DU spectrophotometer. The samples were collected at a point 10 to 50 feet from open furnace kettles containing molten lead. The efficiency of each scrubber was calculated on the assumption that all the lead in the air sample was collected in the three scrubbers. The results (Table II) show that approximately 92% of the lead dust is removed from the air stream in the first scrubber and 98% in the first two. Therefore, two scrubbers connected in series will generally suffice for inorganic lead sampling. For more complete recovery, the use of an impinger tube of higher velocity as described by Greenburg and Smith (*4*) would probably be helpful.

A microscopic examination of the dust particles collected in these tests showed that the majority of the particles ranged from 0.4 to 1.0 micron in diameter (round particles). Most of the remaining particles were less than 0.4 micron. Only a few were larger, ranging up to 4.0 microns.

Table III. Analysis of Known Samples

Color Comparator Readings[a]		
Theoretical	Observed	Difference
1.0	1.0	0.0
2.0	2.0	0.0
3.0	3.0	0.0
4.0	4.0	0.0
4.0	4.5	+0.5
6.0	5.5	−0.5
9.0	10.0	−1.0
10.0	10.0	0.0
11.0	12.0	+1.0
14.0	13.0	−1.0
16.0	18.0	+2.0
18.0	17.0	−1.0
20.0	20.0	0.0
	Mean	0.0
	Standard deviation	0.9

[a] Equivalent to micrograms of lead per cubic foot of air based upon a 4 cubic foot sample.

ANALYSIS OF KNOWN TETRAETHYLLEAD SAMPLES

To determine the accuracy with which the lead collected in the air sampling scrubbers may be estimated in the Hellige comparator, known tetraethyllead samples were prepared and analyzed by the following procedure:

Solutions containing known amounts of tetraethyllead in 1.0 ml. of methyl alcohol were added to comparator tubes containing 10 ml. of 1.0 *N* iodine solution. These mixtures were shaken 30 seconds and allowed to stand for 10 minutes at 80° F. Thirty milliliters of Solution A and 40 ml. of water were added to each. The comparator tubes were stoppered and shaken vigorously for 2 or 3 seconds to ensure complete reduction of the iodine. After addition of 10 ml. of dithizone solution, each mixture was shaken vigorously for 30 seconds and the intensity of the color produced in the chloroform layer was visually estimated in the Hellige comparator. The amounts of lead found agree well with the quantities present (Table III).

OTHER APPLICATIONS

The lead-in-air analyzer was developed primarily as a safety device capable of rapidly detecting dangerous concentrations of

lead compounds in the atmosphere. Generally a sample may be collected and analyzed in less than 10 minutes without a constant error. In the range corresponding to the human tolerance limits (0 to approximately 4 micrograms of lead per cubic foot of air) the precision of the method is ±0.5 microgram; in the higher range up to 20 micrograms the precision is ±10% of the amount present. For greater precision, the lead dithizonate complex may be measured with a suitable photometer instead of with the Hellige comparator which is suitable for field use. In either case the only interfering metals in addition to other forms of lead are bismuth, monovalent thallium, and stannous tin.

The sample size may be reduced to 1 or 2 cubic feet of air if the color of the lead dithizonate is viewed through a wider comparator tube or if the volume of dithizone solution is reduced to 5 ml. However, the collection of smaller samples will decrease the accuracy of the method because less homogeneous samples will be collected and the errors due to lead contamination will be correspondingly larger. The reason for specifying a definite sample size (4 cubic feet) is to reduce the probability of error when the method is used by nontechnical operators.

The lead-in-air analyzer may also be used for the determination of lead in liquids such as water, gasoline, oils, etc.

To determine lead in liquids, place about 0.2 to 0.3 gram of the sample in the color comparator tube and shake with 10 ml. of the iodine solution for about 1 minute. Add 30 ml. of Solution A, and approximately 40 ml. of water, and shake about 2 seconds to destroy the iodine. Add 10 ml. of dithizone solution and shake vigorously for 30 seconds. Visually compare the color of the dithizone solution with the standards in the Hellige comparator. As the readings on the disk are given as micrograms of lead per cubic foot of air, they must be converted to micrograms of lead by multiplying the reading by 4.

A color matching 2 micrograms of lead per cubic foot of air is thus equivalent to 8 micrograms of lead. When the test is applied to the determination of lead in 50 ml. of water, the sensitivity of the method is ±0.05 p.p.m. of lead in water.

Enough dithizone is added to each ampoule to react with a little more than 100 micrograms of lead. When a sample is collected which contains more than this amount of lead, the upper layer (aqueous solution) will appear almost colorless. Normally, this layer appears yellow from an excess of the soluble ammonium salt of dithizone.

If Solution A contains insufficient sulfite to reduce all the iodine present, the residual iodine will oxidize the dithizone solution, as evidenced by a color change from green to yellowish orange. If this color change is observed, Solution A must be discarded and a fresh batch prepared.

For consistently accurate work, a blank test should be run to check the equipment and reagents for lead contamination. The blank test is made by following all steps given under "Procedure" with the exception of collecting the air sample.

ACKNOWLEDGMENT

The authors wish to thank the Hellige Company for cooperation in preparing a suitable color comparator disk for estimating lead concentrations.

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NOTES ON ANALYTICAL PROCEDURES

Ammonium Citrate in the Colorimetric Determination of Copper

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IN RECENT work at this laboratory it was necessary to determine small quantities of copper in a strong solution of mercuric chloride. It was found that large amounts of mercury, 3.5 grams of mercuric chloride in 50 ml. of water, interfered with the determination of copper with sodium diethyldithiocarbamate by giving a yellowish turbidity and no true formation of the copper color. The separation of small amounts of copper from mercury by precipitation is a tedious proceeding and therefore it was decided to determine the blue color of the copper ammonium citrate complex directly in the presence of mercury which was held in solution by ammonium citrate and excess ammonia.

APPARATUS AND PROCEDURE

A Spekker photoelectric absorptiometer was employed for the colorimetric observations, with Ilford 608 red color filters and a tungsten filament lamp. Comparisons of the absorptions were made with a cell containing water.

Reagents for Absorptiometric Calibration. Copper sulfate solutions contained 1 mg. of copper per ml. (No. 1) and 5 mg. per ml. (No. 2). Mercuric chloride solution, 70 grams per liter. Ammonium citrate solution was made by dissolving 200 grams of citric acid in water, adding 270 ml. of ammonia, and making the whole up to 1 liter. Ammonia, C.P. grade, 0.88 specific gravity.

Absorptiometric Calibration. 0 TO 50 MG. OF COPPER. Place varying amounts at 5- or 10-mg. intervals, up to 50 mg., of copper sulfate solution 1 in 200-ml. standard flasks. Add 50 ml. of mercuric chloride solution, 50 ml. of ammonium citrate, and 50 ml. of ammonia. Shake well and make up to 200 ml. Measure the absorption of these solutions in 4-cm. (30-ml.) cells.

0 TO 200 MG. OF COPPER. Place varying amounts at 10-mg. intervals, up to 200 mg., of copper sulfate solution 2 in 200-ml. standard flasks. Add mercuric chloride solution, ammonium citrate, and ammonia as above. Measure the absorption of these solutions in 1-cm. (8-ml.) cells.

CONFORMITY TO BEER-LAMBERT LAW

Plotting both sets of absorptiometer readings gave straight lines, which shows that the color reaction obeys the Beer-Lambert law over the range investigated.

INTERFERING ELEMENTS

Because for this particular case in the test solutions the copper was to be determined in the presence of mercury, it was con-

sidered good absorptiometric practice to make up the calibrating solutions with mercuric chloride, but it was found experimentally that varying the amount of mercury present had no effect on the absorptiometer readings.

Further experiments were carried out on the determination of copper in the presence of other metallic elements that give colorless citrate complexes. It was found that copper could be determined in the presence of silver, zinc, cadmium, magnesium, aluminum, and lead. Varying the amounts of these metal ions had no effect on the copper color. The presence of chloride, acetate, and nitrate ions also had no influence. Iron in amounts of 1 to 2 mg. has no effect on the copper color. Five to 10 mg. of iron will impart a greenish tinge but the correct absorptiometer reading is obtained. Fifty milligrams of iron will give a yellow green color to a solution containing 20 mg. of copper and obviously interferes. Other colored ions such as cobalt, chromium, and nickel will affect the copper color.

DISCUSSION

The present work was carried out with the addition of 50 ml. of ammonium citrate and 50 ml. of ammonia to neutral solutions. These amounts are in excess of those required for the immediate complexing of the quantity of mercury present (3.5 grams of mercuric chloride) but it was found that unless a large excess was present the mercury tended to precipitate on standing. The citrate complex of copper has been studied in detail by Bobtelsky and Jordan (*1*), who found in their photometric measurements that a tenfold excess of citrate did not further affect the oxidation. For many purposes it would be possible to employ ammonium citrate only, in order to retain other ions in solution and to develop a copper color such as the determination of copper in the presence of silver and zinc. It was necessary in the present instance to add a constant excess of ammonia in order to assist in complexing the mercury present.

This colorimetric method could be adapted for the rapid determination of copper in a number of light alloys, brasses, and solders and the actual amount of ammonium citrate and ammonia to be added could best be determined for each particular case. Mehlig (*3, 4*) found that the copper-ammonium color was always dependable, provided that a constant excess of ammonia was present, and was stable for 6 weeks. By the addition of ammonium citrate it is possible to determine copper in the presence of other ions which would otherwise precipitate in a straight ammoniacal solution. The volatility of ammonia, to which some workers object (*2*), is not so great that there would be any serious diminution in the concentration during the length of time taken to carry out colorimetric observations on a number of samples, if the solutions are kept in stoppered flasks. By the use of 4-cm. (30-ml.) cells a calibration graph may be constructed which can be read to ±0.5 mg. of copper and will give greater accuracy than smaller cells with low concentrations of copper. The 1-cm. (8-ml.) cells will, however, give a graph which enables the work to be carried over a wider range and can be read with an accuracy of ±1 mg. of copper.

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Determination of Hydroxyl Groups in Organic Compounds

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ACETYLATION of alcoholic compounds, followed by isolation, purification, and saponification of the acetylation product, is time-consuming as a quantitative procedure. Simpler and more rapid methods for determining hydroxyl groups by back-titration of the excess acetyl chloride or acetic anhydride after acetylating a weighed sample of a hydroxylic compound have become well established in recent years (*1, 5, 6, 7*). The method here described presents a modification of a previously published acetyl chloride procedure, which has been used successfully in this laboratory for a number of years on a variety of alcohols (Table I) and on fats and oils. It is of doubtful value in the case of compounds insoluble in toluene.

Table I. Number of Hydroxyl Groups in Common Alcohols

Alcohol	No. of Tests	Av. of Tests	Individual Results		
n-Butyl alcohol (purified)	8	1.04	1.04	1.18	0.98
			1.06	0.94	..
Benzyl alcohol	6	1.12	1.15	1.06	1.15
			1.16	1.06	1.15
1,3-Propanediol	5	2.11	2.26	2.13	2.07
			2.06	2.05	..
Isobutyl alcohol	6	1.26	1.44	1.27	1.25
			1.34	1.23	1.18
n-Propyl alcohol	5	1.15	1.22	1.07	1.19
			1.17	1.19	..
1,2,3-Propanetriol	3	2.95	2.84	3.09	2.91
	5	3.06	3.11	3.09	2.94
			3.04	3.01	..
1,2-Ethanediol monoethyl ether	5	0.99	0.94	0.94	1.03
			1.11	0.92	..

CHEMISTRY OF PROPOSED METHOD

Acetylation in toluene as the solvent:

$$R—OH + CH_3COCl = CH_3—COOR + HCl \quad (1)$$

Hydrolysis of the excess acetyl chloride:

$$CH_3COCl + HOH = CH_3COOH + HCl \quad (2)$$

Back-titration of the acetic acid and hydrochloric acid:

$$CH_3COOH + NaOH = CH_3COONa + H_2O \quad (3)$$

$$HCl + NaOH = NaCl + H_2O \quad (4)$$

Reaction 1 is carried out in an anhydrous system which is closed by a trap containing a measured volume of standardized sodium hydroxide. Loss of volatile hydrogen chloride or acetic acid is thus prevented.

EXPERIMENTAL REAGENTS

Acetyl chloride dissolved in toluene to make an approximately 1 *N* solution, accurately standardized. Approximately 1 *N* sodium hydroxide, accurately standardized. Phenolphthalein indicator, 1 gram in 100 ml. of alcohol. Recently cooled boiled distilled water, in large quantity.

SPECIAL EQUIPMENT

Kjeldahl flasks (500-ml.) fitted with a side arm as shown in Figure 1. Calcium chloride tubes partly filled with glass beads and indented close to stem outlet to prevent beads from sealing

outlet. Test tubes (1 × 10 inch) fitted with inlet and outlet tubes. These act as condensers in the neck of the Kjeldahl flask (Figure 1).

DIRECTIONS

About 0.1 gram of a sample is allowed to fall as drops to the bottom of the modified Kjeldahl flask from a suitable weighing pipet, and 25 ml. of the acetyl chloride solution are pipetted into the flask. The delivery tip of the pipet should extend almost to the bottom of the flask to localize the acetyl chloride vapor in the bulb of the Kjeldahl flask. The condenser is slipped into the neck of the flask and immediately sealed with melted paraffin.

The side arm of the Kjeldahl flask is prepared by attaching the calcium chloride scrubber tube and allowing exactly 3 ml. of the standard sodium hydroxide solution to flow down over the glass beads and into the elbow of the side arm (the trap). A very small flame is lighted below the Kjeldahl flask and the reaction mixture is allowed to reflux for 2 hours. The gas is then turned off and cold recently boiled distilled water is poured rapidly into the calcium chloride tube without overflow. As each portion of this wash water is drawn into the flask, more is added until nearly one half of the flask is filled with it. After the paraffin seal is broken, the side arm is washed by adding water to it as before, but forcing it this time into the flask with a rubber pressure bulb.

As the condenser is lifted out of place it is washed with a stream of cold recently boiled distilled water. The contents of the flask are now ready for titration; 0.5 ml. of the phenolphthalein indicator is added and the contents are titrated with the standardized sodium hydroxide until a definite pink color permanent for 5 minutes is reached. Because a heterogeneous system exists, shaking must follow every increment of alkali added.

Control determinations should be made occasionally to standardize the acetyl chloride solution.

The paraffin seal is best tested by allowing the heat of the hands to expand the gases in the flask. A perfect seal is denoted by movement of the alkali solution in the side-arm trap.

The fog seen at first in the calcium chloride tube is toluene vapor and is ordinarily free from any acid reaction.

Table II. Acetyl Values Obtained by Holland's and Author's Methods

Sample	Saponification No. Unacetylated	Saponification No. Acetylated	Acetyl Value Holland	Acetyl Value Author	% OH Holland	% OH Author
Castor oil						
1	183.7	359.0	...	179.3	...	...
2	184.6	354.0	...	185.2	...	...
3	184.8	359.9	...	179.1	...	...
4	186.0	358.5	...	182.2	...	...
5	185.5	...	...	...	...	...
6	185.6	...	...	...	...	...
Av.	185.0	357.8	172.8	181.4	5.25	5.48
Cottonseed oil						
1	217.2	250.4	...	28.3	...	...
2	221.3	246.0	...	31.4	...	...
3	218.1	233.3	...	28.3	...	...
4	221.9	241.9	...	25.1	...	...
5	223.4	250.0	...	27.8	...	...
6	221.7	243.0	...	28.7	...	...
Av.	220.6	246.1	25.5	28.3	0.77	0.86
Cottonseed oil (used for melting points)	243.1	311.5	...	67.1		
	237.0	301.2	...	65.3	Not	Not
	235.1	299.1	...	65.8	computed	computed
	234.6	299.9	...	69.8		
	238.8	289.7	...	66.9	...	...
	...	283.0	...	...	...	...
Av.	237.7	297 4	59.7	66.9	...	...

Calculations.

$$\text{OH groups} = \frac{\text{equivalents of base} \times \text{molecular weight (ROH)}}{\text{sample weight (ROH)}}$$

$$\text{Equivalents of base} = \frac{[\text{ml. of NaOH (control)} - \text{ml. of NaOH sample})] \times \text{normality of NaOH}}{1000}$$

Older methods have certain objectionable features(*3, 8*). The method of Holland (*2*) requires two saponifications, an acetylation, and a long washing of the acetylated sample. Marks and Morrell (*4*) showed in 1931 that mixtures of acetic anhydride and pyridine could be used satisfactorily in determining the percentage of hydroxyls in castor oil.

The method as described was applied to the determination of acetyl values of fats and oils of plant and animal origin (Table II). The method of Holland, used as the reference method, expresses the acetyl value as the difference between the saponification numbers of the acetylated and unacetylated samples. Marks and Morrell (*4*) report 5.2% of hydroxyl in castor oil. Glyceryl triricinoleate contains 5.47% hydroxyls. The value of 5.25% hydroxyl obtained by the author using Holland's method is in close agreement with the value found by Marks and Morrell. The author obtained an average of 5.48% hydroxyl in castor oil by the method presented here.

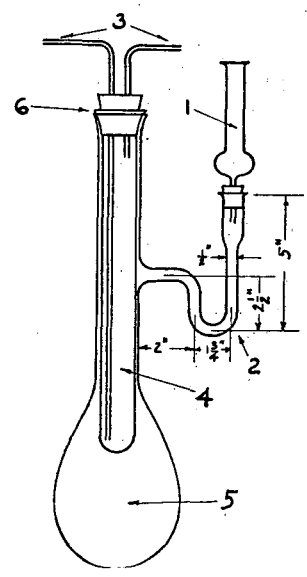

Figure 1. Hydroxyl Group and Acetyl Apparatus for Determining Value

1. Glass bead scrubber
2. Side-arm trap
3. Water inlet and outlet
4. 1 × 10 inch test tube
5. Reaction flask
6. Paraffin seal

The results for cottonseed oil agree with reported values. For the cottonseed oil that had been used for a long time as a high boiling point bath liquid, results are interesting because of the oxidation that probably occurs at points of unsaturation. The author here found slightly elevated hydroxyl values, which possibly reflect increased hydroxyls by oxidation. Decreased losses of acetylated sample in comparison with the method of Holland also may be a factor, inasmuch as the author's method requires no washing.

Eight or more acetyl values may easily be determined in 4 to 5 hours, including all operations.

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RECEIVED July 19, 1946.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

THE description covered this month, α-pyridinesulfonic acid, was completed from data obtained during work on an industrial research project at the Armour Research Foundation. Samples of the pure compound were obtained through the courtesy of Edmond T. Tisza of the Pyridium Corporation.

9. Alpha-Pyridinesulfonic Acid (I)

Crystals of α-pyridinesulfonic Acid (I) can be readily obtained from water either macroscopically or on a microscope slide (Figure 1). The application of fusion methods discloses three polymorphic modifications. The stable form is always obtained from water under ordinary conditions although occasionally crystals of an unstable modification may form and remain momentarily near the edge of the drop. They transform rapidly, however, by solution phase transformation to the stable form. On the other hand, spontaneous crystallization from the melt almost never gives the stable form directly. Further details on the form and stability of these three modifications are presented under Fusion Data.

CRYSTAL MORPHOLOGY (determined and checked by V. Gilpin, W. C. McCrone, and P. T. Cheng).

Crystal System. Orthorhombic

Form and Habit. Prismatic needles, elongated parallel to *c;* showing the forms: prism {210}; brachypinacoid {010}; brachydome {021}; and bipyramid {111}.

Axial Ratio. $a:b:c = 0.624:1:0.519$.

Interfacial Angles (polar): $210 \wedge 2\bar{1}0 = 35° 56'$, $101 \wedge \bar{1}01 = 79° 34'$; $021 \wedge 0\bar{2}1 = 92° 10'$.

X-RAY DIFFRACTION DATA (determined and checked by J. Whitney, I. Corvin, and M. Tull).

Principal Lines

d	I/I_1	d	I/I_1
7.79	0.10	2.794	0.10
5.61	1.00	2.718	weak
4.84	0.63	2.622	0.07
4.61	0.47	2.553	0.14
4.45	0.50	2.458	0.15
3.88	0.94	2.420	0.08
3.73	0.32	2.382	0.08
3.63	0.71	2.323	0.13
3.53	0.51	2.294	weak
3.32	weak	2.250	0.07
3.25	0.37	2.203	0.10
3.04	0.80	2.140	0.05
2.896	weak	2.056	0.05
2.849	weak	2.022	0.06

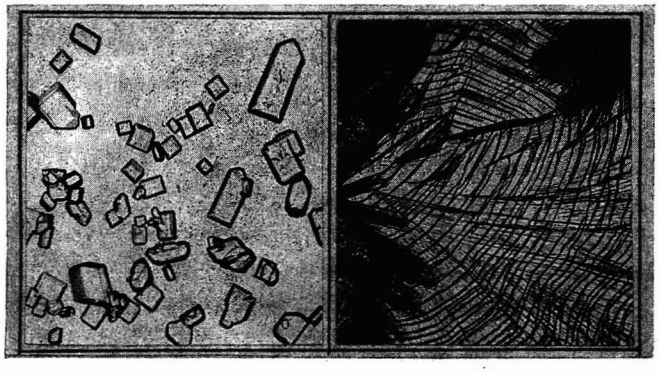


Figure 1. (Left) Crystals of α-Pyridinesulfonic Acid from Water on a Microscope Slide; (Right) Fusion Preparation of α-Pyridinesulfonic Acid Showing Polymorphic forms II and III

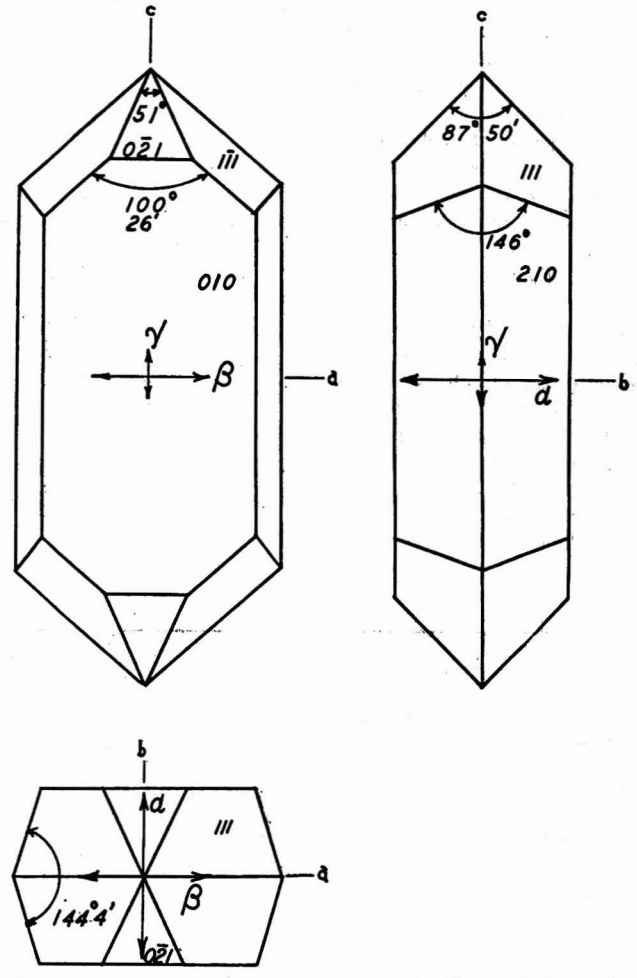


Figure 2. Orthographic Projection of a Typical Crystal of α-Pyridine Sulfonic Acid

Cell Dimensions. $a = 9.73$, $b = 15.60$, $c = 8.10$.

Formula Weights per Cell. 8.

Formula Weight. 159.

Density. 1.710 (buoyancy method); 1.71 (x-ray).

OPTICAL PROPERTIES (determined and checked by V. Gilpin, P. T. Cheng, and W. C. McCrone).

Refractive Indices (5893 Å.; 25° C.). $\alpha = 1.530 \pm 0.002$. $\beta = 1.650 \pm 0.002$. $\gamma = 1.76 \pm 0.01$.

Optic Axial Angles (5893 Å.; 25° C.). $2V = 83°$ (calculated from α, β, and γ). $2H = 92°$.

Dispersion. $v > r$.

Optic Axial Plane. 100.

Sign of Double Refraction. Negative.

Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.644$. R(calcd.) = 40.5. R(obsd.) = 33.7.

FUSION DATA (determined by W. C. McCrone).

The alpha isomer melts at 248° to 250° C., with no sublimation and very slight decomposition. The melt supercools slightly and a few nuclei of a chalky solid (modification II) begin to grow slowly. Within a few seconds, however, the rest of the preparation solidifies very rapidly as modification III. These two polymorphs are easily distinguished by the unaided eye since III scatters very little light and II appears quite chalky due to the many surface markings. Although no further change can be detected at room temperature, careful reheating below the melting

point (about 200°) causes slow growth of II at the expense of III, until most of the slide assumes a chalky appearance.

Modification III grows very rapidly from the melt, with a jagged angular crystal front, and transverse shrinkage cracks. Extinction is approximately parallel to the cracks. An optic axis interference figure can be observed showing 2V approximately 75°; the sign of double refraction is negative, with orthorhombic dispersion, $v > r$.

The centers of the chalky nuclei of II show no definite form, but as the perimeter grows slowly, small bladed crystals become evident. It is almost impossible, however, because of their size to determine their optical character. The appearance of the solid-solid transformation, II to III is shown in Figure 2.

Meltback of the solidified preparation results in further rapid growth of III, and solid-solid transformation from III to II. In most cases, a modification I also appears near the edge of the preparation and nucleating in form III. At any temperature below the melting point, the transformation II to I also takes place fairly rapidly in comparison to that of III to II. Polymorph I grows with a fairly continuous, smooth front, frequently spherulitic in shape, and shows shrinkage cracks parallel to the perimeter. None of the transformations proceeds at a measurable rate at room temperature. Sometimes III does not appear on meltback. Furthermore, unless all nuclei of III are removed by heating the remelted preparation above the melting point for a few seconds, II does not appear, as its rate of growth is so much slower than that of III.

α-Pyridine sulfonic acid is insoluble in melted thymol; hence, a mixed fusion with this compound gives no useful information.

Book Reviews

Electrochemical Analysis with Graded Cathode Potential Control. *Harvey Diehl.* vii + 56 pages. The G. Frederick Smith Chemical Co., 867 McKinley Ave., Columbus, Ohio, 1948. Price, bound, $1.00; paper, gratis.

Few chemists have done as much to develop the theory of electrolytic analysis as the Englishman H. J. S. Sand, who died in 1944. In 1906 he described an electrolytic procedure in which the cathode potential was controlled with the aid of a calomel cell. By such control he was able to prevent evolution of hydrogen gas while bismuth was depositing and thus obtain better deposits. In this way also, he could make electrolytic separations of metals whose discharge potentials are fairly close to one another. Sand was one of the first to adopt stirring during the electrolysis and proposed the use of cylindrical platinum gauze electrodes, with the smaller anode rotating within the large cathode. In most cases the electrolysis was accomplished rapidly but the operator had to watch and regulate the potential drop at the cathode during the analysis.

Recently several investigators have sought to improve the analytical technique by devising apparatus in which the control of the cathode potential is automatic, after the original setting of the instrument. Thus Caldwell, Parker, and Diehl [IND. ENG. CHEM., ANAL. ED., 16, 532 (1944)] devised an apparatus which, in an improved form, is described in this booklet. An ordinary 110-volt lighting circuit is used as the source of the current. By means of a thermionic valve (radio tube), the current is rectified and a suitable step-down transformer is provided. By simply setting a dial, the electrolysis is made to take place within the desirable limits for the cathode potential. Detailed directions are given for the quantitative deposition of silver, copper, bismuth, antimony, lead, tin, nickel, and cadmium and for the complete analysis by electrolysis of bearing metals, brass, and bronze.

The theoretical discussions are not always adequate. Thus on page 3, the Nernst equation $E = E_0 + \frac{RT}{nF} \ln [M^{n+}]$ is given without any hint with respect to its derivation. The significance of E_0, R, F, and T is shown but it is assumed that the reader will guess that $[M^{n+}]$ represents the concentration of the metal ions in moles per liter and that n represents the valence of the cations, or the number of faradays required to react with 1 mole of these cations.

In the same paragraph, three more equations are given, the first of which is $E_{Cu} = +0.345 + \frac{0.059}{2} \log 1 = +0.345\ v.$ There is absolutely no explanation as to how this second equation is derived from the first. Diehl assumes that the reader will know that he has substituted the E_0 value for Cu, has multiplied by 2.303 to change natural logarithms to common logarithms, has assumed the temperature to be 25° C. ($T = 298$), and has inserted the values for R (8.163 watt seconds or joules) and for F (96,500 coulombs).

The booklet is attractively printed and the proof reading has been well done.

WILLIAM T. HALL

Laboratory and Workbook Units in Chemistry. *Maurice U. Ames and Bernard Jaffe.* xx + 275 pages. Silver Burdett Co., 45 East 17th St., New York 3, N. Y., 1947. Price, consumable edition, $1.28; nonconsumable, $1.92.

Division of Analytical and Micro Chemistry Issues List of Speakers

THE following list of speakers is explained and discussed in L. T. Hallett's column in this issue, page 19 A. This is a valuable and timely suggestion list for fall program use. Please see Dr. Hallett's discussion in "The Analyst's Column."

Electroanalysis

HARVEY C. DIEHL, Iowa State University, Ames, Iowa
J. J. LINGANE, Harvard University, Cambridge, Mass.
W. M. MACNEVIN, Ohio State University, Columbus, Ohio
L. B. ROGERS, Massachusetts Institute of Technology, Cambridge, Mass.

Gravimetric and Volumetric Analysis

H. H. WILLARD, University of Michigan, Ann Arbor, Mich.
E. R. CALEY, Ohio State University, Columbus, Ohio
N. H. FURMAN, Princeton University, Princeton, N. J.

Instrumentation

R. H. MÜLLER, Washington Square College, New York University, New York, N. Y.
M. E. DROZ, Precision Laboratories, Pleasantville, N. Y.
V. W. MELOCHE, University of Michigan, Ann Arbor, Mich.

Microchemistry

A. A. BENEDETTI-PICHLER, Queens College, Flushing, L. I., N. Y.
H. W. HERMANCE, Bell Telephone Laboratories, Summit, N. J.
P. L. KIRK, University of California, Berkeley, Calif.

Electron Microscopy

L. L. MARTON, National Bureau of Standards, Washington, D. C.
JAMES HILLIER, Radio Corp. of America, Princeton, N. J.

Polarography

J. J. LINGANE, Harvard University, Cambridge, Mass.
E. F. ORLEMANN, University of California, Berkeley, Calif.
H. A. LAITINEN, University of Illinois, Urbana, Ill.
G. H. TISHKOFF, University of Rochester, Rochester, N. Y.

Nucleonics

C. D. CORYELL, Massachusetts Institute of Technology, Cambridge, Mass.
D. HUME, Massachusetts Institute of Technology, Cambridge, Mass.
J. F. FLAGG, General Electric Co., Schenectady, N. Y.

Chromatography

L. ZECHMEISTER, California Institute of Technology, Pasadena, Calif.
W. H. STEIN, Rockefeller Institute, New York, N. Y.

Distillation
M. R. FENSKE, Pennsylvania State College, State College, Pa.
E. S. PERRY, Distillation Products, Inc., Rochester, N. Y.

Extraction
C. J. RODDEN, National Bureau of Standards, Washington, D. C.
THERALD MOELLER, University of Illinois, Urbana, Ill.
N. H. FURMAN, Princeton University, Princeton, N. J.

X-Ray Spectroscopy
W. PARRISH, North American Phillips Corp., New York, N. Y.
H. A. LIEBHAFSKY, General Electric Co., Schenectady, N. Y.
D. HARKER, General Electric Co., Schenectady, N. Y.

Infrared Spectroscopy
NORMAN WRIGHT, Dow Chemical Co., Midland, Mich.
R. BOWLING BARNES, American Optical Co., Southbridge, Mass.

Ultrasonics
G. H. ROUNDY, Ultrasonic Corp., Cambridge, Mass.
HARVEY C. DIEHL, Iowa State University, Ames, Iowa

Raman Ultraviolet Spectroscopy
WILLIAM WEST, Eastman Kodak Co., Rochester, N. Y.

Emission Spectroscopy
L. W. STROCK, Spectrographic Services and Supplies, Saratoga Springs, N. Y.
W. F. MEGGERS, National Bureau of Standards, Washington, D. C.
N. H. NACHTRIEB, University of Chicago, Nuclear Institute, Chicago, Ill.

Absorption Spectroscopy
WILLIAM WEST, Eastman Kodak Co., Rochester, N. Y.
M. G. MELLON, Purdue University, Lafayette, Ind.
S. E. Q. ASHLEY, General Electric Co., Pittsfield, Mass.

Mass Spectroscopy
A. O. NIER, University of Minnesota, Minneapolis, Minn.
F. J. NORTON, General Electric Co., Schenectady, N. Y.

Fluorescence
W. F. NEUMAN, University of Rochester, Rochester, N. Y.
C. E. WHITE, University of Maryland, College Park, Md.

Statistics Applied to Analysis
G. T. WERNIMONT, Eastman Kodak Co., Rochester, N. Y.

Metallurgical Analysis, Ferrous
A. H. THOMAS, American Rolling Mills, Middletown, Ohio

Metallurgical Analysis, Nonferrous
C. L. LUKE, Bell Telephone Laboratories, Summit, N. J.
D. R. EVANS, Western Electric Co., Kearny, N. J.

Organic Reagents
G. FREDERICK SMITH, University of Illinois, Urbana, Ill.
E. B. SANDELL, University of Minnesota, Minneapolis, Minn.
J. H. YOE, University of Virginia, Charlottesville, Va.
F. J. WELCHER, Indiana University, Bloomington, Ind.
L. A. SARVER, American Viscose Corp., Roanoke, Va.

Electrometric Methods
N. H. FURMAN, Princeton University, Princeton, N. J.
I. M. KOLTHOFF, University of Minnesota, Minneapolis, Minn.
H. H. WILLARD, University of Michigan, Ann Arbor, Mich.
H. A. LAITINEN, University of Illinois, Urbana, Ill.
J. J. LINGANE, Harvard University, Cambridge, Mass.

Optical Methods
T. R. P. GIBB, Metal Hydrides, Boston, Mass.
M. G. MELLON, Purdue University, Lafayette, Ind.

Low Pressure Techniques
L. A. WOOTEN, Bell Telephone Laboratories, Summit, N. J.
R. W. GURRY, U. S. Steel Research Laboratory, Kearny, N. J.
S. E. Q. ASHLEY, General Electric Co., Pittsfield, Mass.

Analytical Chemistry in Crime Detection
A. O. GETTLER, New York University Medical College, New York, N. Y.

Fundamentals of Analytical Chemistry
S. E. Q. ASHLEY, General Electric Co., Pittsfield, Mass.
L. T. HALLETT, General Aniline & Film Corp., Easton, Pa.
P. J. ELVING, Purdue University, Lafayette, Ind.

The Analyst's Calendar

Applications of Chemical Microscopy

THE Division of Analytical and Micro Chemistry has planned a Symposium on Current Developments in the Application of Chemical Microscopy under the chairmanship of Mary L. Willard, Pennsylvania State College, State College, Pa., for presentation at the St. Louis meeting of the AMERICAN CHEMICAL SOCIETY, September 6 to 10. Emphasis is to be given to applications of microscopy in industry.

C. W. Mason, professor of chemical microscopy at Cornell University, will start the symposium with a paper on the microscopist in the technical organization, presenting the justification for a microscopical laboratory, the training and equipment needed, and the strategic position of the chemical microscopist in the plant and in sales service.

J. Mitchell, Jr., Ammonia Department, Du Pont Co., speaking on identification of organic compounds and elements, will emphasize the use of the petrographic microscope for the identification of organic compounds and elements. Leon V. Foster, Bausch & Lomb Optical Co., will discuss ultraviolet, visible, and infrared microscopy with reference to their role in recent developments in the microscopy of unstained material. G. L. Royer, Calco Chemical Division, American Cyanamid Co., will illustrate with Kodachrome photomicrographs the importance of chemical microscopy in the study of dyeing. T. G. Rochow, American Cyanamid Co., will discuss the graphic study of structures in resins and their plastics. W. C. McCrone, Armour Research Foundation, will emphasize application of fusion methods, and John Turkevich, Princeton University, will discuss the application of the electron microscopy to colloidal metals, oxides, and sulfides and show the relation between the work with the electron microscope and with other tools.

The complete program is as follows:

The Microscopist in the Technical Organization. C. W. MASON, Cornell University, Ithaca, N. Y.

Applied Phase Microscopy. OSCAR W. RICHARDS, American Optical Co., Buffalo, N. Y.

Identification of Organic Compounds and Elements. JOHN MITCHELL, JR., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

Color Photomicrography in the Laboratory. ROGER P. LOVELAND, Eastman Kodak Co., Rochester, N. Y.

Ultraviolet, Visible, and Infrared Microscopy. LEON V. FOSTER, Bausch & Lomb Optical Co., Rochester, N. Y.

Microscopic Examination of Metals. J. R. VILELLA, U. S. Steel Corp. of Delaware, Kearny, N. J.

Application of Chemical Microscopy to the Study of Dyeing. G. L. ROYER, Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J.

Resinography. T. G. ROCHOW, American Cyanamid Co., Stamford, Conn.

Application of Fusion Methods in Chemical Microscopy. W. C. MCCRONE, Armour Research Foundation, Illinois Institute of Technology, Chicago, Ill.

Electron Microscopy of Colloidal Systems. JOHN TURKEVICH, Princeton University, Princeton, N. J.

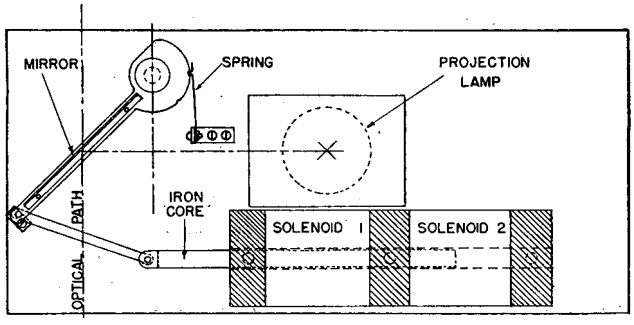
Second Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., March 9 to 12, 1949.

Second Annual Summer Symposium on Analytical Chemistry. Wesleyan University, Middletown, Conn., June 1949.

AIDS FOR THE ANALYST.....

Combined Shutter and Projection Device for Alignment of Electrodes in Spectrographic Analysis. A. Lee Smith and V. A. Fassel, Institute for Atomic Research and Department of Chemistry, Iowa State College, Ames, Iowa.

In quantitative spectrographic analysis the position and spacing of the electrodes are critical if reproducible results are to be obtained. Proper alignment is usually made by means of an auxiliary lens which projects the electrode image on a previously marked screen (*2*, *4*). In some cases the electrodes are quickly positioned after the exposure has been started; for more careful work, the electrodes are aligned before excitation by the use of a manually operated lamp or projection device placed behind the electrodes to project their image (*1*, *3*, *5*). The apparatus is almost instantaneous in action and is operated electrically by conveniently placed push buttons. It also provides an excellent shutter for spectrographs not already so equipped. It operates on the principle that an iron rod placed in a solenoid tends to center itself when alternating or direct current is passed through the windings.



The assembly is mounted on a 0.125-inch brass plate bolted to an optical bench carriage. The double solenoid is made by winding enough No. 32 cotton-covered copper wire on a 4-inch brass tube with Bakelite spacers to give a resistance of about 80 ohms in each section. The soft iron core, 4 inches long and 0.25 inch in diameter, should slide freely in the tube. The polished steel mirror is mounted on an arm which is free to rotate through a 45° angle, thus swinging the mirror in and out of the optical path as the position of the solenoid core changes. A spring bears against the notched disk which is a part of the mirror arm, and ensures the mirror's stopping in the same position each time. A metal stop near the edge of the base plate limits the motion of the mirror. If desired, a circuit contact may be mounted on the mirror arm to turn on the lamp automatically when the mirror is in the projection position.

The lamp used on the instrument described is a 50-watt toy projector bulb of type S-11, although any similar lamp may be used. The socket is mounted on an insulated platform which is fastened to the base plate. A cover may be placed over the whole assembly, with suitable holes cut for the optical path. Electrical connections may be made by means of a tube socket mounted back of the lamp. The two sides of the double solenoid are activated by individual push-button switches (momentary contact) mounted in a convenient place.

ACKNOWLEDGMENT

The authors wish to thank Lael Smith for assistance in the const uction of the instrument.

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(5) Torok, Tibor, *Spectrochim. Acta*, **2**, 300 (1943).

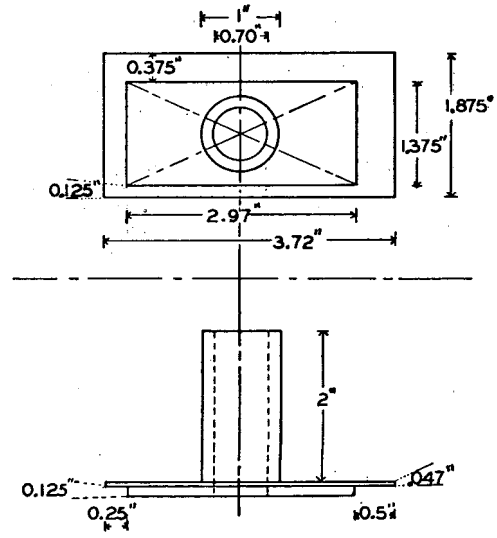
Paper 13 of the Institute for Atomic Research. Work done under Contract W-7405 eng-82, Manhattan District, U. S. Corps of Engineers. F. H. Spedding, project director.

A Colorimeter Tube Adapter for the Pfaltz and Bauer Fluorophotometer, Model B. Richard L. Durst and John B. Lewis, S. F. Durst & Company, Inc., Philadelphia 20, Pa.

In several colorimetric methods used in the control laboratory readings are best made in reaction tubes, to avoid transfer and time losses. With commercial equipment the authors found random variations of considerable magnitude, and it was observed that the taller the chemist the smaller the variation. This was found to be due to variations in diffused light that was reaching the instrument from windows behind the operator. When the colorimeter tubes were wrapped with black tape for 5 cm. (2 inches) above the chamber of the instrument, practically all variations were removed.

The device illustrated has been used with excellent results. The construction and dimensions are self-evident. A drawn copper tube could have been used but would not have been as satisfactory as the heavier machined tube, which more firmly seats the unit in the cuvette housing.

This adapter was made to fit No. 9800 Pyrex tubes 18 × 150 mm., with as little wobble as possible. The adapter furnished by the company as an insert for the cell carrier was found to be unnecessary with well-fitted tubes. Colorimeter tubes were obtained by selecting from several dozen tubes those which fitted the adapter, and classifying them for transmittance into groups of reasonable identity with various filters. The green trade mark is used as the position marker when reading.



The plate was constructed by riveting together two pieces of brass sheet and boring and threading a hole through them. The tube was made by boring a piece of 2.5-cm. (1-inch) brass rod and threading one end to fit the hole. This device was made by the George B. Henne Company, Philadelphia.

To avoid internal reflections the surfaces were blackened (by Plummer and Kershaw, Philadelphia), with optical black, which produces a dull finish integral with the metal.