ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Summer Symposia—A Reality

PLANS have been completed for the first Annual Summer Symposium sponsored jointly by the Division of Analytical and Micro Chemistry and ANALYTICAL CHEMISTRY, a project discussed by analysts in rather vague terms for a number of years. Under the able leadership of Philip J. Elving, chairman of the division, a number of committees are functioning efficiently and a record attendance is expected at Northwestern University on August 13 and 14.

The success of the AMERICAN CHEMICAL SOCIETY, its local sections, and scientific divisions, is due largely to the enthusiasm of its members and a willingness on the part of a large segment to devote time and energy to Society affairs. This fact is evidenced in many ways, by our reviewers, for example, and by acceptance of memberships on committees.

S. C. Lind of Minnesota has accepted the honorary chairmanship of the first symposium; Clement J. Rodden, chief of the Section of Uranium and Related Materials at the National Bureau of Standards, is general chairman; R. K. Summerbell has made available the splendid facilities of Northwestern; and Laurence D. Frizzell is chairman of the committee on local arrangements. Associated with Rodden and Frizzell are committee members widely known in chemical circles. The immediate response of these individuals is indicative of the enthusiasm of AMERICAN CHEMICAL SOCIETY members and the latent interest that has existed for a long time in the idea of an annual symposium on same phase of analytical chemistry.

The subject chosen for the August meeting is a most timely one—"Nucleonics and Analytical Chemistry." Rodden and his associates, G. E. Boyd of the Oak Ridge National Laboratory, D. N. Hume of M.I.T., and I. Perlman of the University of California, are arranging a two-day program of invited papers and a display of equipment and apparatus of special interest in radiochemistry.

Interest in peacetime applications of our present knowledge of nuclear chemistry continues to grow. This is particularly true of the developments in analytical chemistry, but as yet comparatively little has been made available generally to the analytical profession. Certainly the instructive and entertaining talk by N. H. Furman before the Division of Analytical and Micro Chemistry at its dinner meeting in Chicago indicated that a number of noteworthy strides have been made in microanalytical methods, inorganic chromatography, emission spectroscopy, absorption spectroscopy, fluorescence, and the development of varied applications of electrodeposition, polarography, potentiometry, and conductometry. Research will lag in many fields until such knowledge is widely disseminated. The summer symposium at Northwestern will provide the first real opportunity to acquaint chemists with new techniques and a familiarity with a number of new pieces of apparatus.

The large attendances at the recent analytical symposia at Louisiana State University, Pittsburgh, and Minneapolis demonstrate a revived interest in analytical chemistry. To the analysts themselves must go the credit for these successful meetings. Annual symposia jointly sponsored by the Division of Analytical and Micro Chemistry and this publication will assist materially in maintaining sustained interest. We are delighted to add to this announcement of the first in the series the information that the Committee on Annual Symposia, headed by B. L. Clarke, has selected the subject "Organic Reagents in Chemical Analysis" for 1949.

We advise early reservations for the Northwestern meeting, as the number that can be accommodated is limited. The final program will appear in both *Chemical and Engineering News* and ANALYTICAL CHEMISTRY, together with complete details for making reservations.

Awards

THE first recipient of the Fisher Award in Analytical Chemistry is N. H. Furman of Princeton University. His distinguished career will be commented on in detail when the award is actually presented to him at the fall meeting of the Society.

At this time we are happy to announce still another award of interest to analysts—the Fritzsche Award, financed by Fritzsche Brothers, Inc., consisting of a gold medal and one thousand dollars, to be presented annually for outstanding achievement in analysis, research, and new applications of essential oils, essential oil isolates, and related chemicals.

We hope we will be pardoned for a little show of personal pride. The same editorial in ANALYTICAL CHEMISTRY which influenced C. G. Fisher to establish the Fisher Award also initiated the proposal by the officials of Fritzsche Brothers that the AMERICAN CHEMICAL SOCIETY administer an award commemorating the 75th anniversary of that company.

The Mass Spectrometer in Organic Chemical Analysis

R. C. TAYLOR, R. A. BROWN, W. S. YOUNG, AND C. E. HEADINGTON

The Atlantic Refining Co., Philadelphia, Pa.

Mass spectrometer analyses of a number of mixtures such as ethylene oxide, ethylene, and carbon dioxide; alkyl iodides; aliphatic chlorides, ethers, and hydrocarbons; silicanes; and water, ether, and alcohol are presented to show the utility of the instrument when used to analyze organic substances other than the usual hydrocarbons. The analysis of gas samples as small as 10^{-4} ml. and the qualitative identification of polymers and elastomers are discussed, and examples are given of organic elementary analysis for carbon, hydrogen, oxygen, sulfur, and nitrogen.

WITHIN the past few years, many of the conventional chemical methods for analyzing mixtures of organic compounds have been replaced by newer physical methods such as infrared, ultraviolet, Raman, and mass spectrometry. Although first employed for the measurement of isotope abundance ratios and isotope tracer studies, the mass spectrometer has for some time been utilized by the petroleum industry for hydrocarbon analyses previously difficult to perform and extremely time-consuming. The notable success of the instrument in this latter capacity has spurred efforts to extend its application to a wide variety of mixtures of organic compounds in both the liquid and gaseous states. The aim of this paper is to present some examples that are typical of the numerous analytical problems encountered in the organic research laboratory upon which the mass spectrometer can throw much light.

The applications discussed have been chosen to provide a representative but by no means complete picture of the role of the spectrometer in modern analytical practice. To give some indication of the accuracy of analyses, particularly those not checked by known mixtures, a calculated average error for each component has been included in the tables. Fundamental data in the form of mass spectra of the calibrating compounds used, which are not available in the recent literature, are provided in Tables I, II, and III. Each analysis shown is supplemented by the mass spectrum of the mixture as well as an outline of the method used for computation. The operation conditions (Consolidated Engineering Corporation mass spectrometer, model 21-101) used throughout were: ionizing current 46 microamperes; ionizing voltage 50 volts; ion accelerating voltages at m/e 32, 1342 or 2340 volts.

ANALYSES OF GASEOUS MIXTURES

Although a considerable amount of published information is available on the analysis of paraffin and olefin hydrocarbons in petroleum gases by mass spectrometry, the authors have found this instrument equally versatile in analyzing other compounds that are not ordinarily encountered in petroleum refining. For instance the infrared-inactive homopolar diatomic gases such as hydrogen, nitrogen, and oxygen, as well as other nonhydrocarbon gases, can usually be resolved by this means even in complex mixtures. Table IV, for example, shows an analysis of a mixture of ethylene oxide, ethylene, propane, nitrogen, oxygen, and carbon dioxide. In the case of this mixture, although the primary interest was centered about ethylene oxide, the mass spectrometer record provided complete data for a total analysis, which if attempted by conventional chemical methods would have been considerably more time-consuming.

Another example of interest is the analysis of such sulfur compounds as hydrogen sulfide, carbon disulfide, sulfur dioxide, carbonyl sulfide, and methyl mercaptan (methanethiol). A complex mixture containing all five compounds can be easily and quickly resolved by means of the spectrometer in a fraction of the

Table I. Relative Intensities of Principal Peaks in Mass Spectra of Some Oxygenated Compounds

m/e	Water	Ethylene Oxide	Nitrous Oxide	Ethanol	Dimethyl Ether	Diethyl Ether	Ethyl *tert*-butyl Ether	m/e
14	...	42.0	13.8	...	13.3	...	...	...
15	0.00	98.5	...	70.5	91.8	52.2	21.4	15
16	2.32	12.9	6.24	3.55[a]	3.35[a]	2.36[a]	0.90[a]	16
17	24.5	...	...	8.09[a]	2.14[a]	1.51[a]	0.23[a]	17
18	100.0	...	...	12.5[a]	1.75[a]	4.49[a]	0.31[a]	18
19	...	...	...	19.8	0.0	9.19	0.81	19
26	...	10.4	...	39.0	0.54	23.8	3.24	26
27	...	7.33	...	120.	0.77	116.	32.0	27
28	...	2.52	13.6	30.0	1.03	38.7	5.65	28
29	...	172.	0.28	152.	123.	224.	53.0	29
30	...	3.56	35.9	34.1	5.05	12.05	2.06	30
31	...	1.62	0.22	579.	11.5	397.	19.2	31
41	...	2.25	...	24.3	0.49	12.8	50.3	41
42	...	18.1	...	26.0	0.52	8.25	7.66	42
43	...	24.8	...	61.9	3.09	40.4	28.4	43
44	...	100.0	100.0	17.2	1.21	9.68	1.29	44
45	...	2.20	0.73	275.	203.	133.	5.55	45
46	...	...	0.22	100.	100.	5.43	...	46
57	...	...	...	...	...	1.72	93.7	57
58	...	...	...	...	...	1.18	7.25	58
59	...	...	...	...	...	168.	261.	59
73	...	...	...	...	...	9.48	1.71	73
74	...	...	...	...	...	100.	...	74
87	...	...	...	...	...	...	100.	87
Sensitivity of 100% peak (div./micron)	16.1	11.4	13.6	8.17	7.60	6.86	13.5	
Sensitivity of *n*-butane at m/e = 58	5.00	4.70	4.08	7.96	5.00	4.22	3.40	

[a] Peaks uncorrected for presence of water.

Table II. Relative Intensities of Principal Peaks in Mass Spectra of Some Aromatic Compounds

m/e	Benzene	Toluene	Phenyl-acetylene	Styrene	α-Methyl-styrene	m/e
39	11.1	19.3	3.60	7.00	13.5	39
41	0.05	2.19	0.05	0.26	1.96	41
50	14.7	6.04	11.7	10.0	7.46	50
51	17.6	10.5	10.6	21.2	17.8	51
52	16.4	2.69	5.52	7.80	4.43	52
57	0.02	0.04	0.46	0.02	1.95	57
58	0.0	0.01	0.04	0.45	7.75	58
75	1.67	0.81	7.09	3.10	2.90	75
76	5.54	0.52	17.8	3.90	3.30	67
77	12.9	1.45	1.83	16.6	19.6	77
78	100.	0.16	0.85	26.4	27.2	78
91	...	117.	0.79	0.40	16.2	91
92	...	100.	0.06	0.03	1.67	92
102	...	...	100.0	7.85	5.40	102
103	...	...	8.03	37.6	47.6	103
104	...	...	0.25	100.	4.06	104
105	...	...	...	8.40	2.60	105
115	...	...	...	...	18.1	115
116	...	...	...	...	6.52	116
117	...	...	...	...	59.5	117
118	...	...	...	...	100.	118
Sensitivity of 100% peak (div./micron)	252.	184.	342.	282.	187.	
Sensitivity of *n*-butane at m/e = 58	21.8	21.8	21.8	21.8	21.8	

Table III. Relative Intensities of Principal Peaks in Mass Spectra of Some Halides

m/e	Methyl Chloride	Vinyl Chloride	Isopropyl Iodide	*n*-Butyl Iodide	Chloro-trimethyl-silicane	m/e
25	...	10.8	...	...	...	25
26	...	27.6	9.40	19.0	0.50	26
27	...	82.9	89.5	109.	2.20	27
28	...	1.83	8.36	44.8	2.66	28
29	...	0.09	6.00	218.	6.03	29
35	6.45	5.36	0.09	0.10	...	35
36	1.83	1.57	0.67	0.50	...	36
37	2.07	1.80	4.68	2.42	...	37
38	...	0.53	8.97	5.34	...	38
39	...	...	47.3	46.6	...	39
40	...	...	7.67	5.56	...	40
41	...	...	90.4	157.	1.48	41
42	...	...	11.6	8.05	4.14	42
43	...	...	212.	3.87	12.0	43
44	...	...	8.10	0.16	2.71	44
45	...	...	...	...	4.98	45
49	9.95	1.66	...	...	...	49
50	100.	0.53	...	3.14	...	50
51	3.43	0.21	...	3.28	...	51
52	31.5	...	...	1.23	...	52
55	...	...	...	16.5	1.75	55
56	...	...	...	12.6	0.48	56
57	...	...	...	280.	1.03	57
61	...	8.13	...	...	...	61
62	...	100.0	...	...	...	62
63	...	4.64	...	...	17.3	63
64	...	31.3	...	...	2.49	64
65	...	0.63	...	...	16.8	65
73	...	...	...	...	31.8	73
93	...	...	...	...	100.0	93
94	...	...	...	...	7.38	94
95	...	...	...	...	35.2	95
108	...	...	...	...	4.27	108
109	...	...	Magnet current changed from 0.56 to 0.76 amp.		0.37	109
110	...	...	29.0	17.7	1.51	110
127	...	...	0.45	3.60	...	127
141	...	...	2.02	10.4	...	141
155	...	...	0.00	0.23	...	155
169	...	...	100.0	0.0	...	169
170	...	...	...	100.0	...	170
184	...	...	...		...	184
Sensitivity of 100% peak (div./micron)	18.5	117.	46.2	35.4	210.	
Sensitivity of *n*-butane at m/e = 58	4.40	24.6	8.88	8.88	19.9	

Table IV. Analysis of Ethylene Oxide Mixture

Components	Known	Mass Spectrometer Analysis	Observed Error	Predicted Average Error
	Mole Per Cent			
Ethylene oxide	3.8	3.5	−0.3	0.2
Ethylene	6.3	6.1	0.2	0.3
Propane	1.2	1.2	0.0	0.1
Nitrogen	75.2	76.6	1.4	0.5
Oxygen	9.6	9.0	−0.6	0.5
Carbon dioxide	3.9	3.6	−0.3	0.3

Calculation Outline

Component Sequence	Mixture Peak, m/e	Other Components Contributing to Mixture Peak[a]
Propane	39	None
Ethylene oxide	42 (or 43)	Propane
Carbon dioxide	44	Propane and ethylene oxide
Oxygen	32	None
Ethylene	26	Propane and ethylene oxide
Nitrogen	28	Propane, ethylene oxide, carbon dioxide, and ethylene

Mass Spectrum of Mixture

m/e	Divisions of Peak
14	9.6
15	19.2
16	14.4
26	36.8
27	42.0
28	759.
29	43.8
30	0.8
41	2.6
42	4.2
43	8.2
44	62.0
45	2.0
Pressure, microns	40.0
Sensitivity of *n*-butane at mass 58	5.30

[a] Propane and ethylene oxide resolution may be checked by residual peaks at m/e 15 and/or 29.

Table V. Sorption of Water on Glass Inlet System

Pressure of Water, Microns				
Background	Added	Determined (total minus backgrnd.)	Adsorbed (added minus detd.)	Standard Used
0.056	0.156	0.0	0.156	Benzene saturated with water at 24° C. (liquid mixture)[a]
0.069	0.131	0.0	0.131	
0.063	0.137	0.0	0.137	
0.218	0.119	0.045	0.074	
0.256	0.137	0.044	0.093	
0.569	0.106	0.076	0.030	
0.881	0.100	0.070	0.030	
1.70	0.144	0.040	0.104	
3.75	0.212	0.040	0.172	
0.144	1.94	0.737	1.20	Water in *n*-butane (gas mixture)[b]
0.328	0.568	0.193	0.375	
0.250	0.613	0.257	0.356	
0.256	0.175	0.038	0.137	
0.344	0.301	0.131	0.170	
0.419	0.506	0.232	0.274	
0.500	5.37	3.13	2.24	
2.78	5.49	4.19	1.30	
0.18	24.0	20.0	4.0	Pure water (liquid phase)[a]
0.22	68.5	59.2	9.3	
0.27	79.1	72.9	6.2	
0.31	86.9	81.1	5.8	

[a] Measured with microburet.
[b] Measured with vacuum micromanometer.

time required by chemical methods. The same is true for many of the normally liquid sulfur compounds such as higher thiols, sulfides, and disulfides. However, in this instance, as well as in the case of water and alcohol mixtures, accuracies are ordinarily not so good as those obtained with hydrocarbon mixtures, because of the unavoidable loss of certain components due to reaction or sorption in the inlet system. Errors due to this source, however, may frequently be minimized by the application of suitable correction factors. An illustration of this procedure can be given in the case of water, one of the more strongly sorbed substances commonly encountered. Data for its sorption characteristics on the inlet system are given in Table V. These characteristics will vary from system to system and may change even with the same system as the surface conditions alter with time or treatment. Frequent checks should, therefore, be made on a particular system where best accuracy is desired.

The procedure used for obtaining these data consisted of adding measured amounts of water to the inlet system, which were compared, after equilibrium had been attained, to the amounts of water determined by spectrometer analyses. However, because the normal water background of the instrument was in the neighborhood of 0.06 micron, no data could be obtained below this value, and furthermore, at this point, if 0.1 to 0.2 micron of water pressure were added to the inlet system, sorption would occur without increasing the background value measurably. The data in Table V were plotted as the sorption isotherm (Figure 1), where for convenience both ordinate and abscissa are shown as microns' pressure in the inlet system. Before plotting Figure 1, however, three conditions had to be established. (1) The line which passes through these initial background points must have a slope approaching infinity in order to be consistent with the observed fact—i.e., for the amounts of water added—that sorption was practically complete. (2) It was necessary to fix an arbitrary ordinate value for these points, because it was not known how much water had already sorbed on the walls of the inlet system. The value chosen corresponded to the pressure of water lost to the inlet system at these lowest background values. (3) In order to effect a gradual change in slope between these points and those showing measurable increases in pressure upon the addition of water, an arbitrary ordinate value of 0.5 was chosen for the abscissa value of 0.144. In use, the abscissa readings corresponding to background water pressure and total water pressure are subtracted to give the measured pressure of water in the sample. Ordinate values corresponding to the abscissa readings are subtracted to give the pressure of water lost by sorption. This latter value, added to the measured water pressure, gives the corrected pressure of water in the sample.

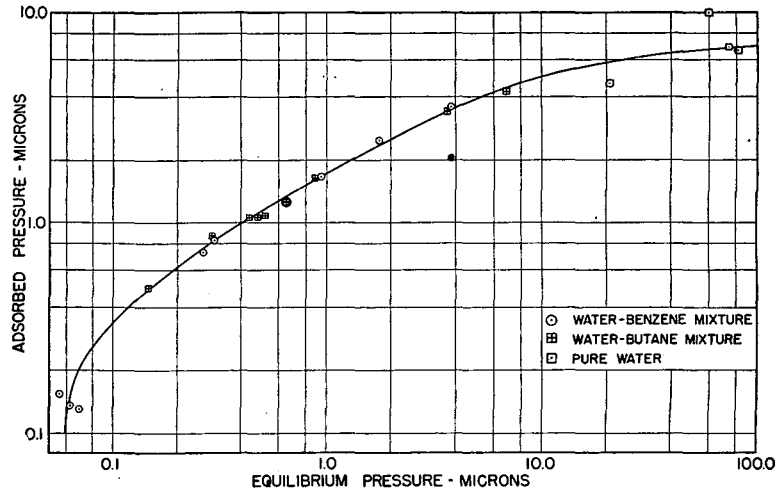

Figure 1. Sorption of Water on Glass Inlet System

One of the more remarkable features of this instrument lies in its suitability for the analysis of extremely small quantities of gas. The amount required for a conventional mass spectrometer analysis is of the order of 0.1 ml. at atmospheric pressure, but samples as small as 0.0001 ml. have been analyzed on a routine basis. In the analysis of these small samples, the chief problem lies in collecting the sample and protecting it from possible alteration by such factors as sorption and contamination. Analysis of such samples must be preceded by a study of the sorption characteristics of the components of the mixture. In this case the determination of water required that the spectrometer inlet system be first conditioned with water, so as to minimize the amount lost by sorption. Using this approach, the minimum detectable quantity of water vapor is 0.0001 ml. at S.T.P. and from 0.0005 to 0.005 ml. may be determined with an average accuracy of ±20%. Contamination of these microsamples arises largely from the desorption of small amounts of gases previously sorbed on the walls of the system which, in the case of macrosamples, would be of no significance. Much can be done to eliminate errors from this source by long pumping prior to analysis and by determining a background on the instrument before scanning the sample. Additional corrections are then determined from the background and applied before a final analysis is calculated.

Hydrocarbon background has been largely eliminated in the authors' instrument by the installation of an inlet system in which the stopcocks have been replaced by mercury cut-off valves. This system in some cases enables one to reduce the background with 5 minutes' pumping to a value one fortieth of that attained after 16 hours of pumping on a similar system containing greased stopcocks.

A typical microanalysis of gas collected from a soil sample associated with geochemical prospecting is shown in Table VI. Because in this type of analysis the quantities of the components are approaching the minimum detectable amounts, errors are large relative to those encountered in samples of normal size. It is noteworthy, however, that such a detailed analysis could be made on approximately 0.001 ml. of sample. It is also notable that such an analysis would reveal the presence and the amounts of nitrous oxide and unsaturated hydrocarbons which might well have gone undetected with other methods of analysis.

Table VI. Analysis of Soil Gas Sample

	Mass Spectrometer Analysis		Predicted Av. Error,
	Gas at S.T.P., Ml.	Mole %	Mole %
Methane	0.00007	5	0.7
Ethene	0.00013	9	0.7
Ethane	0.00017	12	1.4
Propene	0.00004	3	0.7
Propane	0.00014	10	2.2
Butenes	0.00005	4	0.7
Isobutane	0.00001	1	0.7
n-Butane	0.00008	6	1.4
Pentenes and/or cyclopentane	0.00004	3	0.7
Isopentane			
n-Pentane	0.00009	6	1.4
Nitrous oxide	0.00030	22	2.2
Water	0.00026	19	3.6
Total volume, ml.	0.00138		

Mass Spectrum of Mixture

m/e	Divisions of Peak	m/e	Divisions of Peak
15	1.9	53	0.2
16	1.4	54	0.1
17	0.8	55	1.5
18	3.0	56	1.0
26	3.4	57	1.0
27	8.3	58	0.4
28	12.6	70	0.6
29	9.7	71	0.1
30	2.4	72	0.2
39	3.2	Pressure, microns	..
40	0.6	Sensitivity of *n*-butane at mass 58	13.7
41	5.4		
42	3.8		
43	7.1		
44	6.9		

Fundamental method of calculation as previously described (*4*).

ANALYSIS OF VOLATILE LIQUIDS

Using mercury-sealed sintered-disk sample introduction devices previously described (*3*), and substituting mercury cutoff

Table VII. Analysis of Aromatic Mixture

Components	M.W.	Known Mixture: Known	Known Mixture: Mass spectrometer analysis	Known Mixture: Observed error	Known Mixture: Predicted average error	Laboratory Reaction Product: Mass spectrometer analysis	Laboratory Reaction Product: Predicted average error	Laboratory Reaction Product: Ultraviolet analysis	Laboratory Reaction Product: Chemical analysis
		Weight Per Cent							
Benzene	78	17.4	18.9	1.5	1.5	13.2	2.5		...
Toluene	92	...	...	...	...	3.2	0.2		...
Aromatic	106	...	...	...	...	0.9 (18.7)	0.1	15.0	...
Aromatic	120	...	...	...	...	1.4	0.1		...
Styrene	104	63.7	61.0	−2.7	1.7	53.5	2.2	56.0	...
α-Methylstyrene	118	9.6	10.1	0.5	0.5	13.0	0.5	20.0	...
Aromatic[a]	132	...	...	...	...	1.4	0.1		...
Phenylacetylene	102	9.3	10.0	0.7	0.5	7.7	0.7	7.5	7.5
Aromatic[a]	116	...	...	...	...	4.4	0.5	...	...
Aromatic[a]	130	...	...	...	...	1.0	0.1	...	...
Aromatic[a]	128	...	...	...	...	0.3	0.1	...	...

Calculation Outline

Component Sequence	Mixture Peak, m/e	Other Components Contributing to Mixture Peak
α-Methylstyrene	118	None
Styrene	104	α-Methylstyrene
Phenylacetylene	102	α-Methylstyrene and styrene
Benzene	78	α-Methylstyrene, styrene, and phenylacetylene

Mass Spectrum of Mixture

m/e	Divisions of Peak	m/e	Divisions of Peak
39	289	105	167
50	338	106	32.5
51	585	115	49
52	276	116	17
74	143	117	159
75	102	118	268
76	170	119	24.6
77	447	120	17.5
78	1059	Pressure, microns	13.2
91	194		
92	90	Sensitivity of *n*-butane at mass 58	21.8
102	492		
103	841		
104	1825		

[a] Estimated without calibrating compounds.

valves for greased stopcocks to decrease sorption tendencies, the accurate analysis of normally liquid samples becomes as feasible and as rapid as the analysis of samples which are normally gases. The liquids, of course, must have sufficient vapor pressure to give a measurable spectrum, which in general corresponds to compounds more volatile than C_{14} hydrocarbons. Of these, the less volatile compounds require a somewhat different introduction method because of the slowness with which they vaporize from a sintered disk. This requires measuring from a microburet (*3*) a predetermined amount of sample directly into a removable cup attached to the inlet system. After the cup is replaced, the sample is frozen in liquid nitrogen and the system re-evacuated. Warming to room temperature vaporizes the sample, which is then analyzed in the usual fashion.

The analysis of mixtures of C_6, C_7, C_8, and even C_9 hydrocarbons of the paraffin, cycloparaffin, olefin, or aromatic types is in many cases easily accomplished (*2*). In complex systems, however, the analysis must be preceded by a multiplate fractional distillation to limit the number of components to 10 or less, depending upon the similarity of individual mass spectra.

Table VII shows analyses of a laboratory reaction product and a synthetic mixture both containing principally benzene, phenylacetylene, styrene, and α-methylstyrene. Analyses by ultraviolet spectrophotometry and a chemical method are also included for purposes of comparison. The novelty of this analysis lies principally in the amount of information obtainable by the mass spectrometer, which in many cases would provide the research worker with valuable clues concerning reaction mechanisms, which would not have been obtained by other methods. In addition to the components of the synthetic mixture, the laboratory reaction product was shown to contain the following substituted aromatic compounds presumably produced by side reactions: (1) other simple aromatic hydrocarbons (toluene, xylene, and cumene); (2) four-carbon substituted aromatics of molecular weight 132 with one double bond; (3) acetylene or diolefin linkages in both three-carbon and four-carbon substituted aromatics of molecular weights 116 and 130, and (4) four-carbon substituted aromatics of molecular weight 128, having either a triple bond and a double bond, or three double bonds.

The estimation of many of these additional compounds was made without the aid of calibrating standards. Frequently a study of the spectra will reveal the presence of fragments characteristic of portions of the parent substance. These can then be used to elucidate the possible structure of the original material as well as to furnish a semiquantitative measure of the amount present. The presence of dioxane which is a mass isomer of amyl alcohols, butyric acids, four-carbon esters, and five-carbon ethers, was suspected from the mass spectrum of a liquid reaction mixture before the spectra of any of these compounds had been determined experimentally. This followed when consideration of the oxygen isotope ratio of the unknown indicated the presence of two oxygen atoms in the molecule, which established an empirical formula of $C_4H_8O_2$ and eliminated from consideration mass isomers of the alcohol and ether type. Of other possible compounds having this formula, the acids and esters were thought improbable because the mass spectra that are available for such compounds generally show a high ratio between breakdown peaks and the parent mass peak, a condition which did not hold for the unknown. Stable cyclic structures, on the other hand, do show relatively low ratios of breakdown peaks to parent peaks; hence, a structure of the dioxane type was indicated.

Table VIII gives the analysis of an ethanol-ether-water mixture, which shows good agreement with the known composition in spite of the fact that water and alcohol are two compounds

Table VIII. Analysis of Ethanol-Ether-Water Mixture

Components	Known	Mass Spectrometer Analysis 1	Mass Spectrometer Analysis 2	Av. Observed Error	Predicted Av. Error
	Mole Per Cent				
Ethanol	51.1	51.7	52.2	0.9	1.0
Diethyl ether	1.8	1.6	1.1	−0.5	0.2
Ethyl *tert*-butyl ether	32.3	33.4	32.9	0.8	0.8
Water	14.8	13.3	13.8	−1.3	1.3

Calculation Outline

Component Sequence	Mixture Peak, m/e	Other Components Contributing to Mixture Peak
Ethyl *tert*-butyl ether	87	None
Ethyl ether	74	None
Ethanol[a]	46	None
Water[a]	18	Ethyl *tert*-butyl ether, ethyl ether, and ethanol

Mass Spectrum of Mixture

m/e	Divisions of Peak	m/e	Divisions of Peak
15	84.5	43	80.4
16	5.1	44	14.1
17	18.2	45	198.
18	67.8	46	66.8
19	12.1	57	153.
26	32.5	58	11.9
27	138.	59	451.
28	30.6	73	3.5
29	206.	74	4.8
30	26.7	87	163.2
31	465.	Pressures, microns	38.5
41	99.	Sensitivity of *n*-butane at mass 58	3.20
42	30.3		

[a] Corrections for sorption must be made.

Table IX. Analysis of Silicanes

Components	Mass Spectrometer Analysis	Predicted Average Error
	Mole Per Cent	
Chlorotrimethylsilicane	51	0.8
Hexamethylsilicoethane[a]	48	0.8
High molecular weight silicanes[b]	1	0.2

Calculation Outline

Component Sequence	Mixture Peak, m/e	Other Components Contributing to Mixture Peak
Hexamethylsilicoethane[a]	146	None
Chlorotrimethylsilicane	93	None

Mass Spectrum of Mixture

m/e	Divisions of Peak	m/e	Divisions of Peak
31	56.9	73	1273.
32	3.3	85	10.5
40	5.0	86	7.9
41	47.3	87	65.6
42	120.	93	2132.
43	393.	94	163.
44	101.	95	770.
45	318.	101	29.
49	4.6	105	31.
50	2.0	108	88.4
51	36.9	109	10.8
52	96.3	110	31.9
57	54.4	117	43.8
58	54.8	131	169.
59	302.	146	2800.
63	473.	147	613.
64	64.3	148	193.
65	409.	Pressure, microns	18.5
66	411.	Sensitivity of *n*-butane at mass	
71	28.	58	19.5
72	98.4		

[a] Presence of this compound could not be proved conclusively from a study of mixture spectrum, because no calibration standard was available. It is highly probable in view of reaction mechanism involved in preparing mixture. [b] Estimated without calibrating compounds.

strongly sorbed on the walls of the inlet system. Because of this behavior the predicted average error in this case is calculated using the sorption isotherm as well as calibration spectra of the components.

Table IX, showing the analysis of a mixture of silicanes, indicates that good accuracy is also possible with this somewhat unusual sample, and Table X illustrates the analysis of a typical mixture of alkyl iodides and oxygenated compounds. The results in Table XI, were obtained on a fairly complex system consisting of hydrocarbons, aliphatic chlorides, and ethers. In these cases and in many similar ones the spectra of the pure calibrating compounds are all that is required to calculate the predicted average accuracy.

ANALYSIS OF ORGANIC MATERIALS OF HIGH MOLECULAR WEIGHT

A recent publication of the National Bureau of Standards (*1*) reveals the application of mass spectrometry to the identification of high polymeric substances. Advantage is taken of the fact that, in many cases, these substances can be thermally decomposed to yield characteristic volatile fragments. Work done by the authors has shown that natural rubber, Buna-N, and GR-S can all be qualitatively identified in this fashion. Natural rubber gives a gaseous product of which over 92% is isoprene, Buna-N yields butadiene and acrylonitrile, whereas GR-S yields butadiene and styrene. The acryloid polymers give characteristic yields of acroleins, and the polyglycol ethers give appreciable yields of characteristic products.

An interesting extension of this approach is in the rapid analysis of organic substances for such elements as carbon, hydrogen, sulfur, nitrogen, and oxygen. Microquantities of material can be subjected to either oxidation or reduction at high tempera-

Table X. Analysis of Alkyl Iodides

Components	Mass Spectrometer Analysis	Predicted Average Error
	Mole Per Cent	
Isopropyl iodide	74	1.8
n-Butyl iodide	5	0.7
Amyl iodides[a]	3	0.3
Oxygenated compounds[a]	18	1.8

Calculation Outline

Component Sequence	Mixture Peak, m/e	Other Components Contributing to Mixture Peak
n-Butyl iodide	184	None
Isopropyl iodide	170	None
Oxygenated[a]	87	None
Amyl iodide[a]	198	None

Mass Spectrum of Mixture

m/e	Divisions of Peak	m/e	Divisions of Peak
26	149.	58	43.2
27	1355.	59	62.0
28	209.	70	0.6
29	261.	71	3.1
30	10.1	72	7.4
31	3.8	73	1.9
37	66.7	87	9.2
38	124.	101	7.6
39	690.	115	10.8
40	110.	116	7.1
41	1387.	127	393.
42	173.	141	8.1
43	2800.	155	35.3
44	91.2	169	4.5
45	44.7	170	1296.
55	15.0	184	79.2
56	6.4	Pressure, microns	42.8
57	197.	Sensitivity of *n*-butane at mass 58	8.20

[a] Estimated without calibrating compounds.

Table XI. Analysis of Hydrocarbon-Halide-Ether Mixture

Components	Mass Spectrometer Analysis	Predicted Average Error
	Mole Per Cent	
Methane	8.1	0.3
Acetylene	36.2	0.5
Methyl chloride	46.2	0.5
Vinyl chloride	5.9	0.2
Methyl vinyl chloride[a]	0.2	0.02
Dimethyl ether	2.3	0.1
Methyl ethyl ether[a]	1.0	0.05
Diethyl ether	0.1	0.01

Calculation Outline

Component Sequence	Mixture Peak m/e	Other Components Contributing to Mixture Peak
Methyl vinyl chloride[a]	76	None
Diethyl ether	74	Methyl vinyl chloride
Vinyl chloride	62	Methyl vinyl chloride
Methyl ethyl ether[a]	60	Methyl vinyl chloride and viny chloride
Methyl chloride	50	Methyl vinyl chloride and vinyl chloride
Dimethyl ether	46	None
Acetylene	26	Methyl vinyl chloride, vinyl chloride, diethyl, methyl ethyl, and dimethyl ethers
Methane	16	Diethyl, methyl ethyl, and dimethyl ethers

Mass Spectrum of Mixture

m/e	Divisions of Peak	m/e	Divisions of Peak	m/e	Divisions of Peak
14	110.4	36	14.7	62	102.
15	672.	37	16.6	63	4.7
16	128.1	45	39.0	64	31.8
26	1044.	46	15.9	73	0.8
27	139.8	49	80.0	74	1.0
28	137.7	50	804.	Pressure, microns	49.6
29	32.	51	27.8	Sensitivity of *n*-butane at mass	
30	2.5	52	253.5	58	9.5
31	5.4	59	16.7		
32	21.0	60	7.2		
35	42.3	61	7.5		

[a] Estimated without calibrating compounds.

Table XII. Elementary Analysis

Diphenylsulfone				Acetamide			
	Oxygen Pyrolysis at 780° C.		Hydrogen pyrolysis at 780° C.		Oxygen Pyrolysis at 780° C.		Hydrogen pyrolysis at 1140° C.
Element	Known	Determined (av. of 2)	determined (av. of 2)	Element	Known	Determined (av. of 3)	determined, (1 detn.)
	Mole Per Cent				*Mole Per Cent*		
C	66.0	70.1	18.7	C	40.7	42.9	40.9
H	4.62	3.7	...	H	8.54	9.0	...
O	14.7	...	13.7	O	27.1	...	27.1
S	14.7	12.2	10.8	N	23.7	27.8	18.8
Sample size, μg.		301	318			280	243

Pyrolysis Products Determined by Mass Spectrometer				Pyrolysis Products Determined by Mass Spectrometer			
Oxygen pyrolysis		Hydrogen pyrolysis		Oxygen pyrolysis		Hydrogen pyrolysis	
CO_2	23.1	CO_2	4.5	CO_2	39.8	CH_4	11.3
H_2O	72.2	H_2O	36.3	H_2O	49.3	CO	48.9
SO_2	4.5	CO	6.0	N_2	10.9	HCN	39.1
H_2S	0.2	H_2S	19.9			H_2O	0.65
COS	0.01	COS	0.1				
		C_6H_6	9.3				
		$C_6H_5CH_3$	0.05				
		CH_4	23.8				

ture, the choice depending upon the elements to be determined and the necessary condition that volatile products must be formed in order to be detected. A limited amount of work has shown this general technique to be of considerable promise as a rapid semiquantitative as well as qualitative method.

The experimental technique consists of placing a weighed amount of sample, several hundred micrograms in size, in a quartz capillary tube, which is then filled with an excess of either oxygen or hydrogen and sealed off. The tube is placed in a furnace, brought up to the reaction temperature, and held there for 10 to 15 minutes. After cooling, the tube is placed in the spectrometer break-off device and the gas analyzed in the conventional manner.

Table XII shows exploratory results obtained with acetamide and diphenylsulfone subjected to both oxidation and reduction pyrolysis. Although a reaction temperature of 780° C. appears to be satisfactory for oxygen pyrolysis, very poor results were obtained for hydrogen pyrolysis of acetamide at this temperature. Subsequently a temperature of 1140° C. was tried with acetamide, with satisfactory results for both carbon and oxygen. The low results for nitrogen are unexplained, but may result from the formation of ammonia, which is difficult to determine at present because of sorption effects. It is possible that the higher temperature used here might also improve the carbon and sulfur results obtained from the hydrogen pyrolysis of diphenylsulfone.

LIMITATIONS

The mass spectrometer, although extremely versatile, resembles any other analytical tool in that it is limited in its applications. Ammonia, amines, water, and some alcohols, for instance, are so strongly sorbed on the walls of the inlet system that to date they have been analyzed only with difficulty. In the hydrocarbon field the cis-trans isomers of 2-butene cannot be readily resolved and the resolution of 1-butene and 2-butene, except in simple mixtures, is poor unless special techniques are resorted to. Ortho, meta, and para isomers of some aromatic hydrocarbons show nearly identical mass spectra and in some cases it is necessary to group aliphatic hydrocarbon isomers in the C_7 and C_8 range.

Sometimes the shortcomings of the method, however, can be effectively utilized. For example, in a complex hydrocarbon mixture containing all the paraffins and olefins from C_1 through C_5, both the butene isomers and pentene isomers must be grouped and treated as two components, primarily because of the similarity of their mass spectra. The remaining components can then be accurately resolved. If the spectra of the butenes and pentenes were all too dissimilar to treat in this fashion, the large number of components relative to the number of peaks available would prevent the resolution of any of the olefins. This grouping procedure can also be advantageously applied to much more complex systems.

CONCLUSIONS

A factor of great importance to both the analyst and the research worker is the element of speed. Although the time required for an analysis has been shown in previous publications to be highly variable, depending upon the complexity of the mixture (*4*), a further insight into the time requirement may be obtained from a breakdown of the total man-hours required for the analysis of the eight-component mixture shown in Table XI. The calibration time for this mixture was 3.75 hours, after which the first analysis required about 2.5 hours. A group of samples, however, could subsequently be analyzed at the rate of approximately 1.5 hours per sample.

Another interesting and important aspect of mass spectrometry is the ease of obtaining cross checks in the computation of each analysis, which can be done by any or all of three methods. The first method consists of making a second and independent calculation utilizing a different set of peaks in the spectrum. The second method is based on the calculation of residual peak heights (*4*), and the third is very simply done by comparing the total pressure computed for the mixture to that measured in the inlet system. These cross checks greatly reduce the possibility of errors of a qualitative as well as quantitative nature.

In the case of a number of illustrative examples shown in the tables no comparison with a mixture of known compositions is available to indicate the accuracy of the analysis. Because reliable average errors can be calculated, such a known mixture is not needed for this purpose (*5*). Observed data on instrument fluctuations, differences in the spectra of calibrating compounds, and, where necessary, data on the sorption characteristics of the components are all utilized to calculate an average predicted error for each component of a mixture. This procedure has the advantage of giving expected errors for the exact mixture under analysis. For instance, in Table VII the predicted average errors for the laboratory reaction product are appreciably different from the much simpler mixture of known composition. Changes in either the complexity of the mixture or the concentrations of the components will normally affect accuracy. A comparison of calculated errors and observed errors is available in Tables V, VII, and VIII.

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RECEIVED March 28, 1947. Presented before the Division of Petroleum Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Qualitative Organic Analysis and Infrared Spectrometry

R. BOWLING BARNES, R. C. GORE, R. W. STAFFORD, AND V. Z. WILLIAMS
Stamford Research Laboratories, American Cyanamid Company, Stamford, Conn.

The application of infrared spectrometry to qualitative organic analysis and molecular structure determination is discussed as an approach complementary to conventional methods. A chart correlating characteristic absorption frequencies and atomic groups is presented and discussed. Several examples of the infrared approach to typical problems are described in order to illustrate the utility of the method and the nature of the information that can be obtained.

THE last few years have seen a tremendous increase in the use of infrared spectrometry in industry for the solution of chemical problems. Most of these published applications (*4, 5, 7, 8, 10, 13, 15, 16, 20, 24, 25, 27, 32*) have been concerned with the quantitative analysis of mixtures either to replace an existing chemical method or to permit an analysis not previously possible by chemical means. It has been primarily for these applications that several fast, accurate, automatic recording spectrometers have become commercially available since 1940.

Another use of infrared spectrometry, its application to qualitative analysis, has not been so widely publicized although several papers (*1, 2, 6, 9, 12, 14, 21, 26*) have illustrated this use for a particular case or discussed the spectral correlations of certain functional groups. It is a natural step to try to broaden these concepts and to summarize these correlations with a view towards shaping a field of infrared qualitative analysis which may be alternative or complementary to conventional organic analysis.

The potentialities of infrared spectrometry in this direction are fairly well known. The spectrum of an organic material from 4000 cm.$^{-1}$ (2.5 μ) to 400 cm.$^{-1}$ (25 μ) can be obtained in 0.5 to 2 hours. The sample may be studied in solid, liquid, or vapor phase or in a few limited solvents. The amount of material required is small (a few milligrams) and can usually be recovered intact. Such a spectrum forms a permanent, unique characteristic of a material which can be used immediately or brought out at a future time for comparison with a known material or for further detailed information. For the qualitative analysis of an unknown, however, one of the best features of this spectrum is that the absorption or lack of absorption in specific frequency regions can be correlated with specific atomic groups and, in some cases, with the relationship of these groups to the rest of the molecule. Thus, by interpretation of the spectrum it is possible to state that certain functional groups are present in the material and that certain others are absent. With this one datum, the infrared spectrum, the possibilities for the unknown can sometimes be narrowed so sharply that comparison with a library of pure spectra permits identification. In other cases where such information is insufficient to enable a very narrow classification, the necessity of many chemical tests is certainly avoided and a minimum of further chemical knowledge is required to obtain an answer.

It is the purpose of this paper to point out some of these functional group correlations and to illustrate the complementary use of infrared and chemical methods for the identification of some typical unknowns that may be encountered in organic research and production. A brief summary of some of the chemical methods of qualitative analysis has been included in order to compare the two individual methods of approach, and to facilitate further discussion of the unification of the two into a single procedure.

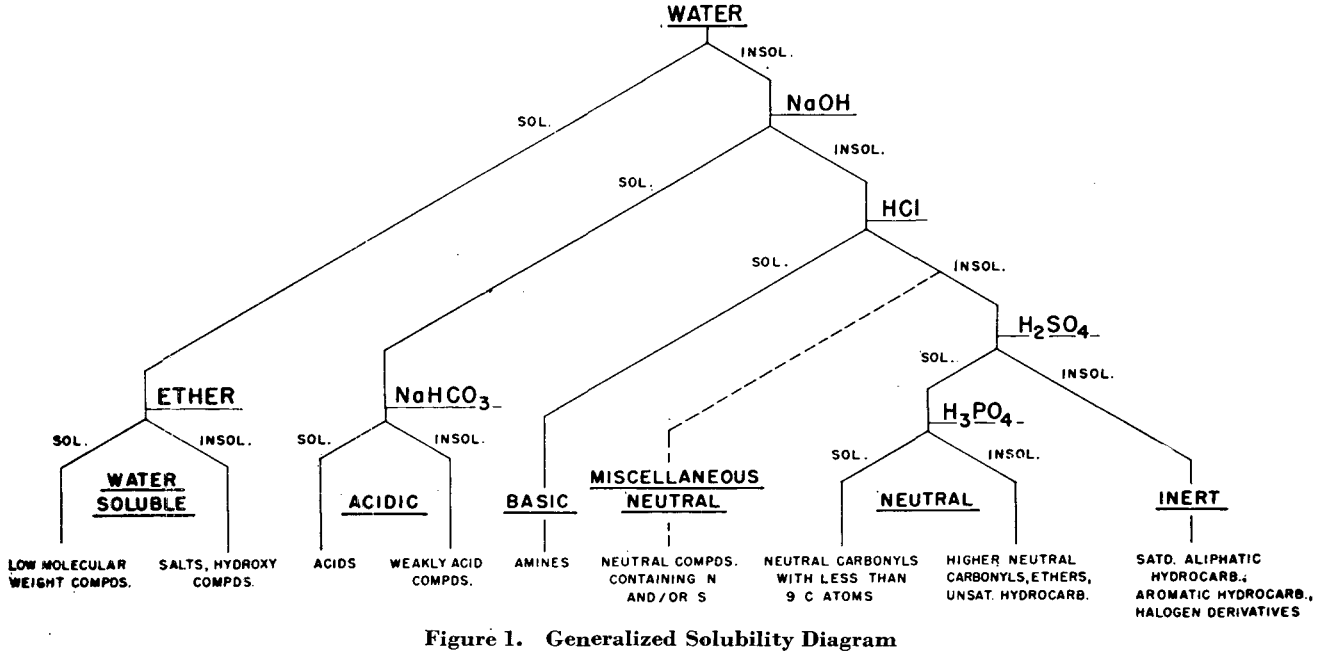

Figure 1. Generalized Solubility Diagram

A modification of more specific diagram by Shriner and Fuson (*28*)

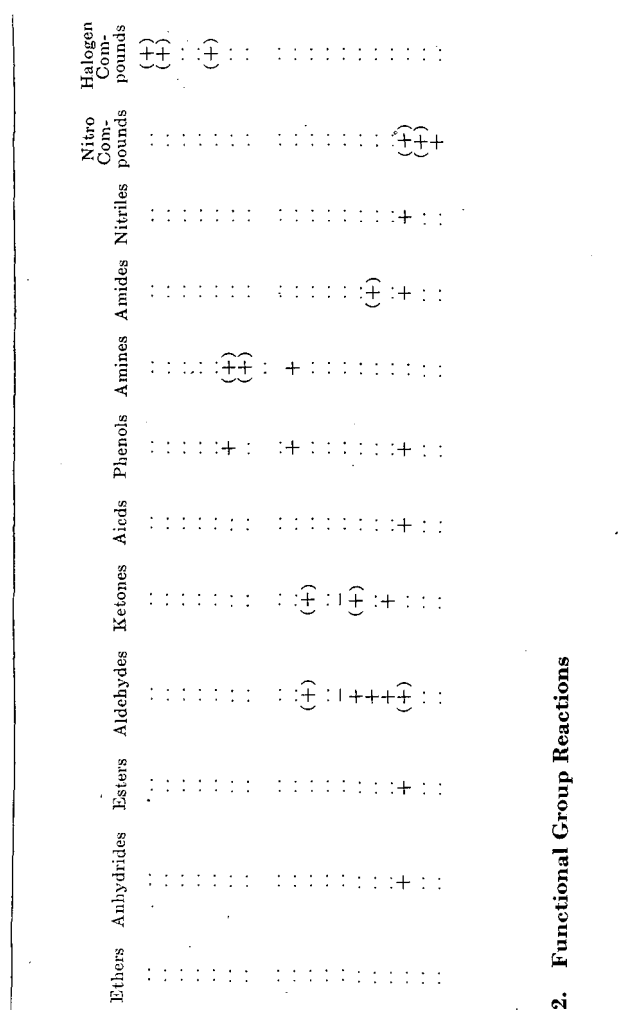

Figure 2. Functional Group Reactions

QUALITATIVE ANALYSIS BY CONVENTIONAL METHODS

Although the techniques of qualitative inorganic analysis have been so thoroughly investigated and developed that the identification of an inorganic unknown has become a relatively simple and routine task, organic analysis presents a somewhat more complicated problem. Organic compounds are considerably more numerous (probably numbering at present between 450,000 and 500,000), they exhibit isomerism, and lend themselves to a wide variety of substitution reactions. Unfortunately, in a large number of cases the proportion of reactive or functional groups to the molecule as a whole is relatively small. Lack of specificity of reagents for identifying molecular structures by means of individual functional groups is, therefore, to be expected. As a result of this overlapping effect, a broad knowledge of organic chemistry is required to interpret the results obtained.

To emphasize the nature of this problem the following brief outline of a typical organic analytical approach is given. The various steps in the examination of an organic unknown, either as received or after preliminary separation, are discussed in detail in such standard texts as Kamm (*19*), Shriner and Fuson (*28*), McElvain (*22*), and Meyer (*23*).

Step 1. Physical Examination. The physical state, the relative degree of hardness, and the homogeneity, color, and odor of the sample are noted. These observations, accompanied by microscopical examination, permit a considerable narrowing of the possibilities, and give some idea of the complexity of the problem. They indicate whether or not separation is necessary and often suggest to the experienced analyst the best means by which this may be carried out. The sample's behavior during ignition and destructive distillation is also observed; degradation products are collected for further examination. Ignition behavior may indicate whether the material is aliphatic or aromatic (the latter type usually burns with a smoky flame) and whether or not any salts are present (as evidenced by an ash). Destructive distillation is valuable in attacking insoluble, infusible products.

Step 2. Physical Constants. The melting and boiling ranges, and also the refractive index and associated properties, the specific gravity, optical rotation, molecular weight, etc., are determined and interpreted. By these procedures a considerable amount of data is collected for use as confirmatory evidence once the general structure of the sample is determined. The results can be used to guide the course of the investigation. In many instances, the optical properties become the primary basis of identification. Weissberger (*31*) has discussed the physical methods of organic chemistry in considerable detail.

Step 3. Elementary Analysis. The sample is analyzed for elements other than carbon, hydrogen, and oxygen. By fusion with metallic sodium, elements such as sulfur, nitrogen, the halogens, and phosphorus may be detected, and their presence taken into consideration. Where such elements are present, the number of possibilities can be reduced sharply. In certain cases ultimate analysis may be desirable to furnish an empirical formula.

Step 4. Solubility. The unknown is subjected to solubility tests by selected solvents, and is then classified in one or more of nine broad solubility groups. Although considerable overlapping may occur, solubility data are very valuable in preliminary classification. Figure 1 represents a simplified diagram included to illustrate some deductions that may be made from solubility data. The simplification consists of the brief generalizations of the types of compounds occurring in the various groups.

Step 5. Functional Groups. The sample is tested for functional groups by the application of appropriate reagents, and classification is still further narrowed. Although not completely specific, the results of these tests may be coordinated to yield a fairly complete structural picture. Some of the more common groups and tests are listed in Figure 2. The tests are intended for the exploration of possibilities suggested by the solubility results. The treatment is necessarily general and omits qualifying remarks. For further details, a standard text, such as Shriner and Fuson (*28*), should be consulted.

Step 6. Correlation. The co-consideration of all the data resulting from the above procedures usually permits the identification of the unknown as a particular member of a certain homologous series. Verification is first sought in the literature [Richter, Beilstein, Huntress and Mulliken (*18*), Meyer (*23*)] and final proof is arrived at through the preparation of one or more derivatives. Hopkin and Williams (*17*) have published a volume confined to reagents for characterization and derivative formation.

It is apparent that, in most instances, the identification of an organic unknown requires a considerable amount of time and ingenuity together with a sizable sample. This is particularly true in the case of mixtures of organic components of technical purity, which is the usual form in which organic compounds are encountered by the analyst. A considerable proportion of this long procedure may often be eliminated by the proper interpretation of an infrared spectrum of a single small aliquot of the unknown.

QUALITATIVE ANALYSIS BY INFRARED METHODS

The basis of infrared qualitative analysis is somewhat simpler than that of chemical analysis, although not nearly so complete at the present time. It has been observed from vibrational analyses of simple molecules and from comparison of the spectra of large numbers of compounds containing the same atomic grouping that certain characteristic or functional groups show absorption bands in definite regions of the infrared spectrum—i.e., the atoms of the group have characteristic mechanical vibration frequencies that are, to a large extent, independent of the rest of the molecule. With a knowledge of these characteristic absorption frequencies it is possible, by inspection of the spectrum of an unknown, to determine with varying degrees of certainty those functional groups that are present and those that are not present. This combined information will often permit an excellent deduction or estimation as to the type and structure of the unknown. Confirmation can be obtained from direct matching of the unknown spectrum with that of a known compound or from further combined chemical tests and infrared study of derivatives, model compounds, etc.

In practice this spectral analysis for functional groups can be made by preparing a transparent master sheet having a frequency calibration along which intervals are marked with the associated atomic groups. The unknown spectrum can be placed over this master sheet on a reading box, the frequency of the observed bands recorded, and the functional groups present or absent designated on the spectrum or on a separate report form. A chart showing these correlations is given in Figure 3, *A* and *B*, where the region of absorption is designated by a horizontal line to the left of the group for which it seems to be characteristic.

The correlations of this chart are presented with considerable reservation. They represent the work of many people, together with results obtained in these laboratories. The assigned regions are necessarily based on observation of a limited number of compounds and such a simple chart does not permit showing qualifications or exceptions. In general, the region shown is slightly wider than that required for the normal occurrence of the groups, but it is felt that the extension of any region to include all atypical cases would weaken the value of the chart by implying that excessive overlapping occurs. The absorptions shown are primarily fundamentals. Although overtone and combination absorption bands may occur anywhere in this spectral range, they are usually weak enough to prevent confusion. There is the further point that, in order to be infrared-active, a vibration must involve a change of dipole moment of the molecule. There are cases—e.g., a fairly symmetrically substituted C=C—where the absorption of a group is weak or nonexistent, resulting in misinterpretation. Such difficulties arise mainly from the fact that the vibration of an atomic group and its appearance in the infrared spectrum are not entirely independent of the rest of the molecule. Although this fact will necessarily be confusing and lead to misinterpretation in early applications of the method, it is to be hoped that ultimately such exceptions or abnormal frequency shifts can be sufficiently understood to give even more information about the group involved—e.g., if vibrations of the attached hydrogens show a C=C to be present yet it is not observed in the double bond region, one can infer that it is symmetrically substituted in a chain or ring.

The chart has been split purposely into two sections to emphasize that there is a sharp difference in the certainty of the assignments higher than 1350 cm.$^{-1}$ and those below this point. The frequency of the vibrations depends primarily on the masses of the atoms involved and the bond force constants connecting them—either radial stretching (ν) or angle bending (δ). For the higher energy vibrations of Figure 3, *A*, the assignments are fairly

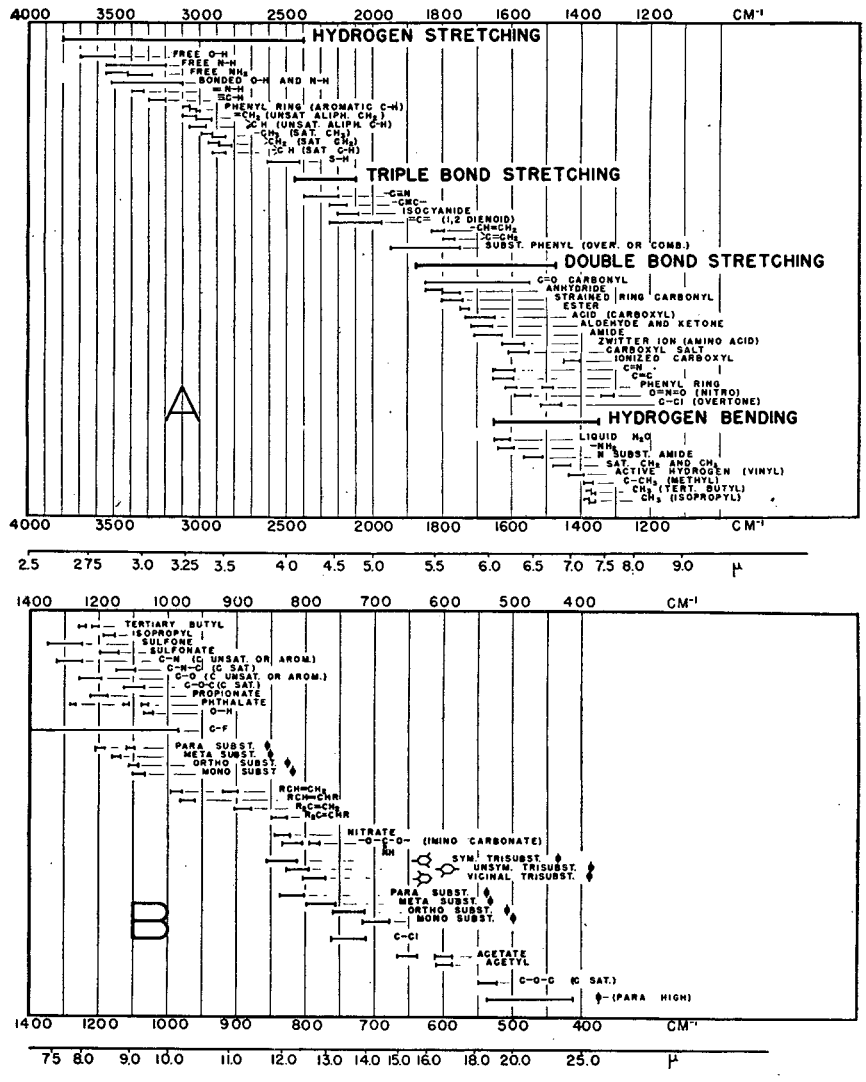

Figure 3. Functional Group Absorption Frequencies

Correlations tentative and based upon best information available to authors at this time

definite because the vibrations resulting from the combined force constants and atomic masses are well separated and there is little likelihood of interactions causing frequency shifts from expected positions. In the lower regions this is not true, for combinations of force constants and atomic masses—i.e., δ (C—H), ν(C—C), ν(C—O), ν(C—N), etc.—can give rise to frequencies in the same region or result in interactions causing unpredictable frequency shifts. (Although this situation is unfortunate from the point of view of qualitative analysis, it is this same fact that often enables identification or quantitative analysis of very similar molecules.) Therefore, the correlations of Figure 3, *A*, are primary in designating the presence or absence of a group; those of Figure 3, *B*, are secondary or confirmatory. The presence of a band below 1375 cm.$^{-1}$ does not necessarily imply the presence of the group shown on the chart, but the absence of a band in a specified region is fairly indicative that the corresponding group is absent. For example, the presence of a strong band near 775 cm.$^{-1}$ does not, by itself, imply the presence of a meta-substituted aromatic ring. The sharp CH triplet from 3060 to 3100 cm.$^{-1}$, the 1600 cm.$^{-1}$ and 1500 cm.$^{-1}$ bands imply the aromatic ring; the presence of the band at 775 cm.$^{-1}$ permits a guess that it is meta-substituted; the absence of a 775 cm.$^{-1}$ band implies strongly that the ring is not meta-substituted.

Figure 3, *A* and *B*, gives only the absorption regions for the various groups. Additional correlational information can be derived from the appearance of the bands—their absorption intensity and breadth—as well as from minor shifts within the region shown. The following section gives a short summary of these additional characteristics of the group absorptions.

These remarks are very general and subject to the same conditions and reservations discussed in connection with the band positions. Moreover, a further qualification must be introduced in connection with a discussion of relative band intensities. In general, the width of infrared absorption bands of liquids and solids is of the same order of magnitude (5 to 15 cm.$^{-1}$) as the spectral resolution of present-day infrared prism spectrometers, so that variations in instrument resolution strongly affect the apparent intensity of absorption bands. This fact, together with the difficulty of measuring sample thickness accurately, has greatly retarded the ideal situation of describing intensities in terms of extinction coefficients.

In obtaining an absorption spectrum the general practice is to regulate the thickness of a sample so that its strongest bands show an absorption in the range 85 to 95%. With this criterion, it is observed that relatively nonpolar materials such as saturated hydrocarbons are usually studied at a thickness of 0.1 mm., while more polar materials such as oxygenated compounds appear best at about 0.01 mm., or thinner. In the subsequent sections the bands are described as weak (5 to 20% absorption), medium (20 to 50% absorption), or strong (50 to 100% absorption) relative to the spectrum in which they appear rather than on any absolute basis. For this reason some of the alkyl correlations, which seem to be fairly consistent for hydrocarbons, may be masked by stronger bands in oxygenated compounds. In spite of this extremely qualitative method of denoting intensities, it is felt that this additional information is of considerable value in the determination of functional groups from spectral correlations.

(O—H) Stretching Frequency. Considerable care must be exercised in the assignment of a hydroxyl band because the strong tendency of this group to "associate" or "hydrogen bond" causes considerable variation in absorption frequency and band width. If a hydroxyl band is suspected, it is best to obtain the spectrum of the material pure and in dilute solution in a nonpolar solvent where the group is essentially free unless the hydrogen bond is intramolecular. A free hydroxyl usually exhibits a sharp band of varying intensity, whereas an associated group shows a broad strong absorption at a lower frequency. The amount of the frequency shift is a function of the strength of the "hydrogen bond."

(N—H) Frequencies. The amine linkage has much less tendency to associate than the hydroxyl group, so that the shift from concentrated to dilute solution is less noticeable. The absorption of an N—H group is usually a sharp medium-intensity band. In the case of primary amines, two stretching vibrations and one bending vibration are usually observed.

(C—H) Stretching Frequency. The intensity of a C—H absorption may vary widely. However, inasmuch as most organic molecules contain many such groups, their summed effect appears as a strong broad band, particularly when studied under low resolution. The regions given in Figure 3, *A*, are based on results primarily obtained by a study of hydrocarbons. The presence of atoms other than carbon or hydrogen adjacent to the carbon atom involved in the C—H linkage in question will shift the above frequencies—for example, the presence of a halogen, particularly fluorine, will shift a C—H frequency to higher values than normal. Again, the stretching CH_3 frequencies shift to progressively lower values in the series N—CH_3, C—CH_3, O—CH_3.

(S—H) Stretching Frequency. This absorption is weak to medium in intensity.

(X≡Y) Stretching Frequencies. The intensity of the triple bond frequencies is medium to strong. They occur in a region which is clear of other fundamental vibrations except that of an *X*-deuterium group. (The expected frequency of a deuterium stretching vibration is about 0.7 times that of the corresponding hydrogen linkage.)

(X=C=Y) Stretching Frequencies. As a result of interaction in this group, the two stretching vibrations are more nearly characteristic of a triple bond and a single bond than of two double bond frequencies. Where *X* and *Y* are either C, N, or O atoms the high-frequency band is usually very strong in intensity and the lower, of somewhat less intensity, occurs from 1000 to 1200 cm^{-1}. Typical structures and their high-frequency bands are as follows: O=C=N, 2270 cm.$^{-1}$; C=C=O and N=C=N, 2150 cm.$^{-1}$; S=C=N and S=C=O, 2075 cm.$^{-1}$; and C=C=C, 1970 cm^{-1}.

$\left(\begin{matrix}H\\H\end{matrix}\right.$C=C$\left.\right)$ **Frequencies.** These bands occur weakly in hydrocarbons near 1800 cm.$^{-1}$ but are greatly enhanced in intensity if the group is conjugated. They are often absent if the molecule contains oxygen or nitrogen, even if such atoms are considerably separated from the terminal unsaturation. These bands may be overtones of the corresponding group of bending (C-H) bands at 910 and 890 cm.$^{-1}$, respectively, although they are often greater than twice the fundamental frequency.

(C=C), (C=O), and (C=N) Stretching Frequencies. The ranges shown for these groups are somewhat wide to allow for the influence of adjoining portions of the molecule—for example, a normal aliphatic ketone or a six-membered ring ketone occurs at 1710 to 1720 cm^{-1}. Strain in the ring moves the band to higher frequencies—1740 cm.$^{-1}$ in cyclopentanone and higher in lactam structures. On the other hand, conjugation will lower the band 10 to 40 cm^{-1}. Similarly, a normal aliphatic C=C occurs at 1640 to 1650 cm.$^{-1}$, conjugation with an aromatic ring lowers the frequency to about 1625 cm.$^{-1}$, and full aliphatic conjugation to near 1600 cm^{-1}. The carbonyl is almost always a very strong band but the strength of the C=C band varies with the symmetry and polarity of the substitution.

Phenyl Frequencies. The aromatic ring bands near 1600 cm.$^{-1}$ and 1500 cm.$^{-1}$ are medium to strongly absorbing, depending upon the character of the substitution groups. In the case of disubstituted isomers, the para band near 1500 cm.$^{-1}$ is shifted to a higher frequency than the corresponding ortho or meta bands. The aromatic ring often shows appreciable overtone or combination bands in the region from 1700 cm.$^{-1}$ to 2000 cm.$^{-1}$.

(NO_2) Frequencies. These are usually strong bands.

(C—H) Bending Frequencies. The concentration of bands at 1450 cm.$^{-1}$ from several linkages causes a strong band. Aromatic molecules often show absorption from 1460 to 1475 cm.$^{-1}$.

An active C—H group is medium to strong. The C—CH_3 near 1380 cm.$^{-1}$ is a sharp band of variable intensity. An isopropyl or *tert*-butyl group splits this frequency into a doublet with 15 to 20 cm.$^{-1}$ separation. In *tert*-butyl the higher frequency band is the weaker of the two, whereas for an isopropyl they are equally strong.

***tert*-Butyl and Isopropyl Frequencies.** Both these assignments hold fairly well for hydrocarbons. The 1250 cm.$^{-1}$ and 1200 cm.$^{-1}$ bands of the *tert*-butyl are medium in intensity with the 1250 cm.$^{-1}$ somewhat the stronger of the two. The 1160 to 1190 band of the isopropyl group is weak to medium in intensity. Both correlations are uncertain for molecules containing oxygen or nitrogen, although this may be partially caused by the fact that the resultant strong bands would probably obscure the weaker alkyl ones.

(C—O) and (C—N) Frequencies. The 1290 C—N and the 1200 to 1250 C—O are thought to be stretching vibrations whose frequency is enhanced over that of the normal single bond stretching motions (1125 cm.$^{-1}$ and 1100 cm.$^{-1}$, respectively) by the unsaturation of the carbon. All the bands are strong and the 1250 cm.$^{-1}$ C—O band is often broad. If the carbonyl of the ester is conjugated with a C=C as in the acrylates, the 1250 cm.$^{-1}$ band disappears and bands occur near 1300 cm.$^{-1}$ and 1200 cm^{-1}. For the lower esters this C—O has been assigned (*29*) as follows: formates, 1185 cm.$^{-1}$; acetates, 1245 cm.$^{-1}$; propionates, 1190 cm.$^{-1}$; butyrates, 1190 cm.$^{-1}$; and isobutyrates, 1200 cm^{-1}.

Bending Vibrations of Hydrogens of an Ethylenic Linkage. The correlations for the various bending vibrations of hydrogen atoms attached to a C=C linkage are consistent even in conjugated systems or molecules containing elements other than C or H. These bands are medium to strong in intensity.

Substituted Phenyl Frequencies. The strongest and most characteristic of the aromatic bands are the series 835 cm.$^{-1}$, 775 cm.$^{-1}$, 745 cm.$^{-1}$, and 700 cm.$^{-1}$ for para-, meta-, ortho-, and mono-substituted phenyls, respectively. The trisubstitution bands shown are also strong. The aromatic bands from 1050 to 1200 cm.$^{-1}$ are of medium intensity and are, therefore, not so useful when the presence of oxygen or nitrogen causes stronger masking bands in the same region. The bands near 500 cm.$^{-1}$ are weak and not particularly useful. As noted on the chart, there is very considerable overlapping in the 500 cm.$^{-1}$ region—the only predominant characteristic evident is that in an isomeric series the para band has the highest frequency.

Miscellaneous Groups. There is not sufficient information available on the absorption of the C—F group to permit any detailed assignment. The task is the more difficult because of the strong interactions between a fluorine atom and the neighboring atoms. A single C—F link usually shows a strong stretching band near 1000 cm.$^{-1}$ but additional fluorines on the same carbon give considerably higher frequencies—i.e., for CF_2 at least one strong band appears near 1200 cm.$^{-1}$, for a few C—CF_3 molecules a band is observed as high as 1325 cm.$^{-1}$, and for a CF_2 in a four-membered ring, a band appears near 1400 cm.$^{-1}$. There is some evidence that a =CF_2 group gives a band at 1340 cm.$^{-1}$ and 1200 cm.$^{-1}$. For a highly fluorinated hydrocarbon intense absorption is observed in the region 1200 to 1325 cm.$^{-1}$.

The C—Cl stretching bands are also strong but show greater constancy and less interaction. A single linkage usually occurs from 725 to 750 cm.$^{-1}$ and the presence of more chlorine atoms on the same carbon may give an absorption in a higher range 770 to 790 cm.$^{-1}$.

There is evidence that a P=S band absorbs strongly near 750 cm.$^{-1}$. However, considerable data are required to substantiate this assignment.

C—S and C—S—C stretching vibrations appear (*30*) to fall in the region 600 to 750 cm.$^{-1}$ although they vary considerably in both position and intensity. There is little definite information on the C=S group, although it should occur near 1400 cm.$^{-1}$.

Si—O and Si—C stretching frequencies (*33*) fall in the ranges *1220* to *1020* cm.$^{-1}$ and 860 to 700 cm.$^{-1}$, respectively. They are strong bands but, like C—C and C—O, these linkages evidently interact sufficiently with adjacent atoms so that a unique assignment to a single bond is dangerous except in the simpler molecules. The methyl vibrations of Si—CH_3 are essentially unchanged from the characteristic C—CH_3 frequencies. There is a strong band at 1260 cm.$^{-1}$ which has been assigned to the methyl group rocking motion in an Si—CH_3 group.

The bands characteristic of sulfones, sulfonates, carbonates, acetates, acetyls, phthalates, etc., are all strong bands.

To summarize, Figure 3, *A* and *B*, and the descriptive section represent an initial step to group these correlations for general qualitative use. They are subject to corrections, refinements, and additions. The authors have, for example, in these laboratories undertaken a rather wide study of the characteristic infrared absorptions associated with various functional groups involving the carbonyl linkage and intend to publish this material as a refinement of the rough assignments shown. Undoubtedly, infrared workers in the petroleum field have made special structural studies of hydrocarbons involving chain branching, position of unsaturation, etc. As the literature expands these necessary changes and refinements can be made and the usefulness of infrared spectrometry to organic chemistry can be enlarged.

COMPLEMENTARY USE OF THE TWO ANALYTICAL METHODS

In considering the complementary use of the above methods, the question naturally arises as to the point in the procedure at which an infrared examination should best be made. On the basis of what has been described, it seems obvious that infrared spectra should be obtained as soon as the analyst feels that he is dealing with a single component or with a mixture representative of the problem. The usefulness of this infrared information may range all the way from the furnishing of a complete and independent solution of certain problems to the discovery in other cases of a tenuous clue which may influence all further work. As pointed out above, a lucky "hit" identification, from infrared interpretation and comparison alone, may preclude the necessity of performing any additional analyses. Failing this, the infrared spectrum should be included as an integral part of Step 1 (physical examination) of the conventional procedure, for it will serve in a general way as a primary guide in the future course of the analysis. For example, there is no point in carrying out a saponification reaction on a material which shows no carbonyl absorption in the infrared. More specifically, infrared studies may be used to obviate some of the elementary analysis of Step 3, to eliminate or narrow very sharply the solubility tests indicated under Step 4 and the functional group tests of Step 5, and to provide a much

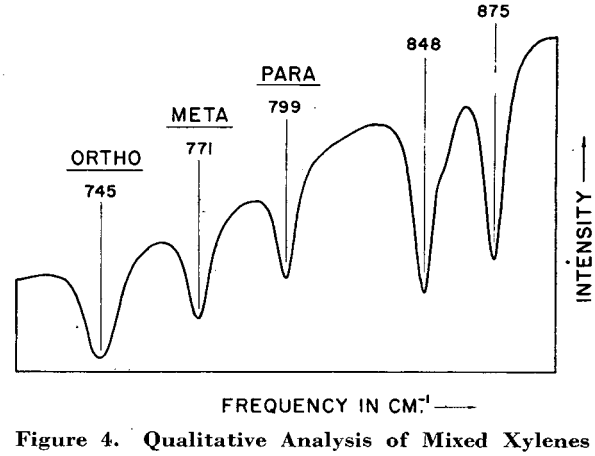

Figure 4. Qualitative Analysis of Mixed Xylenes

Cell thickness 0.1 mm.
Sample diluted 1 to 10 by volume with methyl cyclohexane

simpler confirmatory test (spectral matching with a known) than the preparation of derivatives of Step 6.

In order to illustrate certain of the above-described possibilities, a few illustrative examples of typical qualitative analyses are given.

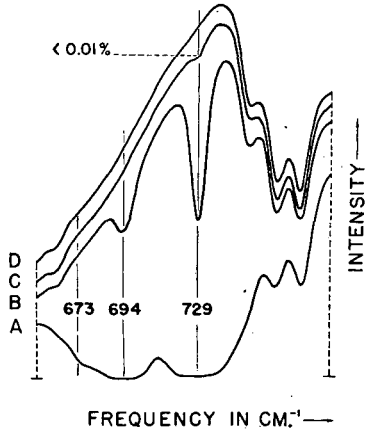

Figure 5. Spectra Illustrating Successive Steps of Purification of Methyl Cyclohexane

Cell thickness 3.0 mm.

Example I. Frequently, the analyst encounters a sample that is known to be, or is strongly suspected of being, one of several definite possibilities. The identity of the sample is required. Such a case presents to the spectroscopist one of the simplest analytical problems, one which he can usually solve without any great difficulty.

A small specimen of rubber hydrocarbon was received together with a statement that it was supposed to be natural, GR-S, or Buna-N. A sample was prepared for infrared examination and its spectrum was compared with the spectra of those of the three suspected types of rubber. This comparison, without the aid of any steps of the conventional analytical procedure, clearly identified the unknown as a sample of natural rubber.

This conclusion was arrived at by spectral matching, with particular reference to the absorption bands of natural rubber that occur at 1375 cm.$^{-1}$ and 820 to 840 cm.$^{-1}$. These bands do not occur in the infrared spectrum of other types of rubber. The absorption at 1720 cm.$^{-1}$ implies the presence of oxidation products; strongly suggesting that the sample was reclaimed rubber.

Example II. A second example of a simple qualitative analysis is the determination of the components present in an unknown mixture.

Figure 4 shows a portion of the spectrum of a sample suspected to contain xylenes. The presence of the *o*-, *m*-, and *p*-xylene bands marked on the figure showed that all three isomers were present. Moreover, comparison of the intensity of the bands with those of pure samples permitted the estimation that the proportions present were ortho, 55 to 65%; meta, 20 to 30%; and para, 10 to 20%. The infrared technique was much more rapid than a distillation analysis and the complete spectrum provided evidence that no unexpected impurities were present.

Example III. The organic research chemist frequently obtains a purified reaction product, which according to a preliminary characterization may be either one of two isomeric forms. With the exception of spatial isomers, these reaction products are characterized by the presence or absence of certain specific reactive groups. A single infrared absorption spectrum of such an unknown frequently furnishes information of the type that might be obtained from Steps 5 and 6 of the conventional method of analysis.

A crystalline material thought to be either a substituted hydroxy ethyl cyanamide or an imino oxazolidine was submitted for analysis. This sample was identified (see Figure 3, *A*) as being in the latter form by the presence of a strong sharp absorption band around 3330 cm.$^{-1}$ (N—H) and 1660 cm.$^{-1}$ (C=N), and the absence of bands around 2300 cm.$^{-1}$ (C≡N) and 3400 cm.$^{-1}$ (associated O—H).

Example IV. One of the most profitable applications of the infrared method is its use as a yardstick for following a purification process. Here, even if the impurity is never identified, its progressive disappearance can be followed and the purity of the final product confirmed.

The purification by fractional distillation of a sample of methylcyclohexane was followed. In the curves of Figure 5 the disappearance of the band at 730 cm.$^{-1}$ and 693 cm.$^{-1}$ with successive purification may be seen. Although not positively identified as such, these bands were thought to indicate the presence of toluene. If this is the impurity, the absorption at 730 cm.$^{-1}$ in curve *D* represents 0.01%. Here infrared seemed preferable to ultraviolet because it would detect saturated as well as unsaturated impurities.

Example V. In this example the infrared spectrum of the unknown served to complete the analytical data furnished by the microscope and to render further analysis unnecessary.

The unknown was submitted as a chlorinated, crystalline, waxy solid. Information on its melting point and approximate chlorine content was supplied with the sample. A microscopical portion was recrystallized from the melt under a cover glass and examined under the "petrographic" microscope. The crystalline habit and available optical properties indicated that chlorinated naphthalenes were possibilities, with the trichloro substitution the most probable. For final proof of identity the "unknown" and known trichloronaphthalene samples were submitted for comparison of their infrared absorption spectra. A comparison of the *A* and *B* curves of Figure 6 shows the two materials to be the same.

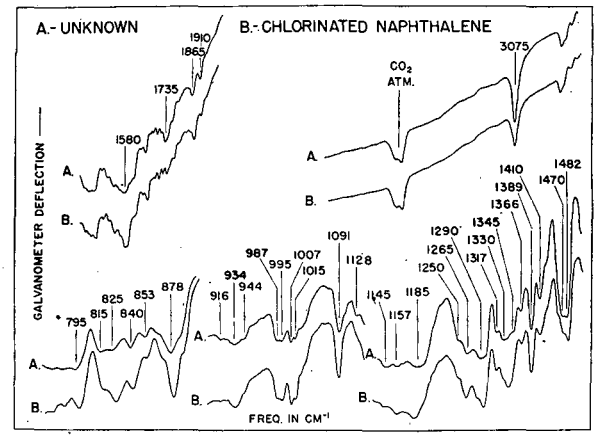

Figure 6. Identification of a Chlorinated Crystalline Waxy Solid

Samples run as melts

Example VI. Another important qualitative application of infrared is its use in connection with mixed melting point studies. Although a depression of the melting point of a mixture may usually be taken to indicate a difference in the two components of the mixture, the converse is sometimes not true.

An unknown organic acid, suspected of being either 5-chlorothiophene carboxylic acid or 5-bromothiophene carboxylic acid, showed no mixed melting point depression with either the chloro or bromo acids. In view of the uncertainty caused by this situation, the sample was submitted for infrared analysis. Its spectrum was recorded (curve *B* of Figure 7) and compared with those of the chloro and bromo compounds (curves *A* and C). A study of the spectra readily indicates that the unknown is the 5-bromothiophene-2-carboxylic acid.

Example VII. This example presents a case where infrared proved useful for both the early detection of certain reactive groups and the final identification of the product.

The unknown was a liquid resin stabilizer, found to be free of water and volatile solvents during preliminary examination. As experience suggested that such stabilizers usually include fatty acid soaps, and as solvent-free liquid soaps are rare, the presence of a high boiling solvent oil was suspected. Infrared analysis indicated that the sample included a carboxy salt, and strong —COOR, —C=C—, and —OH absorption; this confirmed the soap hypothesis and indicated that the solvent was a vegetable oil. After chemical separation and identification of the soap infrared analysis identified the unsaponified high boiling solvent fraction as castor oil by comparison with a known spectrum.

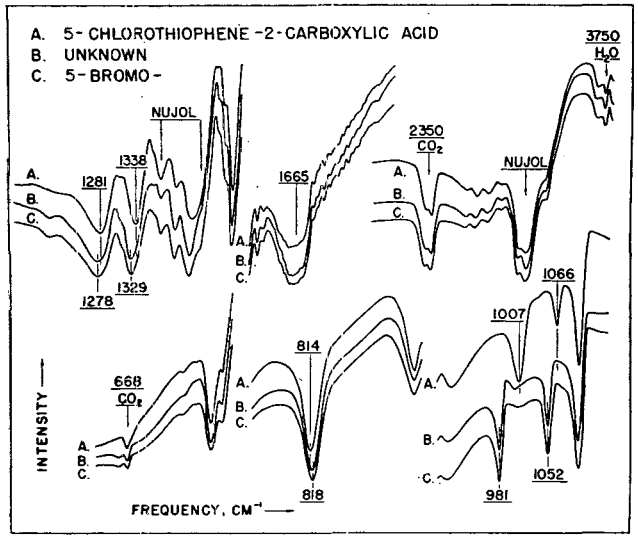

Figure 7. Identification of an Unknown Carboxylic Acid

Samples run as Nujol mulls

Example VIII. A further application of the infrared method is shown by this example, wherein a solvent mixture isolated by distillation from a complex pigment-resin-solvent formulation was submitted for identification of its components. The two fractions, designated, respectively, as high boiling and 115° to 118° C., were submitted for infrared analysis.

In the case of the high boiling fraction bands at the listed frequencies indicated the presence of the following radicals: 3050 cm.$^{-1}$, unsaturation or phenyl C—H; 2840–3000 cm.$^{-1}$, saturated methylene and methyl; 1640 cm.$^{-1}$, X=Y such as C=C; 1466, 1455, 1440 cm.$^{-1}$, three types of methylene; 1379–1367 doublet, isopropyl; 963 cm.$^{-1}$ and 876 cm.$^{-1}$, possibly substituted vinyl groups.

The unsaturated hydrocarbon character of the molecule along with the presence of the isopropyl and substituted vinyl groups implied a terpene of the pinene group. A comparison of the spectra of this high boiling fraction and those of α- and β-pinene indicated that the unknown was mainly α-pinene with some β-pinene present.

The other fraction boiling between 115° and 118° C. was next examined in a similar manner. The spectrum showed only those absorption bands associated with saturated hydrocarbon liquids. This fact, in view of the boiling point, suggested that this fraction might be a petroleum cut. Accordingly, its spectrum was compared with that of a commercial material. The similarity of the spectra confirmed this assumption.

Example IX. This unknown was submitted as a fragment of a molded object which preliminary examination and elementary analysis indicated to be a highly vulcanized rubber.

The per cent sulfur was determined quantitatively. Thermal degradation yielded a dark oil, which was subjected to infrared analysis after clarification with carbon black. Examination of its spectrum (Figure 8, *A*) reveals the following absorption bands associated with the listed radicals: 3090 to 3020 cm.$^{-1}$, phenyl; 2925, 2850, methylene and methyl; 1944, 1870, 1600, 1496, phenyl (not para-substituted); 1710, carbonyl (slight oxidation); 1460, methylene; 1375, methyl; and 793 cm.$^{-1}$, phenyl. The strong aromatic absorption suggested a styrene-butadiene copolymer such as is found in GR-S. Accordingly, a sample of GR-S rubber, vulcanized to the same degree as the unknown, was destructively distilled and a liquid was obtained which yielded the spectrum obtained in curve B. With over twenty-five coincidences in frequency and intensity in the two spectra, the probability of their identity is again very high.

In this instance, chemical work was limited to preliminary examination, elementary analysis, and the preparation of the distillates; a complete analysis of the decomposition products by conventional methods alone would have been a very laborious procedure.

Example X. The unknown in this example was submitted as a water-white liquid monomer.

Infrared analysis was run concurrent with physical examination, determination of physical constants, and elementary analysis. The following observations were made from the spectrum shown in Figure 9, *A:* Absorption at 3080 cm.$^{-1}$ indicated unsaturation or a phenyl radical; 2990, 2900 cm.$^{-1}$ absorption bands indicated methylene and methyl radicals; the broad strong absorption near 1750 cm.$^{-1}$ could be caused by an unconjugated ester and/or a carbonate carbonyl; the 1645 cm.$^{-1}$ band is related to the C=C stretching vibration; the 1450, 1416, and 1375 cm.$^{-1}$ bands indicated methylene, active C—H, and a small proportion of C—CH_3 radicals; 1270 cm.$^{-1}$ absorption confirmed the ester type of carbonyl; and the 971 cm.$^{-1}$ band also confirmed the presence of an internal C=C linkage. The chemical establishment of the double bond position as internal or external would be difficult and time-consuming. However, the infrared evidence shows fairly definitely that it is internal.

A comparison of the spectrum with that of diallyl carbonate revealed significant differences, caused perhaps by additional ester carbonyl and C—O—C— groupings. This led to the consideration of a structure similar to that of a bis (allyl lactate) ester of carbonic acid. This molecule was subsequently synthesized and its spectrum is shown with that of the unknown in Figure 9, *B*. The complete coincidence of over twenty-five absorption frequencies leaves little doubt as to the nature of the unknown.

Example XI. For purposes of final illustration, the analysis of a fragment of clear transparent plastic is discussed in detail.

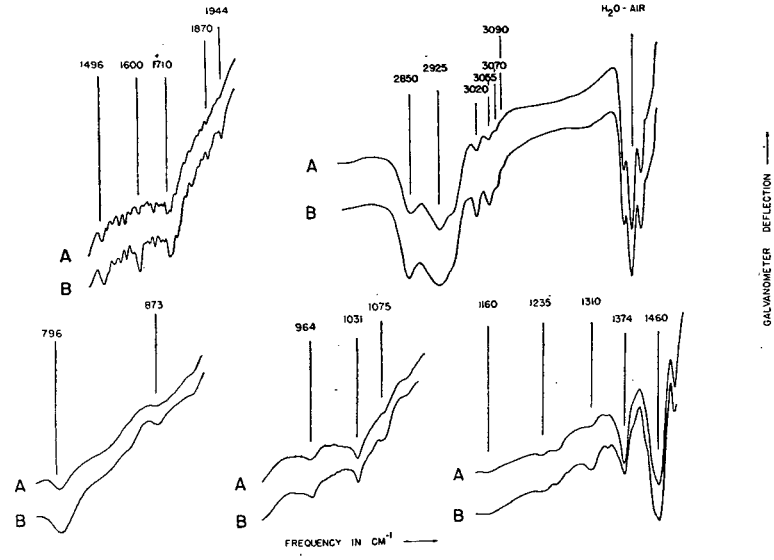

Figure 8. Identification of Destructive Distillation Product of an Unknown Rubber

Samples run as smears
***A.* Distillation product of unknown**
***B.* Distillation product of known**

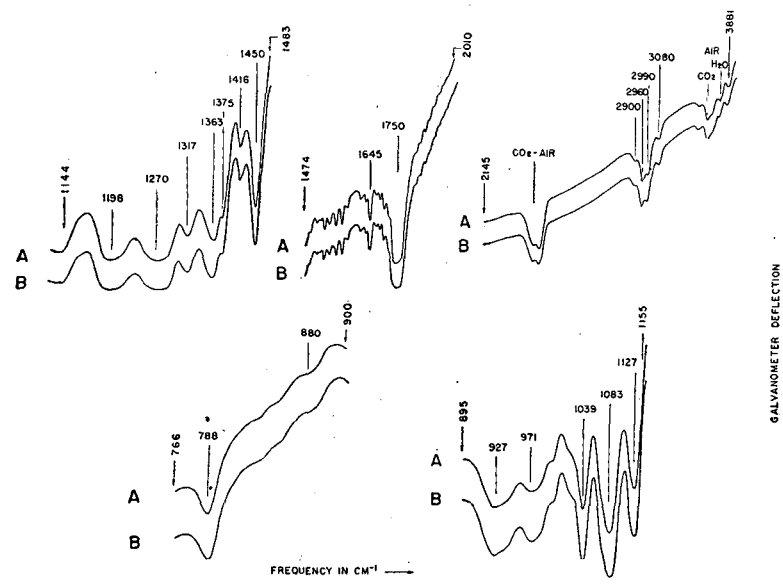

Figure 9. Identification of an Unknown Liquid

Cell thickness 0.025 mm.
***A.* Sample monomer**
***B.* Known monomer**

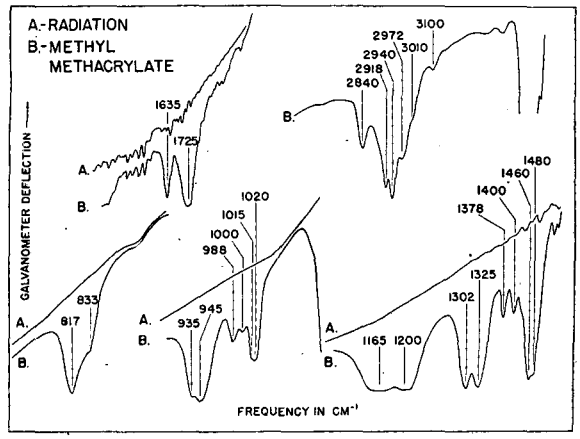

Figure 10. Identification of a Fragment of Clear Plastic

Sample run as a monomer, derived from polymer by distillation. Cell thickness 0.01 mm.

The behavior during physical examination suggested a vinyl-type polymer. Thermal degradation resulted in the separation of a yellow, water-immiscible oil, which was purified by distillation to obtain a sample representative of the plastic itself, and suitable for infrared analysis. The spectrum of the thermal degradation product, the unknown, is shown in Figure 10 and the results of a qualitative analysis for functional groups by comparison with the charts of Figure 3, *A* and *B*, are given below.

Groups Probably Absent. O—H, N—H, ≡C—H, S—H, triple bonds, phenyl, NO_2, gem methyl, C—O—C (C satd.), C—Cl, etc.

Groups Probably Present. The C—H bands between 3000 cm.$^{-1}$ and 3100 cm.$^{-1}$, and at 1400 cm.$^{-1}$ as well as the band at 1637 cm.$^{-1}$ imply the presence of a C=C with the possibility of more than one attached hydrogen. None of the typical unsaturated C—H bending bands assigned in the region 840–1090 cm.$^{-1}$ appear, so the unsaturation is probably not an unconjugated, alkyl-substituted group. The small number of distinct C—H bands between 2800 and 3000 cm.$^{-1}$ implies that the amount of saturated C—H in the molecule is relatively low. The 1375 cm.$^{-1}$ band shows that a C—CH_3 group is present. The carbonyl at 1725 cm.$^{-1}$ could be a low frequency ester, an acid, or a high ketone or aldehyde. The acid is ruled out by the absence of the OH group. As an ester, the low frequency value could be caused by conjugation or as a ketone by its presence in a strained ring. There is no broad strong band near 1250 cm.$^{-1}$ as usually occurs with an unconjugated ester, but there are strong bands at 1300 and 1200 cm.$^{-1}$ which may be caused by an ester carbonyl conjugated with a C=C linkage.

Infrared Summary. The over-all appearance of the spectrum, relatively few broad bands, the lack of characteristic functional groups, and the small number of C—H linkages point to a compound of low molecular weight. The presence of a C=C and a C=O band with their anomalies in the confirmation (Figure 3, *B*), region imply the possibility of an ester with the C=C group closely adjacent or conjugated. With this evidence an acrylate of low molecular weight is a distinct possibility. Identification of the unknown as methyl methacrylate was made by matching with the spectrum of that compound from a library of standard curves. If a spectral match had not been found, it would have been necessary to obtain the spectra of the suspected monomers or degraded polymers. The infrared analysis, therefore, eliminates the necessity of solubility and functional group tests, and derivative preparation.

Had the infrared data not been available, a purely chemical analysis might have involved the performance of the following tests on the purified destructive distillate (assuming no experience in resin analysis; familiarity with commercial high polymers naturally permits many short cuts, and confines the various possibilities within much more narrow limits).

Physical Examination. Water-white liquid; supports combustion on ignition, evolving a sharp, bittersweet odor.

Physical Constants. $n_D^{20°}$ = 1.4165, $d^{20°}$ = 0.934, b.p. = 100° C.

Elementry Analysis. Nitrogen, sulfur, and halogens absent.

Solubility Tests. Soluble in ether, cold concentrated sulfuric acid, and sirupy phosphoric acid; insoluble in water, dilute acid, and alkali. Member of group of indifferent compounds which includes alcohols, aldehydes, methyl ketones, and esters, containing fewer than 9 carbon atoms.

Classification Tests

Reagent	Result	Inference
$KMnO_4$	Positive	Ethylenic linkage
Br_2 in CCl_4	Positive	Ethylenic linkage
Acetyl chloride	Negative	Probably not an alcohol
2,4-Dinitrophenylhydrazine	Negative	Probably not aldehyde or ketene

The remaining possibility, an unsaturated ester of low molecular weight, is confirmed by saponification with alkali to give a saponification value of 561. With monofunctional reactants, a molecular weight of 100 is indicated. This is the more likely possibility, as a difunctional reactant would have a molecular weight of 200, somewhat higher than suggested by the solubility data.

Consultation of Literature. The following unsaturated esters with a molecular weight of 100 are among the possibilities:

Ester	Formula	$n_D^{20°}$	$d^{20°}$	B.P., °C.
Ethyl acrylate	$CH_2{=}CH{-}C(=O){-}OCH_2{-}CH_3$	1.4059	$0.925^{15°}$	100–101
Methyl methacrylate	$CH_2{=}C(CH_3){-}C(=O){-}OCH_3$	1.418	0.936	100
Methyl crotonate	$CH_3{-}CH{=}CH{-}C(=O){-}OCH_3$	.....	$0.9806^{4°}$	118–119
Methyl isocrotonate	$CH_3{-}CH{=}CH{-}C(=O){-}OCH_3$	.....	.....	106–108
Allyl acetate	$CH_2{=}CH{-}CH_2{-}O{-}C(=O){-}CH_3$	1.40488	0.9276	104

Comparison of the determined data with the listed possibilities indicates methyl methacrylate as the most likely. Confirmation may then be obtained by aminolysis of the ester with benzylamine and ammonium chloride as catalyst (*11*) to form a crystalline amide. The melting point of this compound may then be compared with the value reported in the literature, or, should this be lacking, with that of the amide derivative similarly prepared from a known sample of methyl methacrylate.

ACKNOWLEDGMENT

The authors are glad to acknowledge a valuable discussion of Figure 3 with H. W. Thompson of Oxford University.

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RECEIVED November 14, 1947. Presented in part before the Optical Society of America (*3*) and at the Symposium on Molecular Spectroscopy, Ohio State University, June 1947.

Compounds Involved in Production of Synthetic Rubber

Determination of Purity by Measurement of Freezing Points

AUGUSTUS R. GLASGOW, JR., NED C. KROUSKOP, JOAN BEADLE, GERTRUDE D. AXILROD, AND FREDERICK D. ROSSINI

National Bureau of Standards, Washington, D. C.

This report presents a summary of the method, apparatus, procedure, and results obtained in work on the determination of purity by measurement of freezing points of compounds involved in the production of synthetic rubber. Experimental data are given on the lowering of the freezing points of 1,3-butadiene, isoprene, and styrene on the addition of known amounts of the known most probable impurities. Values of freezing points for zero impurity, and of the appropriate cryoscopic constants, are given for 1,3-butadiene, 2-methyl-1,3-butadiene (isoprene), styrene, isobutene, *cis*-2-butene, *trans*-2-butene, *n*-butane, isobutane, and methyl chloride.

ONE of the most powerful tools for evaluating the purity of chemical substances is that involving determination of the freezing point. In connection with the work on hydrocarbons at the National Bureau of Standards, the freezing point, with appropriate observation of the temperature of the liquid-solid equilibrium as a function of the fraction of the sample frozen or melted, has for many years been used as the criterion of the purity of hydrocarbons.

As part of the national synthetic rubber program, this laboratory was requested by the Office of Rubber Reserve, Reconstruction Finance Corporation, to utilize its existing experience and apparatus for the development of suitable methods of test for the precise determination of purity. Appropriate methods of test were prepared and utilized by the Office of Rubber Reserve and its affiliated laboratories during the war years.

The present report gives the results of the work involved in these investigations which have hitherto not been published, with data on 1,3-butadiene, 2-methyl-1,3-butadiene (isoprene), styrene, isobutene, *cis*-2-butene, *trans*-2-butene, *n*-butane, isobutane, and methyl chloride.

PRINCIPLES INVOLVED

For the equilibrium between a crystalline phase consisting of the major component alone and a liquid phase consisting of the major component and one or more other components, the thermodynamic relation (see *11*, *20*, *21* for further details) between the temperature of equilibrium and the composition of the liquid phase, for an ideal or sufficiently dilute solution, is

$$-\ln N_1 = -\ln (1 - N_2) = (\Delta H_{f_0}/RT_{f_0}^2)\,(t_{f_0} - t) \times [1 + (1/T_{f_0} - \Delta C_{p_0}/2\,\Delta H_{f_0})\,(t_{f_0} - t) + \ldots] \quad (1)$$

where

N_1 = mole fraction of major component in liquid phase
$N_2 = (1 - N_1)$ = sum of mole fractions of all other components in liquid phase

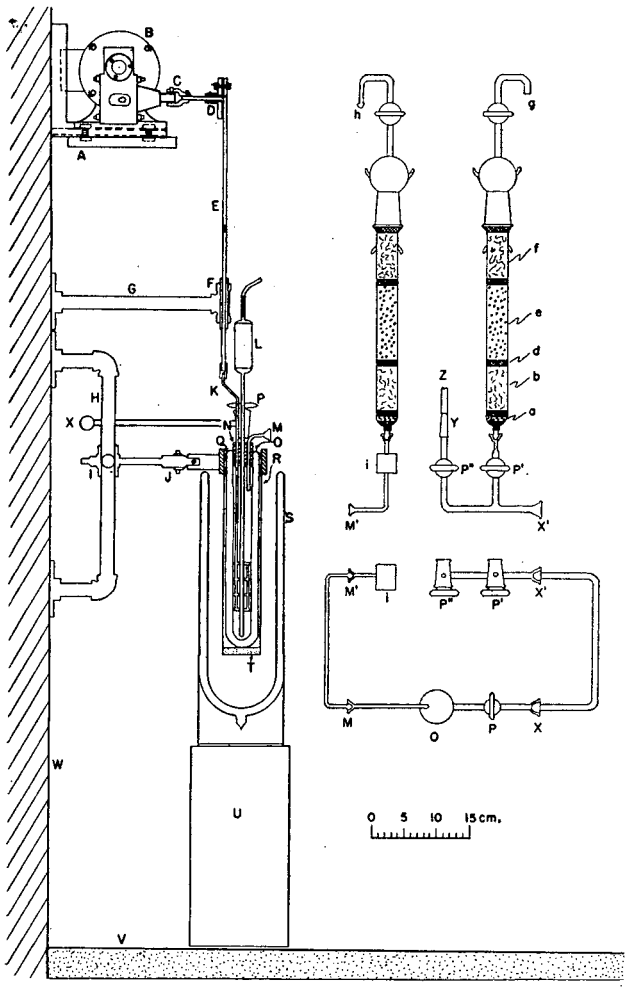


Figure 1. Assembly of Freezing-Point Apparatus

A. Bracket for motor, with rubber pad
B. Motor, with reduction gears, to give 120 r.p.m.
C. Coupling
D. Wheel
E. Steel rod
F. Bearing
G. Support for bearing
H. Support for freezing tube
I. Adjustable clamp holder
J. Clamp for freezing tube
K. Stirrer
L. Thermometer
M. Tube for inlet of dry air with 12/5 spherical joint
M'. 12/5 spherical joint connection to rotameter
N. Cork stopper, with holes as shown, plus a small hole for seed wire
O. Freezing tube, with silvered jacket
P. Stopcock on freezing tube
P'. Stopcock (high vacuum) to drying tube
P''. Stopcock (high vacuum) to vacuum line
Q. Asbestos collar
R. Brass cylinder, 12.5 inches long and 2.125 inches in inside diameter, with Bakelite collar

(When liquid nitrogen is used, metal shield must be provided with suitable openings in sides and bottom. If liquid air is used, metal shield should be constructed to keep hydrocarbon from contact with liquid air.)

S. Dewar flask, for cooling or warming bath
T. Asbestos pad at bottom of cylinder *R*
U. Wood block support
V. Table top
W. Wall
X, X'. Spherical joint, 18/7
Y. Standard metal (copper or brass) to glass taper connections, soldered
Z. Connection to vacuum pump
a. Anhydrous calcium sulfate, with indicator
b. Anhydrous magnesium perchlorate, granular
d. Separating layer of glass wool
e. Ascarite
f. Anhydrous calcium sulfate
g. To air
h. To source of compressed air
i. Flowmeter, for rates of 10 to 20 ml. per minute

t_{f_0} = temperature, in ° C., of freezing point of major component when pure—that is, when $N_2 = 0$
t = given temperature of equilibrium, in ° C
R = gas constant, per mole
$T_{f_0} = t_{f_0} + 273.16$
ΔH_{f_0} = heat of fusion, per mole, of major component in pure state at temperature T_{f_0}
ΔC_{p_0} = heat capacity, per mole, of pure liquid less that of pure solid, for major component in pure state at temperature T_{f_0}.

The three constant terms (t_{f_0}), ($1/T_{f_0} - \Delta C_{p_0}/2\ \Delta H_{f_0}$), and ($\Delta H_{f_0}/RT_{f_0}^2$) in Equation 1 are properties of only the major component, so that the relation between the temperature of equilibrium and the mole fraction of solute is the same for all solutes, provided they remain in the liquid phase and form with the major component an ideal solution.

In those cases where significant departures from the ideal solution law occur, but where the addition of solute to the pure solvent still lowers the freezing point of the solvent, the relation between the temperature of the solid-liquid equilibrium and the composition of the liquid phase can be expressed, in the dilute dilute region, by an equation of the form of (*1*):

$$-\ln N_1 = -\ln(1 - N_2) = A(t_{f_0} - t_f)\,[1 + B(t_{f_0} - t_f) + \ldots] \quad (2)$$

in which constants A and B are determined experimentally for given systems by measuring the lowering of the freezing point of the major component on the addition of known amounts of known impurities (solutes). In Equation 2, constant A has replaced in Equation 1 the term ($\Delta H_{f_0}/RT_{f_0}^2$) and B has replaced the term ($1/T_{f_0} - \Delta C_{p_0}/2\,\Delta H_{f_0}$).

If r is the fraction crystallized of the total number of moles of all components in the system, then, as previously shown (*20*)

$$t = t_{f_0} - a/[\{1 - (b/a)\} - r] \quad (3)$$

where a and b are constants for the given sample. Equation 3 gives the relation between the temperature of equilibrium and the fraction of material crystallized.

When the experiment is performed according to the procedure described in the present report, the rate of crystallization or melting of the major component is substantially constant with time, and, as previously shown (*20*)

$$r = k\,(z - z_f) \quad (4)$$

where k is a constant characteristic of the given experiment, z is any given time, and z_f is the time at which freezing begins or melting is complete. Furthermore,

$$t = t_{f_0} - a'/[1 - k'(z - z_f)] \quad (5)$$

where a' and k' are constants. Equation 5 gives the relation between the temperature of equilibrium and the time during the part of the experiment in which equilibrium between the liquid and solid phases of the major component exists.

DETERMINATION OF FREEZING POINTS

Apparatus. The apparatus used in the present investigation is substantially the same as that described in (*8*) with several improvements. For convenience in the reading of the present report, the assembly of the apparatus is shown in Figure 1, which shows also the drying tubes used to remove water and carbon dioxide from the air going into the vacuum jacket and from the air flowing into the space above the sample in the freezing tube.

In Figure 2 is shown a double helical stirrer [similar to one made independently at the Shell Development Company (*10*)], which is better to use than the aluminum cage stirrer previously described in those cases where the solid-liquid equilibrium is set up sluggishly. Figure 14 shows the successful results of freezing and melting experiments made with the double helical stirrer on a sample of isobutene for which the aluminum cage stirrer is normally used.

In Figure 3 is shown the simple apparatus used to induce crystallization, either by means of a rod cooled below the freezing point of the sample or by producing by hand a small amount of

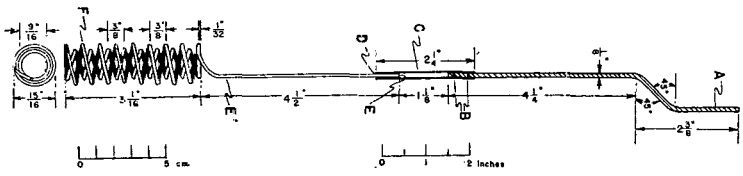


Figure 2. Details of Double Helical Stirrer

A. Stainless steel rod, round
B. Pins
C. German-silver tube, 0.125 inch inside diameter, 1/64 inch wall thickness
D. Place where Nichrome section of shaft is joined to German-silver tube of upper portion of shaft
E, E'. Nichrome section of shaft of double helical stirrer formed by silver soldering this length of two ends of Nichrome wire from inner and outer helix together
F. Double helical stirrer, made by winding 1/16 inch diameter Nichrome wire downwards on a cylinder 9/16 inch in outside diameter to form inner helix, and then upwards over a cylinder 13/16 inch in outside diameter to form outer helix

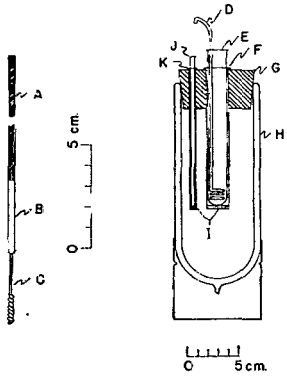


Figure 3. Apparatus for Inducing Crystallization

A. Bakelite rod; 0.125 inch in diameter, 12.5 inches in length
B. German silver tube, sealed to Nichrome wire on one end and sweated on Bakelite rod on other
C. Nichrome wire, 3/64 inch in diameter, with a helical coil on one end
D. Stirrer, Nichrome wire 1/16 to 1/8 inch in diameter, coiled on one end
E. Pyrex test tube
F. Metal shield for precautions in use of liquid nitrogen and liquid air see *R* in legend to Figure 1 and text
G. Cork stopper, with holes as shown
H. Dewar flask, 1-pint size
I. Asbestos padding
J. Pyrex tube closed on one side
K. Metal shield

crystals of the major component and introducing these into the sample at a temperature below the freezing point.

Freezing Experiment. The procedure for performing a freezing experiment is as follows:

The apparatus is assembled, with no refrigerant and no sample yet in place, but with a stream of air, freed of carbon dioxide and water, flowing at a rate of 10 to 20 ml. per minute. The jacket of the freezing tube is filled with air freed of carbon dioxide and water.

As required, the operator must be prepared to induce crystallization in the sample as soon as possible after the temperature has passed below the freezing point of the sample. In some cases, crystallization may be induced by introducing into the sample at the appropriate time a small rod (*ABC*, Figure 3) which has been kept at an appropriate lower temperature (near 0°, −80°, or −180° C.) (*J*, Figure 3). In other cases, crystallization may be induced by introducing into the sample at the appropriate time crystals of the sample on the coiled end of the small rod (*ABC*, Figure 3). These crystals are made by placing several milliliters of the sample in a small test tube (*E*, Figure 3) encased in a thin metal tube, immersed in a refrigerant whose temperature is below the freezing point of the sample. A slurry or mush of liquid and crystals is produced. Rod *ABC*, with wet crystals adhering to the helical coil, *C*, is raised above the liquid level in tube *E* and held in position with a cork stopper until required for "seeding."

The Dewar flask surrounding the freezing tube is filled with the appropriate refrigerant. The thermometer and stopper are temporarily removed and the sample (usually 50 ml. of liquid) is introduced, through a pipet if the material is normally liquid or by pouring the refrigerated liquid sample through the tapered male outlet of the reservoir trap, if the material is normally gaseous. When the sample is volatile or normally gaseous at room temperature, the freezing tube is cooled before introduction of the sample in order to minimize loss by evaporation. The flow of air (freed of carbon dioxide and water) into the freezing tube is continued in order to keep out water vapor. The stirrer is started and the sample is allowed to cool down to within about 15° C. of the freezing point, when evacuation of the jacket of the freezing tube is begun.

The time and the resistance of the thermometer are observed at even intervals of 0.02 to 0.05 ohm (about 0.2° to 0.5° C.) to determine the rate of cooling, which is continually changing as the pressure in the jacket of the freezing tube is reduced. Care must be taken to close the stopcock to the freezing tube when the desired cooling rate is obtained. In case the cooling rate is allowed to become too slow, the pressure and likewise the cooling rate can be increased by "bleeding in" air (freed of carbon dioxide and water) through stopcocks *P'* and *P* in Figure 1. When a cooling rate is obtained that will give a change of 1° in about 2 to 3 minutes in the range of about 5° to 10° above the freezing point, the stopcock controlling the jacket of the freezing tube is closed. The optimum rate of cooling will vary with the material being examined.

When the temperature reaches a point about 5° above the expected freezing point, the time is recorded to 1 second (or 0.01 minute) at which the resistance of the thermometer equals certain preselected values (every 0.1 or 0.05 ohm). At the appropriate time, crystallization is induced. The beginning of crystallization will be accompanied by a halt in the cooling of the liquid. After recovery from undercooling is substantially complete, the resistances, including the reading of the scale of the galvanometer at full sensitivity as well as the scale reading with no current through the galvanometer, are recorded at intervals of about 1 minute. These observations, together with the sensitivity of the galvanometer system in terms of ohms per millimeter of scale reading, yield a sensitivity near 0.0001° C.

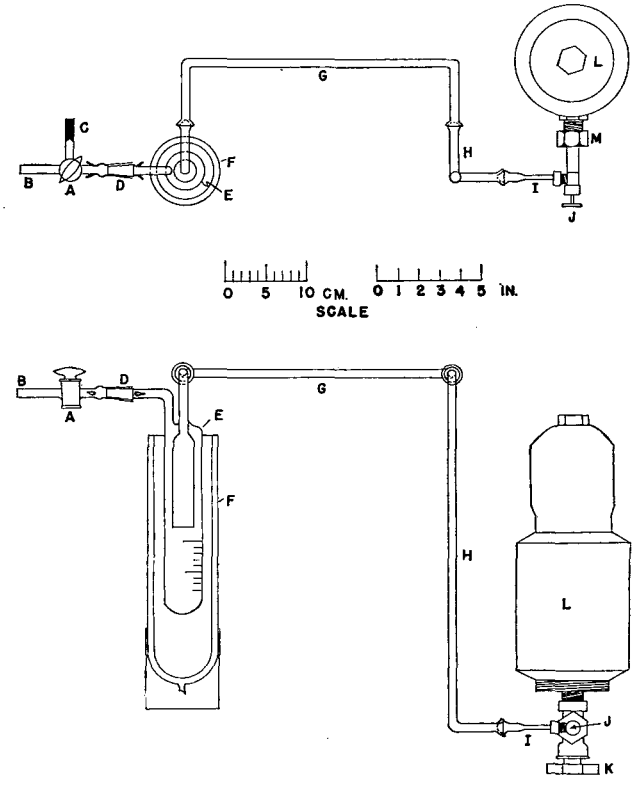


Figure 4. Apparatus for Obtaining Sample

A. Three-way T stopcock, Pyrex (similar to Corning Pyrex No. 7420)
B. Connection to vacuum for purging and for evacuating system *CDEGHI*
C. Capillary tube for venting, to which drying tube is also connected
D. Joint, standard-taper, 12/30 Pyrex
E. Condensing tube, Pyrex
F. Dewar flask, 1-quart size, Pyrex (similar to American Thermos Bottle Co. No. 8645)
G, H. Tubing, Pyrex, 10-mm. outside diameter, with spherical ground-glass joints, 18/7
I. Metal connection, brass, spherical male joint at one end and fitting to connection to needle valve at other end
J. Needle valve, brass
K. Valve on cylinder containing material
L. Standard cylinder containing material
M. Fitting to connect needle valve *J* to valve *K* on cylinder

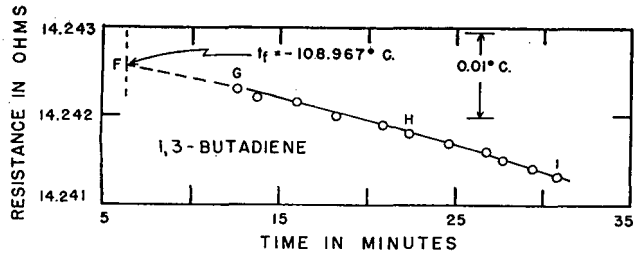


Figure 5. Time-Temperature Cooling Curve for Determining Freezing Point of a Sample of 1,3-Butadiene of Purity 99.82 Mole %

Scale of ordinates gives resistance in ohms of platinum resistance thermometer, and scale of abscissas gives time in minutes. *GHI* represents equilibrium portion of freezing curve. Freezing point *F* is determined as described in text

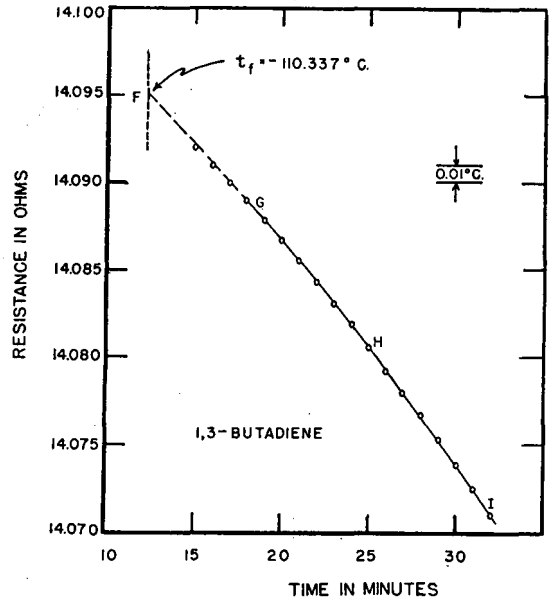


Figure 6. Time-Temperature Cooling Curve for Determining Freezing Point of a Sample of 1,3-Butadiene of Purity 95.17 Mole %

Scale of ordinates gives resistance in ohms of platinum resistance thermometer, and scale of abscissas gives time in minutes. *GHI* represents equilibrium portion of freezing curve

in the measurement of temperature. These observations are continued until the stirrer begins laboring. Then the stirrer is stopped and *N* (normal) and *R* (reverse) readings are compared by use of the commutator (*15*) after several minutes (when a steady rate is obtained). These latter readings are made at fixed intervals of about 1 minute, alternately for *N* and *R*, and the difference between the two at any given instant is determined from a plot of the several values against time.

Melting Experiment. The procedure for performing a melting experiment is exactly the same as for a freezing experiment, up to the point where the stirrer begins laboring.

When the stirrer shows signs of laboring, a comparison of *N* and *R* readings is made through the commutator, as above except that the stirrer is still operating. When the laboring of the stirrer becomes pronounced the freezing experiment (with the stirrer still operating) is changed to a melting experiment.

The energy for melting can be supplied either by a warming bath or by the energy introduced by the stirrer if the heat flow to or from the cooling or warming bath is sufficiently low. In the former case, the cooling bath is replaced by a warming bath and simultaneously the jacket is evacuated for an appropriate short time (3 to 10 minutes), after which the stopcock on the freezing tube is closed. In the latter case, the cooling bath is left in position or replaced by a warming bath and the jacket evacuated as much as possible to give minimum heat flow, leaving the stopcock to the freezing tube open to the vacuum system during the entire melting experiment. Thus, the thermal conductivity across the jacket is so small that the energy introduced by the stirrer is sufficient to provide the energy for melting. Observations of time and resistance are continued along the equilibrium portion of the freezing curve. When melting is substantially complete, as evidenced by a marked change in the rate of change of resistance, observations of time are made at even intervals of 0.05 ohm (0.5° C.). The experiment is concluded when the temperature has gone about 5° to 10° above the freezing point.

Procedure for 1,3-Butadiene. The method of obtaining a sample of 1,3-butadiene for measurement of the freezing point is as follows:

The apparatus shown in Figure 4 is assembled with no lubricant on the ground-glass joints and with the valve at the bottom of the cylinder, so that sampling is from the liquid phase. An absorption tube containing anhydrous calcium sulfate or other suitable desiccant (except magnesium perchlorate) is attached to *C*, so that water is not introduced into the system. (If some water does condense with the 1,3-butadiene, the freezing point will not be affected significantly because of the extremely low solubility of water in 1,3-butadiene at the freezing point of the latter.) Flask *F* is filled, to within about 5 cm. (2 inches) of the top, with the carbon dioxide refrigerant (a slush or slurry of solid carbon dioxide in a solution of equal volumes of carbon tetrachloride and chloroform, temperature about −78° C.). After about 20 or 30 minutes, when the system will have cooled sufficiently, the absorption tube is removed and collection of liquid 1,3-butadiene is begun by opening valve *K* and adjusting needle valve *J* so that the sample is collected at a rate of 1 to 2 ml. (liquid) per minute in condensing tube *E*. When 50 ml. of liquid (temperature about −80° C.) have been collected in the condensing tube, valve *K* is closed and the liquid that has collected at *I* is allowed to warm and be transferred to the condensing tube. (In case the original sample contained water, there will remain at *I* some water which may be discarded after the hydrocarbon portion has been collected as outlined above.) The attaching tubes, *G* and *D*, on the condensing tube are replaced by caps. The liquid sample is now ready for introduction into the freezing tube (*O*, Figure 1).

The liquid butadiene is introduced into the double-walled freezing tube (*O*, Figure 1), when the temperature of the platinum resistance thermometer is near −80° C., by raising the stopper holding the platinum thermometer and pouring about 50 ml. of liquid (temperature about −80° C.) from the condensing tube or receiver. The upper portion of the condensing tube or the receiver is wrapped with a cloth for ease of handling and to prevent the refrigerating liquid from contaminating the sample.

The freezing point of the given sample of 1,3-butadiene is determined from a freezing experiment, with a cooling bath of liquid air or liquid nitrogen, and with a cooling rate of 0.3° to 0.8° C. (0.03 to 0.08 ohm) per minute for the liquid near the freezing point. Crystallization is induced, immediately below the freezing point, by means of a cold rod precooled to the temperature of liquid air or liquid nitrogen.

The freezing point is evaluated by observations of temperature (resistance) and time as described in (*8*) and (*20*).

Figures 5 and 6 show typical time-temperature freezing curves obtained on 1,3-butadiene having a purity of 99.82 and 95.17 mole %, respectively.

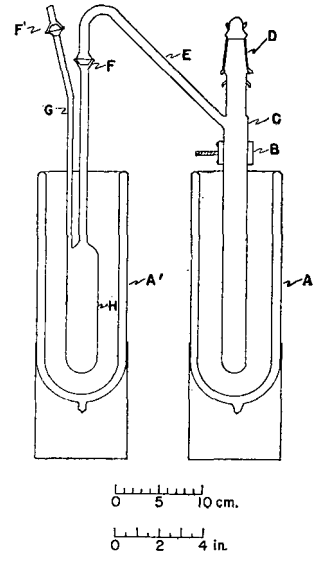


Figure 7. Simple Distilling Apparatus for Normally Gaseous Substances

A, *A'*. Dewar vessel, 1-quart capacity, Pyrex
B. Clamp
C. Distilling tube, Pyrex, 25-mm. outside diameter
D. Standard-taper ground-glass joint, 24/40, Pyrex
E. Tubing, 10-mm. outside diameter, Pyrex
F, *F'*. Spherical ground-glass joints, 18/7, Pyrex
G. Tubing, 6-mm. outside diameter, Pyrex
H. Receiver, 35-mm. outside diameter, 150-mm. length, Pyrex

When it is desired to determine the purity of a sample of 1,3-butadiene with respect to impurities of about the same molecular weight and volatility, the dimer, other C_4 hydrocarbons, and higher polymer are removed before determining the purity.

The apparatus shown in Figure 7 is assembled with a small amount (10 to 100 p.p.m.) of *tert*-butyl catechol or other suitable inhibitor placed in the bottom of the distilling tube, *C*, with no lubricant on the ground-glass joints. It is also desirable to place at the bottom of the distilling tube a piece of Carborundum or other suitable material to prevent bumping. A connection to the atmosphere through an absorption tube is made at *F'*, so that entering air is freed of carbon dioxide and water. A bath of carbon dioxide refrigerant is placed around the distilling tube and around receiver *H*, so that the small entrance and exit tubes of the receiver are covered with at least 5 cm. (2 inches) of the bath. After about 20 to 30 minutes, when the system will have precooled sufficiently, the connection to the atmosphere is disconnected, cap *D* is removed, and the liquid butadiene (temperature near −80° C.) is introduced by pouring through a precooled funnel (such as *Q*, Figure 11, which may be cooled without contamination by liquid air or liquid nitrogen) into the distilling tube. The cap is greased and replaced immediately after the introduction of the sample. The material is then distilled by removing the bath from the distilling tube and allowing it to warm in contact with the air of the room.

Distillation is complete when the distilling tube has warmed to room temperature. The receiver with the bath around it is disconnected and capped at *F* and *F'*. The liquid butadiene (temperature about −80° C.) is kept refrigerated in the Dewar flask until it is introduced into the freezing tube (*O*, Figure 1).

Experiments were performed to ascertain the effectiveness of the simple procedure outlined above for removing 4-vinyl-1-cyclohexene (cyclic dimer of 1,3-butadiene) from 1,3-butadiene. These experiments consisted in determining the freezing point of a given sample of purified butadiene, adding to the sample of 1,3-butadiene about 5 mole % of 4-vinyl-1-cyclohexene, removing the 4-vinyl-1-cyclohexene by the procedure described above, and determining again the freezing point of the sample of 1,3-butadiene. The freezing points were the same within 0.06° C., corresponding to about 0.2 mole % in purity. This procedure was used to determine, by measurement of freezing points, the amount of 1,3-butadiene in a sample of "recycle butadiene" containing 0.5 and 0.4 mole % of styrene and 4-vinyl-1-cyclohexene, respectively. The value obtained from the measurements of freezing points was 83.6 mole % of 1,3-butadiene, which is to be compared with the value 83.4 mole % obtained from a distillation at high efficiency and high reflux ratio (*6*).

Procedure for Isoprene. The method of obtaining a sample of isoprene from a cylinder for measurement of the freezing point is as follows:

The apparatus shown in Figure 4 is assembled, with Apiezon or other suitable lubricant on the ground-glass joints, and with the valve below the body of the cylinder, so that the sample is obtained from the liquid phase. The system is evacuated by connecting opening *B* to a vacuum line through heavy-walled tubing. After evacuation, stopcock *A* is closed to outlets *B* and *C*, and the sample of isoprene (55 ml., liquid, at about −80° C.) is collected in the refrigerated condensing tube, *E*, in which was previously placed a small amount (about 10 to 100 p.p.m.) of *tert*-butyl catechol or other suitable inhibitor. The sample as thus collected will contain substantially all the dimer present in the original material. This sample is used for determination of the purity of isoprene, including the dimer associated with it as an impurity, and is introduced into the freezing tube in the manner previously described for 1,3-butadiene. (Experiments on a sample of isoprene of about 95 mole % over 7 months showed the dimer formation on storage at 8° to 10° C. to be of the order of about 0.1 mole % per month calculated on the basis that the dimer follows the ideal solution laws.)

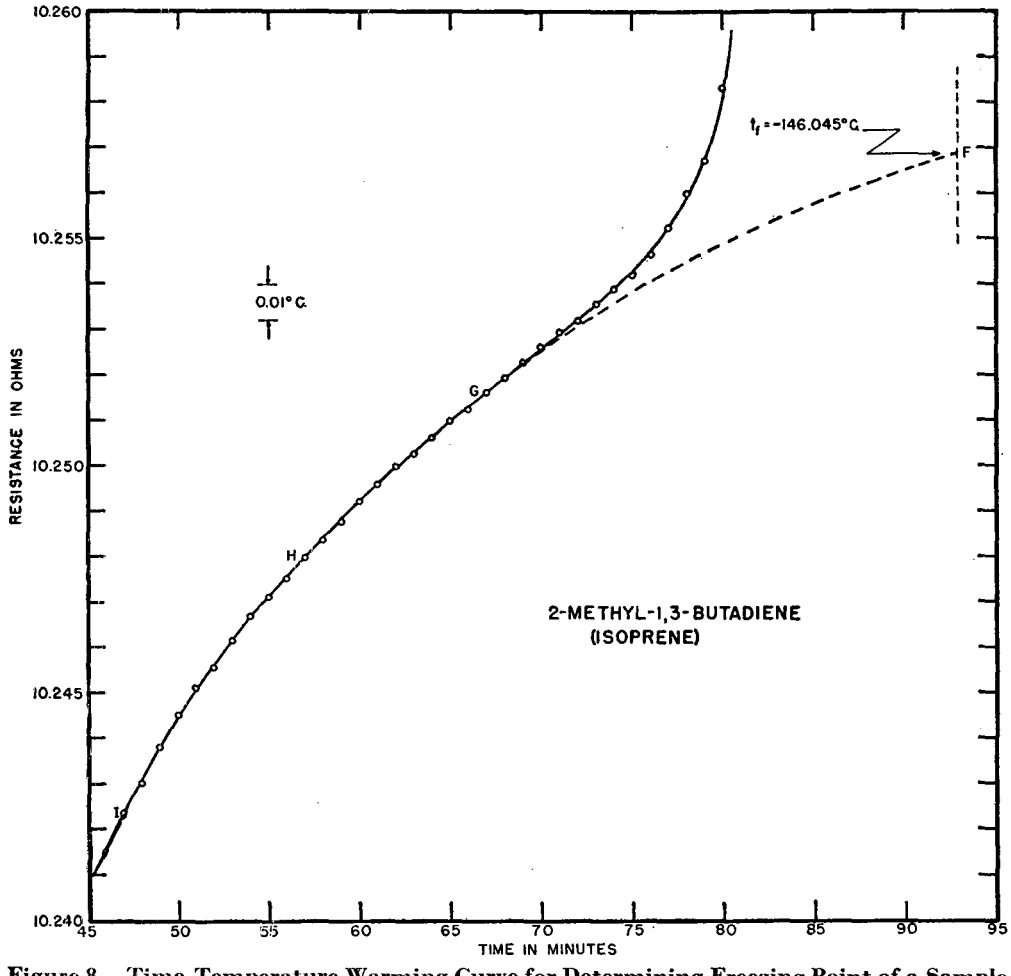


Figure 8. Time-Temperature Warming Curve for Determining Freezing Point of a Sample of Isoprene of Purity 99.66 Mole %

Scale of ordinates gives resistance in ohms of platinum resistance thermometer, and scale of abscissas gives time in minutes. *IHG* represents equilibrium portion of warming curve

The freezing point of the given sample of isoprene, when the purity is greater than about 98 mole %, is determined from melting experiments using the double helical stirrer instead of the aluminum cage stirrer. The slurry of crystals and liquid is obtained by using a cooling bath of liquid air or liquid nitrogen, with a cooling rate of 0.3° to 0.8° C. (0.03 to 0.08 ohm) per minute for the liquid near the freezing point. Crystallization is induced immediately below the freezing point by means of a cold rod, precooled to the temperature of liquid air or

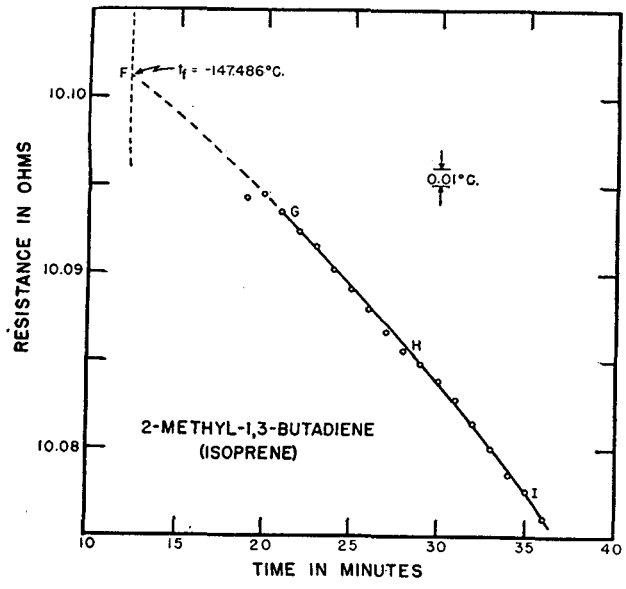


Figure 9. Time-Temperature Cooling Curve for Determining Freezing Point of a Sample of Isoprene of Purity 94.61 Mole %

Scale of ordinates gives resistance in ohms of platinum resistance thermometer, and scale of abscissas gives time in minutes. *GHI* represents equilibrium portion of freezing curve

liquid nitrogen. A warming bath of carbon dioxide refrigerant is used, with the jacket of the freezing tube open to the high vacuum system during the entire melting part of the experiment.

For samples having a purity less than about 98 mole %, the freezing point is determined from freezing experiments as for 1,3-butadiene.

The evaluation of the freezing point from observations of temperature (resistance) and time is made as described in (*8*) and (*20*).

Figures 8 and 9 show typical time-temperature melting and freezing curves obtained on isoprene having a purity of 99.66 and 94.61 mole %, respectively.

When it is desired to determine the purity of a sample of isoprene with respect to impurities of about the same molecular weight and volatility, the dimer and higher polymer are removed before determining the purity.

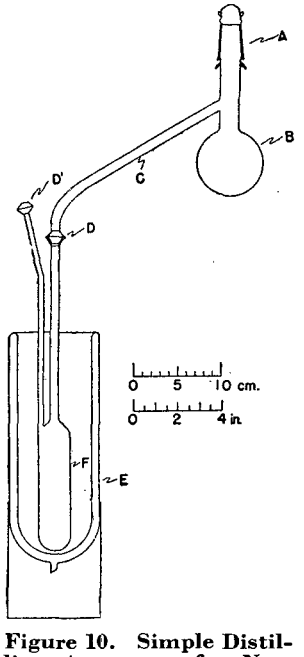


Figure 10. Simple Distilling Apparatus for Normally Liquid Substances

A. Standard-taper, ground-glass joint, 24/40 Pyrex
B. Distilling flask, round-bottomed, 200 ml. capacity, Pyrex
C. Tubing, 10-mm. outside diameter, Pyrex
D, D'. Spherical ground-glass joints, 18/7 Pyrex
E. Dewar flask, 1-quart capacity, Pyrex
F. Receiver, same as *H*, Figure 7

The apparatus shown in Figure 10 is assembled, with no lubricant on the ground-glass joints, *D* and *D'*. A small amount of *tert*-butyl catechol or other suitable inhibitor (about 10 to 100 p.p.m.) is placed in receiver *F* and a larger amount (about 100 to 1000 p.p.m.) in distilling flask *B*. It is also desirable to place at the bottom of *B* a piece of Carborundum or other suitable material to prevent bumping. A cooling bath of water-ice is placed around *B* and a bath containing carbon dioxide refrigerant is placed around *F*. A connection is made to the atmosphere at *D'* through which the air is first freed of carbon dioxide and water, using a tube containing Ascarite and anhydrous calcium sulfate or other suitable desiccant. The sample (at 0° C.) is introduced into *B*, cap *A* is placed in position with some Apiezon or other suitable lubricant between the grindings, and the connection to the atmosphere removed at *D'*. A water bath (at 40° to 50° C.) is placed around *B* and the material distilled into *F*. The distillation is stopped when a small residue remains in *B* or when no more material distills over from *B* with the water bath at 50° C. *F* is then detached at *D* and capped at *D* and *D'* with the bath containing solid carbon dioxide ice still surrounding it.

If the sample contains a very large amount of dimer and polymer, the simple procedure outlined above will not suffice because the required distilling temperature will be too high, and the more complicated procedure outlined under styrene is used with the following alterations: The sample is frozen to a solid, using liquid air or liquid nitrogen for the removal of the air dissolved in the sample. The distilling tube is surrounded by an ice-water bath and the receiver by a bath containing carbon dioxide refrigerant.

Procedure for Styrene. A sample of styrene for measurement of the freezing point is obtained as follows:

A 50-ml. sample (measured at room temperature) is obtained directly from the original container by means of a pipet or by pouring into a graduated cylinder and is introduced into the freezing tube.

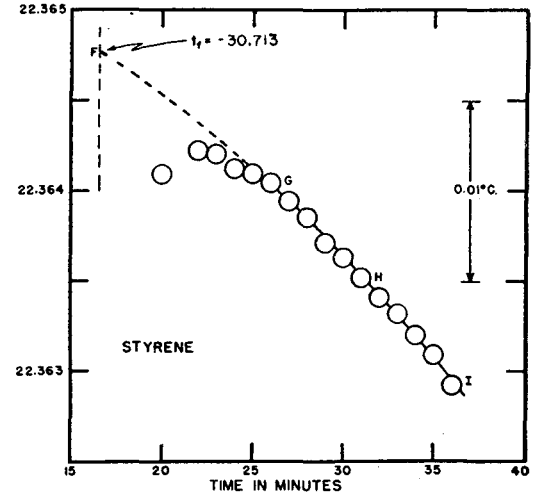


Figure 11. Time-Temperature Cooling Curve for Determining Freezing Point of a Sample of Styrene of Purity 99.81 Mole %

Scale of ordinates gives resistance in ohms of platinum resistance thermometer, and scale of abscissas gives time in minutes. *GHI* represents equilibrium portion of freezing curve

The freezing point of the given sample of styrene is determined from freezing experiments, with a cooling bath of carbon dioxide refrigerant, and with a cooling rate of 0.3° to 0.8° C. (0.03 to 0.08 ohm) per minute for the liquid near the freezing point. Crystallization is induced, immediately below the freezing point, by means of a cold rod, kept near −80° C. in a bath of carbon dioxide refrigerant.

The evaluation of the freezing point from observations of temperature (resistance) and time is made as described in (*8*) and (*20*).

Figures 11 and 12 show typical time-temperature freezing curves obtained on styrene having a purity of 99.81 and 95.77 mole %, respectively.

If the previous treatment or storage condition of the material was such that dimerization or polymerization may have occurred, the dimer or polymer is removed before determining the purity.

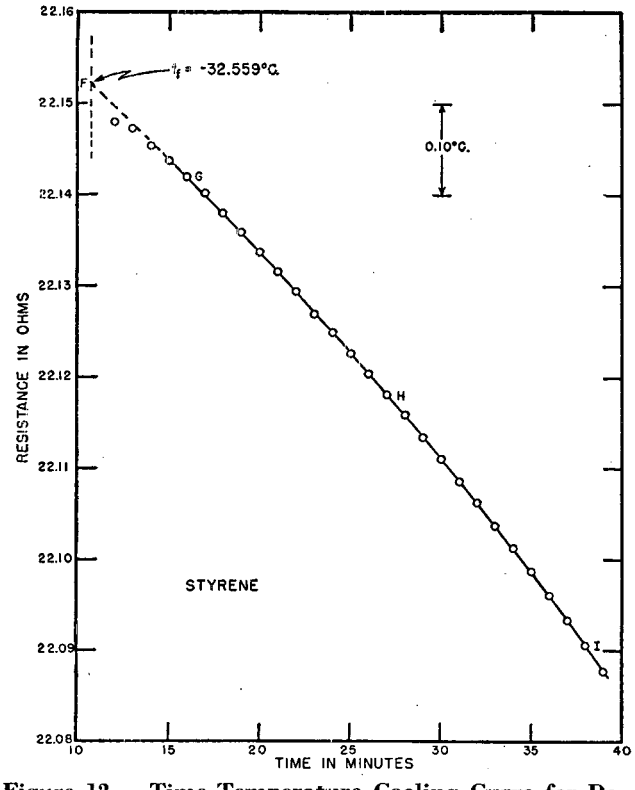


Figure 12. Time-Temperature Cooling Curve for Determining Freezing Point of a Sample of Styrene of Purity 95.77 Mole %

Scale of ordinates gives resistance in ohms, of platinum resistance thermometer, and scale of abscissas gives time in minutes. *GHI* represents equilibrium portion of freezing curve

The apparatus shown in Figure 13 is assembled with inhibitor placed in the distilling tube and receiver as previously described and with all the ground joints except that at *A* lubricated. It is also desirable to place at the bottom of the distilling tube a piece of Carborundum or other suitable material to prevent bumping. A bath of carbon dioxide refrigerant is placed around tube *E*. Air, freed of carbon dioxide and water, is permitted to enter the system through *RH"H'H* in order to compensate for the change in volume. Cap *A* is removed and the sample is introduced through funnel *Q*. *A* is then lubricated and stopcocks *H*, *H'*, and *H"* are closed. A bath of carbon dioxide refrigerant is placed around the condensing tube, *J* (same as *E*, Figure 4), which serves as a trap. After the styrene has solidified, the system is evacuated by opening *H* and *H'* to the vacuum system. *H* and *H'* are closed and the bath removed from *E* to allow the material to melt and release dissolved air. The material is crystallized again and the system evacuated as before. The process is repeated again, if necessary, to remove substantially all the air. (If any hydrocarbon has been caught in trap *J*, it should be distilled back into tube *E*, with *H* open and *H'* closed.) The material is distilled into receiver *E'* by surrounding it with a bath of carbon dioxide refrigerant and allowing distilling tube *E* to warm in contact with the air of the room.

The distillation is stopped when the transfer of material into the receiver has substantially halted, by admitting air (freed of water and carbon dioxide) into the system through *RH"H'H*. The sample is removed from *E* with the withdrawal receiver, *N*. The system *L'MNL*, with *L'* open and *L* closed, is evacuated through *P* and the *L'* is then closed. Receiver *N* is surrounded by a carbon dioxide freezing mixture. The material is removed by inserting the inlet tube at *L* into the receiver and then opening stopcock *L*. This withdrawal procedure avoids preferential fractionation by evaporation of mixtures of components of different volatilities. The material is introduced into the freezing tube by pouring it through the tapered joint, *M*, of the withdrawal receiver.

Procedure for Isobutene, *cis*-2-Butene, *trans*-2-Butene, *n*-Butane, Isobutane, and Methyl Chloride. The method of obtaining a sample of isobutene, *cis*-2-butene, *trans*-2-butene, *n*-butane, isobutane, and methyl chloride for measurement of the freezing point is the same as that described for 1,3-butadiene.

The determination and evaluation of the freezing point are the same as described for 1,3-butadiene.

Figure 14 shows a time-temperature freezing and melting curve obtained on isobutene having a purity of 99.49 mole %. The freezing and melting curves were obtained using the double helical stirrer shown in Figure 2 and the melting conditions described under isoprene.

FREEZING POINTS FOR ZERO IMPURITY

Values of the freezing points for zero impurity in air at 1 atmosphere were determined as described in (*8*) and (*20*), from appropriate freezing or melting experiments made on samples of the highest purity available. For 1,3-butadiene, isoprene, styrene, isobutene, *cis*-2-butene, *trans*-2-butene, *n*-butane, isobutane, and methyl chloride, information is given in Table I.

LOWERING OF FREEZING POINT ON ADDITION OF KNOWN AMOUNTS OF KNOWN IMPURITIES

Method of Evaluating Cryoscopic Constants. Equation 1, relating temperature and the composition of the liquid phase in the solid-liquid equilibrium in an ideal system, involves the two assumptions that all impurities (solutes) (1) remain in the liquid

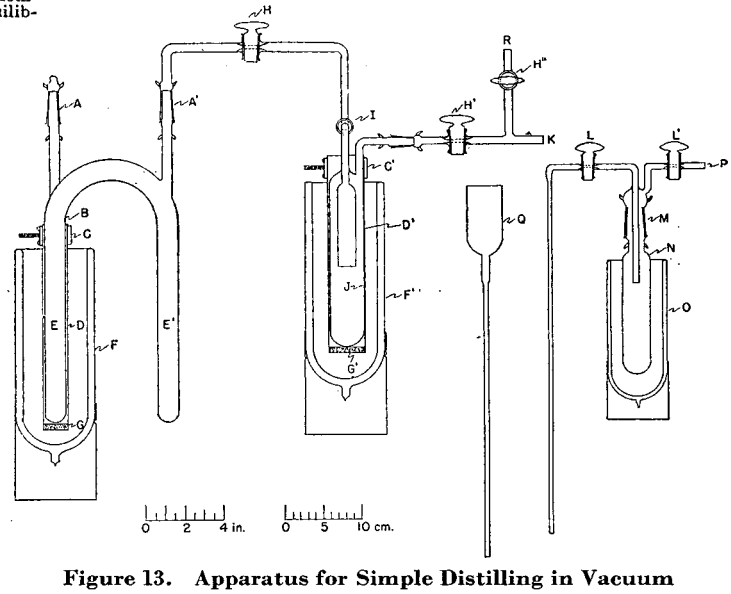


Figure 13. Apparatus for Simple Distilling in Vacuum

A, A'. Standard-taper ground-glass joints, 14/35, Pyrex
B. Tubing, 27-mm. outside diameter, Pyrex
C, C'. Clamp
D. Brass cylinder, 10.75-inch length, 1.125-inch inside diameter (for precautions in use of liquid nitrogen and liquid air, see *R*, Figure 1 and text)
D'. Brass cylinder, 10-inch length, 1.875-inch inside diameter
E. Original sample
E'. Distilled sample
F, F'. Dewar flask, 1-quart capacity, Pyrex
G, G'. Asbestos pad
H, H', H". Stopcock, ground for high vacuum, Pyrex
I. Spherical ground-glass joint, 18/7, Pyrex
J. Condensing tube, used as trap (see Figure 4)
K. Connection to vacuum system
L, L'. Stopcock, ground for high vacuum, Pyrex
M. Standard taper ground-glass joint, 24/40, Pyrex
N. Receiver withdrawal, 36-mm. outside diameter, Pyrex
O. Dewar flask, 1-pint capacity, Pyrex
P. Connection to vacuum
Q. Funnel with extension of 4-mm. inside diameter, Pyrex
R. Connection to drying tube, Pyrex

phase during crystallization and (2) form with the major component a substantially ideal solution. The first assumption requires the absence of mixed crystals. The tendency of formation of mixed crystals is more favorable when the solute molecules have nearly the same size and shape as those of the major component; this permits some of the latter molecules in the crystalline lattice to be replaced by solute molecules without fusion of the crystal. Fortunately, in the case of hydrocarbons of low molecular weight, mixed crystals occur seldom between a given hydrocarbon and the other hydrocarbons that are likely to remain as impurity in the given hydrocarbon after the latter has been subjected to a thorough fractionation. Furthermore, with regard to the assumption requiring that the impurity form with the major component a substantially ideal solution, it is apparent that when a given hydrocarbon has been put through a logical system of purification there will remain as impurity only those hydrocarbons that are very similar in properties to the major component. These latter hydrocarbons are, however, precisely those which are most likely to form with the major component a substantially ideal solution.

In connection with the purposes of the present investigation, it was desirable to ascertain explicitly for 1,3-butadiene, styrene, and isoprene, whether the addition of known amounts of the most probable impurities would produce a lowering of the freezing point in accordance with the ideal relation as given by Equation 1, and, if not, to evaluate the extent of the departures from ideality.

Table I. Values of Freezing Points for Zero Impurity in Air at 1 Atmosphere for Nine Compounds

Compound	Source[a]	Kind of Time-Temperature Observations Used to Determine Freezing Point[b]	Freezing Point in Air at 1 Atm.		Calculated Purity of Actual Sample[d]
			Actual sample	Zero impurity[c]	
			° C.	° C.	*Mole %*
1,3-Butadiene	M-K-H	F	−108.922	−108.915 ± 0.010	99.98
Isoprene	Univ. Ill.	M	−145.967	−145.950 ± 0.020	99.94
Styrene	NBS Rubber	F	− 30.640	− 30.628 ± 0.008	99.97
					99.97[e]
Isobutene	Std. Oil Dev.	F and M	−140.416	−140.350 ± 0.020	99.73
trans-2-Butene	Penn State	F	−105.625	−105.550 ± 0.020	99.69
cis-2-Butene	M-K-H	F	−139.001	−138.910 ± 0.020	99.56
n-Butane	Phillips	F	−138.421	−138.350 ± 0.025	99.78
Isobutane	Std. Oil Co. La.	F	−159.634	−159.600 ± 0.025	99.86
Methyl chloride	Std. Oil Dev.	F and M	− 97.730	− 97.720 ± 0.010	99.97

[a] M-K-H, Mellon-Koppers-Hinckley, compounds prepared by J. A. Hinckley on Koppers fellowship at Mellon Institute of Industrial Research; Univ. Ill., Department of Chemistry, University of Illinois, compounds prepared by R. L. Frank, R. D. Emmick and R. S. Johnson (*5*); NBS Rubber, National Bureau of Standards Rubber Section; Std. Oil Dev., Standard Oil Development Co., Chemical Division, through P. K. Frolich and W. L. Nelson; Phillips, Phillips Petroleum Co., research grade material; Std. Oil Co., La., Standard Oil Co. of New Jersey, Louisiana Division, through C. E. Starr; Penn State, Petroleum Refining Laboratory, Pennsylvania State College, material prepared under supervision of M. R. Fenske.
[b] F freezing, M melting.
[c] For uncertainties, see (*8*, *20*).
[d] See Table II for values of cryoscopic constants.
[e] Value for ethylbenzene and toluene as impurities (solutes).

Equation 2 may be written in the form

$$-\ln N_1/(t_{f_0} - t_f) = A + AB\,(t_{f_0} - t_f) \qquad (6)$$

The values of N_1, the mole fraction of the major component, were determined from the masses and molecular weights of the components used to make up the mixtures, as described in the following section. The value of the constant t_{f_0} was taken as that given above in the discussion of freezing points for zero impurity. The values of t_f, the freezing point, were determined as described in the discussion of determination of freezing points. For high precision the successive values of t_f for different values of the mole fraction of a given major component should be de-

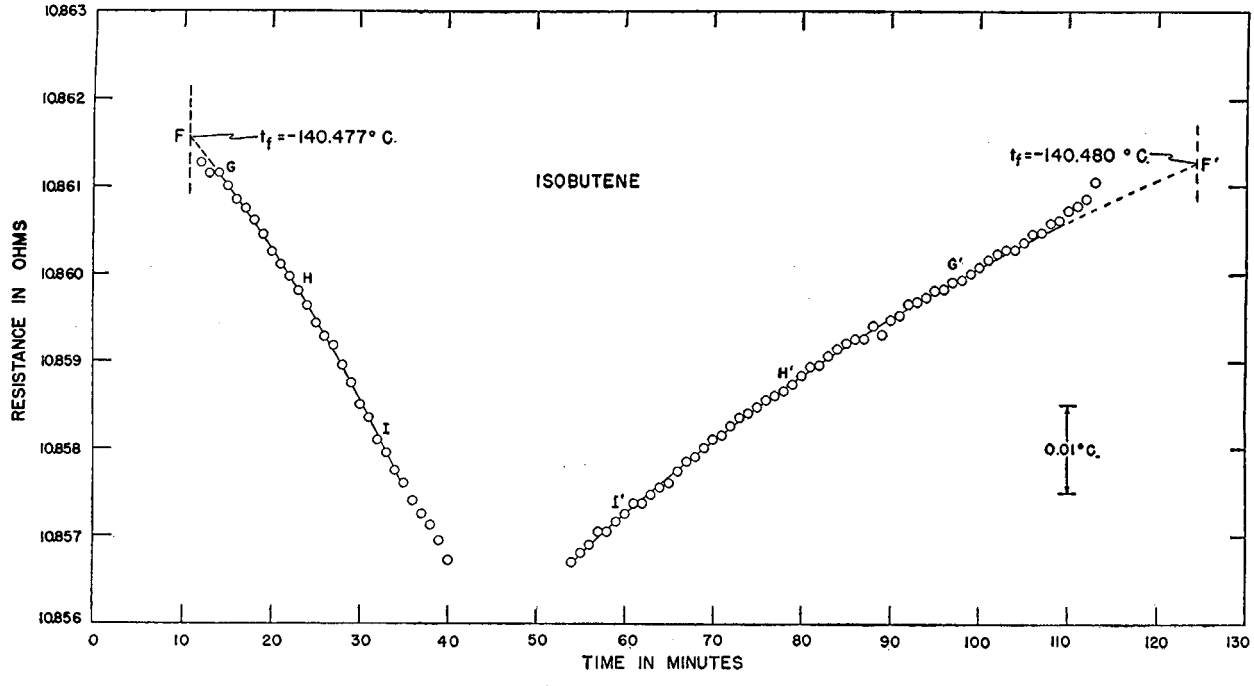


Figure 14. Time-Temperature Cooling and Warming Curves for Determining Freezing Point of a Sample of Isobutene of Purity 99.49 Mole %

Scale of ordinates gives resistance in ohms of platinum resistance thermometer, and scale of abscissas gives time in minutes. *GHI* represents equilibrium portion of freezing curve and *I'H'G'* represents equilibrium portion of warming curve. *F* and *F'* determined as described in text

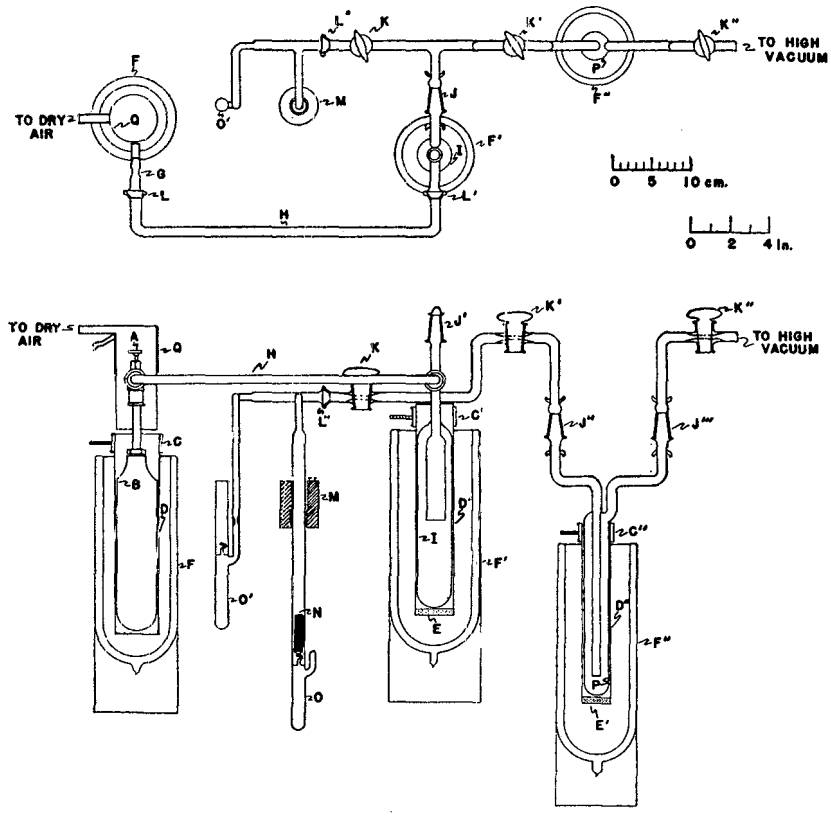


Figure 15. Apparatus for Preparation of Known Mixtures of Volatile Compounds

A, A'. Valve on cylinder
B, B'. Metal cylinder (0.5 pint)
C, C', C". Clamps
D, D', D". Brass cylinder (for precautions in use of liquid nitrogen and liquid air, see *R* in legend to Figure 1)
E, E'. Asbestos pad
F, F', F". Dewar flask, 1-quart capacity, Pyrex
G. Glass-to-metal connection, joined together by soldering ground-glass tapered male joint to brass female joint of same taper, with brass fitting on one end to connect to needle valve of cylinder and a Pyrex spherical male joint 18/7, on other end
H. Tubing, Pyrex, 10-mm. outside diameter, with spherical ground-glass joints, 18/7
I. Condensing tube, Pyrex, graduated in 5-ml. divisions
J, J', J", J'". Joint, standard-taper, 12/30, Pyrex
K, K', K", K'". Stopcock, ground for high vacuum, Pyrex
L, L', L", L'", L"". Spherical ground-glass joint, 18/7, Pyrex
M. Solenoid, about 1200 ampere turns, 110 volts A.C.
N. Iron (mild annealed steel) rod encased in glass
O, O'. Pyrex ampoule with internal vacuum break-off tip
P. Condensing tube used as trap
Q. Brass cylinder, wrapped with insulated Nichrome wire heater, through which stream of dry air is passed
R. Tubing, Pyrex, 10-mm. outside diameter, with spherical ground-glass joints, 18/7
S. Tubing, Pyrex, 10-mm. outside diameter graduated in ml.

termined as precisely as possible, whereas the value selected for t_{f_0} was here of secondary importance, as it was used primarily as a reference point to obtain differences.

In handling the experimental data, values of the quantity, $-\ln N_1/(t_{f_0} - t_f)$, were plotted as ordinates against values of $t_{f_0} - t_f$ as abscissas. The data so plotted can be represented sufficiently well by a straight line, the intercept of which on the ordinate scale at $(t_{f_0} - t_f)$ equal to zero is the value of the first cryoscopic constant, A, and the slope of which is the product of A and the second cryoscopic constant, B.

Table II. Summary of Information Relating to Evaluation of Purity for Measurements of Freezing Points

Compound	Freezing Point for Zero Impurity in Air at 1 Atm.	Cryoscopic Constants[a]		Constants Applicable to Solutes (Impurities)
	° C.	*A* Deg.$^{-1}$	*B* Deg.$^{-1}$	
1,3-Butadiene	−108.915	0.03560	0.0053	Ideal, including 1-butene, *cis*-2-butene, *trans*-2-butene, isobutene, 1,2-butadiene, 4-vinyl-1-cyclohexene, styrene
2-Methyl-1,3-butadiene (isoprene)	−145.950	0.0330	0.0030	Ideal, including *n*-pentane, 1-pentene, *trans*-2-pentene, 2-methyl-1-butene, 2-methyl - 2 - butene, *cis* - 1,3 - pentadiene, *trans* - 1,3 - pentadiene, dimethylacetylene
Styrene	− 30.628	0.02250	0.0044	Ideal, including benzene, *o*-xylene, *m*-xylene, *n*-propylbenzene, isopropylbenzene, 4-vinyl-1-cyclohexene
	− 30.628	0.02480	0.0044	Toluene, ethylbenzene
Isobutene	−140.350	0.04044	0.005	Ideal
trans-2-Butene	−105.550	0.04177	0.0058	Ideal
cis-2-Butene	−138.910	0.04878	0.0052	Ideal
n-Butane	−138.350	0.03084	0.0048	Ideal
Isobutane	−159.600	0.04234	0.0057	Ideal
Methyl chloride	− 97.720	0.02512	0.0051	Ideal

[a] From this report, (*1*), and (*13*).

Apparatus and Method for Making Known Mixtures. The apparatus shown in Figures 15 and 16, was used to prepare mixtures involving gaseous or volatile components.

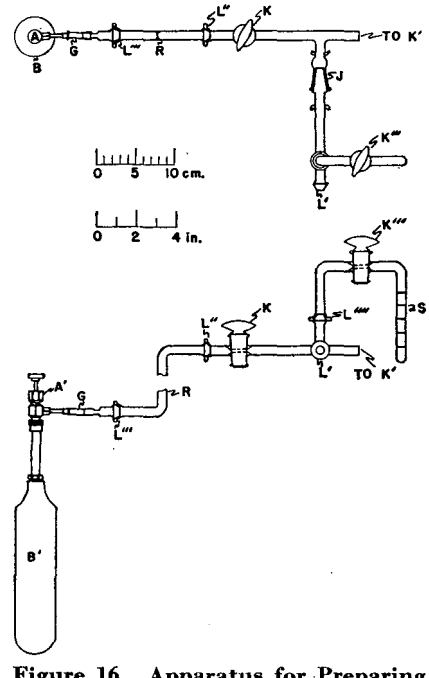


Figure 16. Apparatus for Preparing Known Mixtures of Volatile Compounds

See Figure 15 for key to parts

It consists of a bomb, B, for weighing; a condensing tube, I or S, for collecting component to be introduced into the weighing bomb; a trap, P; and attachments to the ground-glass spherical joint, L'', for adding material to the condensing tube either from the break-off ampoule, O in Figure 15 or from a cylinder, B', as shown in Figure 16.

The steel bomb for weighing was coated with a mixture of aluminum powder in a high-grade spar varnish to protect the bomb from corrosion due to alternate cooling and warming. The valve was kept slightly above room temperature by a stream of hot dry air from the heater, Q. The bomb was evacuated through valve A, with stopcock K closed and stopcocks K' and K'' open. The evacuated bomb was detached at the spherical joint, L, and the lubricant on the ball joint was removed with ether. The bomb was weighed on a large analytical balance with a duplicate bomb as a tare. Blank experiments on the weight of the evacuated bomb, including evacuation, greasing, degreasing, cooling, and warming, indicated constancy of weight of the bomb within about 1 mg.

To facilitate the addition to the bomb of the appropriate weight of gaseous component, condensing tubes I and S were graduated in volumes of 5- and 1-ml., respectively.

In the preparation of known mixtures involving components that are all gaseous, the transfer of the hydrocarbons "in vacuum" was performed substantially as described in (*12*).

The apparatus was assembled as shown in Figure 15 (except that the attachment at L'' was not in place), with Apiezon grease on the ground-glass joints. The bomb (evacuated and weighed to 1 mg.) was placed in position. The system comprising the trap, condensing tube, and glass line to the closed valve of bomb, with stopcock K closed, was evacuated and purged with dry air to remove moisture. Cylinder B' containing the major component to be added was then attached to the socket joint, L'', using attachment R (shown in Figure 16). Liquid air or liquid nitrogen was placed in the Dewar flask, F'', surrounding the trap. The glass line to this cylinder was then evacuated.

(If liquid air is used as a refrigerant, it is imperative that any glass vessel containing hydrocarbon or other combustible compounds and immersed in liquid air be protected with a suitable metal shield. When liquid nitrogen is used as a refrigerant, means must be provided to prevent condensation of oxygen in the space between the glass vessel and the metal sheath and subsequent sealing of the space by ice forming at the top of the sheath. The metal sheath must be provided with suitable openings in the sides and bottom. Failure to do this may result in breakage of the glass container when the liquefied oxygen evaporates within the sealed space.)

The appropriate amount of the major component was then introduced from the cylinder into the graduated condensing tube, refrigerated with liquid air or liquid nitrogen, with K' closed. This transfer was made with the cylinder valve and K open, after which transfer both were closed. The gaseous component now contained as a liquid or solid in the condensing tube was cooled to an appropriate low temperature where its vapor

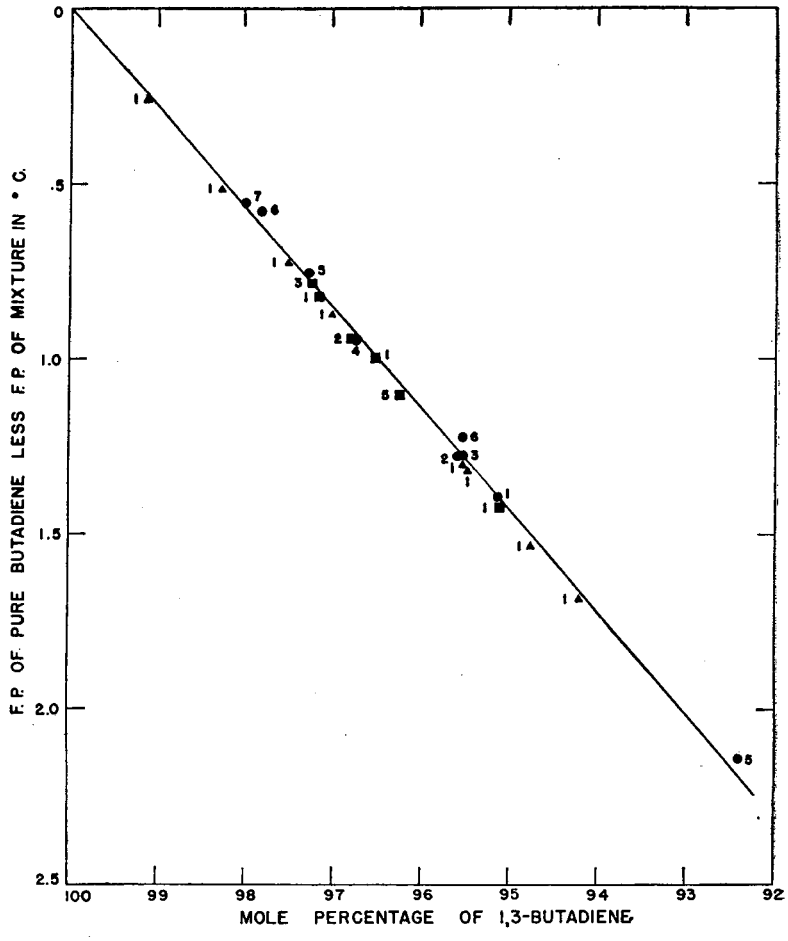


Figure 17. Lowering of Freezing Point of 1,3-Butadiene on Addition of Known Amounts of Known Impurities

Scale of ordinates gives, in ° C., freezing point of 1,3-butadiene of zero impurity less freezing point of mixtures of 1,3-butadiene with known amounts of known impurities. Scale of abscissas gives mole percentage of 1,3-butadiene in mixture. Curve shown is calculated for an ideal solution with 1,3-butadiene as major component, and has a limiting slope of 0.2809° C. per mole %. Experimental values are from following investigations: triangles, Cross, Tamplin, and Shank (*3*); squares, Chemical Division, Standard Oil Development Co. (*9*); and circles, National Bureau of Standards, present report. The solutes used in investigations are identified by following numbers: (1) 1-butene; (2) *cis*-2-butene; (3) *trans*-2-butene; (4) isobutene; (5) 1,2-butadiene; (6) 4-vinyl-1-cyclohexene; (7) styrene

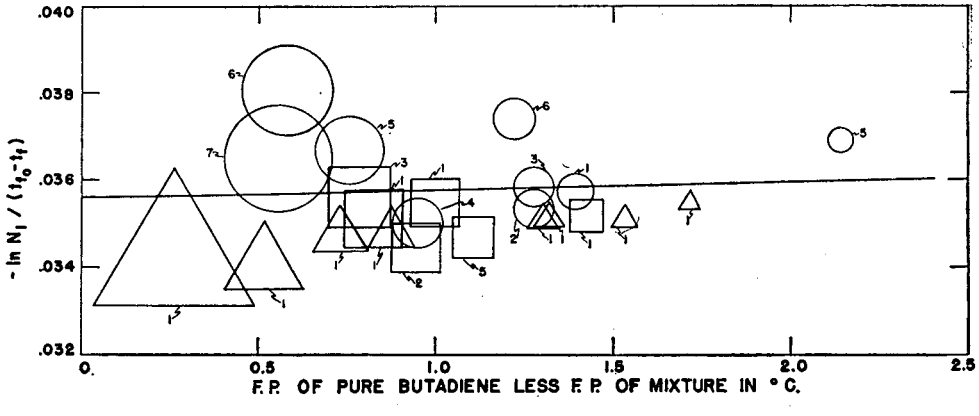


Figure 18. Differential Lowering of Freezing Point of 1,3-Butadiene on Addition of Known Amounts of Known Impurities

Scale of ordinates gives value of $-\ln N_1/(t_{f0} - t_f)$, which is the negative of the natural logarithm of the mole fraction of 1,3-butadiene divided by the difference in freezing points of 1,3-butadiene of zero impurity and 1,3-butadiene with the given impurity. For an ideal solution, $-\ln N_1/(t_{f0} - t_f) = A[1 + B(t_{f0} - t_f)] = A + AB(t_{f0} - t_f)$, where A and B are the first and second cryoscopic constants, respectively (*20*). Scale of abscissas gives value of $(t_{f0} - t_f)$. For a straight line representing data, ordinate at $(t_{f0} - t_f) = 0$ is equal to A and slope is product AB. Experimental data are same as those given in Figure 17

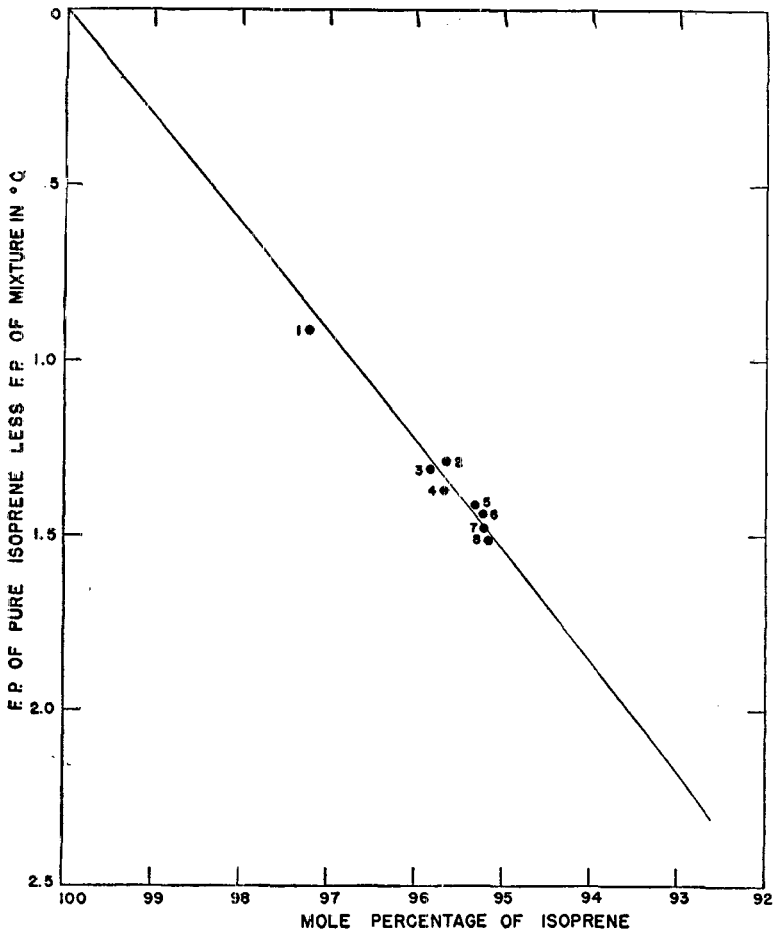


Figure 19. Lowering of Freezing Point of Isoprene on Addition of Known Amounts of Known Impurities

Scale of ordinates gives, in ° C., freezing point of isoprene of zero impurity less freezing point of mixtures of isoprene with known amounts of known impurities. Scale of abscissas gives mole percentages of isoprene in mixtures. Curve shown is that calculated for an ideal solution with isoprene as the major component, and has a limiting slope of 0.303 ° C. per mole %. Circles represent experimental observations of present investigation. Solutes used in this investigation are identified by following numbers: (1) dimethylacetylene; (2) *n*-pentane; (3) *trans*-1,3-pentadiene; (4) 2-methyl-1-butene; (5) *trans*-2-pentene; (6) 1-pentene; (7) 2-methyl-2-butene; (8) *cis*-1,3-pentadiene

pressure was negligible, and the system was degassed. Repeated melting, crystallization, and degassing served to remove all the air from the sample, as described in (*12*).

The sample was transferred from the condensing tube (which was allowed to warm to room temperature after the refrigerating bath was removed) to the refrigerated bomb (previously evacuated and weighed) through the cylinder valve maintained above room temperature as previously described. The refrigerating bath surrounding the bomb was removed and the cylinder allowed to warm to room temperature with the hot air blast from the heater, *Q*. The difference in weight of the bomb before and after addition of the component gave the weight of the added component. A check on the weight of the added component was obtained from the loss in weight of the cylinder, *B'*. Other gaseous components to the bomb from cylinders were added in a similar manner after the previously added component (contained in the bomb) was cooled by refrigeration to a low or negligible vapor pressure. In some cases, the gaseous component may also be added as a refrigerated liquid directly to the condensing tube through cap *J'*.

Known mixtures of volatile liquid components contained in cylinders were prepared in the same manner as gaseous components. Liquid samples contained in ampoules with internal vacuum break-off tips were handled as described in (*12*). Samples in containers of other types, such as bottles, plain ampoules, etc., were introduced into the condensing tube directly (after slight cooling to reduce losses by evaporation).

Known mixtures of gaseous components with volatile liquid components were prepared in the same manner as gaseous components. A mixture of a gas in a liquid of low or moderate vapor pressure was prepared as follows:

The gas under its own vapor pressure was weighed in the bomb, the liquid containing air was weighed in the condensing tube, the liquid was freed of dissolved air (as previously described), and then the gas was transferred to the condensing tube and refrigerated.

Lowering of Freezing Point of 1,3-Butadiene. For these experiments, the compounds used had the following purity, in mole per cent, as determined from measurements of freezing points unless otherwise noted:

1,3,-Butadiene, 99.77, and 1-butene, >99 [by mass spectrometer (*14*)], from the Phillips Petroleum Company, Research Grade; *cis*-2-butene, 99.56, *trans*-2-butene, 98.2 (with impurity largely the cis isomer), and isobutene, 99.78, prepared by J. A. Hinckley on the Koppers Fellowship, Mellon Institute of Industrial Research; styrene, 99.73, from the Dow Chemical Company; 1,2-butadiene 99.92, N.B.S. standard sample; 4-vinyl-1-cyclohexene, 99 (estimated), from A.P.I. Research Project 6, National Bureau of Standards.

The results of the experiments on the lowering of the freezing point of 1,3-butadiene on the addition of

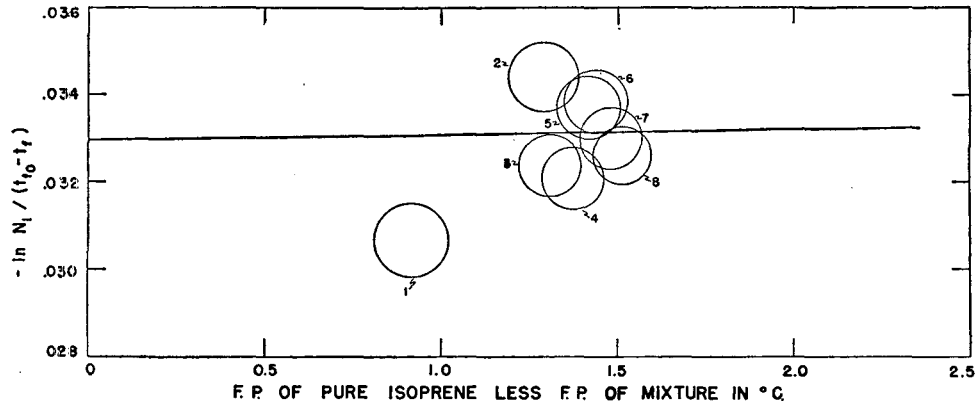
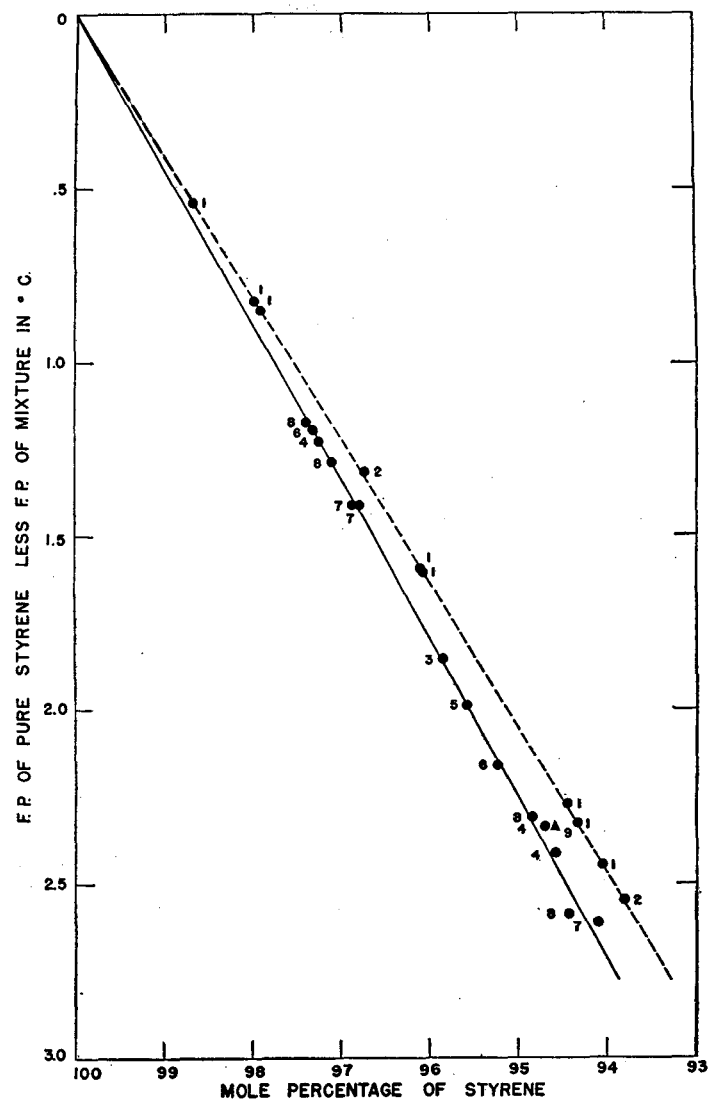


Figure 20. Differential Lowering of Freezing Point of Isoprene on Addition of Known Amounts of Known Impurities

Scale of ordinates gives value of $-\ln N_1/(t_{f_0} - t_f)$, which is the negative of the natural logarithm of the mole fraction of isoprene divided by the difference in freezing points of isoprene of zero impurity and isoprene with the given impurity. For an ideal solution, $-\ln N_1/(t_{f_0} - t_f) = A[1 + B(t_{f_0} - t_f)] = A + AB\ (t_{f_0} - t_f)$, where A and B are the first and second cryoscopic constants, respectively (*20*). Scale of abscissas gives value of $(t_{f_0} - t_f)$. For a straight line representing data, ordinate at $(t_{f_0} - t_f) = 0$ is equal to A and slope is product AB. Experimental data are same as in Figure 19

Figure 21. Lowering of Freezing Point of Styrene on Addition of Known Amounts of Known Impurities

Scale of ordinates gives, in ° C., freezing point of styrene of zero impurity less freezing point of mixtures of styrene with known amounts of known impurities. Scale of abscissas gives mole percentages of styrene in mixtures. Solid curve shown is calculated for an ideal solution with styrene as major component, and has a limiting slope of 0.4444° C. per mole %. Dashed curve is calculated using cryoscopic constants determined in this investigation for styrene with ethylbenzene and toluene as solutes, and has a limiting slope of 0.4032° C. per mole %. Circles and triangle represent experimental observations of present investigation. Solutes used in investigation are identified by following numbers: (1) ethylbenzene; (2) toluene; (3) benzene; (4) 4-vinyl-1-cyclohexene; (5) *n*-propylbenzene; (6) isopropylbenzene; (7) *o*-xylene; (8) *m*-xylene; (9) ethylbenzene, 2.975 mole %, plus 4-vinyl-1-cyclohexene, 2.445 mole %



known amounts, up to about 6 mole %, of each of the four butenes, 1,2-butadiene, styrene, and 4-vinyl-1-cyclohexene, are shown in Figures 17 and 18. The seven solutes selected to serve as impurities are the most probable ones as determined in an exhaustive analysis of "recycle butadiene" (*6*, *19*). Included in the plots in Figures 17 and 18 are some additional data from the Carbide and Carbon Chemical Corporation (*3*) and the Standard Oil Development Company (*9*).

The lines in Figures 17 and 18 are given by the ideal values of the cryoscopic constants, as follows: $A = 0.03560$ deg.$^{-1}$; $B = 0.0053$ deg.$^{-1}$ (*1*, *16*, *18*). The experimental data on the lowering of the freezing point of 1,3-butadiene on the addition of known amounts of its most probable impurities are in accord, within the limits of uncertainty, with the relations called for by the ideal solution law.

Lowering of the Freezing Point of Isoprene. For these experiments, the compounds used had the following purity, in mole per cent, as determined from measurements of freezing points unless otherwise noted:

2-Methyl-1,3-butadiene (isoprene), 99.79, *n*-pentane, 99.89, from A.P.I. Research Project 6, National Bureau of Standards; *cis*-1,3-pentadiene, greater than 99, and *trans*-1,3-pentadiene, 85 (with the impurity substantially all the cis isomer), from the University of Illinois *5*); 2-methyl-2-butene, 99.93, and 2-methyl-1-butene, 99 (estimated), from the Atlantic Refining Company; *trans*-2-pentene, 80 (with impurity substantially all the cis isomer), from the Standard Oil Company (Indiana); 1-pentene, 99.34, from the Phillips Petroleum Company, Research Grade; 2-butyne (dimethylacetylene), 99.93, N.B.S. standard sample.

The results of the experiments on the lowering of the freezing point of isoprene on the addition of known amounts, up to about 5 mole %, of *n*-pentane, *trans*-1,3-pentadiene, *cis*-1,3-pentadiene, *trans*-2-pentene, 1-pentene, 2-methyl-1-butene, 2-methyl-2-butene, and dimethylacetylene, are shown in Figures 19 and 20. With the exception of *n*-pentane, all these compounds are ones that are most likely to occur in commercially prepared isoprene (*10*, *14*).

The lines in Figures 19 and 20 are given by the following values of the cryoscopic constants: $A = 0.0330$ deg.$^{-1}$; $B = 0.0030$ deg^{-1}. The value of B was calculated (*1*) from the calorimetric data from (*2*) combined with the value of t_{f_0} from the present investigation. With the value of B thus fixed, the value of A, the first or main cryoscopic constant, was determined from the

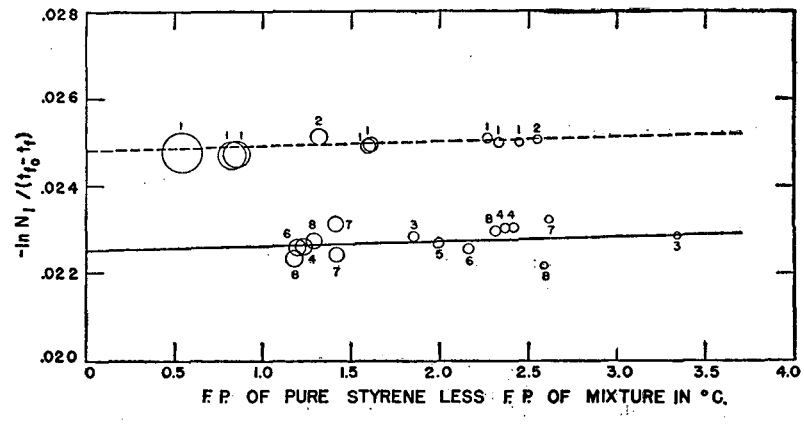


Figure 22. Differential Lowering of Freezing Point of Styrene on Addition of Known Amounts of Known Impurities

Scale of ordinates gives value of $-\ln N_1/(t_{f_0} - t_f)$, which is the negative of the natural logarithm of the mole fraction of styrene divided by the difference in freezing points of styrene of zero impurity and styrene with the given impurity. For an ideal solution, $-\ln N_1/(t_{f_0} - t_f) = A[1 + B(t_{f_0} - t_f)] = A + AB(t_{f_0} - t_f)$, where A and B are first and second cryoscopic constants, respectively (*20*). Scale of abscissas gives value of $(t_{f_0} - t_f)$. For a straight line representing data, ordinate at $(t_{f_0} - t_f) = 0$ is equal to A and slope is product AB. Solid line represents experimental observations for benzene, 4-vinyl-1-cyclohexene, *n*-propylbenzene, isopropylbenzene, *o*-xylene, and *m*-xylene as solutes. Dashed line represents data for ethylbenzene and toluene as solutes. Experimental data are same as in Figure 21

experimental data of the present investigation. The calorimetrically determined heat of fusion of isoprene reported in (*2*) was obtained on the then best available isoprene, which however, contained a significant amount of impurity; and the value of A calculated with the reported heat of fusion differs from that determined by direct measurement in the present investigation by an amount which is probably due to the impurities in the sample reported in 1937.

The experimental data of the present investigation show that all the solutes measured, including compounds of the paraffin, mono-olefin, diolefin, and acetylene classes, produce substantially the same lowering of the freezing point of isoprene.

Lowering of Freezing Point of Styrene. For these measurements, the compounds used had the following purity, in mole per cent, as determined from measurements of freezing points unless otherwise noted:

Styrene, 99.73, from the Dow Chemical Company; 4-vinyl-1-cyclohexene, 99 (estimated), from A.P.I. Research Project 6, National Bureau of Standards; benzene, 99.96, toluene, 99.90, ethylbenzene, 99.54, *o*-xylene, 99.90, *m*-xylene, 99.71, *n*-propylbenzene, 99.72, isopropylbenzene, 99.96, from A.P.I. Research Project 6, National Bureau of Standards (*4*).

The results of the experiments on the lowering of the freezing point of styrene on the addition of known amounts, up to 6 mole %, of benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, each of the two propylbenzenes, and 4-vinyl-1-cyclohexene, are shown in Figures 21 and 22. The compounds selected as solutes include the ones most likely to occur in commercial styrene as shown by an exhaustive analysis of "recycle styrene" (*7*).

The solid lower line in Figure 21 and the lower line in Figure 22 are given by the ideal values of the cryoscopic constants, as follows: $A = 0.02250$ deg.$^{-1}$; $B = 0.0044$ deg.$^{-1}$ (*1*, *16*, *17*). The experimental data involving all the solutes except ethylbenzene and toluene are seen to be in accord, within the limits of uncertainty, with the relations required by the ideal solution law.

The dashed upper line in Figure 21 and the dashed upper line in Figure 22, representing the data for ethylbenzene and toluene, are given by the following values of the cryoscopic constants: $A = 0.02480$ deg.$^{-1}$; $B = 0.0044$ deg.$^{-1}$. The value of B was taken the same as for the ideal system, and the value of A was fixed by the data for ethylbenzene and toluene from the present investigation. Ethylbenzene and toluene, for a given mole fraction in styrene, produce a lowering of the freezing point of styrene about 10% less than that required by the ideal solution law.

EVALUATION OF PURITY FROM FREEZING POINTS

For calculating the purity of a given compound from measurement of the freezing point, it is convenient to transform Equation 2 to the following form:

$$\log_{10} p = 2.00000 - (A/2.30259)\,(t_{f_0} - t_f)\,[1 + B\,(t_{f_0} - t_f)] \quad (7)$$

In Equation 7, p is the purity in mole per cent; t_f is the freezing point, in air at 1 atmosphere, determined as described in discussion of determination of freezing points; t_{f_0} is the value of the freezing point for zero impurity, in air at 1 atmosphere, as given in discussion of freezing points for zero impurity; and A and B are the first and second cryoscopic constants, as described in discussion of lowering of freezing point.

In those cases where the value of $t_{f_0} - t_f$ is not large and the sample is of high purity, the second cryoscopic constant, B, may be neglected without significant error, and Equation 7 reduces to

$$\log_{10} p = 2.00000 - (A/2.30259)\,(t_{f_0} - t_f) \quad (8)$$

For very small values to $t_{f_0} - t_f$, Equation 8 may be further reduced to

$$p = 100\,[1 - A(t_{f_0} - t_f)] \quad (9)$$

In Table II are summarized the values of the several constants, t_{f_0}, A, and B, which may be used for evaluating the purity from measurements of freezing points of 1,3-butadiene, isoprene, styrene, isobutene, *cis*-2-butene, *trans*-2-butene, *n*-butane, isobutane, and methyl chloride.

When the impurity in the styrene is a mixture of ethylbenzene plus toluene, on the one hand, and other hydrocarbons that give a substantially ideal lowering, on the other hand, the proper value of the cryoscopic constant, A, to use varies in proportion to the relative fraction of the impurity which is ethylbenzene plus toluene—that is, if x is the relative fraction (0 to 1.0) of the impurity (in the styrene) which is ethylbenzene plus toluene, the appropriate value of the cryoscopic constant is $A = (1 + 0.102x)\,(0.02250)$ deg.$^{-1}$ (*10*).

ACKNOWLEDGMENT

The authors wish to express their gratitude to the several laboratories and individuals named above for supplying materials for use in this investigation.

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RECEIVED August 28, 1947. Presented before the Division of Analytical and Micro Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Intercomparison of Beckman Spectrophotometers

GALEN W. EWING[1]
Sterling-Winthrop Research Institute, Rensselaer, N. Y.
AND
THEOPHILUS PARSONS, JR.
E. I. du Pont de Nemours and Co., New Brunswick, N. J.

A comparative study has been made of ten Beckman quartz spectrophotometers using potassium acid phthalate as a standard reference material. The samples have been weighed and dissolved by an identical procedure in the several laboratories. It is found that the precision to be expected of any one instrument is considerably better than the agreement with other similar instruments. An assay to be valid should be compared with a standard determined on the same spectrophotometer, or corrected by a factor determined in advance for that instrument.

IN THE course of routine analyses on chemical intermediates, it was found that precise checks could not be obtained between determinations in two laboratories using standard solutions and presumably identical Beckman spectrophotometers (*1*). To track down the source of the discrepancies, a series of careful measurements was made on one sample of standard material on both instruments, and this work was later extended to other laboratories to cover a total of ten instruments.

Potassium acid phthalate, which was chosen as the reference material, shows the following advantages: It is easily purified and is stable enough to retain its purity for an indefinite period under usual laboratory conditions. It has a single, rather broad absorption maximum at approximately 281 mμ wave length, and a well defined, broad minimum at 264 mμ, both in the range of convenient operation of the spectrophotometer, and both sufficiently broad that small differences in slit widths and small inaccuracies in the wave-length scale will not have an appreciable effect on the result. The extinctions of the maximum and minimum are close enough together, so that both points can be measured on the same solution, with optical densities all within the range 0.4 to 0.7 (*2*). The sample studied was Mallinckrodt's primary standard analytical reagent. It was checked spectrophotometrically against Bureau of Standards material, from which it showed no appreciable difference.

Portions of this material in the solid state were sent to each participating laboratory where they were weighed out and dissolved by an identical procedure.

Weigh out accurately, directly from the bottle without drying, a sample of approximately 0.4000 ± 0.0015 gram. Dissolve in 100-ml. of distilled water in a 100-ml. volumetric flask. After thorough shaking withdraw by pipet a 10-ml. sample and place in another 100-ml. volumetric flask. Make up to volume with distilled water and shake well. From the second volumetric flask withdraw by pipet a 25-ml. sample and dilute by a similar procedure to 100 ml. Thus the final concentration is about 0.1000 gram of potassium acid phthalate per liter. Run this last solution in the Beckman spectrophotometer against a water blank, using 1-cm. silica cells. Use wave lengths of 264, 265, 266, 280, 281, and 282 mμ. Adjust the slit to give a band width of 1 mμ, or as near this value as possible. Calculate a constant, K, for each wave length by the formula

$$K = D/c$$

where D is the optical density and c is the concentration in grams per liter.

In each case corrections for the particular absorption cells used were obtained by filling both reference and test cells with distilled water and determining the optical density of the test with respect to the reference cell at both 264 and 281 mμ wave lengths. The figure so obtained was subtracted from the observed densities of the phthalate solutions.

No attempt was made to control the temperature of the apparatus and solutions. A rough calculation shows that the effect of normal variations in room temperature with respect to the expansion coefficients of glassware, etc., will be negligible. To test the change in the absorption of phthalate with small temperature changes, an experiment was performed with a solution of potassium acid phthalate in special absorption cells provided with jackets for the circulation of water of controlled temperature (Table I). These cells were 100 mm. in length; hence the solution used was one tenth the concentration specified for the comparative experiments. Any differences due to temperature changes are entirely within the limits of experimental error. The actual values of $K_{max.}$ in this experiment are considerably lower than in the comparative experiments described. This is due to failure of Beer's law over the tenfold concentration change involved; it does not invalidate the comparative determinations, which are all made at the same concentration.

The maximum error which might be introduced into the measurements through cumulative errors in glassware and

[1] Present address, Department of Chemistry, Union College, Schenectady, N. Y.

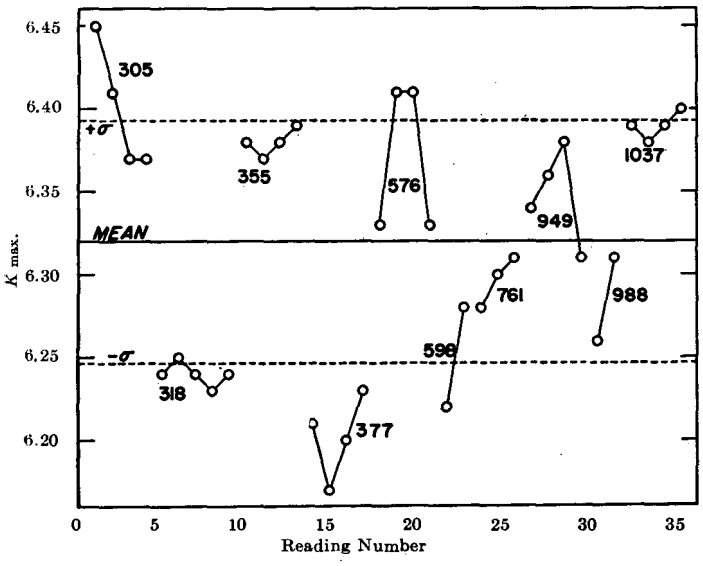

Figure 1. Extinction Coefficients of Potassium Acid Phthalate at Wave Length of Maximum Absorption

Serial number of the spectrophotometer is indicated adjacent to each group of determinations

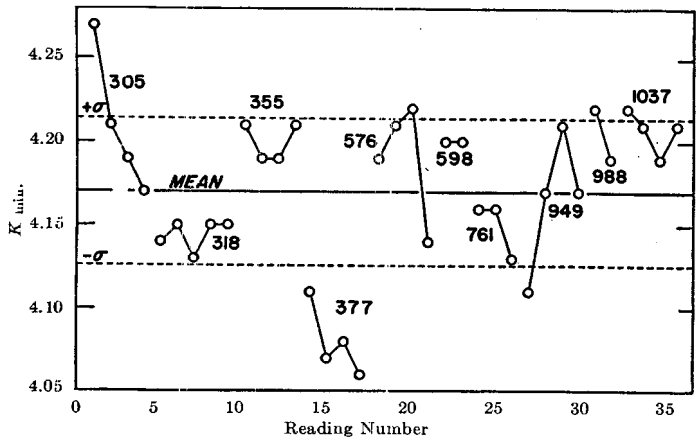

Figure 2. Extinction Coefficients of Potassium Acid Phthalate at Wave Length of Minimum Absorption

Serial number of spectrophotometer is indicated adjacent to each group of determinations

analytical balance is considerably less than 0.5%, based on the following specifications:

10-ml. pipet, tolerance	±0.02 ml.
25-ml. pipet, tolerance	0.03 ml.
100-ml. flask, tolerance	0.08 ml.
Balance, precision	0.05 mg.

The data obtained on the ten instruments are given in Table II, arranged according to serial numbers. The original data are given for each of the several solutions made up from the crystalline material in each laboratory, averaged in each case in the columns headed "Mean." The columns headed "Dev." show the deviation of the mean of the readings for each instrument from the mean of the individual means for all the instruments. The average deviations of the individual means from the mean of the means are, for the minimum, 0.77%, for the maximum, 0.95%, and for the ratio, 0.63%.

These thirty-six individual readings have been plotted in Figures 1 and 2 for the maximum and minimum, respectively. The values of the arithmetic means and of the standard deviations —i.e., $\pm\sigma$—are indicated by horizontal lines. The standard deviations were calculated according to the method of Worthing and Geffner (*4*).

CONCLUSIONS

Both table and graphs indicate that the agreement among the several readings taken on any one instrument is usually much better than the agreement among the various instruments. The constancy of the ratio of maximum to minimum extinction is only slightly better than the absolute values themselves.

It is evident from these results that any absolute assay which is undertaken using the Beckman spectrophotometer will be subject to an uncertainty greater than that indicated by the limits of precision of each individual instrument. This is presumably due to slight differences of manufacture inevitable in a sensitive instrument of this type. Error from this source may be avoided by comparing each instrument with the statistical mean of a large number of instruments, to determine a correction factor. In the absence of any such general statistical mean, two laboratories may eliminate these instrumental errors by using an appropriate secondary standard, such as potassium acid phthalate, as a basis for comparison. A continuing comparison would also furnish evidence of procedural or instrumental changes taking place in either of the laboratories.

The present study is in general agreement with the findings of the Vitamin Oil Producers Institute (*3*), which has reported data taken on nineteen Beckman spectrophotometers as well as other types of apparatus. Of these nineteen, six fall consistently within ±1.5% of the mean, six fall between ±1.5 and ±3.0%, and the remaining seven are erratic in that some of the five samples studied showed good agreement and some poor.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the cooperation of the following individuals and of their organizations in making determinations for this study; in many cases they have made valuable sugges-

Table I. Effect of Temperature on Extinction Coefficients of Potassium Acid Phthalate

Wave Length, mμ	$K^{20°}$	$K^{30°}$
264, min.	4.24	4.27
281, max.	5.70	5.72

Table II. Extinction Coefficients of Potassium Acid Phthalate Determined on Various Beckman Spectrophotometers

Serial No.	Sample No.	$K_{min.}$ Detns.	$K_{min.}$ Mean	$K_{min.}$ Dev.	$K_{max.}$ Detns.	$K_{max.}$ Mean	$K_{max.}$ Dev.	$K_{max.}/K_{min.}$ Ratio	$K_{max.}/K_{min.}$ Dev.
305	1	4.27	4.210	0.035	6.45	6.385	0.071	1.517	0.002
	2	4.21			6.41				
	3	4.19			6.37				
	4	4.17			6.31				
318	1	4.14	4.144	0.031	6.24	6.240	0.074	1.506	0.006
	2	4.15			6.25				
	3	4.13			6.24				
	4	4.15			6.23				
	5	4.15			6.24				
355	1	4.21	4.200	0.025	6.38	6.377	0.063	1.518	0.006
	2	4.19			6.37				
	3	4.19			6.38				
	4	4.21			6.39				
377	1	4.11	4.080	0.095	6.21	6.203	0.111	1.520	0.008
	2	4.07			6.17				
	3	4.08			6.20				
	4[a]	4.06			6.23				
576	1	4.19	4.190	0.015	6.33	6.370	0.056	1.520	0.008
	2	4.21			6.41				
	3	4.22			6.41				
	4[b]	4.14			6.33				
598	1	4.20	4.200	0.025	6.22	6.250	0.064	1.488	0.024
	2	4.20			6.28				
761[c]	1	4.16	4.150	0.025	6.28	6.296	0.018	1.517	0.005
	2	4.16			6.30				
	3	4.13			6.31				
949[d]	1	4.11	4.165	0.010	6.34	6.348	0.034	1.524	0.012
	2	4.17			6.36				
	3	4.21			6.38				
	4	4.17			6.31				
988[e]	1	4.22	4.205	0.030	6.26	6.282	0.032	1.494	0.018
	2	4.19			6.31				
1037[f]	1	4.22	4.207	0.032	6.39	6.390	0.076	1.519	0.007
	2	4.21			6.38				
	3	4.19			6.39				
	4	4.21			6.40				

[a] Same weighed sample used for determination of data for Table I, appropriately diluted for 10-mm. cells. [b] Average of four determinations made 7 months later than others reported for this instrument. [c] Same solutions as on No. 377, same cells, same operator. [d] Same solutions as on No. 305. [e] Same solutions as on No. 598, different operators. [f] Same solutions as on No. 355.

tions as well. The serial numbers of the spectrophotometers are included in parentheses:

A. Black, E. R. Squibb and Sons, New Brunswick, N. J. (598, 988)
Irving M. Klotz, Northwestern University, Evanston, Ill. (318)
Kenneth Morgareidge, National Oil Products Co., Harrison, N. J. (305, 949)
J. M. Vandenbelt, Parke, Davis and Co., Detroit, Mich. (355, 1037)
Instruments 377 and 761 are in the laboratory of the Sterling-Winthrop Research Institute, and No. 576 is in that of E. I. du Pont de Nemours and Co.

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RECEIVED July 18, 1946.

Preparation of Standard Chromous Sulfate or Chromous Chloride Solutions of Determinate Concentration

JAMES J. LINGANE AND ROBERT L. PECSOK
Department of Chemistry, Harvard University, Cambridge 38, Mass.

A simple technique for preparing standard chromous sulfate solutions by complete reduction of chromic ion in very dilute sulfuric acid with zinc is described. The solution is stored under hydrogen in contact with amalgamated zinc in the same vessel in which it is reduced and is stable for several weeks. Chromous sulfate (or chromous chloride) solutions of an exactly specified concentration, which do not require standardization, may be prepared determinately from pure potassium dichromate. The potentiometric titration of +2 copper in 4 to 6 *N* hydrochloric acid with 0.1 *N* chromous ion is discussed, and data show that it is accurate to ±0.1% or better. The direct potentiometric titration of dichromate ion with chromous ion in dilute sulfuric acid is not a satisfactory standardization method, but excellent results are obtained by adding excess ferrous ion and titrating the resulting ferric ion.

THE standard potential of the couple $Cr^{+++} + e = Cr^{++}$ is -0.40 volt *vs.* the standard hydrogen electrode (*5*, *6*), and chromous ion is the most powerful reductant used in the form of a standard solution in volumetric analysis. Because of this fact the preparation and storage of chromous solutions necessitate special care, and the preparation of a standard solution of an exactly determinate strength has not heretofore been described.

Chromous ion is so very easily air-oxidized

$$4Cr^{++} + O_2 + 4H^+ = 4Cr^{+++} + 2H_2O \qquad (1)$$

that the solution must be stored and delivered from the measuring buret under an inert gas (usually hydrogen), and the solution being titrated must also be scrupulously freed from dissolved air. Furthermore, chromous ion is a powerful enough reductant to reduce hydrogen ion

$$2Cr^{++} + 2H^+ = 2Cr^{+++} + H_2;\ K = 10^{+13} \qquad (2)$$

and acid chromous solutions are metastable. The oxidation of chromous ion by hydrogen ion is very slow when the solution in dilute sulfuric or hydrochloric acid is composited from very pure materials, but Reaction 2 is strongly catalyzed by various substances, particularly platinum and other finely divided metals (*1*, *9*). Hence chromous solutions must be prepared from very pure materials that are free of foreign heavy metals.

Forbes and Richter (*5*), who made the first reliable measurement of the chromic-chromous potential, prepared chromous chloride by reducing resublimed chromic chloride with hydrogen at 400° C. and dissolving the solid salt in appropriate acid solutions under carbon dioxide. Dimroth and Frister (*4*), who introduced standard chromous solutions in volumetric analysis, also employed chromous chloride. Traube, Burmeister, and Stahn (*16*), Grube and Schlecht (*7*), and Asmanow (*1*) prepared chromous salts by electrolytic reduction of chromic solutions; Asmanow precipitated chromous sulfate pentahydrate from the reduced solution with alcohol, and used this salt to prepare standard solutions. Buehrer and Schupp (*3*) started with potassium dichromate which they first reduced to the chromic state with hot, concentrated hydrochloric acid; the diluted chromic solution was finally reduced to the chromous state with pure zinc and then transferred to the storage bottle under a layer of kerosene. Buehrer and Schupp reported that their solution decreased in titer at the rate of about 1% per week.

Rienäcker (*12*, *17*) first prepared pure chromous acetate by reducing chromic solutions with zinc and then precipitating the resulting chromous solution with sodium acetate. The chromous acetate was then washed thoroughly with water, finally dissolved in only a very slight excess of hydrochloric acid, and transferred to the storage bottle under hydrogen. Rienäcker found that a solution thus prepared decreased in titer only about 0.1% per week. The Rienäcker procedure appears to be the best of those recommended up to 1932, but the fact that all the manipulations involved must be carried out under hydrogen renders it inconvenient.

Thornton and Sadusk (*14*) prepared chromous sulfate solutions in dilute sulfuric acid (approximately 0.18 *N*) by reducing solutions of potassium dichromate with amalgamated zinc in a Jones-type reductor; they obtained only 67% reduction to the chromous state, but reported that the solution "did not undergo an appreciable change of titer during a period of 2 months" when stored under carbon dioxide in the storage apparatus of Thornton and Wood (*15*). The reduction of chromic solutions by flowing them under carbon dioxide through a Jones-type reductor containing amalgamated zinc was later investigated by Stone and Beeson (*13*), who found that violet chromic solutions in dilute sulfuric acid were reduced more rapidly by zinc than solutions containing the green modification, and that 90 to 100% yields of chromous chromium could be obtained when violet chrome alum solutions were used instead of dichromate solution.

The method described herein employs reduction of chromic solutions by amalgamated zinc, but it possesses the following advantages over previous procedures: The solution is reduced and stored in the same vessel, and the apparatus is much simpler than any previously described; a solution containing the chromium entirely in the +2 state is obtained; and by starting with pure potassium dichromate (or any chromic salt of known purity) a

solution of an exactly specified titer, which does not require standardization, may be prepared.

APPARATUS AND TECHNIQUE

The apparatus shown in Figure 1 consists of a 1-liter round-bottomed flask, one half to two thirds full of the purest amalgamated mossy zinc, connected to a 50-cc. dispensing buret by a long glass tube and a short length of gum rubber tubing closed by a screw clamp (not a pinch clamp). The solution is kept under pure hydrogen obtained from a small Kipp generator, and freed from oxygen by passage through a U-tube containing a little chromous sulfate solution in approximately 0.1 N sulfuric acid in contact with amalgamated mossy zinc. Very little hydrogen is required.

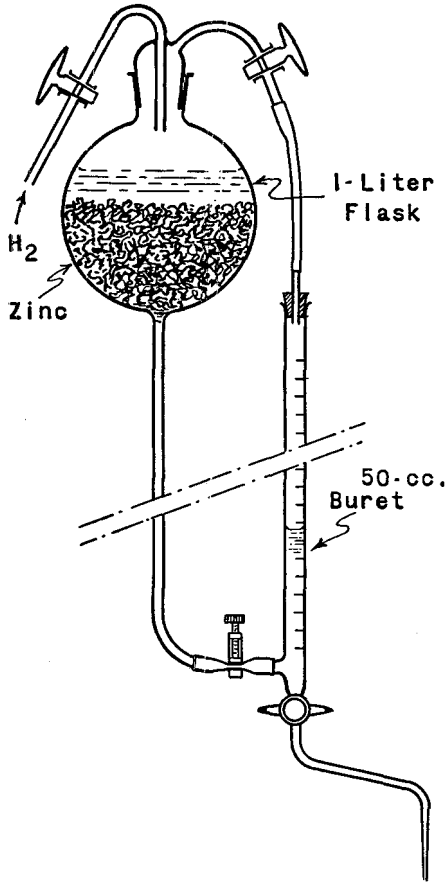

Figure 1. Apparatus for Preparation, Storage, and Dispensing of Standard Chromous Sulfate Solutions

The buret is easily removable for cleaning without disturbing the solution in the storage flask. It is partly for this reason, and also because the flow of solution past a greased stopcock quickly fouls a buret, that connection to the delivery tube from the storage flask is made with a short length of rubber tubing and screw clamp, rather than by an all-glass line.

The storage flask is supported on a rubber-covered ring, and the buret by two buret clamps, one at the very top and the other just below the 50-cc. mark. The apparatus is held by a heavy tripod-base support rod, and is easily portable.

The zinc used must be very pure, and it should be of the mossy variety. Before use it is amalgamated with about 1% mercury by stirring for a few minutes in a mercuric chloride solution in dilute hydrochloric acid and then washing with pure water. Merck or Mallinckrodt "reagent quality" zinc free from arsenic, lead, and iron, was found to be satisfactory. About 1 kg. is required to half-fill a 1-liter storage flask, and about 200 cc. of solution are contained in the interstices among the pieces, so that 600 to 700 cc. of solution can be placed in the flask at each filling.

To prepare an exactly 0.1000 N chromous sulfate solution in 0.1 N sulfuric acid, dissolve 29.421 grams of pure, dried potassium dichromate in about 500 cc. of water in a 2-liter Erlenmeyer flask and acidify with 27.8 cc. of concentrated (36 N) sulfuric acid. Place a long-stemmed funnel in the neck of the flask and add slowly in several small portions about 75 cc. (twofold excess) of pure 30% hydrogen peroxide, which reduces the dichromate to chromic ion. Heat the solution to incipient boiling until evolution of oxygen ceases (about 20 minutes). Cool to room temperature, transfer to a 2-liter volumetric flask, and dilute to the mark. This solution will be approximately 0.1 N (0.05 M) in respect to sulfuric acid. Rinse out the storage flask with two 100-cc. portions of the solution, and then fill it almost full with the solution. Close the flask and connect it to the Kipp generator. It is not necessary to sweep out the solution or storage flask with hydrogen; oxygen is very quickly consumed by the solution itself as it undergoes reduction, and hence only a very small amount of hydrogen is required to keep the solution protected from air and to replace the solution withdrawn.

If a solution that is 1 N rather than 0.1 N in respect to sulfuric acid is desired, add 50.0 cc. more of concentrated sulfuric acid before the final dilution to 2 liters.

Solutions of chromous chloride in 0.1 N hydrochloric acid were prepared in the same manner by using the appropriate amount of hydrochloric acid instead of sulfuric acid.

The time required for complete reduction depends on the relative volumes of solution and zinc. If the solution just covers the zinc, reduction is complete in about 30 minutes. When the flask is nearly filled with solution, reduction is complete after a few hours if the flask is shaken occasionally to promote contact with the zinc. Complete reduction is easily judged by the appearance of the characteristic pale sky blue color of chromous ion without any tinge of green.

Solutions prepared in this way were stable for about a week when the initial concentration of sulfuric or hydrochloric acid was 0.1 N, and for about 3 weeks when the initial sulfuric acid concentration was 1 N. The period of stability is limited by the gradual reduction of hydrogen ion by the zinc, which finally raises the pH to such a value that hydrolytic precipitation of the chromous ion occurs. The solutions are so easily prepared that long storage is not an important consideration.

Because of the deep green color of chromic ion, titrations with 0.1 N chromous solutions must be carried out potentiometrically, but a very simple potentiometer suffices because the potential change at the end point is usually very great.

An ordinary 200-cc. three-necked balloon flask serves as a convenient titration vessel, as shown in Figure 2. The gas inlet tube used to bubble carbon dioxide or other inert gas through the solution to exclude air is held by a two-hole rubber stopper in the central neck, and a small thermometer placed in the second hole of the stopper is convenient when titrations are made at an ele-

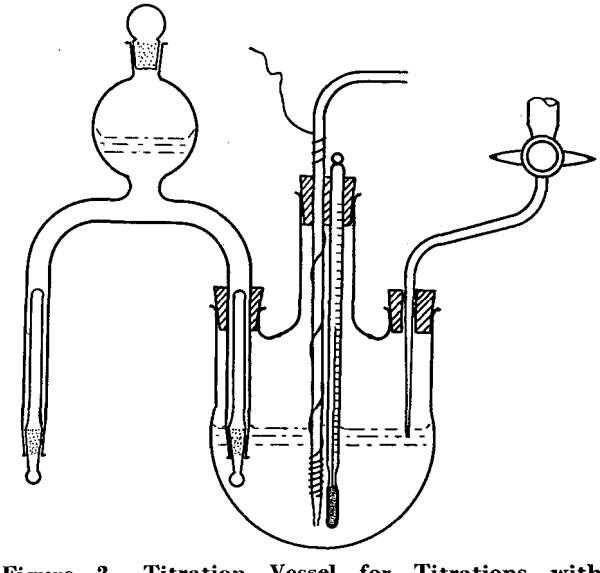

Figure 2. Titration Vessel for Titrations with Chromous Solutions

vated temperature. The platinum wire which serves as indicator electrode is wound as a spiral on the bottom of the gas inlet tube. One of the side necks carries a salt bridge with ends closed by internal ground-glass plugs of the type described by Irving and Smith (*8*), for connection to the saturated calomel reference electrode. Usually this bridge was filled with 1 to 2 *N* sulfuric acid. The outer end of the bridge is placed in a saturated potassium chloride solution in a small beaker, in which the side arm of a saturated calomel electrode dips. The buret tip passes through a slightly oversize hole in a one-hole stopper in the third neck; the oversize hole serves as the gas exit.

The carbon dioxide used to remove dissolved air from the titration vessel is freed from traces of oxygen by passage through a large U-tube half full of amalgamated mossy zinc containing some chromous sulfate solution in very dilute sulfuric acid. The solution to be titrated is swept out with carbon dioxide for 15 to 20 minutes before the start of a titration, and the carbon dioxide stream serves for stirring during the titration. When precipitates are produced mechanical stirring is desirable.

STANDARDIZATION

To verify the fact that a chromous solution prepared as described above does not require standardization, an exactly 0.1000 *M* solution was prepared and standardized against copper and potassium dichromate. As far as the writers are aware this is the first time that the stoichiometric accuracy of these titrations has been established directly, because chromous solutions used by previous investigators were not made up determinately, but were standardized against some one substance and then used to titrate others.

Standardization against Copper. In agreement with Rienäcker (*12, 17*), the authors found that the titration of +2 copper (present as $CuCl_4^{--}$ ion) in a solution containing a large concentration of chloride ion yields excellent results. The complex tetrachlorocuprate ion is reduced first to a chloro complex of the +1 state (either $CuCl_2^-$ or $CuCl_3^{--}$ ion) and then to metallic copper,

$$CuCl_4^{--} + e = CuCl_2^- + 2Cl^- \quad (3)$$

$$CuCl_2^- + e = Cu + 2Cl^- \quad (4)$$

A typical titration curve obtained in a titration at room temperature in a solution containing 5 *N* hydrochloric acid at the start, and about 4 *N* hydrochloric acid at the second end point, is shown as curve 1 in Figure 3. Both stages of reduction are very clearly defined; the change in potential after the completion of Reaction 3 is very great and the end point can be located precisely. The change in potential at the completion of Reaction 4 is much smaller, and hence it is less suitable for analytical purposes.

Rienäcker (*12*) claimed that Reaction 4 was not quantitative and that the second end point could not be observed. Contrary to this conclusion, the authors found that the second end point does occur exactly at the stoichiometric equivalence point for the completion of Reaction 4. Rienäcker's failure to observe the second end point was probably due to the fact that he titrated too rapidly and did not wait for the attainment of steady potentials; the titration curves he shows appear to be symmetrical at the first end point, whereas actually the curve at the first end point is highly unsymmetrical (see Figure 3). After the large decrease in potential corresponding to completion of Reaction 3, the potential remains almost constant (actually increases very slightly) until the second end point is closely approached, and the titration curve displays a very sharp right-angle bend as seen in Figure 3.

This is the expected behavior, because during the second stage of the titration, when the chloride ion concentration is so large that it is virtually constant, the potential is governed entirely by the activity of the $CuCl_2^-$ ion, rather than by a ratio of activities. Furthermore, when the potential has decreased to a negative value against the standard hydrogen electrode—i.e., after Reaction 3 is complete—hydrogen ion tends to be reduced by $CuCl_2^-$ ion at the surface of the platinum indicator electrode,

$$2H^+ + 2CuCl_2^- + 4Cl^- = H_2 + 2CuCl_4^{--} \quad (5)$$

This reaction causes the observed potential to be somewhat too positive, and may also be partly responsible for the virtual constancy of the potential beyond the first end point.

The stoichiometric equivalence point of Reaction 3 does not correspond exactly with the position of the sharp bend, because at the bend Reaction 3 has been completed and Reaction 4 has already begun to proceed, so that the true equivalence point potential is more positive than the potential at the bend. The titration curve shows that the potential of Reaction 3 in 6 *N* hydrochloric acid is very near +0.48 volt against the standard hydrogen electrode, and the chromic-chromous potential appears to be close to −0.33 volt. Therefore, the equivalence point potential must be close to +0.07 volt (with a probable uncertainty of ±0.05 volt) against the standard hydrogen electrode, whereas the bend occurs between −0.1 and −0.15 volt.

The potential becomes steady very quickly during the titration, except right at the end point, where 2 to 3 minutes are required. The titration to the first equivalence point can be completed rapidly and precisely by simply titrating to an end-point potential of −0.15 ± 0.05 volt against the saturated calomel electrode (0.10 ± 0.05 volt against the standard hydrogen electrode).

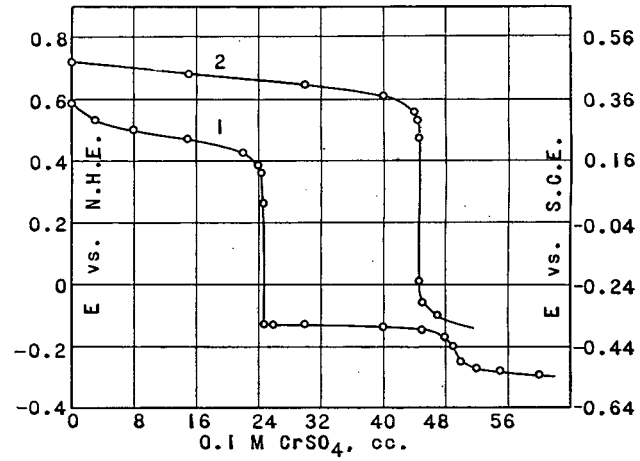

Figure 3. Titration Curves

1. Cupric copper in 5 *N* hydrochloric acid at room temperature
2. Ferric iron in 1 *N* sulfuric acid, obtained in standardization of chromous sulfate solution against potassium dichromate to which excess ferrous ammonium sulfate had been added

A standard copper solution was prepared by dissolving exactly 0.1000 mole of pure cupric sulfate pentahydrate in water and diluting to exactly 1 liter. Electrogravimetric determination of the copper in this solution by a method previously described (*11*) showed a molarity (normality for Reaction 3) of 0.10005. Portions of this solution (50 cc.) were mixed with 50 cc. of concentrated (12 *N*) hydrochloric acid and titrated at room temperature with the chromous sulfate solution under carbon dioxide. In two titrations, 50.06 and 49.97 cc. of the chromous solution were required, compared to the theoretical 50.03 cc. Thus the apparent normality of the chromous sulfate solution against copper agreed with the determinate normality to within ±0.1%.

Standardization against Potassium Dichromate. Although the direct titration of dichromate ion in 3 to 5% sulfuric acid has been recommended for the standardization of chromous solutions (*2, 3, 10, 18*), it is the authors' experience that this titration is unsatisfactory for this purpose. Because the dichromate ion–chromic ion couple does not function at all reversibly, the titration curve at the equivalence point is very asymmetrical, and no warning of the approach to the end point is obtained. Furthermore, in several titrations the potential underwent large and very erratic fluctuations near the equivalence point, and the latter could not be located with the degree of precision desirable in a

standardization. Heating the solution to 65° during the titration caused only a slight improvement. Therefore, the standardization against potassium dichromate was carried out by adding a slight excess of ferrous ion to the dichromate solution in 1 *N* sulfuric acid, and titrating the resulting ferric ion with the chromous solution. The titration of ferric ion is an excellent one, steady potentials are quickly established, and the titration curve at the equivalence point is symmetrical, so that the end point can be determined accurately from the maximum value of $\Delta E/\Delta V$. A typical titration curve is shown as curve 2 in Figure 3. The equivalence point potential is 0.00 volt versus the saturated calomel electrode (+0.24 volt against the standard hydrogen electrode).

The ferrous salt which is used must, of course, be free from ferric ion. The authors used Mallinckrodt reagent quality ferrous ammonium sulfate hexahydrate, which had been very carefully assayed in connection with another investigation and found to have a purity factor of 99.90%.

A 0.2200-gram portion of pure, dried potassium dichromate was dissolved in approximately 100 cc. of 1 *N* sulfuric acid in the titration vessel, and, after the flow of carbon dioxide was started, 2 grams of ferrous ammonium sulfate hexahydrate were added. The titration was made after carbon dioxide had been passed through the solution for 30 minutes. In two titrations, 44.81 and 44.82 cc. of the chromous sulfate solution were required, corresponding to an apparent normality for the chromous sulfate solution of 0.1001, which agrees very well with the theoretical 0.1000.

Equally satisfactory results were obtained in the preparation of a 0.02 *M* chromous sulfate solution.

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RECEIVED April 26, 1947.

Apparatus for Measurement of Vapor Pressure

R. R. LEGAULT, BENJAMIN MAKOWER, AND W. F. TALBURT
Western Regional Research Laboratory, Albany, Calif.

A compact, portable glass apparatus has been designed for the determination of aqueous vapor pressure of dehydrated agricultural products. The apparatus includes a mercury manometer of the Dubrovin type, which has a sensitivity about seven times that of an ordinary U-tube mercury manometer. The apparatus will be found useful for vapor pressure measurements in general.

IN STUDIES on food dehydration and related problems, equilibrium vapor pressure measurements are frequently required. Makower and Myers (*4*) proposed that aqueous vapor pressure rather than per cent moisture content be used as the index of degree of dehydration, and described a manometric apparatus for such vapor-pressure measurements. A slightly modified form of this apparatus has been described by Fischbach (*2*). In both units, the manometric fluid is an oil of low vapor pressure. The first-mentioned apparatus has proved entirely satisfactory, both as to accuracy and precision, provided the precautions outlined by Makower and Myers (*4*) are followed. However, it is inconvenient for routine measurements because of its fragility and lack of compactness, and because of the necessity of continuous pumping on the reference arm of the oil manometer. A device described by Vincent and Bristol (*5*) employs a U-tube mercury manometer and, in consequence, has not the sensitivity required for precise work. These disadvantages have been eliminated in a new design, incorporating a mercury manometer of the Dubrovin type (*1*) that has a sensitivity about seven times as great as a U-tube mercury manometer.

The new apparatus, shown in Figure 1, is rugged, compact, easily portable, and identical in principle to the previously described unit (*4*). It consists essentially of a sample flask, *A*, connected through the trap, *T*, with the modified Dubrovin manometer, *M*. The manometer rests on the ring seal, *V*, at the bottom of tube *Z*.

OPERATION OF THE GAGE

The procedure for making a measurement is as follows: Flask *A* containing the material—for example, ground dehydrated vegetable—the vapor pressure of which is to be determined, is attached to the apparatus by means of the ground joint and immersed in a constant-temperature bath. The flask containing the sample and the rest of the apparatus are evacuated through the open stopcocks, *D* and *C*, to less than 0.1 mm. of mercury pressure with an oil pump. During the evacuation, which requires about 2 minutes, trap *T* is kept cold by means of a solid carbon dioxide–alcohol mixture contained in a Dewar flask. Stopcock *D* is then closed, the cold bath is removed from trap *T*, and the small amount of ice collected is evaporated by warming the trap slightly above room temperature. Approximately 0.5 hour is allowed for temperature and pressure equilibrium to become established, as indicated by a constant gage reading. If the evacuation is not thorough, or if the material in the flask releases adsorbed gases or volatile decomposition products during the measurement, the pressure reading will not represent water vapor alone.

The pressure of noncondensable gases (air or carbon dioxide) may be easily determined by closing stopcock *C* to isolate the sample, then freezing out the water vapor in the gage by immersing trap *T* in a solid carbon dioxide–alcohol bath. The aqueous vapor pressure may then be taken as the difference between the first and second pressure readings. When the apparent pressure of noncondensable gases exceeds 0.5 mm. of mercury, the rate of diffusion of water vapor into the cold trap will be greatly retarded. To avoid errors in measuring the pressure of noncondensable gases, it is best in those cases to re-evacuate the whole system in the manner previously described. When evacuation is applied to samples containing finely ground materials, the fine powder is frequently sprayed from the sample bottle into the trap and the

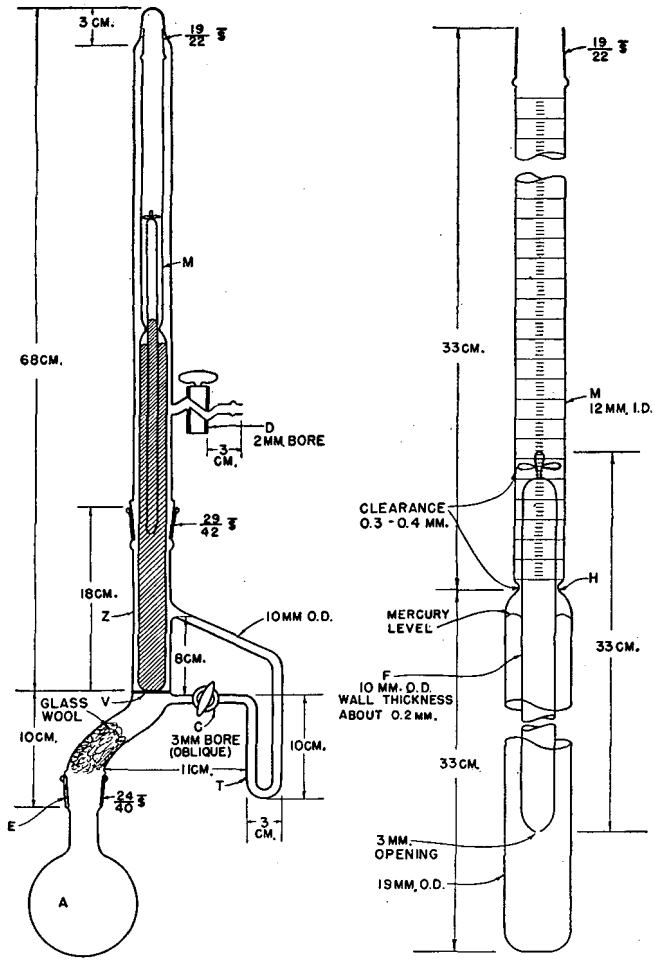

Figure 1. Vapor Pressure Apparatus (Left), Dubrovin Manometer (Right)

No scale

manometer. To prevent this occurrence, it is convenient to place a plug of glass wool in the tube immediately above the joint that connects the sample bottle to the gage.

CONSTRUCTION AND ASSEMBLY OF GAGE

Detailed construction of the Dubrovin manometer is shown in Figure 1.

It consists of an open tube, *M*, containing mercury and an evacuated tubular float, *F*, which is free to move up and down with variation in pressure. The position of the top of the float is the index of pressure. This position is read on an arbitrary scale engraved on tube *M*, and the reading is interpreted in pressure units from a calibration described below. A convenient way of securing a scale is by the use of the graduated part of either a buret or a pipet for the top portion of *M*. A complete description of the theory of the gage has been given by Germann and Gagos (*3*), who prefer metal floats because of their mechanical strength. The present authors found it expedient to use glass floats, inasmuch as metal ones of the desired characteristics were not readily available. Furthermore, glass floats were found to be very sturdy and are simple to construct. To avoid breakage of the float when the gage is brought to atmospheric pressure, the reservoir tube, *M*, is made long enough to allow for about 2-cm. clearance between the base of *M* and the bottom of *F* when the latter is completely full of mercury.

Most of the authors' manometers were designed to cover a range from zero to about 30 mm. of mercury; in a few cases the range was extended to about 60 mm. by increasing the length of the float. Float *F* was constructed from soft-glass tubing of fairly uniform diameter (obtained from the Kimble Glass Co.); its approximate dimensions were: length, 33 cm.; outside diameter, 10 mm.; wall thickness, 0.3 mm. or less. The float moved a distance of about 7 mm. for a change in pressure of 1 mm. of mercury. Since a change of 0.3 mm. in the position of the float could readily be perceived with the eye, the sensitivity of the gage was of the order of 0.04 mm. of mercury.

The construction of an all-glass manometer requires attention to details, some of which have not been previously described. Float *F* must be kept in a vertical position and must be free to move up and down with minimum friction. To guide the tube, three glass prongs were affixed to the top of the float and a constriction was made in tube *M* at point *H*. Guide prongs were not used near the bottom of the float because of excessive friction arising from glass-to-glass contact under a mercury surface. For the same reason the mercury level was maintained below *H* in *M*.

The choice of the dimensions of the float was in some measure governed by observations made during calibration of earlier models. Some gages exhibited a phenomenon of "hysteresis"—that is, the position of the float was different (lower) when approached from the high-pressure side than when the same pressure was attained from the low-pressure side. This effect was reduced by the use of float tubes of large diameter and by maintaining a wide annular space between the float and *M* (at the point where the float enters the mercury). With a float having a 5-mm. inside diameter, the maximum hysteresis effect was equivalent to 1.0 mm. of mercury; with a larger float, 10 mm. in diameter, it was reduced to a maximum of 0.5 mm. Hysteresis was practically eliminated (less than 0.05 mm. of mercury) by coating both walls of the float tube and the inside wall of *M* with a thin layer of colloidal graphite, applied by wetting the meticulously cleaned walls with a dilute water suspension (approximately 1 to 100) of the colloidal graphite, draining, and allowing the adhering film to dry on the glass surface. The function of the graphite is not clearly understood. It is not known whether it functions by virtue of its electrical conductivity (eliminating any static charges) or whether it has the effect of equalizing the advancing and receding contact angles of mercury on glass.

In assembling the Dubrovin manometer, it is important that *F* be thoroughly evacuated. Any air that is left, or that may accumulate in the float when the gage is in use, will be compressed when the float falls, and consequently the sensitivity of the gage will decrease with increasing applied pressure. To conduct the evacuation, a special all-glass adapter was constructed, as shown in Figure 2.

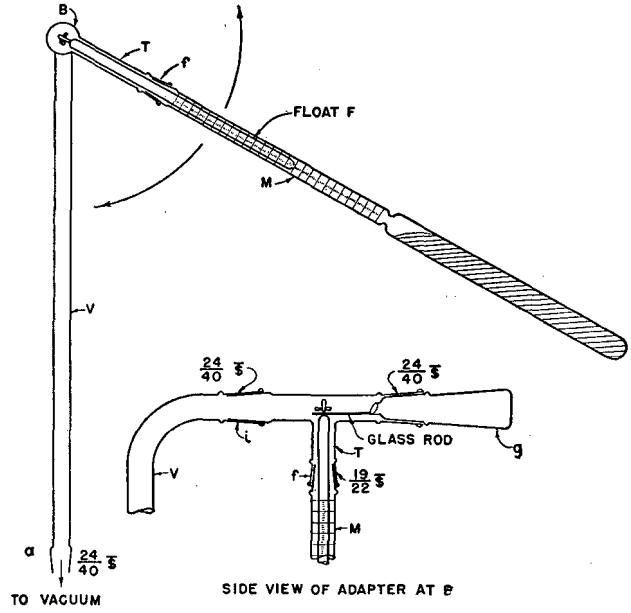

Figure 2. Adapter for Evacuation of Manometer

The reservoir tube, *M*, was attached to the adapter by means of ground joint *f*. At the same time the float, *F*, was kept out of the mercury by supporting it, under the prongs, with a glass rod attached eccentrically to ground-glass stopper *g*. The adapter was attached to a vacuum system (manifold shown in Figure 3) through its joint, *a*. The whole manometer was then rotated to a nearly horizontal position around the ground-glass swivel joint,

Table I. Precision of Gage at Various Pressures

Pressure, Mm. Hg	No. of Dubrovin Gage Readings[a]	Standard Deviation from Average of Pressure Values, Mm. Hg
4.58	10	±0.03
12.94	10	±0.04
17.86	5	±0.01

[a] Readings made alternately from high- and low-pressure sides.

i, in order to boil the mercury under vacuum without excessive bumping. After thorough evacuation, boiling, and cooling, the float was released by turning g and allowed to slide into the mercury as the manometer was slowly rotated to a vertical position.

CALIBRATION OF THE GAGES

The gages were calibrated against a U-shaped oil manometer. For this purpose one or more units similar to that illustrated in Figure 1 were attached through joint E to a glass manifold, as indicated in Figure 3. A low-vapor-pressure oil of measured density (Octoil, obtained from Distillation Products, Inc.) was used as the reference manometric fluid. Preliminary to the calibration, the system (Figure 3) was pumped down overnight to a pressure of about 5 microns, measured with a Pirani gage. Corresponding readings of the Dubrovin gages were considered the zero pressure points. Stopcock d (Figure 3) was closed and pumping was continued on the reference limb of the oil manometer during the whole calibration process. Comparative readings of the Dubrovin gages and of the oil manometer were made following each successive addition of a small increment of gas to the system through stopcock D (Figure 1), until the pressure range of the gages was covered. Subsequently, another series of readings was obtained through progressive decrease of the pressure down to the zero point with an auxiliary pump connected to D (Figure 1). In most cases the empirical calibration curve obtained in that manner followed a linear relationship within the limit of error of the calibration. Occasionally, a deviation from linearity appeared at the high-pressure end of the calibration curve. It could usually be shown that this deviation was caused by improper evacuation of the float tube. The calibration may be expressed in terms of the equation:

$$g = A + Bp$$

where g is the gage reading, p is the pressure in millimeters of mercury, and A and B are constants.

The results for one gage, calibrated with dry air, carbon dioxide, and water vapor, are given as an illustrative example.

From the reading errors of the oil manometer and of the Dubrovin gage (±0.04 mm. each) the maximum reading error of the calibration was estimated to be ±0.08 mm. of mercury. The value found for slope B was 6.81 for the three gases. The magnification factor of the gage, 6.95 (the change in gage reading in millimeters per millimeter of mercury change in pressure) was obtained by multiplying B by 1.02, the factor for converting to millimeters the arbitrary unit intervals of the scale of the Dubrovin gage. The values of intercept A (calculated reading of the gage at zero pressure) were 16.5, 16.9, and 17.1 for dry air, water vapor, and carbon dioxide, respectively. It is not possible to say whether the slight differences in the A values signify real differences in the behavior of the gage toward the three gases. Practically, they are not important, as they would give rise to differences in calculated pressures of not more than ±0.04 mm. of mercury if the average value A = 16.8 were used.

Reproducibility of vapor-pressure values obtained with the gage was determined by repeated measurements at each of three definite pressures. The pressures used were those of water vapor in equilibrium with liquid water. The water was contained in flask A (Figure 1) and was held within ±0.02° C. during three different experiments at 0.00°, 15.18°, and 20.30° C. The summarized results in Table I show that the precision of the gage, as expressed by standard deviation, is ±0.04 mm. of mercury or less.

The gage readings at known vapor pressures of water, referred to in Table I, constitute an independent calibration. The three points fall on a straight line whose intercept, 16.3, is in excellent agreement with the experimentally determined value, 16.5. The slope, 6.90, is about 1% higher than the value obtained from calibration against the oil manometer. The discrepancy is apparently due to errors inherent in the oil manometer used for the calibration. Although calibration against the known vapor pressure of water is undoubtedly the more accurate procedure, it has the disadvantage that it is conveniently applicable over a limited pressure range, up to the saturation pressure of water vapor, or about 20 mm. of mercury at room temperature. For this reason, calibration against an oil manometer is more convenient for routine work.

It is of interest to know how the calibration of the gage is affected by changes in ambient temperature of the room. An increase in temperature causes the level of the mercury to rise in the reservoir and raises the position of F with respect to the graduations on tube M (Figure 1). This rise is partially offset by the fact that F sinks deeper because of decreased density of the mercury. The net effect for a rise in temperature of 5° C. is an error of about 0.03 mm. of mercury in the pressure reading.

Dimensional changes, with temperature, of the various glass units are negligibly small and have not been included in these calculations. However, in addition to this correction, the pressure readings obtained with the Dubrovin gage are subject to the same temperature corrections as those for any mercury manometer.

ACKNOWLEDGMENT

The assistance of Elisabeth Nielsen in making the calibrations is gratefully acknowledged.

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RECEIVED September 8, 1947.

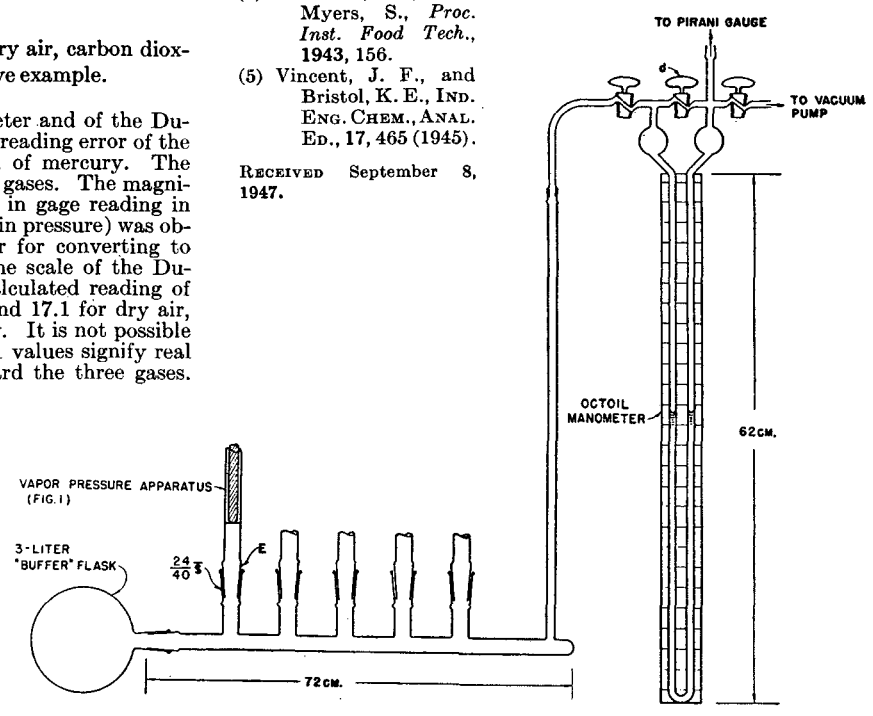

Figure 3. Manifold for Calibration of Gages

Quantitative Methods for Certain Organic Sulfides

JOHN W. SEASE[1], THOMAS LEE[2], GEORGE HOLZMAN[3], ERNEST H. SWIFT, AND CARL NIEMANN
California Institute of Technology, Pasadena, Calif.

The reactions of bromine, chloramine-T, iodate, and iodoplatinate with organic sulfides, particularly bis(2-chloroethyl) sulfide and thiodiglycol, have been critically examined in respect to their application to analytical methods for the determination of these latter substances.

DURING the war considerable effort was expended in developing analytical methods for the determination of mustard gas and a number of these methods were based upon characteristic reactions of the sulfide-sulfur atom. Because these latter reactions are of some general interest, this report discusses the principles of the various methods and points out their advantages and disadvantages. No attempt has been made to present detailed analytical procedures, as such procedures are useful only for a particular set of conditions and are available for those interested (*27*). Although all the experimental work was done on mustard gas or thiodiglycol, the findings are applicable to numerous other organic sulfides.

REACTION WITH BROMINE

The reaction of organic sulfides in dilute acidic aqueous solution with bromine is described by the equation (*11*):

$$R_2S + Br_2 + H_2O \longrightarrow R_2SO + 2H^+ + 2Br^-$$

Northrop (*16*) developed a simple procedure for the estimation of mustard gas and of thiodiglycol, by titrating a 0.1 formal sulfuric acid solution of the sulfide with bromine water and determining the end point either by the Pinkhof potentiometric method (*12*) or through the use of methyl red as an indicator. Because mustard gas, in common with many organic sulfides, is not very soluble in water, 50% aqueous acetic acid and a two-phase system of diethyl phthalate and 50% aqueous acetic acid may be used in lieu of the 0.1 formal sulfuric acid.

The direct titration with bromine water is simple of execution and has been shown to have a precision of ±0.2 microgram of sulfide-sulfur over the range of 5 to 100 micrograms (*27*). Its principal disadvantages are instability of the bromine solution, and a lack of specificity even if consideration is limited to sulfur-containing compounds. While bis(2-chloroethyl) sulfoxide, bis-(2-chloroethyl) sulfone, 2-chloroethylvinyl sulfone, divinyl sulfone, thiodiglycol sulfoxide, thiodiglycol sulfone, and thioxane sulfone do not react with bromine under the conditions specified, the di- and higher bis(2-chloroethyl) sulfides cause serious interference. The rate of reaction of bromine with the latter compounds is, however, significantly slower than the rate of reaction of bromine with bis(2-chloroethyl) sulfide or with thiodiglycol. If the amount of bromine consumed is expressed in terms of equivalent moles of bis(2-chloroethyl) sulfide, values found for the disulfide were 4.4 to 4.55, for the trisulfide 4.3 to 5.8, and for the pentasulfide 2.6 to 10.3, depending upon whether or not allowance was made for the slower rate of reaction in the case of the higher sulfides. In view of these facts it is obvious that use of bromometric methods for the determination of organic sulfides is clearly limited to those cases where di- and higher sulfides, as well as mercaptans (thiols), are absent.

In a number of instances conditions were such as to permit the use of a bromometric method and attention was directed to finding procedures more convenient than the direct titration with bromine water. Among the procedures investigated were (*a*) the addition of an excess of bromine water and back-titration with standard solutions of methyl red, (*b*) formation of bromine *in situ* by titration with a standard solution of chloramine-T in the presence of bromide ion, and (*c*) formation of the bromine *in situ* by the electrolysis of bromide ion and determination of the end point either potentiometrically or amperometrically. The first two procedures are no more convenient than the direct titration with bromine water, whereas the last procedure has many attractive features including particular adaptability to instrumentation. An instrument utilizing the principle of procedure (*c*) has been described (*20*).

REACTION WITH CHLORAMINE-T

An organic sulfide, such as bis(2-chloroethyl) sulfide or thiodiglycol, may react in aqueous solutions with chloramine-T to give the sulfilimine, the sulfoxide, or the sulfone, depending on concentration of reagents, acidity, and other factors (*2, 3, 7, 10, 14, 17, 22*). The reactions can be represented by the following equations:

$$CH_3C_6H_4SO_2NCl^- + R_2S \longrightarrow CH_3C_6H_4SO_2NSR_2 + Cl^-$$

$$CH_3C_6H_4SO_2NCl^- + R_2S + H_2O \longrightarrow CH_3C_6H_4SO_2NH_2 + R_2SO + Cl^-$$

$$2CH_3C_6H_4SO_2NCl^- + R_2S + 2H_2O \longrightarrow 2CH_3C_6H_4SO_2NH_2 + R_2SO_2 + 2Cl^-$$

Experiments were conducted to find which of the above reactions occur in dilute aqueous solutions of varying acidities and in 50% acetic acid. An excess of chloramine-T solution was added to the solution of sulfide and after appropriate periods the excess chloramine-T was determined by iodometric titration. It was found that 1 mole of chloramine-T reacted with 1 mole of either thiodiglycol or bis(2-chloroethyl) sulfide in neutral aqueous solution, in sulfuric acid solutions up to 3 formal, or in acetic acid solutions up to 50%. The reaction was faster in acid than in neutral solutions, and thiodiglycol reacted more rapidly than did bis(2-chloroethyl) sulfide.

In the presence of hydrochloric acid more than one mole of chloramine-T reacted per mole of sulfide. Potentiometric titration of either of the sulfides with chloramine-T in the presence of 2 formal hydrochloric acid showed that exactly 2 moles of the reagent reacted with 1 mole of the sulfide, presumably with the formation of the sulfone. This result may be interpreted as proceeding through the intermediate formation of chlorine which then oxidized the sulfide, inasmuch as it is known that chloramine-T will react with hydrochloric acid to form chlorine (*18, 21*) and that a dilute solution of chlorine will oxidize bis(2-chloro-

[1] Present address, Hall Laboratory of Chemistry, Wesleyan University, Middletown, Conn.

[2] Present address, School of Chemistry, Institute of Technology, University of Minnesota, Minneapolis, Minn.

[3] Present address, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass.

ethyl) sulfide or thiodiglycol, first to the corresponding sulfoxide and finally to the sulfone (*17*).

When 1 mole of chloramine-T reacts with 1 mole of sulfide in dilute solutions containing no hydrochloric acid, the product may be either the sulfilimine or sulfoxide. The relative amounts of these two substances in reaction mixtures prepared by adding equimolar quantities of chloramine-T and sulfide was determined by adding hydrochloric acid and titrating with chloramine-T to a potentiometric end point. Under these conditions the sulfoxide is quantitatively oxidized to the sulfone but the sulfilimine does not react. The sulfilimine hydrolyzes only very slowly at room temperature in 2 formal hydrochloric acid or in other aqueous solutions. It was found that when thiodiglycol or bis(2-chloroethyl) sulfide reacted with chloramine-T in acetic acid solutions up to 50%, or in sulfuric acid solutions up to 3 formal, 90 to 100% of the reaction product was the sulfoxide. In neutral solution a somewhat smaller fraction (70 to 80%) of the product was the sulfoxide.

The direct potentiometric titration of the sulfides in 50% acetic acid with chloramine-T (1 mole of chloramine-T used per mole of sulfide) and in 2 formal hydrochloric acid (2 moles of chloramine-T per mole of sulfide) were investigated as methods of determination but were found inferior to the potentiometric titration with bromine, principally because of the slowness of the reactions and the difficulty of obtaining a reversible electrode system.

British investigators (*24*, *28*) had developed a colorimetric method for the estimation of thiodiglycol and bis(2-chloroethyl) sulfide which was based upon the oxidation of the sulfide to the sulfoxide by chloramine-T and subsequent determination of the excess of the latter reagent by reaction with *o*-tolidine. The conditions used by these investigators—i.e., initial reactions of the chloramine-T and the sulfide in 30 to 45 volume % aqueous acetic acid followed by the addition of *o*-tolidine and hydrochloric acid giving a solution approximately 0.04 formal in the latter reagent—were such as to permit the formation of only the sulfoxide or the sulfilimine. Despite the advantage of stoichiometric reaction with 1 mole of chloramine-T consumed per mole of sulfide present, it was recognized that the method was an empirical one because of the complex nature of the reaction of the excess chloramine-T with *o*-tolidine (*4-6*, *9*, *23*). It was found that the results obtained with the original British procedure varied with the acetic acid concentration and that this difficulty could be eliminated by adding sufficient sulfuric or perchloric acid to give a reaction mixture which was approximately 0.5 formal in either acid. While this procedure permitted the added complication of partial oxidation of sulfoxide to sulfone because of higher acidity, the latter procedure actually was more convenient than the original method and results obtained were independent of the acetic acid concentration.

The precision of the modified procedure may be judged by the fact that results within 0.3 microgram of the true value were obtained throughout the range of 0 to 20 micrograms of sulfide sulfur per 10 ml. of acetic acid solution with only infrequent deviations of as much as 0.6 microgram. As might be expected from the nature of the reactions involved, the specificity of the modified colorimetric method, based upon the use of chloramine-T, was not greatly different than that observed in the case of bromometric methods. No reaction was observed in the case of bis(2-chloroethyl) sulfone, divinyl sulfone, thiodiglycol sulfone, and thioxane sulfone. Expressing the amount of chloramine-T consumed in terms of equivalent moles of bis(2-chloroethyl) sulfide per mole of substance, the following values were obtained with the modified colorimetric method: bis(2-chloroethyl) sulfoxide, 0.18 to 0.21; bis(2-chloroethyl) disulfide, 3.23 to 3.70; bis(2-chloroethyl) trisulfide, 0.40 to 0.44; and bis(2-chloroethyl) pentasulfide, 0.79 to 1.41. With the original British procedure no reaction was observed with bis(2-chloroethyl) sulfoxide, bis(2-chloroethyl) sulfone, 2-chloroethylvinyl sulfone, divinyl sulfone, thiodiglycol sulfoxide, thiodiglycol sulfone, and thioxane sulfone. The following values were observed for the higher sulfides: bis(2-chloroethyl) disulfide, 2.30; bis(2-chloroethyl) trisulfide, 3.51; and bis(2-chloroethyl) pentasulfide, 1.36 to 1.58.

Table I. Qualitative Observations on Reaction of Iodoplatinate with Sulfur Compounds

Test Compound	Solution Decolorized	Iodine Formed[a]
None	No	No
Hydrogen sulfide	Yes	No
Sulfur dioxide	Yes	No
tert-Butyl mercaptan	Yes	No
Benzyl mercaptan	Yes	No
Isobutyl sulfide	Yes	Yes
Benzyl sulfide	Yes (slowly)	Yes
Ethyl selenide	Yes	Yes
Isobutyl disulfide	Yes	Yes
Benzyl disulfide	Yes (slowly)	Yes
Di-β,β′-chloroethyl disulfide	Yes	Yes
Di-β,β′-chloroethyl trisulfide	Yes (slowly)	Yes
Di-β,β′-chloroethyl sulfoxide	No	No
Di-β,β′-chloroethyl sulfone	No	No

[a] Noted by extraction of the reaction mixture with CCl_4 and subsequent test for I_2 in this extract with starch-iodide.

REACTION WITH IODATE

Bis(2-chloroethyl) sulfide was titrated potentiometrically with a standard solution of potassium iodate in 90% acetic acid. The solvent for the sulfide was 60% acetic acid, 35% dibutyl phthalate, and 5% water and was 0.4 formal in hydrochloric acid and 0.005 formal in iodine monochloride. Under these conditions the iodate was reduced by the sulfide to unipositive iodine and the sulfide probably oxidized to the corresponding sulfoxide, as two equivalents of iodate were consumed per mole of sulfide present. Iodine monochloride was used because at the end point the iodine was unipositive and, therefore, no correction for the amount originally added was necessary. The titration gave quantitative results but no detailed information was obtained in regard to sensitivity and specificity.

REACTION WITH IODOPLATINATE

Organic sulfides are known to react with solutions of chloroplatinite ion to give precipitates of the formula $[Pt(R_2S)_4]^{++}$ $PtCl_4^{--}$ and with bromoplatinite ion to give the very much less stable precipitate $[Pt(R_2S)_4]^{++}$ $PtBr_4^{--}$ (*26*). Tschugaeff and Benewolensky (*25*) treated organic sulfides with an excess of chloroplatinate and obtained precipitates which they identified as complex salts containing both dipositive and tetrapositive platinum—i.e., $[Pt(R_2S)_4]^{++}$ $[PtCl_6]^{--}$. To explain the partial reduction of platinum they suggested that a portion of the sulfide was oxidized to the sulfoxide.

The above suggests that a partial explanation of the experimental fact that organic sulfides decolorize iodoplatinate ion with the formation of iodine is to be found in the reaction

$$[PtI_6]^{--} + 4R_2S \longrightarrow [Pt(R_2S)_4]^{++} + 4I^- + I_2$$

though it is clear that a second reaction

$$[PtI_6]^{--} + 2R_2S \longrightarrow [Pt(R_2S)_2I_2] + 2I^- + I_2$$

is also of importance (*1*). In fact, it has been observed that the slope of a plot of the iodoplatinate consumed versus the amount of sulfide present is not linear throughout the entire range of sulfide concentrations but that after a certain sulfide concentration is attained the slope decreases. Thus it appears that both the above reactions are involved. The behavior of the compounds given in Table I appears to be in general agreement with the above formulations. In a spectrophotometric study of the reaction of bis(2-chloroethyl) sulfide in 50% aqueous acetic acid with iodoplatinate ion it was observed that destruction of

iodoplatinate ion, as indicated by a decrease in extinction at 500 mμ, was invariably associated with an increase of the triiodide maxima, and in the presence of starch, an increase in extinction at wave lengths above about 570 mμ.

The aqueous iodoplatinate reagent used was prepared by a fourfold dilution of a stock solution which was 0.003 formal in chloroplatinic acid hexahydrate and 0.040 formal in sodium iodide. The dilute solution of the reagent is much less stable than the more concentrated stock solution. The effects of concentration, light, and heat upon the stability of aqueous solutions of iodoplatinate ion previously noted (*8*, *15*) were verified and in addition it was found that adjustment of the solution to pH 6.0 to 6.8 led to no marked improvement in respect to stability. When an aqueous solution of the iodoplatinate reagent was added to 10 volumes of 50% aqueous acetic acid it was observed that the extinction value at 500 mμ decreased rather slowly with time, reaching a steady value of approximately 40% of the original value after 30 minutes. This decrease in extinction on change of solvent may be due to the dissociation of iodoplatinate ion,

$$[PtI_6]^{--} \longrightarrow PtI_4 + 2I^-$$

or to a shift in the equilibrium between iodoplatinate and chloroplatinate ions,

$$[PtI_6]^{--} + 6Cl^- \longrightarrow [PtCl_6]^{--} + 6I^-$$

Although it was possible to overcome this instability of iodoplatinate solutions upon change of solvent by preparing the reagent in 50% aqueous acetic acid or by the addition of iodide ion, neither of these alternatives was found to be necessary, as the decrease in extinction becomes smaller and a constant extinction value is obtained within 5 minutes after mixing if as little as 1 microgram of sulfide sulfur is present per ml. of solution. With higher concentrations of sulfide sulfur a steady value is attained in still shorter time.

The application of the iodoplatinate reagent to the quantitative estimation of bis(2-chloroethyl) sulfide and thiodiglycol was suggested originally by British investigators (*1*), who developed a procedure depending upon visual estimation of the change in color of the iodoplatinate-starch system brought about by the presence of organic sulfides. This procedure was studied, as were the following modifications: photometric estimation using the iodoplatinate-starch system, determination of the excess iodoplatinate remaining after reaction by titration with standard solution of thiosulfate, visual estimation of the amount of iodoplatinate consumed using only iodoplatinate, and the photometric variant of this latter procedure.

The original British procedure was subject to the usual errors of visual colorimetric methods. While a single estimate was often accurate to within 0.2 to 0.4 microgram of sulfide sulfur, frequent poor estimates reduced the reliability to about ±2 micrograms when the amount of sulfide sulfur was below 10 micrograms and ±4 micrograms when the amount was in the range of 10 to 20 micrograms.

The substitution of a photometric determination for the visual comparison of the immediately preceding method might be expected to increase the reliability of the procedure. Theoretically either the increase in extinction at wave lengths above 570 mμ or the decrease in extinction between 400 and 570 mμ might be measured. However, it was observed that the increase in extinction due to the formation of the blue starch-triiodide complex partially balanced the decrease in extinction due to the decomposition of iodoplatinate ion and with an instrument such as the Pulfrich photometer using filters S57 and S61 little advantage was gained over direct visual comparison. The authors' experience on this point coincides with that of Lamour and Wheat (*13*).

It was thought that the iodine formed by the reaction of an organic sulfide with iodoplatinate ion might be titrated potentiometrically in the presence of excess iodoplatinate even though the latter substance is reduced by thiosulfate. Although definite differences were observed in a potentiometric titration of iodoplatinate in 50% aqueous acetic acid in the presence and absence of 20 micrograms of sulfide sulfur, taken as bis(2-chloroethyl) sulfide, no sharp potential breaks were encountered. In view of these unpromising results no further work was attempted.

The observation that much greater changes in extinction values in the region of 500 mμ occur when 100 micrograms of bis(2-chloroethyl) sulfide are added to solutions of iodoplatinate ion than when the same amount of the sulfide is added to solutions containing both iodoplatinate ion and starch suggested that it might be possible to devise a simple visual comparison method using only iodoplatinate. However, after numerous experiments all observers agreed that the omission of starch made any visual comparison much more difficult. It was, therefore, concluded that the original visual method using both iodoplatinate and starch was superior.

The photometric estimations of bis(2-chloroethyl) sulfide and thiodiglycol by reaction with solutions containing only iodoplatinate ion were investigated rather extensively. Four variants of a single basic method were studied, each of which involved the addition of the iodoplatinate reagent to the acetic acid solution of the sulfide and photometering the solution after it had been allowed to stand for a definite time at either 25° or 30° C.

The combinations of time and temperature employed were: (I) 25° C. for 30 minutes; (II) 25° C. for 3 minutes; (III) 30° C. for 3 minutes; and (IV) 30° C. for 1 minute (*19*). A Klett-Summerson photometer equipped with a No. 54 (green) filter which transmitted in the region of 500 to 700 mμ was used.

Method I was discarded for reasons of low sensitivity and low reproducibility. Method II proved to be particularly useful for low concentrations of sulfide, and below 3 micrograms of sulfide sulfur results were obtained with a precision of ±0.3 microgram. Methods III and IV gave results that did not differ greatly, though the shorter period of heating, which required precise timing to within ±5 seconds, was slightly more sensitive. In the range below 5 micrograms of sulfide sulfur the precision was ±0.3 microgram and in the range of 6 to 20 micrograms ±0.6 microgram. Therefore, of all the methods using iodoplatinate, the greatest precision was obtained by the above photometric methods.

In respect to specificity the iodoplatinate reagent was found superior to all the other reagents considered in this report. Whereas with bromine or chloramine-T considerable interference was encountered in the case of the polysulfides, this interference was minimal even with methods based upon measurement of iodoplatinate consumed. With the photometric procedure using only iodoplatinate, the following compounds reacted to the extent indicated, where the results are expressed in terms of equivalent moles of bis(2-chloroethyl) sulfide per mole of compound: bis(2-chloroethyl) sulfoxide, 0.02; thiodiglycol sulfoxide, 0.02; bis(2-chloroethyl) sulfone, 0.02; thiodiglycol sulfone 0.01; 2-chloroethyl vinyl sulfone, 0.01; divinyl sulfone, 0.02; thioxane sulfone, 0.01; bis(2-chloroethyl) disulfide, 0.24 to 0.41; bis(2-chloroethyl) trisulfide, 0.32 to 0.55; and bis(2-chloroethyl) pentasulfide, 0.55 to 1.06. In the case of the last three compounds the value observed was dependent upon the absolute concentration of the substance, the lower values being obtained in solutions of greatest dilution. For this reason when appreciable amounts of polysulfides are thought to be present it would appear advisable to dilute the samples to a point where 4 micrograms or less of sulfide sulfur are present. As with chloramine-T, iodoplatinate was observed to react more slowly with bis(2-chloroethyl) sulfide than with thiodiglycol.

CONCLUSION

Of the methods studied, the one based upon the reaction of the organic sulfide with iodoplatinate ion is superior from the

standpoint of specificity, although it is not so sensitive as the methods dependent upon the use of bromine or chloramine-T. Of the latter two methods, which possess comparable specificity the former is probably the more useful from the viewpoint of convenience.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to Edward L. Bennett, David H. Brown, Warren G. Schlinger, Carl T. Redemann, and Clark W. Gould, Jr., for their contributions to this investigation.

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RECEIVED October 27, 1947. Contribution 1152 from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology. Based on work done for the Office of Scientific Research and Development under Contract OEMsr-325 with the California Institute of Technology.

Identification of Pennsylvania Lubricating Oils

R. E. HERSH, M. R. FENSKE, H. J. MATSON, E. F. KOCH, E. R. BOOSER, AND W. G. BRAUN
School of Chemistry and Physics, The Pennsylvania State College, State College, Pa.

Extensive surveys of the lubricating oils derived from various crude oils indicate that distillation fractions from Pennsylvania oils, in general, have lower optical activity than those of oils from other crude sources. Pennsylvania lubricating oils show a more definite absorption band in the infrared region at 10.3 microns and, when clay-filtered in a standard manner, they show a higher rate of change in absorption with wave length in the ultraviolet region around 250 millimicrons than oils from other crude sources. Procedures utilizing these characteristics and other properties relating to composition are suggested for identifying Pennsylvania oils and carrying out comparative studies regarding the source and recognition of oils or crudes. The methods utilizing optical activity and ultraviolet absorption are to a large extent independent of the previous history of the oil and the presence of additives.

IT HAS been recognized that "Pennsylvania grade oils," commonly known as Pennsylvania oils, are those oils refined from a grade of crude oil produced in a well-defined geographical region located in Western Pennsylvania, Southwestern New York, Eastern Ohio, and West Virginia. A definition of Pennsylvania crude oil acceptable to the refiners would be one that is produced in the above region and which yields, by refining methods being practiced in the Pennsylvania industry, the amounts and the quality of products typical of such recognized Pennsylvania crude oils as Bradford, Buckeye, or Eureka crudes. This latter definition would be of importance in any consideration of new pools which may be an extension of present areas. Additional crude reserves are desired by refiners in all the producing areas of the country.

It is also recognized in the industry that Pennsylvania lubricating oils are characterized by distinctive physical properties or chemical compositions which are different from oils produced from other crude sources, and these differences are such that various methods of identification of Pennsylvania lubricating oils have been established. Some of the most obvious methods of differentiation include the examination of the viscosity index (*2*, *9*) and gravity index (*12*) of the lubricating oil. For conventionally refined Pennsylvania oils, the values for viscosity index and gravity index are around 100, while oils prepared similarly from crudes produced in other regions average considerably below this value. Although the viscosity index or gravity index may not be adequate to identify an oil of unknown source, in many instances these constants are useful for sorting and rough classification.

Large quantities of lubricants of high viscosity index were consumed during the war, and the demand for these products is still large. The supply of high viscosity index lube stocks is now not very plentiful. Refiners need to find more high quality crudes, or devise other ways of making high viscosity index oils, such as by new or improved refining methods, by synthesis, or by employing viscosity index additives.

A more comprehensive method of identification of Pennsylvania lubricating oils utilizes the maximum optical rotation shown by any 10% fractions distilled from the sample. It has been pointed out (*7*) that the optical activity of Pennsylvania oil fractions is considerably less than that of fractions derived from oils of other sources, particularly for those oil fractions having average molecular weights in the range of about 375 to 425. In a

Table I. Comparison of Peak Rotations on 10% Fractions of Oils Obtained from Various Refiners at Different Times

		Samples of 1936–41 Survey			Samples of 1945 Survey			
Refiner	Sample Codes	Visc. at 100° F., S.U.S.	Visc. Index	Max. $+\alpha_D^{20}$	Visc. at 100° F., S.U.S.	Visc. index	Max. $+\alpha_D^{20}$	Change in α_D^{20}
1	32–3048	182.9	99	0.25	182.4	99	0.29	+0.04
2	06–3124	147.8	105	0.22	178.7	106	0.24	+0.02
3	35–3097	183.3	99	0.16	181.3	107	0.14	−0.02
4	05–3085	147.8	110	0.11	143.3	110	0.17	+0.06
	36–3086	183.4	102	0.14	183.0	109	0.18	+0.04
5	39–3065	185.6	95	0.24	176.9	105	0.19	−0.05
6	02–3136	140.5	111	0.14	135.3	112	0.15	+0.01
	27–3135	180.6	104	0.22	179.9	108	0.17	−0.05
7	04–3087	147.2	111	0.21	137.8	115	0.22	+0.01
	23–3078	177.8	102	0.23	189.3	102	0.26	+0.03
8	15–3185	158.4	105	0.21	142.4	101	0.23	+0.02
	33–3186	183.0	97	0.24	170.1	98	0.21	−0.03
9	38–2923	184.6	96	0.26	187.8	96	0.33	+0.07
	66–2942	1593	105	0.18	1526	102	0.23	+0.05
10	45–3098	187.3	100	0.22	146.2	104	0.25	+0.03
	46–3099	188.0	97	0.26	180.5	98	0.28	+0.02
11	03–3101	146.9	104	0.22	145.5	109	0.22	0
	24–3089	177.8	101	0.21	183.4	106	0.20	−0.01
12	07–3088	148.9	111	0.18	142.1	113	0.15	−0.03
	48–3093	193.2	107	0.13	179.3	109	0.12	−0.01
13	41–3081	186.0	108	0.13	182.1	106	0.10	−0.03
14	44–3094	186.6	104	0.19	143.5	110	0.19	0
	51–3095	198.9	104	0.17	178.4	108	0.16	−0.01

survey of oils manufactured by every known refiner of Pennsylvania crude oil (*4*), it was concluded that the data "seem to indicate that lubricants subjected to distillation under the conditions detailed are not entirely of Pennsylvania origin if they give fractions with optical rotations exceeding about 0.26°" (observed dextrorotation per 10-cm. length of oil using sodium light). This does not infer that a sharp line of demarcation exists between oils of different sources. In the few special cases where optical rotation alone may permit uncertainty, other procedures of identification that are described in this paper may be used. Such cases include synthetic oils, hydrogenated mineral, animal, and vegetable oils, mixtures of oils from different sources, and even some Pennsylvania oils as pointed out below.

In order to verify the above relation after about a 5-year period, a series of neutral stocks obtained from 14 different Pennsylvania refiners was examined and the results were compared with the data obtained in the previous survey (*4*) on similar samples from the same refiners. This comparison is shown in Table I. The oils were distilled by a standardized procedure into 10% fractions (*4*). The maximum optical rotation for any 10% fraction observed with sodium light for a 10-cm. length of oil is recorded in Table I. In most cases the peak rotations observed on the 1945 samples agree with those obtained in the 1936–41 survey almost within experimental reproducibility, the arithmetic average change for the 23 samples being ±0.03° and the algebraic change being +0.01°. However, the peak rotations observed on three oils from refiners 1, 9, and 10 were +0.29°, +0.33°, and +0.28°, respectively, all of which are above the value of "about +0.26°" observed in the previous survey. The reproducibility of measurements on a given sample is about ±0.02° on the fractions, assuming that the distillations into fractions are conducted in the same standardized manner. The minor changes noted are evidently due to the inclusion of different proportions of materials having higher or lower optical activity. Possible causes for this might include (1) extension of the production area, (2) shifting of the proportions withdrawn from different pools since most refiners obtain their crude supply from an extensive gathering system so that material run to the stills may vary slightly in source and characteristics from day to day, (3) intrinsic changes in the crude supply due to more extensive drilling and recovery practices, or (4) slight changes in the concentration of the optically active materials by some refining processes. Of these possibilities, items (1) and (2) are probably the only ones that apply in the present case.

OILS FROM ALLEGANY COUNTY, N. Y., CRUDE

Inasmuch as the samples in Table I that show slightly higher than usual optical activity were all manufactured from crude oil coming from the same region, Allegany County, N. Y., a more complete survey of the oils derived from this region was undertaken. The examination of about 60 samples of commercial oils prepared from these crude oils showed maximum optical rotations of 10% fractions ranging from +0.23° to +0.33°, the average being +0.28° per 10-cm. length of oil.

As a further survey of this region, crude oil samples from individual wells and from the gathering areas or pumping stations collecting crude oils from the different water sheds in the region were examined.

These crude oils were separated into primary fractions by simple distillation in a modified Engler apparatus having a charging capacity of 2.5 liters and a 25.4-cm. (10-inch) column of 3.1-cm. (1.25-inch) standard pipe containing a series of six 14-mesh Monel metal screen disks as packing. The pressure in the system was reduced at intervals, preceded by cooling periods, to avoid thermal decomposition of the oil during distillation. The scheme employed can be summarized as follows:

Fraction	Operating Pressure, Mm. Hg	Cut Point (Vapor Temp.), ° F. At operating pressure	Cut Point (Vapor Temp.), ° F. Equiv. temp. at atm. pressure
Gasoline	760	385	385
Kerosene	50	363	540
Gas oil	3	385	700
Wax distillate	1	490	870

The wax distillate fraction produced by this procedure included more of the cylinder stock than is usual in commercial refining. The end points were purposely set high to ensure the inclusion of the material of about 400 molecular weight showing high optical activity. The wax distillate was then dewaxed at 0° F. using a solvent-oil volume ratio of 5 to 1, the solvent employed being methyl isobutyl ketone. This gave dewaxed oils having pour points of −10° to +10° F.

The dewaxed fractions were subjected to the standardized vacuum distillation in a small glass apparatus operated at about 0.1 mm. of mercury absolute pressure in order to obtain a series of 10% fractions for optical rotation measurements. The optical activities of these fractions were measured on a Schmidt & Haensch polarimeter and reported as observed optical rotations per 10-cm. length of oil using sodium light. The polarimeter can be read to ±0.01°. Measurements were usually made at room temperature, 20° to 30° C. (68° to 86° F.); precise control was unnecessary because the rotations of mineral oils appear to be unaffected by change in temperature. In experiments conducted on different types of oils over the range 10° to 90° C. (40° to 194° F.) no appreciable difference in the observed optical rotations were detected.

Typical analytical data obtained by the above procedure on individual well samples are presented in Table II. Data obtained on pumping station samples are summarized in Table III. Several duplicate samples taken on different dates are included to determine whether any fluctuations occurred. The peak rotations on the 10% fractions from the dewaxed distillates derived from the Allegany crudes varied from +0.17° to 0.32° per 10-cm. length. The arithmetic average of the peak values for the 16 different oils was +0.26° or, including the four duplicate samples, +0.27. The arithmetic average, however, may not agree with the rotations of products from specific refineries using these crudes, because of variations in the proportions of the above samples in the crude oil charge to any refinery.

Table II. Analysis of Crude Oils from the Allegany County, N. Y., Region

	Crude Oil No. K-34	K-107	K-132	K-133
	Designation: Wiflets & Paul Lease, Lot 8, Genesee Twp.	Oscar Potter Lease, Independence Twp.	Canneld Lease, Town of Wirt	Almy Lease, Town of Wirt
	Date of Sampling: Mar. 1936	6/20/45	5/2/46	5/3/46
Gravity of crude oil, °A.P.I.	44.3	42.3	39.2	40.9
Crude Oil Analysis				
Gasoline (E.P. = 385° F. at 760 mm.), wt. %	32.1	31.2	32.8	34.4
Kerosene (E.P. = 365° F. at 50 mm.), wt. %	14.2	9.9	10.0	9.3
Gas oil (E.P. = 385° F. at 3 mm.), wt. %	18.8	20.7	19.2	20.9
Wax distillate (E.P. = 490° F. at 1 mm.), wt. %	13.4	16.7	14.9	12.6
Cylinder stock, wt. %	17.1	18.7	16.9	21.2
Visc. of cylinder stock, S.U.S. at 210° F.	180.5	259.2	243.5	178.6
Dewaxing of wax distillate at 0° F.				
Yield, based on crude, wt. %	9.9	11.7	10.4	8.8
Gravity, °A.P.I.	29.8	29.2	28.7	29.6
Viscosity at 210° F., cs., S.U.S.	5.91 45.5	7.79 51.7	6.33 46.9	6.14 46.3
Viscosity at 100° F., cs., S.U.S.	40.61 188.4	63.07 291.6	45.82 212.2	43.26 200.4
Viscosity index	96	94	93	95
Gravity index	96	97	92	96
Pour point, ° F.			−5	0
Optical rotations of 10% fractions, $+\alpha_D^{20}$ per 10-cm. length				
Fraction 1	...	...	...	...
2	...	...	...	...
3	...	0.14	...	...
4	...	0.15	0.25	...
5	...	0.17	0.29	0.26
6	0.22	0.16	0.29	0.28
7	0.23	0.13	0.29	0.27
8	0.24	...	0.25	0.26
9	0.18	...	...	...

FREQUENCY OF OCCURRENCE OF ANY GIVEN VALUE OF OPTICAL ROTATION

Since the oils produced in the Allegany County, N. Y., region comprise only a portion of the total production of the Pennsylvania area, it is appropriate to examine the range and order of magnitude of the optical rotations of other Pennsylvania oils. From the data available on optical rotations of 10% fractions for 372 different Pennsylvania oils, it is possible to determine the frequency of occurrence of any given maximum value. The oils employed in this survey include 75 of the oils from various refiners that were investigated previously (*4*), 45 additional samples received from Pennsylvania refiners since that time, 24 oils received directly from jobbers, and 228 commercial oils marketed between January 1943 and August 1946, by refiners and jobbers of Pennsylvania grade oil. All these oils had pour points below about +30° F. Some of the commercial oils may have contained additives—for example, the highest value observed on 10% fractions was +0.34° on two samples known to contain additives. The possible effect of these materials is discussed in another section of this paper.

The arithmetic mean of the maximum optical rotations observed on the 10% fractions from the 372 Pennsylvania oils was +0.200°, and the standard deviation of the optical rotation values, σ, was 0.0594. The frequency distribution of the maximum observed optical rotations for 10% fractions within various limits is shown in Figure 1. The heights of the bars indicate the number of oils having maximum observed rotations within the different groups, each group including a range of 0.20°. Superimposed on this graph is the theoretical normal frequency curve computed from the data, indicating that the data agree reasonably well with the normal probability function. Actually, the frequency distribution has a very slight positive skewness,

$$\Sigma x^3/N\sigma^3 = 0.24$$

Based on the theoretical normal distribution curve for the optical rotation data of random samples of Pennsylvania oils, it is possible to compute that about 85% of the oils will show maximum optical rotations of 10% fractions below +0.27°, or that the odds against the occurrence of peak rotations of +0.27° or greater are a little more than 5 to 1. The chances increase to over 100 to 1 that a peak rotation of +0.34° or greater will not occur in the examination of random samples of Pennsylvania oils. In actual practice, the occurrence of relatively high values may be even less favorable, because the distribution curve shown may be influenced unduly by the fact that a considerably larger number of samples of relatively high optical activity were included than would be expected from normal production of oils in the total Pennsylvania region.

FACTORS AFFECTING OPTICAL ACTIVITY

In addition to the variation of optical activity of oils with geological occurrence it is of interest to determine if this property is altered by refining procedures, treating conditions, production methods, etc. The persistence of the optical rotation characteristics of oils through various refining procedures such as solvent extraction, aluminum chloride treatment, clay filtration, and acid treatment, and after use in engines has been demonstrated (*7*). As more information regarding the chemical composition of the optically active materials in oils becomes available, a better understanding of the chemical stability may be obtained. Most speculations to date indicate that optical activity in oils is imparted by the presence of certain steroids. The optical activity of petroleum may be useful in understanding better how petroleum is formed and where it might be found. The American Petroleum Institute Research Project 43A is studying some of these problems. In the present paper, the studies of some factors that may have an influence on the optical rotations of oils have been extended to include the possible effects of water flooding of wells during secondary recovery operations, dewaxing to different extents, treatment with various types of adsorbents, and the use of additives.

Water Flooding. On the theory that an increase in optical activity of Pennsylvania crude oils might occur after water flooding is started, due to displacement from the sands of materials of relatively high optical activity which were retained during natural flow or pumping of the wells, three pairs of crude oils were examined. Each pair was obtained from adjacent or nearby wells operating in the same pool, one crude oil of each pair being natural flow with pumping and the other being water flooded with pumping.

Table III. Optical Activity of Crude Oils from Different Pumping Stations in Allegany County, N. Y., Region

(Maximum observed optical rotations on 10 volume % distillation fraction of the dewaxed neutrals separated from the crude oils)

Sample No.	Pumping Station	Date of Sampling	Viscosity of Dewaxed 0-Pour Neutral at 100° F., S.U.S.	Max. Optical Rotation of 10% Fractions from Neutral, α_D^{20}
K-117	Obi, Clarksville Twp.	11–12–45	333.8	+0.30
K-134	Obi, Clarksville Twp.	5–10–46	244.5	+0.31
K-118	Rock City, Genesee Twp.	11–12–45	247.3	+0.23
K-119	Horse Run, Bolivar Twp.	11–12–45	252.1	+0.23
K-120	Nile, Wirt Twp.	11–12–45	286.0	+0.30
K-135	Nile, Wirt Twp.	5–10–46	239.4	+0.30
K-121	Wirt Center, Wirt Twp.	11–12–45	274.1	+0.29
K-110	S. Bolivar, Tank 7	11–12–45	260.6	+0.29
K-111	Bolivar, Tank 10	11–12–45	251.5	+0.26
K-112	Scio, Tank 31	11–12–45	242.9	+0.22
K-113	Petrolia, Tank 2	11–12–45	275.8	+0.27
K-114	Allentown, Tank 5	11–12–45	266.4	+0.30
K-130	Allentown, Tank 5	5–3–46	233.3	+0.30
K-131	Allentown, Tank 5	5–4–46	235.3	+0.32
K-115	Stannards, Tank 21 (Ford Brook District)	11–12–45	231.5	+0.25
K-116	Sinclair Refinery, Tank 80 (Madison Hill crude)	11–14–45	263.9	+0.29

Table IV. Effect of Water Flooding on Optical Activity of Petroleum Fractions

Pool	Allegany		Bradford		Tiona	
Sand formation	Richburg Sand		Bradford Third Sand		Clarendon Sand	
Crude oil No.	K-102	K-101	K-103	K-104	K-106	K-105
Method of production	Natural pumping	Water flood	Natural pumping	Water flood	Natural pumping	Water flood
Well No.	258	43	77	97	. .	. .
Location	"Rolls" Lease, Allentown, Allegany Co., N. Y.	Harder & Cochran, Allentown, Allegany Co., N. Y.	Whipple Property, Foster Twp., McKean Co., Pa.	Enterprise B & P Propty., Foster Twp., McKean Co., Pa.	Sharp & Seavy Lease, Clarendon, Pa.	Davis Farm, Clarendon, Pa.
Age of well, years	0.2	17	32	4	23	10
Depth to top of sand, feet	1401	1059	1085	1237	990	1125
Depth of well, ft.	1470	1170	1145	1328	1022	1143
Date of sampling	4/45	4/45	4/45	4/45	5/45	5/45
Gravity of crude oil, °A.P.I.	41.5	40.0	46.3	46.7	46.1	45.9
Crude oil analysis:						
Yield of gasoline (E.P. 385° F. at 760 mm.), wt. %[a]	30.2	30.9	36.6	37.7	38.8	37.7
Yield of kerosene (E.P. 363° F. at 50 mm.), wt. %	11.2	11.0	12.1	10.6	12.2	12.3
Yield of gas oil, (E.P. 385° F. at 3 mm.), wt. %	20.3	21.1	18.8	18.1	19.9	19.1
Yield of wax distillate (E.P. 490° F. at 1 mm.), wt. %	16.7	14.2	15.5	14.8	13.9	13.9
Yield of cylinder stock, wt. %	18.1	20.2	12.4	14.2	12.6	13.2
Viscosity of cylinder stock, S.U.S. at 210° F.	268.7	212.2	273.8	249.5	194.7	211.7
Dewaxing of wax distillate at 0° F.						
Yield, based on crude, wt. %	11.6	10.2	10.8	10.8	10.5	10.4
Gravity, °A.P.I.	28.2	28.3	29.2	29.4	30.6	30.6
Viscosity at 210° F., cs.	7.27	7.18	7.82	7.28	6.95	6.64
Viscosity at 100° F., cs.	58.29	57.14	64.34	56.71	51.59	47.68
Viscosity index	89	89	91	94	98	100
Gravity index	92	92	96	97	103	102
Optical rotations of 10% fraction, $+\alpha_D^{24°C.}$ per 10-cm. length						
Fraction 1	. . .	. . .	. . .	. . .	. . .	. . .
2	. . .	. . .	. . .	. . .	. . .	. . .
3	0.28	. . .	0.18	0.16	. . .	0.10
4	0.31	0.29	0.20	0.19	0.10	0.11
5	0.32	0.32	0.21	0.20	0.13	0.12
6	0.30	0.31	0.19	0.20	0.12	0.11
7	0.26	0.28	0.18	0.18	0.10	0.09

[a] E.P. = end or cut point. S.U.S. = Saybolt Universal seconds viscosity. cs. = centistoke viscosity.

The analytical data obtained on the six crude oils by the method previously described are given in Table IV. With the three sets of crude oils investigated, the use of water flooding produced no change in the optical activity of the crude oil. Crude oil K-101 came from a well that had been under water flood operations for more than 10 years, while crude oil K-102 was obtained from a well less than a quarter mile away in the same sand formation where flooding had not yet been practiced; the particular well was only about 2 months old at the time of sampling. The length of time water flooding was employed on the wells which produced crude oils K-104 and K-105 is not known, but from the data available it would appear that temporary changes in optical activity at the start of flooding also would be negligible.

Dewaxing. The removal of paraffin wax in the dewaxing process tends to increase the optical rotation of the dewaxed oil, because the wax itself has little or no rotatory power (*7*), and the removal of this "diluent" serves to concentrate the optically active compounds in the remaining oil. This can be illustrated by the experimental data given in Table V on the effect of dewaxing a raw wax distillate having a pour point of 80° F. to pour points of +25° and −5° F.

Table V. Effect of Dewaxing on Optical Rotation

	Wax Distillate of 80° F. Pour Point	Dewaxed to +25° F. Pour Point	Dewaxed to −5° F. Pour Point
Viscosity at 100° F., S.U.S.		165.7	164.4
Optical rotations of 10% fractions, $+\alpha_D^{25}$			
Fraction 5	0.15	0.27	0.27
6	0.21[a]	0.28	0.29
7	0.24[a]	0.25	0.28
8	0.22[a]	. . .	0.24

[a] Measured at about 90° C. to keep wax in solution.

The maximum optical rotation of 10% fractions is the same, within experimental reproducibility, for both the +25-pour and the −5-pour oils. The effect of dewaxing might be expected to diminish on approaching extremely low-pour oils, as the optically active compounds in an oil probably also cover a range in melting points and the separation of the low-melting "waxes" by successive dewaxing steps or other fractional crystallization processes would be expected to include some of the optically active materials. For example, in dewaxing a sample of a +15° F.-pour 180 neutral to a pour point of −15° F. the maximum optical rotations of 10% fractions from the +15-pour and the −15-pour oils were +0.28° and +0.29°, respectively, and the optical rotation of the so-called "wax" fraction, which was fluid at room temperature, showed a rotation of +0.20° for the over-all sample, indicating that

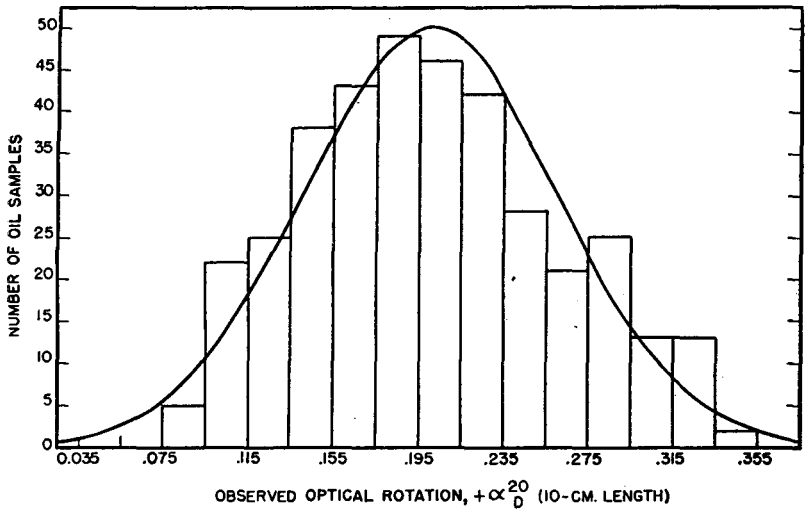

Figure 1. Frequency Distribution of Maximum Optical Rotation Values Observed on 10% Fractions from 372 Pennsylvania Oils

Table VI. Comparative Studies of Effect of Five Adsorbents on a Pennsylvania 150 Neutral

(Oil percolated at room temperature through a 26-inch height of adsorbent in a 0.75-inch diameter steel tower, treatment 3 pounds of adsorbent per gallon of oil charged. Filtration and drainage by gravity)

Type of Adsorbent	None	Fuller's Earth (30/60-Mesh)	Bauxite (30/60-Mesh)	Silica Gel (28/200-Mesh)	Brockman Anhydrous Al_2O_3	Granular Bone Charcoal (J. T. Baker 1557)
Recovery from filter, wt. % of original	...	63.2	80.0	86.5	69.3	82.2
Properties of over-all oil						
Gravity, °A.P.I.	30.3	31.1	30.7	32.0	31.2	30.6
Viscosity at 100° F., cs.	30.59	30.16	30.02	27.62	29.25	29.97
S.U.S.	143.5	141.6	141.0	130.4	137.6	140.8
Viscosity at 210° F., cs.	5.04	5.00	4.98	4.83	4.94	5.00
S.U.S.	42.73	42.60	42.54	42.05	42.41	42.60
Viscosity index	105	105	105	114	108	107
Gravity index	96	96	97	103	98	96
Color, N.P.A.	2-3/4	1	1-3/4	1-3/4	1	1-3/4
Density, d_4^{20}	0.8712	0.8664	0.8686	0.8619	0.8661	0.8693
Refractive index, n_D^{20}	1.4845	1.4815	1.4831	1.4779	1.4803	1.4829
Refractivity intercept, n-d/2	1.0489	1.0483	1.0488	1.0469	1.0472	1.0482
Specific refraction	0.3287	0.3288	0.3289	0.3283	0.3283	0.3285
Specific dispersion	111	109	109	103	105	109
Aniline point, ° C.	96.6	98.5	97.0	100.5	98.8	97.4
Molecular weight (calcd.)	376	380	380	374	376	380
Waterman analysis						
Wt. % aromatic rings	9	8	9	6	7	9
Wt. % naphthene rings	16	16	17	18	19	17
Wt. % paraffin chains	75	76	74	76	74	74
No rings per molecule	1.6	1.5	1.6	1.4	1.6	1.6
In Formula C_nH_{2n+x}						
n	27.1	27.5	27.4	26.9	27.1	27.4
x	−4.2	−5.0	−4.1	−2.7	−4.3	−4.1
Optical rotation at 20° C., α_D^{20}	+0.20	+0.21	+0.17	+0.18	+0.19	+0.18
Optical rotation of 10% distillation fractions, $+\alpha_D^{20}$						
Fraction 5	0.21	0.20	...	...	...	...
6	0.26	0.26	0.22	0.21	0.20	0.23
7	0.27	0.26	0.25	0.26	0.24	0.24
8	0.25	0.23	0.22	0.24	0.23	0.22
9	0.21	...	...	...	...	...

fractional crystallization processes tend to remove some of the optically active compounds when extended to low temperatures.

Treatment with Adsorbents. The effect of using different types of adsorbents in the refining of lubricating oils has been extended to include fuller's earth, bauxite, silica gel, anhydrous aluminum oxide prepared by Brockman procedure (Merck and Company, Inc.), and granular bone charcoal. A reference Pennsylvania 150 neutral (No. 3076) showing relatively high optical activity was percolated through a 66-cm. (26-inch) layer of the adsorbent supported in 21.9-cm. (0.75-inch) diameter steel tower, the quantity of oil charged being determined in each to give a treatment of 360 grams of adsorbent per liter of oil charged (3 pounds per gallon). The rate of percolation varied from 0.4 to 0.75 ml. per minute. The treatments were conducted at room temperature, 75° to 88° F. In the case of silica gel it was necessary to dilute the oil with a low-boiling naphtha in order to expedite the filtration, and remove the diluent by distillation after treatment.

The properties of the treated oils are given in Table VI. The relatively heavy treatments did not produce an appreciable effect on the maximum optical rotations of the 10% fractions. Silica gel was most effective in changing the chemical composition of the oil, as evidenced by the viscosity index and the hydrocarbon-type analysis calculated by the Waterman procedure (*16*), but did not materially change the optical rotation.

Additives. The manufacturers of lubricating oil additives for use in improving oxidation or bearing corrosion stability, detergency, rust-preventive characteristics, etc., sometimes use materials containing mineral oil (such as petroleum sulfonates) and also disperse or dissolve the active ingredients of the additive in a mineral oil carrier to facilitate handling and easy solution in the oils to be compounded. For economic reasons or because of solubility requirements, the oil carrier chosen is usually a naphthene-base oil having relatively high optical activity. The active ingredient rarely has optical activity. The observed optical rotations of 10% fractions distilled from several commercial additives are listed in Table VII. As a number of these fractions were dark in color, optical rotations, measured in 5-cm. tubes and converted to 10-cm. lengths of oil, are probably accurate to only ±0.02°.

Table VII. Observed Optical Rotations of Fractions Distilled from Several Commercial Additives

(Unless otherwise indicated, fractions are 10% by volume of over-all additive)

Commercial Additive	Lubri-Zol Additives					Paranox	
	728	737	738	746	749	62	105
Fraction No. 1	+0.04 (7%)	−(5%)	....	+1.18	+4.24	−0.04 (5%)	−0.02 (5%)
2	.....	+0.15	+0.22	+0.80	....	.....	.....
3	.....	+0.28	+0.49	....	....	.....	.....
4	.....	+0.46	+0.73	....	....	.....	.....
5	.....	+0.62	+1.04	....	....	.....	.....
6	.....	+1.00	+1.06	....	....	.....	.....
7	.....	+1.22	....	....	....	.....	.....
8	.....	+1.32	....	....	....	.....	.....

Because of thermal decomposition of the additives during distillation it was difficult to obtain an accurate estimate of the boiling range of these materials. In most cases decomposition started at 350° to 400° F. at 0.5-mm. pressure, but where the distillation was continued beyond this initial "cracking" phase, the boiling points of the additive-oil carrier mixtures were comparable to those of commercial neutrals. Inasmuch as the property of optical rotation is essentially additive, the use of some of these additives in relatively high concentrations may have an appreciable effect on the optical rotations of fractions from an oil whose boiling range matches that of the oil carrier in the additive. For example, the observed optical rotations of 10% fractions distilled from a Pennsylvania 150 neutral containing high concentrations of several of the above additives are shown as follows:

Fraction No.	Pa. 150 Neutral (0182)	Blend 89.4 Wt. % 0182 10.6 Wt. % L-Z 737	Blend 91.9 Wt. % 0182 8.1 Wt. % L-Z 738
1	+0.08	+0.10	+0.09
2	0.08	0.13	0.11
3	0.09	0.12	0.14
4	0.10	0.13	0.14
5	0.12	0.17	0.17
6	0.17	0.20	0.22
7	0.18	0.24	0.21
8	0.16	0.25	0.22
9	0.12	0.22	0.19

Table VIII. Properties of Lubricating Oil Fractions Derived from Various Crude Petroleums

Sample No.	Type of Crude	State	County	Township or Area	Sand Zone or Formation	Depth, Ft.	Properties of Dewaxed Distillate: Yield from crude, wt. %	Gravity, °A.P.I.	Pour point, °F.	Visc., 210° F.	S.U.S. 100° F.	Visc. index	Gravity index	Observed opt. rot. per 10-cm., $+\alpha_D^{20}$
K-31	Allegany	N. Y.	Allegany	Scio	Scio	800	9.1	31.1	+15	43.9	163.2	99	101	0.15
K-34	Allegany	N. Y.	Allegany	Genesee	Richburg	1230	8.8	30.2	+10	44.0	163.2	99	96	0.17
K-50	Bradford	N. Y.	Cattaraugus	Allegany	Bradford	2022	9.2	31.4	+10	42.5	141.5	103	101	0.15
K-27	Bradford	Pa.	McKean	Otto	Bradford	1700	8.0	30.6	+20	43.6	157.2	101	98	0.17
K-49	Bradford	Pa.	McKean	Bradford	Bradford	1900	9.5	31.5	+10	42.7	145.1	101	101	0.15
K-51	Bradford	Pa.	McKean	Lafayette	Clarendon	1640	8.7	29.9	+15	43.8	163.5	96	95	0.28
K-76	Bradford	Pa.	McKean	Bradford Area	Bradford	[a]	6.7	30.1	+10	43.0	152.0	97	96	0.21
K-89	Bradford	Pa.	[a]	Bradford Area	[a]	[a]	8.6	30.4	+10	42.4	143.7	100	95	0.19
K-33	Middle Dist.	Pa.	Crawford	Titusville	3rd Venango	750	8.0	31.0	+15	43.3	152.4	102	100	0.12
K-53	Venango	Pa.	Venango	Mineral	Raymilton 3rd	800	7.3	31.9	+15	40.7	121.3	99	101	0.15
K-59	Franklin	Pa.	Venango	Sugar Creek	Franklin, 1st sand	715	16.8	30.5	−5	44.8	176.3	98	98	0.12
K-55	.....	Pa.	Butler	Petrolia	3rd sand	1291	10.9	32.5	+10	43.6	152.4	108	107	0.06
K-72	.....	Pa.	Butler	Parker	30-ft. oil sand	1195	10.1	31.1	+10	44.5	166.4	106	102	0.11
K-60	Bessemer	Pa.	Lawrence	Beaver	Berea grit	610	9.9	33.2	+15	44.2	163.2	105	110	0.01
K-58	McDonald	Pa.	Allegany	S. Fayette	5th sand	2341	11.4	32.9	+10	44.2	158.4	110	109	0.05
K-57	Washington	Pa.	Washington	Buffalo	5th sand	2677	9.8	33.2	+15	43.4	149.7	108	109	0.04
K-61	.....	W. Va.	Pleasants	Washington	Cow Run	639	9.1	32.4	+15	42.7	144.6	102	105	0.09
K-62	Harrisville	W. Va.	Ritchie	Grant	Keener	2173	10.3	32.1	+10	44.8	171.7	104	107	0.08
K-63	Greenwood	W. Va.	Doddridge	Central	Maxon	1934	10.6	31.7	+10	43.8	160.4	100	103	0.09
K-64	Greenwood	W. Va.	Doddridge	Central	Big Injun	1944	11.0	31.8	+5	44.6	173.3	100	105	0.09
K-71	Lincoln	W. Va.	Lincoln	Washington	Berea	2694	5.9	32.1	+15	42.3	142.4	97	103	0.10
K-91	Eureka	W. Va.	[a]	[a]	[a]	[a]	10.7	31.4	0	43.4	152.8	103	102	0.13
K-65	Buckeye	Ohio	Perry	Sec. 36, Coal	Clinton	3725	10.3	32.0	+15	44.1	160.5	106	105	0.04
K-66	Buckeye	Ohio	Meigs	Sec. 34, Sutton	Berea	1711	10.0	30.3	+15	45.4	196.2	91	99	0.23
K-67	Buckeye	Ohio	Belmont	Spec. 21, Warren	Berea	1632	9.2	32.0	+10	43.0	156.3	97	104	0.11
K-68	Buckeye	Ohio	Muskingum	Sec. 5, Blue Rock	Medina	4152	10.7	32.6	+15	43.6	154.9	105	107	0.03
K-70	Buckeye	Ohio	Washington	Warren	Cow Run	683	8.7	31.9	+15	43.1	147.2	106	103	0.12
K-79	Buckeye	Ohio	Perry	Clayton	Clinton	3269	8.6	31.2	+10	44.4	165.8	104	102	0.06
K-86	Buckeye	Ohio	[a]	[a]	[a]	[a]	9.5	31.2	+10	42.7	144.4	103	100	0.11
K-87	Buckeye	Ohio	Perry	Madison	Clinton	3366	6.5	30.5	+15	41.9	136.7	99	95	0.16
K-88	Buckeye	Ohio	Perry	Madison	Clinton	3267	8.9	30.7	+15	41.8	133.2	102	97	0.13
K-90	Buckeye	Ohio	Perry	Madison	Clinton	3371	5.8	30.4	+15	41.9	134.3	103	95	0.18
K-92	Buckeye	Ohio	Morgan	Bloom	Clinton	4198	11.1	32.1	+10	43.1	146.1	109	105	0.04
K-93	Buckeye	Ohio	[a]	[a]	[a]	[a]	9.8	30.9	+10	44.6	171.2	101	101	0.09
K-94	Buckeye	Ohio	Muskingum	Falls	Clinton	3494	6.5	32.0	+10	43.0	147.7	104	104	0.09
K-95	Buckeye	Ohio	[a]	[a]	[a]	[a]	9.5	31.3	+10	42.6	142.3	104	100	0.09
K-77	Corning	Ohio	Muskingum	Newton	Clinton	3409	5.2	30.4	0	43.1	153.6	97	96	0.18
K-78	Corning	Ohio	Muskingum	Newton	Clinton	3435	5.1	30.9	0	42.3	150.1	99	97	0.17
K-80	Corning	Ohio	Coshocton	Perry	Clinton	3193	4.2	27.6	+5	43.1	164.1	79	80	0.39
K-81	Corning	Ohio	Meigs	Orange	1st Berea (Mississippian)	1699	4.6	29.5	+5	41.7	138.7	89	90	0.28
K-109	Corning	Ohio	Fairfield	Walnut	.....	...	6.9	24.0	[b]	53.1	370.1	66	64	2.14[c]
K-73	Illinois	Ill.	White	Phillips	Benoist or Paint Creek	2712	8.3	24.3	0	52.3	397.5	41	64	1.22
K-74	Illinois	Ill.	Jasper	Fox	McKloskey	2800	8.0	26.2	+5	48.3	263.9	68	78	0.69
K-82	Somerset	Ky.	Martin	.....	1st Maxon	1468	5.8	28.6	+5	42.4	147.5	90	86	0.22
K-83	Somerset	Ky.	Lawrence	.....	Berea	1786	12.2	27.9	+15	44.4	186.6	77	84	0.34
K-3	Louisiana	La.	Acadia	85 Rge. 3E	Mire (Middle)	8085	7.1	26.5	+5	48.2	262.2	70	80	1.05
K-4	Louisiana	La.	Cameron	13S Rge. 8W	Miocene (or Marine)	5642	16.0	24.9	−25	49.5	316.2	50	69	0.98
K-5	Louisiana	La.	St. Landry	85 Rge. 3E	Dominque (Lower)	8716	9.0	27.0	+5	46.6	226.8	74	82	0.99
K-6	Louisiana	La.	Acadia	85 Rge. 3E	Discorbis (Middle)	7849	7.2	26.9	+20	46.3	219.9	77	81	0.93
K-7	Louisiana	La.	Acadia	85 Rge. 3E	Richard (Middle)	8620	10.8	26.8	+15	46.9	231.8	75	80	0.96
K-99	Lost Soldier	Wyo.	.....	.....	Iakota and Dakota	...	16.7	24.4	[b]	46.8	244.2	62	62	1.30[c]
K-10	New Mexico	N. M.	Lea	24-S Rge. 36E	Big Time	3504	8.8	24.3	−30	50.5	343.8	47	64	1.23
K-24	New Mexico	N. M.	Lea	.....	Limestone	4200	6.6	24.7	+10	48.8	287.5	63	66	1.28
K-12	Oklahoma	Okla.	Kay	.....	Tonkawa, Wilcox, Layton	Various Depths	7.3	25.6	+5	47.8	261.3	65	73	0.61
K-30	Oklahoma	Okla.	Seminole	7-N Rge. 6E	Simpson	4422	8.1	25.8	+5	47.3	258.0	58	73	0.74
K-108	Oklahoma	Okla.	[d]	[d]	Hunton Lime	6850–7100	11.2	27.2	[b]	50.6	301.9	78	84	0.58[c]
...	Oklahoma	Okla.	Kay	25-N	Wilcox	3872	8.4	26.2	+10	44.1	181.2	79	70	0.45
K-75	Big Lake Pool	Texas	Reagen	University Land	Ellenburger (Lower Ordovician)	8405	9.3	30.8	+10	43.4	154.9	100	107	0.26
K-98	West Texas	Texas	Winkler	Keystone (Ellenburger Field)	Keystone Ellenburger (Ordovician)	9835	9.4	28.3	−5	43.1	150.1	101	85	0.22
K-85	Turner Valley	Alberta, Canada	.....	L.S. 11 of 32-18-2W5	Dolomite (Madison in Mississipian)	7690	5.4	23.7	0	45.5	217.9	62	52	0.35

[a] Composite pipe-line sample from numerous wells.
[b] Approximately +5° F.
[c] Maximum value for 10% fractions of dewaxed distillate obtained by simple distillation.
[d] Composite sample of crude oil from West Edmond, Okla., field, obtained by Toronto Pipe Line Co., Oklahoma City, from Sohio-Western Pipe Line, 9–17–45.

In most practical applications of additives, however, no serious problem in the identification of Pennsylvania oils is presented because of the relatively low concentrations employed. The presence of additives in lubricating oils usually can be readily detected and, if necessary, the effect on optical rotations can be determined from a knowledge of the type and concentration employed.

OILS FROM VARIOUS SOURCES

The observed optical rotations of fractions from lubricating oils derived from sources other than the Pennsylvania region are

Table IX. Optical Activity of Various Oils Derived from Foreign Crude Oils

	Viscosity at 100° F. Cs.	Viscosity at 100° F. S.U.S.	Viscosity Index	Gravity Index	Observed Optical Rotation $+\alpha^t_D$	Observed Optical Rotation Temp. of meas., t° C.
Russia 00 oil[a]	200.7	927	38	56	2.00	30
Russia No. 1 engine[a]	99.30	459	49	68	1.86	30
Russia No. 2 spindle[a]	37.53	174.6	66	62	1.20	30
Russia bright stock[a]	528.4	2441	84	97	1.04	30
Rumania No. 1[a]	23.09	110.7	33	..	0.86	30
Rumania No. 2[a]	486.4	2247	−36	..	3.48	30
Waxy fraction (2.9%) from Bukkszeki-Lispei crude, Hungary[b]	....	....	About 82	..	0.50	90
Fraction (5.1%) from Gbely crude, Czechoslovakia[b]	....	....	About 72	..	2.52	18
German aviation oil	265.9	1229	107	118	0.40	25
Dewaxed fraction from Eakring crude, England[c]	63.76	294.7	70	81	1.24[d]	22
Dewaxed fraction from Duke's Wood crude, England[c]	75.26	347.7	77	93	0.45[a]	22
Dewaxed fraction from Kelham Hills crude, England[c]	149.4	690	28	22	1.18[d]	22
Dewaxed fraction from Caunton crude, England[c]	83.97	388	42	50	1.22[d]	22
Dewaxed fraction from Formby crude, England[c]	50.71	234.7	93	92	0.56[d]	22
Iraq solvent-treated oil	533.8	2466	96	100	1.54[d]	25
Japanese SAE 30 oil	122.8	567	25	16	2.15	20

[a] Oils described by Dow, McCartney, and Fink (*6*).
[b] Crude oils supplied by Universal Oil Products.
[c] Fields from which these crudes were obtained are described by Lees and Taitt (*11*).
[d] Maximum values observed on 10% fractions.

usually considerably higher than those from Pennsylvania oils (*4, 7*). Continuation of the studies on this subject has resulted in the accumulation of data on fractions of lubricating oils from various parts of the world. In most cases the data are based on fractions from given crude oils derived from individual wells or small pipe lines collecting from known areas. A summary of some of the pertinent data obtained on the lubricating oils derived by a procedure similar to that outlined above from various crude oils produced in the United States and Canada is presented in Table VIII (*3, 10, 17*). Similar data on other United States crude oils are available in the literature (*4*). Optical rotations of oils derived from several other foreign crude oils are listed in Table IX.

In these surveys of lubricating oils from widely different sources, several interesting facts are noted. Although in general the optical rotations of non-Pennsylvania oils are considerably higher than those of Pennsylvania oils, a few of the non-Pennsylvania oils show relatively low optical activity, bordering on or within the range of values typical of Pennsylvania oils. This immediately suggests the application of other identifying tests for the "borderline" cases. The optical rotation values given in Table VIII are for the over-all dewaxed distillate fraction from the crude oil and the maximum values for 10% fractions of the oils, which are commonly used for comparison, would be somewhat higher. The extent to which these values would be increased depends upon the boiling range of the oil and the optical activity of the oil, the difference between the peak rotation of 10% fractions and the rotation of the over-all oil varying from about 0.05° to 0.07° for oils with over-all rotations around 0.20°, and from about 0.10° to 0.12° for oils around 0.30°, to much greater differences for oils of about the same boiling range and higher over-all optical activity.

No premature conclusions based on the viscosity index of the dewaxed distillate fractions should be drawn regarding the source or quality of the crude oils, as such an evaluation would require more complete information on such items as the nature of all the products derived from the crude and the type and extent of refining necessary to obtain marketable products.

INFRARED ABSORPTION CHARACTERISTICS OF OILS

Another method for identification of Pennsylvania oils is based on the infrared absorption spectra of oils in the vicinity of 10.3 microns. The different absorption characteristics of Pennsylvania oils and non-Pennsylvania oils at this wave length was noted by Armour Research Foundation (*13*). Typical absorption spectra of lubricating oils over the range 2 to 15 microns are illustrated in Figure 2. At a wave length of 10.31 microns there is a large difference in the absorption characteristics of Pennsylvania and non-Pennsylvania oils. In this region the absorption of non-Pennsylvania oils is weak. The exact molecular configuration causing the absorption at 10.31 microns in Pennsylvania oils is not known at the present time. In the lower molecular weight ranges absorption bands at this wave length are noted for hydrocarbon of various types—e.g., isopentane, *n*-hexane, methylcyclohexane, 2-octene, *m*-xylene, etc. (*1*). This information, however, cannot be safely extended to the more complex composition of the lubricating oils.

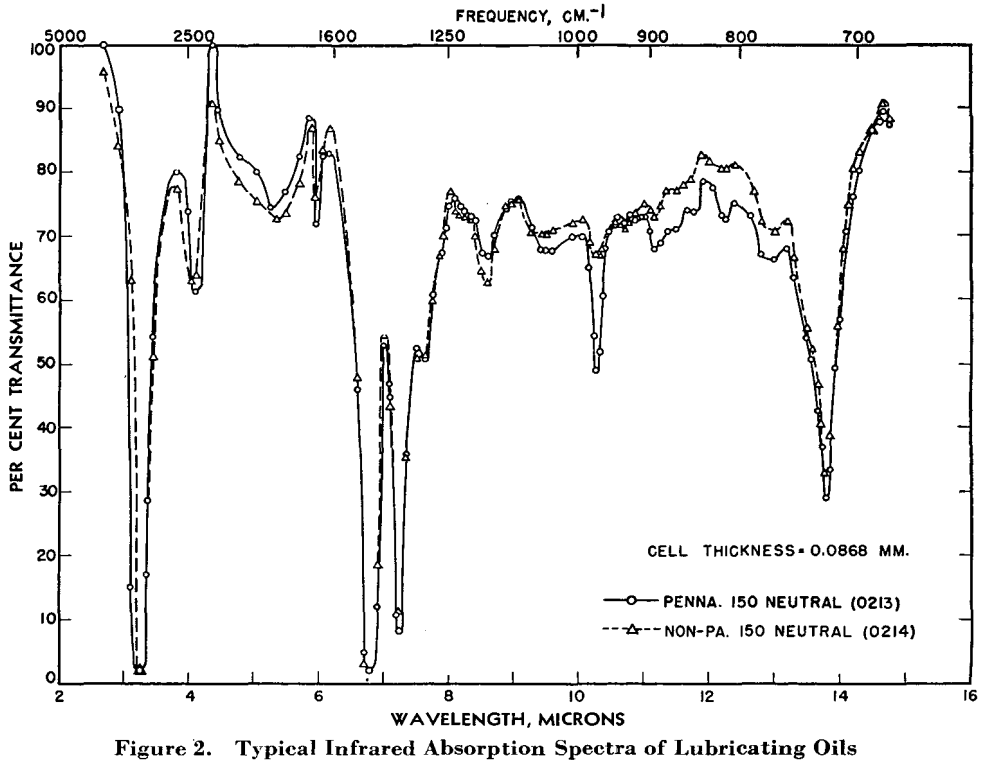

Figure 2. Typical Infrared Absorption Spectra of Lubricating Oils

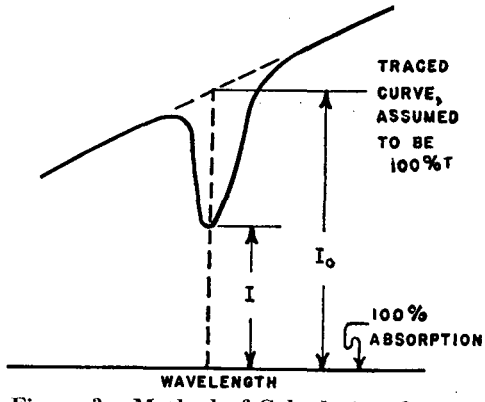

Figure 3. Method of Calculating Qualitative Values of % Transmittance

$\%T = (I/I_0)\,100$

The infrared absorption data were obtained on a Perkin-Elmer Corporation Model No. 12A infrared spectrometer having a specially built amplifier and recorder which produced a tracing of the spectra in terms of arbitrary units of galvanometer deflection. By bracketing the runs on the oils with runs on carbon tetrachloride and carbon bisulfide under the same conditions of light source, cell thickness, and slit-width schedule, it was possible to determine the galvanometer deflections for 100% transmittance, which were then used to compute the percentage transmittance of infrared light for the oils over the range investigated.

For the purpose at hand a more practical method of expressing the magnitude of the absorption bands was found satisfactory. This consisted of the calculation of a qualitative measure of the transmission directly from the recordings of galvanometer deflections by the method outlined in Figure 3.

By this procedure the "% transmittance" of the Pennsylvania oil and the non-Pennsylvania oil are 70.1 and 92.2, respectively, compared to actual transmittance of 49 and 67%, respectively, as given in Figure 2. In other words, the traced curve represents about 70 to 75% transmittance in this region instead of the 100% assumed in the qualitative method. The relative order of magnitude, however, is not changed, and the simplified method of calculation expedites the analytical work with about the same degree of accuracy as the more cumbersome procedure.

Typical values for the "transmittances" at 10.31 microns of a series of Pennsylvania oils and a series of non-Pennsylvania oils are listed in Table X. These values were calculated directly from the tracings produced when a cell thickness of 0.0868 ± 0.0005 mm. and a slit width of 0.202 mm. were used (theoretical resolution about 0.046μ). Considering the series of Pennsylvania oils, several items of interest are indicated: (1) Commercial solvent extraction apparently removes very little of the material causing absorption at 10.31 microns (samples L170 and L171). (2) Heavy acid treatment to water-white specifications, however, removed practically all these materials (samples 42832 and 42831). In similar studies it is also found that filtration through clays in normal commercial treatments causes little effect, but if very heavy treatments are employed to produce water-white oils the absorption at 10.31 microns may be slightly reduced in some cases. (3) This characteristic absorption band noted for Pennsylvania lubricating oils is also present in the crude oil itself and in all the fractions derived from it by distillation (sample series K-131). (4) Excluding the extract (L171) having a viscosity index of −96 and acid-treated water-white nonviscous neutral (42,831), the values of $(I/I_0)100$ for the Pennsylvania oils range from about 67 to 82. (5) The values obtained on oils derived from Allegany County, N. Y., crude oils, which showed slightly higher optical activity than the average for Pennsylvania oils (see Table III), are well within the limits defined for Pennsylvania oils.

For the series of non-Pennsylvania oils the range in values for $(I/I_0)100$ at 10.31μ appears to average from about 85 to 96. A

Table X. Infrared Absorption Data at 10.31 Microns for Oils from Various Sources

Sample No.	Description	Viscosity at 100° F., S.U.S.	Viscosity Index	Gravity Index	"% Transmittance" at 10.31μ, $(I/I_0)100$
	Pennsylvania Grade Oils				
3076	Neutral, refiner 1	143.5	105	96	71.5
3085	Neutral, refiner 4	143.3	110	104	79.4
3087	Neutral, refiner 7	137.8	115	106	69.8
3096	Neutral, refiner 3	142.9	111	102	78.6
3098	Neutral, refiner 10	146.2	104	97	71.6
3101	Neutral, refiner 11	145.5	109	99	69.5
3136	Neutral, refiner 6	135.5	112	103	76.3
3185	Neutral, refiner 8	142.4	101	97	71.4
L167	Unfiltered, neutral, refiner 5	176	98	..	72.4
L170	90% raffinate of L167	157.5	109	..	72.1
L171	10% extract of L167	1211	−96	..	86.3
L148	Lab. extracted neutral	154	115	..	74.8
082	Bright stock, refiner 11	2522	99	107	70.2
0199	SAE 30, Refiner 11	413	104	102	70.4
0211	Bright stock, refiner 5	2004	102	..	68.5
0213	Neutral, refiner 8	141.9	113	107	70.1
0215	Pressed distillate, refiner 4	88.3	124	..	81.1
0217	Straight-run gas oil, refiner 4	47.6	136	..	81.3
0218	SAE 30, refiner 11	405.2	106	..	70.2
K51N	Dewaxed distillate from crude (Table VIII)	163.5	96	95	82.2
K51CS	Cylinder stock from crude	2907	91	101	82.3
K131	Allegany crude oil (Table II)	....	..	..	72.8
K131G	Gasoline from crude	....	..	..	72.3
K131K	Kerosene from crude	....	..	..	76.8
K131GO	Gas oil from crude	....	..	..	73.1
K131N	Dewaxed distillate from crude	235.4	87	91	72.1
K131CS	Cylinder stock from crude	203/210°	..	..	67.8
K134N	Dewaxed distillate from crude (Table II)	244.6	89	92	71.7
K135N	Dewaxed distillate from crude (Table II)	239.4	89	92	71.6
42832	Nonviscous neutral	70.0	128	..	78.1
42831	Same, acid treated water white	64.6	148	..	92.2
	Non-Pennsylvania Oils				
0117	Rodessa extd. neutral	192.0	113	106	89.2
0118	Mid-continent extd. neutral	211	89	89	87.3
0122	Mid-continent extd. neutral	207	116	113	88.0
0134	Rodessa neutral	181	103	95	94.6
L211	Hydrolube SAE 20	254	100	..	91.4
L212	Big Lake, Texas, SAE 30	450	99	..	91.0
0214	Mid-continent, extd. neut.	148.4	119	112	92.2
M	Water white mid-continent	209.9	91	101	89.8
M20	Water white mid-continent	69.4	142	112	92.1
K11N	Distillate from Corning crude	164.5	92	94	86.3
K75N	Distillate from Big Lake (Texas) crude	154.9	100	107	92.7
K77N	Distillate from Corning crude	153.6	97	96	78.7
K78N	Distillate from Corning crude	150.1	99	97	79.2
K82N	Distillate from Kentucky crude	147.5	90	86	84.8
K83N	Distillate from Kentucky crude	186.6	77	84	72.2
K85N	Distillate from Canadian crude	217.9	62	52	95.0
K85CS	Cyl. stock from Canadian crude	4208	38	37	95.4
K98N	Distillate from Ellenberger (Texas) crude	157.9	101	85	93.8
K123N	Distillate from English crude (Table IX)	294.7	70	81	84.9
K124N	Distillate from English crude (Table IX)	347.7	77	93	91.0
K108G	Gasoline from Okla. crude	....	..	..	82.7
K108K	Kerosene from Okla. crude	....	..	..	91.5
K108GO	Gas oil from Okla. crude	....	..	..	93.7
K108N	Distillate from Okla. crude	301.9	78	84	91.9

few values below 85 are noted, indicating that this method would not screen out all of the non-Pennsylvania oils. The oils from the Ohio crudes, K77N and K78N, which show low optical activity (Table VIII) and values of $(I/I_0)100$ of 78.7 and 79.2, respectively, were undoubtedly classified as of Corning grade because of the poor quality of the cylinder stock which these crudes yield. The oil from the Kentucky crude (K83N) would not be considered as of Pennsylvania origin because of the low viscosity index and high optical activity; the optical rotations of the over-all neutral was +0.34° and values for 10% fractions would probably reach a maximum of about +0.45°.

The several non-Pennsylvania oils that showed relatively low optical activity (0212, K75N, K82N, and K98N) are now properly classified by their infrared absorption characteristics, since the values obtained for $(I/I_0)100$ are all above about 85. Only a very weak absorption band occurs at 10.31 microns in other fractions derived from non-Pennsylvania crude oils, even in the gasoline fraction (sample series K108).

The absorption band at other wave lengths in the infrared region are also useful in determining the general character of crude oils or petroleum fractions, and various correlations among these bands or with certain physical properties can be employed in identification work. For example, infrared absorption data may provide a convenient method for producers to determine the similarity of crudes coming from adjacent wells or from different levels in the same well.

ULTRAVIOLET ABSORPTION SPECTRA OF OILS

Extensive investigations of the optical properties of lubricating oils have yielded other useful correlations and methods applicable to the problem of oil identification. The ultraviolet absorption spectra of conventionally refined lubricating oils generally show an absorption band in the vicinity of about 260 mμ which varies in magnitude in almost direct relation to the concentration of aromatic compounds present in the oil (*5*, *14*). Removal of the greater portion of the aromatics by the use of a selective adsorbent causes a shift of this maximum from 260 to about 270 mμ and more sharply defines the minimum occurring at around 250 mμ.

Typical ultraviolet absorption spectra are shown in Figure 4 for several oils before and after treatment with fuller's earth to remove all coloring matter. The ultraviolet adsorption data were obtained with a Beckman quartz spectrophotometer using a hydrogen discharge tube for a light source. Pure refractionated "iso-octane" was employed as a reference and diluent, the concentration being expressed as grams of oil per liter of solution for the calculation of extinction coefficients. The absorption minimum of the water-white Pennsylvania oil at about 250 mμ is much sharper than that of the non-Pennsylvania oil. Expressing this as the percentage increase in the extinction coefficient, k, in going from 250 to 246 mμ, the numerical values are 53 and 8%, respectively.

Similar data for a series of oils of different origin are given in Table XI, which includes complete information on the clay treatment and the effect of this treatment on the physical and chemical properties of the oils. The Pennsylvania oils listed here represent typical products manufactured from crude oils in the New York, Pennsylvania, Ohio, and West Virginia regions. The non-Pennsylvania oils were selected to typify present-day manufacture of high-viscosity index mid-continent neutrals, which are not readily distinguishable from Pennsylvania oils by the usual inspection methods.

Maximum optical rotation of 10% fractions, however, would serve to differentiate these oils, the values being as follows:

Oil 0117	+0.84
Oil 0118	+1.21
Oil 0122	+0.77
Oil 0214	+0.46
Oil M	+1.57
Oil M20	+0.82

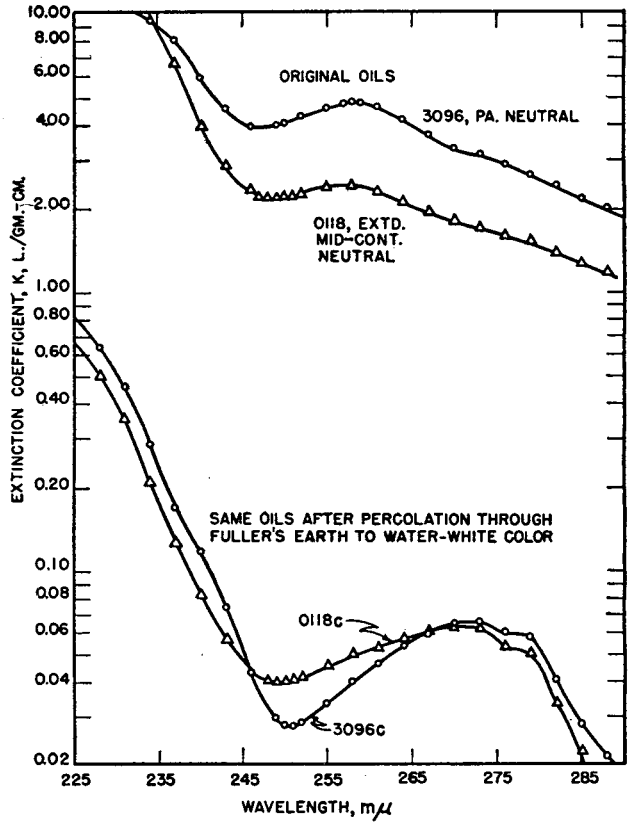

Figure 4. Typical Ultraviolet Absorption Spectra for Lubricating Oils before and after Clay Treatment

The data in the last column of Table XI, together with similar figures calculated for the range 250 to 240 mμ, may be summarized as follows:

Oil No.	% Increase in k, 250 to 246 mμ	% Increase in k, 250 to 240 mμ
Pennsylvania oils		
3096c	53	327
3076c	32	229
3101c	42	234
3087c	39	235
3085c	30	245
3098c	34	202
3185c	37	253
3136c	30	235
42831	57	826
Non-Pennsylvania oils		
0117c	16	132
0118c	8	104
0122c	12	121
0214c	−1	38
M	−8	136
M20	5	52

Before clay treatment to a water-white color, the oils do not show this differentiation, but on reducing the extinction coefficient by a standardized filtration to relatively low values at 250 mμ or by examining successive filtration fractions, the characteristics described can be utilized in identification work. For example, the highly extracted mid-continent neutral, 0214, showed an observed optical rotation per 10-cm. length of +0.32° before clay treatment, and +0.24° for the 4% water-white fraction. While the examination of the optical activity of 10% distillation fractions would also classify this oil properly, the ultraviolet absorption spectra in the region of 250 mμ differentiates this oil from those of Pennsylvania origin when the over-all oil is treated to a water-white color.

HYDROCARBON-TYPE ANALYSIS

The properties listed in Table XI include a comparison of the hydrocarbon-type analyses calculated by the Waterman proce-

Table XI. Physical Properties of Commercial Neutral Stocks and Water-White Oils Prepared from Them by Percolation through Fuller's Earth

Sample No.	Source	Clay Treatment, Lb./Gal.	Filtrate Taken, Wt. % of Charge	Gravity, °A.P.I.	Viscosity at 100° F., S.U.S.	Visc. Index	Gravity Index	Color[a] N.P.A.	Specific Magnetic Opt. Rot., $[\omega]_D^{20}$	Molecular Magnetic Rotation, $[\Omega]_D^{20}$	Hydrocarbon Type Analysis: Waterman Method: Wt. % arom.	Wt. % naph.	Wt. % paraff.	Mag. Opt. Rot. Method: Wt. % arom.	Wt. % naph.	Wt. % paraff.	Ratio, Naph. Paraff. (Mag. Opt. Method)	"% T" at 10.31μ, $(I/I_0)100$	Extinction Coeff., k, at 250 mμ	Increase in k from 250 to 246 mμ
									Pennsylvania Oils											
3096	Refiner 3			31.5	142.9	111	102	2¾	1.26	30.7										
3096c		5.8	4.07	34.5	131.0	118	117	WW	1.12	28.3	6	16	78	12	21	67	0.313	78.6	4.02	−2
3076	Refiner 1			30.3	143.5	105	96	2¾	1.27	30.4	0	20	80	2	17	81	0.210	83.5	0.0274	53
3076c		4.1	3.11	34.0	132.0	118	113	WW	1.13	28.6	9	16	75	12	22	66	0.333	71.5		
3101	Refiner 11			31.0	145.5	109	99	2½	1.25	30.7	0	23	77		24	74	0.311	76.7	0.0821	32
3101c		9.4	3.61	34.3	135.5	119	114	WW	1.11	28.0	8	15	77	2	24	74	0.271	69.5		
3087	Refiner 7			32.5	137.8	115	106	1¾	1.19	29.6	0	19	81	11	19	70	0.269	80.7	0.0497	42
3087c		6.6	3.86	34.3	137.2	117	114	WW	1.12	28.4	5	24	71	6	21	73	0.288	69.8		
3085	Refiner 4			31.8	143.3	110	120	3¼	1.23	29.8	0	19	81	1	21	78	0.253	80.5	0.0325	39
3085c		4.3	4.03	34.7	130.0	104	117	WW	1.13	28.5	6	17	77	7	17	76	0.223	79.9		
3098	Refiner 10			30.4	146.2	104	97	3	1.27	30.7	0	19	81	2	22	76	0.289	82.2	0.0383	30
3098c		9.3	3.93	34.2	135.1	115	114	WW	1.12	28.2	0	17	74	12	23	65	0.354	71.6		
3185	Refiner 8			30.7	142.4	101	97	2½	1.26	30.6	0	21	79	1	21	78	0.269	81.5	0.0360	34
3185c		6.7	3.89	34.1	129.2	116	113	WW	1.13	27.4	0	21	79	2	23	75	0.333	71.4		
3136	Refiner			32.0	135.3	112	103	2¾	1.23	30.1	7	15	78	9	20	71	0.307	77.3	0.0607	37
3136c		5.9	3.90	34.7	127.5	121	117	WW	1.12	28.2	0	19	81	1	21	78	0.269	76.3		
42832	Nonviscous			34.6	70.0	128	96	1¾	1.20	23.8	4	16	80	8	18	84	0.214	82.4	0.0439	30
42831		[b]		38.1	64.6	148	111	WW	1.09	21.6	0	6	94	0	22	78	0.282	78.1		
																		92.2		
									Non-Pennsylvania Oils											
0117	Extd. Rodessa			32.2	192.0	113	106	3	1.17	32.3	2	23	75	4	23	73	0.315	89.2		
0117c		5.8	3.14	33.2	206	115	111	WW	1.13	32.7	0	21	79	2	21	77	0.273	88.6	0.107	16
0118	Extd. M-C			29.0	211	89	89	3¼	1.22	31.3	6	26	68	8	24	68	0.375	87.3	2.19	5
0118c		5.8	3.93	32.0	173.9	105	105	WW	1.12	29.2	0	26	74	1	26	73	0.356	87.7	0.0395	8
0122	Extd. M-C			30.7	207	99	98	2½	1.19	31.1	4	25	71	6	26	68	0.383	88.0		
0122c		6.8	3.93	32.7	182.1	106	108	WW	1.12	29.9	0	22	78	1	24	75	0.320	88.4	0.0201	12
0214	Extd. M-C			33.7	148.4	119	119	1½	1.13	29.8	0	20	80	2	22	76	0.289	92.2	0.333	9
0214c		6.7	3.99	34.4	148.7	112	115	WW	1.11	29.4	0	13	87	0	23	77	0.299	89.9	0.0286	−1
M	Comm. white	[b]						WW										89.8	0.00122	−8
M20	Comm. white	[b]			69.4	142		WW										92.1	0.00279	−5

[a] WW = water-white.
[b] Prepared commercially by acid-treatment.

dure (*14*) and by a method utilizing the specific and molecular magnetic optical rotations. The latter method is based on experimental correlations of these properties with molecular weight for pure hydrocarbons (*8*). The two methods are in reasonable agreement, although the magneto-optic method tends to give slightly higher values for aromatic rings than obtained by the Waterman procedure. The ratio of the weight per cent aromatic rings to the weight per cent naphthene rings (A/N) or the ratio of the latter to the weight per cent paraffin chains (N/P) as calculated by the magneto-optic method for the untreated oils vary over a wide range, but when considered in conjunction with other physical properties such as the viscosity, viscosity index, refractive index, etc., the ratio becomes of some use in identifying highly naphthenic oils, even though the physical properties of the oils have been greatly altered by drastic refining procedures. For example, solvent extraction of non-Pennsylvania oils to match the physical properties of conventional Pennsylvania oils greatly reduces the ratio of aromatics to naphthenes but has a lesser effect on the ratio of naphthenes to paraffins.

OTHER METHODS

In addition to the methods of identification described above, physical and chemical characteristics of successive or selected fractions of the lubricating oil separated by distillation, solvent extraction, treatment with adsorbents, etc. For example, successive distillation fractions from Pennsylvania oils show greater uniformity in their derived constants such as viscosity index, gravity index, viscosity-gravity constant, etc., than distillate fractions of oils from other sources (*7*).

Similarly, methods may be employed which are based on differences in certain physical constants, particularly refractive index, boiling point, and density, of narrow-out fractions from oils of various sources when the fractions are matched in viscosity or average molecular weight, or when the oils have been treated by a standardized procedure to obtain specific fractions. A possible method utilizing the differences in behavior of treated oils from various sources when subjected to extreme pressures has been demonstrated (*15*).

ACKNOWLEDGMENT

The authors wish to thank the many refiners and producers who cooperated in furnishing samples of lubricating oils and crude oils for this study. They are particularly indebted to George W. Holbrook of the New York State Crude Oil Producers Association, Wellsville, N. Y., for supplying samples and crude oils from the New York region, and to D. T. Ring of the Preston Oil Company, Columbus, Ohio, for supplying numerous Ohio crude oils. The assistance of various members of the Pennsylvania Grade Crude Oil Association and of the staff of the Petroleum Refining Laboratory is acknowledged. The financial assistance of the association and permission to publish this article are appreciated.

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Received May 2, 1947. Previous articles on this subject have been published in *Industrial and Engineering Chemistry* (*4*, *7*).

Composition of Rosin Size Precipitates

Analyses of Standard Size Precipitates

DONNA PRICE

Hercules Experiment Station, Hercules Powder Company, Wilmington 99, Del.

Petroleum ether, in contrast to other solvents, did not change the composition of fresh rosin size precipitate extraction residue, even when used in the presence of excess water, and fresh size precipitates contained little or no oxidized resin acids that are insoluble in petroleum ether. Therefore, this solvent was used to study the composition of rosin size precipitates. Extraction with it isolated material of the composition of aluminum diresinate from laboratory preparations of rosin size precipitate, as well as from rosin-sized papers. For the analyses, the ratio carbon-aluminum was used instead of the more customary ash determination. A knowledge of the free rosin content of the original size and the use of a proposed size–aluminum sulfate reaction mechanism accounted for all size precipitate compositions observed. Preparations were made from sizes of 0, 20, and 75% free rosin.

A PRECEDING paper (*4*) mentioned that there is no satisfactory method available for the analysis of rosin size precipitate. Most attempts at analysis have been confined to a determination of the ash, and to solvent extraction of the precipitate (*1*, *6*). The ash values reported have been 4.78 to 5.69% of the size precipitate, which has frequently been interpreted to mean that the material was essentially aluminum triresinate, for which the theoretical ash is 5.5%.

In the present work more complete analysis was obtained by considering the carbon-aluminum ratio (C/Al). This value is independent of the moisture content of the precipitate, and it has served as a guide in determining the composition of precipitates from sizes of various free rosin contents, as well as in identifying sizing materials extracted from papers. Petroleum ether was used as a solvent for extraction of freshly prepared precipitates because it did not change the precipitate residue composition, as other solvents did, and because the fresh precipitates seemed to contain practically no oxidized resin acids that are insoluble in this solvent.

Based on data obtained from extractions and analyses of various size precipitates, a mechanism for the reaction between size and aluminum sulfate is suggested.

EXPERIMENTAL

The preparation of standard size precipitates was described in the first paper of this series (*4*). Flocs obtained by this standardized procedure were concentrated by decantation and then filtered in a Büchner funnel. The precipitate was washed in the funnel three times with distilled water, air-dried, then ground to pass a 60-mesh screen and dried in a vacuum desiccator.

The high dilution of the standard preparation corresponds to size concentrations in standard papermaking (*5*). For large samples—e.g., 20 grams of precipitate—filtration of the large volumes required several days. However, several pairs of analyses showed that the additional time of exposure of the precipitate to the liquor of the system in which it was prepared did not detectably affect its composition. For example, a sample of precipitate exposed for 4 hours and a sample of the same precipitate exposed for 24 hours gave respective carbon-aluminum values of 34.4 and 34.2.

Solvent extractions were carried out in a glass-stoppered, 250-ml. Erlenmeyer flask. Approximately a 5-gram sample of precipitate was placed in the flask, shaken with the solvent, and allowed to settle. The solvent was changed 7 to 10 times over a period of several weeks. The suspended material in the extract was removed and rinsed by centrifuging; it was then added to the residue in the flask. The residue of the extraction was air-dried and then dried to constant (or only slightly increasing) weight at 100° C. The extracted material was recovered by low-temperature evaporation of the solvent, and was finally dried at 100° C. Most extract solutions became cloudy either with age or upon heating.

The apparatus used for the continuous petroleum ether extraction of large samples of paper consisted of a distilling flask in which the extract was distilled and a large flask containing the paper sample through which the distillate was continuously circulated. The extract from the treatment flask was returned to the distilling flask. Cork stoppers sealed with sodium silicate were used in this apparatus. The paper sample was cut into narrow strips for extraction, and for each load the continuous process was carried on for 48 hours.

Chemicals. Size A was a paste of 70% solids; it contained 20% free rosin, based on the solids.

Size B was a dry, neutral (no free rosin) size.

Size C was an emulsion-type size of 40% solids; it contained 75% free rosin based on the solids.

The aluminum sulfate used was iron-free papermakers' alum, essentially $Al_2(SO_4)_3.18\ H_2O$.

The ethyl ether used was Merck, C.P., which had been dried and stored over metallic sodium. The petroleum ether was a commercial grade with specific gravity 0.6460 (20°/20°) and refractive index 1.3710 (n_D^{20}) and contained no nonvolatile component.

All other reagents used were C.P. grade.

Analyses. An analysis of size precipitates for total resin content was attempted by a procedure similar to that used in the paper analysis (*8*). Although size precipitate in the acidified alcohol specified by this method readily gave a clear solution, the residue by evaporation of this solution could not be completely

extracted with ether within a reasonable time. Instead of an analysis for total resin, therefore, the per cent carbon and per cent hydrogen of the precipitates were determined by the semimicroprocedure described by Fieser (*2*).

The ash determinations were, unless otherwise indicated, direct ashes at 1200° C. by both a macro and a semimicromethod, according to the size of the sample available. This high ignition temperature was necessary to decompose any aluminum sulfate to alumina.

"Benzene-insoluble" material in rosin size precipitate was determined in a few instances by treating a 2-gram sample with 50 ml. of warm anhydrous benzene, filtering the mixture through a weighed Selas crucible, drying the crucible at 100° C., and reweighing.

Moisture content was estimated in two ways: by drying at 100° C. for 16 hours, and by the Karl Fischer determination.

The methoxyl determination was made by a method based on the original procedure of Zeisel.

BEHAVIOR OF SIZE PRECIPITATE TOWARD VARIOUS SOLVENTS

The solubility behavior of rosin size precipitates is varied. Robinson (*6*) reported that aluminum abietate dried in a vacuum oven at 50° C., was soluble in benzene, chloroform, and absolute ether, insoluble in absolute alcohol and acetone. He also found that a small amount of water in the ether, heating the precipitate at 80° C., or boiling a benzene solution of the precipitate, resulted in an insolubilization of some of the material. Spurlin and Vandenberg (*7*) prepared a rosin size precipitate and dried it at 50° C. in a vacuum oven. This material dissolved readily in hydrocarbon solvents to give a clear solution but, after several hours' or days' standing (depending on the concentration), a considerable amount of precipitate formed or the solution gelled. The same behavior was observed for ethyl ether solutions. Spurlin and Vandenberg also refluxed a toluene solution of rosin size precipitate for 24 hours, and recovered from it only 0.1% water. They concluded that the presence of water was not responsible for the insolubilization of the size precipitate in aged toluene solutions. Spurlin and Vandenberg found that material began to precipitate from the toluene solution at 65° C.; Robinson found precipitation from boiling benzene (80° C.), but none from boiling chloroform (61° C.).

In the present work, freshly prepared size precipitate, dried at 45° to 50° C. in a vacuum oven, gave apparently clear solutions in benzene, but ultramicroscopic examination of such solutions showed the presence of many particles of about 1-micron diameter. A portion of the clear solution, sealed in a test tube, developed a gel-like precipitate in 24 hours. It seemed likely, therefore, that some material in the size precipitate, though easily dispersed, was not truly soluble. The ease of dispersion was found true for petroleum ether, ethyl ether, alcohol, and other solvents. Clear extracts generally formed a precipitate if they aged sufficiently, and precipitates of apparently 100% solubility when fresh showed only partial solubility after aging for a month in a desiccator. In view of this variable solubility behavior, no attempt was made to remove inorganic salts left in the precipitate after washing, as Robinson (*6*) did, by solvent treatment.

It was also found that size mixtures containing aluminum hydroxide could be dispersed in benzene so that ordinary filtration did not remove the alumina. The "benzene-insoluble" matter from a standard washed precipitate was negligible in amount (0.15%).

No very satisfactory method was found for determining the moisture on small samples of a size precipitate after the drying procedure used. Results obtained by oven-drying were in the same range as those obtained by Karl Fischer titrations. Precipitate mixtures showed as much as 5% weight loss when heated in a vacuum oven at 100° C., and the per cent water computed from the ash of benzene-insoluble material was also in this range. The data indicated that ordinary size precipitate prepared by the standard procedure (*4*), after the drying procedure used here, contained about 1% moisture.

In order to avoid more strenuous drying and its possible effect on the size precipitate, the carbon-aluminum ratio, independent of the water content, was used for all analytical study of the precipitates. The value of this ratio and other theoretical values for three aluminum resinates are given in Table I. The carbon-aluminum ratio of the data of Table II shows that, although the residue from a solvent extraction of size precipitate was about the same for wet and dry precipitate when petroleum ether was used, ethyl ether extraction resulted in the removal of a larger quantity of organic material from the extraction residue, and consequently, in a change in its composition. However, the ethyl ether residue from dry precipitate had the same composition as that obtained with petroleum ether; this indicated that there is a negligible amount of oxidized resin acids insoluble in petroleum ether in the freshly prepared size precipitates. This conclusion is also supported by the fact that ethyl ether extracted no more material from dry size precipitate than petroleum ether did (Table II). More polar solvents—e.g., anhydrous alcohol—showed a change in the composition of the extraction residue even on the dried precipitate. For this reason petroleum ether was used for solvent extraction, despite the fact that oxidized resin acids are insoluble in this material.

Table I. Theoretical Composition of Aluminum Resinates

Aluminum Resinates	C, %	Ash, %	Al, %	C/Al
$Al(C_{20}H_{29}O_2)(OH)_2$	66.3	14.1	7.46	8.88
$Al(C_{20}H_{29}O_2)_2OH$	74.3	7.89	4.18	17.8
$Al(C_{20}H_{29}O_2)_3$	77.4	5.49	2.91	26.6

Table II. Effect of Moisture on Solvent Extraction of Size Precipitates

Precipitate from Size A	Condition	Solvent	Analysis of Residue after Extraction: C, %	Ash, (1200° C.), %	Al, (calcd.), %	C/Al
1	Wet	Petroleum ether	69.1 69.5 69.2	8.65[a]	4.58	15.1
			69.3			
1	Wet	Ethyl ether	56.8 56.9	15.72 15.50		
			56.8	15.61	8.26	6.8
2 31.2% residue	Nearly dry[b]	Petroleum ether	70.4 70.5	8.20 8.20		
			70.5	8.20	4.38	16.2
3 33.8% residue	Dry[c]	Ethyl ether	67.3 67.1	7.99 8.10		
			67.2	8.06	4.27	15.7
4 33.6% residue	Dry[c]	Ethyl ether	67.8 67.8	8.25 8.24		
			67.8	8.24	4.36	15.6

[a] Sufficient material for only single determination.
[b] Vacuum desiccator 48 hours.
[c] Vacuum oven at 50° C. 2 hours.

The mechanism of solvent effect on the precipitate residues is not clear. However, in view of the fact that its severity increases with solvent polarity and hydrophylicity, hydrolysis seems a possible explanation. Thus, anhydrous ethyl ether had no effect, but water-saturated ether resulted in appreciable change of composition. Water-saturated petroleum ether (no change) would contain only about 5% of the amount of water in saturated ethyl ether. On the other hand, the water affinity of ethyl alcohol is so great that it is almost impossible to keep this solvent anhydrous. Moreover, in this case, alcoholysis might take place as well as hydrolysis.

Extractions of the wet precipitates reported in Table II were carried out on precipitate suspensions prepared in the standard manner (*4*). Instead of the usual procedure of filtering, washing, and drying the precipitate, the suspension was transferred to a separatory funnel and treated with the solvent.

The carbon-aluminum value for the residue from the petroleum ether extraction was 15.1 for the wet precipitate, as compared to 16.2 for the dry, and 17.8 (theoretical) for aluminum diresinate. Since the wet precipitate was not subjected to any

Table III. Analysis of First Precipitate from Solvent Extract of Wet Size Precipitate

Precipitate from Size A	Solvent	C, %	Ash (1200° C.), %	Al (Calcd.), %	C/Al
1	Petroleum ether	72.3 72.5	7.62 7.53		
		72.4	7.58	4.01	18.0
1	Ethyl ether	68.4[a]	10.21[a]	5.41	12.6

[a] Sufficient material for only single determination.

washing, whereas the dry one was treated in the standard manner, retention of water-soluble materials by the former seems most likely to be responsible for the difference in ratios.

The fact that the residue has a carbon-aluminum value less than the theoretical 17.8 may be explained by one or more of the following:

1. Ash-contributing, inorganic salts left in the precipitate by incomplete water washing.
2. Formation of a small amount of aluminum hydroxide (about 0.3%) which could not be detected analytically in the initial precipitate (*4*), and which would be concentrated in the residue of a petroleum ether extraction.
3. Formation of a small amount of aluminum monoresinate, which would be concentrated in the extraction residue.
4. Concentration of oxidized materials in the extraction residue. It will be shown in a subsequent paper that oxidation lowers the carbon-aluminum ratio of size precipitates.

Of these possibilities, the first seems both the simplest and the most probable. The size precipitates were difficult to wash and to filter. The washing procedure adopted certainly did not remove all water-soluble material. A precipitate prepared in distilled water containing 470 p.p.m. of sodium sulfate gave 0.10 to 0.15% sodium, and a precipitate prepared in distilled water was found to contain 0.27% sulfate. Both these figures give a maximum estimate of 0.4% sodium sulfate in the standard size precipitate. Hence, despite the low benzene-insoluble (0.15%), as much as 0.4% of the ash could have been due to sodium sulfate that had not been completely washed from the precipitate. This amount, a maximum, in view of its concentration in the residue of the extraction, is more than sufficient to lower the carbon-aluminum ratio for the residue from 17.8 to 16. It seems the most probable cause of the low carbon-aluminum ratio in the case of petroleum ether extractions. While the precipitate prepared in distilled water contained 0.27% sulfate (nitric acid digestion), the amount of sulfate that could be recovered from the 1200° C. precipitate ash, after three fusions, was less than 0.01%, based on the weight of the original precipitate. This suggests that the sulfate present in the precipitate was due to the incomplete hydrolysis of the aluminum diresinate in washing, rather than to adsorbed sodium sulfate.

In the solvent extraction of the wet size precipitate, both the ether and the petroleum ether extracts were initially clear. On concentration and exposure to air, precipitates formed as usual for size precipitate "solutions." The first material precipitated from each extract was filtered off, thoroughly washed with solvent, dried, and analyzed (Table III). The material precipitated from petroleum ether had the composition of aluminum diresinate. This confirms the suggestion that petroleum ether does not affect the precipitate residue chemically. On the other hand, the material similarly precipitated from ethyl ether had a carbon-aluminum ratio not only lower than 17.8 (theoretical), but also higher than the ratio found for the residue from this extraction. This confirms the previous suggestion of composition change of the wet precipitate by the ethyl ether.

COMPOSITION OF PRECIPITATES OBTAINED FROM VARIOUS SIZES

Analyses of precipitates prepared from sizes A, B, and C are shown in Table IV. Those for size B (neutral, no free rosin) agree with many previous reports for precipitates prepared from sodium abietate and sodium resinate in having the over-all composition of normal aluminum resinate (carbon-aluminum ratio, 25; theoretical, 26.6). Precipitates from sizes containing free rosin, however, reflected the presence of that free rosin in the carbon-aluminum ratio value of their precipitates. The present data indicate that the amount of aluminum sulfate required to form the precipitate depends on the amount of sodium resinate present and is independent of the free resin acids.

In general, aluminum was determined on the ash of a 0.5-gram sample. Hence, for this determination, the ash was subjected to a sodium carbonate fusion, subsequent acid solution, and precipitation of the aluminum. Precipitations of aluminum as the hydroxide, as the basic succinate, and by the 8-hydroxyquinoline method were investigated. Of these, the first was most satisfactory, and the standard R_2O_3 method was adopted for this determination. Because of the many operations involved and the difficulty of handling a gelatinous precipitate such as aluminum hydroxide it is evident that the aluminum determination is less accurate than the ash, particularly for small amounts of ash. Hence, while the carbon-aluminum value of 63 for the precipitate from size C in Table IV is appreciably lower than might be expected for a 75% free rosin size, it is considered more valid than the value of 300 obtained from direct aluminum analysis of a similar precipitate.

Further confirmation that the reaction involved in the formation of size precipitate is one between aluminum sulfate and the sodium resinate of the size was found in the alum requirements of the three sizes when used in papermaking. Typical data appear in Table V; they show that the alum requirement increased with increased sodium resinate content of the size or decreased free rosin content, as would be expected.

The data for petroleum ether extraction of two precipitates are given in Table VI. In both cases, the composition of the residue was approximately that of aluminum diresinate, despite the original free rosin content of the size. The material extracted obviously contains free rosin and neutral bodies, as well as some aluminum compound, possibly soluble diresinate. The amount

Table IV. Composition of Precipitates from Various Rosin Sizes

Size Precipitate	Size Used	Free Rosin in Size, %	C, %	Ash (1200°C.), %	Al (Calcd.), %	C/Al
5	B	0	72.9 73.0	5.46 5.43		
			73.0	5.44	2.88	25.3
6	B type[a]	1.8	73.2 72.8	5.58 5.54		
			73.0	5.56	2.94	24.8
2	A	20	74.4 74.5	4.40 4.40		
			74.5	4.40	2.33	31.9
7	A	20	75.0 75.6	4.54 4.64		
			75.3	4.59	2.43	31.2
8	A	20	71.7 71.6	3.95 3.97		
			71.6	3.96	2.10	34.1
9[b]	A	20	72.4 72.0 72.2	4.07 4.02 ..		
			72.2	4.05	2.14	33.7
10	A	20	75.7 75.7	4.16 4.19		
			75.7	4.18	2.21	34.2
11[c]	C	75	74.8 74.8	2.20 2.30		
			74.8	2.25	1.19	62.7

[a] Prepared from resin acids of acid No. 185; no rosin neutral bodies present.
[b] Required 12.6 ml. of 10% alum per 3 grams of size.
[c] Required 3.3 ml. of 10% alum per 3 grams of size.

Table V. Aluminum Sulfate Required by Various Sizes in Papermaking

[Conditions. 3.0% size. Burgess standard bleached sulfite pulp at 750 ± 10 Schopper-Riegler. Distilled water pH 5.3 to 5.6 to which nonalkaline salts were added to obtain total hardness of 450 p.p.m. $CaCO_3$. Standard papermaking procedure (*5*)]

Size	10% $Al_2(SO_4)_3$ Added to Sizing Crock[a]	Free Rosin in Size
	Ml.	%
A	17	20
B	19	0
C	13	75

[a] Average over eight hard waters used.

Table VI. Petroleum Ether Extraction of Size Precipitates

Material	C, %	Ash (1200° C.), %	Al (Calcd.), %	C/Al	Data in
		Size C			
Precipitate 11	74.8	2.25	1.19	62.7	Table III
Residue of extraction (23%)	64.9 65.0	6.70 6.80			
	65.0	6.75	3.57	18.1	
Extracted (78%)	75.4 75.1	0.26 0.25			
	75.2	0.26	0.14	539	
		Size A			
Precipitate 2	74.5	4.40	2.33	31.9	Table III
Residue of extraction (31%)	70.5	8.20	4.38	16.2	Table VII
Extracted (75%)	73.4 73.3	2.70 2.69			
	73.4	2.70	1.43	51.2	

of this material in the precipitate of size C is very small because size C is a high free-rosin size. The ash indicates only about 3% soluble aluminum diresinate in the original precipitate. In contrast to this, the material extracted from size A precipitate amounts to 26%, calculated as soluble aluminum diresinate.

ALUMINUM DIRESINATE IN SIZE PRECIPITATE

It has been shown that the residue of petroleum ether extraction of size precipitate (wet or dry) has a composition suggestive of aluminum diresinate, $Al(C_{20}H_{29}O_2)_2OH$. Moreover, the first material insolubilized and precipitated from a petroleum ether extract of size precipitate also had this composition. These findings suggest that an appreciable portion of size precipitate consists of this particular compound, and that in the fresh precipitate, a portion of the aluminum diresinate is "soluble" (or at least dispersible) in petroleum ether. Of this soluble portion a fraction became insolubilized, possibly by oxidation of the resin acids, as well as by some other mechanism such as coagulation.

Further evidence of the presence of aluminum diresinate in size precipitates was obtained by the continuous petroleum ether extraction of large samples of freshly prepared rosin-sized papers. Both laboratory papers (standard preparation, *5*) and commercial papers were used and the extractions were carried out on 900-gram samples. In both extractions a precipitate appeared in the extract at the end of 3 to 4 hours' operation. This material was removed and washed with solvent by centrifuging. After being dried in the usual manner, it was analyzed (Table VII). Comparison of the value for the carbon-aluminum ratio with theoretical values for the aluminum resinates (Table I) shows that this material has the composition of the diresinate. In both cases the amount obtained was about 0.14% of the bone-dry paper or about 10% of the size on the paper. However, a portion of the size precipitate remained on the paper, and another portion (ash-containing) remained soluble in the petroleum ether. Hence, the amount recovered in this fashion is no indication of the total amount present.

The aluminum diresinate recovered in this manner did not seem to be affected by alcohol or cold acidified alcohol, but it was somewhat swollen by carbon tetrachloride, and fused when it was heated in a flame.

After removal of the insolubilized aluminum diresinate, the clear extract was concentrated on a steam bath. It remained clear during this process, but formed a gelatinous precipitate when diluted with fresh solvent. This precipitate disappeared upon further concentration. The extract residue, a stiff paste, was dried to constant weight at 83° C. but, even so, it probably contained some petroleum ether residues. The dried paste was partially soluble in petroleum ether and alcohol, apparently completely so in carbon tetrachloride and in a 50-50 xylene-alcohol mixture. The incomplete solubility in petroleum ether might be caused by the oxidation and consequent insolubilization of some of the material or by coagulation of colloidal matter. The residue from the extract of the waterleaf paper had a consistency similar to soft wax and was completely soluble in the ether.

Analyses of the materials extracted from the papers, as well as analyses of the paper before and after extraction (shown in Table VIII), confirm the expected result that petroleum ether extraction removed a certain amount of free rosin and most of the nonacid rosin materials from the sized paper. The increase in the acid number of the resinous material (*8*) of the petroleum ether–extracted paper over that from the unextracted paper indicated that the petroleum ether removed some nonacidic material from the resin in the paper. The methoxyl content of rosins is low, whereas that of natural pulp resins and of rosin neutral bodies is high. Hence the high methoxyl content of material extracted from the sized paper (corrected for pulp resins) also indicated the removal of rosin neutral bodies by petroleum ether extraction. It also removed some aluminum compound which remained soluble in the petroleum ether extract during evaporation (see ash values in lower half of Table VIII). This is analogous to results obtained for the extraction of size precipitates. In fact, the carbon-aluminum ratio of material extracted from paper made with size A was approximately the same as that for material extracted from size A precipitate (see Table VI).

Table VII. Material Precipitated from Petroleum Ether Extracts of Rosin-Sized Papers

Paper Prepared with Size A	C, %	Ash (1200° C.), %	Al (Calcd.), %	C/Al
Laboratory sample	73.4 72.9	7.64 7.68		
	73.2	7.66	4.06	18.0
Mill sample	73.4 73.5	7.46 7.45		
	73.5	7.46	3.95	18.6

While the petroleum ether extractions described above indicate experimentally the occurrence of aluminum diresinate in size precipitate, there is a theoretical reason for expecting such a compound. The formation of $Al(C_{20}H_{29}O_2)_2{}^+HSO_4{}^-$ would account very readily for the positive charge of rosin size precipitate at papermaking pH's. Moreover, such a compound would be expected to hydrolyze with water-washing to give $Al(C_{20}H_{29}O_2)_2OH$, which would no longer have a positive charge, and which seems to be the material obtained from the size precipitates prepared in the standard manner.

SIZE PRECIPITATE COMPOSITION AND MECHANISM OF REACTION OF SIZE WITH ALUM

The present information about the composition of standard size precipitate may be summarized as follows:

1. Aluminum triresinate, the normal salt, hydrolyzes immediately upon contact with water to the diresinate and resin acid

Table VIII. Petroleum Ether Extraction of Sized Paper

Paper	Petroleum Ether Extracted	Paper Analyses: H_2SO_4 ash (1200° C.)[a], %	Paper Analyses: Extractable[b], %	Acid No. of Resin Extracted by TAPPI Method	Acid No. Corrected for Waterleaf
Waterleaf [no $Al_2(SO_4)_3$ used]	No	0.37	0.40	7	
			0.35	9	
			0.38	8	...
Waterleaf [no $Al_2(SO_4)_3$ used]	Yes	0.37	0.35	21	
		0.37	0.25	21	
		0.37	0.30	21	...
Sized paper	No	0.54	1.90	112	
			1.85	118	
			1.88	115	142
Sized paper	Yes	0.53	1.30	129	
		0.53	1.20	131	
		0.53	1.25	130	165

Material in Clear Petroleum Ether Extract

From	Material Extracted (Based on Pulp), %	C, %	Ash (1200° C.), %	Methoxyl, %	Al (Calcd.), %	C/Al
Waterleaf [no $Al_2(SO_4)_3$ used]	0.114	79.6		0.77		
		80.2		0.80		
		79.9	0.15	0.78	..	..
Sized paper	0.77	76.8		0.43		
		76.9		0.46		
		76.8	2.82	0.44	1.49	51.5
Sized paper (corrected for waterleaf)	0.66	76.3	3.28	0.38[c]	1.68	45.4

[a] Paper moistened with concentrated H_2SO_4 before ashing.
[b] TAPPI Method T408-m-44.
[c] For gum rosins, 0.10 to 0.14% methoxyl.

(7). Therefore, the normal salt cannot be expected in size precipitate.

2. Size precipitate contains no alumina [$Al(OH)_3$] as such. If present, the amount is negligible (4).

3. It contains a material of the composition of aluminum diresinate which can be isolated from sized paper.

4. It contains free resin acids and rosin-neutral bodies. This follows from items 1 and 3 above, and the fact that precipitate from neutral size has the over-all composition of aluminum triresinate. This is also indicated by the composition of material extracted by petroleum ether.

5. The amount of aluminum sulfate required to form the precipitate is determined only by the amount of sodium resinate in the size; the free resin acids are carried into the precipitate unchanged. [Bialkowsky (1) has shown that sodium resinate undergoes very little hydrolysis at beater concentrations and at moderate temperatures (30° C.), and that the presence of pulp affects the hydrolysis of sodium resinate. The effect of cellulosic materials on size precipitate composition will be considered in a subsequent paper.]

6. The residues from petroleum ether extractions of size precipitates have a composition approximating that of aluminum diresinate.

In the case of item 6 in three out of the four residues examined, the carbon-aluminum ratio was lower than the theoretical value; reasons for this have been discussed above. Because small amounts of sodium sulfate, alumina, or aluminum monoresinate (computed as 0.3, 0.3, and 3%, respectively) in the original precipitate, as well as oxidation, could account for this low carbon-aluminum value, the residues are assumed to be the diresinate.

The material removed by the petroleum ether contained free resin acids and neutral bodies of the rosin. It has been assumed that the carbon content of these materials will be about the same as for resin acids, and their ashes zero. This view is supported by rosin analyses, by the analysis of resinous materials removed from pulp (Table VIII), and by the nearly identical analyses of precipitates from size B and from a size containing no neutral bodies (Table IV).

The aluminum-containing material removed from the size precipitates by petroleum ether could not be the normal salt or alumina. As aluminum monoresinate would be expected to be even less soluble in petroleum ether than the diresinate, a further assumption was made that this material was soluble (or dispersible) aluminum diresinate. The absence of aluminum monoresinate was confirmed by the analysis of the material first insolubilized upon concentrating the extract (analysis of the diresinate).

Assumptions. To explain the mechanism of the aluminum sulfate–size reaction, the following assumptions were made on the basis of present information:

1. Residues of petroleum ether extraction of size precipitate are aluminum diresinate.
2. Resin acids and rosin neutral bodies have about the same per cent carbon and no ash.
3. The aluminum-containing material removed from size precipitate by petroleum ether is a soluble (or dispersed) aluminum diresinate.

The present information and these assumptions point to the reaction

$$Al^{+++} + 3\ Res^- + H_2O \longrightarrow AlRes_2OH\downarrow + HRes\downarrow \quad (1)$$

as the mechanism whereby size precipitate is formed. (The symbol Res is used to represent the various resin acid radicals present in the rosin size solution.) Nonacid material and free rosin, originally present in the size, will be carried down unchanged with the reaction products of Equation 1. The precipitate formed by the reaction will be 97.5% by weight of the sodium resinate added, and will consist of 32% resin acids and 68% aluminum diresinate, by weight. This corresponds very closely to the composition and mechanism suggested by Neugebauer (3) 25 years ago. Table IX contains the theoretical values of carbon-aluminum for various mixtures, obtained by the use of assumption 2 and Equation 1. Table IX indicates that in every case the carbon-aluminum ratio is a good approximation to (though slightly lower than) the theoretical value based on Equation 1. The small discrepancy is probably due to inadequate washing of the precipitate.

Table IX. Composition of Mixtures of Resin Acids and Aluminum Diresinate

Aluminum Diresinate, %	Resin Acids + Neutral Bodies, %	C, %	Al, %	C/Al
0	100	79.5	0	...
10	90	79.0	0.418	189
20	80	78.5	0.836	93.9
30	70	77.9	1.25	62.0
40	60	77.4	1.67	46.2
50	50	76.9	2.09	36.8
60	40	76.4	2.51	30.4
70	30	75.9	2.93	25.9
80	20	75.3	3.34	22.5
90	10	74.8	3.76	19.9
100	0	74.3	4.18	17.8

Precipitate Composition According to Proposed Mechanism[a]

Size	Aluminum Diresinate, %	Others, %	Actual C/Al Found
B	63	37	24.8–25.3
A	50	50	31.2–34.2
C	17	83	62.7

[a] 7.0% nonacid material assumed in size.

CONCLUSIONS

Size precipitate was easily dispersible in many solvents. No true solution should be assumed, despite visual clarity, unless the material can be shown to be a solution by ultramicroscopic examination.

Many solvents changed the composition of the size precipitate extraction residue, possibly by hydrolysis caused by traces of water. Ethyl ether and alcohol showed this effect.

Fresh size precipitates, prepared by a standard method, contained little or no oxidized resin acids. The standard preparation was made by adding 25 ml. of 3.0% size to 1 liter of dis-

tilled water and adding aluminum sulfate to obtain a final pH of 4.5.

Petroleum ether extraction did not change the composition of the precipitate residue, even in the presence of a water phase.

A material of the composition of aluminum diresinate was isolated from size precipitates and sized papers by petroleum ether extraction.

Petroleum ether extraction of size precipitates prepared from sizes of 0 to 75% free rosin left a residue of the approximate composition of aluminum diresinate in each case.

The reaction

$$Al^{+++} + 3\ Res^- + H_2O \longrightarrow AlRes_2OH\downarrow + HRes\downarrow$$

and the free rosin content of the original sizes used can account for the size precipitate compositions observed.

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RECEIVED August 28, 1947. Presented before the Division of Cellulose Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

Quantitative Separation of Calcium, Barium, and Strontium

SILVE KALLMANN, *Ledoux & Co., 155 Sixth Ave., New York, N. Y.*

A method for the quantitative separation of calcium, barium, and strontium is exclusively based on the difference in solubility of the chlorides of the three alkaline earths in *n*-butyl alcohol containing hydrogen chloride and in hydrochloric acid containing *n*-butyl alcohol. From the butyl alcohol solution of the perchlorates of calcium, barium, and strontium, a 20% solution of hydrogen chloride in *n*-butyl alcohol (Willard and Smith reagent) precipitates chlorides of barium and strontium; calcium chloride is very soluble. From an aqueous solution of the chlorides of barium and strontium, a 4 to 1 mixture of 11.0 *N* hydrochloric acid and *n*-butyl alcohol precipitates barium but not strontium chloride.

NO really good methods for the quantitative separation of calcium, barium, and strontium from each other are described in the literature.

From the anhydrous nitrates calcium may be extracted with absolute alcohol (*12*), or preferably, with a mixture of equal volumes of absolute alcohol and anhydrous ether (*3*, *10*) or with concentrated nitric acid (*9*). As these methods are based on extraction, retreatment of the filtered nitrates of barium and/or strontium, with intermittent solution in water, is required in dealing with larger amounts of the alkaline earths.

Far superior therefore is the precipitation method suggested by Willard and Goodspeed (*13*) in which barium and strontium are precipitated as nitrates by the addition of concentrated nitric acid to the aqueous solution of the mixed nitrates. Barium nitrate appears to be practically insoluble in 76% nitric acid, affording a clean-cut separation from calcium. Strontium nitrate, being appreciably soluble in the 76% nitric acid, requires an 80% acid concentration; this necessitates reprecipitation of the strontium nitrate in dealing with more than 25 mg. of calcium.

Less satisfactory are the methods for separating barium and strontium from each other. The favored procedure consists in precipitating barium chromate at a pH of about 4.6 in a buffered acetic acid–acetate solution. Reprecipitation of the barium chromate is usually necessary, and recovery of the strontium in the filtrate is equally tedious and involved. Considerable has been published from 1890 (*2*) to 1947 (*1*) regarding the details of this method, much of it contradictory.

On the other hand, the separation of barium and strontium with the aid of a 4 to 1 mixture of 10.6 *N* hydrochloric acid and ether (*4*) has scarcely been critically discussed in the literature. This writer carried out a number of tests (Table I), but was unable to confirm the findings of Gooch and Soderman, who claim that their method affords a clean-cut separation of the two alkaline earths.

In the first place, the solubility of barium chloride appears to be considerably greater than can be gathered from the data submitted by the authors (*4*), who state that the solubility of barium chloride in 75 ml. of a 4 to 1 mixture of 33% (10.6 *N*) hydrochloric acid and ether at 20° C. amounts to less than 0.5 mg. Mar (*8*), on the other hand, has shown that the solubility of barium chloride in a 4 to 1 mixture of concentrated (12.0 *N*) hydrochloric acid and ether at 20° C. amounts to approximately 1.3 mg. of barium chloride per 100 ml. of solution and states that the "solubility increases very rapidly with the diminuation in strength of acid." From the data in Table I it would appear that the solubility of barium chloride in a 4 to 1 mixture of 10.6 *N* hydrochloric acid and ether amounts to about 4 mg. per 100 ml. of solution, and that a marked tendency toward coprecipitation of strontium chloride with the barium chloride may offset part or all of the solubility losses of the barium chloride or may even cause high barium results.

Table I. Solubility of Barium Chloride in a 4 to 1 Mixture of 10.6 *N* Hydrochloric Acid and Ether in Presence of Strontium Chloride at 20° C.

$BaCl_2$ Taken	$SrCl_2$ Taken	$BaCl_2$ Found	Volume Exclusive of Washings	Error
Gram	*Gram*	*Gram*	*Ml.*	*Gram*
0.0500	....	0.0490	25	−0.0010
0.1000	....	0.0981	50	−0.0019
0.3000	....	0.2980	50	−0.0020
0.3000	....	0.2971	75	−0.0029
0.2500	0.0500	0.2482	75	−0.0018
0.2500	0.1000	0.2494	75	−0.0006
0.2500	0.2000	0.2513	75	+0.0013
0.2500	0.2500	0.2547	75	+0.0047
0.2500	0.3000	0.2591	75	+0.0091
0.0500	0.2000	0.0509	75	+0.0009

WILLARD AND SMITH REAGENT

In earlier papers (*6*, *7*, *14*) the effect of a 20% solution of hydrogen chloride in *n*-butyl alcohol (Willard and Smith reagent)

upon the butyl alcohol solution of the perchlorates of a number of elements was discussed.

It was shown that sodium is precipitated as chloride, that potassium which is insoluble as the perchlorate in *n*-butyl alcohol is partly converted into the equally insoluble chloride, and that many other perchlorates, notably those of aluminum, calcium, magnesium, and iron, are not precipitated.

Barium and strontium are precipitated, presumably quantitatively, by the Willard and Smith reagent, and data are presented here on the application of this reagent for the quantitative separation of barium and strontium from calcium. In addition, a new reagent, a 4 to 1 mixture of 11.0 *N* hydrochloric acid and butyl alcohol, is introduced for the separation of barium from strontium.

REAGENTS REQUIRED

1. Anhydrous *n*-butyl alcohol, $d_4^{25} = 0.8065$, boiling range 116° to 117.7° C., is readily available on the market.
2. Willard and Smith reagent, 20% solution of hydrogen chloride in *n*-butyl alcohol, is prepared by passing hydrogen chloride gas into *n*-butyl alcohol (*6*, *7*).
3. A 10% solution of hydrogen chloride in butyl alcohol is prepared by mixing equal volumes of reagents 1 and 2.
4. Mixture of 4 to 1 hydrochloric acid (11.0 *N*) and butyl alcohol. To 440 ml. of concentrated (12.0 *N*) hydrochloric acid are added 40 ml. of water. The solution is cooled to below 20° C. and 120 ml. of *n*-butyl alcohol are added.

Reagents Used in Experiments. Barium chloride free from calcium and strontium was prepared by dissolving the reagent-grade salt in water and precipitating the barium chloride with reagent 4.

Strontium nitrate free from calcium, barium, and alkali metals was prepared by dissolving the reagent-grade salt, which contained less than 0.005% barium but approximately 0.1% of calcium and 0.5% alkali metals, in water and precipitating the strontium nitrate by the addition of concentrated nitric acid (*13*).

A solution of calcium chloride was prepared by acidifying reagent-grade calcium carbonate, of very high purity, with hydrochloric acid.

PROCEDURE

Separation of Barium and Strontium from Calcium. Add 5 ml. of perchloric acid to the nitric or hydrochloric acid solution of calcium, barium, and strontium, contained in a 50-ml. beaker, and evaporate to dryness on a hot plate at a temperature not over 180° C. Cool, add 10 ml. of *n*-butyl alcohol, and heat with intermittent agitation just to boiling. A clear solution should result. Immediately add 10 ml. of the Willard and Smith reagent, the first 2 ml. dropwise, and continue the boiling for about a minute to facilitate the formation of a coarse crystalline precipitate.

[Inasmuch as the solubility of strontium chloride in a 10% solution of hydrogen chloride must not be neglected (see Figure 1) smaller amounts of butyl alcohol and Willard and Smith reagent should be used whenever feasible, particularly when dealing with small quantities of the alkaline earths.]

Digest at about 50° C. for about 15 minutes, cool to below 20° C., and decant the supernatant liquid into a dry but unweighed Gooch crucible, arranging the filtering apparatus so that the filtrate can be directly caught in a 250-ml. beaker. Transfer the precipitate to the Gooch crucible, police the beaker, and wash the crucible five to eight times with 1-ml. portions of the 10% solution of hydrogen chloride in butyl alcohol. Reserve the filtrate for the determination of calcium.

Dry the Gooch crucible for 1 hour at 110° C., and finally for 15 minutes in a muffle at 350° C. Cool in a desiccator and weigh. Dissolve the mixed chlorides of barium and strontium in a small amount of hot water, receiving the filtrate and washings directly in a 100-ml. beaker. Dry the crucible for 1 hour at 110° C., cool, and weigh. The loss in weight represents the combined chlorides, of barium and strontium. (The Gooch crucible, after ignition over a free flame, may be held for the subsequent separation of barium and strontium.)

Determination of Barium. Evaporate the solution of the mixed chlorides to dryness. Dissolve in 5.0 ml. of warm water (50° to 60° C.), then add with constant stirring 55 ml. of concentrated (12.0 *N*) hydrochloric acid and 15 ml. of *n*-butyl alcohol. Warm to about 75° C., then cool to below 20° C.

(The quantities of water, acid, and alcohol specified are intended for amounts of mixed chlorides not exceeding 500 mg. and containing not more than 250 mg. of strontium chloride. When the weight of the mixed chlorides is less than 250 mg., containing less than 125 mg. of strontium chloride, only half the amount of water, acid, and alcohol should be used.)

Decant the clear supernatant liquid through a weighed Gooch crucible (the one held from the previous operation may be used), receiving the filtrate directly into a 150-ml. beaker, transfer the barium chloride onto the crucible, police the beaker, and wash the crucible five to eight times with 1- to 2-ml. portions of reagent 4. Place the crucible on a small cover glass, dry for 1 hour at 110° C., and finally in a muffle for 15 minutes at 350° C. Cool in a desiccator and weigh as anhydrous barium chloride.

This weight should be corrected in accordance with the data presented in Table III. The solubility of barium chloride in 75 ml. of the water, acid, and alcohol mixture amounts to 2.0 mg. This solubility of barium chloride, however, is partly compensated by coprecipitation of strontium chloride. Therefore, for amounts of barium chloride up to 50 mg., add 2.0 mg., irrespective of the amount of strontium chloride present (up to the limit of 250 mg.). For amounts of barium chloride from 50 to 150 mg., add 2.0 mg. minus 0.4 mg. for every 100 mg. of strontium chloride present. For amounts of barium chloride from 150 to 500 mg., add 2.0 mg. minus 0.6 mg. for every 100 mg. of strontium chloride present.

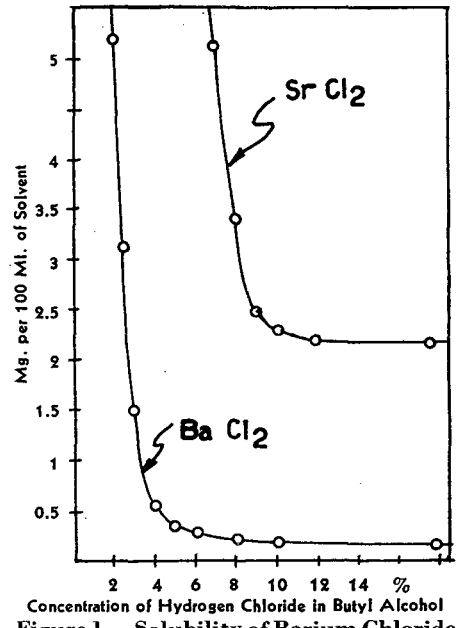

Figure 1. Solubility of Barium Chloride and Strontium Chloride in *n*-Butyl Alcohol Containing Willard and Smith Reagent at 20° C.

Determination of Strontium. Strontium chloride may be calculated by difference from the weight of the mixed chlorides, but for greater accuracy it can be easily determined with little extra work.

Evaporate the filtrate from the barium chloride to a small volume, transfer to a 50-ml. beaker, and evaporate to dryness. Add 5 ml. of water, 1 ml. of nitric acid, and 3 ml. of perchloric acid and evaporate to complete dryness on a hot plate at a temperature not higher than 180° C. To the cold strontium perchlorate add just sufficient *n*-butyl alcohol to cause complete solution when heated to boiling; 5 ml. of the alcohol should be ample for most work.

To the boiling solution add an equal volume of the Willard and Smith reagent, the first 2 ml. dropwise, and continue the boiling for about 0.5 minute to facilitate formation of a coarse crystalline precipitate. Cool to below 15° C., and filter on a dry tared Gooch crucible, policing the beaker and washing the crucible twice with 1-ml. portions of 10% hydrogen chloride in *n*-butyl alcohol. Dry the Gooch crucible for 1 hour at 110° C., then for 15 minutes at 350° C., and weigh as anhydrous strontium chloride after cooling in a desiccator. Correct this apparent weight of strontium chloride for the solubility of barium chloride in the hydrochloric acid–butyl alcohol mixture and for the coprecipitation of strontium chloride, in accordance with the instructions given above.

Table II. Separation of Barium and Strontium from Calcium

$BaCl_2$ Taken	$SrCl_2$ Taken	$CaCl_2$ Taken	$BaCl_2$ plus $SrCl_2$ Found	Error	CaO Found Equivalent to $CaCl_2$
Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
0.0210	....	0.1250	0.0211	+0.0001	0.1254
0.0421	....	0.2500	0.0418	−0.0003	0.2497
0.0421	....	0.5000	0.0424	+0.0003	0.5008
0.0421	0.0188	....	0.0606	−0.0003	....
0.0421	0.0754	....	0.1173	−0.0002	....
....	0.0019	0.2500	0.0014	−0.0005	0.2497
....	0.0188	0.2500	0.0183	−0.0005	0.2506
....	0.0754	0.2500	0.0750	−0.0004	0.2505
0.2104	....	0.2500	0.2103	−0.0001	0.2498
....	0.1884	0.2500	0.1881	−0.0003	0.2502
0.2104	0.0754	0.2500	0.2855	−0.0003	0.2505
0.2104	0.1884	....	0.3982	−0.0006	0.0003
0.2104	0.1884	0.5000	0.3986	−0.0002	0.5000
0.2104	0.1884	0.7500	0.3990	+0.0002	0.7497
0.2104	0.1884	1.0000	0.3999	+0.0011	0.9979
0.4208	0.3768	0.0500	0.7980	+0.0004	0.0498

Table III. Separation of Barium from Strontium

$BaCl_2$ Taken	$SrCl_2$ Taken	$BaCl_2$ Found	$BaCl_2$ Corrected	$SrCl_2$ Found	$SrCl_2$ Corrected	Volume of Precipitant
Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Ml.*
0.0210	....	0.0200	0.0210	0.0008	....	38
0.0421	....	0.0412	0.0422	0.0008	....	38
0.2104	....	0.2084	0.2104	0.0018	....	75
0.2104	....	0.2085	0.2105	0.0020	....	75
0.4208	....	0.4188	0.4208	0.0022	0.0002	75
0.0421	0.0754	0.0410	0.0420	0.0766	0.0756	38
0.0421	0.1884	0.0402	0.0422	0.1903	0.1883	75
0.0421	0.2826	0.0408	0.0428	0.2835	0.2815	75
0.0842	0.0754	0.0826	0.0843	0.0768	0.0751	75
0.0842	0.1884	0.0830	0.0843	0.1897	0.1884	75
0.0842	0.2826	0.0835	0.0844	0.2833	0.2824	75
0.1684	0.0754	0.1670	0.1686	0.0768	0.0752	75
0.1684	0.1884	0.1675	0.1684	0.1890	0.1881	75
0.1684	0.2826	0.1680	0.1683	0.2825	0.2822	75
0.2104	0.0754	0.2086	0.2102	0.0769	0.0753	75
0.2104	0.1884	0.2093	0.2102	0.1892	0.1883	75
0.2104	0.2826	0.2100	0.2103	0.2828	0.2825	75
0.4208	0.0754	0.4199	0.4214	0.0764	0.0749	75

Determination of Calcium. The filtrate from the strontium chloride may contain traces, but seldom more than 0.1 to 0.3 mg. of calcium chloride, which may have escaped the first separation of calcium from barium and strontium. For extreme accuracy, it should therefore be combined with the solution containing the bulk of the calcium.

Dilute the butyl alcohol–hydrogen chloride solution containing the calcium with one third its volume of water and evaporate on the water bath in such a way as to avoid condensation on the upper part of the beaker (*6*, *7*). When completely dry (prolonged heating should be avoided), add 10 ml. of water, 2 ml. of nitric acid, and 10 ml. of perchloric acid. Fume until fumes of perchloric acid escape. Cool and dilute with water. In the absence of elements of the R_2O_3 group precipitate the calcium in the usual way as oxalate, weighing it finally as the oxide or determining it volumetrically with potassium permanganate. In the presence of interfering elements, use separations described in standard analytical textbooks (*5*, *11*).

EXPERIMENTAL

Solubility of Barium and Strontium Chlorides. To determine the solubility of barium chloride and strontium chloride in varying concentrations of the Willard and Smith reagent, measured quantities of barium chloride and strontium nitrate were fumed to dryness with an excess of perchloric acid. To the dry salts measured amounts of butyl alcohol were added, the solutions heated to boiling, and the barium and strontium precipitated as chlorides by the addition of varying measured amounts of the Willard and Smith reagent. After cooling, the solutions were filtered through Gooch crucibles and evaporated to dryness on the water bath with the usual precautions, and after fuming with nitric and sulfuric acids, barium and strontium were determined as the sulfates. The resulting data are presented in Figure 1.

It is apparent that the solubility of barium chloride in the 5 to 6% solution of hydrogen chloride in butyl alcohol, used in earlier work for the determination of the alkali metals, is very small. On the other hand, the solubility of strontium chloride in the same medium is much greater, and minimum solubility is approached only when the concentration of the hydrogen chloride is increased to 10%. Therefore, a 10% solution of hydrogen chloride in butyl alcohol has been used throughout this work. A solubility of 2.2 mg. of strontium chloride in 100 ml. of the 10% solution of hydrogen chloride in butyl alcohol may seem prohibitive for quantitative analysis. However, 20 ml. is the maximum quantity of solution required, exclusive of washings, even in operating upon a mixture of 0.3 gram of each of the three alkaline earths. Even smaller volumes may be used with minor quantities of the three elements.

Separation of Barium and Strontium from Calcium. Mixtures of known quantities of the alkaline earths were fumed to dryness with an excess of perchloric acid and subjected to the recommended procedure for the separation of barium and strontium from calcium. The resulting data, presented in Table II, indicate that the proposed method affords a complete separation of the two alkaline earths from calcium. This should prove an attractive feature not found in the earlier extraction methods. Even the excellent precipitation method of Willard and Goodspeed (*13*) requires retreatment of the strontium salt in dealing with more than 25 mg. of calcium.

Separation of Barium from Strontium. Mixtures of known quantities of barium and strontium were subjected to the proposed procedure for the separation of barium from strontium (Table III). Solubility corrections were applied in accordance with instructions given in an earlier part of this paper.

Interfering Elements. In an earlier paper (*7*) the effect of the Willard and Smith reagent upon the perchlorates of a number of elements was discussed; it is apparent that only a limited number of elements interfere with the method described in this paper.

Sodium, potassium, and large amounts of ammonium salts should be absent. They may be removed by precipitating the alkaline earths with ammonium carbonate, followed by their conversion into the perchlorates. Small amounts of potassium, but not sodium, may be removed as perchlorate from the butyl alcohol solution prior to addition of the Willard and Smith reagent.

The sulfate ion should be absent. When dealing with a mixture of the sulfates of the alkaline earths, preliminary steps would involve fusion of the sample with potassium carbonate, solution of the filtered and washed carbonates in hydrochloric acid, precipitation with ammonium carbonate, and conversion of the filtered and washed carbonates into perchlorates; or the ammonium carbonate separation may be omitted and any potassium, retained by the alkaline earths, be removed as perchlorate prior to addition of the Willard and Smith reagent.

Lead should be absent and may be removed by precipitation with hydrogen sulfide in a nonoxidizing dilute hydrochloric acid solution, or preferably, by electrolysis in a nitric acid solution.

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RECEIVED June 19, 1947.

Determination of Water in Dry Food Materials

Karl Fischer Method

C. W. SCHROEDER AND J. H. NAIR, *Continental Foods, Inc., Hoboken, N. J.*

In a search for a more reliable method for determining moisture in food materials of a low residual moisture content (ca. 5%), the Karl Fischer titrimetric procedure has been investigated over a period of years. The advantages and disadvantages of this method have been explored. Certain modifications of technique are described and evidence is presented of side reactions occurring with certain food materials. Data developed on a number of dried foodstuffs and low-moisture food mixtures are presented in tables and graphs.

THE measurement of the moisture content in food materials is carried out by a variety of methods; drying in an air oven or in a vacuum oven is probably most widely used. For certain purposes, such as determining moisture in grains, electrical methods of measurement are used. Distillation of the material with an immiscible liquid such as toluene has been adapted for a number of food materials. When these methods are applied to foodstuffs having residual moisture contents of 5% or less, they frequently yield inaccurate or nonreproducible results. Electrical methods depend on changes in conductance or capacitance with moisture content. In the region of low moistures these changes are small and difficult to measure. The extreme sensitivity of readings to temperature variations imposes distinct limitations under some conditions.

The Association of Official Agricultural Chemists has standardized (*4*), generally, on the use of an electrically heated and thermoregulated vacuum oven for moisture determinations in food materials. This affords a greater protection against decomposition of the foodstuffs than is possible with air ovens, because the use of vacuum increases the rapidity of moisture removal and consequently reduces required drying times and temperatures. For many foodstuffs approximately 6 hours at 70° C. is accepted as the end point for vacuum oven drying. In some instances a current of dry air is passed slowly through the oven under an absolute pressure less than 100 mm. of mercury. Drying in the vacuum oven as well as in the air oven (*5*) is due to a combination of conduction, radiation, and convection. Temperatures used in air oven drying are rather arbitrary, varying with the product between 100° and 135° C. It is commonly found that with oven methods the material never comes to constant weight.

Homogeneity of particle size is extremely important to obtain even removal of moisture. Decomposition, loss of volatile constituents other than water, and oxidation of certain components may all cause changes in weight other than those due to moisture removal. Distillation with an immiscible organic liquid such as toluene or xylene has the advantage that the water present is distilled over, condensed, and collected in a calibrated receiving trap and the moisture content of the material is calculated directly from the volume of water. The method is empirical and is hazardous with food materials that char or decompose at the boiling point of the liquid.

The various difficulties encountered in the application of such methods for the determination of moisture in dehydrated materials led to an investigation of the Karl Fischer volumetric method, which has been applied to a large number of foodstuffs (*11, 15, 16, 18, 33, 40, 43*) as well as to various technical products (*1, 2, 3, 8, 9, 10, 13, 17, 18, 20–25, 31, 32, 34*). It has been employed to determine compounds other than water by indirect means (*6, 14, 26, 27, 29, 30, 37, 38, 39*). One group of investigators has reported on the nature of the reagent and interferences developing with certain compounds (*7, 8, 28, 36*). In some work the reagent itself has been used to indicate the end point visually (*10, 14, 18, 34, 36*), but many investigators have proposed a variety of electrical arrangements to indicate the end point regardless of any interfering colors (*1, 3, 9, 15, 19, 21, 23, 32, 40, 42*). It has been recommended that direct titration with the reagent be used (*1, 9, 10, 18, 34, 36*) or that an excess be added and back-titrated with methanol containing a known quantity of water (*2, 3, 23, 32, 40–43*).

The accuracy of the Karl Fischer procedure is difficult to demonstrate with most foodstuffs because of the lack of any method that is known with certainty to give correct results for moisture content. In principle, a method that depends upon the total quantity of moisture in the sample should be more reliable and exact than one that measures only loss of weight, which is assumed to be due to the evaporation of moisture. Bryant *et al.* (*8*) have shown that the Fischer reagent reacts completely even with the water of hydration of certain inorganic salts. Their results have been corroborated in this laboratory. When quick results on a given sample are required, the Fischer titration method is advantageous, as a determination may be carried out in about 15 minutes. On the other hand, the method must be applied

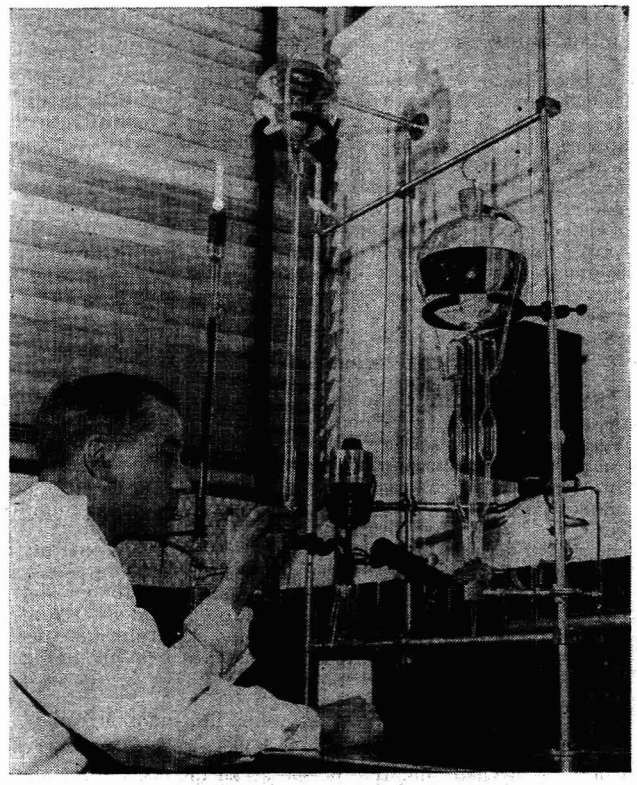

Figure 1. General Arrangement of Apparatus

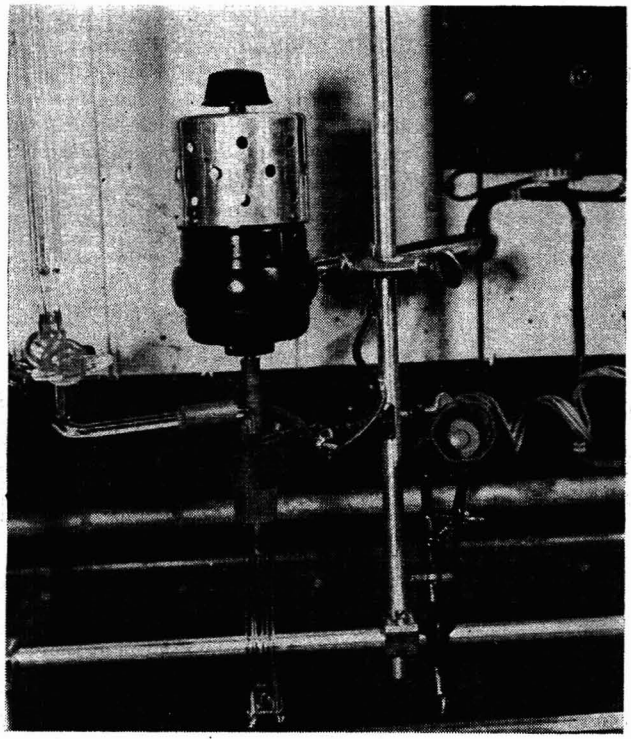

Figure 2. Magic Eye Tube

with caution because of the evidence of side reactions with some food materials. This method is believed to be the only one suitable for the determination of moisture in materials such as volatile spice oils on a salt or dextrose base where the volatility of the oil nullifies the oven method and the low moisture content (about 0.1%) makes a distillation procedure impractical. In all cases the ease of duplicating results is a definite advantage.

APPARATUS

The arrangement used in this laboratory of a Bakelite stopper, fitted to a No. 27 standard-taper 250-ml. glass-stoppered Erlenmeyer flask, and through which pass the electrodes, glass stirrer, and buret tip, is similar to that described by Wernimont and Hopkinson (*42*). The end point, except when visual titration is used, is detected by means of the Serfass rectified titrimeter circuit (*35*, Figure 2) modified by elimination of *R*-5 and *S*-1 and changing *R*-3 to 100,000 ohms. This arrangement has given satisfactory results for several years on either alternating or direct current.

The Fischer reagent is dispensed from an automatic buret with attached reservoir (Machlett No. A8-470). The standard water-in-methanol solution is delivered from another automatic buret (Machlett No. A8-525) altered by lateral extension of the delivery tip about 10 cm. (4 inches) to one side to facilitate passing it through the Bakelite stopper of the titration assembly. Anhydrous methanol for extraction of the moisture from the samples is delivered from an automatic 25-ml. pipet (Machlett No. 71-645). The burets and pipet are protected by drying tubes containing Drierite. General arrangement of the apparatus is shown in Figure 1, and details, including the 6E5 Magic Eye tube which serves as the end-point indicator, in Figure 2. The burets are interchangeable in the titration assembly, so that either direct or indirect titrations can be made.

REAGENTS

The Fischer reagent and standard water-in-methanol solution have been prepared according to the directions of Wernimont and Hopkinson (*42*) with one exception: the use of double the quantity of methanol in the Fischer reagent, which prevents the separation of crystalline derivatives on standing. The Fischer reagent loses strength at the rate of about 1% a day, being somewhat less stable when first prepared. The loss of strength has been attributed to the reduction of iodine to iodide with simultaneous formation of quaternary methyl pyridinium salts (*36*). For this reason the titer must be checked daily, either against the standard water-in-methanol solution or against weighed amounts of water in methanol blanks. The standard water-in-methanol solution is checked weekly, although it has been found unchanged.

Direct titration with the reagent to an electrometric end point, as described below, is used for standardizations. Duplicate 25-ml. methanol blanks contained in dried flasks are first titrated with the Fischer reagent, following which approximately 0.1-gram portions of water are weighed to 0.1 mg. from a weighing bottle into other duplicate 25-ml. blanks, and these are titrated. The titer of the Fischer reagent is then readily calculated. Ten to 15-ml. portions of the standard water-in-methanol solution may then be titrated to determine its water content.

Commercial anhydrous methanol was found to contain 0.02 to 0.08% water and for most purposes was not redried. When redrying was required for special purposes, the procedure of Gilman and Blatt (*12*) was used.

METHODS

A 2-gram sample is used for materials containing up to 10% moisture. When higher moisture contents are encountered the sample size should be correspondingly reduced to avoid excessive use of reagent. Solid samples must be ground to pass a 40-mesh screen.

The sample is transferred to a glass-stoppered titration flask which has previously been dried 1 hour at 100° C. and cooled with stopper in place, 25 ml. of anhydrous methanol are added, and the mixture is immediately refluxed 5 minutes or longer to extract the bulk of the moisture. The condensers are fitted at the tops with rubber gas expansion bags (Eimer and Amend No. 10-675) to prevent loss or gain of moisture through the condenser. The flasks are heated in baths at 90° C. containing Carbowax 1000 (Carbide and Carbon Chemicals Corp.). This product, being water-soluble, nonvolatile, and nonhygroscopic, is more satisfactory than the usual bath materials.

Following refluxing, the flask is cooled to room temperature in a water bath while still attached to the condenser. It is then wiped dry, detached, and connected to the titration assembly. The mixture is titrated directly with the Fischer reagent, using the modified Serfass circuit, until the Magic Eye tube remains open at least 30 seconds. In order to avoid a false end-point, care must be excercised with solid samples from which the moisture is only slowly extracted to avoid adding the reagent at a rate much exceeding the extraction rate. After subtracting the methanol blank, the percentage of moisture is calculated directly from the titer of the reagent.

Table I. Titration of Water of Hydration of Inorganic Salts

	Direct Visual Titration	Direct Electrometric Titration	Indirect Electrometric Titration
	Moles	*Moles*	*Moles*
$CuSO_4.5H_2O$	4.53	4.50	4.49
	4.53	4.54	4.49
	..	4.51	..
$Fe(NH_4)_2(SO_4)_2.6H_2O$	..	5.98	..
	..	5.96	..

Direct electrometric titration has the advantage over visual titration of allowing dark-colored samples to be run. It is preferred to electrometric back-titration with standard water-in-methanol solution, following addition of excess Fischer reagent, because it reduces the likelihood of side reactions, requires less reagent, and simplifies the calculations.

A series of tests was run to determine whether the above three methods gave the same results and to evaluate the accuracy of the general method. Copper sulfate pentahydrate and ferrous ammonium sulfate hexahydrate were titrated and gave theoretical results in each case (Table I). However a correction must be applied to the results with copper sulfate. As pointed out by Mitchell, Smith, Ashby, and Bryant (*28*), the cupric ion reoxidizes reduced iodine equivalent to 0.5 mole of water, so that an apparent water content of 4.5 moles is found. When allowance is made for this side reaction the water of hydration is seen to be completely titrated.

Dehydrated food products were run by direct visual titration and indirect electrometric titration (Table II). In almost all cases results by the two methods checked within a few hundredths of a per cent and the averages on all samples were the

Table II. Water in Dehydrated Food Products

Sample	Direct Visual Titration	Indirect Electrometric Titration
	%	%
Mixed vegetables	2.45	2.45
	2.36	2.34
Pea soup mix	3.55	3.58
Pea powder	2.99	2.90
	4.50	4.46
Anhydrous dextrose	0.05	0.02
Soy protein	4.89	5.21
Beans (cooked)	3.94	3.93
Noodles	7.91	7.93
Thyme oleoresin on salt base	0.07	0.08
Clove oleoresin on salt base	0.04	0.04
Bread crumbs	1.05	1.00
Wheat flour	3.74	3.74
Tomato flakes	3.87	3.77

same by either method. Lower results by the direct method on soy protein may be due to slow extraction of water.

In many cases the apparent moisture content of samples run by the Fischer method increases as the period of refluxing with the methanol is increased. This may be due to partial decomposition at the temperature of boiling methanol. Preliminary refluxing, however, is desirable with ground solid samples in order to expedite the extraction of water; low results are obtained with room temperature extraction unless the sample is allowed to stand in methanol for a period of hours or days.

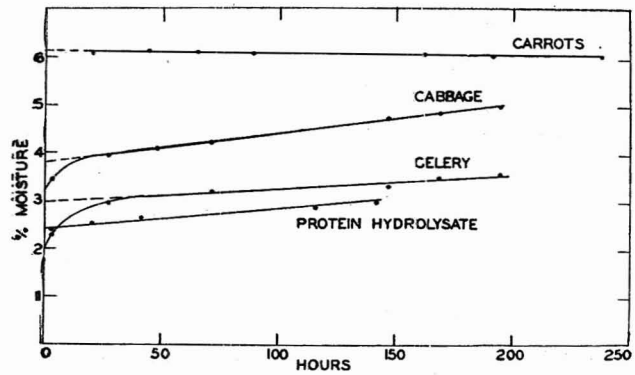

Figure 3. Representative Results by the Intermittent Titration Method

Two modifications of the Fischer method which did not involve heating were used to determine the proper time of refluxing.

The first, designated as the Fischer intermittent method, involved titration at intervals to electrometric end points of duplicate or triplicate 2-gram samples suspended in 25 ml. of methanol. A corresponding number of methanol blanks were run at the same time in order to correct for moisture absorption from the air or deterioration of the slight excess of reagent present after each titration. The samples and blanks were contained in dried, glass-stoppered flasks, the stoppers of which were greased with dried silicone stopcock grease. In general, samples and blanks were titrated daily for 6 to 12 days and the results plotted. After the initial titration a fraction of a milliliter of Fischer reagent was generally sufficient to restore the end point, but with some samples, notably ground noodles, additional free iodine was formed on standing as indicated by deepening of the brown color of the mixture and titration with standard water-in-methanol solution was necessary. No attempt was made to remove the slight amount of reagent that adhered to the electrodes and stirrer after each titration, the blank value being relied upon to correct for this loss. It was considered that holding the sample in an anhydrous medium in this manner for an extended period would ensure the complete removal of water at room temperature.

The results when plotted against the time of standing showed a steep rise in the first few hours, followed by a gently sloping straight-line portion which gave no indication of leveling off. The slope of the straight-line portion varied with the samples, being greatest with cabbage and negative with noodles and carrots. This portion of the curve was interpreted as indicating slow side reactions. Consequently, the value used was that obtained by extrapolating the straight-line portion back to its intersection with the vertical axis. If it be assumed that all side reactions proceed at a much slower rate than that with water, this value should approximate the true moisture content of the sample. Representative results by this method on a number of samples are plotted in Figure 3.

Table III. Titration of Dried Foods

Samples	Intermittent Titration (Extrapolated)	Allowed to Soak in Methanol: Low moisture CH_3OH	Allowed to Soak in Methanol: High moisture CH_3OH	Refluxed with Methanol, Minutes: 2	5	15	30
	%	%	%	%	%	%	%
Onion powder	3.95	3.97	3.93	3.90	3.90	3.84	4.01
Protein hydrolyzate	2.41	2.56	2.52	2.47	2.44	2.51	2.54
Carrots	6.13	6.22	6.19	6.13	6.20	6.26	6.32
Peppers	3.58	3.61	3.68	3.35	3.49	3.61	3.78
Cabbage	3.82	4.37	4.25	3.96	4.00	4.07	4.19
Celery	2.99	2.97	2.96	2.79	2.84	2.91	2.99
Tomato	3.20	3.25	3.19	3.04	3.07	3.16	3.32

The second modification of the Fischer method which did not involve heating consisted of allowing samples to soak in methanol at room temperature for varying periods before titrating. Samples were run in duplicate and duplicate blanks run at the same time to correct for any moisture pickup from the air as well as that originally present in the methanol. Two or more series were run with each sample, using methanol of differing initial moisture contents, to determine whether the amount of moisture initially present in the methanol affected the completeness of extraction. Increasing the moisture content of the methanol from levels of 0.03 to 0.06% to the range of 0.06 to 0.54% made little difference in the final results obtained. Sufficient samples and blanks were set up in each case so that titrations could be continued until the last few sets of duplicates showed no further upward trend. The time necessary to reach this equilibrium varied from a few hours to about 2 days. Further titrations were then carried out at intervals up to a total soaking time of 6 to 11 days to ensure that no further increase took place in the moisture content as determined. The final value was derived by averaging all figures obtained after the plateau was reached.

Table III compares results on a number of dried foods by these modifications and by the standard method involving refluxing. All were ground to pass 40-mesh unless initially finer.

PRECISION

The precision of the method is indicated by the close checks generally obtained on duplicate samples. Results in duplicate, selected at random, are given in Table IV.

Table IV. Precision of Method

Sample	% Moisture	
Cornstarch	13.9	13.9
Dextrose, anhydrous	0.06	0.05
Corn sirup solids	3.45	3.39
Noodles	7.88	7.94
Onions, dehydrated	3.79	3.83
Carrots, dehydrated	6.18	6.22
Peppers, dehydrated	3.47	3.51
Cabbage, dehydrated	4.02	3.98
Celery, dehydrated	2.85	2.83
Tomato, dehydrated	3.09	3.04
Chicken meat solids	3.39	3.42
Protein hydrolyzate solids, 1	2.51	2.51
Protein hydrolyzate solids, 2	3.05	2.94, 3.04
Monosodium glutamate monohydrate	9.91	9.89

A survey of the literature indicates that most investigators have obtained a precision within ±1 mg. of water. The authors' results fell in the same range and dictated the use of a normal sample weight of 2 grams in order to obtain results checking within 0.1%.

COMPARATIVE RESULTS BY DIFFERENT METHODS

Determinations by oven-drying methods and by distillation with toluene have been compared with the Fischer results on

Table V. Moisture in Foods

Sample	Fischer Method	Vacuum Oven 70° C., 6 hours	Vacuum Oven 70° C., 16 hours	Vacuum Oven 100° C. to constant weight	Air Oven, 130° C.	Toluene Distillation
	%	%	%	%	%	%
Mixed vegetables						
20-mesh	6.48	5.77	5.96	..	..	...
40-mesh	6.50	5.93	6.00	..	..	...
60-mesh	6.44	6.00	6.02	..	..	...
Onion	3.24	2.95	..	..	..	...
Carrots	6.20	4.22	5.42	..	..	....
Peppers	3.49	3.25	3.56	..	..	...
Cabbage	4.00	3.43	3.69	..	..	...
Celery	2.84	2.37	2.66	..	..	...
Tomato	3.07	2.57	3.20	..	..	...
Protein hydrolyzate	2.44	2.48	2.41	..	..	2.40
Cornstarch	13.9	..	..	14.1 (7 hours)	14.0 (1 hour)	13.2
Monosodium glutamate monohydrate	9.79	..	0.7	..	..	...
	0.3[a]	..	..	..	..	...
Dry soup mix 1	1.85[a]	..	1.79	..	..	...
Dry soup mix 2	1.38[a]	..	1.27	..	..	...

[a] Results corrected for water of hydration of monosodium glutamate monohydrate.

various types of foods (Table V). All samples were ground to pass 40-mesh except where noted.

Results by the Fischer method, particularly on dehydrated vegetables, tend to run higher than by the oven methods. The dehydrating power of the reagent is also made evident by its ability to remove the water of hydration from monosodium glutamate, whereas the vacuum oven at 70° C. bled with a current of dry air removes little more than the adsorbed moisture.

Table VI. Evidence of Side Reactions

	Per Cent Moisture	
	Onion	Protein hydrolyzate
Standard Fischer method	3.78	3.30
In contact with excess reagent	(Approx. 10-ml. excess)	(Approx. 20-ml excess)
0.5 hour	..	4.33
1 hour	..	4.50
2 hours	4.92	5.22
4 hours	..	5.98
19 hours	6.28	..
46 hours	6.50	..
118 hours	7.19	..
Held for constant time with excess reagent	96 Hours	4 Hours
Approx. 10-ml. excess	6.98	4.65
Approx. 20-ml. excess	7.50	5.90
Approx. 30-ml. excess	7.83	6.83
Approx. 40-ml. excess	7.98	7.69

EVIDENCE OF SIDE REACTIONS

The experiment has been tried of allowing samples of dehydrated onion powder and protein hydrolyzate solids to stand for varying times in an excess of Fischer reagent, followed by back-titration with standard water-in-methanol solution. In both cases the apparent water content increased considerably on prolonged standing with the reagent. Blank determinations were run at the same time to correct for deterioration of the reagent or moisture pickup from the air.

Somewhat similar experiments were run in which the amount of excess reagent was varied while the time of standing was held constant. In this case the apparent moisture content increased almost in proportion to the amount of excess reagent (Table VI).

Although these results evidently demonstrate that the reagent is by no means specific for the moisture in onion powder or protein hydrolyzate, the standard method the authors have used, which avoids contact of the sample with any appreciable excess of Fischer reagent, minimized the likelihood of side reactions interfering with the accuracy of results.

CONCLUSIONS

In determining the moisture of extremely dry food materials the Karl Fischer method offers certain advantages over the more commonly used procedures. The ease of duplicating results and the short time required for an individual determination make it a useful technique in food laboratories. No reliable reference method for moisture in food materials is known by which to verify the absolute accuracy of the method. The Karl Fischer method has been applied to a wide variety of dehydrated food materials with good results.

Disadvantages of the method are the necessity of restandardizing the reagent daily or whenever used, the fairly high cost of the reagent, and the somewhat longer time per sample required of the analyst. The advantages mentioned above are considered to outweigh these drawbacks and the Karl Fischer method has been adopted as a routine standard procedure in this laboratory.

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RECEIVED September 26, 1947.

Determination of Aromatics and Olefins in Hydrocarbon Mixtures

ACID SOLUBILITY TEST

CLYDE BERG AND F. D. PARKER[1]
Union Oil Company of California, Los Angeles, Calif.

A modified acid solubility method offers improved accuracy in the determination of aromatics and olefins in hydrocarbon mixtures. The method is simple and rapid and errors due to incomplete solution of olefins and partial solution of saturates have been considerably reduced. The test is carried out by adding 15% fuming sulfuric acid to a solution of the sample in glacial acetic acid. Tests on various known mixtures have determined the optimum glacial acetic acid–fuming sulfuric acid ratio and have shown the method to be superior to the 98% sulfuric acid method.

THE sulfuric acid extraction method has been used extensively to determine the amount of aromatic and olefinic hydrocarbons present in gasoline, kerosene, and coal-tar distillates. In this determination the aromatics and olefins are dissolved in the acid, leaving an insoluble residue of naphthenes and paraffins. By combining the results of this test with a determination of the olefin content such as the bromide-bromate method (*4, 7, 10, 13*), both the aromatic content and olefin content can be evaluated. The residue from the acid treat is sometimes used for analysis of paraffins and naphthenes. Ordinarily, 98% sulfuric acid (*3, 14*) is used for the combined olefin-aromatic determination; however, acid strengths ranging from 96% (*5*) to 25% fuming (*13*) as well as dimethyl sulfate (*12*) have been used. Many methods employ two treatments with acid, one with weak acid to remove the olefins, the second with strong acid to remove the aromatics (*1, 2, 5, 6, 8, 9, 14, 15*). Most of these methods employ a distillation after the olefin extraction to remove the polymerized olefins.

The solubility method using 98% acid is subject to error due to incomplete solution of the olefins because of polymerization reactions or formation of saturated hydrocarbons through other complex reactions (*11, 16*). The extent of this error depends on what particular olefins are present in the sample. Diisobutylene and diamylene are particularly difficult to dissolve. Treatment with several strengths of acid and distillation to remove the products of polymerization will reduce this error but not eliminate it. The considerable increase in labor and time required to carry out such determinations is objectionable and Fisher and Eisner (*3*) have shown that the olefin contents indicated by such sulfuric acid extraction methods are unreliable.

A second error, usually of lesser magnitude in the case of 98% acid, results from partial solution of the more active saturated hydrocarbons. This error increases with the strength of the acid; 98% acid will dissolve 3% iso-octane, whereas 100% acid will dissolve 13% of the iso-octane (*3*). Fuming acid shows even greater solution of saturates (*13*).

DEVELOPMENT OF METHOD

It has been fairly well established that the use of sulfuric acid of any strength in the solubility test will be attended by olefin polymerization and solution of saturated hydrocarbons. To reduce or eliminate these errors there remains the alternative of using addition agents or nonaqueous diluents with the acid.

Preliminary investigation of possible nonaqueous diluents indicated that mixtures of glacial acetic acid and fuming sulfuric acid were effective in dissolving olefins. The most successful method was to add the fuming sulfuric acid to a solution of the hydrocarbons in acetic acid. The alternate method of adding a mixture of fuming sulfuric acid and glacial acetic acid to the hydrocarbon gives only partial solution of aromatic compounds. Fuming sulfuric acid, 15%, was more effective in dissolving the olefins than acid of lower strengths.

In order to determine the optimum ratio of sulfuric and acetic acids and establish the accuracy of the method, a series of tests was carried out on saturated hydrocarbons, aromatics, and olefins, using various ratios of the two acids.

SOLUBILITY OF SATURATED HYDROCARBONS

The solubility of iso-octane (2,2,4-trimethylpentane) was determined for four different ratios of acids. Iso-octane was used for this test because it is one of the most sensitive saturated hydrocarbons. The tests were carried out in 60-ml. standard Stoddard solvent test bottles (A.S.T.M. designation, D484–40). After addition of the acid, the bottles were rotated in an agitation machine at 30 r.p.m. for 15 minutes and allowed to stand 2 hours before being read. In reading the solubility, 0.5% was added to the top meniscus reading to correct for the difference in curvature between the top and bottom menisci.

Results of these tests, given in Table I, show that the mixed acid reagents were more mild in their action on iso-octane than 98% sulfuric acid. The negative solubility (increase in volume) obtained with the weakest acid mixture is due to partial solution of acetic acid in the hydrocarbon layer. To prevent this, a minimum ratio of 15 ml. of fuming sulfuric acid to 25 ml. of glacial acetic acid must be used.

SOLUBILITY OF AROMATICS

Benzene is the most difficultly soluble in sulfuric acid of the lower aromatics and was therefore used to test the effectiveness

Table I. Solubility of Iso-octane

Glacial Acetic Acid	15% Fuming H_2SO_4	Soluble
Ml.	*Ml.*	%
30	10	−0.5
25	15	+0.5
20	20	0.5
15	25	0.5
30 ml. of 98% sulfuric acid		3.0

Table II. Solubility of Benzene

Glacial Acetic Acid	15% Fuming H_2SO_4	Soluble
Ml.	*Ml.*	%
25	15	62.0
20	20	100
15	25	100
30 ml. of 98% sulfuric acid		100

[1] Present address, 375 Dalkeith Ave., Los Angeles, Calif.

Table III. Solubility of Olefins

Composition of Sample, Per Cent by Volume[a]						Acid Solubility		
n-Heptane	Iso-octane[b]	Di-isobutylene	Diamylene[c]	Iso-octene[d]	Toluene	Total aromatics and olefins	Fuming H_2SO_4, glacial HOAC	98% H_2SO_4
100.0	..	....	...	....	...	0.0	0.0	0.0
....	..	100.0	....	....	...	100.0	80.0	42.0
...	..	...	100.0	....	...	100.0	93.0	45.0
...	..	...	.....	100.0	...	100.0	99.0	49.5
...	...	...	.....	...	100.0	100.0	100.0	100.0
50.00	...	30.0	...	...	20.0	50.0	50.0	32.0
50.00	..	...	30.0	...	20.0	50.0	50.0	31.0
50.0	..	...	...	30.0	20.0	50.0	50.0	33.5
50.0	..	10.0	....	...	40.0	50.0	50.0	45.0
50.0	..	...	10.0	..	40.0	50.0	50.0	45.0
50.0	..	...	....	10.0	40.0	50.0	50.0	50.0
20.0	20.0	10.0	10.0	10.0	30.0	60.0	60.0	48.5

[a] Hydrocarbons used were of best commercial grade, freshly redistilled, and analyzed over 99% olefinic, aromatic, or saturated by conventional tests.
[b] 2,2,4-Trimethylpentane.
[c] Mixture of 3,5,5-trimethylheptene-2 and 3,4,5,5-tetramethylhexane-2.
[d] Mixture of isomeric octenes.

Table IV. Analysis of Gasolines

Gasoline	Olefin[a]	Solubility	
		In 98% H_2SO_4	In fuming H_2SO_4 and glacial HOAC
	%	%	%
Straight-run gasoline from Los Angeles Basin crude oil	1.0	14.5	14.5
200–240° F. cut from thermally reformed Los Angeles Basin raffinate	21.4	22.0	29.0
Thermally reformed gasoline from Santa Maria Valley crude oil[b]	32.0	28.0	37.0
Catalytic polymer gasoline (from C_3-C_4 olefins)	76.0	38.5	74.5

[a] By bromide-bromate determination.
[b] 0.44% sulfur.

of the acid mixtures in dissolving aromatics. Table II gives the data on solubility of benzene. These data show that the reagent composed of 15 ml. of sulfuric and 25 ml. of acetic acid will not dissolve benzene completely. Although 20 ml. of sulfuric and 20 ml. of acetic acid dissolved benzene completely, this solution did not occur appreciably until the bottle was rotated in the agitating machine. It is undesirable that this reaction should take place during agitation, because heat is generated and the mixture may overheat. The most desirable reagent for this test is therefore 25 ml. of fuming sulfuric acid and 15 ml. of glacial acetic acid.

SOLUBILITY OF OLEFINS

The ability of this mixed acid reagent to dissolve olefins was compared with that of 98% sulfuric acid, using test mixtures containing diamylene, diisobutylene and iso-octene. These olefins are some of the most difficult to dissolve and so serve as a good standard of comparison. The data of Table III show that the mixed acid reagent gives much better solution of the olefins than 98% acid; 98% acid dissolves less than 50% of these olefins, whereas the mixed acid reagent dissolves the olefins completely in mixtures and dissolves from 80 to 99% of the pure olefin samples. Since the acetic-sulfuric acid reagent dissolves these difficultly soluble olefins from the mixtures, as noted, it was concluded that the olefins occurring in petroleum and coal-tar distillates would be dissolved with a high degree of effectiveness.

PROCEDURE

Reagents. Fuming sulfuric acid containing 15% sulfur trioxide. Glacial acetic acid.

Apparatus. Ten 60-ml. standard Stoddard solvent test bottles (A.S.T.M. designation, D484-40), two 250-ml. burets, one 10-ml. pipet, one battery jar, and one agitation machine (capacity 10 bottles), rotation rate, 30 r.p.m.

The acid solubility is determined by adding fuming sulfuric acid to a solution of the hydrocarbon sample in glacial acetic acid. The bottle containing the mixture is rotated in an agitator for 15 minutes, and filled to level with acid, and the reading is taken after 2 hours.

Add 15 ml. of glacial acetic acid to the solvent test bottle, pipet 10 ml. of the sample into the bottle, and add 25 ml. of fuming sulfuric acid in 5-ml. portions to the mixture, shaking the bottle in a bath of ice water after each addition until no further heat is generated. Crushed ice and water in a battery jar serves well as the cooling bath. If the sample is very volatile, the mixture should be chilled in crushed ice prior to the addition of acid in order to reduce the possibility of loss by vaporization. Rotate the bottle in an agitator for 15 minutes at speed of 30 r.p.m. Fill the bottle to level with fuming sulfuric acid and allow to stand 2 hours before reading the solubility. Add 0.5% to the upper meniscus reading to correct for the difference in curvature between the top and bottom menisci.

TYPICAL ANALYTICAL RESULTS

The increased absorption of olefins obtained by this method of analysis is particularly advantageous when cracked gasolines are analyzed. The 98% sulfuric acid test frequently presents the anomaly of a lower acid absorption than the olefin content as determined by the bromide-bromate method (*4*, *7*, *10*, *13*). The olefin content determined by bromine number is fairly reliable and the discrepancy is usually due to incomplete absorption of the olefins in 98% acid. As is shown in Table IV, the acetic-sulfuric acid reagent eliminates this difficulty and the aromatic content of these cracked gasolines can be ascertained by the difference between the acid solubility and the olefin content with good accuracy.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the management of Union Oil Company of California for permission to make public the results of this investigation.

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RECEIVED May 9, 1947. Presented before the Division of Analytical and Micro Chemistry at the 111th Meeting of the AMERICAN CHEMICAL SOCIETY, Atlantic City, N. J.

1,2-CYCLOHEXANEDIONE DIOXIME

A Reagent for Nickel

ROGER C. VOTER, CHARLES V. BANKS, AND HARVEY DIEHL

Institute for Atomic Research and Department of Chemistry, Iowa State College, Ames, Iowa

The compound 1,2-cyclohexanedione dioxime, nioxime, is similar to dimethylglyoxime in yielding scarlet and yellow precipitates with nickel and palladium, respectively, which can be used for the gravimetric determination of these metals. It is soluble in water in contrast to dimethylglyoxime, so that its use is theoretically unaccompanied by danger of contaminating the precipitates with excess reagent or of solubility loss by the addition of alcohol. On the other hand nioxime is a more powerful reducing agent than dimethylglyoxime, and this introduces some complications in its use. One part of nickel in 10,000,000 may be detected with the reagent. The precipitation of nickel nioxime is complete at pH values of 3 and greater, and may be made from a solution of various anions: chloride, sulfate, perchlorate, nitrate, acetate, tartrate, and sulfosalicylate. The precipitation effectively separates nickel from a variety of metals including zinc, beryllium, uranium, aluminum, the alkali and alkaline earth metals, manganese, cadmium, antimony, and arsenic. Attempts to separate nickel from iron failed, as no suitable complexing agent for the latter was found which would prevent precipitation of the iron and not interfere in the determination of the nickel.

THE unique and useful applications of the 1,2-dioximes to analytical chemistry were extensively reviewed in 1940 (*2*). That 1,2-cyclohexanedione dioxime yielded a scarlet precipitate with nickel and in fact was a very sensitive qualitative test for nickel was early discovered by Wallach (*10*). Feigl (*3*) pointed out that 1,2-cyclohexanedione dioxime should be the ideal reagent for nickel, inasmuch as its solubility in water would be a significant advantage over dimethylglyoxime, which must be made up in alcohol or acetone. Although this attracted the attention of various analytical chemists, the great difficulty in synthesizing 1,2-cyclohexanedione dioxime precluded a detailed study of its properties and uses as an analytical reagent; indeed, numerous unreported attempts to prepare the reagent met with signal failure, and it was only in 1945 that Rauh, Smith, Banks, and Diehl (*8*) succeeded in obtaining sufficient material to make possible its investigation. One of their methods of preparation was later greatly improved by Hach (*5*), and the compound is now available at moderate prices (Hach Chemical and Oxygen Company, Ames, Iowa). A common name, nioxime, has been proposed for the reagent.

The uses of nioxime in the analytical chemistry of nickel and palladium were investigated by Banks (*1*). This work and also some by Voter, both interrupted for war service, have now been continued and form the subject matter of this paper. In the meantime work was done at Purdue University by Mellon and Griffing (*4*) on the use of the reagent in the colorimetric determination of nickel and iron. Before their work could be published a short paper by certain English workers (*6*) dealing with the gravimetric and colorimetric uses of nioxime appeared.

PHYSICAL PROPERTIES

Nioxime is a white, crystalline material, melting at 189–190° C., which when properly prepared, is free from any pink coloration due to contamination by iron and remains white indefinitely. Its molecular weight is 142.16.

The solubility of nioxime in water was determined by precipitating a measured volume of a saturated solution with an excess of nickel: 0.32 gram per 100 ml. of water at 21.5° C.

REAGENTS

An 0.8% aqueous solution of nioxime was used. This solution keeps indefinitely.

A standard nickel solution was prepared from Mond nickel obtained from the International Nickel Company; it was analyzed spectrographically and found to contain only traces of iron and cobalt. A weighed amount of this nickel was dissolved in aqua regia and the solution evaporated to dryness five times with concentrated hydrochloric acid to eliminate nitrate ions. By weighing the diluted nickel chloride solution, the weight of nickel per weight of solution was found to be 0.001990 gram of nickel per gram of solution. This nickel concentration was checked by an electrolytic determination of the nickel, the sample being measured by a weight buret. The average value of four such determinations was found to be 0.001994 gram of nickel per gram of solution with an average deviation of 0.000001 gram of nickel per gram of solution. The residual liquid was tested with nioxime to ensure the complete deposition of the nickel. The value obtained by electrolysis was used.

A 20% solution of ammonium acetate was prepared from reagent grade salt and filtered.

SENSITIVITY

Ten nickel solutions were made up in 100-ml. volumetric flasks with concentrations ranging from 1 to 10 parts in 10,000,000. To each solution were added 3 drops of 0.8% nioxime solution followed by vigorous shaking. Observations made less than 2 minutes after the addition of the reagent showed that each solution exhibited a color ranging from red for the more concentrated to pink for the 1 part in 10,000,000. At the end of 1 hour, a red precipitate was found in each flask. The pH of the solutions as determined with a glass electrode was 6.4. A comparative test was performed using dimethylglyoxime (1% in ethanol) on solutions of like concentrations at pH 8. No visible coloration was detected until 30 minutes after addition of the reagent and then only in solutions of higher concentration. The sensitivity of the nioxime for nickel determined this way is greater than that reported by Wallach (*10*) and the English workers (*6*), who reported 1 part in 2,000,000 and 1 part in 5,000,000, respectively.

GRAVIMETRIC DETERMINATION OF NICKEL

Because hydrogen ions are liberated on the formation of the nickel derivatives of the 1,2-dioximes, it is necessary to buffer the solution or otherwise control the pH by the addition of ammonia. Whereas nickel dimethylglyoxime is generally precipitated from a mildly ammoniacal medium, it was found best to precipitate nickel nioxime from a slightly acid solution. This is an advantage because it makes possible the separation of nickel from certain metallic ions without the use of complexing agents. Quantitative precipitation of nickel was obtained at a pH of 3 or higher.

The rate of precipitation and the pH of the solution determine the ease of filtration of the nickel nioxime precipitate. If the nickel was precipitated by the dropwise addition of the nioxime solution to the buffered nickel solution, the precipitate formed clogged the filtering crucible and filtration was difficult. If the

pH of the solution was slowly raised from a point where the nickel nioxime would not precipitate to a pH of about 4.5, the precipitate was filtered without trouble. This slow precipitation was effected by the dropwise addition of a 20% solution of ammonium acetate with constant stirring. Precipitates formed at pH values above 7 were somewhat gelatinous, were exceedingly difficult to filter, and exhibited a bluish coloration. The best results with respect to accuracy and precision were obtained by precipitation from a slightly acid solution.

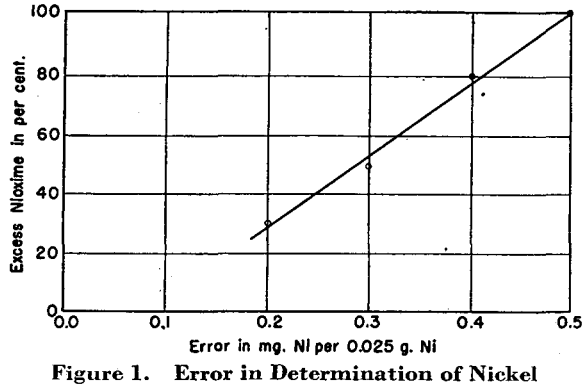

Figure 1. Error in Determination of Nickel

Nickel nioxime is formed by the union of one nickel atom with two molecules of nioxime with the liberation of two hydrogen ions, and the nickel derivative, $Ni(C_6H_9O_2N_2)_2$, molecular weight 340.99, should contain 17.21% nickel. The results of nearly a hundred determinations indicate, however, that the nickel content is slightly lower than the theoretical. It has been shown that this departure is independent of the anion present in the solution, of any foreign cations present, and in general of any diverse ions present in the solution. The various reagents and distilled water were checked as possible sources of contamination and found to be without fault. A determination made on the nickel chloride in water without the addition of acetate gave the same results. The data all indicated that the positive error was caused by the coprecipitation of the reagent.

A series of determinations in which the percentage excess of the nioxime was varied showed that the error was nearly a linear function of the concentration of the excess nioxime for a given weight of nickel (Figure 1) as the volume of the solution was constant in these determinations, the percentage excess was proportional to the nioxime concentration and thus determined the amount of nioxime coprecipitated by the nickel nioxime precipitate. Washing the nickel nioxime precipitate with 95% ethanol, in which nioxime is fairly soluble and the precipitate insoluble, did not free the precipitate of the reagent nor did precipitation from 10% alcohol solution change the result.

Because the magnitude of this error was a linear function of the excess nioxime, an empirical equation was easily developed by which the correct results for nickel could be calculated from the weight of the precipitate and the total reagent added. The weight of nioxime used is the weight of the nickel precipitate multiplied by the factor 0.834; the volume of 0.8% solution used is then

$$\text{Vol. used} = \frac{0.834\ (\text{weight of Ni nioxime ppt.})}{0.008} = 104\ (\text{weight of Ni nioxime ppt.})$$

Knowing the volume added the per cent excess of reagent is calculated. The correction is calculated by

$$\text{Correction in grams of Ni} = (0.0002)\ (\%\ \text{excess nioxime})\ (\text{weight of Ni nioxime ppt.})\ (0.1721)$$

The factor 0.0002 results from the observation that each 20% excess of nioxime caused a positive error of 0.1 mg. per 25 mg. of nickel present. The result of the analysis is then calculated in the usual way:

$$\%\ \text{Ni} = \frac{(\text{weight of Ni nioxime})\ (0.1721) - (\text{correction, grams to Ni})\ (100)}{\text{weight of sample}}$$

As the correction is small, the per cent excess of nioxime need only be approximately known and it is sufficient merely to measure the volume of nioxime solution added in a graduated cylinder.

Determinations conducted on a series of samples of different sizes indicated that amounts of nickel from 5 to 25 mg. could be successfully determined. The correction for the reagent carried down need be applied only for amounts of nickel above 15 mg. The precipitation of small amounts of nickel, less than 2 or 3 mg., is slow. In determination 1 of Table I the solution was refiltered after 2 hours through the filter crucible containing the first precipitate.

The nickel nioxime precipitate was dried at various temperatures from 100° to 155° C.; no further loss in weight occurred at the higher temperatures. At temperatures above 155° C., however, the precipitate turned brown and lost weight rapidly. The precipitate could not be ignited to the oxide for weighing, as sublimation occurred before the decomposition could be effected.

RECOMMENDED PROCEDURE

Adjust the volume of the solution containing about 25 mg. of nickel to approximately 250 ml. If a complexing agent is needed to prevent the precipitation of other metals, it should be added before continuing with the procedure. Add 8 ml. of a solution of 0.8% nioxime for each 10 mg. of nickel present, measuring the volume of reagent added with a graduated cylinder. Slowly add with stirring enough concentrated hydrochloric acid to cause any red precipitate of nickel nioxime to dissolve, then add ammonia dropwise until a faint red coloration persists. Heat the solution to about 60° C. From a buret, add dropwise and with constant stirring, 25 ml. of the 20% ammonium acetate. Digest the solution with occasional stirring for 30 to 40 minutes at 60°. Filter through a weighed filter crucible of medium porosity and wash with five portions of hot water. Dry at 110° for 1 hour and weigh. Calculate the results as described above. The pH of the solution just before filtration will be between 4 and 5, if the procedure is carefully followed.

Table I. Determination of Various Amounts of Nickel

Detn.	Nickel Taken *Gram*	Weight of Precipitate *Gram*	Nickel Found *Gram*	Error *Mg.*
1	0.0018	0.0110	0.0019	+0.1
2	0.0049	0.0292	0.0050	+0.1
3	0.0107	0.0631	0.0108	+0.1
4	0.0144	0.0847	0.0145	+0.1
5	0.0203	0.1186	0.0203	0.0

The effect of various anions upon the determination of nickel was studied. The weighed nickel chloride solution was evaporated with sulfuric acid, nitric acid, or perchloric acid to eliminate the chloride ions and the nickel nioxime precipitated by the addition of the nioxime reagent to the acid solution and then neutralizing with a 20% solution of ammonium acetate. Further determinations were made in which tartrate, acetate, and sulfosalicylate had been added as the ammonium salts to the nickel chloride solution. Typical results in Table II indicate that these anions do not interfere in the determination of nickel.

Nickel was determined in the presence of uranyl, manganous, sodium, potassium, lithium, barium, calcium, strontium, magnesium, cadmium, arsenite, beryllium, and zinc ions. When aluminum and antimonite ions are present with the nickel, tartrate must be added as a complexing agent to prevent coprecipitation of aluminum hydroxide and antimonous hydroxide with the nickel nioxime. Nickel was determined in the presence of beryllium both with and without sulfosalicylic acid as a complexing agent for the beryllium ion. As beryllium hydroxide does

Table II. Effect of Various Anions upon Determination of Nickel

Anion Present	Anion	Nickel Taken	Weight of Precipitate	Nickel Found	Error
	Grams	*Gram*	*Gram*	*Gram*	*Mg.*
Acetate[a]	4 5	0 0265	0 1554	0 0266	+0.1
Chloride	0.5	0.0229	0.1346	0.0230	+0.1
Sulfate	1.0	0.0246	0.1440	0.0246	0.0
Nitrate	0.2	0.0255	0.1497	0.0256	+0.1
Perchlorate	0.2	0.0264	0.1531	0.0262	−0.2
Tartrate[a]	8.0	0.0238	0.1389	0.0237	−0.1
Sulfosalicylate[a]	5.7	0.0239	0.1400	0.0239	0.0

[a] Small amount of chloride present.

Table III. Determination of Nickel in the Presence of Various Cations

Cation Present	Cation	Nickel Taken	Weight of Precipitate	Nickel Found	Error
	Gram	*Gram*	*Gram*	*Gram*	*Mg.*
Uranyl	0.55	0.0236	0.1385	0.0237	+0.1
Uranyl	0.23	0.0234	0.1368	0.0234	0.0
Manganous	0.50	0 0236	0 1381	0.0236	0.0
Manganous	0.10	0.0231	0.1351	0.0231	0.0
Sodium, Potassium, Lithium, Barium	0.10 each	0.0235	0.1375	0.0235	0.0
Calcium, Strontium	0.10 each	0.0255	0.1491	0.0255	0.0
Magnesium, Cadmium	0.20 each	0.0220	0.1291	0.0221	+0.1
Antimonite[a]	0.14	0.0221	0.1301	0.0222	+0.1
Aluminum[b]	0.20	0.0233	0.1362	0.0233	0.0
Arsenite	0.34	0.0234	0.1373	0.0235	+0.1
Beryllium	0.1	0.0242	0.1409	0.0241	−0.1
Beryllium	0.3	0.0233	0.1363	0.0233	0.0
Zinc	0.1	0.0210	0.1226	0.0210	0.0
Zinc	1.0	0.0252	0.1474	0.0252	0.0

[a] Complexed with 0.2 gram of tartrate.
[b] Complexed with 1.2 grams of tartrate.

not precipitate below a pH of 5.7 (*11*), the complexing agent is not necessary when the determination is conducted in the manner suggested. The precipitate from determination 11 was decomposed with nitric acid and ignited to the oxide. Spectrographic analysis revealed the presence of less than 100 p.p.m. of beryllium, indicating a successful separation. The data from these separations are shown in Table III.

Copper yields a brownish green color with nioxime, whereas cobalt gives a brown color and is coprecipitated with nickel; this is in agreement with the findings of other workers (*6*).

A method for the satisfactory quantitative separation of nickel from iron was not found. The difficulty lay in finding a complexing agent for the ferric iron. The possibility of using tartrate and citrate was exhaustively investigated. However, these anions are known to reduce iron to the ferrous state (*9*) which forms a very stable complex with nioxime and incomplete precipitation of the nickel results. Nioxime will also reduce ferric to ferrous iron. Ferric sulfosalicylate complex inhibits the precipitation of the nickel nioxime and postprecipitation occurs as much as 24 hours after filtration.

Fluoride, phosphate, and pyrophosphate precipitate ferric iron whereas dextrose, glycerol, salicylate, D-mannitol, and thiocyanate do not prevent hydrous ferric oxide from precipitating under the conditions of the recommended procedure. Ferrocyanide was ruled out, because nickel ferrocyanide is insoluble. 2,2′-Bipyridine complexes nickel as well as ferrous iron (*7*) and nickel nioxime is not precipitated in its presence.

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RECEIVED June 6, 1947. Presented in part at the 15th Midwest Regional Meeting, AMERICAN CHEMICAL SOCIETY, Kansas City, Mo. Much of this work was done under Contract No. W-7405 eng-82 with the Manhattan Project at the Department of Chemistry, Iowa State College. Contribution 16 of the Institute for Atomic Research, Iowa State College, Ames, Iowa.

Determination of Asphaltenes, Oils, and Resins in Asphalt

RETHEL L. HUBBARD AND K. E. STANFIELD

Petroleum and Oil-Shale Experiment Station, Bureau of Mines, Laramie, Wyo.

THE present development of analytical methods does not permit determination of the individual compounds of which asphalt is composed. The best that can be done is to segregate its similar compounds into groups. Probably the most widely accepted grouping is that proposed by Marcusson (*1*, *16*), who separated asphalt into three major fractions—asphaltenes, asphaltic resins, and oily constituents—by means of different solvents and by adsorption on fuller's earth. Many modifications (*3*, *11*, *13*, *14*, *15*, *18*, *22*, *23*) of the Marcusson method and numerous other methods (*2*, *4–7*, *9*, *10*, *12*, *17*, *24*) based entirely upon the solubility of the asphalt constituents in different solvents have been proposed. None of these methods is entirely satisfactory; each one fails in one or more of the following respects:

The methods are too time-consuming. Analyses of asphalts by many of the published methods require several days to several weeks for completion, and during these periods the constituents may undergo undesirable chemical alterations.

Extractions are made at the boiling points of the solvents rather than at specified reproducible temperatures. The boiling-point temperatures of the solvents are difficult to reproduce in different localities because of differences in barometric pressure.

The reagents used are mixtures of uncertain composition rather than chemical compounds of fixed characteristics. The petroleum naphtha solvents of specified boiling ranges and the fuller's earth adsorbents are mixtures of variable composition and characteristics, depending upon their source and method of preparation. These mixtures are accordingly less satisfactory as reagents than chemical compounds of reproducible purities and properties.

The yields of certain constituents, usually the resins, are calculated by difference and are, thus, in error by the accumulated experimental errors of all the constituents. This practice has been followed because fuller's earth adsorbs resins so tenaciously they generally cannot be quantitatively recovered by eluting agents and, thus, each of the asphalt constituents is not obtained for further examination.

No precautions are taken to avoid the possibility of oxidizing the asphalt constituents during the analyses. Such oxidation may cause erroneously high yields.

A method of analysis is described here whereby the objections made to other methods are largely overcome and the results provide comparative information on the composition of asphalts

A method is described for the quantitative determination of the three groups of constituents—asphaltenes, oils, and resins—in petroleum and native asphalts. The asphaltene (benzene-soluble, pentane-insoluble) fraction is obtained by extracting the asphalt with *n*-pentane. The pentane-soluble portion is further fractionated by adsorption on anhydrous alumina and extraction first with pentane to obtain the oil fraction and then with a methanol-benzene solution to obtain the resin fraction. In contrast to most proposed methods, the three asphalt constituents are recovered and may be used for further study. A complete analysis can be made in one 8-hour day after the sample has been dispersed in pentane and allowed to stand approximately 12 hours or overnight.

from different crude oils. The asphalt is extracted with pentane (*n*-pentane) to obtain a pentane-soluble petrolene fraction and a pentane-insoluble asphaltene fraction. The pentane-soluble petrolene portion is further fractionated by dispersing it on anhydrous alumina and recovering the unadsorbed oils by extraction with pentane. The resins adsorbed on the alumina are recovered by eluting with benzene and methanol.

BUREAU OF MINES METHOD

Apparatus. Oil centrifuge tubes, California-type, 100-ml. capacity, graduated in divisions of 1 ml.

Goetz phosphorus centrifuge tubes, 100-ml. capacity.

Glass stirring rods, screw type, 25 cm. long by 6 mm. in diameter, formed by flattening a 5-cm. section of the rod from a point 1 cm. above the bottom, then twisting this flattened portion in the form of a screw.

Boiling flasks, 300-ml. flat bottoms, with 24/40 female joints.

Glass adapter (Figure 1) with 24/40 male joint and two opposite side tubes, for removing solvents and drying extracts at atmospheric or reduced pressure. In use, flushing gas passes through one side tube onto the surface of the extract and is vented through the opposite tube.

Pyrex Gooch crucibles, 50-ml. capacity, with porous-disk bottoms 40 mm. in diameter. The disks should be sufficiently porous to permit the acetone-filled crucibles to filter 15 to 20 ml. of acetone in 30 seconds. Before use, the crucibles are cleaned in hot sulfuric acid, washed by suction with water and acetone, and then dried.

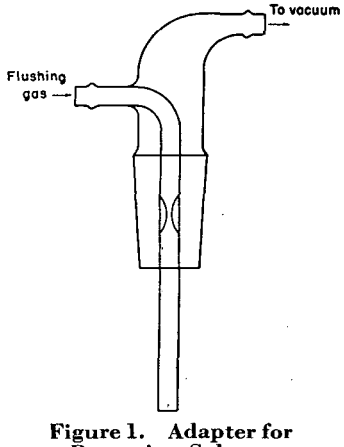

Figure 1. Adapter for Removing Solvent

Constant-temperature extraction apparatus, shown in Figure 2, is designed to make continuous liquid extractions of solid or semisolid materials in an inert atmosphere at specified liquid temperatures. Water bath *D*, surrounding the solvent-cooling coil, is maintained at the desired temperature by water circulating from a thermostatically controlled bath of larger capacity. The mercury-sealed paddle stirrer, *H*, is operated by an oscillating motor, *G*, of the type commonly used on motor cars to operate the windshield wiper. The liquid trap, *K*, in the solvent-return line aids in observing the color of the extract and retaining the few particles of alumina which may be washed out of the extraction crucible.

Reagents. *n*-Pentane, C.P. grade. Different lots of a petroleum fraction of *n*-pentane had properties within the following limits:

Density, d_4^{20}	0.6255 to 0.6262
Refractive index, n_D^{20}	1.35752 to 1.35758
Boiling point at 760 mm., ° C.	36.10 to 36.15

Benzene, C.P. grade.

Anhydrous methanol, C.P. grade.

Anhydrous aluminum oxide, technical grade, screened to pass a 100-mesh per inch sieve and be retained upon a 200-mesh per inch sieve. The alumina is heated for 2 hours at 700° C. (1292° F.) and cooled in a desiccator before being used.

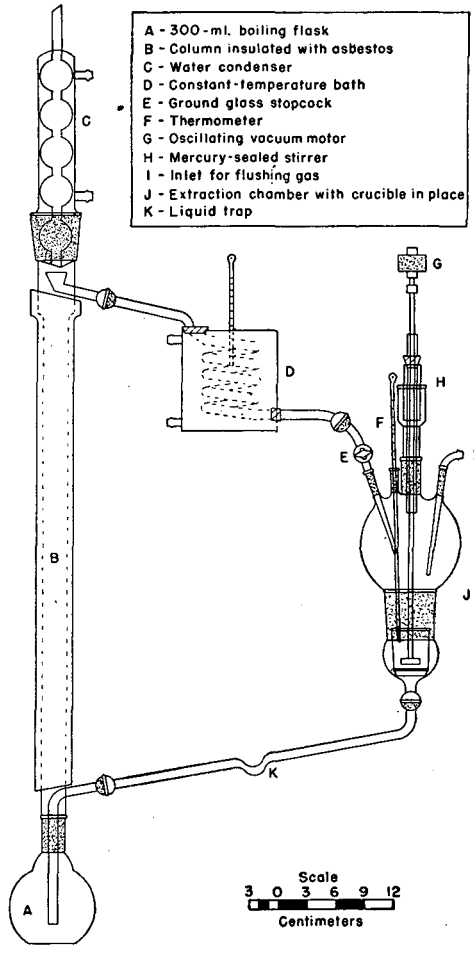

Figure 2. Constant-Temperature Extraction Apparatus

Preparation of Asphalt Sample. A sample containing 1.25 to 1.5 grams of asphalt (the smaller sample is used for asphalts containing unusually large proportions of resins or asphaltenes) is weighed accurately into a 100-ml. oil centrifuge tube. The tube is warmed to soften the asphalt, which is then distributed evenly about the lower part of the tube.

Determination of Asphaltenes. Forty milliliters of *n*-pentane per gram of asphalt are added to the sample in the centrifuge tube. The sample is dispersed by hand with a screw-type stirring rod. The tube is then supported in a water bath at 15.6° C. (60° F.) and the sample stirred by rotating the rod at approximately 2500 r.p.m. for 10 minutes. The stirring rod is removed, and the tube is stoppered and allowed to stand approximately 12 hours or overnight in darkness or in subdued light. After standing the required time, the tube and its contents are again placed in the constant-temperature bath (at 15.6° C.) for 20 minutes. At the end of this time, the sample is stirred for 10 minutes in the manner described above and then centrifuged for 5 minutes at a relative centrifugal force approximately 975 times gravity. The clear pentane solution is decanted into a 300-ml. boiling

flask and the approximate volume of pentane-insoluble residue is noted. Twenty-five milliliters of pentane per milliliter of residue are added; the mixture is stirred for 10 minutes at 15.6° C., then centrifuged for 5 minutes in the manner previously described. This washing process is repeated three times (a total of four washings); the same volume of pentane is used each time. The pentane washings are added to the original pentane extract in the 300-ml. boiling flask.

The pentane-insoluble asphaltene fraction in the centrifuge tube is dissolved in benzene and filtered through filter paper into a weighed boiling flask. In this filtration, a few drops of methanol are helpful in eluting the final traces of asphaltenes adsorbed on the filter paper. The benzene and methanol extract is distilled to dryness on a steam bath while flushing gas, as carbon dioxide, nitrogen, or helium, is passed over the material at a reduced pressure of approximately 5 to 10 inches of water. The adapter (Figure 1) is used for this distillation. The outside of the flask is washed and, by means of the adapter, the extract is dried 25 minutes under reduced pressure in an oven at 105° C. (221° F.) while the flushing gas is passed through the flask. After drying, the flask is cooled in a vacuum desiccator, then flushed with air and weighed to determine the yield of the asphaltene fraction.

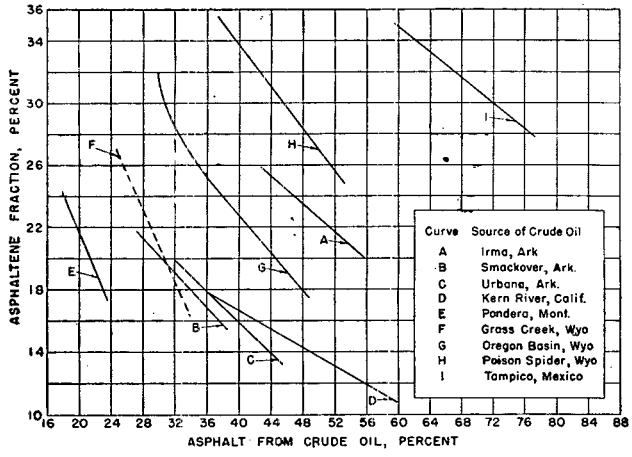

Figure 3. Asphaltene Content of Asphalts from Different Crude Oils

Determination of Oils. The combined pentane extract and washings from the extraction of the original sample are distilled on a water bath to remove most of the pentane solvent. (If this extract is allowed to stand for an hour or longer before further treatment, the air in the flask is displaced with flushing gas, and the flask is stoppered.) The concentrated extract is evaporated to approximately 5 ml. and poured evenly over 25 grams of anhydrous alumina in a Gooch crucible. Three- to 5-ml. portions of pentane are used to wash out the flask and transfer the pentane-soluble material to the alumina. This washing is continued until the bottom of the crucible becomes slightly moistened with solvent. When properly done, the upper portion of the column of alumina is dark and the lower portion is colorless.

The crucible is placed in the extraction chamber of the apparatus shown in Figure 2, and the discolored portion of the mixture is dried to a powder by stirring it gently in a stream of the flushing gas. *n*-Pentane, which has been dried by passing it through a column of anhydrous alumina, is placed in boiling flask *A* (Figure 2). The flushing gas is adjusted to bubble slowly through the solvent, which is heated to boiling by a hot-water bath. By adjusting stopcock *E*, *n*-pentane, at 15.6° C. from the cooling coil, is added slowly to moisten the sample; the flow of pentane is then regulated to keep the crucible one half to three fourths full of liquid. The initial percolate from the crucible should be colorless or only slightly yellow. If it is more distinctly colored, the analysis is repeated with a smaller sample of asphalt. This significant color indicates that the petrolenes have been improperly dispersed over the alumina or there is insufficient alumina to adsorb the resins completely. If the first percolate indicates that the resins have been properly adsorbed, the stirrer is started and the extraction continued for one hour. The extraction chamber and return line are then washed with pentane into the boiling flask, and the extract is distilled to approximately 25 ml. of liquid. The concentrated oil extract is filtered through filter paper, dried, and weighed as described for the asphaltene fraction. (Occasionally the oil fractions from certain asphalts, particularly those having penetrations over 200 at 25° C., are partially volatilized by the usual method of drying. These more volatile oils are dried at atmospheric rather than at reduced pressure.)

Determination of Resins. The Gooch crucible containing the mixture of resins and alumina is removed from the extraction chamber and mounted on a filter funnel, and the resins are eluted by alternately stirring the material with 10- to 20-ml. portions of a methanol-benzene solution (10 ml. of anhydrous methanol plus 90 ml. of benzene) and withdrawing the elutrient by means of suction. The resin extract is distilled to a small volume, centrifuged in a 100-ml. Goetz phosphorus tube to remove fine particles of alumina, and filtered through filter paper into a weighed boiling flask. The resin extract is then distilled, dried, and weighed as described for the asphaltene fraction.

The determination of the asphaltene, oil, and resin fractions can be completed in one 8-hour day after the sample has been dispersed in pentane and allowed to stand overnight.

EXPERIMENTAL RESULTS

The yields of asphaltenes, oils, and resins from 117 asphalts prepared in the laboratory from 25 crude oils were determined by the method described above. The composition data for the 25 series of asphalts will be available in a forthcoming publication of the Bureau of Mines (*21*). The composition data for 47 asphalts prepared from 9 of the crude oils studied are shown graphically in Figures 3, 4, and 5, which give the yields of each con-

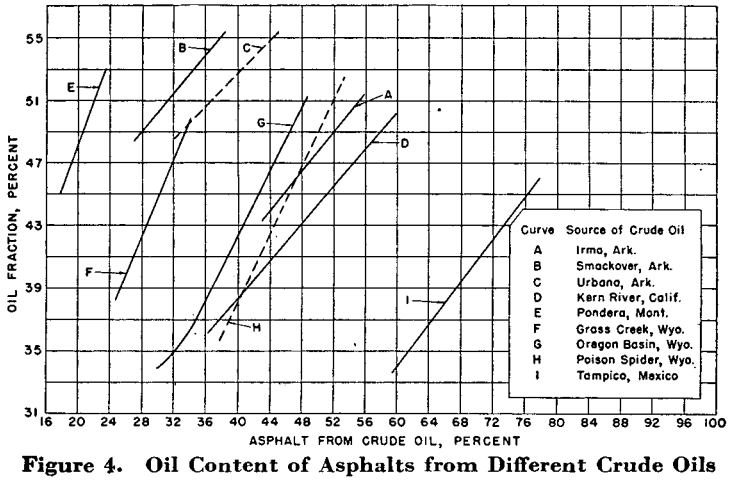

Figure 4. Oil Content of Asphalts from Different Crude Oils

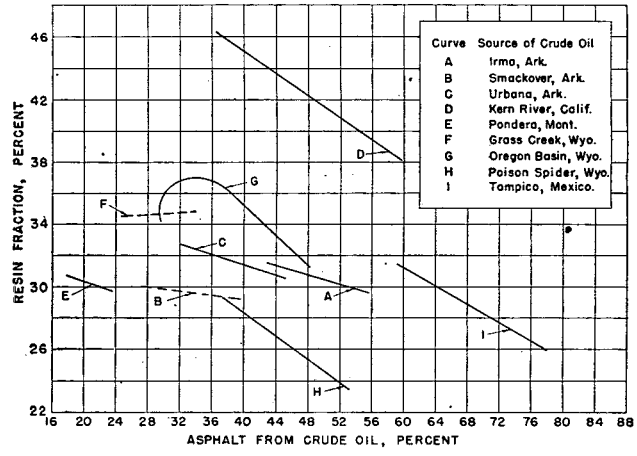

Figure 5. Resin Content of Asphalts from Different Crude Oils

stituent plotted against yield of asphalt from the crude oil for each series of asphalts. These straight-run asphalts were prepared as described in previous publications (*19*, *20*) by topping the crude oil by distillation at atmospheric pressure and removing the heavy distillate or lubricating oil fractions at decreased pressure. The penetrations of the asphalts in each of the series are given in Figure 6.

Thus (Figures 3, 4, 5, and 6) an 80-penetration asphalt from Oregon Basin, Wyo., crude oil (Figure 6, *G*) yielded 20.5% asphaltenes (Figure 3, *G*), 46.0% oils (Figure 4, *G*), and 33.5% resins (Figure 5, *G*).

For asphalts from the same crude oil, the yields of asphaltenes and in most cases the yields of resins increased with decrease in yield or penetration of the asphalt. On the other hand, the yields of oils generally decreased with decrease in asphalt yield and penetration. These findings are in general agreement with data presented by Hoiberg, Hougen, and Zapata (*8*).

From the graphs of the constituent yields, as illustrated by Figures 3, 4, and 5, the composition of the 100-penetration asphalt of each of the 25 series was estimated. Figure 7 shows a comparison of the estimated composition of these 100-penetration asphalts, arranged in the order of their increasing asphaltene content.

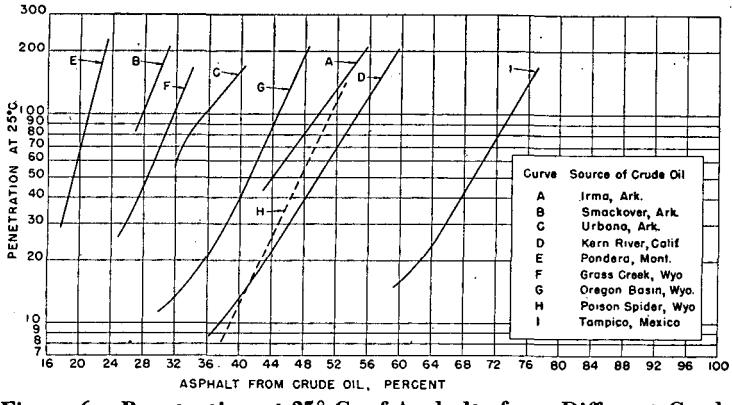

Figure 6. Penetration at 25° C. of Asphalts from Different Crude Oils

Characteristics of Fractions. The asphaltene fractions were black solids which intumesced and softened on heating. The oil fractions were viscous liquids at room temperature and ranged in color from light yellow to light orange. The resin fractions of the asphalts were dark, reddish-brown to brownish-black semi-solids, which softened readily on heating. Some of the resins were slightly tacky at room temperature.

PRECISION OF METHOD

The precision of the method based upon a total of 41 analyses of 16 asphalts is given in Table I. These results show that the yields of oils and resins deviated approximately twice as much as the yields of asphaltenes. Little difficulty was experienced in obtaining consistent yields of asphaltenes within these limits.

A comparison of the compositions of coating (air-blown) asphalts by this method and by a method developed by Strieter (*22*) is given in Table II. The coating asphalts and their analyses by the Strieter method were furnished by O. G. Strieter of the National Bureau of Standards. The average yields of the asphaltene, oil, and resin fractions by the Bureau of Mines method were 0.6% higher, 1.9% lower, and 1.2% higher, respectively, than the average yields of the corresponding fractions by the Strieter method. The differences in the oil and resin yields by the two methods are attributed largely to the different adsorbents used—namely, anhydrous alumina and fuller's earth.

DISCUSSION

As there are no abrupt changes in the characteristics of the series of hydrocarbons constituting asphalt, its separation into arbitrary groups of constituents such as asphaltenes, oils, and resins must be made under carefully controlled experimental conditions if reproducible results are to be obtained. In the method described, it is

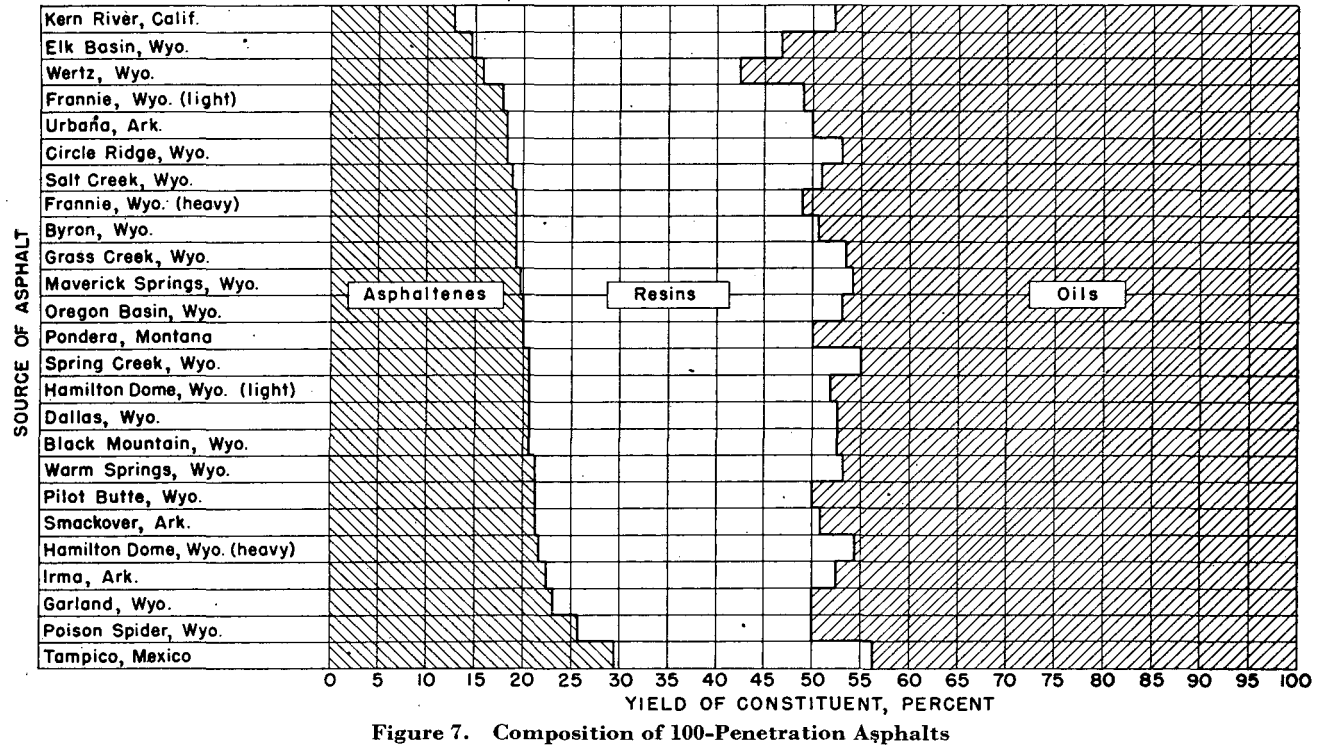

Figure 7. Composition of 100-Penetration Asphalts

Table I. Precision of Method Based upon 41 Analyses of 16 Asphalts

	Asphaltenes	Oils	Resins
Average deviation from mean of each asphalt, grams per 100 grams of sample	0.19	0.40	0.43
Maximum deviation from mean of each asphalt, grams per 100 grams of sample	0.6	1.3	1.2
Percentage of samples with deviations greater than ±0.50%	2	20	32

essential that the extraction times, the temperatures, and the amounts and characteristics of the reagents be standardized.

n-Pentane is used as the principal solvent, as it is available in high purity from the refining of petroleum. *n*-Hexane is possibly more convenient to use as a solvent for these extractions because of its higher boiling point; however, at present, petroleum fractions containing *n*-hexane also contain varying amounts of impurities which cannot be removed effectively by fractionation alone. For example, a petroleum-derived hexane used in preliminary asphalt studies had an A.S.T.M. distillation range at 760-mm. mercury pressure of 65.0° to 67.0° C. (149.0° to 152.6° F.) and by distillation analysis showed 12.3% (by volume) 2-methylpentane, 27.2% 3-methylpentane, 1.6% benzene, and 56.9% *n*-hexane, with a 2% distillation loss. A comparison of the yields of constituents of asphalts from two crude oils (Poison Spider, Wyo., and Tampico, Mexico) by extraction with *n*-pentane and with petroleum-derived hexane is given in Table III. The total yields of the constituents as obtained by the hexane solvent were over 100% and were greater than those obtained for the pentane solvent. These high total yields were due in part to unstable compounds in the *n*-hexane solvent, which consistently yielded an oil-like residue upon evaporation to dryness.

It is essential to allow the initial asphalt-pentane dispersions to stand approximately 12 hours or overnight as directed in the method. The immediate determination of the asphaltenes from several samples reduced their yields by an average of 1.3%. If the petrolene fractions of these samples were allowed to stand overnight, a dark, reddish-brown precipitate collected on the sides of the flask. This precipitate did not redissolve by the addition of fresh pentane, which indicates it should have been removed as a part of the asphaltene or pentane-insoluble fraction.

Washing the asphaltenes four times with 25 ml. of pentane per milliliter of residue as directed in the method does not completely remove all the pentane-soluble material, but the amount of pentane-soluble material removed by each additional washing after four is slight. Consequently, four washings are considered adequate for comparing the yields of asphaltenes.

Water must be effectively removed from the pentane, alumina, and extraction apparatus, as it decreases the adsorption of resins and causes the oil yields to be correspondingly high.

Except for the series of asphalts from Kern River, Calif., crude oil and for several other hard asphalts which contained more than 35% resins, 25 grams of alumina were adequate to adsorb the resins completely from 1.5-gram samples of asphalt. Complete adsorption is indicated if the initial pentane extract is colorless or has only a slight yellow color. The above ratio of alumina to resins should be used unless the resins are incompletely adsorbed, in which case the ratio of alumina to resins is increased by using a smaller sample of asphalt (1.25 to 1.4 grams). A large excess of alumina should be avoided, as the oil yields are reduced thereby. For example, a 15% increase in the amount of alumina over that required to adsorb the resins completely caused, on the average, a decrease in oil yields and a corresponding increase in resin yields amounting to 1% of the asphalt.

The method has not been used for asphalt samples weighing over 1.5 grams. It is possible that larger samples can be accommodated by enlarging the equipment, if the standard extraction procedure and the ratios of solvent and alumina to sample are not significantly altered.

Care should be taken to prevent oxidation of the asphalt fractions by conducting the extraction and drying operations in an oxygen-free atmosphere. Contact with oxygen during these procedures may cause oxidation, with a resulting high yield.

The method described is more rapid than most of the proposed methods for the quantitative determination of the compositions of petroleum and native asphalts in terms of the three groups of constituents—asphaltenes, oils, and resins. It has been found useful in comparing the compositions of asphalts obtained from different sources and by different methods of preparation. By this

Table II. Comparison of Composition of Coating Asphalts (Air-Blown Asphalts) by Bureau of Mines and Strieter Methods of Analysis

Asphalt No.	Softening Point, ° F.[a]	Penetration at 77° F.[a]	Yield of Fraction, Per Cent: Asphaltene M[b]	Asphaltene S[a]	Asphaltene M-S[c]	Oil M	Oil S	Oil M-S	Resin M	Resin S	Resin M-S	Total Yield of Fractions, % M	Total Yield of Fractions, % S
34	232	12	35.8	35.8	0.0	33.6	32.2	1.4	30.6	31.1	−0.5	100.0	99.1
1	225	15	36.7	35.8	0.9	37.1	41.3	−4.2	26.1	23.2	2.9	99.9	100.3
15	212	16	36.4	35.5	0.9	38.7	39.7	−1.0	25.0	24.7	0.3	100.1	99.9
10	213	17	38.8	38.3	0.5	37.6	37.5	0.1	23.8	24.3	−0.5	100.2	100.1
44	214	18	37.4	36.2	1.2	39.5	44.3	−4.8	23.0	19.5	3.5	99.9	100.0
6	216	21	32.1	31.8	0.3	43.0	46.1	−3.1	24.6	22.7	1.9	99.7	100.6
Average value					0.6			−1.9			1.2	100.0	100.0
Maximum value					1.2			−4.8			3.5	100.2	100.6

[a] Softening points, penetrations, and yields of constituents by Strieter method were determined by O. G. Strieter, National Bureau of Standards, Washington, D. C.
[b] By Bureau of Mines method.
[c] Yield of fraction by Bureau of Mines method minus yield of fraction by Strieter method.

Table III. Comparison of Yields of Asphalt Constituents by Extraction with *n*-Pentane and with Petroleum-Derived Hexane

Asphalt	Asphalt from Crude Oil, %	Penetration of Asphalt at 77° F.	Yield of Fraction, Per Cent: Asphaltene P[a]	Asphaltene H[b]	Asphaltene P-H	Oil P	Oil H	Oil P-H	Resin P	Resin H	Resin P-H	Total yield of Fractions, % P	Total yield of Fractions, % H
Poison Spider, Wyo.													
1	37.6	7.8	35.5	29.2	6.3	35.6	46.2	−10.6	29.4	25.2	4.2	100.5	100.6
2	39.4	11	35.0	28.3	6.7	36.1	45.1	−9.0	28.8	27.1	1.7	99.9	100.5
5	43.3	23	31.5	26.3	5.2	41.5	50.1	−8.6	27.2	24.3	2.9	100.2	100.7
3	47.0	49	27.9	22.7	5.2	46.8	54.9	−8.1	25.5	22.7	2.8	100.2	100.3
4	53.2	138	24.8	20.4	4.4	51.8	60.8	−9.0	23.6	19.5	4.1	100.2	100.7
Tampico, Mex.													
4	59.3	15	35.0	28.2	6.8	33.5	44.6	−11.1	31.6	27.3	4.3	100.1	100.1
1	63.6	21	33.5	26.7	6.8	36.4	47.9	−11.5	30.0	25.8	4.2	99.9	100.4
2	70.8	64	29.8	24.1	5.7	42.3	53.0	−10.7	27.7	23.0	4.7	99.8	100.1
3	77.2	176	28.2	23.2	5.0	45.2	55.5	−10.3	26.4	21.8	4.6	99.8	100.5
Average value					5.8			−9.9			3.7	100.1	100.4
Maximum value					6.8			−11.5			4.7	100.5	100.7

[a] By extraction with *n*-pentane.
[b] By extraction with petroleum-derived hexane.

method, each asphalt constituent is recovered and may be used for further study. The method has the disadvantage whichis common to other proposed methods, that only arbitrary fractions are obtained.

ACKNOWLEDGMENT

This report represents the results of work done under a cooperative agreement between the Bureau of Mines, United States Department of the Interior, and the University of Wyoming.

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RECEIVED May 30, 1947. Approved for publication by the Director, Bureau of Mines.

Vitamin A Acetate as a Vitamin A Standard

N. B. GUERRANT, M. E. CHILCOTE, H. A. ELLENBERGER, AND R. A. DUTCHER
The Pennsylvania State College, State College, Pa.

Batches of crystalline vitamin A acetate having uniform extinction coefficients were prepared repeatedly from a high-potency halibut viscera oil distillate by the procedure suggested by Meng. Purification studies with the crystalline material revealed that a product having a constant extinction value and a constant melting point could be obtained through two or three recrystallizations. Stability studies with the undissolved crystals and with the crystals dissolved in refined deodorized cottonseed oil, in corn oil, and in peanut oil showed this ester of the vitamin to be relatively stable when stored in vacuum at room temperature and under refrigeration and also when stored in nitrogen under refrigeration. Both the crystalline vitamin and its oily solutions were less stable in an atmosphere of nitrogen than in vacuum when stored at room temperature. Data thus far obtained indicate that vitamin A acetate offers definite advantages, as a vitamin A standard, over U.S.P. reference cod liver oil and over commercially available crystalline vitamin A alcohol and β-carotene. Studies are being continued.

NUMEROUS scientific articles have been published during the past decade which contained experimental evidence that cod liver oil is not a satisfactory vitamin A standard, even though the oil has been selected with great care and has been handled and stored under conditions favoring vitamin retention. Observations reported as early as 1939 (*8*) indicated that the U.S.P. reference cod liver oil No. 1 had been inaccurately standardized against the international standard or that its vitamin A potency had deteriorated with time.

Studies reported by McFarlan *et al.* (*9*) further indicated that while freshly opened bottles of U.S.P. reference cod liver oil No. 1 yielded absorption values in good agreement with those reported by other experimenters, there was a continuous change in the absorption value of the oil during its use in the laboratory. Subsequent studies by Coy *et al.* (*4*) showed that this decrease in the ultraviolet absorption value of the oil, in the partially filled bottles, took place even when the bottles were flushed with carbon dioxide and stored under refrigeration.

Soon after U.S.P. reference cod liver oil No. 2 became official, data began to appear in the scientific literature which showed that this oil was also unsuitable as a vitamin A standard [Morgareidge (*11*), Coy *et al.* (*5*), Oser *et al.* (*13*), Zscheile *et al.* (*17*), and Callison *et al.* (*3*)]. These reports indicated that this particular oil was unsuitable as a vitamin A standard because of its apparent variability as measured by biological assay and by ultraviolet absorption measurements. As a result, U.S.P. reference cod liver oil No. 3 became official during the latter part of 1944. Like U.S.P. reference oils 1 and 2, oil No. 3 also appears unsatisfactory as a vitamin A standard. Thus a more stable and reliable standard was needed.

Gridgeman (*7*) has summarized the objections to cod liver oils as a vitamin A standard: Cod liver oils are difficult to assay spectrographically because of the presence of much extraneous absorption; even when the extraneous absorption is removed, the residual spectroscopic characteristics are not necessarily those of normal vitamin A; and very strict precautions are necessary if cod liver oils are to be kept without change for any length of time. Because of these seemingly inherent characteristics of cod liver oils it appears that fish liver oils, in general, are unsuited for use as a vitamin A standard; hence one must look elsewhere for

a more reliable standard. The present report describes some of the studies which have been carried out in search of such a standard.

β-CAROTENE

In 1931, the Permanent Commission on Biological Standardization of the League of Nations (Health Section) set up an international unit of vitamin A potency which was based on the biological activity of 1 microgram of crystalline carotene, the pooled product of seven different laboratories. With the advance in the knowledge of the chemistry of the carotenoids, the international unit of vitamin A potency became more specific in 1934 when the above organization defined the unit as the biological activity of 0.6 microgram of pure β-carotene. As β-carotene has been used as the international standard of vitamin A potency from 1934 and Callison and Orent-Keiles (*3*) had recommended it as a standard in preference to U.S.P. reference cod liver oil No. 2, it appeared to be the logical material for further investigation. This seemed to be true regardless of the fact that β-carotene is difficult to obtain and to preserve in the pure form and that much remains to be learned concerning the conversion of β-carotene to vitamin A in the animal body.

Owing to the difficulty encountered by various investigators in preparing absolutely pure β-carotene in the crystalline state, the present studies were restricted to a commercially available crystalline product which had been packed under vacuum in small glass ampoules. Because of its supposedly high degree of purity, its commercial availability, and its rather general usage in biological laboratories, this material seemed to offer possibilities as a vitamin A standard. Therefore, samples of this carotene were obtained and subjected to spectrophotometric examination in order to ascertain its uniformity and, if possible, its stability. The carotene was purchased directly from the commercial source, usually in quantities ranging from one to six (10-mg.) ampoules.

The various shipments of carotene were examined spectrophotometrically when received at the laboratory or soon thereafter. In the meantime the ampoules of carotene remained stored in a refrigerator in the absence of light.

In carrying out the spectrophotometric measurements, the carotene was immediately removed from the opened ampoule, weighed on a microbalance, dissolved in petroleum ether (boiling point 35° to 69° C.), and made to volume with that solvent. From the stock solution thus prepared, a series of ten dilutions was made which were calculated to range in concentration from 0.5 to 3.5 micrograms of carotene per ml., using petroleum ether as the diluent. Caution was taken not to expose the crystalline carotene or the carotene solutions to light or to prolonged standing before carrying out the absorption measurements. The absorption at 450 mμ of each of the ten solutions of carotene was measured by means of a Beckman quartz spectrophotometer, using matched corex cells and a slit width of 0.2 mm. From the absorption data the $E^{1\%}_{1\,cm.}$ 450 mμ was calculated. The mean computed values for some of the samples of carotene examined are given in Table I.

When it became apparent that the first ampoule of carotene [control A, ampoule 1(a)] under investigation showed an $E^{1\%}_{1\,cm.}$ 450 mμ considerably lower than that attributable to pure β-carotene, spectrophotometric measurements were carried out on a second series of solutions made from another portion of crystals from the same ampoule. Because the mean absorption value of the second portion of crystalline carotene was in good agreement with that of the first portion, it was concluded that the experimental technique employed was reasonably reliable, at least in so far as reproducibility of data was concerned. As the result of examining sixteen different ampoules of this carotene, over a period of 18 months, the variability of the product became more fully apparent, with the carotene from some ampoules showing approximately 26% less absorption than that from other ampoules. Only two ampoules of the carotene, and these bearing the same control number (*B*), showed an absorption value approaching that ascribed to pure β-carotene. While there were variations in the absorption value of carotene from ampoules bearing the same control number, the greatest variations appeared to be between the absorption values of the ampoules of carotene bearing different control numbers. Although the data do not explain why the absorption characteristics of the carotene samples were so variable, it is evident that present supplies of carotene will not serve as a reliable vitamin A standard.

VITAMIN A ALCOHOL

Because the esterified form of vitamin A as well as the vitamin A derived from the provitamin (carotene) apparently passes through the alcohol form in the process of metabolism in the animal body, it would seem that pure vitamin A alcohol should constitute an ideal vitamin A standard, from the spectrophotometric as well as the biological standpoint, provided this form of the vitamin possessed the required characteristics as to purity and stability. Inasmuch as crystalline vitamin A alcohol was available from commercial sources, it was decided that a series of absorption measurements should be made on different ampoules of this material for the purpose of ascertaining the uniformity of the available product, although biological tests in the laboratory had already indicated that it was unstable when used under assay conditions comparable to those recommended by the U. S. Pharmacopoeia XII.

Thirteen ampoules of crystalline vitamin A alcohol were purchased during a period of 15 months for the absorption tests. At least one ampoule bearing each control number was examined immediately on being received at the laboratory, while other ampoules of the crystalline vitamin were stored under refrigeration and examined spectrophotometrically at a later date.

In carrying out the absorption measurements, a portion of the crystalline vitamin was taken from the freshly opened ampoule, weighed on a microbalance, dissolved in isopropanol, and made to volume with this solvent. From this stock solution, ten dilutions were prepared having a calculated concentration of vitamin A alcohol ranging from 0.5 to 3.5 micrograms per ml., using isopropanol as the diluent. The usual precautions were taken to avoid exposure of the crystalline vitamin or the prepared solutions to light or to prolonged standing before the absorption measurements were carried out. The absorption measurements were made on a Beckman quartz spectrophotometer at a wave length of 328 mμ, while using matched quartz cells and a slit width of 0.4 mm. The instrument was so adjusted that the solvent gave a 100% transmittance. From the absorption data the $E^{1\%}_{1\,cm}$ 328 mμ values were calculated. The mean value for the ten dilutions prepared from each vial of the crystalline vitamin is likewise presented in Table I.

Table I. Variations in Extinction Coefficients of Ampoules of Crystalline β-Carotene and Crystalline Vitamin A Alcohol Determined by Beckman Quartz Spectrophotometer[a]

β-Carotene in Petroleum Ether				Vitamin A Alcohol in Isopropyl Alcohol			
Control No.	Ampoule No.	Date of assay	$E^{1\%}_{1\,cm.}$ 450 mμ	Control No.	Ampoule No.	Date of assay	$E^{1\%}_{1\,cm.}$ 328 mμ
A	1(a)	8/ 1/44	1900	N	1	7/7/44	1794
A	1(b)	8/ 2/44	1880	O	1	7/13/44	1428
A	2	8/ 9/44	1896	O	2	7/14/44	1460
A	3	12/30/44	1958	O	3(a)	7/31/44	1652
B	1	12/27/43	2554	O	3(b)	7/31/44	1670
B	2	12/28/44	2536	O	4(a)	6/24/47	1444
C	1	12/28/44	2423	O	4(b)	6/24/47	1462
C	2	12/28/44	2330	P	1	10/18/45	1574
D	1	12/28/45	2405	P	2	10/30/45	1484
D	2	12/28/45	2347	P	3	11/8/45	1346
E	1	12/28/45	2366	P	4	11/14/45	1420
F	1	12/29/45	2407	P	5(a)	6/23/47	1362
F	2	12/29/45	2381	P	5(b)	6/23/47	1326
F	3	12/29/45	2458	Q	1	12/28/44	1741
F	4	12/29/45	2170	Q	2	12/28/44	1780
F	5	12/29/45	2223	Q	3(a)	6/23/47	1653
F	6	12/29/45	2390	Q	3(b)	6/23/47	1666

[a] Average $E^{1\%}_{1\,cm.}$ values for ten dilutions of each sample ranging in concentration from 3.5 to 0.5 microgram per ml.

Table II. Effect of Recrystallization of Vitamin A Acetate on Extinction Coefficient and Melting Point

No. of Crystallizations	$E^{1\%}_{1\text{ cm.}}$ 325 mμ[a], Crystallized from		Melting Point Range, Crystallized from	
	Ethyl formate	Methyl alcohol	Ethyl formate	Methyl alcohol
1	1153	..	.....	.....
2	1279	1385	55.0–56.0	54.3–56.3
3	1512	1519	57.7–58.7	.....
4	1516	1518	57.8–59.0	57.6–58.9
5	..	1515	...	57.4–58.8

[a] Absorption measurements made with vitamin ester dissolved in isopropyl alcohol.

Here again duplicate determinations on a portion of the crystalline vitamin from the same ampoule [portions (a) and (b)] yielded absorption data which show reasonably good agreement. Of the thirteen ampoules of vitamin A alcohol examined, only three were found to have absorption values equal to that ascribed to pure vitamin A alcohol (1750); two of these ampoules bore one control number and the third bore a second control number. Here again the absorption data show a somewhat greater variation between the ampoules of the crystalline vitamin bearing different control numbers than between the different ampoules of the vitamin bearing the same control number. Ampoules of the crystalline vitamin which had been stored at 35° F. for almost 3 years yielded absorption values equal to or only slightly lower than those of the contents of comparable ampoules when purchased. While the absorption data do not explain why the different ampoules of crystalline vitamin vary so widely in this physical characteristic, they do suggest that the purity of the crystalline product was not the same when placed in the various ampoules or that perhaps deterioration had taken place. It was concluded that this source of crystalline vitamin A alcohol would not constitute a reliable vitamin A standard, owing to the variability of the contents of different ampoules.

VITAMIN A ACETATE

Inasmuch as Baxter and Robeson (*1*) had found vitamin A acetate to be rather easily prepared from the vitamin A alcohol and to be the most stable crystalline ester of vitamin A thus far reported, attention became focused on this ester as a possible vitamin A standard. This interest was increased when Meng (*10*) reported a procedure for preparing the acetic ester of the vitamin directly from high-potency natural ester distillates, thereby increasing the possibility of conducting detailed collaborative studies relative to the physical and biological properties of vitamin A acetate.

In order to confirm the findings of Baxter and Robeson (*1*) and to ascertain the suitability of this ester as a vitamin A standard, a supply of natural vitamin A ester concentrate, prepared by the molecular distillation of halibut viscera oil (supplied through the courtesy of Distillation Products, Inc., Rochester, N. Y.) was obtained as the starting material. The potency of the concentrate as determined by the Carr-Price reaction and by direct spectrophotometric measurement was found to be approximately 1,000,000 U.S.P. units per gram. Through experimentation it was found that the formation and the isolation of crystalline vitamin A acetate could be most effectively carried out by following a procedure essentially as outlined by Meng (*10*) and by applying some of the techniques described in detail by Baxter and Robeson (*2*). The general procedure was as follows:

The vitamin A concentrate was saponified, the nonsaponifiable matter was recovered and dissolved in methyl alcohol (15% solution), and the alcoholic solution was subjected to a two-stage desterolation. (Ethyl formate, the solvent suggested by Meng, did not prove especially useful in desterolating this particular vitamin A concentrate, owing to the presence of a dark red oily fraction which subsequently interfered with the crystallization of the vitamin A acetate. This dark red oily fraction was found to be insoluble in cold methyl alcohol.) The first stage of desterolation was effected at a temperature of −20° C. and the second stage at a temperature of −70° C. The methyl alcohol was removed from the desterolated concentrate by vacuum distillation and the resulting concentrate dissolved in pyridine for acetylation. The acetylation was carried out by adding successive small portions of acetic anhydride and cooling so as to minimize destruction of the vitamin. The acetylated concentrate was then maintained at a temperature of 60° to 65° C. for about 30 minutes, then diluted with water, and the acetylated vitamin was removed by a series of extractions with diethyl ether. The combined ether extract was purified by washing with water, dilute hydrochloric acid, and sodium bicarbonate solution as suggested by Meng (*10*). The ether solution of the concentrate was dried over anhydrous sodium sulfate and the ether subsequently removed by vacuum distillation. The yield at this stage was usually about 75% of the vitamin A originally present in the concentrate.

The ether-free concentrate from the acetylation reaction was dissolved in a mixture consisting of equal parts of ethyl formate and methyl alcohol at the rate of 3 parts of concentrate to 2 parts of the solvent. The resulting solution was cooled, seeded with crystals of vitamin A acetate, and placed in a freezing unit at −20° C. until crystallization took place. This usually required about 5 days for completion.

Recrystallization of Vitamin A Acetate. Because vitamin A acetate of maximal purity was desired, and the initial crystallization produced a yellow colored amorphous precipitate which had a relatively low $E^{1\%}_{1\text{ cm.}}$ 325 mμ when dissolved in isopropanol, it was necessary to subject the product to recrystallization. Consequently, several recrystallization experiments were carried out, and the purity of the crystals from each crystallization was tested by their ultraviolet absorption characteristic when dissolved in isopropanol. The melting point of each crop of crystals was also determined.

In the first experiment, a batch of previously crystallized vitamin A acetate (one crystallization) was recrystallized three times from ethyl formate (1.5 ml. of solvent per gram of crystals) at −20° C. In other experiments, a similar batch of previously crystallized vitamin A acetate was recrystallized four times from methyl alcohol (6 ml. of solvent per gram of crystals) at 5° C. The crystals were always removed from the mother liquor by filtering through a precooled Büchner funnel, washed with a small amount of the cold solvent, and dried under a moderate vacuum, and the desired portions of crystals were transferred to 9 mm. × 15 cm. glass ampoules. These ampoules were sealed onto a glass manifold-type vacuum system, and the system was evacuated to a pressure of 3 to 5 microns, as measured by a McLeod gage. After being held at this low pressure for at least 0.5 hour, the capsules were sealed off and stored at 5° C. in the dark.

The data presented in Table II show that in both experiments three successive crystallizations of the product brought about the maximal purity attainable under these conditions, as indicated by the ultraviolet absorption and by melting point measurements. In both experiments the color of the crystals diminished with subsequent recrystallization up to and including the fourth crystallization. The use of ethyl formate as the solvent resulted in the production of more lightly colored (pale lemon yellow) crystals.

The final over-all yield after three crystallizations amounted to approximately 7.5% of the original vitamin A present in the concentrate. Baxter and Robeson (*1*) had previously noted that production of crystalline vitamin A acetate from distilled ester concentrates resulted in sharply reduced yields as compared to production from crystalline vitamin A alcohol. Perhaps this may be due, in part, to the presence of neo-vitamin A in the concentrate (*14*).

Reproducibility of Product. Close conformance to a definite set of physical, chemical, and biological characteristics is a necessary attribute of a satisfactory assay standard. To test the relative purity of the various batches of crystalline vitamin A acetate thus prepared, ultraviolet absorption measurements were made on portions of five batches of crystals. One of the five batches had been recrystallized from a combination of odd-lot portions of previously (one crystallization) crystallized vitamin

Table III. Reproducibility of $E^{1\%}_{1\ cm.}$ 325 mμ of Vitamin A Acetate

Batch No.	Date of Crystallization	Crystallized from	$E^{1\%}_{1\ cm.}$ 325 mμ in Isopropanol
1	12/17/46	Ethyl formate	1515
2	1/ 2/47	Methyl alcohol	1517
3	1/29/47	Ethyl formate	1533
4	1/29/47	Ethyl formate	1514
5	2/14/47	Ethyl formate	1521[a]
	Arithmetic mean		1520
	Standard deviation		7.7
	Coefficient of variation		0.5

[a] Average of twelve values obtained by testing twelve different samples of crystals from this batch (coefficient of variation = 0.7).

Table IV. Some Physical Constants of Vitamin A Acetate

Source of Data	$E^{1\%}_{1\ cm.}$ 325 mμ Isopropyl alcohol	$E^{1\%}_{1\ cm.}$ 325 mμ Ethyl alcohol	$L^{1\%}_{1\ cm.}$ 620 mμ Carr-Price Reaction[a]	Melting Point, ° C.
This laboratory, A[b]	1520	1545	4210	57.6–58.9
This laboratory, B[c]	1510	1545	4220	57.7–58.8
Baxter and Robeson[d]	...	1510	4090	57.0–58.0
Oser *et al.*[d]	1570	..	..	...

[a] Calculated as vitamin A alcohol equivalent of crystalline acetate.
[b] Own preparation.
[c] Supplied for collaborative studies.
[d] Absorption measurements made at 328 mμ.

A acetate from five different batches of concentrate. The resulting data, included in Table III, show that very close agreement was attained as regards the tested characteristics.

Data on a typical sample of crystalline vitamin A acetate prepared in this laboratory, and on the samples of crystalline vitamin A acetate supplied for the U.S.P. collaborative study (*16*) and on crystalline vitamin A acetate as reported by other investigators, are given in Table IV.

Stability of Vitamin A Acetate. As a satisfactory vitamin A standard must withstand storage during the period of assay without deterioration, it seemed desirable to test the stability of vitamin A acetate under conditions simulating those actually encountered during biological assay.

Portions of the vitamin crystals (4 to 7 mg.) as well as oil solutions (250 mg.) of the crystals were sealed in glass ampoules and stored in the absence of light at room temperatures (25° to 30° C.) and at 5° C. For these studies, solutions of the crystalline vitamin A acetate in purified cottonseed oil (Wesson oil), corn oil (Mazola oil), and peanut oil were made up so as to contain approximately 10,000 U.S.P. units of vitamin A per gram. This particular concentration of the vitamin had been recommended by Embree (*6*). In each instance, some of the ampoules were highly evacuated as previously described, while others were filled with nitrogen. To achieve the latter condition the ampoules were either attached to the previously mentioned evacuation manifold by means of rubber connections or were evacuated and charged by means of a vacuum desiccator. In both instances the ampoules were evacuated and refilled with nitrogen four successive times to ensure complete removal of oxygen. The nitrogen used had been previously scrubbed by passing successively through a tower of alkaline pyrogallol, a tower of concentrated sulfuric acid, and tubes of anhydrous calcium chloride and indicating Drierite (W. A. Hammond Drierite Co., Yellow Springs, Ohio). The data obtained to date in the stability studies are presented in Table V.

DISCUSSION

The results of the foregoing studies show that the current sources of the so-called pure β-carotene and of crystalline vitamin A alcohol cannot be relied upon to yield reproducible data when used as vitamin A standards, owing to impurities in the crystalline product when placed in the ampoule or to degradation products formed after the crystals were packaged. On the other hand, batches of crystalline vitamin A acetate were repeatedly prepared from a fish oil concentrate, and on subsequent recrystallization possessed physical characteristics that indicated a high degree of purity. Preliminary stability studies on the prepared vitamin A acetate, in the crystalline state and in its oily solutions, in vacuum and in nitrogen, at room temperature and under refrigeration, indicated that the acetate ester of the vitamin was reasonably stable. This form of the vitamin was found to be stable when stored in vacuum and when stored in nitrogen under refrigeration. However, when stored in nitrogen at room temperature there was marked evidence of deterioration with time, especially when the crystalline acetate was dissolved in peanut oil. Although efforts were made to purify the nitrogen used in these studies, there is a remote possibility that traces of oxygen remained in the gas and thereby contributed to the instability of the vitamin. The peanut oil employed may not have been representative of the most satisfactory peanut oil obtainable for this particular purpose. It was purchased through a chemical supply source.

As a whole, the data thus far obtained strongly indicate that vitamin A acetate possesses some of the most significant characteristics desired in a vitamin A standard. It appears to offer distinct advantages in these respects over the U. S. P. reference cod liver oil, the currently available crystalline β-carotene, and the crystalline vitamin A alcohol.

Table V. Stability of Vitamin A Acetate in Crystalline State and in Solution as Indicated by Extinction Coefficients ($E^{1\%}_{1\ cm.}$ 325 mμ)

Time in Storage, Days	Stored at Room Temperature: Under nitrogen	Stored at Room Temperature: In vacuum	Stored in Refrigerator: Under nitrogen	Stored in Refrigerator: In vacuum
	In crystalline state			
0	1522[a]	1522	1522[a]	1522
30	...	1538	1528, 1512[a]	1510
60	1466, 1474[b] 1268, 1254[a]	1522	1532[a]	..
120	..	1529, 1522	1489[b] 1464, 1462[a]	1511, 1516
	In cottonseed oil			
0	5.31	5.31	5.31	5.31
31	5.18	5.32	5.29	5.34
61	4.53, 4.93 4.90	5.27	5.16, 5.24	..
117	4.61, 4.82	5.19	5.21	5.16
	In corn oil			
0	5.44	5.44	5.44	5.44
30	5.42	5.39	5.33	5.35
60	5.29	5.31	...	..
101	4.41, 4.59	5.23	5.35	...
120	4.87, 4.70	5.27, 5.26	5.34, 5.31	5.30, 5.31
	In peanut oil			
0	5.06	5.06	5.06	5.06
30	4.24, 4.32	4.74	...	...
73	3.24, 3.69	4.79, 4.69	4.90, 4.89	4.94, 5.02
90	3.08, 3.20	4.71, 4.69	4.87, 4.87	5.02

[a] Filled with nitrogen by means of desiccator.
[b] Filled with nitrogen individually.

Oser *et al.* (*13*), however, appear to favor the use of a distilled ester concentrate in preference to crystalline vitamin A acetate as an internal standard in their proposed modification of the Carr-Price procedure for the determination of the vitamin A content of biological materials. A prime reason for the choice seems to have been based on the probability that crystalline vitamin A acetate contained oxidized vitamin A. This deduction was drawn from the results of a previously conducted forced oxidation study (*12*) of both the crystalline vitamin A acetate and the alcohol in solution in ethyl laurate. These authors noted, in effect, that the ratio of the $L^{1\%}_{1\ cm.}$ 620 mμ to the $E^{1\%}_{1\ cm.}$ 325 mμ of the vitamin A in the freshly prepared ethyl laurate solution was lower than the corresponding ratio for the vitamin A in a distilled ester concentrate. This was interpreted as indicating partial oxidation of the crystalline vitamin A acetate and alcohol, as both they and Robinson (*15*) had noted that in the initial stages of forced oxidation of vitamin A there was a greater decrease in chromogenic power in the antimony trichloride reaction than in its capacity to absorb ultraviolet radiation at 325 mμ.

In the authors' studies, however, a distilled ester concentrate (obtained from Distillation Products, Inc.) gave an $E^{1\%}_{1\,cm.}$ 620 mμ value that agrees well with both the reported value for vitamin A acetate (*1*) and with the value obtained for the crystalline acetate prepared in this laboratory. This $E^{1\%}_{1\,cm.}$ 620 mμ value of 4240 for the distilled ester concentrate was calculated from its content of vitamin A as determined by reference of its $E^{1\%}_{1\,cm.}$ 325 mμ value to the corresponding value for pure crystalline vitamin A acetate.

The investigation is being continued. It will eventually include, in addition to the studies already mentioned, stability studies on vitamin A acetate when dissolved in those organic solvents most commonly used in spectrophotometric tests and other studies relating to the acceptability of this form of the vitamin as a standard in biological and in chemicophysical methods of assay.

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RECEIVED September 5, 1947. Authorized for publication on August 14 1947, as Paper 1388 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Supported in part by a grant from The Nutrition Foundation.

Rapid Colorimetric Determination of Copper in Tin-Base Alloys

GEORGE NORWITZ, *Material Laboratory, New York Naval Shipyard, Brooklyn 1, N. Y.*

A rapid colorimetric method for the determination of copper in tin-base alloys is described. After the sample has been dissolved in hydrochloric and nitric acids, phosphoric acid is added and the solution is heated. Water and ammonia are added, and the resultant blue copper amine color is measured on a photoelectric colorimeter. The addition of phosphoric acid prevents precipitation of the tin when the solution is made ammoniacal.

THE author undertook to develop a rapid colorimetric procedure for the determination of copper in tin-base alloys. Two A.S.T.M. methods (*1*, *2*), neither one of which is particularly rapid, are commonly used for the analysis of copper in tin-base alloys. In one of these procedures (*1*) the copper is electrolyzed, after volatilization of the tin with hydrobromic acid, or hydrobromic acid and bromine. In the other (*2*) the copper is electrolyzed in a nitric acid–hydrofluoric acid medium, stripped from the cathode, and replated.

The rapid colorimetric method described in this paper is novel, yet extremely simple. After the sample has been dissolved in hydrochloric and nitric acids, phosphoric acid is added and the solution is heated. Water and ammonia are added, and the resultant blue color is measured on a photoelectric colorimeter. The addition of the phosphoric acid prevents the precipitation of the tin when the solution is made ammoniacal.

APPARATUS

Klett-Summerson photoelectric colorimeter. Rectangular 4-cm. glass absorption cell. Klett-Summerson filter No. 59 (transmittance maximum at 580 millimicrons).

METHOD

Transfer 1.000 gram of the sample to a 500-ml. Erlenmeyer flask that has a mark indicating the 500-ml. level. Add 10 ml. of concentrated hydrochloric acid and 10 ml. of concentrated nitric acid in that order, and heat on a hot plate for a minute or two to dissolve the sample. Add 30 ml. of concentrated phosphoric acid (85%) and heat strongly on a hot plate until the solution is a clear green (12 to 15 minutes). Remove the flask from the hot plate and allow the solution to cool somewhat. Add about 200 ml. of cold water, and then add cautiously 120 ml. of concentrated ammonium hydroxide while swirling the flask. Cool to room temperature. Dilute to the 500-ml. mark with water, stopper the flask, and shake well. Read the blue copper amine color at 580 millimicrons on a colorimeter that has been set to zero with distilled water. Convert the colorimeter readings to percentage copper by consulting a curve previously prepared by the use of tin-base samples of known copper content.

The author ran National Bureau of Standards samples 54a and 54b in triplicate and obtained the results shown in Table I.

Table I. Determination of Copper

Sample	Cu Present, %	Cu Found, %	Average Cu Found, %
54a[a]	3.75	3.78 3.70 3.73	3.74 ± 0.03
54b[b]	3.19	3.20 3.19 3.23	3.21 ± 0.02

[a] Contains 88.61 Sn, 0.21 Pb, 7.32 Sb, 0.02 Bi, 0.04 Fe, 0.04 As.
[b] Contains 87.48 Sn, 1.81 Pb, 7.39 Sb, 0.029 Bi, 0.028 Fe, 0.052 As.

DISCUSSION

No mention was found in the literature of the use of phosphoric acid to prevent precipitation of tin in ammoniacal solution. The author originally believed that the pyrophosphoric acid formed by heating the phosphoric acid (*4*) prevented precipitation of the tin. Experiments showed that if no heat were

applied after addition of the phosphoric acid, a clear solution was still obtained when the ammonia was added. Better colorimetric results were obtained when the solution was heated to drive off the nitric and hydrochloric acids.

The colorimetric reference curve obtained using tin samples of known copper content was a straight line.

As excellent results were obtained by using a Klett-Summerson filter No. 59 (*6*) no spectrophotometric study was made of the blue copper amine color. The spectrophotometric curves published by previous investigators (*5*, *6*, *7*) may not be applicable to the author's method, because the hue of the blue copper amine color is changed by the presence of phosphoric acid (*5*).

The method described is designed for the routine determination of copper in tin-base alloys that contain 1 to 10% copper.

The blue copper amine color will keep 24 hours without change if the flasks are tightly stoppered to prevent the escape of ammonia.

Nickel, cobalt, and chromium interfere with the blue copper amine color (*5*). These elements, however, are rarely present in tin-base alloys. Iron up to 5% was found not to interfere with the determination.

An attempt to hold up the tin in ammoniacal solution merely by the presence of tartaric acid without the presence of phosphates was not successful (*3*).

It would seem that considerable tin and antimony would be volatilized in driving off the hydrochloric and nitric acids. Actual analysis, however, revealed that most of the tin and antimony remained in solution.

The author has made a study of the effect of phosphoric and pyrophosphoric acids on the precipitation of the common metals in ammoniacal solution.

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RECEIVED September 2, 1947. The opinions expressed in this article are those of the author, and are not to be construed as representing the official views of the Navy Department.

Analysis of Chloroacetic Acid

Determination of Dichloroacetic and Acetic Acids

GEORGE A. DALIN AND JEROME N. HAIMSOHN
Stauffer Chemical Company, New York Research Laboratory, New York, N. Y.

The accuracy of the determination of dichloroacetic acid in chloroacetic acid is shown to be dependent on the quantity of reagent used. Regulation of the amount of reagents used and the addition of manganous sulfate bring the accuracy within the range required by material specifications. Determination of the acetic acid content by difference is shown to be unsatisfactory and a direct method involving titration after distillation with an added solvent is described.

CHLOROACETIC acid achieved prominence during World War I as a raw material in the manufacture of chloroacetophenone and analytical methods were established for army use (*2*). More recently, chloroacetic acid has become of importance in manufacturing 2,4-dichlorophenoxyacetic acid and carboxymethylcellulose, products which will be used on a large scale. Specifications for chloroacetic acid have been issued by purchasers and have included analytical methods similar to those described in the army report. This method in turn goes back to work done by Pool (*1*) and in brief depends on the following procedure:

A sample is dissolved in water, caustic is added, and the solution is refluxed until the chloride is completely hydrolyzed. The monochloroacetic acid yields glycolic acid; dichloroacetic acid yields glyoxylic acid, which in the presence of alkali disproportionates to sodium glycolate and sodium oxalate. From an aliquot, the oxalate is precipitated out with calcium acetate, and is determined by permanganate titration. This procedure gives the dichloroacetic concentration.

The reactions involved are:

$$2CHCl_2CO_2H + 6NaOH \rightarrow 2CH(OH)_2CO_2Na + 4NaCl + 2H_2O \quad (1)$$

$$2CH(OH)_2CO_2Na + NaOH \rightarrow Na_2C_2O_4 + CH_2(OH)CO_2Na + 2H_2O \quad (2)$$

$$CH_2ClCO_2H + 2NaOH \rightarrow CH_2OHCO_2Na + NaCl + H_2O \quad (3)$$

A second aliquot is taken and the total chlorine is determined by the Volhard method. The chlorine as dichloroacetic acid is subtracted from the total chlorine to give the amount as monochloroacetic acid and thus the monochloroacetic acid concentration. Another portion of the sample is dissolved in water and is titrated with standard alkali. Subtraction of the monochloroacetic and dichloroacetic acid values from the total acid, as determined with standard alkali, gives the acetic acid concentration. The determination of trichloroacetic acid is not involved because the concentration is negligible under the usual conditions of preparation.

In order to test the method for the determination of dichloroacetic acid, samples were prepared and analyzed. The results were invariably low by factors ranging from 15 to 85%. Because calcium oxalate is soluble to the extent of approximately 7 mg. per liter at 25° C. it seemed likely that part of the discrepancy was due to the use of too much water during the procedure. It was noted that when the total quantity of permanganate used was small, it was very difficult to recognize the end point. Satisfactory titration with permanganate requires that manganous ion be present. When the titer is large, this ion is produced during the first of the titration. In the revised procedure, as described below, these difficulties were satisfactorily eliminated by scaling down the quantities of reagents and wash liquids used, and by adding manganous sulfate immediately before the titration.

DETERMINATION OF DICHLOROACETIC ACID IN MONOCHLOROACETIC ACID

A sample weighing from 2 to 3 grams is dissolved in 40 ml. of water, and 14 grams of sodium hydroxide pellets are added. The mixture is refluxed, under a water-cooled condenser, for 1 hour. After cooling, the solution is made acid with concentrated hydrochloric acid, then neutralized with concentrated aqueous ammonia, and 3 ml. of excess ammonia are added. The silica which

is precipitated at this point can be filtered off, but this was found to be unnecessary. The solution (or filtrate) is concentrated to the saturation point, using glass beads to prevent bumping. After concentrating, and while the solution is still hot, 11 ml. of freshly prepared 5% calcium acetate solution are added, followed by 5 ml. of concentrated aqueous ammonia. The mixture is allowed to stand, with intermittent stirring, for 1 hour, after which time the precipitate is filtered off by gravity on No. 40 Whatman paper. The flask and the precipitate are then washed successively with 20 ml. of dilute ammonia (1 to 10), 20 ml. of 1% calcium acetate, and 20 ml. of water, following which the filter paper and precipitate are returned to the flask. No attempt is made to remove the last traces of precipitate from the flask, as it is subsequently used for the titration. Just before the titration of each sample 200 ml. of 1 to 10 sulfuric acid are added, the mixture is heated to 70° C., and then 3 ml. of manganous sulfate solution (containing 2 grams per liter) are added. Standard permanganate (approximately 0.1 *N*) is used for the titration. A buret of 5-ml. volume, graduated in 0.01 ml. and fitted with a reservoir, has been found convenient.

Blank determinations are simultaneously carried through the entire procedure, with the same amounts of reagents. If there is any delay between the addition of the sulfuric acid and the titration, excessive attack on the filter paper takes place, resulting in an irregular blank. The concentration of dichloroacetic acid is calculated as follows:

$$\% \text{ dichloroacetic acid} = \frac{(\text{ml. of titer} - \text{ml. of blank}) \times \text{normal factor} \times 12.895}{\text{weight of sample}} \quad (4)$$

Table I shows a comparison of results when varying quantities of reagents are used.

In this procedure, approximately 30 mg. of calcium oxalate would be formed from a 3-gram sample containing 2% of dichloroacetic acid. With 100 grams of water used as solvent and for washing, the loss of calcium oxalate would be approximately 0.7 mg. If the army method were used, the loss would be about 3 mg., which is about 10% of the total oxalate present. If an excessive amount of wash water is used the loss could be much greater.

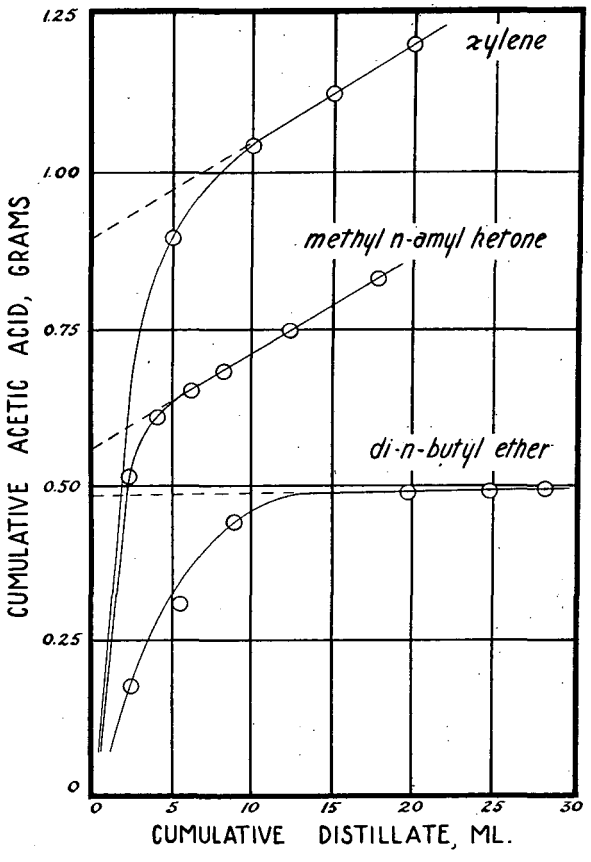

Figure 1

Table I. Effect of Quantity of Reagents Used on Dichloroacetic Acid Results

Quantity of Reagents[a]	No. of Replicates	Dichloroacetic Acid Concentration, Known, %	Dichloroacetic Acid Concentration, Found, %
1	6	2.40	2.38 ± 0.02
1	4	2.06	2.09 ± 0.04
1	5	100[b]	102 ± 1[b]
1	5	2.4	2.30 ± 0.02
1	4	2.05	2.05 ± 0.02
1	3	3.50	3.48 ± 0.02
4.5	4	2.05	1.76 ± 0.04
4.5	5	3.00	2.52 ± 0.13
5	2	2.06	0.72 ± 0.04
4[c]	3	3.50	0.5

[a] Multiples of amounts given in method. Also includes diluent and wash liquids.
[b] Analysis of recrystallized dichloroacetic acid.
[c] Result obtained in another laboratory.

DETERMINATION OF ACETIC ACID IN CHLOROACETIC ACID

The specifications which have been issued recently limit the dichloroacetic acid content to approximately 2% and the acetic acid content to approximately 1%. If the error in determining chloroacetic acid is of the order of 1%, the concentration of acetic acid as determined by the difference between two quantities, each of which is approximately 100% (the weight of the sample and the combined weights of the chloroacids), will be completely indeterminate. In fact, it is surprising that negative values for the acetic acid content have never been reported.

Obviously, acetic acid must be determined directly and not by difference. It was decided to investigate separation by distillation, but the comparatively large holdup in any sort of column and distilling head ruled out the use of any of the standard techniques.

As an alternative, the effect of adding an inert, intermediate-boiling material was investigated.

Xylene (boiling point 137° to 140° C.), methyl *n*-amyl ketone (boiling point 150° C.), and di-*n*-butyl ether (boiling point 142° C.) were selected for study because their boiling points are well removed from those of acetic acid (boiling point 118° C.) and chloroacetic acid (boiling point 189° C.). Synthetic mixtures were prepared and distilled through a column 60 cm. (2 feet) long and 10 mm. in inside diameter, packed with $^1/_{16}$ inch helices. The reflux ratio was held constant at approximately 10 to 1. The distillate was collected in small fractions (each of which was extracted with water if the inert material was water-immiscible). The fractions were titrated with standard alkali, and the acid was calculated as acetic acid. Fractions were collected until the ratio of acid value to size of fraction became constant. The cumulative acid values were then graphed against the cumulative distillate.

With xylene and the ketone as additives, the pot residues were discolored and the distillates fumed on exposure to air, indicating that decomposition had taken place during the distillation. Since the pot temperature rose to approximately 170° C. in each case, decomposition was inevitable. The ether was added in much larger quantity, as a result of which the pot temperature rose only to 150° C.; the residue was colorless and no fuming was noted. Undoubtedly the same result could have been obtained by using about the same proportion of xylene or the ketone.

Graphs of the xylene and ketone runs (Figure 1) showed that acid is continuously evolved. If the assumption is made that acid (other than the original acetic acid) was evolved at a constant rate throughout the distillation, the effect of the additional acid can be eliminated by extrapolating the straight portion of the curves back to zero distillate. Table II gives the results obtained in this way.

The value obtained with xylene as the additive is somewhat low, but it is likely that in this, the first such experiment run, the distillation should have been carried further. It is also possible

Table II. Acetic Acid Determination

Added Solvent	Acetic Acid Known	Acetic Acid Found	Chlorinated Acid Content	
Ml.	*Gram*	*Gram*	*Grams*	
50 Xylene	0.993	0.905	50	Monochloroacetic acid
			1	Dichloroacetic acid
40 Methyl *n*-amyl ketone	0.564	0.564	50	Monochloroacetic acid
100 Di-*n*-butyl ether	0.497	0.485	50	Monochloroacetic acid

that the acid was not completely extracted from the xylene. The procedure using the ketone is the simplest in that the ketone is soluble in a reasonable quantity of water, and extraction is therefore unnecessary. This is especially interesting because it was impossible to prepare a completely neutral sample of the ketone by distillation from calcium oxide.

Apparently the precision of the method can be improved to any extent desired by increasing the size of the sample and amount of additive, as the acetic acid is very easily separated.

The method should be capable of application to any series of homologous compounds for which a series of inert additives can be found. It would have to be established that relative volatilities were not too much altered.

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RECEIVED August 25, 1947.

Determination of Alcohol-Insoluble Solids and Sugar Contents of Vegetables

JAMES C. MOYER AND KENNETH C. HOLGATE
New York State Agricultural Experiment Station, Geneva, N. Y.

With the aid of a Waring Blendor and 85% alcohol, sugars are extracted from frozen vegetables. The alcohol-insoluble solids content is determined by filtering the macerate and drying the residue. The alcohol in a small aliquot of the filtrate is evaporated and an aqueous solution of the residue is clarified by Somogyi's barium hydroxide–zinc sulfate procedure. For estimation of the total sugar content, an aliquot of the clarified extract is inverted with invertase. The sugar content before and after inversion is determined by Nelson's colorimetric method, using Somogyi's new copper reagent.

DURING a study of the losses of soluble constituents in the processing of vegetables, the need arose for a rapid method of determining the reducing and total sugar contents. The desirability of combining the sugar determinations with that of alcohol-insoluble solids also became apparent; there were indications that the calculation of the constituent losses based on the dry weight or total solids content would be erroneous because of the altered composition of the solid material after contact with water. To avoid this error, Lee (*4*) suggested that the alcohol-insoluble solids content of a vegetable may be used as a reliable basis for the computation of vitamin losses during blanching. This investigation was undertaken to devise a procedure whereby the reducing and total sugar contents and alcohol-insoluble solids determinations might be combined and appreciably shortened.

The formulation of such a procedure requires that consideration be given to the rapid and thorough extraction of the sugars from the vegetable, the removal of pigments and interfering compounds which would normally react with the chromogen of a colorimetric procedure, the hydrolysis of sucrose for estimation as reducing sugars, and the formation of a reproducible and stable color which is proportional in intensity to the sugars present over a fairly wide range.

EXTRACTION

Sugars are usually extracted from plant tissues with ethyl alcohol, which, if present in sufficient concentration, precipitates polysaccharides and proteinaceous materials and inhibits enzymatic action. Thorough alcoholic extraction is greatly aided by a maceration of the tissues in a Waring Blendor (*2, 5*). The following extraction procedure employs the Waring Blendor and a resulting alcohol concentration of at least 75%, depending on the moisture content of the vegetable.

As a means of preserving the samples until a convenient time for analysis, the vegetables are frozen and stored at −23° C. This also affords an opportunity for obtaining a representative sample for analysis by grinding one or more pounds of the frozen material twice in a food chopper at −23° C. Twenty grams of the finely ground vegetable are weighed into a 100-ml. beaker in the cold room. At room temperature the ground sample is washed into a Blendor cup (500-ml. capacity, having a rubber gasket under the screw top) with 150 ml. of 85% ethyl alcohol (specific gravity 0.850). After 5 minutes of maceration, the contents of the Blendor cup are washed into a 600-ml. beaker with 85% ethyl alcohol from a wash bottle. The solids are allowed to settle and the contents of the beaker are poured onto a weighed 5.5-cm. No. 40 Whatman filter paper in a Büchner funnel inserted through a two-holed rubber stopper placed in the mouth of a 500-ml. Kohlrausch sugar flask. Where heavy-walled or Pyrex Kohlrausch flasks are not available, a 500-ml. Pyrex volumetric flask can be adapted by sealing onto the neck a piece of tubing, 37 mm. in outside diameter and 6 cm. long. The use of a Kohlrausch flask and the danger of possible collapse of the flask by the vacuum can be avoided by collecting the filtrate in a 500-ml. volumetric flask under the high-form glass cover of a Fisher Filtrator.

Suction is applied to the interior of the Kohlrausch flask through a small piece of glass tubing bent at right angles and inserted into the other hole of the rubber stopper. By decanting off most of the alcoholic solution before adding the solids to the funnel, the filtration is greatly accelerated. The beaker is then rinsed out with more 85% ethyl alcohol which is poured into the Büchner funnel. The residue is washed with alcohol three or four times. After each washing the residue is allowed to become partially dry but care must be taken that the precipitate is not too thoroughly dried or the filter paper will pull away from the sides of the funnel and the next addition of alcohol will wash the solids into the flask. When the volume of the filtrate approaches the 500-ml. mark, the residue is allowed to dry and carefully removed to a weighing dish for complete drying at 95° C. overnight. The weight of the dried residue represents the alcohol-insoluble solids content of the sample. The volume of the filtrate is then made up to the 500-ml. mark with the 85% alcohol and the contents of the flask are thoroughly mixed.

The completeness of the extraction was tested by further extracting several residues with alcohol and no sugar was found. The precision of this alcohol-insoluble solids method was determined on ten replicate samples of frozen lima beans and an average value of 29.37% was obtained with a standard deviation of ±0.16 for a single determination.

CLARIFICATION

An alcoholic extract of vegetable tissues usually contains nonsugar reducing substances and pigments which would interfere in a colorimetric procedure. These coloring materials can usually be removed by adsorbents. In preliminary experiments with such decolorizing agents as Nuchar XXX, Nuchar WA, Darco G60, Norite A, activated charcoal, Hyflo-Supercel, and Celite in the form of filter beds on Hirsch funnels, it was hoped that the green and yellow pigments would be adsorbed and the alcohol in the filtrate would evaporate rapidly in the vacuum. Under these conditions only Norite A and Nuchar XXX completely removed the pigments, and some of the filtrate was lost by bumping of the alcohol during evaporation. Subsequent recovery experiments indicated that the Norite A and Nuchar XXX had to be washed with unduly large amounts of water if all the reducing sugar was to be reclaimed.

Because of the difficulties in the removal of pigments from an alcoholic solution by adsorption and the realization that the nonsugar reducing materials might be removed more conclusively by coprecipitation, the use of lead and barium salts was studied. As representatives of nonsugar reducing materials present in vegetable extracts, ascorbic acid and glutathione solutions were treated with neutral lead acetate and potassium oxalate. The glutathione was removed completely by lead precipitation, whereas the ascorbic acid remained untouched. As many of the barium salts of organic acids are insoluble, the barium-zinc procedure of Somogyi (*9*) was tested for its effectiveness in removing ascorbic acid and glutathione. As in the case of lead precipitation, the glutathione was quantitatively removed while approximately 60% of the ascorbic acid was eliminated. Increasing the concentration of barium hydroxide or prolonging the time the ascorbic acid remained in contact with the barium did not further appreciably the reduction in ascorbic acid content. Ascorbic acid and glutathione at concentrations likely to be encountered in vegetables—i.e., 50 and 10 mg., respectively, per 100 grams—possessed only 60 and 10% of the reducing power of corresponding weights of glucose when measured by the colorimetric procedure described herein. The resulting interference from ascorbic acid, then, is of limited importance with those vegetables where the reducing sugar content is low, and is negligible in the measurement of total sugar content.

The authors have obtained reproducible results and experienced good reducing sugar recoveries with the following adaptation of Somogyi's procedure.

Reagents. Barium hydroxide (56 grams) is mixed with 2 liters of hot, boiled distilled water and the mixture is then filtered into a storage bottle through a small Büchner funnel. The stopper in the bottle carries a soda-lime tube and a syphon tube to a 10-ml. buret having a three-way stopcock.

Zinc sulfate heptahydrate (100 grams) is dissolved in 2 liters of distilled water. The final concentration of zinc sulfate is adjusted so that a 10-ml. aliquot mixed with 50 ml. of water requires 9.5 ml. of barium hydroxide, added dropwise, to give a faint pink end point with phenolphthalein that is stable for one minute. Somogyi states that the zinc sulfate solution neutralizes the barium hydroxide, volume for volume, but the authors have found that this degree of neutralization leaves zinc in the filtrate which results in subsequent erratic colorimetric readings.

Procedure. Ten milliliters of an alcoholic extract are pipetted into a 100-ml. beaker and the contents evaporated to near dryness on a steam bath. The walls of the beaker are washed down with approximately 5 ml. of water and 2 ml. of barium hydroxide solution are added, followed by 2 ml. of zinc sulfate solution with constant agitation during the addition of each reagent. The contents of the beaker are washed into a funnel 50 mm. in diameter and the filtrate is collected in a graduate test tube. The precipitate is washed with a fine stream of water until a filtrate of 35-ml volume is attained. For the determination of the reducing sugar content, a 2-ml. aliquot of this filtrate is used in the colorimetric procedure. When determining the total sugar content, a 5-ml. aliquot of the filtrate is used for inversion.

INVERSION FOR TOTAL SUGAR CONTENT

Of the two methods commonly used to hydrolyze sucrose, the authors have found enzymatic action better suited for use with the colorimetric procedure. With acid hydrolysis the concentration of acid necessary for inversion required very careful neutralization and did not lend itself to routine analyses.

Reagents. To prepare the sodium acetate buffer, 13.6 grams of sodium acetate trihydrate are dissolved in distilled water, 8 ml. of glacial acetic acid are added, and the mixture is diluted to 500 ml.

For the invertase solution, 200 mg. of Wallerstein Laboratories Blue Label invertase scales are dissolved in 100 ml. of distilled water and the solution is kept in the ice box under a layer of toluene. This solution contains an excess of invertase activity for the amounts of sugar encountered in the analysis. Information supplied by the manufacturer indicates that the solution will have a k value in the neighborhood of 0.02, and can be tested by the procedure (*1*).

Procedure. A 5-ml. aliquot of the clarified extract is pipetted into a graduated test tube. Then 2 drops of the sodium acetate buffer and 5 drops of the invertase solution are added to the test tube before incubation overnight at 35° C. The contents of the test tube are diluted to 35 ml. with water before a 2-ml. aliquot is used for color development. The overnight incubation period has been used for convenience. A much shorter time at a higher temperature would undoubtedly accomplish the same degree of inversion.

COLORIMETRIC PROCEDURE

By evaporating only a small aliquot (10 ml.) of the alcoholic extract, the time required to complete a series of analysis is appreciably shortened, though it necessitates the use of a sensitive colorimetric method to determine the limited quantity of sugar present. Initially, the colorimetric procedure of Folin (*3*) was used but although the color intensity was proportional to the glucose present, the color faded very rapidly. In search of a stable color, the method of Polis and Sortwell (*7*) was tried, and it was found that although the color intensity remained unchanged over a long period, there was not a linear relationship between the color intensity and the range of glucose concentrations encountered in the analyses of vegetables. The colorimetric method of Nelson (*6*) in combination with Somogyi's new copper reagent (*8*) has been found satisfactory, as it can be used over a sufficiently wide range of sugar contents (0.01 to 0.30 mg.) and the color is reasonably stable. Nelson preferred to read the color density at 500 mu, but it has been the authors' experience that variations in the amount of excess arsenomolybdate reagent influences the readings at this wave length and that at 600 mu the yellow arsenomolybdate exerts little effect on the readings. The color stability is apparently greater at 500 mu than at 600 mu, although at the latter wave length the readings are constant over a period of 2 hours, which is sufficiently long to permit the examination of numerous samples.

This colorimetric procedure is sensitive and apparently minor variations in technique may result in variable readings, which, when multiplied by the high dilution factor, greatly lessen the precision of the results. For this reason, the combined colorimetric procedures of Nelson and Somogyi are described in detail.

Reagents. For Somogyi's copper solution 56 grams of anhydrous disodium phosphate are slowly added with stirring to 1400 ml. of distilled water. Then, with continued stirring, 80 grams of Rochelle salts are added, followed by the slow addition of 200 ml. of 1 N sodium hydroxide. A cupric sulfate solution is prepared by dissolving 16 grams in 160 ml. of distilled water, and this solution is added to the phosphate-tartrate mixture. Finally, 360 grams of anhydrous sodium sulfate are slowly added with stirring. The mixture is diluted with water to the 2000-ml. mark and allowed to stand for 2 days before filtration.

Nelson's Arsenomolybdate Solution. One hundred grams of ammonium molybdate are dissolved with stirring in 1800 ml. of distilled water. Then 84 ml. of concentrated sulfuric acid are slowly added with continued agitation and finally 12 grams of sodium arsenate heptahydrate are added. When the arsenate is dissolved, the solution is diluted to 2000 ml. with water and stored at 37° C. for 48 hours. At the end of this period, the solution is filtered and stored in a brown bottle.

Procedure. A 2-ml. aliquot of the clarified extract (for reducing sugars) or of the inverted extract (for total sugars) is placed in a Folin-Wu blood sugar tube with an Ostwald pipet. Then, 2 ml. of Somogyi's copper reagent are added from a 25-ml. buret and the tube is placed in boiling water for 20 minutes. (When a number of samples are being analyzed, sufficient space must be provided around each tube to permit adequate circulation of the boiling water. Wire test tube supports serve this purpose very well.) The sugar tubes are then cooled in water at room temperature and 2 ml. of Nelson's arsenomolybdate soution are added from a 25-ml. buret. Because the copper reagent has a high specific gravity, the solutions are most effectively mixed by moderate vertical agitation with a small knob on the end of a glass rod. This rod is washed and the contents of the sugar tube are diluted to the 25-ml. mark with water before shaking to ensure thorough mixing. It has been found advantageous to allow the tubes to stand for 15 minutes to permit maximum color development before the solutions are read at 600 mu in a photoelectric colorimeter or spectrophotometer which has been adjusted to give 100% transmittance with distilled water. With each series of unknown samples, tubes containing 2 ml. of water for a blank and 2 ml. of standard solutions having 0.10 and 0.20 mg. of glucose are treated in a similar manner to obtain a standard reference graph.

The precision of the analyses was determined with ten replicate samples of ground frozen lima beans where the reducing sugar content was found to be 0.068% and the standard deviation of a single determination was ±0.003. After inversion of aliquots of the same extracts, the total sugar content was 2.43% with ±0.07 as the standard deviation of a single determination.

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RECEIVED June 6, 1947. Journal Paper 715, New York State Agricultural Experiment Station, Geneva, N. Y.

Simplified Absolute and Differential Manometer

ROGER GILMONT
The Emil Greiner Company, New York, N. Y.

A new manometer for either absolute or differential measurements based upon a combination of new and previously described design elements is presented. The closed end of the manometer is fabricated with a U-type loop and cut-off stopcock. This construction eliminates the difficult process of filling a closed-end manometer and still ensures a perfect seal by virtue of the manometric fluid trapped in the loop. It also permits rapid changeover from absolute to differential measurements. The ratio of the diameter of the manometer tube to the diameter of the reservoir is chosen so that a single reading on the manometer tube gives a direct pressure reading in millimeters of mercury at 0° C. The theoretical basis of this design is discussed as well as the tolerances premitted in the above ratio to maintain a given precision; thus, high precision is obtainable with reasonably large tolerances. A plastic scale having a temperature coefficient of linear expansion of the same order of magnitude as the volumetric coefficient for mercury eliminates errors due to changes in ambient temperature. The permissible tolerances which give a desired precision are theoretically derived for the choice of plastic. Other factors, such as surface tension, are considered in order to establish tolerances in dimensions which preserve high precision. The manometer is easily filled and very convenient to use, especially since a single reading gives, with high precision, the true corrected pressure, which normally has to be obtained by suitable correction calculations.

THE need for an absolute manometer which can be easily filled and cleaned has presented a constant problem for the laboratory scientist and technician. Several schemes have been proposed for surmounting this problem, such as special devices and techniques for filling closed-end manometers (*1, 2, 3, 7, 10, 12*). To simplify filling and cleaning, some experimenters have used a stopcock to make the closed end (*4, 11*), but this unfortunately leads to uncertain readings because the stopcock cannot be relied upon to be absolutely leakproof. The use of a loop to cut off the closed end is the basis of the Zimmerli gage (*13*), and more recently of a gage proposed by Robertson (*9*).

If the idea of a loop to cut off the closed end is combined with a stopcock to close off the loop, a simplified type of absolute manometer is possible; the loop forms a mercury seal which prevents any gas from entering the closed end even if the stopcock should fail to be leakproof. Moreover, the presence of the stopcock makes it possible to use the manometer for differential pressure measurements, and also permits any desired height of gage to be conveniently constructed. In Figure 1, a working drawing is shown for such a gage designed to read a maximum of about 200 mm.

In the reading of the ordinary U-type manometer, two columns are observed, necessitating subtraction to obtain the pressure differential. Some investigators have employed a very large reservoir, so that its change in level could be neglected, and thereby have eliminated a double reading and subtraction. For precise work, as in barometers, the scale is adjusted to read from the level in the reservoir, but this again introduces an additional operation, which sometimes leads to more trouble than it is worth. Thus, for precise work, unless an impractically large reservoir is used, the change in level cannot be neglected. Nevertheless, it is still possible to obtain precise results with a single reading by making use of a specially contracted scale which accounts for the change in level of the reservoir. The tolerances required in the bore of the tubing employed are not too critical to obtain precise results, if a reasonably large reservoir is used. In view of the fact that precise pressure readings with a mercurial manometer must be corrected to read a height of mercury

at 0° C., it is possible to design a manometer using a regular scale and a suitably sized reservoir (the dimensions of which are determined below) which by means of a single reading gives the pressure reading in millimeters at 0° C., directly. Furthermore, if a plastic scale having a coefficient of thermal expansion of the same order of magnitude as mercury is used, the single reading can be automatically corrected for changes in the ambient temperature. The design in Figure 1 is for a manometer having these features. The theoretical basis for its design is described in the following section.

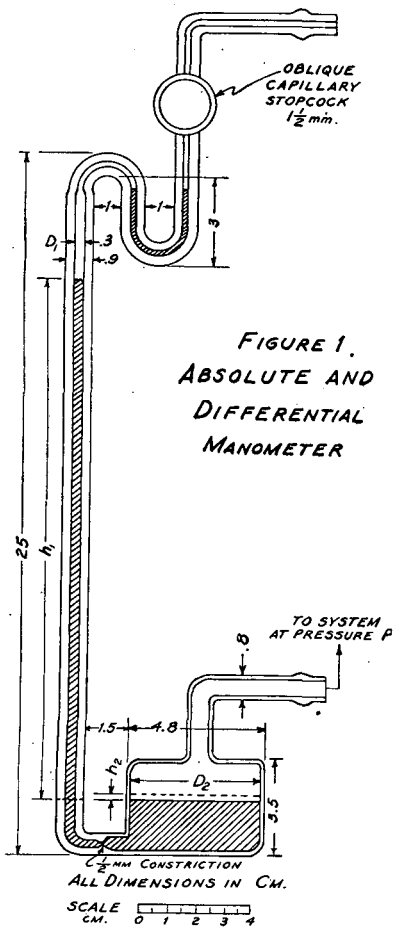

FIGURE 1. ABSOLUTE AND DIFFERENTIAL MANOMETER

THEORY

The basis for choosing the size of the manometer tube and reservoir is the fact that the contraction in the height of the mercury column due to the change in level of the reservoir can be exactly neutralized by the expansion of the mercury column due to the increase in temperature from 0° C. to the ambient room temperature. Thus, using the symbols of Figure 1:

Equality of volume displacement yields

$$\frac{\pi D_1^2 h_1}{4} = \frac{\pi D_2^2 h_2}{4}$$

and

$$h_2 = h_1 \left(\frac{D_1}{D_2}\right)^2 = h_1 R^2 \qquad (1)$$

The total height of mercury column is given by

$$h = h_1 + h_2$$

and substituting h_2 from Equation 1,

$$h_1 = \frac{h}{(1 + R^2)} \qquad (2)$$

The height of the mercury column reduced to 0° C., which is the true pressure by definition, is expressed by

$$h_0 = \frac{h}{(1 + \beta t)} \qquad (3)$$

where h_1 = height of mercury column in manometer tube above zero pressure differential position
h_2 = depth of mercury column in reservoir below zero pressure differential position
h = height of mercury column in manometer tube above level in reservoir at temperature t° C. (corrected for capillarity)
h_0 = above height reduced to mercury at 0° C. exerting the same pressure
$R = \frac{D_1}{D_2}$ = ratio of diameters of manometer tube to reservoir
β = coefficient of volumetric thermal expansion of mercury for the interval 0° to t° C.

The volumetric expansion coefficient of mercury is used, since this correctly accounts for the change in height of the column due to temperature as shown below. (The thermal expansion of the glass is not involved in so far as the change in length of the mercury column with temperature is concerned, since this is affected by density changes alone. The change in the diameters of the glass tubes with temperature would be proportional and not affect the value of R.)

$$h = Pv$$

where h = height of mercury of specific volume, v, exerting a pressure, P, at its base

For constant P,

$$\frac{dh}{dt} = P\frac{dv}{dt} = \frac{h}{v}\frac{dv}{dt}$$

Therefore

$$\frac{1}{h} \times \frac{dh}{dt} = \frac{1}{v} \times \frac{dv}{dt} = \beta \qquad (4)$$

Equation 4 demonstrates that the fractional change in mercury height of column with temperature is measured by the volumetric coefficient of expansion, which is the fractional change of specific volume with temperature.

The condition required to make $h_1 = h_0$ is obtained by combining Equations 2 and 3 as follows:

$$(1 + \beta t) = (1 + R^2).$$

or

$$R^2 = \beta t \qquad (5)$$

Basing the calculations on a room temperature of 25° C. and using an average value of 18.2×10^{-5} per ° C. for the volumetric coefficient of thermal expansion of mercury, taken from the Handbook of Chemistry and Physics (*5*), one obtains a value of:

$$R = 0.0675 \qquad (6)$$

If a 3-mm. bore capillary tubing is used for the manometer tube, a reasonably sized reservoir 44.4 mm. in inside diameter is obtained from Equation 6, which corresponds to a standard wall Pyrex tubing 48 mm. in outside diameter. The permissible variation in R to obtain a given desired precision may be determined as follows:

Since an ordinary scale can be read with the naked eye to no better than about 0.1 mm., a precision of 0.1 mm. in 200 mm. will be assumed—i.e., $\Delta h_1 = 0.1$ mm. and $h_1 = 200$ mm.

From Equation 2, it follows by differentiation that for constant h:

$$\frac{\Delta R}{R} = -\frac{\Delta h_1}{2h_1}\left(1 + \frac{1}{R^2}\right) =$$

$$-\frac{0.100}{400} \times 221 \times 100 = -5.5\% \qquad (7)$$

Thus, a deviation of as much as 5.5% can be made in the selection of the ratio of diameters and still maintain a precision of 0.1 mm. in 200 mm. Experience has shown that these tubes may be selected within a precision of 1% of their respective sizes, even for sizes as small as 3-mm. capillaries.

When tubing of small diameter is used for manometric measurements, the effect of surface tension must be considered. Although the capillary depression for a tubing 3 mm. in diameter may be as high as 4 mm., this is accounted for in setting the zero position of the scale at zero pressure differential. However, the variation in this correction must be considered. For the 3-mm. capillary, the permissible variation in its diameter, to maintain a precision of 0.1 mm., may be calculated as follows from the surface tension and density of mercury:

Table I. Data on Plastics

Property	Vinyl Chloride Flexible, Unfilled	Vinylidene Chloride	Polyvinal Butyral, Rigid	Styrene Elastomer	Poly-ethylene	Cellulose Compound (Acetate)	Phenol-Formaldehyde Cast, No Filler
Thermal Expansion, 10^{-5}/° C.	7–25	19	8–22	18–23	18	8–16	5–15
Modulus, lb. per sq. inch, × 10^5	3.5–4.1 (rigid)	0.4–0.8	3.5–4.0	0.4	0.15	1–3.5	4
Water absorption in 24 hours, 1/8 inch, %	0.05–0.15 (rigid)	<0.1	1.0–3.0	0.2–0.3	0.01	2–4	0.02–2.0
Commercial	Chemaco Corp., Berkeley Heights, N. J. Geon, B. F. Goodrich Chemical Co., Cleveland, Ohio Tygon, U. S. Stoneware Co., Akron, Ohio Vinylite, Bakelite Corp., New York, N. Y.	Geon, Goodrich Saran, Dow Chemical Co., Midland, Mich.	Butacite, E. I. du Pont de Nemours & Co., Arlington, N. J. Butvar, Shawinigan Products Corp., New York, N. Y. Monsanto Chemical Co., Springfield, Mass. Saflex, Monsanto Vinylite, Bakelite	Styraloy, Dow	Bakelite Du Pont	Bakelite Chemaco Hercules Powder Co., Wilmington, Del. Monsanto Celanese Corp. of America, New York, N. Y. Nixon Nitration Works, Nixon, N. J. Du Pont Tennessee Eastman Corp., Kingsport, Tenn.	Bakelite Catalin Corp., New York, N. Y. Gemstone, A. Knoedler Co., Lancaster, Pa. Marblette Corp., Long Island City, N. Y.

The depression in level due to capillarity is given by

$$d = \frac{4\gamma}{\rho D} \qquad (8)$$

where d = depression in level
γ = surface tension
ρ = density
D = diameter of tube

Equation 8 assumes a maximum contact angle of 180°. LeRoy (*6*) has pointed out that using the correct value for the contact angle increases the tolerance to approximately 2.5%.

The variation of d with respect to D is obtained by differentiating Equation 8:

$$\Delta d = -\frac{4\gamma}{\rho} \times \frac{\Delta D}{D^2} \qquad (9)$$

which rearranges to give:

$$\frac{\Delta D}{D} = -\frac{\Delta d}{4\gamma} \times \rho D \qquad (10)$$

where d = 0.01 cm.
D = 0.3 cm.
ρ = 13.6 grams per cc.
γ = 0.50 gram per cm.

$$\text{Then } \frac{\Delta D}{D} = -\frac{0.01\ (0.3)\ 13.6}{4\ (0.50)} \times 100 = -2\% \qquad (11)$$

Thus, the effect of variations in capillary depression due to variation in diameter of tubing can be made negligible under the above prescribed conditions, providing the variation in the diameter of the manometer tubing does not exceed 2%. This requirement is easily met, since standard capillary tubing can be selected to give less than 1% variation.

The final consideration is the permissible values of the temperature coefficient of linear expansion for the plastic scale to maintain the precision of 0.1 mm. in 200 mm. The change in length of mercury column as read by the plastic scale due to temperature fluctuation may be expressed as follows:

$$\Delta h_1 = h_1 (\beta - \alpha)\ \Delta t \qquad (12)$$

where α = linear coefficient of thermal expansion of the plastic
Δt = temperature change

Assuming a maximum Δt of 10° C., the permissible difference between the two expansion coefficients is

$$\beta - \alpha = \frac{\Delta h_1}{h_1 \Delta t} = \frac{0.1}{200 \times 10} = 5 \times 10^{-5} \text{ per ° C.} \qquad (13)$$

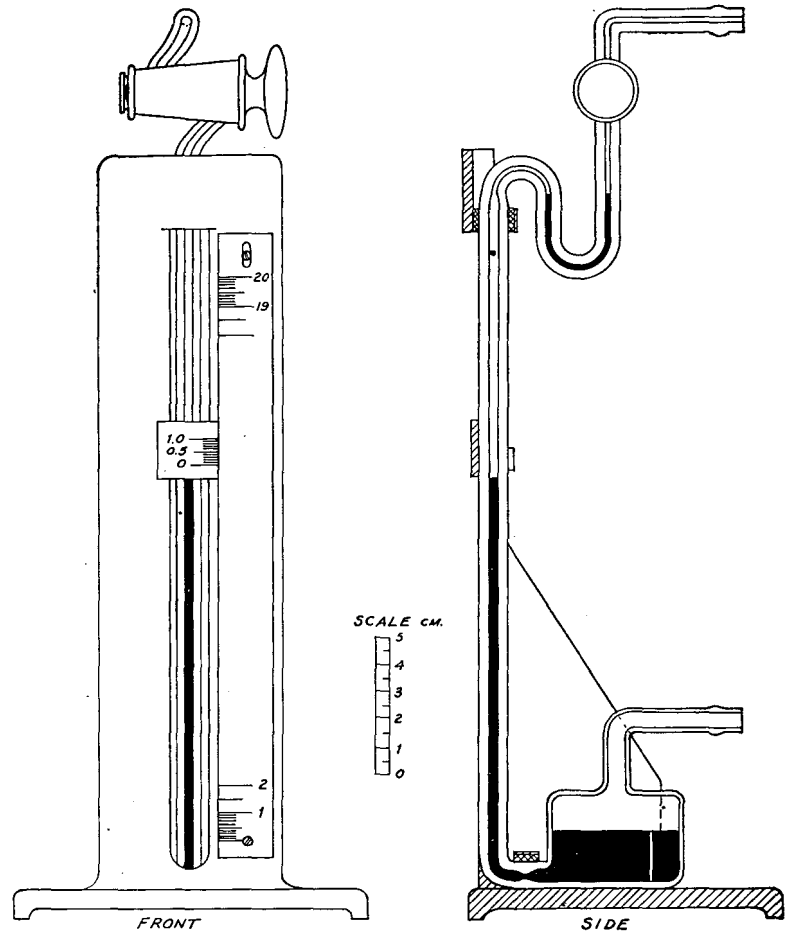

Figure 2. Manometer Assembly

For a temperature change of 5° C., $\beta - \alpha = 10 \times 10^{-5}$/°C. — that is, the permissible difference is inversely proportional to the temperature change. For most room conditions a variation of ±5° C. is sufficient, although variations of ±10° C. are encountered in extreme cases. Many plastics are available to permit variations of at least ±10° C. in room temperature and maintain a precision of 0.1 mm. in 200 mm. (Table I). These data were abstracted from the Modern Plastics Encyclopedia (*8*). Vinylite scales, printed and laminated, were found to be suitable for a maximum change of ±5° C. in ambient temperature and still maintain a precision of 0.1 mm. in 200 mm., or a maximum of ±10° C. if the tolerance in precision were increased to 0.2 mm. in 200 mm.

OPERATION

The use of the manometer described is almost self-evident from its construction. In Figure 2 is shown a possible construction of a finished instrument mounted on a stand and ready for use in the laboratory.

After the glassware has been scrupulously cleaned and dried, which is a simple matter since the tube is open at both ends, and the stopcock has been carefully greased with a low vapor pressure lubricant, clean dry mercury (preferably reagent grade) is poured into the reservoir, until the level in the manometer tube corresponds to a zero reading on the scale (with the use of a vernier, the mercury level should just reach the lower edge of the vernier when set to read zero). Differential pressure readings can now be made with the stopcock open and the higher pressure connected to the reservoir.

To use this manometer as an absolute gage, mercury must be drawn up the manometer tube into the trap and the stopcock sealed off. This may be done with vacuum, pressure, or by simply tilting the stand. The recommended procedure is to use vacuum connected to both outlets simultaneously with a three-way cut-off stopcock in the connection to the reservoir. After the system is pumped out, the cut-off stopcock is carefully closed and turned to the air, permitting mercury to rise gradually in the manometer tube. When the mercury level reaches the stopcock above the trap, it is closed off. The gage is now ready for absolute measurements on connecting the system to the reservoir outlet and reading the resulting mercury level in the manometer tube on the scale. The amount of mercury held up in the trap has negligible effect on the zero setting of the instrument, as can be seen from the calculation below, in which the length, L, of the mercury column in the trap is taken as approximately 70 mm.:

$$\Delta h_1 = L\left(\frac{D_1}{D_2}\right)^2 = 70\left(\frac{1.5}{44.4}\right)^2 = 0.08 \text{ mm.}$$

CONCLUSION

A simplified absolute and differential manometer has been found both accurate and more convenient than any of the ordinary types used in the laboratory. Besides the smaller gage, a larger one reading about 800 mm. has been used with comparable accuracy and ease of manipulation, cleaning, and observation.

ACKNOWLEDGMENT

Thanks are due R. E. Bader of the Emil Greiner Company for his kind assistance in making the literature survey, and to the Emil Greiner Company for permission to publish this information.

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RECEIVED May 24, 1947. Presented before the Division of Physical and Inorganic Chemistry at the 113th Meeting of the AMERICAN CHEMICAL SOCIETY, Chicago, Ill.

Determination of Small Amounts of Carbon in Steel

Evaluation of Low-Pressure Combustion Apparatus

JOHN J. NAUGHTON[1] AND HERBERT H. UHLIG[2]

General Electric Co., Schenectady, N. Y.

The discrepancy between carbon values for low-carbon steels as determined by the low-pressure combustion method and the standard combustion method has been investigated and possible causes of the differences have been checked. All results show the reliability of the low-pressure combustion method, especially on low-carbon steels, where accuracy to 0.001% carbon is sought.

THE low-pressure combustion method has been investigated by several laboratories and has proved satisfactory for the determination of carbon in low-carbon iron, even for use on a routine basis. The limitations of low-carbon determinations by standard methods using an absorption train were pointed out by Yensen (*6*), who proposed measuring the pressure of the carbon dioxide resulting from carbon combustion instead of weighing it, a method which was extended by Ziegler (*7*). Modification of their method culminated in the low-pressure method of carbon analysis of Wooten and Guldner (*5*), reported on by Gurry and Trigg (*1*), and used with further modification by Murray and Ashley (*2*) for routine determinations.

An extension of this method was reported by Stanley and Yensen (*4*). Following the form suggested by Murray and Niedrach (*3*), an apparatus has been set up for investigation in this laboratory.

[1] Present address, Department of Chemistry, University of Hawaii, Honolulu, T. H.

[2] Present address, Department of Metallurgy, Massachusetts Institute of Technology, Cambridge, Mass.

These investigators all reported low values on determinations by this method run on Bureau of Standards sample 55A. This was checked by the authors, and similar low results were also obtained with Bureau of Standards samples 55B and 8G.

This paper reports the investigation of two aspects of this method: lack of agreement on carbon content of steels with values reported by the standard methods of carbon analysis (Bureau of Standards samples), and source of blank.

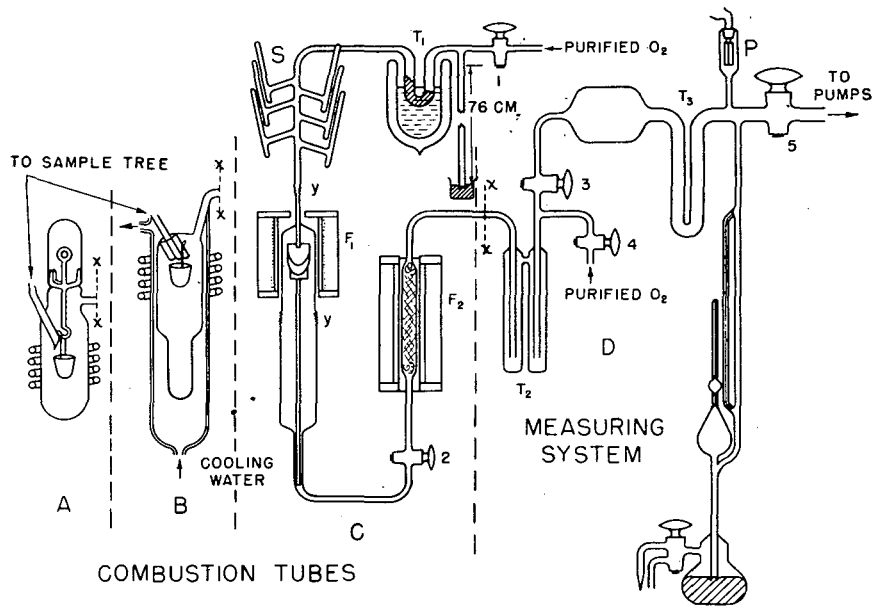

Figure 1. Types of Combustion Chambers and Measuring System

APPARATUS

The apparatus is essentially that described by Murray and Niedrach (*3*). The sample is burned in an all-glass apparatus, the carbon dioxide is collected by condensation in a low-temperature trap (liquid nitrogen–cooled), and, after the excess oxygen has been pumped out the carbon dioxide is determined by a pressure measurement in a calibrated volume. The weight of sample used depends on the carbon content; thus, for low carbon values in this apparatus, the weight used is 0.5 gram, and for higher carbon contents it is proportionally less.

The sample is burned in oxygen at 15 to 20 cm. of mercury pressure in a beryllia or alumina crucible, which is contained in a platinum crucible heated by high frequency. The equipment is shown in Figure 1 (furnace *A* and measuring system *D*). After combustion is completed (5 minutes), the excess oxygen containing the gaseous products of combustion is pumped out through the water trap, T_2, and the carbon dioxide trap, T_3.

When the pressure in the system has been pumped to a value of 10^{-4} mm. or better, as indicated qualitatively by the thermocouple gage, *P*, stopcocks 3 and 5 are closed, the carbon dioxide trap is allowed to warm up, and the pressure of the carbon dioxide released in the known volume of the system is measured by means of the McLeod gage. A simple calculation converts this to per cent carbon in the original steel sample. Carbon dioxide collected in the U-type trap at liquid nitrogen temperatures can be quickly released by immersion of the trap in water. Such traps have been shown by experiment to remove carbon dioxide completely from an oxygen stream under conditions of the test and their use greatly speeds up the determination.

Water vapor is removed in trap T_2 by the use of a frozen acetone bath (obtained by pouring liquid nitrogen into acetone–solid carbon dioxide) which cools the trap to −95° C. At this temperature, water vapor is removed efficiently, as the corresponding vapor pressure of ice is only 2.7×10^{-5} mm. of mercury.

CARBON LOSS

That the low-pressure combustion method gives uniformly lower results than standard combustion-weighing technique when applied to low-carbon samples, has been checked by many experimenters. In the authors' work an effort was made to trace systematically any nonevident source of error in the low-pressure method.

The lower results might be due to the existence of some oxidized carbon as noncondensable carbon monoxide, incomplete oxidation of carbon in the sample, or inadvertent removal of some carbon dioxide en route to the measuring device.

Existence of Carbon Monoxide. Introduction of a glowing platinum filament or of copper oxide (400° C.) into the path of the combustion gases obtained in the normal manner failed to show any evidence that carbon monoxide was present.

Incomplete Oxidation of Carbon. The oxide from burned samples was ground and carbon redetermined. As much as 0.01% carbon for a 0.5-gram sample was always found. The source, however, was found to be not incompletely oxidized carbon, but carbon dioxide absorbed from the air by both the oxide and the ceramic crucible. Indeed, any ceramic crucible or boat alone, ordinarily used for carbon determinations, was found greatly to increase its blank on exposure to the atmosphere—for example, absorbed carbon dioxide was equivalent to an average of 0.003% carbon based on 0.5-gram sample weight. This extraneous source of carbon on exposure of ceramic materials to the atmosphere could be the source of the higher results in the standard combustion method, as has been suggested by Wooten and Guldner (*5*). [Stanley and Yensen (*4*) successfully used a nickel boat previously decarburized in wet hydrogen at 1200° for 50 hours.]

As an alternative test, the burned residue from a sample was reduced by palladium-diffused hydrogen at 700° C. in situ for 10 minutes. Examination of another specimen treated in like manner showed it to be completely reduced. The sample, together with the hydrogen used in the reduction, was then burned again. No additional carbon dioxide beyond the normal blank value was obtained from the second burning.

Samples have also been burned with and without tin as a flux, but without any appreciable difference in the results. If carbon is held in the iron oxide residue, one would expect some difference of available carbon dioxide in this case because of the different oxide environment.

The conclusion seems to be in order, therefore, that no carbon is left in the residue which results from the burning of low-carbon steels in oxygen. Any carbon found probably results from adsorption of carbon dioxide from the atmosphere on oxide and ceramic.

Loss of Carbon Dioxide. The possibility of adsorption of the carbon dioxide resulting from the combustion was examined. This could happen in the ceramic crucible and on the walls of the furnace.

Crucibles made of many different materials with many different treatments and of many sizes and shapes were used. The blank varied with crucible material but the determined values of the carbon content for samples were all consistent. Increased precision resulted from better crucibles. The best material was fused alumina or beryllia. No result indicated that the hot crucibles adsorbed sufficient carbon dioxide to account for the dif-

ference in determined values between the low-pressure and the standard methods.

After several combustions an appreciable film of platinum (confirmed by x-ray) collected on the glass walls of the combustion chamber. This film probably results from the formation and possible later decomposition of an oxide of platinum. The suspicion was entertained that this platinum film, acting as a sort of "getter," might adsorb some of the carbon dioxide present in the system during a run or during an initial blank run. It was also conceivable that the slow desorption of some of this carbon dioxide would account for the blank.

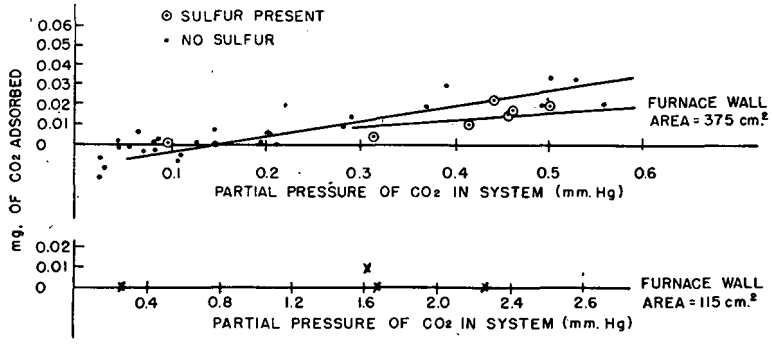

Figure 2. Effect of Pressure of Carbon Dioxide in Measuring System and Area of Furnace Wall on Carbon Dioxide Adsorbed

The suspicion of appreciable adsorption was confirmed by the observation that if the walls of the combustion chamber were strongly heated (400° to 500° C.) after such a platinum film had collected, considerable quantities of gas were driven off. Qualitatively, this desorption had the following characteristics:

No appreciable gas was obtained by heating the glass walls without the platinum coating.

The amount of gas driven off was roughly proportional to the number of runs made before heating the film.

Vapor pressure–temperature distillation curves run for the gas obtained from a heated platinum film that accumulated during actual runs showed that it consisted of carbon dioxide and sulfur dioxide, and some noncondensable gas which was not carbon monoxide (probably oxygen).

The amount of carbon dioxide desorbed seemed to vary inversely with the sulfur content of the sample.

The amount of carbon dioxide adsorbed (and desorbed on heating the film) depended on the sulfur content of samples run prior to the sample in question.

Greater desorption of carbon dioxide was found on heating the film in oxygen than in vacuum.

Adsorption of carbon dioxide could be appreciable for high pressures of carbon dioxide (from high-carbon samples) and in the absence of sulfur dioxide.

These facts lead one to postulate that carbon dioxide under these conditions is either physically adsorbed or chemisorbed, and that sulfur dioxide is similarly adsorbed and in preference to the carbon dioxide.

Better to evaluate the quantitative aspect of this process, a small known quantity of pure carbon dioxide was admitted to the system. Oxygen was also admitted and a run was carried out in the normal manner with the crucible heated and other factors the same as during an actual carbon determination. The carbon dioxide was measured after the run and compared with the amount introduced. The results, shown graphically in Figure 2, essentially substantiate the conclusions reached from the experiments on desorption. The adsorption of carbon dioxide varies with the furnace wall area, as would be expected.

While these experiments are significant in showing that some carbon can be lost under these conditions, the amount of carbon involved is insignificant in comparison with the total amount of carbon being determined, even for high-carbon steels where the samples of small weight increase the percentage error. In the range of carbon contents included by the authors' work the maximum carbon per specimen released as carbon dioxide is about 0.4 mg. Maximum adsorption for the corresponding carbon dioxide pressure (0.3 mm.) is 0.005 mg. of carbon (0.02 mg. of carbon dioxide), or about 1% loss, which is within the deviation for such samples. For low-carbon samples, where disagreement exists with the Bureau of Standards' results, the adsorption is still less (total carbon per specimen released as carbon dioxide = 0.050 mg., corresponding to 0.03 mm. of carbon dioxide, at which pressure adsorption is negligible). To bring about agreement with the Bureau of Standards' sample 55B, for example, we would have to account for the large loss of 0.012 mg. of carbon (0.04 mg. of carbon dioxide).

A series of experiments was also run using an oxygen flow method. The equipment is shown in Figure 1, *C* and *D*.

Oxygen at 1 atmosphere flowed over a beryllia crucible contained in a quartz combustion tube heated by a platinum-wound furnace. This eliminates the troubles resulting from the platinum film in contact with the carbon dioxide released from the sample. The samples could be dropped into the furnace for combustion without opening the system. The gases resulting from the combustion were pumped over a platinum catalyst to oxidize the sulfur dioxide and through a frozen acetone freeze-out trap to remove water vapor. Finally, the carbon dioxide was condensed in a liquid nitrogen trap and, after removal of the excess oxygen, was released into a known volume and its pressure was measured. Thus, the quantity of carbon could be determined, as in the low-pressure method.

The results are in agreement with the low-pressure method (Table I). A conventional horizontal furnace and boat arrangement gave comparable results if provision was made for filling the boat without exposure to the air. The procedure was clumsy and the vertical furnace was preferable.

All this leads to the conclusion that while the platinum film collected on the furnace walls does adsorb some carbon dioxide,

Table I. Carbon Values Obtained by Combustion at Low Pressure and at 1 Atmosphere Pressure

Sample	No. of Detns.	% Carbon, Std. Method	Condition of Run	Average Blank C, Mg.	Average Blank C, %	Average % Carbon	Standard Deviation, %
55A	15	0.014	Low pressure combustion	1.5×10^{-3}	0.0003, 0.5-g. sample	0.0108	0.0003
55A	20	0.014	Low pressure combustion - cooled walls	0.53×10^{-3}	0.00008, 0.5-g. sample	0.0108	0.0003
55A	11	0.014	Atmospheric pressure-O_2 flow	3.1×10^{-3}	0.0006, 0.5-g. sample	0.0105	0.0003
55B	10	0.012	Low pressure combustion	1.8×10^{-3}	0.0003, 0.5-g. sample	0.0095	0.0003
55B	4	0.012	Low pressure combustion-Sn flux	1.2×10^{-3}	.....	0.0098	0.0003
8G	14	0.069	Low pressure combustion	0.6×10^{-3}	.....	0.063_5	0.0011
14C	13	0.791	Low pressure combustion	0.7×10^{-3}	0.0015, 0.05-g. sample	0.78_5	0.008
Sucrose	2	42.1	Low pressure combustion	0.7×10^{-3}	0.025, 0.003-g. sample	42.0	0.1
Piano wire	7	0.88	Low pressure combustion	1.6×10^{-3}	0.003, 0.05-g. sample	0.88_1	0.0007

the amount is not sufficient to affect the results of low carbon determinations.

If all the sulfur dioxide was not removed by the platinum catalyst in the oxygen flow method outlined, higher values in agreement with those of the Bureau of Standards were obtained for 55A. This removal of sulfur dioxide was difficult, and depended on careful preparation of the catalyst and the presence of sufficient catalyst area. Gas was taken from a commercial combustion setup which is in everyday use for the determination of carbon in steels, at the point where pure carbon dioxide is supposed to exist. The gas was analyzed by running a vapor pressure–temperature curve, and 5% sulfur dioxide by volume was found (using sample 55A).

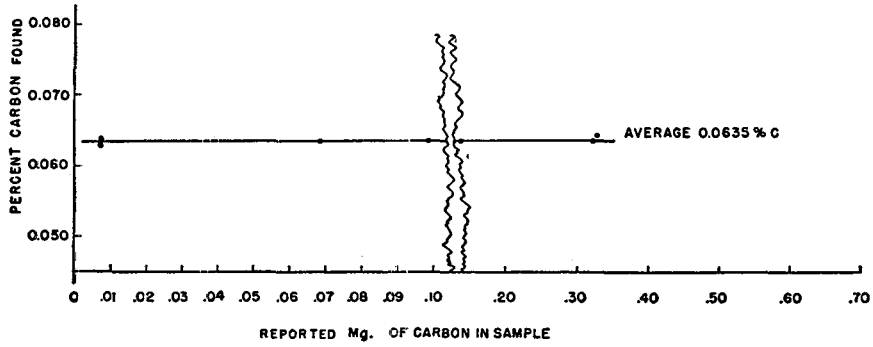

Figure 3. Variation of Per Cent Carbon Found with Size of Sample

Bureau of Standards steel 8G, 0.069% C

BLANK

The small amount of carbon dioxide adsorbed by the platinum film can probably account for the blank in the low-pressure equipment. This is indicated by the following results. Thorough degassing of the walls by heating in the presence of oxygen reduces the blank 3- to 10-fold. A special furnace tube was built (Figure 1, *B*) which was designed in such a way that all surfaces that would collect platinum could be water-cooled. The operation of this equipment was identical with that of the apparatus previously used. The purpose of the water cooling was to reduce desorption, and thus reduce the blank if the latter was due to desorbed carbon dioxide. The blank was found to be lower by a factor of about 3 (see Table I).

Consequently, to obtain low blank values and increase precision, it is best to keep the walls of the combustion chamber as free of platinum film as possible. A cleaning every ten runs will suffice. The use of fused alumina crucibles also leads to increased precision in the results.

SIZE OF SAMPLE AND CARBON CONTENT

Finally, another experiment having special significance was performed (Figure 3). Bureau of Standards steel 8G (0.069% carbon) was analyzed in samples of weight varying from 0.005 to 1.1 grams. This gives a variation in carbon from 0.003 to 0.70 mg. The average carbon content of the controversial samples 55A and 55B weighing 0.5 gram is 0.050 mg., well inside this range. All results are in agreement (0.0635%, standard deviation 0.0011%). This is additional proof that the equipment can handle all ranges of carbon contents without appreciable loss or trend, depending on size of sample or carbon content.

DISCUSSION

Results of a series of runs are shown in Table I. No difficulties or unusual deviations were experienced in making determinations on small samples (as low as 0.005 gram) of high-carbon steel. This was true for both the commercial steel examined and the Bureau of Standards steels, and does not substantiate the claim that all high-carbon samples are too inhomogeneous to give good precision under these circumstances.

Another feature of the authors' results that can be noted in Table I is the agreement between the results by the standard method and by the low-pressure combustion method for high-carbon samples. The writers believe that this agreement is the result of a corresponding lower percentage error in the standard method, introduced, for example, by the amount of carbon dioxide absorbed by the boat and liner material on exposure to the air, or to sulfur dioxide that might be entrapped with the carbon dioxide. The errors thus introduced are small when compared with the large total amount of carbon dioxide resulting from the combustion of the relatively large sample used in the standard procedure. For example, 0.01 to 0.05 mg. of carbon (average 0.02 mg.) can be absorbed from the air by an alumina boat. Therefore an 0.8% carbon sample, factor weight 1.36 grams, would give 10.9 mg. of carbon with a corresponding error of 0.2%. However, a 0.01% carbon sample, factor weight 2.72 grams, would give 0.272 mg. of carbon and involve an error of approximately 7%.

The difficulty of the difference between carbon results by the two methods can be resolved easily by recognizing that the limitations of the combustion-weighing method sets the apparent limit of accuracy of this method to not better than 0.003% carbon for the factor weight. For low-carbon determinations the discrepancy falls within this error (sample 55B, 0.012 *vs.* 0.0095%). On the other hand, were each method equally accurate and the error in each case of an indeterminate nature and purely random, the mean of many determinations by each method should more nearly coincide. The observed discrepancy for low-carbon determinations dealt with here is based on such averages in each case.

The magnitude of discrepancy indicates a fundamental determinate or method error. Examination of the low-pressure method has been, in effect, a search for the source of this error which, however, has not been found. On the other hand, it has been shown that exposure of boats with liners to the air, and possible trapping of some sulfur dioxide in carbon dioxide traps might be, at least partially, the source of error in the standard combustion-weighing method. It is the authors' conclusion, therefore, that the low-pressure combustion method meets present requirements for analyses of low-carbon materials with respect to both high precision and accuracy.

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RECEIVED August 26, 1947.

CORRECTION. In the article on "Application of Corrections in Viscometry of High-Polymer Solutions" [ANAL. CHEM., **20**, 155 (1948)], Equation 1 should have a multiplication sign, and not a minus sign, before the bracketed quantity. R. H. WAGNER

Eastman Kodak Co.
Rochester, N. Y.

Determination of Nitrogen in Biological Materials

Improved Kjeldahl-Nessler Method

GAIL LORENZ MILLER AND ELIZABETH ESHELMAN MILLER

The Institute for Cancer Research and The Lankenau Hospital Research Institute, Philadelphia 30, Pa.

The conditions of digestion, colorimetric aspects, factors of technique, and reproducibility of the micro-Kjeldahl method of Koch and McMeekin have been studied in detail. Tests have been carried out with amino acids, pyrimidines, purines, nucleic acid, creatine, vitamins and allied substances, extracts of normal muscle and muscle tumor, and purified proteins. The importance of a period of digestion before treatment with hydrogen peroxide and of repeated additions of peroxide is demonstrated. "Short" and "long" methods requiring total digestion periods of 9 and 15 minutes, respectively, and differing only in number of treatments with peroxide, have been developed. The choice of method and of number of replicas is shown to depend on the substance to be determined and the accuracy desired. A statistical range of error of ±1.94% for 19 out of 20 determinations is possible with duplicate samples. The practical value of a flexible method for determination of nitrogen is pointed out.

IT IS practical in research to contribute to an analysis only the amount of work required to maintain the error within appropriate limits. This principle is pertinent to measurements of nitrogen in biological materials. For example, in determinations of crude fractions of tissue extracts, errors up to ±5% might be quite acceptable; in determinations of purified substances, on the other hand, an accuracy of better than ±1 or 2% is usually desired. A method that provides such a flexibility has special value, particularly if it also provides simplicity of operation and economy of time and material.

The authors have found that Koch and McMeekin's micro-Kjeldahl method (*15*) can be made to fulfill the above requirements. The rapidity and convenience of this method are well known. It appeared necessary, however, to substantiate its reliability, as a number of nitrogenous substances of biological interest have been reported to be refractory to Kjeldahl digestion. Conspicuous among these substances are lysine (*7, 18, 21*), tryptophan (*14, 18, 21*), histidine (*7, 22*), tyrosine (*3, 18*), creatine (*23*), nicotinic acid (*1, 20*), and certain alkaloids (*1, 8*). Difficulty in digestion of specific amino acids may also lead to difficulty in digestion of proteins containing them (*7*). For complete liberation of the nitrogen of resistant materials, 2- to 12-hour periods of digestion with accessory agents such as copper, mercury, selenium, and persulfate have been reported necessary (*1, 3, 7, 8, 14, 18, 20–23*). However, Koch and McMeekin, using short digestion with the aid of hydrogen peroxide, obtained excellent recoveries of nitrogen for tryptophan, histidine, and caffeine.

In the present investigation, tests were carried out with amino acids, pyrimidines, purines, yeast nucleic acid, creatine, vitamins and allied substances, extracts of normal muscle and of muscle tumor, and purified proteins. By a detailed study, conditions of primary importance to complete liberation of nitrogen were determined. Studies also were made of colorimetric aspects of the method. Improved reproducibility was realized through control of technical factors known to affect the results. Finally, the actual degree of reproducibility that might be attained was established by a statistical analysis. Modified procedures based on the findings yielded satisfactory results for all the substances tested, including the refractory substances referred to above. Only a few of the vitamins, containing cyclic nitrogen, were found to yield results 3 to 5% low.

Errors of the method arise from two principal sources—incompleteness of digestion and irreproducibility of colorimetry. The errors from the first source can be delimited by the number of treatments with hydrogen peroxide, those from the second source by the number of replicas that are run.

As a result of the study, specific procedures were evolved to meet varying requirements, depending on the substance to be determined and the precision desired.

DESCRIPTION OF METHOD

Test Materials. Most of the test materials, of commercial origin, were supplied by Gerrit Toennies, Dorothy Leaf Gallant, and Mary Adelia Bennett. Glutamic acid hydrochloride, four times recrystallized, was supplied by J. J. Kolb. Cystine, 99.7% pure by optical rotation, was put at the authors' disposal by Dr. Toennies. Thymus nucleoprotein, obtained by the procedure of Carter and Hall (*5*), was supplied by H. M. Winegard. Extracts of muscle tumors and normal muscle tissue were prepared by Mr. Kolb. The tumor (a transplantable rhabdomyosarcoma) and the normal muscle tissue from which the extracts were made were obtained from mice and were supplied by Elizabeth Ufford Green.

As a rule, 30 mg. of test material were weighed and dissolved in 25 ml. of water to which sufficient 1 to 1 sulfuric acid was added, when necessary, to dissolve the sample. One- or 2-ml. aliquots were used for analysis; account was taken of the amount of sulfuric acid present when more was added later for the digestion. The nucleic acid was dissolved in very dilute sodium hydroxide, the edestin and insulin in very dilute hydrochloric acid, and the thymus nucleoprotein in distilled water. Tumor and normal muscle proteins were extracted with 0.45 molar potassium chloride containing small amounts of other salts. Samples of edestin and insulin were weighed on an air-dried basis and their nitrogen values were corrected for moisture and ash. Moisture was determined by drying the proteins in vacuo at 93° C. and ash by incineration at 600° C. The nucleoprotein was dried to constant weight in vacuo at 93° C. before being weighed for analysis. Of the other test samples, dried in a vacuum desiccator over calcium chloride before weighing, the only ones affected were creatine, which lost its water of crystallization, and nucleic acid which lost appreciable weight, presumably moisture.

Reagents. The reagents used comprised 1 to 1 sulfuric acid, Merck 30% hydrogen peroxide (Superoxol), Hartman-Leddon Nessler's reagent prepared according to Koch and McMeekin (*15*), and a stock ammonium sulfate solution containing 2 mg. of nitrogen per ml. The ammonium sulfate solution was subsequently diluted further as required for use as a standard.

Apparatus. An electric digester, shown in Figure 1, was used to provide even heating and to minimize bumping. It accommodates six 30-ml. micro-Kjeldahl flasks and is similar to the one described by Sanigar and Allen (*19*). Clark (*8*) and a group of English workers (*10*) also described electric digesters for Kjeldahl determinations. The important feature of the present one is the wire racks placed on the platform between heater openings to support the flasks while they are cooled before addition of peroxide. The inconvenience of removing flasks from the digester is thus avoided, yet sufficient cooling is permitted within a short period of time to prevent the violent reaction and loss of sample that may occur when peroxide is added directly to digests that are too hot.

The base of the digester and the supports for the manifold are made of wood. A sheet of Transite (portland cement bonded

asbestos board) is placed over the base. The heater box is made of Transite. The center shelf on which the coils rest is placed 3.1 cm. (1.25 inches) from the top of the box. The center vertical strip, used to separate the coils, is fastened only to the center shelf. The outside vertical strips are fastened only to the pieces comprising the top shelf. The top shelf with attached side strips is constructed to fit loosely inside the outside box, so that it can be easily removed when necessary for replacement of coils. Openings in the top are rounded out to accommodate the bulbs of the digestion flasks. Wood screws, $^5/_8$ inch, No. 2, are used for fastening together the different pieces of Transite after holes of a suitable size are drilled. The wire racks are fitted into holes drilled into the top shelf.

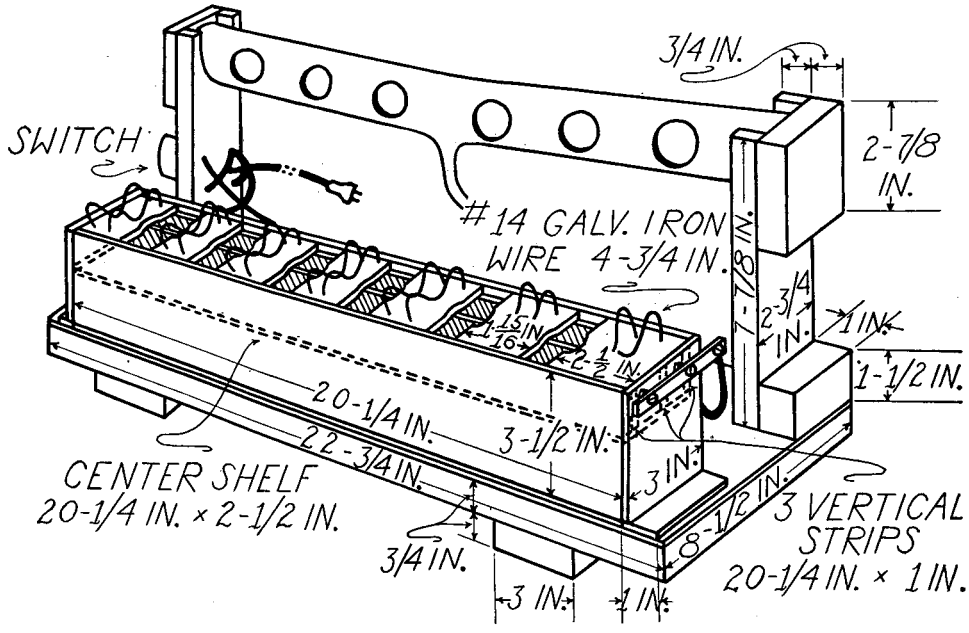

Figure 1. Diagram of Electric Digester

The heating unit, the coils of which are not shown in the figure, is wired as described by Sanigar and Allen (*19*). No. 22 nickel-chrome wire, 1.01 ohms per foot, is suitable for the coils, and may be purchased from the Wilbur B. Driver Co., Newark, N. J. Terminals of the coils are joined at one end by a brass bar. The heating unit is used ordinarily at high heat, although the lower heats may be useful for some purposes. At high heat, the temperature of the digests approaches the boiling point of sulfuric acid. The glass exhaust manifold may be obtained from the Arthur H. Thomas Co., Philadelphia, Pa.

An apparatus for swirling the flasks at a constant rate during addition of the Nessler reagent, shown in Figure 2, assures a more reproducible color development than is possible by manual swirling. A Bodine adjustable-speed electrical stirrer, useful for the purpose, may be obtained from Harshaw Scientific Co., Philadelphia, Pa. A cup, made from a No. 9 rubber stopper, is used to support the flasks at the bottom; a loose-fitting, U-shaped piece of brass supports the flasks at the neck. The motor is adjusted to rotate the flasks at 600 r.p.m. The flasks are easily placed on, or taken off, the assembly while the motor is in operation.

Colorimetric measurements are made with a Klett-Summerson photoelectric colorimeter. Although maximum absorption is obtained at about 420 mμ a No. 54 color filter provides a more suitable range of sensitivity.

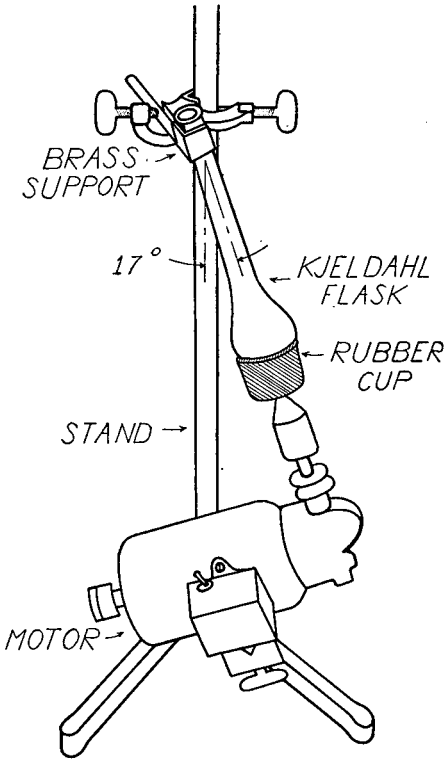

Figure 2. Diagram of Apparatus for Swirling Flasks

Procedure. The method of adding a known amount of water directly to the digest instead of transferring the digest to a volumetric flask and diluting to the mark has been used (*17*) in an earlier modification of Koch and McMeekin's method.

1. Sufficient unknown to contain 0.1 to 0.3 mg. of nitrogen or of standard to contain 0.2 mg. of nitrogen is transferred to a micro-Kjeldahl flask. To eliminate pipetting errors when highest accuracy is desired, aliquots of unknown and standard are delivered from the same pipet. To minimize bumping and foaming, the volume of sample is kept as small as possible. If the sample is expected to foam, 1 drop of caprylic alcohol is added, and further drops are added when needed. The same amount of caprylic alcohol is added to standards as to unknowns.
2. Next 0.4 ml. of 1 to 1 sulfuric acid is added.
3. The flask is placed on the digester, and the water is evaporated until white fumes appear.
4. Preliminary digestion—that is, digestion after evaporation of water but before addition of hydrogen peroxide—is carried out for 5 minutes.
5. The bulb of the flask is lifted onto an adjacent wire rack and allowed to cool for 30 seconds.
6. Two drops, approximately 0.1 ml., of hydrogen peroxide are introduced down the side of the flask, which is held nearly horizontal. The same amount is added to standards as to unknowns, as small amounts of nitrogen are usually present as an impurity in the peroxide.
7. The bulb of the flask is returned to the heater and digestion is carried out for 2 minutes.
8. Steps 5 to 7 are repeated once for the "short" method, four times for the "long" method, and six times for the "extra long" method.
9. The flask is removed from the digester and allowed to cool to room temperature.
10. Twenty milliliters of distilled water are added and allowed to wash down the walls of the flask.
11. The flask is placed on the mixing machine and allowed to spin for 5 seconds.

12. Five milliliters of Nessler's reagent are run down the sides of the flask while it is in motion.
13. The flask is spun 5 seconds longer after all the reagent is added.
14. After the resulting solution has stood for 2 minutes or longer, the color intensity is read in the colorimeter.

When several unknowns and standards are being run simultaneously, the Nessler reagent is added to the different samples at 1-minute intervals and readings are made at 1-minute intervals in the same order. Samples containing 0.2 mg. of nitrogen will give a reading of about 200 scale units. Over the scale range of 100 to 300, within which the samples should fall, readings are estimated to three significant figures at whole number intervals. When more than one replicate is run, the means are calculated to four figures, the fourth figure being understood to have limited significance.

In special cases it may be desirable to determine the nitrogen of comparatively large samples of material. This becomes necessary when a sample is insoluble in ordinary solvents and a microbalance is not available for weighing out small samples, or when large samples of inhomogeneous, insoluble material must be taken in order to ensure representative sampling. For such materials, the following procedure may be followed.

In a micro-Kjeldahl flask 120 mg. of material are placed, 3 ml. of 1 to 1 sulfuric acid are added, and digestion is carried out for 5 minutes after the water has been boiled off and the sample appears to have dissolved. Hydrogen peroxide is added 2 drops at a time at 2-minute intervals until the mixture becomes clear, although not necessarily colorless. The digest is transferred to a 100-ml. volumetric flask and diluted to volume. One-milliliter aliquots of the diluted samples are now carried through the regular procedure described above, 0.37 ml. of sulfuric acid being added for the digestion. This variation of the method was found very satisfactory. Results obtained with its use are not, however, described in the present paper.

DEVELOPMENT OF METHOD

Stability of Color and Proportionality Relationships. The rate of development and stability of color at different levels of nitrogen are shown in Figure 3. The colors approach maximum intensity within 2 minutes, increasing at diminished rates thereafter. The increase on a percentage basis after 2 minutes is slightly greater at higher nitrogen levels than at lower ones.

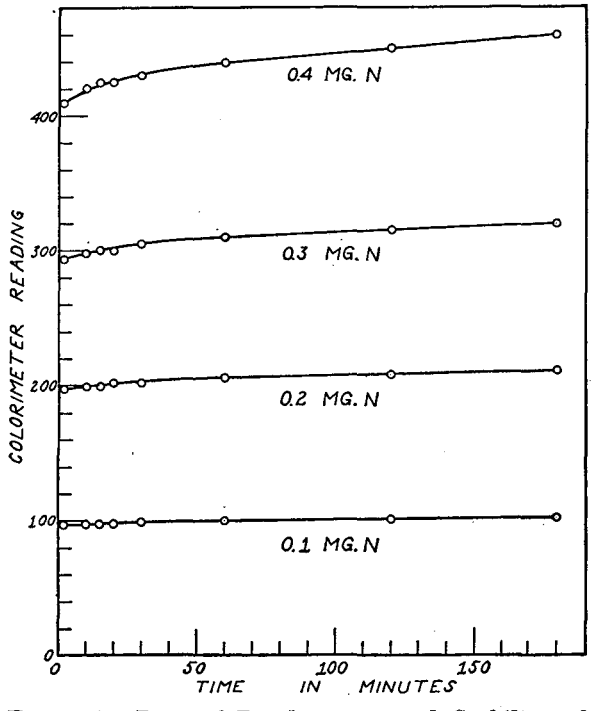

Figure 3. Rate of Development and Stability of Color at Different Levels of Nitrogen

The relationship between color intensity and nitrogen level is shown in Figure 4. The readings are not corrected for the blank reading. The points on the line represent triplicate determinations. Failure to obtain strict proportionality has also been observed by other workers (*16*).

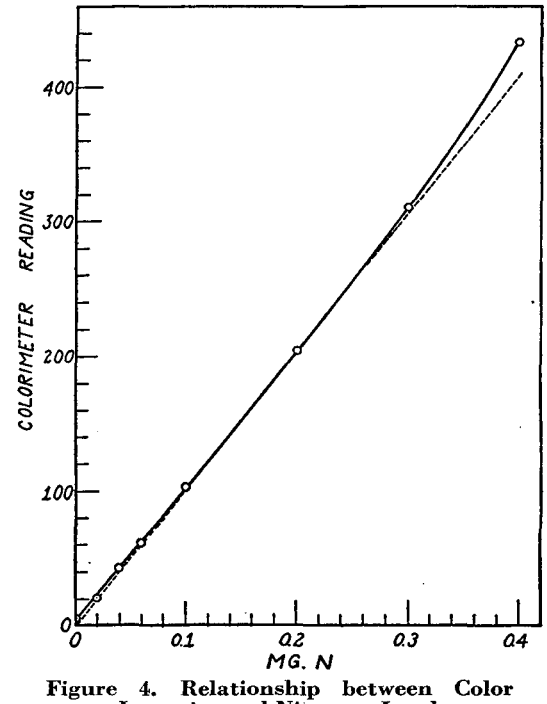

Figure 4. Relationship between Color Intensity and Nitrogen Level

On the basis of the above results, 0.2 mg. of nitrogen appeared the most useful standard and 0.1 to 0.3 mg. of nitrogen, a practical limit in range of nitrogen. Under these conditions, strict proportionality without correction for the blank could be assumed as is shown by the dotted line of Figure 4 drawn through the origin and the point corresponding to 0.2 mg. of nitrogen. Because of the slight but significant change in color intensity with time, readings of unknowns and standards are best made after exactly the same time interval. Although color intensities at higher nitrogen levels increase more rapidly than those at lower levels, errors no greater than about 2% are likely to be encountered at extremes of the nitrogen range, provided readings of unknowns and standards are made after the same time interval and within 30 minutes after addition of the Nessler reagent. When readings are made at 1-minute intervals, 30 samples of unknowns and standards can thus be determined. Inaccuracy due to the effect of time can be eliminated altogether if unknowns and standards are adjusted to the same nitrogen level. Readings can then be begun at any interval between 2 and 180 minutes or probably even later.

Effect of Rate of Addition of Nessler Reagent and Rate of Mixing on Color Intensity. Provided there is sufficient mixing, the more rapidly the Nessler reagent is added, the lower the color intensity which results. This was demonstrated by an experiment in which mixing was carried out by revolving the flasks at 600 r.p.m. and the rate of addition of reagent was varied by the use of two different pipets, one delivering its contents in 10 seconds, the other in 25 seconds. Ten replicas with standards containing 0.2 mg. of nitrogen were run by each method. The mean colorimeter readings were 185 and 190, respectively. The corresponding standard deviations from the means were 1.14 and 0.95%. That the difference between these standard deviations is too small to be significant indicates that under the conditions of the experiment, the reproducibility was as good at one rate as the other. The

actual differences in the mean values of the readings, however, were significant at a high level of probability.

If the rate of addition of reagent is maintained constant, the color intensity is found to vary inversely with the rate of mixing, and if mixing is too slow, opalescence and even precipitation may be produced at will. With the aid of the electrically driven apparatus for swirling flasks at constant speed, errors from this source are effectively minimized.

Table I. Effect of Salts on Color Development and on Digestion

Experiment No.	Substance Tested	Amount Used	Colorimeter Readings: Standard	Colorimeter Readings: Nucleoprotein
		Millimoles		
1	None	...	197	206
2	Sodium chloride	0.5	201	211
3	Potassium chloride	0.5	203	212
4	Disodium hydrogen phosphate	0.2	195	206
5	Calcium chloride	0.1	204	Opalescent
6	Magnesium chloride	0.0002	203	207
7	Calcium chloride plus disodium hydrogen phosphate	0.1 0.0000017	Opalescent	

Effect of Temperature on Color Intensity. Temperature was found to affect color intensity, higher intensities being obtained at elevated temperatures. For example, at 30° C. a standard containing 0.2 mg. of nitrogen gave a colorimeter reading of 193; at 20° C. it gave a reading of 173. This effect has also been noted by other investigators (*12*). A standard that is reproducible from day to day cannot, therefore, readily be obtained, and standards must accordingly always be measured simultaneously with unknowns. Local temperature differences also lead to inaccuracies. For example, when the colorimetry was carried out in close proximity to the electric digester, the temperature of some of the samples was altered more than others and irregular results were produced.

Effect of Possible Interfering Substances on Color Development and Digestion. Sodium chloride, potassium chloride, disodium hydrogen phosphate, calcium chloride, and magnesium chloride were added in varying concentrations to standards containing 0.2 mg. of nitrogen and were carried through the digestion process by the short method. They were also added to aliquots of nucleoprotein and the digestion process was again carried out as described. From the results of the first six experiments of Table I it may be seen that the color intensity of a 0.2-mg. nitrogen standard was increased not more than 4% in any case. Furthermore, except for calcium chloride the salts in the concentrations indicated did not interfere with digestion of nucleoprotein. In experiment 7, an amount of phosphate equivalent to that present in the nucleoprotein was added to a nitrogen standard to which calcium chloride also was added. The results of this experiment indicate that phosphate originating in the nucleoprotein was responsible for the difficulty encountered in the determination of nucleoprotein in the presence of calcium chloride. In further study it was found that larger amounts of magnesium chloride and calcium chloride produced serious opalescence with standards. The upper limits of sodium and potassium chlorides and disodium hydrogen phosphate were not determined. The data indicate that special precautions ordinarily have to be taken only for magnesium or for 0.1 *M* calcium when phosphate is present.

Effect of Single Addition of Hydrogen Peroxide on Completeness of Digestion. Koch and McMeekin (*15*) state that after sulfuric acid is added to the sample and the water has been boiled off, the digestion tube "is heated over the microburner until filled with dense, white fumes of sulfuric acid. After it has been allowed to cool for 15 to 30 seconds, 1 to 5 drops of 30% hydrogen peroxide solution are added and the heating is continued over the microburner. If the material remains colorless after it has again been heated until white fumes form, gentle boiling is continued for 2 to 5 minutes. If the fluid again becomes discolored, the addition of several drops of 30% hydrogen peroxide and the heating are repeated. After complete digestion and cooling, the solution is transferred to a 100-cc. volumetric flask."

In the first experiments carried out in the present study with substances of known nitrogen content, 6 drops of hydrogen peroxide, added at one time, were found to clarify the digests permanently, and further peroxide was, therefore, not added. Preliminary digestion was not given particular consideration, as its importance was not realized. Effective preliminary digestion was probably inadvertently carried out in some instances, not in others. After addition of the peroxide, subsequent digestion was carried out for 5 minutes in one set of experiments, 15 minutes in another. The digests were then cooled to room temperature, diluted with water, and nesslerized. Each analysis was carried out in duplicate. The results obtained are shown in Table II under "Preliminary Tests." For ease of comparison, the data are presented in terms of per cent deviation of the observed nitrogen values from the theoretical ones. Arginine, lysine, tryptophan, guanine, riboflavin, thiamine, and creatine appear to show significantly low results when judged on the basis of the results obtained with the other substances. Except for guanine and creatine, these substances did not give appreciably higher recovery of nitrogen on 15- than on 5-minute digestion. Histidine and tyrosine, determined with difficulty in other procedures (*7*, *14*, *18*), showed no resistance to this one.

Table II. Results of Tests on Known Nitrogenous Materials

Test Material: Class	Test Material: Name	Deviation from Theoretical Value: Preliminary Tests 5-min. digestion, %	Preliminary Tests 15-min. digestion, %	Final Tests Short method, %	Final Tests Long method, %
Amino acids	Glycine[a]	+1	−3	+1	+1
	dl-Alanine[a]	+3	+1	+1	+1
	dl-Valine[a]	+1	0	+1	0
	dl-Leucine[a]	−1	+2	+1	0
	dl-Isoleucine[a]	0	−1	0	−2
	dl-Serine[a]	−1	+2	+1	−1
	dl-Threonine[a]	+1	−1	0	0
	dl-Aspartic acid[a]	0	−1	−1	0
	l-Glutamic acid[a]	−1	−1	+1	0
	l-Proline[a]	0	+1	−1	0
	Hydroxy-*l*-proline[b]	...	...	+2	+1
	dl-Methionine[a]	−2	−2	+1	−1
	l-Cystine[a]	+2	0	+3	+2
	dl-Phenylalanine[a]	+2	0	−3	−2
	l-Tyrosine[a]	+2	+1	−1	−3
	l-Histidine monohydrochloride[a]	−2	0	−2	+1
	l-Arginine monohydrochloride[a]	−5	−6	0	−3
	l-Lysine monohydrochloride[a]	−13	−16	−5	−2
	dl-Tryptophan[a]	−24	−23	−7	−1
Pyrimidines and purines	Uracil[c]	+1	−2	+1	−1
	Thymine[c]	+2	−3	0	0
	Adenine sulfate[c]	+1	−1	−1	−1
	Guanine[d]	−12	−6	−2	−2
	Uric acid[a]	+3	0	−1	+1
	Yeast nucleic acid[c]	...	...	0	0
Vitamins and allied substances	Pyridoxine hydrochloride[a]	...	...	0	0
	Calcium pantothenate[a]	...	...	0	0
	p-Aminobenzoic acid[a]	...	...	−2	0
	Riboflavin[a]	−12	−10	−3	−3
	Thiamine hydrochloride[a]	−7	−11	−4	−5
	Folic acid[e]	...	...	−7	−3
	Choline chloride[a]	...	...	−14	−4[f]
	Nicotinic acid[a]	...	...	−30	−8[f]
Miscellaneous	Creatine[g]	−18	−8	−1	0

[a] Merck.
[b] Univ. of Ill.
[c] Schwarz Lab.
[d] Hoffmann-La Roche.
[e] Lederle (folvite).
[f] By extra long method, deviations for choline and nicotinic acid were reduced to −2 and 0%, respectively.
[g] Eastman.

Table III. Effect of Preliminary Digestion and of Repeated Addition of Hydrogen Peroxide on Completeness of Digestion of Lysine

Experiment No.	Conditions of Treatment of Lysine	Deviation from Theoretical Value
		%
1	No preliminary digestion, 2 drops of peroxide added once, 5-min. digestion	−1
2	5-min. preliminary digestion, 2 drops of peroxide added once, 5-min. digestion	−15
3	15-min. preliminary digestion, 2 drops of peroxide added once, 5-min. digestion	−14
4	30-min. preliminary digestion, 2 drops of peroxide added once, 5-min. digestion	−15
5	5-min. preliminary digestion, 2 drops of peroxide added 3 times, 5-min. digestion each time	−7
6	5-min. preliminary digestion, 2 drops of peroxide added 6 times, 5-min. digestion each time	−1
7	5-min. preliminary digestion, 2 drops of peroxide added once, 30-min. digestion	−10

Effect of Preliminary Digestion and of Repeated Addition of Hydrogen Peroxide on Completeness of Digestion. Of the refractory materials, lysine was chosen first for detailed study. When lysine was subjected to preliminary digestion, low nitrogen values were obtained, but when it was treated with peroxide immediately after evaporation of the water, close to the theoretical recovery of nitrogen resulted. This is shown by the results of experiments 1 and 2 of Table III, based on duplicate analyses. If loss of nitrogen occurred during preliminary digestion, it appeared that longer preliminary digestion might enhance the loss. However, as shown by experiments 3 and 4, increasing the period to 15 and 30 minutes did not alter the result. To determine whether the nitrogen not determinable after preliminary digestion might be recovered by repeated additions of peroxide, experiments 5, 6, and 7 (Table III) were carried out. The results indicate that this nitrogen can be recovered by such treatment, and that the recovery is due more to the action of the peroxide than to the lengthened period of digestion. The similarity of the results of experiment 2 of Table III, obtained with 2 drops of peroxide, to previous ones of Table II with 6 drops of peroxide, suggests that the larger amount of peroxide has no advantage. It appears further that 6 drops of peroxide added at one time are of less value than the same amount added 2 drops at a time at intervals.

It was next of interest to determine whether some of the other refractory substances, in particular tryptophan, arginine, guanine, and creatine, might behave similarly to lysine. Histidine, tyrosine, adenine, thymine, and uracil also were studied for any information they might reveal. Four experiments were carried out on each substance: experiment A, designed to show the effect of a single addition of peroxide following preliminary digestion of the sample; experiment B, the effect of a single addition of peroxide without preliminary digestion; experiment C, the effect of repeated additions of peroxide following preliminary digestion; experiment D, the effect of repeated additions of peroxide without preliminary digestion. The analyses were made in duplicate.

The results, shown in Table IV, are presented in groups based on similarities of behavior. Tryptophan behaved like lysine giving a low recovery after preliminary digestion when peroxide was added but once, and high recoveries in the absence of preliminary digestion or on repeated additions of peroxide. The high recovery obtained for tryptophan in the absence of preliminary digestion was not, however, always reproducible, as it was in the case of lysine. It was concluded that the good recoveries obtained for tryptophan by Koch and McMeekin were probably the result of their use of repeated additions of peroxide.

Guanine, adenine, arginine, and creatine, on the other hand, behaved unlike lysine and tryptophan. They yielded correct nitrogen values only after preliminary digestion, and this was true following a single addition of peroxide as well as several additions. Without preliminary digestion, they gave low results which, moreover, were not raised by repeated addition of peroxide. Histidine, tyrosine, uracil, and thymine yielded their nitrogen without difficulty, regardless of whether or not preliminary digestion was carried out.

Of greatest practical importance was the finding that only the conditions of experiment C—namely, 5 minutes of preliminary digestion with five treatments with peroxide—yielded good results for each and every one of the test materials of Table IV. Although the pyrimidine, cytosine, was not available for the present study, one would expect that it also would respond satisfactorily to these conditions.

The similarity in behavior of guanine, adenine, arginine, and creatine is doubtless related to their similarity in chemical structure. Lysine and tryptophan, though superficially dissimilar, may yield analogous intermediates on digestion. It appears that preliminary digestion of the latter yields intermediates that are less susceptible to oxidation by hydrogen peroxide than the original amino acids. Guanine, adenine, arginine, and creatine, on the other hand, appear more readily oxidizable after preliminary digestion. This may be due to irreversible oxidation of nitrogen itself, which may occur to a certain extent when these substances are treated with hydrogen peroxide before preliminary digestion.

Effect of Time on Completeness of Digestion. Certain results shown in Table II and III have minimized the importance of time of digestion after addition of peroxide. It appeared, therefore, that a 2-minute period of digestion might be as effective as the 5-minute period used in the experiments of Table IV. By means of tests with lysine and tryptophan, the results of which are shown in Table V, this supposition was substantiated. Such a modification affords an appreciable saving of time, particularly when the addition of peroxide is repeated several times. However, tryptophan and tyrosine digests do not become clear after a single addition of peroxide and one 2-minute period of digestion. A minimum of two treatments is necessary.

Two minutes of digestion are not sufficient to remove excess peroxide. This is revealed by the appearance of a yellow color when only a portion of the Nessler reagent is added. Color from this source does not, however, cause interference, for as soon as

Table IV. Effect of Preliminary Digestion and Repeated Additions of Hydrogen Peroxide on Completeness of Digestion of Lysine, Tryptophan, Guanine, Adenine, Arginine, Creatine, Histidine, Tyrosine, Uracil, and Thymine

Test Group	Material Name	Deviation from Theoretical Value			
		Expt. A[a]	Expt. B	Expt. C	Expt. D
		%	%	%	%
I	Lysine	−16	−1	−1	−1
	Tryptophan	−13	−2	0	−1
II	Guanine	− 1	−7	−2	−7
	Adenine	− 1	−8	0	−6
	Arginine	0	−6	0	−5
	Creatine	− 1	−9	−1	−6
III	Histidine	0	0	−1	0
	Tyrosine	+ 1	−1	0	+1
	Uracil	0	0	+1	+2
	Thymine	− 1	−2	−3	−2

[a] Expt. A. 5-min. preliminary digestion, 2 drops of peroxide added once, 5-min. digestion.
Expt. B. No preliminary digestion, 2 drops of peroxide added once, 10-min. digestion.
Expt. C. 5-min. preliminary digestion, 2 drops of peroxide added 5 times, 5-min. digestion each time.
Expt. D. No preliminary digestion, 2 drops of peroxide added 5 times, 5-min. digestion 4 times, 10-min. digestion 5th time.

Table V. Effect of Time on Completeness of Digestion of Lysine and Tryptophan

Substance	Time of Digestion after Each of 5 Additions of Peroxide	Deviation from Theoretical Value
	Min.	%
Lysine	5	−2
	2	−2
Tryptophan	5	−3
	2	+1

sufficient reagent is added to make the solution alkaline, the color due to peroxide is bleached out, and that due to ammonia begins to develop. The color due to the ammonia is unaffected by the small amounts of peroxide that may be present.

Significance of Clarification of Digests to Completeness of Digestion. As was found by workers using other catalysts (*2*, *7*, *9*), clarification of digests with hydrogen peroxide likewise did not necessarily indicate completeness of digestion. For example, lysine, on heating with sulfuric acid, turned dark, but after a few minutes became clear. The cleared digest treated once with 2 drops of hydrogen peroxide gave, however, only 85% of its total nitrogen. Tryptophan charred on digestion with sulfuric acid, clarified completely on addition of 6 drops of peroxide, but under these conditions gave up only 76% of its nitrogen. Nicotinic acid did not become colored on digestion, but after a single addition of peroxide still gave up only 70% of its nitrogen. Repeated additions of peroxide to clarified digests of the above substances are required to release all their nitrogen. Tyrosine, by contrast, was found to char on heating with sulfuric acid and to clarify only partially on a single addition of 2 drops of peroxide, yet appeared to yield all its nitrogen.

APPLICATION OF FINAL METHOD

Tests with Known Biological Materials. The above findings determined the conditions under which the final tests were made. Preliminary digestion of 5 minutes' duration was carried out and the effect of addition of 2 drops of peroxide with 2 minutes' digestion, repeated twice, was compared with the same treatment repeated 5 times. The former procedure was called the "short" method; the latter, the "long" method. Duplicate analyses were made on the amino acids, pyrimidines, purines, vitamins, and creatine studied in the preliminary tests and also on hydroxyproline, nucleic acid, and additional vitamins. The results are shown as final tests in Table II.

The improvement in data for arginine, lysine, tryptophan, guanine, creatine, riboflavin, and thiamine is very marked. Lysine and tryptophan, the only refractory amino acids remaining, yielded 93 and 95%, respectively, of their nitrogen by the short method, essentially all of their nitrogen by the long method. The results for riboflavin and thiamine, however, still appeared somewhat low, as also were those for folic acid, choline, and nicotinic acid. In further studies, it was found that by repeating the addition of peroxide a total of seven times it was possible to bring the data of choline and nicotinic acid down to deviations of only −2 and 0%, respectively. Riboflavin, thiamine, and folic acid appeared to remain slightly refractory even on further treatment, and showed respective deviations of −5, −2, and −4%. Lysine and tryptophan were found to yield the same results with seven and five peroxide treatments.

Tests with Purified Proteins and Biological Mixtures. Tests by the short and long methods were next made on more complex substances and mixtures of substances—namely, insulin, edestin, nucleoprotein, extracts of muscle tumor and normal muscle, blood plasma, and urine. It was assumed that proteins, such as those mentioned, would be rapidly hydrolyzed during the digestion procedure, and that if quantitative nitrogen values were obtainable for amino acids, pyrimidines, and purines, they should also be obtainable for proteins and nucleoproteins. It was realized that oxidative degradation might to a certain extent precede hydrolysis, but, as oxidative degradation constitutes the principal process requisite to digestion, it appeared of possibly little consequence at what point it took place. To remove any question as to errors due to incompleteness of hydrolysis, analyses were also made on protein hydrolyzates obtained by refluxing the proteins with 6 *N* sulfuric acid for 6 hours. Furthermore, to determine whether maximum nitrogen liberation occurred in the long method when applied directly to unhydrolyzed proteins, a number of analyses were made by seven treatments with hydrogen peroxide, called the extra long method. The final results are summarized in Table VI.

In the duplicate analyses carried out on urine and blood plasma, no significant differences were revealed by the short and long methods. Tests of the remaining substances, made with a larger number of replicas, revealed significantly higher nitrogen values by the long method for muscle extract and nucleoprotein, but not for tumor extract, insulin, and edestin. The determination of significance of differences was based on statistical considerations presented below.

Values obtained by the long method appeared essentially maximal, as shown by close agreement with results obtained by the extra long method. Furthermore, determinations by the long method made after hydrolysis did not differ significantly from the corresponding determinations made without hydrolysis, demonstrating that complete hydrolysis occurred during digestion by the long method or that its occurrence before oxidative degradation was not of primary importance.

The nitrogen values obtained for nucleoprotein, insulin, and edestin are in reasonable agreement with the respective values of 16.73, 15.54, and 18.7% reported elsewhere (*5*, *7*) for these substances. It is felt that the discrepancies, in so far as they exist, should not be ascribed altogether to the use of different methods of analysis, because factors such as moisture determination and purity of material could account for at least part of such small differences. Different nitrogen methods when applied to complexes such as proteins should properly be compared on samples of the same preparations of test material. The authors feel that their data are self-consistent and are reliable for the particular samples of test materials used.

Table VI. Nitrogen Content of Purified Proteins and Biological Mixtures

	Direct Determination				Determination after Hydrolysis	
Test Material	No. of replicas	Short method	Long method	Extra long method	No. of replicas	Long method
		Mg./ml.	*Mg./ml.*	%		%
Urine	2	14.76	14.53	..	..	..
Blood plasma	2	9.24	9.16	..	..	..
Tumor extract[a]	12	0.2138	0.2130	..	..	..
Muscle extract[a]	12	0.1687	0.1718	..	..	..
		%	%			
Nucleoprotein	12	17.21	17.35	17.32	3	17.37
Insulin[b]	12	15.42	15.47	15.44	3	15.43
Edestin[c]	12	18.27	18.37	18.44	3	18.35

[a] Arbitrary dilutions.
[b] Lilly.
[c] Hoffmann-La Roche.

Using available data for the lysine and tryptophan contents of insulin (*6*, *13*), edestin (*4*, *6*), nucleoprotein (*11*), and muscle protein (*4*), together with the data shown in Tables II and III for recovery of the nitrogen of lysine and tryptophan by the short and long methods of analysis, the authors attempted to estimate the recoveries to be expected for the nitrogen of these proteins by the short method. The recoveries, thus estimated, ranged from 0.05 to 0.79% low. The corresponding observed recoveries by the short method, calculated from the data of Table VI on the basis of the assumption that the results obtained by the long method represented 100% recovery, ranged from 0.32 to 1.80% low. Although in either instance only the larger errors are of sufficient numerical magnitude to become statistically significant, it appears from the fact that the observed recoveries by the short method were lower than those estimated, that protein structures are probably more resistant to Kjeldahl breakdown than the individual amino acids out of which they are composed.

Statistical Study of Reproducibility of Method. The large number of replicas carried out under the uniform conditions of the tests in Table VI were used for statistical measurement of reproducibility. To obtain data on substances of known purity,

Table VII. Standard Deviations of Colorimeter Readings for Digests of Different Materials

Test Material	Short Method			Long Method			Extra Long Method		
	Unknown	Standard	Unknown/standard	Unknown	Standard	Unknown/standard	Unknown	Standard	Unknown/standard
	%	%	%	%	%	%	%	%	%
Glutamic acid	0.84	1.53	1.28	1.12	1.05	1.17	..	..	..
Cystine	1.32	0.71	1.03	0.90	1.19	1.75	..	..	..
Tumor extract	1.50	1.22	1.35	1.39	1.69	1.90	..	..	..
Muscle extract	1.31	1.10	1.88	1.23	0.92	1.56	..	..	..
Nucleoprotein	1.08	0.81	1.13	0.76	0.97	1.12	0.80	0.71	1.65
Insulin	0.95	0.82	0.62	0.86	0.91	1.33	1.19	1.08	1.71
Edestin	1.21	0.90	1.45	1.18	0.85	1.25	0.74	0.74	1.11
Av.	1.17	1.01	1.25	1.06	1.08	1.44	0.91	0.84	1.49
Over-all average for unknowns and standards									1.05
Over-all average for unknowns/standards									1.37

measurements by the short and long methods were also made on specially purified preparations of glutamic acid hydrochloride and cystine.

The statistical data are summarized in Table VII, in which are presented the standard deviations of the readings of the unknowns, of the readings of the standards, and of the ratios of the readings of the unknowns to those of the standards. The standard deviations were calculated from the expression, $\sigma = \left(\sqrt{\frac{\Sigma x^2}{N-1}}\right)\left(\frac{100}{\overline{X}}\right)$ where σ represents standard deviation in %, x repesents individual deviations from the mean, N represents the number of different tests in the sample, and $\overline{X}$ represents the value of the mean.

The reproducibility as measured by the numerical value of the standard deviation is essentially the same in the short, long, and extra long methods. That it is also the same for unknowns as for standards shows that the variability in degree of digestion of unknowns was insufficient to be detectable. To determine how much of the deviation was the result of colorimetric errors alone, replicate measurements were made on 44 separate aliquots of a standard ammonium sulfate solution containing 0.2 mg. of nitrogen per ml., the digestion with addition of peroxide being omitted. The standard deviation of these readings was 1.03%, which agreed closely with the over-all average value of 1.05% given in Table VII. This showed that most of the variability observed in the analyses of test materials was due to the limited reproducibility in the colorimetry, and, accordingly, not to losses of material during addition of peroxide or other handling.

The over-all average standard deviation of ratios of unknowns to standards, shown in Table VII—namely, 1.37%—is a direct measure of the reproducibility to be expected for analyses in which single standards are run with single unknowns. Twice this standard deviation, or ±2.74%, is the range of error within which the results of 19 out of 20 such analyses should occur. The corresponding ranges of error for duplicate or triplicate analyses are ±1.94 and ±1.58%, respectively, calculated from the expression, $\frac{\sigma_{\overline{U}}}{\overline{S}} = \frac{\sigma_U}{\overline{S}} \Big/ \sqrt{N}$ where $\frac{\sigma_{\overline{U}}}{\overline{S}}$ represents the standard error of the mean ratio of unknowns to standards, $\frac{\sigma_U}{\overline{S}}$ is the standard deviation of single ratios, and N is the number of ratios in the sample—that is, the number of replicas.

On the basis of a level of probability of 19 out of 20 as a suitable test for significance of differences, the above statistics also signify that the chances are 19 out of 20 that differences of 2.74% between the results of two single analyses, differences of 1.94% between the two sets of duplicate analyses and differences of 1.58% between two sets of triplicate analyses are significant. For sets of twelve replicas, such as those on which certain data of Table VI were based, differences of 0.79% are significant.

The values obtained for the nitrogen of glutamic acid were 7.61% by the short method and 7.65% by the long method. The values for cystine were 11.74% by the short method and 11.69% by the long method. These data correspond closely to the theoretical values of 7.64 and 11.67% nitrogen, respectively.

CONCLUSIONS

For routine determinations of nitrogen in biological fluids and extracts, duplicate determinations by the short method provide sufficient accuracy. The maximum error due to incompleteness of digestion, as exemplified by the results with muscle extract, might be about −2%. Error due to colorimetry would fall within the range of ±1.9% for 19 out of 20 determinations. Combining the errors from the two sources one can, therefore, expect, under least favorable conditions, a range of error of approximately −4 to 0% for 19 out of 20 determinations; under most favorable conditions, a range of −2 to +2%.

For analyses of purified substances, triplicate determinations by the long method yield satisfactory data. Except for some of the most refractory substances, one may assume that no error arises from incompleteness of digestion. The remaining error, that due to colorimetry, is ±1.6% for 19 out of 20 determinations.

Any desired degree of accuracy higher than the above may, of course, be obtained by increasing the number of replicas.

Refractory substances requiring special consideration are riboflavin, thiamine, and folic acid, which may be 3 to 5% low, and choline and nicotinic acid which require at least seven additions of hydrogen peroxide for complete recovery of nitrogen.

ADDENDUM

The authors have observed commonly the formation of a small amount of granular red precipitate after nesslerization of samples containing less than 0.1 mg. of nitrogen. Since the completion of this study Leitch [*J. Franklin Inst.*, **245**, 355 (1948)] has shown that this precipitation can be minimized if 0.3 ml. of 1 to 1 sulfuric acid is used in place of 0.4 ml., and that with the aid of suitable filters the range of nitrogen can thereby be extended to very low levels. In order to establish, however, whether the use of 0.3 ml. would prove satisfactory for the digestion of nitrogenous compounds, the authors have carried out additional analyses on two of the most refractory substances tested earlier—lysine and muscle extract—each by both short and long methods and in replicas of 12. The final data, subjected to statistical analysis, were found to be the same with either amount of acid. Furthermore, the reproducibility and the proportionality were unchanged. It is, therefore, concluded that 0.3 ml. of 1 to 1 sulfuric acid is satisfactory from the standpoints of completeness of digestion and accuracy of results, and finally, in view of its advantage in preventing red precipitate formation at low levels of nitrogen, its use is to be recommended.

ACKNOWLEDGMENT

The authors acknowledge valuable discussions of statistical aspects of the problem with Gordon L. Walker, Department of Mathematics, Temple University, Philadelphia, Pa.

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RECEIVED September 17, 1947. Aided by funds in memory of Ruth C. Joyce.

Microdetermination of Tellurium V

R. H. DEMEIO[1], *Harvard School of Public Health, Boston, Mass.*

To determine tellurium in air dust, the sample is dissolved with the aid of sulfuric and nitric acids and hydrogen peroxide, and the tellurium is precipitated by boiling with hydrazine dihydrochloride and stannous chloride. The precipitated tellurium is dissolved in warm 1 to 1 nitric acid, the solution evaporated to dryness, the residue taken up in concentrated hydrochloric acid, and tellurium precipitated again with stannous chloride in presence of gum arabic. The degree of light absorption produced by the suspension is determined with the Klett-Summerson photoelectric colorimeter using light filter No. 42. The method allows the determination of 5 to 140 micrograms of tellurium with an error of 10%.

IT IS to Lenher that we owe a great deal of our knowledge concerning the chemical behavior of tellurium. Most of the macromethods for its determination in use at present are either his methods or modifications of them (*1*).

The few micromethods developed include the colorimetric technique of Volkov (*4*), that of Kronenberg and Setterlind (*2*) for 50 to 700 micrograms of tellurium, applied to air dust analysis in iron foundries, and the method used by Steinberg, Massari, Miner, and Rink (*3*) for 5 to 50 micrograms used in the determination of tellurium in air dust samples and urine. All three methods are based on the reduction to elementary tellurium.

This paper deals with the application of the same principle to the determination of minute quantities of the element with the use of the Klett-Summerson photoelectric colorimeter.

REAGENTS

Concentrated hydrochloric acid. Distilled water, particle-free, filtered through sintered glass funnels F. A 5% solution of gum arabic, freed by centrifugation from suspended particles.

A 10% solution of stannous chloride, prepared by adding 1 ml. of concentrated hydrochloric acid and 10 ml. of distilled water for each gram of stannous chloride, and centrifuging to free it from turbidity.

A 15% solution of hydrazine dihydrochloride.

Standard tellurium solution, prepared by dissolving 50 mg. of elementary tellurium in warm 1 to 1 nitric acid and evaporating to dryness. The residue is dissolved in a few drops of 10% sodium hydroxide and brought to 500 ml. with filtered distilled water (1 ml. = 100 micrograms of tellurium). This solution will be stable for six, or probably more, months. A standard solution prepared by dissolving the residue of the nitric acid solution in diluted hydrochloric acid was less stable; in time a white precipitate, probably tellurium dioxide, settled out.

PRELIMINARY TREATMENT OF SAMPLE

Steinberg and co-workers (*3*) and Kronenberg and Setterlind (*2*) have developed methods for air sampling in iron foundries. The authors have analyzed air samples from a selenium and tellurium plant.

The atmospheric dust was collected on filter paper by means of a filter paper sampler. Whatman No. 52 paper was used and air drawn through the paper at a measured rate in the range from 30 to 40 liters per minute. The rate of flow was measured by the use of an orifice at the intake and a water manometer and calibrated with a standard rotameter and gas meter. A uniform procedure for analysis cannot be used, as the concentration of tellurium in air varies in different parts of the plant. The volume of air collected varied from 400 to 3000 liters and tellurium per cubic meter of air varied from 0 to 12 mg. Consequently, an appropriate aliquot had to be used for every sample.

The dust sample was treated with 5 ml. of concentrated sulfuric acid, concentrated nitric acid, and 30% hydrogen peroxide, or with concentrated hydrochloric acid and potassium chlorate, according to Scott (*1*). In the presence of selenium, this element should be separated first by volatilization according to Lenher and Smith (*1*).

ISOLATION AND DETERMINATION OF TELLURIUM

After the above treatment, 50 ml. of distilled water followed by 50 ml. of approximately 3 *N* hydrochloric acid were added, and the residue was brought into solution if necessary, by heating.

After addition of 6 ml. of 15% hydrazine dihydrochloride and 10 ml. of 10% stannous chloride, the solution was boiled for 15 minutes. In this manner complete precipitation of tellurium was achieved. The tellurium precipitate was collected in a Selas

Table I. Recovery of Tellurium

Te Added, Micrograms	Te Found, Micrograms	Error, %
10	8.8	−12
20	18	−10
30	27	−10
40	39	−2.5
50	47	−6
100	102	+2
120	110	−8.3
140	135	−3.6

[1] Present address, Department of Experimental Medicine, The Jefferson Medical College, Philadelphia, Pa.

porcelain crucible, with the use of suction. It was washed first with a few milliliters of approximately 3 *N* hydrochloric acid to avoid precipitation of basic tin salt, and then with hot distilled water until it was free from chloride. During filtering, the precipitate was kept always covered by the solution in order to avoid possible oxidation and, as a consequence, solution of the tellurium.

The crucible was placed on a new suction flask and the beaker, in which the precipitation had been carried out, was washed with a few milliliters of warm 1 to 1 nitric acid which was then transferred into the crucible. More warm 1 to 1 nitric acid was added to the crucible until tellurium was completely dissolved. The tellurium solution was transferred from the suction flask to a 50-ml. beaker and evaporated to dryness on a hot plate. The residue was taken up with 3 ml. of concentrated hydrochloric acid, transferred to a graduated test tube of 1.2-cm. diameter, brought to a volume of 8 ml. with filterd distilled water, and mixed by stirring with a glass rod. One milliliter of 10% stannous chloride was added to this solution with immediate and vigorous stirring and this was followed by the addition of 1 ml. of 5% gum arabic, with equally vigorous and immediate stirring. This concentration of gum arabic stabilizes the tellurium suspension for about 6 hours. Deviations from this standardized technique led to irregular results. Obviously the efficiency of mixing affected the particle sizes in the tellurium suspension. By adding stannous chloride before the gum arabic is added, a higher light absorption and a higher sensitivity are attained. Tellurate is not reduced by stannous chloride under the conditions of this method.

The absorption was compared in the Klett-Summerson photoelectric colorimeter, using light filter No. 42, with that of a blank. The concentration may be read off a graph or computed by multiplying the reading by the factor $\frac{\text{micrograms of tellurium}}{\text{reading}}$, which in this case yielded 0.346. Graph and factor are readily obtained from a series of controls. Plotting of the amount of tellurium, between 5 and 140 micrograms, against the colorimeter reading gave a straight line, which shows that Beer's law is satisfied.

Table I gives a few of the results obtained and shows that tellurium can be determined with an error of ±10%.

Application of the method to the analysis of urine was attempted. Urine blanks show absorption with the filter employed. This difficulty may be overcome by removal of the tellurium by centrifuging after the colorimetric value has been determined and subtracting the reading obtained with the centrifugate.

With the same light filter (No. 42) in the Klett-Summerson photoelectric colorimeter, one may use the method also for the determination of selenium.

ACKNOWLEDGMENT

The author is indebted to John N. Abersold for the description of the air-sampling technique.

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RECEIVED May 2, 1947.

NOTES ON ANALYTICAL PROCEDURES

Direct-Reading Contact Scale for Analysis of X-Ray Spectrometer Charts

WILFRID R. FOSTER, *Ceramic Division, Champion Spark Plug Company, Detroit 11, Mich.*

THE recording x-ray spectrometer (Norelco Geiger-counter x-ray spectrometer) which has recently become commercially available is proving a boon to industrial research and control laboratories because of the speed with which it yields results. By its use a complete diffraction pattern can ordinarily be obtained in a small fraction of the time required with the usual diffraction-camera equipment. The pattern so obtained is analogous to the microphotometer trace of the photographic pattern, but is superior in sensitivity and resolution. Proper interpretation of such a spectrometer pattern involves, of course, the determination of the *d*-value (interplanar spacing) corresponding to each peak, the evaluation of the relative intensities of the peaks, and a check of these data against the A.S.T.M. card file of x-ray diffraction data.

In an industrial testing laboratory, where the spectrometer may be in continuous service, the analysis of the steadily accumulating charts can consume a considerable amount of time. To obviate the tediousness of repeated solution of the Bragg equation by actual calculation, it has become standard practice to use tabulated data (*2*) relating Bragg angle to *d*-value for the particular target material used. This results in a distinct saving of time. However, errors in estimating the value of the Bragg angle or in reading and interpolating the tabulated data are not infrequent.

A time-saving and accuracy-increasing device is a combined two-theta and *d*-value scale which, when appropriately aligned with the spectrometer pattern, allows one to read directly the *d*-values of the peaks. Two-theta, or twice the Bragg angle, is chosen as the unit of measurement of this scale, because the angular motion of the Geiger counter is so measured. Since for most work the speed of the Geiger counter is such as to give a 45-inch chart for 90° of two-theta, the scale is made up on the basis of 2° of two-theta per inch. Since for most work the copper-target x-ray tube is employed, the scale is made up from the data of the appropriate tables for copper Kα radiation.

The scale may be constructed in two sections, the one covering the two-theta range 0° to 45°, the other the range 45° to 90°. Each section will thus be 22.5 inches long. If available, a pair of 24-inch rulers of wood, metal, or plastic, divided into 20 divisions per inch, furnishes an excellent starting point for the construction of these scales. A strip of paper is attached down the center of the ruler so as not to obscure the divisions. Along the center of the strip the values of two-theta are inserted at 5° (2.5-inch) intervals, or at shorter intervals if desired. Along the bottom edge of the strip of paper, opposite every second division of the scale—that is, at intervals of 0.1 inch—the appropriate *d*-values from the tabulated data for copper Kα radiation are inserted. The same procedure is followed along the top edge of the paper, but in this case the *d*-values are inserted for the intervening divisions, those not indexed along the bottom edge.

If rulers of suitable size and scale are not available, two thin unmarked strips of wood, metal, or plastic, and measuring 22.5 by 1.5 inches are secured. Strips of graph paper are cut and pieced together to fit these blank rulers exactly. The two-theta and *d*-values data are entered on the graph-paper strips, in the manner described above. The graph-paper strips are then affixed to the blank rulers by means of Scotch tape.

The accuracy required of these scales is such that any slight variation due to expansion or contraction of the graph paper may be considered negligible. Figure 1 represents a portion of one of the completed scales against the background of a portion of a

typical spectrometer chart. As described and pictured, the scales present complete *d*-value data for successive increments of 0.1° of two-theta, or of 0.05° of theta. By interpolation it is a simple matter to obtain the *d*-values for still smaller increments of Bragg angle. If desired, similar scales may be constructed for use on spectrometer charts obtained by using radiation other than copper Kα, or on charts obtained by using a different speed of Geiger-counter travel.

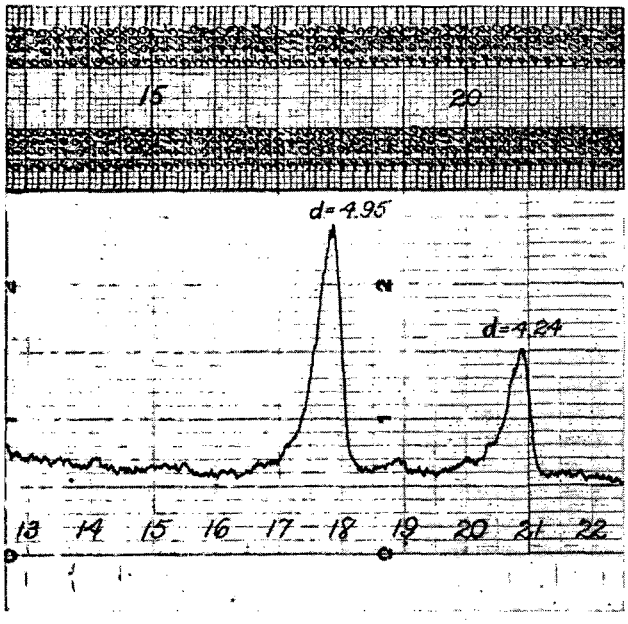

Figure 1. Portion of Completed Scale on Spectrometer Chart

The use of such scales involves a 50% reduction in the time required to analyze an x-ray spectrometer chart, and fewer errors are made in obtaining the *d*-values corresponding to the peaks of the chart. After the scale is set on the chart and carefully matched with the major 5° lines, the *d*-value of a given peak is read directly from the scale. This direct reading of *d*-values greatly reduces the fatigue involved in analyzing spectrometer charts. Accurate estimation of two-theta, conversion to theta, and reference to the tabulated *d*-values, as usually practiced, are no longer necessary. Possible errors in completing any of these steps are thereby eliminated.

Subsequent to the development and use of such scales by the writer, and during the preparation of this paper, a description of somewhat similar scales was published by Brown (*1*). His scales differ somewhat in application from those of the writer. The charts described by Brown are designed to serve merely as an improvement over the usual tabulated data in eliminating the necessity for continually solving the Bragg equation. Those described in this paper, on the other hand, are contact scales, intended primarily for use on spectrometer charts. Thus applied, they possess the additional advantage of giving the *d*-values directly, without necessity for measuring the Bragg angles. Should the user prefer, the system of *d*-value intervals employed by Brown could be substituted for that used by the writer in subdividing such contact scales.

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RECEIVED September 22, 1947. Contribution from the Research Laboratories of the Ceramic Division of the Champion Spark Plug Company.

Identification of Alcohols with 2,4-Dinitrophenylhydrazine

FREDERICK R. DUKE AND ROBERT C. WITMAN
Kedzie Chemical Laboratory, East Lansing, Mich.

THE procedure described here allows the rapid identification of primary and secondary alcohols, and the presence of water does not interfere. The method depends upon the oxidation of the alcohol to the corresponding carbonyl compound, which is identified as the 2,4-dinitrophenylhydrazone.

Reagents. A saturated solution of potassium permanganate in 2 *M* sulfuric acid. A saturated oxalic acid solution. A 1% solution of 2,4-dinitrophenylhydrazine in 2 *M* perchloric acid.

Procedure. To 10 ml. of the permanganate solution is added 0.5 ml. of the alcohol or the equivalent. The mixture is shaken and presently the permanganate color gives way to the brown precipitate of manganese dioxide. Sufficient oxalic acid is added to the mixture to reduce the manganese dioxide. Then 20 ml. of the 2,4-dinitrophenylhydrazine reagent are added. After dilution with 25 ml. of water, the hydrazone is filtered off, washed with water, and recrystallized from alcohol. The melting point identifies the carbonyl compound corresponding to the alcohol.

The procedure was successfully tested on the monohydric primary and secondary alcohols through the pentanols, and in addition, ethyl isopropyl carbinol, 1-hexanol, 1-heptanol, 1-octanol, and 2-octanol. Tertiary alcohols reduce the permanganate very slowly and the products are mixtures of carbonyl compounds. Any easily oxidized substances or carbonyl compounds interfere with the procedure.

Perchloric acid is used in preparing the 2,4-dinitrophenylhydrazine reagent for two reasons: The reagent is readily soluble in aqueous perchloric acid without the aid of organic solvents; and the precipitation of the hydrazone is more rapid and more complete when perchloric acid is used as a catalyst. There is no danger unless the solution is heated for extended periods.

RECEIVED July 24, 1947.

Corrections

In the article on a "Procedure of Determination of the Bromine Number of Olefinic Hydrocarbons" [ANAL. CHEM., 19, 869 (1947)] the titles for the three figures on page 869 should be: Figure 1. Effect of Excess Reagent on Bromine Number. Figure 2. Effect of Temperature on Bromine Number. Figure 3. Effect of Time after Addition of 1-Ml. Excess Reagent on Bromine Number.

On page 871, column 2, line 9 should read Figure 2 instead of Figure 1. Line 18 refers to Figure 3 instead of Figure 2. Line 24 should read "compounds gave *ab*sorptions very close to theoretical." Line 25 should begin Figure 1 instead of Figure 3. Line 37 should read Figures 1 and 4 instead of Figures 3 and 4.

HERBERT L. JOHNSON
RICHARD A. CLARK

Sun Oil Co.
Norwood, Pa.

In the article "Evaluation of Catalysts for Catalytic Cracking" [Rescorla, Ottenweller, and Freeman, ANAL. CHEM., 20, 196 (1948)] reference (1) should read: Alexander, Julian, Jr., and Shimp, H. G., *Natl. Petroleum News*, 36, R537 8 (1944).

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

THIS, the third in a series of monthly summaries of crystal data, is the first to include data contributed by an outside laboratory. The data included were first determined in the Bell Telephone Laboratories by W. L. Bond and were subsequently checked in the Central Research Laboratory of the Canadian Industries Limited by F. W. Matthews and in the Armour Research Foundation Laboratory.

The data reported this month cover two compounds: ethylenediamine *d*-tartrate and dipotassium tartrate hemihydrate. Because both compounds are piezoelectric, a great deal of time and effort have been spent developing methods for the preparation of large single crystals. Nearly all this work has been done in the Bell Telephone Laboratories by A. N. Holden and A. C. Walker. The information included below on solubilities and methods of crystallization were obtained from these two men.

4. Ethylenediamine *d*-Tartrate

Ethylenediamine *d*-tartrate has been grown on a production basis as single crystals weighing up to several pounds (Figure 1); single crystals well over a foot long and weighing over 50 pounds have been prepared on a laboratory scale. In every case these crystals have been grown by slow cooling of saturated aqueous solutions. The general procedure is as follows:

The aqueous solution is saturated at a temperature well above room temperature and the pH is adjusted to very slightly alkaline, using ethylenediamine. The bath is then heated a few degrees and held at this temperature in order to dissolve all crystal nuclei completely. Seed crystals are added and the bath temperature is lowered slowly. The seed crystals dissolve slightly during this period before the solution reaches saturation. This is desirable in order to dissolve extraneous crystalline material from the surface. Finally, the solution is cooled under carefully controlled conditions at a rate of about 0.2° to 0.3° C. per day, so that the solution is always just slightly supersaturated. In general, the process requires from several weeks to several months for commercially useful crystals. The habit varies slightly with pH and a very slightly alkaline solution has been used in order to obtain the proper shape.

Ethylenediamine *d*-tartrate possesses a monohydrate which is stable at temperatures below 41° C. It is essential, therefore, that anhydrous material be grown at temperatures above 41° C. unless great care is taken to avoid nucleation of the monohydrate.

CRYSTAL MORPHOLOGY (determined by W. L. Bond; checked by W. C. McCrone).

Crystal System. Monoclinic sphenoidal.

Form and Habit. Tablets lying on the basal pinacoid {001} showing the forms: orthopinacoid {100}; prisms {110} and {1$\bar{1}$0}; right clinodome {011} and positive hemiorthodome {10$\bar{1}$}.

Axial Ratio. $a:b:c = 1.0194:1:0.6769$.

Interfacial Angles (polar). $110 \wedge \bar{1}10 = 91°\,0'$; $011 \wedge 01\bar{1} = 66°\,14'$.

Beta Angle. $105°\,30' \pm 1'$.

Twinning Plane. 100.

Cleavage. 001 (excellent); 100 (very difficult).

X-RAY DIFFRACTION DATA (determined by W. L. Bond; checked by F. W. Matthews, I. Corvin, and M. Dobbie).

Principal Lines

Index	*d*	I/I_1
100	8.66	0.09
110	6.18	0.29
001	5.78	0.12
...	5.09	Weak
0$\bar{1}$1	4.84	0.11
$\bar{1}$11	4.67	0.69
020	4.42	1.00
101	4.26	0.23
$\bar{2}$01	4.00	0.71
111	3.85	0.53
...	3.74	0.04
$\bar{2}$11	3.65	0.23
$\bar{1}$21	3.45	0.41
201, 220	3.08	0.20
$\bar{1}$02	2.97	0.51
300	2.89	0.36
11$\bar{2}$	2.82	0.02
012	2.73	0.64
...	2.63	0.15
...	2.59	0.22
102	2.53	0.20
112	2.46	0.34
022	2.41	0.28
$\bar{2}$22	2.35	0.24
...	2.30	0.03
...	2.26	0.43
040	2.21	0.03
400	2.16	0.19
202	2.13	0.04
...	2.10	0.09
330	2.06	0.14
402	2.01	0.08
240	1.96	0.05
003	1.91	0.09
013	1.87	0.02
303	1.84	0.06
4$\bar{2}$2	1.82	0.04

Figure 1. Crystals (1, 4, and 5) and Cut Sections (2 and 3) of Ethylenediamine *d*-Tartrate

Space Group. $P2_1$.

Cell Dimensions. $a = 8.974$ Å; $b = 8.803$ Å; $c = 5.959$ Å.

Formula Weights per Cell. 2.

Formula Weight. 210.

Density. 1.538.

OPTICAL PROPERTIES (determined by W. L. Bond and checked by W. C. McCrone).

Refractive Indices (5893 Å.; 25° C.). $\alpha = 1.5086$. $\beta = 1.5893$. $\gamma = 1.5930$.

Optic Axial Angles. (5893 Å.; 25° C.). $2V = 21°$. $2E = 42°$.

Dispersion. Strong horizontal $v > r$.

Optic Axial Plane. $\perp$ to 010 with $\alpha \wedge c = 25°$ in acute β.

Acute Bisectrix. α.

Extinction. $\alpha \wedge c = 25°$ in acute β.

Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.56$. R (calcd.) = 43.95. R (obsd.) = 42.29.

Optical Rotation. Levorotatory, 3.5° per mm. for Hg 5461 Å.

ELECTRICAL PROPERTIES (determined by W. L. Bond).
Piezoelectricity. $+y$ is $-$ on compression. $+y$ is $+$ on compression perpendicular to (001).
THERMAL PROPERTIES (determined by W. C. McCrone).
Ethylenediamine *d*-tartrate decomposes on melting and can be made to crystallize from the melt only with difficulty.

5. Dipotassium Tartrate Hemihydrate

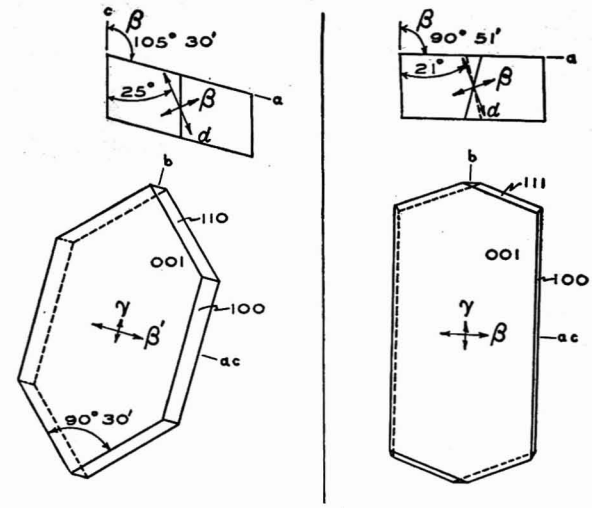
Dipotassium tartrate hemihydrate has also been crystallized at the Bell Telephone Laboratories in the same general manner as ethylene diamine tartrate.

CRYSTAL MORPHOLOGY (determined by W. L. Bond: checked by W. C. McCrone).
Crystal System. Monoclinic sphenoidal.
Form and Habit. Slightly elongated parallel to *b* with the orms: orthopinacoid {100}, basal pinacoid {001}, clinopinacoid {0$\bar{1}$0}, orthodome {101}.
Axial Ratio. $a:b:c$ = 3.068:1:3.981. ($a:b:c$ = 3.0869:1:3.9701) (*1*).
Interfacial Angles (polar). 101 Λ 001 = 51° 45′; $\bar{1}$01 Λ 001 = 52° 50′; 111 Λ 001 = 76° 0′.
Beta Angle. 90° 51′.
Cleavage. 100 (perfect); 001 (perfect).
X-RAY DIFFRACTION DATA (determined by W. L. Bond; checked by L. W. Matthews, I. Corvin, and M. Dobbie).

Principal Lines

Index	*d*	I/I_1
002	10.27	Very weak
...	8.37	Very weak
202	6.06	Very weak
...	5.61	Very weak
...	4.95	0.084
111	4.62	0.19
...	4.19	0.04
400	3.88	0.33
402	3.53	0.19
006	3.33	0.02
404	3.05	1.00
...	2.65	0.06
...	2.60	0.26
600	2.56	...
008	2.49	0.24
...	2.43	0.12
603	2.38	0.02
222	2.32	0.02
009	2.26	0.11
224	2.15	0.14
420	2.11	0.02
422	2.06	0.02
606	2.00	0.07
800	1.94	0.17
...	1.87	0.09
620	1.80	0.08
...	1.77	0.08
...	1.74	...
...	1.71	...
...	1.68	...
...	1.66	...
...	1.62	...
330	1.59	...

Space group. F2 (≡*C*2).
Cell Dimensions. a = 15.490 Å.; b = 5.049 A.; c = 20.101 A



Figures 2 and 3. Orthographic Projection of Simplified Principal Views for Correlation with Optical Properties

2 (left). Ethylenediamine *d*-tartrate
3 (right). Dipotassium tartrate hemihydrate

Formula Weights Per Cell. 8.
Formula Weight. 235.
Density. 1.987 (1.975) (*1*).
OPTICAL PROPERTIES (determined by W. L. Bond; checked by W. C. McCrone).
Refractive Indices (5893 Å.; 25° C.). α = 1.494. β = 1.526. γ = 1.535.
Optic Axial Angles (5893 Å.; 25° C.). $2V$ = 56° (about 62°) (*1*). $2E$ = 100° (102° 16′, red; 104° 21′, green; 106° 21′, violet (*1*).
Dispersion. Very slight horizontal dispersion. $v > r$.
Optic Axial Plane. ⊥ 010 with αΛ c = 21° in acute β.
Acute Bisectrix. α.
Extinction. αΛc = 21° in acute β (21° 21′) (*1*).
Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.518. R (calcd.) = 35.15. R (obsd.) = 35.34.
Optical Rotation. Dextrorotatory, 6.8° per mm. for Hg 5461 Å.
ELECTRICAL PROPERTIES (determined by W. L. Bond).
Piezoelectricity. + is − on compression.
THERMAL PROPERTIES (determined by W. C. McCrone).
Dipotassium tartrate hemihydrate decomposes on melting and crystallizes from the melt only with difficulty.

LITERATURE CITED

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CORRESPONDENCE

Solvents for Extraction of Tocopherol

SIR: In the extraction of tocopherols to be determined by the Emmerie-Engel procedure [Stern and Baxter, ANAL. CHEM., 19, 902 (1947)], Skellysolve B has been the most commonly used solvent. This solvent must be separated from unsaturated hydrocarbons by washing with concentrated sulfuric acid, a tedious and unpleasant procedure at best. Some California petroleum ethers are totally unsuitable, as after several weeks of washing, there are still present measurable amounts of unsaturates as measured by the Emmerie-Engle technique, in glacial acetic acid as solvent.

Commercial grade *n*-hexane or pure grade *n*-heptane, available from the Phillips Petroleum Company, Bartlesville, Okla., has proved satisfactory as a solvent without further purification. Hexane as submitted contained about 1 microgram of reducing material calculated as tocopherol per milliliter or 0.16 microgram calculated as hexene by the Emmerie-Engle procedure, in glacial acetic acid, compared with hexane which had been previously treated with sulfuric acid, washed with potassium hydroxide, and redistilled. A 30-minute color development was employed. After 18 hours only three times the above quantity of reducing material was apparent. Heptane was found satisfactory by comparison with hexane.

S. SHANKMAN

Shankman Laboratories,
Los Angeles, Calif

Joint Meeting of A.S.X.R.E.D. and Crystallographic Society

RALPH H. MÜLLER, Contributing Editor

THE joint meeting of the American Society for X-Ray and Electron Diffraction and the Crystallographic Society of America at Yale University, March 31 to April 3, 1948, occasioned an intensive three-day program of fifty-three invited contributions. A list of titles, authors, and sources of the researches is included in this report for the benefit of analysts who may wish to obtain detailed information about each contribution from the authors. As might be expected from such a very complete and broad treatment of these fields, papers dealt with the properties of specific compounds and systems, new or improved techniques, and new instrumental approaches. In addition, there was a joint symposium on organic structures; in one of the sessions of this symposium, computational methods and the assimilation of data were discussed, and the second was concerned with organic structure determinations.

S. G. Gordon described a simple gnomic transporteur for x-ray Lauegrams which is essentially a simple angle bisector that can be used for converting gnomograms to stereograms. This is an extension of the Clark and Gross system for plotting 90 degrees minus theta.

Prevot and Schwarz solved the problem of detecting small quantities of a light substance either pure or present in a mixture in low concentration. Instead of avoiding preferred orientation, they tried to produce it in order to favor one of the intense lines of the substance present in low concentration. They succeeded in creating this preferred orientation by dissolving the substance under investigation and having the material recrystallized on the surface of the sample holder by evaporation of the solvent. The contribution to background intensity due to the holder was almost completely eliminated by using a very thin collodion film on a wire frame as a support for the sample.

Hamacher and Parrish described an improved Geiger counter spectrometer. Mica to metal seals have made possible the manufacture of improved sealed-off x-ray and Geiger tubes. It is feasible to use 0.0005-inch mica windows which have the advantage of being flat and reduce the inactive length of the Geiger tube to about 2.5 mm. This permits the use of higher pressure and consequently greater absorption in the tube. The performance of the Norelco spectrometer is markedly improved with the new tubes. The use of chlorine to replace methylene bromide as the quenching agent as suggested by H. Friedman has the advantage of a larger sensitive area extending nearly to the cathode, thus affording much better resolution with no sacrifice of intensity. The same authors described a high intensity Geiger counter spectrometer with extended angular range. The water-cooled x-ray tube having four mica windows, which permit viewing the target from 0 to 6 degrees, is run at about seven times greater power input than an air-cooled x-ray tube. The higher intensity permits faster scanning speed or improved precision for the same scanning speed due to faster averaging of the counting statistics. Full wave rectification and the use of an electronic voltage regulator furnish a greater linear range in the intensity measurements with a Geiger tube.

Pepinsky described the design and operation of a grid-controlled fine focus x-ray tube for the stroboscopic diffraction studies of periodic lattice distortions. The control grid turns the tube current on and off and the fine focal spot provides great increase in the net intensity of a parallel collimated beam of small cross section.

At the symposium on computational methods, Donohue and Schomaker discussed the calculation of structure factors by a punched card method. As a typical example of their scheme, 600 (*hkl*) reflections for a crystal with space group $p2_12_12_1$, eight nonequivalent atoms in the general positions of the cell, were calculated. The time required for this calculation is about 24 hours or approximately one tenth of that required for doing the calculation with a hand-operated calculator.

The enormous time saving promised by the electronic digital computor was described by H. A. Goldstine. Speeds many orders of magnitude greater than existing measurements are inherent in these devices and the description of their possibilities evoked the customary headshaking of those who are addicted to, or saddled with, conventional computations.

A startling development by Pepinsky concerning the electronic Fourier synthesis created not only interest but applause. Accurate contour maps of electron densities or Patterson functions can be delineated directly on the screen of a cathode ray tube by triggering coincidence circuits, set at predetermined voltage levels, directly from the synthesized voltage signal formerly applied for intensity modulation to the CR tube grid. The circuits produce 1-microsecond pips each time the synthesized signal crosses one of the preset levels, and these are applied to the CR tube grid in place of the entire synthesized signal. Any desired number of these contours can be delineated simultaneously on a map and they can be differentiated from one another by brightening or dotting various lines. Among other uses, Fourier coefficients for two dimensional functions can be computed.

CRYSTALLOGRAPHIC SOCIETY OF AMERICA

X-Ray Crystallographic Study of the Hydrazides of Some *n*-Aliphatic Acids. E. C. LINGAFELTER AND L. H. JENSEN, University of Washington.

Preliminary Report on the Structure of Tourmaline. G. HAMBURGER AND M. J. BUERGER, Massachusetts Institute of Technology.

Structural Crystallography of Lazulite and Veszelyite. L. G. BERRY, Queen's University, Kingston.

Atacamite Twinning. A Notorious Case on Appeal. J. D. H. DONNAY, The Johns Hopkins University.

Analysis of Twinned Intergrowths of Crystals. C. B. SLAWSON, University of Michigan.

Some Crystalline Hemoglobins. DOROTHY WRINCH, Smith College.

Collection and Publication of Crystallographic Data. W. C. MCCRONE, Armour Research Foundation.

Index of Refraction Measurements on Isometric Opaque Minerals. A. L. HOWLAND AND M. D. QUIGLEY, Northwestern University.

Graphical Method for Transforming Hexagonal Bravais-Miller Indices and Rhombohedral Miller Indices. L. S. RAMSDELL, University of Michigan.

Simple Gnomonic Projector for X-Ray Lauegrams. S. G. GORDON, Academy of Natural Sciences of Philadelphia.

Permanent Polarization of a Barium Titanate Single Crystal. A. P. DEBRETTEVILLE AND H. ESTELLE, Signal Corps Engineering Laboratories.

Crystallography of the Polymorphic Forms of Barium Titanate. T. EVANS, JR., AND R. D. BURBANK, Massachusetts Institute of Technology.

Ferroelectric Activity of Barium Titanate. B. T. MATTHIAS, Massachusetts Institute of Technology (now at Bell Telephone Laboratories).

Progress in Silicate Structures. Address of the Retiring President (C.S.A.). J. W. GRUNER, University of Minnesota.

Crystal Structures of Ammonium and Potassium Molybdotellurates. HOWARD T. EVANS, JR., Massachusetts Institute of Technology.

Crystallization of Simple Salt Solutions. H. THIELSCH, Lehigh University.

Heat of Crystallization of Potassium Nitrate. R. M. GARRELS, V. C. WILLIAMS, AND I. O. STINE, Northwestern University.

Some Data on the Photoelastic Study of Transparent Solids. C. C. WEST AND A. S. MAKAS, Polaroid Corp.

Behavior of Punch Figures in Thallium Halide Crystals. J. W DAVISSON AND B. HENVIS, Naval Research Laboratory.

The Lattice of $AlPO_4$. A. P. DEBRETTEVILLE, JR., Squier Signal Laboratory.

Crystal Chemical Relations in Inorganic Piezoelectric Mate-

rials. S. ZERFOSS, L. R. JOHNSON, P. EGLI, AND P. L. SMITH, Naval Research Laboratory.

The Vibrations of Crystals. N. CHAKO, Alabama Polytechnic Institute.

Observations on Piezoelectric Crystals. K. S. VAN DYKE, Wesleyan University.

Piezoelectric Effects in Some Unipolar Crystals. HANS JAFFE, Brush Development Co.

A.S.X.R.E.D.

Achromatization of Diffraction Lines. H. EKSTEIN AND S. SIEGEL, Armour Research Foundation.

Statistical Fluctuation of Intensity in Debye-Scherrer Lines Due to Random Orientation of Crystal Grains. H. EKSTEIN, Armour Research Foundation.

Preferred Orientation and Sample Preparation for the Geiger Counter Spectrometer. ANNETTE PREVOT AND G. SCHWARZ, The Johns Hopkins University.

Improved Geiger Counter Spectrometer. E. A. HAMACHER AND W. PARRISH, Philips Laboratories, Inc.

High Intensity Geiger Counter Spectrometer with Extended Angular Range. W. PARRISH AND E. A. HAMACHER, Philips Laboratories, Inc.

Design and Operation of a Grid-Controlled Fine-Focus X-Ray Tube. R. PEPINSKY, Alabama Polytechnic Institute.

Observations on Geiger Counter Characteristics by Means of a Grid-Controlled X-Ray Tube. R. PEPINSKY AND H. M. LONG, JR., Alabama Polytechnic Institute.

A Curved Crystal Monochromator. A. GUINIER AND G. FOURNET, Laboratoire d'Essais, Paris (read by I. FANKUCHEN).

Water Uptake of Collagen as Evidenced by Its Low Angle X-Ray Diffraction. BARBARA A. WRIGHT, United Shoe Machinery Corp.

Nature of the Order of Large Size Exhibited by Collagen Fibrils. R. S. BEAR AND O. E. A. BOLDMAN, Massachusetts Institute of Technology.

The Crystal Structure of a Metanilamidopyrimidine. J. SINGER AND I. FANKUCHEN, Franklin Institute of Pennsylvania and Polytechnic Institute of Brooklyn.

Structure of Crystalline Dekaborane. J. S. KASPER, C. M. LUCHT, AND D. HARKER, General Electric Co.

A New Modification of Sodium. C. S. BARRETT, University of Chicago.

The Electron Shift in SiO_2 Due to Its Chemical Bond. R. BRILL, Squier Signal Laboratory.

Electron Diffraction Studies of Manganese Precipitation in Magnesium Alloys. I. STURKEY, Dow Chemical Co.

A New Procedure for Calculating Radial Distribution Curves from Electron Diffraction Data. JEROME AND ISABEL KARLE, Naval Research Laboratory.

Apparatus for Obtaining a Powder Diffraction Pattern from a Single Crystal. F. W. MATTHEWS AND A. O. MCINTOSH, Canadian Industries, Ltd.

Use of Microwave Diffraction in Structure Analysis. W. L. ROTH, General Electric Co.

JOINT C.S.A. AND A.S.X.R.E.D.

Crystals Based on the Silica Structures. M. J. BUERGER, Massachusetts Institute of Technology.

Hypothetical Disorder and Its Use in Crystal Structure Determination. D. HARKER, General Electric Co.

Growing Crystals from Solution. A. N. HOLDEN, Bell Telephone Laboratories.

Crystal Chemistry of the Elements from Actinium to Americium. W. H. ZACHARIASEN, University of Chicago.

Calculation of Structure Factors by a Punched Card Method. J. DONOHUE AND V. SCHOMAKER, California Institute of Technology.

Punched Card Methods of Fourier Analysis. L. H. THOMAS, Watson Scientific Computing Laboratory, Columbia University.

Electronic Digital Computer. H. H. GOLDSTINE, Institute for Advanced Study.

The Electronic Fourier Synthesizer. R. PEPINSKY, Alabama Polytechnic Institute.

Phase Determination with the Aid of the Implication Theory. M. J. BUERGER, Massachusetts Institute of Technology.

Relations between the "Phase Inequalities," the Patterson Function, and Buerger Implications. D. HARKER, General Electric Co.

Morphological and Optical Characterization of Organic Crystals. W. C. MCCRONE, Armour Research Foundation.

Application of Fourier Transforms to X-Ray Structure Analysis. DOROTHY WRINCH, Smith College.

Groping Stages in Some Organic Crystal Structure Determinations. J. D. H. DONNAY AND C. P. FENIMORE, The Johns Hopkins University.

A Complete Structure Determination. BARBARA ROGERS-LOW, Harvard Medical School.

Annual Symposia of Division of Analytical and Micro Chemistry

The Division of Analytical and Micro Chemistry has appointed the following Committee on Annual Symposia, under the chairmanship of Beverly L. Clarke, Merck & Co., Rahway, N. J.:

Philip J. Elving, Purdue University
Lawrence T. Hallett, General Aniline & Film Co.
I. M. Kolthoff, University of Minnesota
Jesse W. Stillman, E. I. du Pont de Nemours & Co.
Edward Wichers, National Bureau of Standards
H. H. Willard, University of Michigan

At a recent meeting in Chicago the committee made tentative plans for a Symposium on Organic Reagents in Chemical Analysis, to be held in June 1949 at a college or university in southern New England. S. E. Q. Ashley, Research Laboratories, General Electric Co., Pittsfield, Mass., has been chosen to act as chairman of the symposium.

Book Reviews

Probit Analysis. A Statistical Treatment of the Sigmoid Response Curve. *D. J. Finney.* xiii + 256 pages. Macmillan Co., 60 Fifth Ave., New York 11, N. Y. (London, Cambridge University Press), 1947. Price, $3.75.

The subtitle of this book is "A Statistical Treatment of the Sigmoid Response Curve." Since data obtained in toxicological, insecticidal, and fungicidal tests plot as sigmoid curves, this treatise is of interest to chemists, entomologists, and pharmacologists. In this first book on the subject the author aims "to give a systematic account of the theory and practice of probit analysis, including as much as possible of the most recent extensions and refinements, in such a form that it may be understood by biologists, chemists, and others who have some knowledge of elementary statistical procedure."

To many the term "probit" (meaning in effect, "probability unit") is new. Finney shows that the underlying principle was recognized by psychophysicists in 1860 but the subsequent history was one of discovery and repeated rediscovery until 1933. The numerous applications developed since then attest to the value of the probit "transformation" which rectifies the natural sigmoid curve into a straight line amenable to simple algebraic expression. Finney originated many of these procedures and gives 33 practical examples ranging from the simplest to the most complex situations. One such example shows how to make allowance in insecticide tests for the deaths occurring in the control group from natural causes. The examples are down-to-earth and focus attention on a frequently overlooked fact that when the basic data are good only to the nearest 1%, approximations are sufficient. No one should expect to find solved examples of all the problems to be encountered in quantitative toxicology but this volume is a handbook for every entomologist who tests insecticides. Chemists synthesizing economic poisons may reasonably insist that their products be tested by the methods Finney describes.

The author devotes a chapter to factorial design to emphasize the necessity for proper planning with respect to the many variables that may affect insecticidal experiments.

The book is virtually free of typographical errors, one benefit resulting from the otherwise regrettable delay of two years in publishing the work. Several useful tables are included.

LLOYD C. MILLER

Method of Semi-Quantitative Spectrographic Analysis. *C. E. Harvey.* 285 pages. Applied Research Laboratories, Glendale, Calif., 1947. Price, $10; reference sample, $15.

This book describes a spectrographic method of semiquantitative analysis that requires no prepared standards and provides approximate quantitative analysis of most elements which can be detected spectrographically in the direct current arc source. The last 258 pages are tables of sensitivity factors for five groups: nonferrous, ferrous, alkali, refractory oxides, and rare metal.

THE ANALYST'S CALENDAR . . .

Symposium on Electron and Light Microscopy

The following preliminary program has been arranged for the symposium emphasizing the supplementary relationship between electron and light microscopy, sponsored by Armour Research Foundation of the Illinois Institute of Technology and the Physics Department of the institute. Meetings are to be held at the Stevens Hotel, Chicago, Ill., June 10, 11, and 12.

Thursday, June 10

9:00 to 10:00 A.M. Registration, North Ballroom Corridor, 3rd Floor, Stevens Hotel

Morning Session, 10:00 to 12:00

Presiding, P. L. Copeland, Physics Department, Illinois Institute of Technology

H. T. HEALD, President, Illinois Institute of Technology.
H. A. LEEDY, Acting Director, Armour Research Foundation of Illinois Institute of Technology.
W. KINSINGER, Hercules Powder Co.
C. W. MASON, Cornell University.
ROBLEY C. WILLIAMS, University of Michigan.

Afternoon Session, 2:00 to 5:00

Presiding, A. L. Ellis, International Harvester Co.

Special Techniques Including Dark Field Electron Microscopy. C. E. HALL, Massachusetts Institute of Technology.
Surface Studies. R. D. HEIDENREICH, Bell Telephone Laboratories.
Phase Microscopy. C. P. SAYLOR, National Bureau of Standards.
Fiber Studies. C. MARESH, American Cyanamid Co.
Structure of Fibers. C. W. HOCK, Hercules Powder Co.

Evening Session, 8:00 to 10:00

Presiding, W. Kinsinger, Hercules Powder Co.

Resinography. T. G. ROCHOW, American Cyanamid Co.
Microradiography. E. A. WOOD, Bell Telephone Laboratories.
Applications of Microscopy to Polymorphism of Tristearine Type Fats. O. T. QUIMBY, Procter & Gamble.

Friday, June 11

Morning Session, 9:00 to 12:00

Presiding, C. P. Saylor, National Bureau of Standards

Particle Size. P. L. COPELAND, Illinois Institute of Technology.
Shapes of Particles by Electron Microscopy. A. F. KIRKPATRICK, American Cyanamid Co.
Electron Microscopic Studies of Photographic Gelatin. F. A. HAMM, General Aniline and Film Corp.
Biological Applications. T. F. ANDERSON, Johnson Foundation, University of Pennsylvania.
Ultraviolet, Visible, and Infrared Microscopy. K. F. HEINICKE, Bausch & Lomb Optical Co.

Afternoon Session, 2:00 to 4:30

Presiding, Robley C. Williams, University of Michigan

Objective Lens Design. L. V. FOSTER, Bausch & Lomb Optical Co.
Polarized Light. E. E. JELLEY, Eastman Kodak Co.
Sample Preparation for Microtomy. E. F. FULLAM, General Electric Research Laboratory.
Metal Films. P. G. WILKINSON, Naval Research Laboratory.

Friday Evening, Banquet in North Ballroom

Panel Meetings

On Saturday, June 12, the symposium will be devoted to panel sessions conducted concurrently and separately.

I. Instrumentation for Electron Microscopy. (High vacuum techniques, electron diffraction, electron optics.)
II. Instrumentation for Crystal Optics.
III. Applications of Microscopy to Biology.
IV. Preparation of Metal Surfaces for Microscopic Examination.
V. Problems in High Speed Microtomy.

Participating in the discussion will be:

L. S. Birks, Naval Research Laboratory.
C. I. Reed, University of Illinois.
G. E. Pellissier, Carnegie Illinois Steel Research Laboratory, Pittsburgh, Pa.
J. R. Vilella, U. S. Steel Corp. Research Laboratory, Kearny, N. J.
Perry C. Smith, Radio Corp. of America.
Philip Nolan, Farrand Optical Co., Inc.
A. L. Ellis, International Harvester Co.
M. Baeyertz, Armour Research Foundation, Metals Division.
R. R. Allen, Custom Scientific Instruments.
W. Fullam, Hemsdale International, Inc.

General Electric Co., Distillation Products, Inc., National Research Corp., and North American Philips have expressed a desire to send representatives who will participate in the panel discussions.

An instrument display containing equipment of interest to workers in microscopy will be an additional feature of the symposium. The following organizations have expressed interest in this display:

American Optical Co.
Bausch & Lomb Optical Co.
Custom Scientific Instruments
Distillation Products, Inc.
Eastman Kodak Co.
General Aniline and Film Corp.
General Electric Co.
Hemsdale International, Inc.
National Research Corp.

Arrangements have been made to exhibit photomicrographs and electron micrographs near the meeting place. Anyone wishing to exhibit should submit material to Howard T. Betz, Armour Research Foundation, 35 West 33rd St., Chicago 16, Ill., before June 6, 1948.

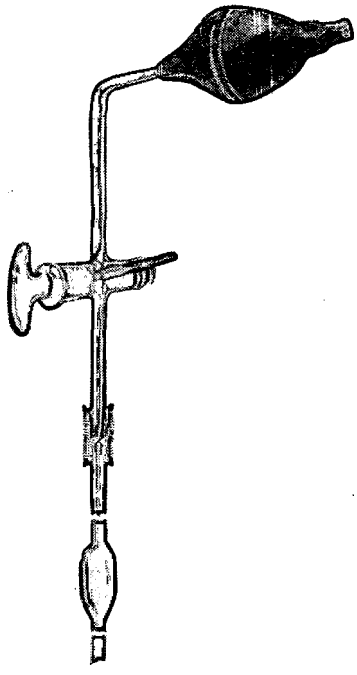
Further information on the program may be obtained from W. C. McCrone or C. F. Tufts, Armour Research Foundation, Chicago.

Symposium on Spectroscopic Equipment. Polytechnic Institute of Brooklyn, Brooklyn, N. Y., May 22.
International Congress of Analytical Chemistry. Utrecht, Netherlands, June 1 to 3, 1948.
Symposium on Electron and Light Microscopy. Armour Research Foundation and Physics Department of Illinois Institute of Technology, Chicago, Ill., June 10, 11, and 12.
Symposium on Nucleonics and Analytical Chemistry. Division of Analytical and Micro Chemistry, Northwestern University, Evanston, Ill., Aug. 13 and 14.

AIDS FOR THE ANALYST

A Safety Pipetter. A. A. Singer and Morris B. Jacobs, Chemical Laboratory, Department of Health, New York, N. Y.

MANY safety pipetters (*2–8, 11–13*) have been proposed, patented, and marketed commercially. They are, however, inadequate for many corrosive liquids, particularly reagents such as bromine, Hanus solution, Wijs solution, and standard iodine solutions. In pipetters in which the pipet is held by a rubber bushing (Fisher Scientific Co.) the bushing often deteriorates relatively rapidly In those in which a rubber aspirator is placed directly in the line (*9, 10,* and automatic pipet, Scientific Glass Apparatus Co.) the aspirator bulb generally deteriorates and both bulb and valves have to be replaced in entirety. Those having a glass syringe (interjoint safety pipet, Scientific Glass Apparatus Co.) generally seize after three or four transfers and have to be rewashed; while those having equivalent three-way stopcocks (*1*) often present a difficult problem of adjustment to the mark.



In order to avoid these difficulties the safety pipetter illustrated has been devised. It consists of a three-way stopcock equipped with a ⅌ externally ground connecting tube, a rubber aspirator bulb, and a capillary tube on each orifice. The apparatus is mounted on a stand.

Operation. A pipet, preferably one with a standard-taper, internally ground connection, is attached to the connecting tube and its tip is inserted into the reagent or other corrosive solution. The stopcock is turned to connect with the aspirator bulb and a few compressions and successive releases bring the solution above the graduated mark. The stopcock is turned to connect with the capillary orifice. Depending on the size of the capillary and the viscosity of the reagent or liquid being transferred, the reagent is adjusted to the mark rapidly or slowly. If a larger capillary is used the rate of adjustment can be controlled by the index finger. The aspirator bulb can be equipped with the usual valves, but only one exit valve is necessary. Standard-taper connections are not essential, for with rubber tubing ordinary pipets serve equally well. The standard-taper externally ground connecting tube can be sealed directly to the three-way stopcock, but a rubber hose connection permits greater flexibility. In an analogous way the glass capilary can be sealed directly to the three-way stopcock, but the rubber hose connection permits ready exchange of capillaries of various sizes.

This device has been in use in one laboratory for several months and has required no repair or replacement of parts It can be easily disassembled for cleaning when necessary.

ACKNOWLEDGMENT

The authors acknowledge the able assistance of Anna V. Marshall in performing tests with this device.

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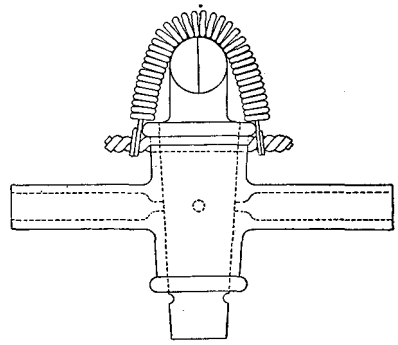
Spring Clamp for a Pressure Stopcock. W. M. Langdon and W. W. Shuster, Rensselaer Polytechnic Institute, Troy, N. Y.

SEVERAL methods [Connelly, J. A., IND. ENG. CHEM., ANAL ED., **15**, 200 (1943); Hamilton, R. H., *Ibid.*, **19**, 360 (1947)] have been described for clamping a glass stopcock for pressure work. The method described here, which may be applied to equipment in place, requires simply the use of a piece of stiff wire and a small spring (or rubber band).



The clamp consists of a stiff wire loop fastened loosely to the upper part of the stopcock barrel. A wire spring (or rubber band) is held over the handle by means of the wire ears. In use, the spring is adjusted to remain perpendicular to the handle, so that stopcock setting is not disturbed. No difficulty has been occasioned in sensitive adjustments, such as those required in still-head reflux control.

The ease of applying the spring clamp compensates for its more awkward action when compared to other devices. A rubber band, which may be cut from Gooch tubing, is more convenient to use if the stopcock does not run hot, which would cause deterioration of the rubber The strength of the band can be varied by changing its width. As grease is gradually forced out of the stopcock in extended use, the barrel should be greased every few days.