ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Lundell Receives the Fisher Award

CHEMISTS everywhere are rejoicing at the signal honor bestowed on G. E. F. Lundell, former chief of the Chemistry Division of the National Bureau of Standards, in Atlantic City this month.

Second recipient of the Fisher Award in Analytical Chemistry, Dr. Lundell richly deserves the plaudits of his fellow scientists, not only for his many and varied scientific contributions, but for the inspiring guidance afforded younger associates both in and out of the bureau.

Much of the growth and influence of the National Bureau of Standards must be attributed to Dr. Lundell. Sacrificing an assured brilliant future as a teacher at Cornell, Dr. Lundell heeded the call of the renowned W. F. Hillebrand to assist in enlarging the scope of the bureau's work in analytical chemistry, and particularly the standard sample program. To a great extent the present international reputation of the bureau is due to the caliber of the man who headed its Chemistry Division.

Dr. Lundell is more than just an able administrator. His unofficial title, "dean of the world's inorganic analytical chemists," has been earned by his scientific accomplishments, universally recognized by thousands, in the profession of analytical chemistry.

He is not only an administrator and a scientist, but one of the world's foremost authors in the field of analytical chemistry. "Applied Inorganic Analysis," begun in 1923 in co-authorship with Dr. Hillebrand, was completed by Dr. Lundell in 1929 and is familiarly referred to as the "analysts' bible." "The Chemical Analysis of Iron and Steel," written jointly with H. A. Bright and J. I. Hoffman, and "Outlines of Methods of Chemical Analysis," jointly authored with Dr. Hoffman, are standard works of reference in every analytical laboratory.

A more recent volume, "A.S.T.M. Methods of Chemical Analysis of Metals," does not bear his name as author but owes its existence, nevertheless, largely to Dr. Lundell's efforts in organizing Committee E-3 of the A.S.T.M. and to his leadership of the committee over a period of 13 years.

The citation accompanying the Fisher Award to Dr. Lundell states in part: "for his direct contribution to applied inorganic analysis by devising new and improved old methods, and for his ability to train young men in the ways of analytical chemistry."

His almost uncanny ability to select able associates has been demonstrated again and again, very dramatically in the accomplishments of his staff during World War II, when methods were developed for analyzing and purifying graphite and uranium, contributions that opened the way to the use of these materials in the first atomic pile.

Several important honors have come to Dr. Lundell over the years; indicating the great esteem with which he is held by his fellow scientists. In 1932 he received the Hillebrand Prize of the Chemical Society of Washington; in 1941 he was awarded an honorary doctor of science by Fordham University; he is a past president of the A.S.T.M. For a number of years he served as an associate editor of the *Journal of the American Chemical Society*, and was one of the editors of the glass division of the American Ceramic Society.

Dr. Lundell's active support of the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY, now ANALYTICAL CHEMISTRY, is well known. His sustained interest in the work of the Advisory Board and his constructive influence have added to the stature of the publication.

Your editor personally is indebted to Dr. Lundell for many acts of direct assistance and guidance. His calm, deliberate, and objective thinking was in evidence at the meetings of the Advisory Board and rare, indeed, were the occasions when he could not lay aside the immediate and pressing responsibilities of his work at the Bureau of Standards in order to attend the meetings of the board. It is characteristic of the man never to assume an office without fulfilling the obligations attached to it.

Dr. Lundell retired from the exhausting duties as chief of the Chemistry Division of the National Bureau of Standards in the latter part of last year. His retirement leaves a void not only in the bureau, but in every gathering of analytical chemists. His inspiring influence, his kindly and gentle attitude toward his fellow workers, his special interest in the younger men of the profession, will remain for years a heritage all will treasure.

Mass Spectrometer Analyses of Oxygenated Compounds

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DURING the past several years there has been an increasing need in industry for rapid, accurate methods of analyzing complex mixtures of organic chemical compounds. Conventional chemical and distillation procedures are frequently tedious and time-consuming, and in many cases are not capable of positive identification or measurement of individual compounds with the accuracy needed for material balance studies. In addition, the large quantities of sample required by distillation and some chemical procedures limit the usefulness of these techniques for small scale operations. In the case of oxygenated compounds, the available methods are usually limited to the determination of functional groups, so that even when distillation is employed as an auxiliary tool for analysis of individual components, the frequent formation of constant-boiling mixtures throws some doubt on the validity of the data.

Although the mass spectrometer was developed originally for use in the analysis of gases, it is finding increasing utilization in the determination of heavier hydrocarbons and volatile organic chemicals (*3*, *6*, *7*). In this laboratory, the application of both mass and infrared instruments to the analysis of oxygenated compounds has been studied. To date, infrared spectrometry in this field has been concerned primarily with the analysis of oxygenated compound types (*1*); although these procedures have been found very useful, they are in general restricted to the determination of classes of compounds and hence are subject to many of the same limitations as are chemical analyses. Mass spectros-

Table I. Polyisotopic Mass Spectra

Compounds	Ketones			Aldehydes										
	Acetone	Methyl ethyl ketone	Methyl propyl ketone	Acetaldehyde	Propionaldehyde	*n*-Butyl aldehyde	Isovaleric aldehyde	Dimethyl Acetal	Acetaldehyde Diethyl Acetal	Methyl	Ethyl	*n*-Propyl	Isopropyl	*n*-Butyl
Purity	99.5	95	95	98	98	95	95	95	98	99.5	99.5	95	99	95
Source[b]														
m/e	1	2	3	2	2	2	2	2	2	1	4	2	1	2
26	0.300	0.318	0.196	0.292	0.614	0.235	0.148	0.227	0.308	...	0.461	0.401	0.166	0.194
27	0.472	1.08	1.316	0.199	2.16	1.907	1.59	0.694	1.23	0.036	1.46	1.68	1.205	1.70
28	0.224	0.462	0.430	0.468	3.36	0.780	0.432	1.280	0.726	0.669	1.04	1.17	0.257	0.799
29	0.295	2.24	2.00	5.38	4.79	1.959	2.30	2.70	2.00	3.13	2.01	1.919	0.645	1.247
30	0.005	0.051	0.037	0.050	0.275	0.030	0.048	0.258	0.139	0.583	0.472	0.268	0.030	0.080
31	0.070	0.100	0.041	0.030	0.201	0.131	0.105	2.910	1.83	7.43	8.61	14.01	0.592	3.56
32	...	...	...	...	...	...	...	0.771	...	5.33	0.097	0.335	...	0.061
33	...	...	...	...	...	...	...	0.083	...	0.082	...	0.164	...	0.356
37	0.155	0.053	0.042	...	0.093	0.096	0.061	...	...	...	...	0.109	0.133	0.062
38	0.178	0.069	0.087	...	0.100	0.167	0.193	...	...	...	0.007	0.165	0.210	0.109
39	0.319	0.282	0.579	0.021	0.286	0.878	1.23	...	...	...	0.068	0.648	0.730	0.773
40	0.070	0.017	0.073	0.048	0.058	...	0.041	0.005	...	...	...	0.178	0.177	0.191
41	0.188	0.186	1.041	0.351	0.150	2.13	3.49	0.107	0.093	...	0.119	1.203	1.13	3.53
42	0.648	0.598	0.500	0.745	0.165	0.488	0.732	0.306	0.300	...	0.388	0.752	0.758	1.67
43	9.84	14.67	12.72	2.59	0.206	2.99	3.03	1.577	2.56	...	1.09	0.542	2.21	3.25
44	0.232	0.477	0.381	4.91	0.063	3.72	4.34	0.127	2.77	...	0.902	0.140	0.446	0.759
45	0.030	0.130	0.162	0.133	0.117	1.89	0.554	0.087	3.57	...	4.49	0.725	11.83	0.390
46	...	...	...	...	...	...	...	...	0.421	...	2.12	0.044	0.275	0.023
47	...	...	...	...	...	...	...	0.481	...	...	...	...	...	...
50	...	0.082	0.022	...	...	0.036	0.101	...	...	...	...	...	...	0.060
51	...	0.050	0.005	...	...	0.012	0.095	...	...	...	...	...	...	0.049
52	0.010	0.016	...	...	0.014	...	0.039	...	...	...	...	...	...	0.016
53	0.032	0.075	0.062	...	0.048	0.036	0.156	...	...	...	...	...	...	0.081
54	...	0.029	...	...	..	0.053	...	...	...	...	...	...	...	0.062
55	0.036	0.090	0.114	...	0.130	0.061	0.334	...	...	...	...	0.118	0.028	0.885
56	0.006	0.026	0.120	...	0.063	...	1.74	...	...	...	...	0.085	0.055	5.21
57	0.113	1.06	3.33	...	0.953	0.869	1.56	0.093	0.044	...	...	0.384	0.050	0.520
58	4.05	0.025	1.177	...	3.65	0.018	2.34	1.38	...	...	...	0.452	0.255	0.035
59	0.132	...	0.038	...	0.128	...	...	5.67	...	...	...	2.132	0.537	0.009
60	...	...	...	...	...	...	...	0.196	...	...	...	1.512	0.056	...
61	...	...	...	...	...	...	...	0.018	...	...	...	...	...	...
68	...	...	...	...	...	...	0.020	...	...	...	...	...	...	...
69	...	...	...	...	...	0.020	0.029	...	...	...	...	...	...	...
70	...	...	...	...	...	0.024	0.095	...	...	...	...	...	...	0.089
71	...	0.157	1.169	...	...	0.268	0.728	...	...	...	...	...	...	0.040
72	...	3.64	0.033	...	...	2.77	0.046	...	1.41	...	...	...	...	0.351
73	...	0.168	...	...	...	0.106	...	...	1.71	...	...	...	...	0.109
74	...	0.004	...	...	...	...	0.014	...	0.062	...	...	...	...	0.044
75	...	...	...	...	...	...	...	1.96	0.100	...	...	...	...	...
84	...	...	...	...	...	...	...	...	...	...	...	...	...	...
85	...	...	...	...	...	...	0.051	...	...	...	...	...	...	...
86	...	...	3.21	...	..	...	0.352	...	...	...	...	...	...	...
87	...	...	0.174	...	...	...	...	...	...	...	...	...	...	...
88	...	...	...	...	...	...	...	0.094	...	...	...	...	...	...
102	...	...	...	...	...	...	...	...	0.448	...	...	...	...	...
103	...	...	...	...	...	...	...	...	...	...	...	...	...	...

[a] Expressed as divisions of peak height per division of mass 78 peak height from benzene for mixtures containing 1 part of benzene and 20 parts (by volume) of oxygenated compound.
[b] 1. J. T. Baker Chemical Company. 2. Eastman Kodak Company. 3. Paragon Testing Laboratories. 4. U. S. Industrial Chemicals, Inc.

Through the use of special techniques, including the utilization of liquid internal standards and operation of the gas-handling system at elevated temperatures, mass spectrometric methods have been developed for the quantitative determination of individual oxygenated compounds containing five or less carbon atoms per molecule. In mixtures containing six or less components the average accuracy (deviation from the true composition) is better than ±1% based on the total sample; for more complex mixtures, the accuracy is in the order of ±2% or less. Water in moderate concentrations has a negligible effect on the accuracy of the oxygenated compound determinations, and water itself is determined with an average deviation of ±1%. Time requirements for a complete analysis of a six-component mixture are about 2 hours, and only a few drops of sample are needed. Calibration data for ten alcohols, three ketones, six aldehydes, three esters, four acids, and two ethers are presented.

copy, on the other hand, has been found useful for the identification and measurement of individual oxygenated compounds in complex mixtures.

In samples containing six or less components, such as are normally obtained from precise distillations, the individual oxygenated compounds can be determined with an average deviation of ±1% or less from the true composition, even in the presence of moderate quantities of water; water itself can be determined with a deviation of about ±1%. More complex samples containing up to eleven components can be determined with a deviation of ±2% or less. Total time requirements for an analysis range from 2 to 3 hours, depending upon their complexity, and sample requirements are only a few drops of liquid. Although the method has been applied thus far only to oxygenated compounds containing five or less carbon atoms, it is believed that the range can be extended to include compounds of higher molecular weight.

APPARATUS AND EXPERIMENTAL TECHNIQUES

General operating principles of the mass spectrometer have been described by Hipple (*5*) and Washburn (*9*). Initial work on the analysis of oxygenated compounds and especially alcohols was attempted in this laboratory on the Consolidated Type 21-101

of Oxygenated Compounds[a]

Alcohols					Esters			Acids				Ethers	
Iso-butyl 95	*sec*-Butyl 95	*tert*-Butyl 95	*n*-Amyl 95	Iso-amyl 95	Methyl acetate 95	Ethyl acetate 99	Ethyl pro-pionate 95	Formic 90	Acetic 99.5	Pro-pionic 95	Butyric 98	Iso-propyl 95	Diethyl 99
2	2	2	2	2	2	1	2	1	1	2	1	2	1
0.146	0.220	0.140	0.140	0.087	0.044	0.195	0.353	...	0.054	0.434	0.081	0.058	0.267
1.85	1.12	0.616	1.072	1.011	0.069	0.692	1.620	...	0.222	1.325	0.376	0.912	1.527
0.545	0.541	0.517	0.588	0.532	0.531	0.629	1.323	1.349	0.891	2.62	0.417	0.184	0.523
0.976	1.13	0.722	2.24	1.753	0.815	1.526	7.77	1.276	0.483	1.91	0.332	0.220	3.41
0.056	0.044	0.021	0.081	0.051	0.084	0.062	0.258	0.050	0.034	0.326	0.017	...	0.155
2.64	1.64	1.871	2.03	1.193	0.584	0.133	0.280	0.093	0.263	0.108	...	0.372	8.63
0.065	0.008	0.018	0.034	0.012	0.177	0.008	...	...	0.005	...	...	...	0.089
2.96	0.022	...	0.032	0.051	...	...	...	...	0.026	...	...	...	...
0.084	0.044	0.095	0.041	0.039	...	...	...	0.004	0.007	0.017	0.003	0.013	...
0.163	0.079	0.168	0.081	0.092	...	...	...	0.013	0.022	0.037	...	0.052	...
1.00	0.573	1.96	0.743	0.901	0.101	0.016	0.022	0.052	0.128	0.063	...	0.665	0.035
0.227	0.092	0.236	0.147	0.148	...	0.003	...	...	0.064	0.008	0.219	0.101	...
4.04	2.00	3.48	2.74	2.83	0.138	0.069	0.089	0.113	0.468	0.123	0.016	1.594	0.452
3.46	0.264	0.267	4.53	2.71	1.40	0.576	0.159	...	0.872	0.131	0.257	0.445	0.597
6.17	2.01	0.761	1.37	2.49	16.12	11.37	0.510	...	3.09	0.262	0.229	5.37	0.596
0.289	1.04	0.054	1.19	0.723	0.496	0.371	0.112	5.80	1.065	1.09	0.268	0.460	0.300
0.300	11.06	0.035	0.335	0.768	0.218	1.761	1.189	0.969	3.03	1.52	0.425	13.21	3.24
...	0.248	...	0.022	0.235	...	0.044	0.037	1.618	0.047	0.248	0.373	0.293	0.068
...	...	...	...	...	...	...	...	...	...	...	...	...	...
0.046	0.109	0.162	0.059	0.063	0.115	...	...	...	...	0.024	0.025	0.003	...
0.040	0.090	0.133	0.070	0.070	0.132	...	...	...	...	0.029	0.022	0.004	...
0.001	0.024	0.032	0.027	0.018	0.171	...	...	...	...	0.037	0.027	0.003	...
0.086	0.134	0.159	0.123	0.174	...	...	...	...	0.019	0.049	...	...	...
0.010	0.047	0.056	0.073	0.062	...	...	0.007	...	0.006	0.022	...	...	...
0.460	0.542	0.584	3.34	3.68	...	...	0.134	...	0.254	0.413	0.144	0.024	...
0.489	0.667	1.396	0.850	1.285	...	...	0.356	...	0.082	0.418	0.054	...	...
0.281	0.449	0.656	1.33	2.04	...	...	9.30	...	0.156	0.658	0.077	0.015	...
0.033	0.060	0.054	0.449	0.436	...	...	0.308	...	0.039	...	...	0.082	...
0.352	2.47	7.03	0.051	0.085	0.976	0.104	0.059	...	0.024	...	...	1.402	4.05
0.033	0.086	0.237	0.067	...	...	...	0.007	...	3.09	0.178	1.15	0.036	0.118
...	...	...	...	...	...	1.794	...	...	...	...	...	...	...
...	...	...	0.019	0.013	...	...	...	...	...	...	...	...	...
...	...	...	0.281	0.216	...	...	...	...	...	...	...	...	...
0.078	...	...	2.54	2.94	...	1.187	...	...	...	...	...	0.432	...
0.020	...	...	0.166	0.307	...	0.047	...	...	...	...	...	0.009	...
0.337	0.278	...	0.016	0.031	...	...	...	...	...	...	...	...	...
0.156	0.193	...	0.002	0.024	...	0.575	0.669	0.211	...	1.34	0.495	...	0.270
0.784	0.038	...	...	0.007	3.32	0.019	1.128	0.115	...	2.06	...	...	2.623
0.016	...	...	...	...	0.126	...	1.363	...	...	0.069	...	...	...
...	...	...	...	0.020	...	...	0.132	...	...	...	...	...	...
...	...	...	0.004	0.004	...	...	...	...	...	...	...	...	...
...	...	...	0.011	0.054	...	...	...	...	...	...	...	...	...
...	...	...	0.011	0.007	...	0.043	0.190	...	...	...	...	...	...
...	...	...	...	...	...	0.782	...	...	...	...	0.014	2.94	...
...	...	...	...	...	...	...	1.538	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	0.071	...

Table II. Analyses of Water-Free Synthetic Mixture

Compound	Key Mass	Syn. Compn., Liq. Vol. %	M.S. Analyses, Liq. Vol. %				Av. Dev. from Av.	Av. Dev. from Synthesis
Acetone	58	25.0	27.0	25.7	25.4	23.9	±0.7	±0.9
Methyl ethyl ketone	72	5.0	4.8	4.8	4.8	4.5	±0.1	±0.3
n-Propionaldehyde	29	15.0	16.1	15.3	13.2	15.3	±0.8	±0.8
Ethyl alcohol	46	20.0	19.1	19.1	19.9	19.1	±0.4	±0.6
Isopropyl alcohol	45	15.0	15.3	15.1	15.6	15.1	±0.2	±0.4
n-Butyl alcohol	56	20.0	17.7	20.0	21.1	22.1	±1.1	±1.1
Total		100.0	100.0	100.0	100.0	100.0		

mass spectrometer without temperature control of the ionization chamber and with the entire gas-handling system at room temperature. This type of operation did not furnish satisfactory reproducibility of patterns or sensitivities; the irregularity was probably caused primarily by adsorption of the oxygenated compounds on the cold walls of the gas-handling system. Furthermore, excessive time was required for removing the oxygenated compounds from the mass spectrometer tube at the end of a run.

In an effort to overcome adsorption and pumping difficulties, a Westinghouse type mass spectrometer was arranged for elevated temperature operation of the entire vacuum and gas-handling systems. Grease-containing stopcocks were removed from the introduction system and replaced by packless metal valves (special type manufactured by Westinghouse) which were capable of operation at elevated temperatures. Temperatures at which various parts of the vacuum system were maintained by individual electrical circuits were as follows: metal valves, 130° C.; glass manifold on metal valves, 200° C.; glass expansion volume, 200° C.; leak and inlet lines, 140° C.; mass spectrometer tube ionization chamber, analyzer, entrance, and exhaust vacuum lines, 215° C. Temperature changes in the ionization chamber of the mass spectrometer tube resulting from changes in room temperature and/or filament emission characteristics were eliminated by use of manually controlled Variacs in the heating circuit of the ionization chamber. The absolute temperature levels listed above do not appear critical; they were found to be well within the range of the heating elements installed on the various units and hence were adopted as standard conditions for obtaining both calibration and mixture spectra data.

In order to improve the operability of the original Westinghouse mass spectrometer, auxiliary oil diffusion and fore-vacuum pumps plus a separate sample introduction system were added. A modified type of Westinghouse pen and ink recorder was used in which had been installed four linear sensitivity scales in the ratio of 1:3:10:30. This instrument during the high temperature operating conditions outlined above gave satisfactory pattern stability and pump-out times for all oxygenated compounds boiling below 100° C.

During the latter phases of this investigation, efforts again were made to perform analyses of oxygenated compounds on a Consolidated mass spectrometer. For this study, a Model 21-102 instrument was used and the leak, leak line, and analyzer portion of the mass spectrometer tube were equipped with electrical heaters for elevated temperature operation. Results obtained during operation of these portions of the gas-handling system at a temperature of 235° C. indicate that the Consolidated instrument is also satisfactory for performing analyses of oxygenated compounds.

It has been found recently (*4*) that these portions of the inlet system need not be heated for oxygenated compounds if certain modifications are made in the "leak" and its location is changed. These modifications consist of replacing the all-glass leak with a gold foil leak and placing the leak very near the ionization chamber of the mass spectrometer tube.

All oxygenated compounds discussed in this paper were introduced into the mass spectrometers as liquid samples. Introductions were made by means of sintered-glass disks as described by Taylor and Young (*8*). Sample introduction lines were heated to within a few inches of the mercury pool which covered the sintered-glass disks. A rough measurement of the amount of liquid sample introduced was obtained by visual observation of the fall in liquid level inside a microburet from which the sample was admitted to the sintered disk.

DEVELOPMENT OF METHOD

The analysis of some liquid hydrocarbons by mass spectrometry has been described by Brown, Taylor *et al.* (*3*, *7*). Because the compounds of higher molecular weight possess relatively low vapor pressures, special techniques are required in obtaining relative sensitivity coefficients in the mass spectrometer. Manometer pressure measurements of the vapor have been found satisfactory for C_5 and lighter hydrocarbons, but this procedure is unsatisfactory for materials of lower volatility than pentanes. In the study of aromatics of higher molecular weight, Johnsen (*6*) has used the peak height ratio method of relating sensitivity coefficients. Young (*11*) has been successful in measuring vapor pressures of higher molecular weight hydrocarbons with a mercurial micromanometer. In order to eliminate the need for either the peak height ratio method or the micromanometer pressure method, the use of an internal standard has been developed in this laboratory in obtaining sensitivity coefficients for compounds of higher molecular weight in the mass spectrometer.

Benzene has been found satisfactory for use as an internal standard for all oxygenated compounds tested to date. Benzene was chosen because its parent mass (78) has not been observed in the spectrum of any of the oxygenated compounds investigated. Furthermore, all masses other than its parent mass are relatively low in sensitivity and hence offer only a minimum of interference with masses to be measured in the spectra of the oxygenated compounds.

Table III. Analyses of Synthetic Mixtures Containing Water

(4 analyses)

Compound	Key Mass	Syn. Compn., Liq. Vol. %	M.S. Analyses, Liq. Vol. %[a]			Av. Dev. from Synthesis		
			Water-free	With added water	Salted out material[b]	Water-free	With water	Salted out material
Acetone	58	7.0	7.3 ± 0.2	6.5 ± 0.5	7.9 ± 0.6	±0.3	±0.5	±0.9
Methyl ethyl ketone	72	3.0	3.0 ± 0.2	2.4 ± 0.3	3.1 ± 0.2	±0.2	±0.6	±0.2
n-Propionaldehyde	29	6.0	5.6 ± 0.3	5.3 ± 0.6	1.5 ± 1.0	±0.5	±0.8	±4.6
Ethyl alcohol	46	50.0	49.2 ± 0.6	50.3 ± 0.4	50.2 ± 2.1	±1.0	±0.9	±2.1
Isopropyl alcohol	45	12.0	12.8 ± 0.4	12.2 ± 0.4	13.5 ± 0.9	±0.8	±0.5	±1.6
n-Propyl alcohol	60	12.0	11.9 ± 0.2	11.6 ± 0.7	13.2 ± 0.6	±0.2	±0.9	±1.2
n-Butyl alcohol	56	10.0	10.2 ± 0.2	11.7 ± 0.5	10.6 ± 0.9	±0.2	±1.7	±0.9
Water	18	0.0	0	82.4 ± 0.4[c]	0	...	±0.9	...
Total		100.0	100.0	100.0	100.0			

[a] Average of four determinations, ± average deviation from average.
[b] Potassium carbonate used for salting out.
[c] Sample contained 83.3% water; data normalized to water-free basis for comparison.

Table IV. Analyses of Complex Synthetic Mixture Containing Water

Compounds	Key Mass	Syn. Compn., Liq. Vol. %	M.S. Analyses, Liq. Vol. %					Av. Dev. from Av.	Av. Dev. from Synthesis
Acetone	58	6.0	3.7	4.8	5.0	5.3	6.2	±0.7	±0.9
Methyl ethyl ketone	72	5.0	4.0	5.0	5.1	5.1	5.4	±0.4	±0.5
Acetaldehyde	44	10.0	9.0	10.0	8.2	6.8	7.5	±0.9	±1.5
n-Propionaldehyde	29	7.0	7.8	7.8	8.0	7.6	7.1	±0.2	±0.7
n-Butyraldehyde	41	2.0	3.5	2.5	2.7	2.9	2.3	±0.3	±0.8
Methyl alcohol	32	2.0	3.7	3.7	3.8	2.3	1.5	±0.8	±1.2
Ethyl alcohol	46	33.0	31.4	31.0	32.5	33.0	34.2	±1.0	±1.1
n-Propyl alcohol	60	25.0	27.1	25.7	24.0	24.6	27.2	±1.1	±1.3
n-Butyl alcohol	56	3.0	3.1	3.1	2.8	2.6	2.8	±0.2	±0.2
Ethyl acetate	88	2.0	0.8	0.9	1.9	1.8	0	±0.6	±0.9
Water	18	5.0	5.9	5.5	6.0	8.0	5.8	±0.8	±1.2
Total		100.0	100.0	100.0	100.0	100.0	100.0		

Table V. Summarized Data on Analyses of Synthetic Mixtures, Liquid Volume Per Cent

Compounds	Key Mass	Blend Number 1 • Three Analyses Syn. Compn.	1 • Three Analyses M.S.	2 Three Analyses Syn. Compn.	2 Three Analyses M.S.	3 Syn. Compn.	3 M.S.	4 Syn. Compn.	4 M.S.	5 Four Analyses Syn. Compn.	5 Four Analyses M.S.	6 M.S.	6 M.S.[a]	7 Syn. Compn.	7 M.S.
Acetone	58	...	...	...	...	5.6	5.7 ± 0.1	...	...	4.2	3.9 ± 0.4	7.2 ± 0.1	6.9 ± 0.1	...	...
Methyl ethyl ketone	72	6.0	5.0 ± 0.4	0	0.7 ± 0.1	11.1	11.0 ± 0.1	4.1	4.1 ± 0.2	7.0		3.0 ± 0.2	3.2 ± 0.2	...	...
Methyl *n*-propyl ketone	86	...	...	...	...	5.6	5.6 ± 0.1	4.0	4.1 ± 0.3	3.0		...	...	3.1	2.4 ± 0.6
Acetaldehyde	44	...	...	...	...	5.6	5.5 ± 0.1	...	...	...		...	...	...	...
n-Propionaldehyde	29	...	...	...	...	2.2	2.1 ± 0.1	...	...	6.0		5.6 ± 0.5	7.5 ± 0.1	...	...
n-Butyraldehyde	41	5.3	5.3 ± 0.8	...	...	2.2	2.3 ± 0.1	...	...	...		...	...	...	...
Dimethyl acetal	75	...	...	...	...	1.1	1.2 ± 0.2	...	...	...		...	...	...	...
Ethyl alcohol	46	74.7	76.2 ± 0.5	83.4	83.7 ± 0.6	33.3	33.8 ± 0.7	4.0	3.5 ± 0.1	...		...	...	...	...
Isopropyl alcohol	45	6.0	5.5 ± 0.2	4.5	4.8 ± 0.1	33.3	32.8 ± 0.9	4.0	4.7 ± 0.1	50.0		49.2 ± 0.9	49.7 ± 0.3	...	...
n-Propyl alcohol	60	8.0	8.0 ± 0.2	4.9	4.6 ± 0.2	...	...	59.8	58.7 ± 0.4	12.0		12.8 ± 0.3	12.0 ± 0.2	...	...
sec-Butyl alcohol	45	...	...	...	...	...	...	4.1	4.6 ± 0.3	66.7	68.4 ± 0.9	12.0 ± 0.2	11.5 ± 0.1	...	...
Isobutyl alcohol	33	...	...	0	0.7 ± 0.2	...	...	7.0	6.7 ± 0.3	...	...	...	...	3.0	2.7 ± 0.3
n-Butyl alcohol	56	...	...	...	...	...	...	8.0	8.7 ± 0.5	8.3	7.8 ± 0.6	10.2 ± 0.2	9.2 ± 0.2	74.9	74.9 ± 0.8
Iso- and *n*-amyl alcohol	55	...	...	...	...	...	...	...	...	20.8	19.9 ± 0.9	...	...	10.0	11.2 ± 0.7
Water	18	0	0	7.2	5.5 ± 0.5	0	0	5.0	4.9 ± 0.2	0		0	0	4.0	4.3 ± 0.3
Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Analyses made on Type 21-102 Consolidated mass spectrometer with leak line and envelope at 235° C.

In the use of a liquid internal standard it is convenient to prepare calibration blends of the internal standard and pure compound on a liquid volume basis; this, of course, results in analyses being computed to liquid volume per cent instead of the usual mole per cent as obtained on pressure basis sensitivity coefficients. Calibration data for water relative to benzene were obtained from the two binary blends of (1) water and alcohol and (2) alcohol and benzene.

General methods of computing mass spectrometer data have been described by Brewer and Dibeler (*2*). The procedure employed in computing data from the internal standard method is essentially the same as that used with pressure or peak height ratio methods.

Calibration Data. Obtain good pattern data for all masses on the compound to be used as an internal standard. Normalize all peak heights in terms of 100 for the peak (78 in the case of benzene) which is to serve as a reference mass.

Prepare binary blends of the internal standard and each pure compound for which analysis is to be made. Each binary should contain exactly the same volume per cent of internal standard; sufficient internal standard should be used to produce a reference mass peak height of approximately 100 recorder scale divisions for a "normal" sample introduction of the binary mixture. (One part of benzene to 20 parts of oxygenated compound was used in this study.)

Obtain mass spectra on all binary mixtures of internal standard and pure compounds. From a measurement of the reference mass compute the peak heights for all masses in the internal standard spectrum and subtract these peak heights from the total binary mixture pattern. Divide the remaining peak heights by the reference peak height in obtaining sensitivity coefficients (A values) for the pure oxygenated compound.

Mixture Data. Blend each mixture to be analyzed with the internal standard. (The same liquid volume per cent of internal standard should be used in all mixtures as was added to each pure compound in obtaining calibration data.)

Obtain mass spectra on the unknown mixture after addition of the internal standard.

Compute the internal standard spectrum from the reference mass and subtract all internal standard peak heights from the total mixture (internal standard plus unknown mixtures) spectrum peak heights.

Divide the remaining peak heights by the reference mass peak height for obtaining mixture sensitivity coefficients (M values).

Computation of Liquid Volume Per Cent. From the calibration data (A values) and the mixture spectra (M values) set up simultaneous equations as follows:

$$A_{11}X_1 + A_{12}X_2 + A_{13}X_3 + \ldots\ldots = M_1$$

where A_{11} is the calibration data coefficient for mass 1 of component 1, X_1 is the liquid volume per cent to be computed for component 1, A_{12} is the calibration coefficient for mass 1 of compound 2, X_2 is the liquid volume per cent to be computed for compound 2, etc., and M_1 is the mixture coefficient for mass 1.

Similar equations are set up for as many masses as there are components present. These equations may be solved for liquid volume per cents by the methods of successive approximations, electrical computer, or rigid mathematics. If either successive approximations or the electrical computer method is used, particular care should be exercised in the choice and arrangement of masses and compounds in the matrix. The main diagonal coefficients, A_{11}, A_{22}, A_{33}, etc., are called the key mass coefficients; for rapid convergence to a solution the key mass values should be larger than any other coefficient in the same row or column of the matrix. Furthermore, the data should be arranged so that the coefficients above and to the right of the main diagonal are as small as possible.

The internal standard method can be used for computing only one or two specific components in a complex mixture if the spectra of the unidentified and unmeasured compounds do not interfere with the masses being used in the partial analysis. If a complete analy is is made, it is not necessary to use an internal standard with the mixture. In this case the mixture peak heights are divided by some constant such as 100 (approximate peak height of mass 78 when benzene is used) prior to computations and the resulting liquid volume per cents must be normalized to a total of 100%.

EVALUATION OF THE METHOD

Some mass spectrometer data on oxygenated compounds have been reported by Washburn *et al.* (*10*). Polyisotopic mass spectra for oxygenated compounds obtained in this laboratory are shown in Table I. These data were obtained using benzene as an internal standard with a liquid volume ratio of 1 part of benzene to 20 parts of oxygenated compound, and using

the Westinghouse instrument under the high temperature operating conditions described previously; the source and indicated purity of the compounds used in this study are listed also in Table I. These sensitivity data are not expressed in the usual units of divisions of peak height per micron of pressure, but as divisions of peak height per division of the reference mass 78 from benzene.

In Table II are shown Westinghouse mass spectrometer analyses of a six-component oxygenated compound mixture containing no water. The average deviations from the average and from the true composition for this mixture were about ±0.7, based on the total sample. In Table III are shown mass spectrometer analyses of a synthetic seven-component oxygenated compound mixture with and without the addition of 82.4% of water. The presence of water in this large concentration increased the errors on some components slightly but not significantly, while the water was also determined accurately. Also shown in Table III are analyses of the synthetic sample after addition of the water followed by recovery of the oxygenated compounds from the water by salting out with potassium carbonate. The use of the salting-out technique resulted in large errors, especially in the case of *n*-propionaldehyde, apparently because of chemical reaction. As the data demonstrated that the presence of water had no appreciable effect on accuracy, the investigation of salting out as a supplementary technique was discontinued.

In Table IV are presented data obtained on a mixture of eleven oxygenated compounds plus water. The accuracies obtained on samples of this complexity, although acceptable, are somewhat poorer than those obtained in the case of the less complex mixtures.

In Table V are summarized analytical data obtained on various synthetic mixtures of oxygenated compounds using the Westinghouse instrument and in one case (blend 6) by the Westinghouse and Consolidated (Type 21-102) mass spectrometers. The good agreement between the two instruments indicates that equivalent results can be obtained with either type.

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Mass Spectrometer Analysis of Alcohols and Other Oxygenated Derivatives

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THE ever-expanding production of chemicals from petroleum fractions and the increased use of these chemicals in industry have created a demand for faster and more effective means of analyzing aqueous and anhydrous mixtures of alcohols, ethers, ketones, and other oxygenated compounds. This demand has become more pronounced as a result of the inception of the industry-wide synthetic gasoline and petrochemical programs. The synthetic processes most popularly being considered for full-scale manufacturing involve the separation and purification of large quantities and varieties of oxygenated compounds, and analyses must be made of mixtures ranging from a conglomeration of many compounds in aqueous solution to relatively pure concentrations of each. In between these extremes are numerous mixtures encountered in the purification processes. Analyses of this type have in the past been largely accomplished by determination of individual components and impurities using chemical methods, by separation of components and constant boiling fractions using distillation, and by determination of A.S.T.M. distillation range. Increasing attention is now being directed toward the newer physical methods of analysis, to supplement and expand past and current techniques.

Prominent among the physical methods of analysis is mass spectrometry, which has often proved its usefulness in handling gas mixtures, complex hydrocarbon mixtures, and organic chemicals (*2–5, 9, 10*). However, one of the limitations of mass spectrometers has been inability to analyze samples containing appreciable quantities of alcohols or quantitatively to determine water. Such work has been accomplished upon occasion (*6*), but with difficulty and with a generally increased time and a lowered standard of precision because of erratic behavior of these materials in a mass spectrometer. Other classes of oxygenated compounds, such as ketones, aldehydes, and esters, do not exhibit such erratic behavior and are therefore handled with less difficulty; however, samples containing these materials frequently also contain alcohols or water, or both.

To overcome the limitations of the mass spectrometer in regard to alcohols and water, a program of investigation was undertaken in connection with the Model 21-102 mass spectrometers manufactured by Consolidated Engineering Corporation. This program was designed to establish the cause of the anomalous variations in the alcohol spectra; to determine which parts of the Consolidated instrument contributed most to this cause; and, finally, to find means of eliminating or minimizing it.

LOCALIZING MAJOR ELUTION SOURCE

The first indication that alcohols and water did not behave conventionally in the mass spectrometer had been the failure of the spectra and sensitivities to reproduce except when one ma-

The mass spectrometer analysis of alcohols, or mixtures of oxygenated compounds in which alcohols or water are present, has been difficult because of strong sorption tendencies of these materials. During an investigation of the underlying cause of the erratic behavior of the alcohol spectra, it was found possible to minimize the effect of sorption and the resultant elution of foreign materials by minor changes in the mass spectrometer sample inlet system. These changes, combined with proper techniques for conditioning the system with the sample to be analyzed, proved sufficient to permit analysis of mixtures of alcohols and water. The results of the analysis of synthetic mixtures of oxygenated compounds are given. It is shown by alternate analyses of known mixtures widely varying in concentration of light and heavy alcohols that the different materials can be separated in the mixture spectra, and that any effect of one sample upon a succeeding one is insignificant. Analysis of a synthetic mixture of the four butanol isomers shows that alcohol isomers are also readily resolved. That the errors in the analyses are relatively small demonstrates that sufficient accuracy can be obtained in the separation of alcohols to meet normal analytical needs. Analysis of the four butanol isomers mixed with water demonstrates that the presence of water does not prevent obtaining precise results and that water can be determined accurately. Analyses of complex mixtures show that other oxygenated compounds can be determined with adequate precision in the presence of alcohols and water.

terial only—e.g., methanol—was run repeatedly. Closer study showed that when runs of other materials were interpolated, materials previously run appeared in the current spectrum to a concentration of several per cent, even though an insignificant background had been obtained after the pump-out of the preceding sample. In mixtures, certain components were depleted, only to appear in later runs. The cause was apparently surface adsorption, and subsequent elution either in the inlet system where sample pressure is about 40 microns, or in the low-pressure (10^{-5} mm. of mercury) section following the leak. Considerable experimentation showed that the major offender was the portion of the gas inlet line between the leak and the ionization chamber, possibly including the leak as well.

INSTRUMENTAL AND OPERATIONAL CHANGES

With the difficulty thus localized, materials of construction and surface coatings for the offending section of glass line were tested. In addition, the effect of elevating the temperature of the line, which is reported by Thomas and Seyfried (*8*) to be effective, was tried. However, the change finally adopted was to reduce to an absolute minimum all surfaces enclosing gas at low pressure, on the inlet side of the ionization chamber, by moving the leak to a point as close as possible to the chamber.

To do this conveniently, the glass leak was removed and one of gold foil inserted, in which orifices were punched with a needle. This foil was sealed in the short section of glass tubing which joins the inlet line to the ionization chamber, and thus was mounted inside the glass envelope that encloses the chamber and analyzer tube. The number and size of orifices punched in the foil were adjusted, after testing by trial, to give a leak rate comparable to that of the original glass leak. For the analyses which follow, the thickness of the gold foil was 1.2 mils, and two holes of 1.7-mil diameter were punched in it.

Operating procedures were also altered somewhat. A pump-out time, after each run, of approximately 8 minutes was adopted in place of the usual 5 minutes used for hydrocarbons. In addition, each sample was passed through the system for 2 minutes, pumped out for 1 minute, and then reintroduced. The second sampling was in all cases used for obtaining the mass spectrum. The additional pump-out and the conditioning with each sample added about 6 minutes to the normal running time of the sample. Under these conditions mass spectra can be obtained at a rate of about three per hour.

ANALYTICAL CHECKS OF REVISED SYSTEM

The next step was to prove that with the mass spectrometer altered as described, and with the revised operating procedure, quantitative analyses of alcohol mixtures might be obtained, with acceptable accuracy, without resorting to the use of correction factors (or sorption coefficients), such as those reported by Taylor, Brown, Young, and Headington (*6*), or to any external heating of the glass sample system. Accordingly, a program was set up to establish more fully the effectiveness of the new development, and, at the same time, to gain further knowledge concerning the mass spectra of alcohols. This program was designed to answer the following questions:

1. Are the mass spectra of the various alcohols sufficiently different to permit quantitative analysis?
2. Is an alcohol analysis affected by the preceding run?
3. Does the presence of water affect the analysis?
4. Can water be quantitatively determined?
5. Can alcohol isomers be successfully determined?
6. Can other oxygenated materials be determined accurately in the presence of alcohol?
7. Can traces of oxygenated compounds be determined in dilute aqueous solutions?

Tables I to X and the following discussion show how these questions were answered.

LIQUID INTRODUCTION METHOD

In all the work involving calibration spectra or mixture analysis, the liquid samples were introduced into the mass spectrometer by means of a micropipet and mercury-covered sintered disk as described by Young *et al.* (*7*). The liquid volume read on the pipet was calibrated against microns pressure by running *n*-pentane, introduced by two methods, on the mass spectrometer. First, the *n*-pentane was introduced through the conventional system—that is, metered in a small volume at about 35 mm. of mercury and then expanded into the primary sample bottle where the sample pressure was about 40 microns. The height of the 72 peak obtained from this gas was then compared with that obtained when a small liquid volume of *n*-pentane was introduced from the pipet through the sintered disk, directly to the primary sample bottle, by-passing all pre-expansion volumes. The liquid level in the pipet was read before and after touching the sintered disk, and net scale divisions admitted were noted. A conversion figure of microns pressure per scale division of the pipet was obtained from this comparison for *n*-pentane. From this figure, similar factors were obtained for other compounds, using liquid densities and molecular weights. Liquid volumes could have been used directly, but mole-percentage results were desired for comparison with past performance. Sensitivities were therefore computed in divisions per micron, using the converted liquid volume measurement read on the pipet.

CALIBRATIONS

In Table I are given the calibrating spectra or relative intensities of the alcohols from methanol through the butanols, as well as the spectra of methyl ethyl ketone and water. The spectra are normalized, using the largest peak as base 100. The operating conditions used in obtaining these spectra and in performing the analytical work are noted at the bottom of the table. Inspection shows that the spectra differ materially, a factor that enhances the accuracy with which one compound may be determined in the presence of others.

Table I. Mass Spectra of C_1 to C_4 Alcohols, Methyl Ethyl Ketone, and Water

(Patterns: largest peak used as base peak)

m/e	Methyl Alcohol	Ethyl Alcohol	*n*-Propyl Alcohol	Isopropyl Alcohol	*n*-Butyl Alcohol	*sec*-Butyl Alcohol	*tert*-Butyl Alcohol	Isobutyl Alcohol	Methyl Ethyl Ketone	Water	Mixture Spectrum[a]
15	35.48	9.44	3.77	10.70	8.39	6.80	13.30	7.47	13.36	..	...
16	0.86	0.51	...	0.47	0.37	0.32	0.31	0.27	0.22	..	...
17	1.47	1.13	0.58	0.73	0.68	...	...	1.56	...	23.61	...
18	1.37	2.32	1.79	2.11	2.18	0.23	0.49	2.05	...	100.00	5.6
19	0.29	3.13	0.90	6.51	3.53	0.77	0.38	0.89	...	..	...
25	...	1.52	0.42	0.29	0.21	...	...	0.21	0.44	..	1.4
26	...	7.78	2.89	2.20	5.52	2.72	1.83	3.62	3.90	..	24.7
27	...	21.62	15.20	15.50	50.89	15.87	9.87	42.02	13.27	..	188.9
28	4.95	5.12	5.08	0.91	16.19	2.98	1.67	5.94	2.46	..	42.8
29	58.80	21.24	14.14	9.49	29.90	13.94	12.65	21.17	23.15	..	143.2
30	7.50	5.73	2.10	0.60	2.10	0.72	0.41	1.73	0.52	..	8.2
31	100.00	100.00	100.00	5.75	100.00	20.31	35.53	63.10	0.45	..	368.6
32	68.03p	1.14	2.25	...	1.68	0.25	0.46	1.62	...	..	6.2
33	0.98	...	1.11	...	8.50	...	...	53.40	...	..	92.1
39	...	...	4.00	5.52	15.63	3.36	7.70	19.03	1.79	..	76.8
41	...	0.60	5.80	6.54	61.57	10.13	20.82	55.68	1.36	..	241.5
42	...	2.85	7.50	3.90	32.36	1.64	3.32	60.46	4.40	..	142.2
43	...	7.45	3.18	16.76	61.36	9.83	14.45	100.00	100.00	..	292.7
44	...	1.74	0.61	3.45	4.25	8.43	0.74	3.78	2.46	..	37.8
45	...	37.33	4.39	100.00	6.59	100.00	0.59	5.03	1.44	..	322.6
46	...	16.23p	...	2.25	0.54	2.25	...	...	...	..	7.9
47	...	0.45	...	0.21	...	0.21	...	...	...	..	0.8
50	...	...	...	...	0.61	0.37	0.48	0.43	...	..	3.6
51	...	...	...	...	0.66	0.38	0.52	0.48	...	..	4.0
52	...	...	...	...	0.21	...	...	...	...	..	1.2
53	...	...	0.20	...	1.03	0.49	0.59	0.94	...	..	5.5
54	...	...	...	...	1.07	...	...	0.32	...	..	2.4
55	...	...	0.42	...	12.29	2.06	1.55	4.35	0.50	..	32.6
56	...	...	...	...	90.58	1.02	1.47	2.46	...	..	126.7
57	...	...	1.42	0.30	6.68	2.74	9.02	3.89	6.83	..	44.0
58	...	...	0.36	...	...	0.52	0.78	0.54	0.26	..	4.8
59	...	...	9.61	3.58	0.26	17.78	100.00	4.98	...	..	301.5
60	...	...	6.36p	0.44p	...	0.64	3.26	0.57	...	..	10.3
71	...	...	...	...	...	...	...	...	0.76	..	...
72	...	...	...	...	...	...	...	...	19.84p	..	...
73	...	...	...	...	1.49	1.20	...	1.82	...	..	8.1
74	...	...	...	...	0.79p	0.29p	p	9.06p	...	..	14.8
Sensitivity, div./μ	8.76	17.98	26.51	23.47	11.51	26.98	20.93	12.05	30.35	8.94	...

p = parent peak (molecular mass)
Operating conditions:
Electron current (catcher) = 9 μa
Ionizing voltage = 70 volts
m/e = 18 scanned at 2100 volts

[a] Mixture spectrum for which analysis is shown in Tables VI, run 1, and VII.

The spectra of the various alcohols exhibit many interesting features. Although a complete discussion of these features is beyond the scope of this paper, one of the most interesting is the existence of several peaks which would not be predicted from a simple study of the molecular structure. The butanol peaks at m/e 33 are the most obvious of these. An interesting possible explanation has been advanced by R. A. Brown of Atlantic Refining Company:

It is of interest to note that among the alcohols a different type of rearrangement occurs from that encountered in the case of hydrocarbons. For instance, it is considered that the mass 32 fragment for *n*-butyl alcohol in Table I consists of $[(CH_2OH)H]^+$, corresponding to a rearrangement peak, such as methane $[(CH_3)H]^+$ in the spectrum of isobutane. The mass 33 fragment, however, is visualized as being $[(CH_2OH)H_2]^+$—a fragment in an associated state. In general the spectra of alcohols and mercaptans show a marked similarity in regard to the presence of rearrangement peaks. Alcohol peaks are higher as a rule, which may be the result of the greater electronegativity of oxygen and its consequent greater affinity for H^+ ions (*1*).

For routine analysis the chief point of interest is that these anomalous peaks are not due to impurities, so that the spectra can be trusted for calibration. It was established that repeat runs of calibrating materials were reproducible, variations being, in general, about twice those found in repeat calibrations of light hydrocarbons, yet still within usable limits. Calibrations were taken daily and used for the mixture analyses of that day. No attempt was made at this time to determine long-range stability of spectra. The patterns listed are a typical day's run.

Table II shows the mass spectra of a number of other oxygenated materials. These, too, showed good reproducibility and no complication such as elution was encountered.

EFFECT OF PRECEDING SAMPLE

A major difficulty in the systems exhibiting elution has been the appearance of small quantities of each sample on the following run. To determine the extent to which such hangover might still be present, two samples of binary mixtures of methanol and ethanol were synthesized. One contained about 90% ethanol and the other about 90% methanol. The mixtures were then run alternately and their analysis was compared with their known compositions. The results of this test are shown in Table III. Also shown are the results of a similar test in which alternate samples of binary mixtures of isopropyl alcohol and *n*-propyl alcohol were run.

If there were appreciable hangover, the concentration of the minor component as determined on the mass spectrometer in runs 2, 3, and 4 of each test would be abnormally large, because of the large concentration of the same component in the previous sample run. With the possible exception of runs 2 and 4, sample D, such a bias is not observed. The preponderance of data indicates that there is no significant effect of one sample on the next.

EFFECT OF WATER

The next step was to determine the effect of water in the sample on the mass spectrometer analyses. This was done by preparing a known mixture of methanol, ethanol, and methyl ethyl ketone (Sample E, Table IV), and analyzing it dry. Then, after the addition of enough water to make up about two thirds of the total sample, it was run again and reanalyzed, and the results were computed on a dry basis.

The errors obtained, as shown in Table IV, are somewhat smaller in the wet than in the dry sample—a fortuitous circumstance,

Table II. Mass Spectra of Various Esters and Ketones

(Patterns: • largest peak used as base peak)

m/e	Methyl Formate 1	Ethyl Formate 2	Methyl Acetate 3	*n*-Propyl Formate 4	Ethyl Acetate 5	Methyl Propionate 6	*n*-Propyl Acetate 7	Isopropyl Acetate 8	Ethyl Propionate 9	Methyl Butyrate 10	*n*-Butyl Acetate 11	*n*-Propyl Propionate 12	Ethyl Butyrate 13	*n*-Propyl Butyrate 14	Acetone 15	Methyl Propyl Ketone 16	Diethyl Ketone 17	Mesityl Oxide 18	Methyl Isobutyl Ketone 19
27	0.61	43.17	0.84	29.14	13.06	36.72	14.76	13.96	34.52	43.72	15.56	38.63	63.10	49.27	8.43	21.32	37.18	44.24	13.37
28	7.14	72.66	1.94	8.11	4.06	15.77	3.79	1.58	15.82	9.03	6.55	11.12	13.99	8.48	1.75	3.97	10.81	7.67	1.95
29	63.05	65.80	10.50	30.52	25.47	100.00	8.77	6.42	100.00	17.10	14.91	57.46	85.54	15.13	4.29	22.20	99.53	47.55	14.87
30	6.91	5.49	1.27	3.50	1.33	3.99	1.25	0.37	3.72	1.83	0.78	2.94	3.27	1.48	...	0.59	2.48	1.14	0.44
31	100.00	100.00	3.43	100.00	1.73	7.23	17.77	2.99	3.62	5.54	6.21	7.15	3.47	8.79	0.56	1.20	1.01	1.12	0.45
32	34.04	1.51	...	1.82	...	0.32	0.38	..	...	...	...	..	...	..	...	...	...	...	...
33	0.63	0.21	...	0.58	...	1.12	..	..	...	1.50	0.40	..	...	..	...	...	...	...	...
41	...	0.77	1.09	19.61	0.66	1.06	8.45	11.33	0.85	32.79	17.24	14.44	29.58	34.20	2.18	10.42	2.58	13.75	19.17
42	...	2.32	10.24	71.88	5.92	3.07	11.16	8.56	2.47	19.03	5.41	11.22	22.03	26.41	7.01	5.54	3.92	7.01	6.21
43	0.23	7.51	100.00	10.11	100.00	1.86	100.00	100.00	6.49	100.00	100.00	27.03	100.00	100.00	100.00	100.00	4.16	93.01	100.00
44	1.46	1.90	2.86	0.80	2.80	0.57	2.75	3.53	0.58	6.35	2.88	1.11	4.66	3.92	2.28	2.78	1.00	2.27	2.46
45	1.44	28.69	1.22	4.10	13.49	5.01	2.66	31.92	10.44	4.84	1.19	0.89	20.25	2.74	0.22	1.80	0.47	0.53	0.71
46	0.36	5.17	...	...	0.35	...	..	0.72	0.34	...	..	..	0.50	..	...	...	...	...	...
47	...	5.98	...	7.53	...	...	..	..	...	...	..	..	0.99	0.28	...	...	...	...	...
56	...	1.67	...	0.85	0.51	2.42	0.50	..	2.39	0.56	33.62	2.49	1.16	1.77	...	0.76	3.45	5.91	1.01
57	...	...	...	4.26	...	75.23	1.29	..	64.45	1.81	4.45	100.00	3.04	4.30	0.71	19.05	100.00	0.53	19.06
58	...	...	...	0.82	...	2.66	0.36	1.85	2.26	...	1.32	3.59	0.27	0.64	22.65p	7.34	3.54	0.91	32.27
59	0.48	0.72	5.71	3.86	...	24.58	4.97	6.83	0.48	24.69	..	4.85	0.52	6.03	0.81	1.58	0.33	0.23	2.02
60	27.94p	0.25	...	0.72	0.61	0.95	1.66	0.58	...	0.91	0.22	0.20	20.90	14.79	...	...	...	...	...
61	0.74	...	...	0.28	9.89	0.26	19.08	11.89	...	0.58	10.14	..	8.91	7.52	...	...	...	0.49	...
62	...	...	...	...	0.22	...	0.46	0.27	...	...	0.24	..	0.28	0.24	...	...	...	0.71	...
63	...	...	...	...	...	...	...	..	...	...	..	..	...	..	...	...	...	0.91	...
69	...	...	...	...	...	...	..	..	...	1.82	..	..	0.70	0.66	...	...	...	0.31	0.47
70	...	...	...	...	4.71	...	..	..	...	0.96	..	..	6.03	0.96	...	...	...	1.62	...
71	...	...	...	...	0.21	...	..	..	...	54.85	1.06	...	90.37	96.24	...	6.68	0.64	...	...
72	...	...	...	...	...	...	1.02	..	...	2.47	0.27	..	4.08	4.54	...	0.32	...	...	0.33
73	...	0.67	...	0.97	2.99	...	9.27	..	3.45	0.26	11.19	0.85	16.18	8.46	...	0.39	...	...	...
74	...	7.07p	15.21p	...	...	...	0.35	..	5.14	69.16	0.49	2.57	1.21	0.53	...	...	...	0.26	...
75	...	0.30	0.54	...	...	...	..	..	7.17	2.50	0.26	32.12	0.28	0.49	...	...	...	...	...
76	...	...	...	...	...	...	..	..	0.28	0.33	..	1.12	...	..	...	...	..	...	...
77	...	...	...	...	...	...	..	..	...	...	..	..	...	0.35	...	...	...	0.71	...
78	...	...	...	...	...	...	..	..	0.42	..	..	..	0.53	2.48	...	...	...	...	...
79	...	...	...	...	...	...	...	..	...	...	..	..	..	..	...	...	...	2.17	...
80	...	...	...	...	...	...	...	..	...	...	..	..	...	..	...	...	...	0.21	...
81	...	...	...	...	...	...	...	..	...	...	..	..	...	..	...	...	...	0.22	...
82	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	1.35	...
83	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	100.00	...
84	...	...	...	...	...	...	..	..	0.57	...	..	..	...	..	...	...	...	5.60	...
85	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	0.35	0.34	10.02
86	...	...	...	...	...	...	...	..	...	...	0.53	0.65	...	..	...	14.95p	17.32p	...	0.55
87	•	...	...	...	0.22	1.59	0.44	6.05	1.02	17.10	0.96	6.51	0.88	1.06	...	0.93	1.03	...	...
88	...	...	...	0.33p	3.95p	21.29p	..	0.27	...	1.05	..	0.30	43.62	3.89	...	...	...	...	...
89	...	...	...	...	0.24	1.03	..	..	...	...	..	..	12.14	51.26	...	...	...	...	...
90	...	...	...	...	...	...	..	..	...	...	..	..	0.64	2.32	...	...	...	...	...
91	...	...	...	...	...	...	..	..	...	...	..	..	...	0.34	...	...	...	...	...
92	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
93	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
94	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
95	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
96	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
97	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
98	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	52.70p	...
99	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	3.70	...
100	...	...	...	...	...	...	..	..	...	...	..	..	...	0.81	...	...	...	...	10.54p
101	...	...	...	...	...	...	..	..	...	0.90	..	0.29	5.40	4.60	...	...	...	...	0.65
102	...	...	...	...	...	...	p	p	6.91p	1.86p	..	..	0.48	3.62	...	...	...	...	...
103	...	...	...	...	...	...	..	..	0.45	0.22	..	..	...	0.22	...	...	...	...	...
114	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
115	...	...	...	...	...	...	..	..	...	...	..	..	...	0.23	...	...	...	...	...
116	...	...	...	...	...	...	..	..	...	...	p	p	4.36p	..	...	...	...	...	...
129	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
130	...	...	...	...	...	...	..	..	...	...	..	..	...	p	...	...	...	...	...
131	...	...	...	...	...	...	..	..	...	...	..	..	...	..	...	...	...	...	...
Sensitivity	47.72	41.04	73.60	61.54	90.27	54.75	127.51	112.46	109.31	34.13	124.25	94.36	31.91	42.10	86.01	67.95	55.80	26.88	73.62

p = parent peak (molecular mass)

Operating conditions:
Electron current (catcher) = 9 μa
Ionizing voltage = 70 volts

Spectra 1-6, mass 27 scanned at 1650 volts
Spectra 7-19, mass 27 scanned at 2400 volts

All spectra taken on Consolidated Engineering mass spectrometer, Model 21-102

Table III. Effect of Preceding Sample

	Known Compn. (Calcd. from Synthesis)	Mass Spectrometer Analysis			
Test 1. Alternate analyses of methanol-ethanol mixture samples. Analysis computed from peaks of m/e 32 and 46					
		Sample A			
		Run 1	% error	Run 3	% error
Ethanol, %	89.5	89.8	+0.3	90.2	+0.7
Methanol, %	10.5	10.2	−0.3	9.8	−0.7
		Sample B			
		Run 2		Run 4	
Ethanol, %	10.3	9.8	−0.5	10.0	−0.3
Methanol, %	89.7	90.2	+0.5	90.0	+0.3
Test 2. Alternate analyses of samples of propanol-isomer mixtures. Analysis computed from peaks of m/e 39 and 59					
		Sample C			
		Run 1	% error	Run 3	% error
Isopropyl alcohol, %	89.8	90.3	+0.5	90.5	+0.7
n-Propyl alcohol, %	10.2	9.7	−0.5	9.5	−0.7
		Sample D			
		Run 2		Run 4	
Isopropyl alcohol, %	10.2	10.7	+0.5	10.7	+0.5
n-Propyl alcohol, %	89.8	89.3	−0.5	89.3	−0.5

Instrument time, 25 min.
Computation time, 15 min.

which indicates, however, that the presence of water does not decrease the accuracy of analysis.

DETERMINATION OF WATER

After it was ascertained that the presence of water had no appreciable effect on the analysis of the other components, two more samples (F and G) were prepared containing measured amounts of water. These were analyzed for all components including the water, using a water calibration pattern for the computation. The results of these tests are also shown in Table IV. Again the errors in the analyses are within reasonable limits, the water fractions showing about the same accuracy as the other components.

Table IV. Mass Spectrometer Analysis of Alcohol and Methyl Ethyl Ketone Mixtures

Sample E, Run 1, Dry	Known compn., mole %	Analysis, mole %	Error, mole %
Methanol	8.5	8.2	−0.3
Ethanol	72.2	73.1	+0.9
Methyl ethyl ketone	19.3	18.7	−0.6
Run 2, Wet (about ⅔ H_2O), Computed on Dry Basis			
Methanol	8.5	8.4	−0.1
Ethanol	72.2	72.1	−0.1
Methyl ethyl ketone	19.3	19.5	+0.2

Computed from m/e = 32, 46, and 57.
Instrument time, 25 min.
Computation time, 20 min.

Sample F, Sample Wet, Water Computed Using Water Calibration Pattern	Known compn., mole %	Analysis, mole %	Error, mole %
Methanol	4.7	4.8	+0.1
Ethanol	39.8	40.3	+0.5
Methyl ethyl ketone	10.7	10.6	−0.1
Water	44.8	44.3	−0.5
Sample G			
Methanol	3.5	3.6	+0.1
Ethanol	29.6	30.2	+0.6
Methyl ethyl ketone	7.9	7.8	−0.1
Water	59.0	58.4	−0.6

Computed from m/e = 18, 32, 46, and 57.
Instrument time, 25 min.
Computation time, 25 min.

Table V. Mass Spectrometer Analysis of Alcohol Mixtures

(3 runs of four-component mixture)

Component	Known Compn., Mole %	Run 1 Analysis, mole %	Run 1 Error, mole %	Run 2 Analysis, mole %	Run 2 Error, mole %	Run 3 Analysis, mole %	Run 3 Error, mole %
Methanol	2.0	2.4	+0.4	1.9	−0.1	2.0	0
Ethanol	8.1	7.0	−1.1[a]	7.7	−0.4	7.6	−0.5
n-Propyl alcohol	20.0	20.2	+0.2	20.2	+0.2	19.6	−0.4
Isopropyl alcohol	69.9	70.4	+0.5	70.2	+0.3	70.8	+0.9

Computed from m/e = 32, 39, 46, and 59.
Instrument time, 25 min.
Computation time, 30 min.

[a] Sensitivity of ethanol high.

Table VI. Mass Spectrometer Analysis of C_4 Alcohol Isomer Mixtures

	Known Compn., Mole %	Analysis, Mole %	Error, Mole %
Run 1, dry			
n-Butyl alcohol	24.9	24.4	−0.5
sec-Butyl alcohol	24.8	24.8	0
tert-Butyl alcohol	25.2	25.0	−0.2
Isobutyl alcohol	25.1	25.8	+0.7
Run 2, wet (about ⅔ H_2O) computed on dry basis			
n-Butyl alcohol	24.9	24.7	−0.2
sec-Butyl alcohol	24.8	24.3	−0.5
tert-Butyl alcohol	25.2	24.0	−1.2
Isobutyl alcohol	25.1	27.0	+1.9
Run 3, wet (about ⅔ H_2O), computed on dry basis			
n-Butyl alcohol	24.9	24.6	−0.3
sec-Butyl alcohol	24.8	24.1	−0.7
tert-Butyl alcohol	25.2	25.3	+0.1
Isobutyl alcohol	25.1	26.0	+0.9
Run 4, wet, water computed using H_2O calibration pattern			
n-Butyl alcohol	8.4	8.4	0
sec-Butyl alcohol	8.3	8.2	−0.1
tert-Butyl alcohol	8.5	8.5	0
Isobutyl alcohol	8.4	8.8	+0.4
Water	66.4	66.1	−0.3

m/e = 45, 56, 59, and 74 used in computations.
Instrument time, 25 min.
Computation time, 25 min. (after inverse matrix is computed)[a]

[a] 40 min. required to invert matrix not included, as under routine conditions one reciprocal matrix is used for many analyses and pro-rated time per analysis is small.

DETERMINATION OF ISOMERS

The problem of resolving the alcohol isomers was next attacked, because it was known that some isomeric compounds, such as unsaturated hydrocarbons, can be separated only with relatively lower precision. A simple case of separating two isomers has been shown in Table III, in which *n*- and isopropyl alcohol were determined with about the same accuracy as methanol and ethanol. This C_3 mixture was next complicated by the addition of methanol and ethanol. The results of analysis shown in Table V demonstrate that this four-component mixture, containing two isomers, is readily analyzed with acceptable accuracy.

Next a sample was prepared containing nearly equal portions of the four butanol isomers. As before, this was analyzed first dry and then after the addition of a large, measured amount of water and results were computed on a dry basis. One drawback to this latter procedure is that analysis discrepancies are multiplied by the dilution factor, which in this case was nearly 3. Table VI shows the results of these analyses. Runs 2 and 3 are seen to have abnormally large errors, whereas the mole per cent errors in run 4, in which water was determined using the water calibration spectra, are considerably smaller. Actual accuracies, in terms of percentage of component, are comparable in all three cases. The important point is, however, that the data indicate that no difficulty was encountered in resolving these alcohol isomers.

The comparatively favorable accuracy obtained for the butyl isomers is explained by the wide differences in the spectra. Generally in mass spectrometer analysis, greatest accuracy and speed of computation result when each component contributes

substantially to a mass to which no other component ionizes. In the case of isomeric mixtures this favorable circumstance seldom holds. The aim is then to find peaks to which only one compound is a major contributor. Fortunately, this can be done with the butanol spectra. Using normalized coefficients from Table I at m/e's of 45, 56, 59, and 74, the equations required can be set up and the analysis carried out as shown in Table VII.

These four equations have good "leverage" because of the large diagonal and small off-diagonal coefficients. With acceptable pattern reproducibility and no differential sorption or elution of the mixture components, this means that accuracy of analysis will be comparatively good. An average accuracy of about ±0.5% is expected, with about equal accuracy on all components. The errors shown in Table VI bear out these expectations.

ANALYSIS OF MIXED CLASSES OF OXYGENATED COMPOUNDS

To extend the investigation further, mixtures of known composition were prepared with pure samples of alcohols, ketones, aldehydes, ether, and water. The results of three separate mass spectrometer analyses of one such mixture are shown in Table VIII. The results of two separate analyses of another are shown in Table IX. The errors shown in Table VIII are reasonably low, whereas those in Table IX are even smaller than could normally be expected for any type of mixture.

ANALYSIS OF TRACE QUANTITIES OF SOLVENTS

One of the remaining unanswered questions concerned the possibility of analyzing small quantities of oxygenated materials in dilute aqueous solution. Four samples of known composition containing butanol, acetone, and ethanol were, therefore, prepared by an independent investigator and submitted as unknowns for mass spectrometer analysis. The known composition of each of these samples, together with the results of three separate determinations made on each sample, are shown in Table X. All samples contain more than 99 mole % water, and two contain nearly 99.9 mole % water. The reproducibility is seen to be good, and accuracy is also good in most cases. In general, the results are consistently slightly low, but the reason for this was not determined.

None of the figures shown in these analyses necessarily represents the minimum quantity of materials detectable, for no special techniques were employed. Using special techniques, such as increased sample pressure, the threshold of detection can be lowered, and accuracy can frequently be improved.

CONCLUSIONS

Mixtures of alcohols, at least through C_4's, can be analyzed speedily and with useful accuracy. By proper design of the inlet system, sorption and consequent elution can be reduced to an insignificant level. The questions that were being investigated can now be answered as follows:

1. Patterns are reproducible and sufficiently different to permit analyses.
2. Alcohol analyses through C_4 are not affected by preceding runs—i.e., there is negligible sample hangover.
3. The presence of water does not decrease accuracy.
4. Water can be quantitatively determined with about the same accuracy as the alcohols.
5. C_3 and C_4 alcohol isomers have widely different spectra, and, hence, mixtures of them can be successfully analyzed.
6. Mixtures containing both alcohols and other oxygenated materials are readily analyzed.
7. Oxygenated materials, including alcohols, can be detected in very dilute solution.

Table VII. Analysis of Butanol Mixture

1. Equations are set up from coefficients of Table I ($\times 10^{-2}$) at 4 masses:

$0.9058x_1 + 0.0147x_2 + 0.0102x_3 + 0.0246x_4 = M_{56}$ = mixture peak, m/e = 56

$0.0026x_1 + 1.0000x_2 + 0.1778x_3 + 0.0498x_4 = M_{59}$ = mixture peak, m/e = 59

$0.0659x_1 + 0.0059x_2 + 1.0000x_3 + 0.0503x_4 = M_{45}$ = mixture peak, m/e = 45

$0.0079x_1 + 0x_2 + 0.0029x_3 + 0.0906x_4 = M_{74}$ = mixture peak, m/e = 74

x_1 = divisions of base peak due to *n*-butyl alcohol
x_2 = divisions of base peak due to *tert*-butyl alcohol
x_3 = divisions of base peak due to *sec*-butyl alcohol
x_4 = divisions of base peak due to isobutyl alcohol

2. Matrix of coefficients is inverted, yielding:

$x_1 = 1.107080M_{56} - 0.016246M_{59} - 0.007442M_{45} - 0.287682M_{74}$
$x_2 = +0.013914M_{56} + 1.000832M_{59} - 0.176742M_{45} - 0.455327M_{74}$
$x_3 = -0.068305M_{56} - 0.004887M_{59} + 1.003128M_{45} - 0.535607M_{74}$
$x_4 = -0.093932M_{56} + 0.001569M_{59} - 0.031662M_{45} + 11.079754M_{74}$

3. Peaks from mixture spectrum are substituted in (2), yielding number of divisions of base peak due to each component.
4. Number of divisions of base peak for each component is divided by corresponding sensitivity, yielding number of microns partial pressure of each component.
5. Each partial pressure is divided by total computed pressure, yielding mole %.

Component	m/e of Base Peak	3 No. Divisions Base Peak	4 Partial Pressure, Microns	5 Mole %
n-Butyl	31	128.71	11.18	24.4
tert-Butyl	59	239.76	11.46	25.0
sec-Butyl	45	305.55	11.33	24.8
Isobutyl	43	142.34	11.81	25.8
Total			45.78	100.0

Table VIII. Mass Spectrometer Analysis of Oxygenated Compounds

(3 runs of five-component mixture)

Component	Known Compn., Mole %	Run 1 Analysis, mole %	Run 1 Error, mole %	Run 2 Analysis, mole %	Run 2 Error, mole %	Run 3 Analysis, mole %	Run 3 Error, mole %
Ethanol	40.1	39.3	−0.8	39.6	−0.5	39.5	−0.6
Acetone	25.0	24.5	−0.5	24.6	−0.4	24.3	−0.7
Propionaldehyde	16.7	17.0	+0.3	17.4	+0.7	17.2	+0.5
Isopropyl ether	2.5	2.8	+0.3	2.8	+0.3	2.8	+0.3
Water	15.7	16.4	+0.7	15.6	−0.1	16.2	+0.5

Table IX. Mass Spectrometer Analysis of Oxygenated Compounds

(2 runs of five-component mixture)

Component	Known Compn., Mole %	Run 1 Analysis, mole %	Run 1 Error, mole %	Run 2 Analysis, mole %	Run 2 Error, mole %
Ethanol	80.4	80.4	0	80.4	0
n-Propyl alcohol	6.7	6.7	0	6.7	0
Isopropyl alcohol	4.9	5.2	+0.3	5.0	+0.1
Butyraldehyde	3.8	3.6	−0.2	3.7	−0.1
Methyl ethyl ketone	4.2	4.1	−0.1	4.2	0

Table X. Analysis of Dilute Aqueous Solutions

Sample 1	Known Compn., Mole %	Run 1, Mole %	Run 2, Mole %	Run 3, Mole %
n-Butyl alcohol	0.262	0.23	0.23	0.24
Acetone	0.185	0.16	0.14	0.16
Ethanol	0.118	0.15	0.08	0.12
H_2O	99.435	99.46	99.55	99.48
Sample 2				
n-Butyl alcohol	0.267	0.27	0.24	0.26
Acetone	0.033	0.03	0.03	0.04
Ethanol	0.309	0.29	0.27	0.30
H_2O	99.391	99.41	99.46	99.40
Sample 3				
n-Butyl alcohol	0.066	0.06	0.06	0.06
Acetone	0.049	0.04	0.04	0.04
Ethanol	0.029	0.02	0.03	0.02
H_2O	99.856	99.88	99.87	99.88
Sample 4				
n-Butyl alcohol	0.066	0.06	0.06	0.06
Acetone	0.009	0.01	0.01	0.01
Ethanol	0.078	0.08	0.08	0.09
H_2O	99.847	99.85	99.85	99.84

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LITERATURE CITED

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Mass Spectrometer Analysis of Some Oxygen-Containing Compounds

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In an attempt to analyze some oxygenated compounds by means of the mass spectrometer, using the same procedures as in the analysis of gaseous and liquid hydrocarbons, several difficulties were encountered. Thus, extremely long evacuation times were necessary to remove some compounds from the tube, and, even more serious, large variations in peak heights occurring during successive scanning of the sample indicated that the instrument was unable to maintain calibration. Some of these difficulties were partly overcome by using a heated tube and a new trap design. Results obtained with mixtures of methanol, formic acid, methyl formate, and methylal are included. In general, the results do not attain the precision and reproducibility of hydrocarbon analyses.

AN INVESTIGATION of the composition of gases and liquids which were produced by vacuum pyrolysis of certain formaldehyde polymers suggested the use of the mass spectrometer for the analysis. Preliminary investigations revealed that mainly oxygen-containing compounds were present. In an attempt to analyze these mixtures by following the general already well-established procedure used in the analysis of liquid hydrocarbons (*2*), several peculiarities were noticed which seem common to compounds of this type. It was therefore felt necessary to investigate some of the complicating factors more closely and, if possible, reduce them to a minimum. Simple mixtures were chosen for this study and from it an experimental procedure was evolved.

ANALYTICAL DIFFICULTIES

With the mass spectrometer tube at room temperature many of the oxygen-containing compounds, once introduced, required extended pumping to remove them from the system, and it has long been recognized that such a condition is intolerable for rapid successive analyses. In some cases more than 0.5 hour of evacuation was required to reduce the peaks to 2% of their original values, and any further reduction is even slower, because the pressure–pumping time curve follows an exponential law.

The peaks in the spectra did not have the reproducibility necessary to calculate, with some accuracy, the compounds in a mixture from previous calibrations on pure substances. Even major peaks varied considerably in successive scannings, although all externally controlled conditions were kept constant. Under such circumstances unreliable calibration spectra were obtained. In some cases certain high masses appeared in the spectrum which could not readily be ascribed to the molecule being studied, as they were not present in other runs. These peaks usually decreased during prolonged operation of the instrument and seemed to be correlated with the past history of the tube. Only in cases where higher masses reappeared consistently in a definite proportion could they be attributed to impurities present in the sample.

It was anticipated that the instability of the dissociation pattern might be associated with the method of handling the sample in the mass spectrometer rather than with a change in cracking pattern of the compound.

In instruments available for analytical purposes the sample is admitted as a continuous gas flow through the tube at a rather low pressure and the gas is intercepted at a certain region by a well-defined beam of electrons (*7*). In order to obtain reproducible results the concentration of the gas in the ionization and dissociation region must be constant, which means a steady gas flow through the tube at all times. To obtain such a continuous flow, the sample is usually introduced from a relatively high pressure reservoir through a leak, which, in the instrument used, was a small orifice in a thin platinum foil. The behavior of such a leak is well understood (*5*); much less defined is the passage of the gases and vapors through the tube and especially the pumping system which maintains the pressure gradient necessary for the flow of the gas.

Because the sample has to flow through a vacuum envelope of considerable area, some substances can be adsorbed on the walls and subsequently eluted by other samples of greater adsorptive power, by compounds of higher concentration, or by increased temperature. In such a case considerable time has to be allowed for the new sample to flush the tube, in order that a steady state may exist when the mass spectrum is taken. A strong adhesion to the walls could therefore account for the excessive evacuation

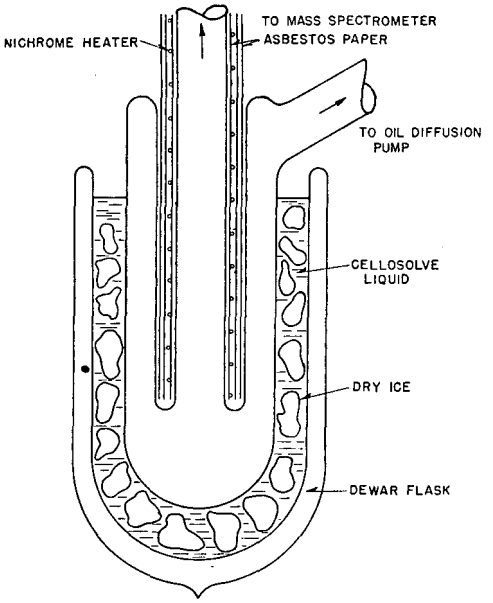

Figure 1. Liquid Air Trap for Eliminating Condensation on Inner Member

time. If such is the case, these phenomena should be influenced by the temperature of the mass spectrometer envelope and the inlet tubing. This was verified by heating the system to about 200° C.

Another serious difficulty seems to be with the pumping system itself. The arrangement consists of a mechanical pump and a multiple-stage oil-diffusion pump. In order to keep the stray molecules of the pumping fluid out of the mass spectrometer, especially since oil gives rise to many peaks over a wide mass range, a cold surface in the form of a vapor trap is introduced between the pump and the tube. Unfortunately, such a trap not only condenses the vapors from the diffusion pump, but very often condenses on its walls the compounds passing through the tube. Now if the cooled zone in a conventional re-entrant trap is diminished, the condensate re-evaporates, probably from the inner member, and the liberated molecules have a chance to diffuse back into the instrument. The trap acts as a source for substances condensed on its wall. To eliminate this difficulty a trap design was installed which was insensitive to changes of the coolant level; by its use, the memory effect was almost completely eliminated.

THE INSTRUMENT

The mass spectrometer used was of the sectored magnetic field type with a 90° deflection of the ions on a 12.5-cm. (5-inch) radius. Some features of the instrument and recorder have been described (*6*). The ion source was at ground potential and the analyzer section at about 600 volts. This arrangement eliminates all metastable peaks and some diffuse background due to loss of kinetic energy (*4*). The entire glass envelope was wrapped in sections with asbestos and a Nichrome heating element, so that the whole system from the leak to the trap could be maintained at an elevated temperature. In this way the temperature of any section could be individually controlled and maintained at any desired level, as indicated by a number of thermocouples on the tube and pumping system.

The diffusion pump vapor trap was of all-glass construction, the result of several attempts to design a trap that would be insensitive to the coolant level. In order to avoid condensation of the gases on the inner member, the evacuation line is heated for a distance far into the cooled zone of the outer member. Figure 1 shows the actual construction. The heater in the annular well is adjusted to keep the surfaces warm enough to prevent gases from condensing on this surface. All the condensation, then, takes place on the outer cylinder and any molecule which might be evaporated when the coolant level drops will have to pass down the trap and strike a cold surface before passing into the inner member, thus making recondensation more probable than diffusion back into the tube.

In the experiments discussed a refrigerant mixture of dry ice and Cellosolve (ethylene glycol monoethyl ether) and a three-stage self-fractionating oil-diffusion pump using Dow Corning DC 703 silicone pumping fluid were used. The diffusion pump was adjusted for maximum pumping speed by introducing argon into the tube and regulating the heater current until the lowest peak height resulted.

PREPARATION AND INTRODUCTION OF SAMPLES

As calibration samples, and for preparing the known blends, substances of the highest grade commercially available were used without further purification. Because these compounds undoubtedly contain some impurities, the spectra indicated in Figure 4 might not represent that of a chemical individuum. Blends of the liquid components were prepared by weighing the amount of individual liquids to be mixed. The blending was done in bottles with self-sealing rubber stoppers (50-ml. penicillin bottles) and the components were injected in the desired amounts by means of a hypodermic syringe and long needle. After the subsequent weighing of each component, the mole per cent composition of the mixture was calculated. Checks made on some mixtures which had stood for a few weeks in the bottles showed no change in composition.

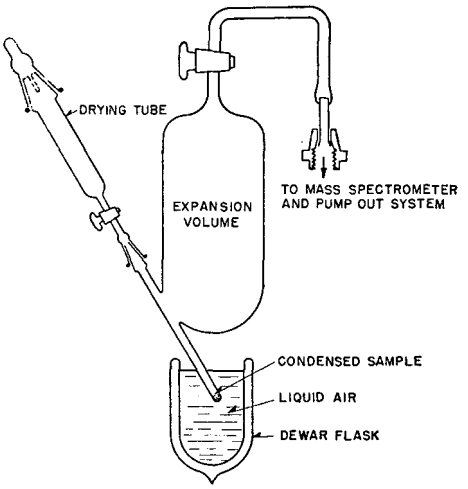

Figure 2. Sample-Introducing System for Liquids Using Freezing-Out Method

In introducing the liquid mixture into the instrument it is of course, necessary to evaporate the sample completely; otherwise, the relative volatility of the components could cause a difference in composition between the liquid and the gaseous phase. It seemed desirable also that the evaporation should proceed first into a larger volume where the gases could easily mix by thermoconvection and attain the necessary homogeneity, rather than directly into the measuring volume of the instrument where subsequent mixing by diffusion in the narrow tubing would be a rather slow process.

The porous membrane mercury seal (*6*), excellent for some liquids, does not work well for the more viscous ones and was therefore replaced by a more direct method of introduction. In one case a sample bottle was constructed as indicated in Figure 2. The 0.5-liter flask can be attached to the instrument by a flare fitting.

The side arm of the tube was closed by a cup with a stopcock to which a drying tube filled with Drierite and Ascarite was attached. After the flask was evacuated, air was admitted through the drying tube to remove the water vapor and carbon dioxide; then the cup was disconnected, the sample was injected by a syringe and needle into the extension arm, the side tube was covered, and the sample was solidified with liquid air. The air was pumped out above the solid sample, and then the sample was evaporated. This procedure was repeated twice. Then the lower end of the tube was immersed in hot water to set up convection

currents which would mix the gas thoroughly. The sample was then introduced into the measuring volume of the mass spectrometer, the pressure was read by a precision manometer, and a definite portion was expanded into the storage vessel behind the leak.

This procedure of introducing the samples was working satisfactorily until liquid air was replaced by liquid nitrogen. It was observed that more cycles were needed to free the sample from oxygen, which made the method of introduction slow; hence a new method was tried.

A sample bottle was used as indicated in Figure 3. The short side arm was covered with a self-sealing rubber stopper such as is used in the blending bottles. After evacuation the sample was simply injected by a needle having a locking stopcock, and the amount of sample injected was assayed by the size and length of the needle. Because this injection does not introduce a measurable amount of air, the sample can be used without any freezing operation. Experience has shown that over fifty injections can be made into the same self-sealing rubber stopper without any sign of leaking. The top of the stopper was always slightly greased with an Apiezon lubricant.

A definite procedure was followed in taking the mass spectra of the calibrating substances and of the blends. About 5 minutes were allowed for each sample to pass through the system in order to condition the tube and then two runs were made on the same sample 10 minutes apart. If these two scannings agreed closely, the condition was taken as favorable for the computation. By using the precautions described, the pumping-out time, even for oxygen-containing substances, was no longer than for hydrocarbons. Operating at an elevated temperature stabilized the peak height of all substances used, so that an analysis was feasible.

Table I. Analysis of Mixtures

Compound	Mole % Synthesis	Mole % Analysis	Mole % Synthesis	Mole % Analysis	Mole % Synthesis	Mole % Analysis	Mole % Synthesis	Mole % Analysis	Mole % Synthesis	Mole % Analysis	Mole % Synthesis	Mole % Analysis
Methanol	91.8[a]	91.9	...	..	...	..	...	..	37.1[a]	38.6	...	..
	73.4[b]	72.6	...	..	...	..	...	..	47.8[b]	48.3	...	..
Formaldehyde	...	..	90.7[a]	90.2	...	..	...	..	...	..	...	..
	...	..	84.4[b]	83.0	...	..	...	..	...	..	...	..
Formic acid	...	..	...	..	15.6[a]	17.2	...	..	...	..	27.4[a]	28.2
	...	..	...	..	13.0[b]	10.4	...	..	...	..	15.3[b]	16.2
Methyl formate	8.2[a]	8.1	...	..	84.4[a]	82.8	75.5[a]	75.9	41.2[a]	40.1	50.4[a]	49.2
	26.6[b]	27.4	...	..	87.0[b]	89.6	63.9[b]	63.3	31.2[b]	30.8	39.6[b]	40.1
Methylal	...	..	9.3[a]	9.8	...	..	24.5[a]	24.1	21.7[a]	21.3	22.1[a]	22.6
	...	..	15.6[b]	17.0	...	..	36.1[b]	36.7	20.8[b]	20.9	45.1[b]	43.7

[a, b] Different blends containing same compounds whose mole fractions have been varied.

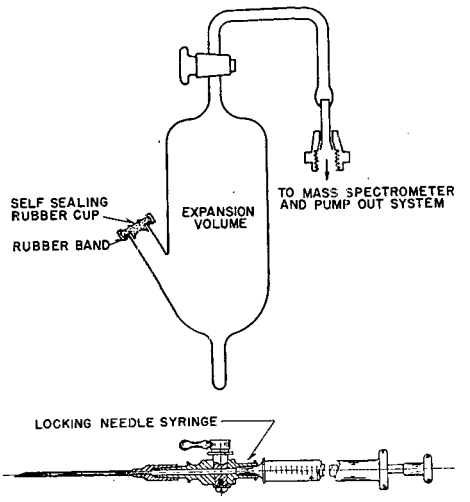

Figure 3. System for Introducing Liquid Samples Using Syringe and Self-Sealing Rubber Stopper

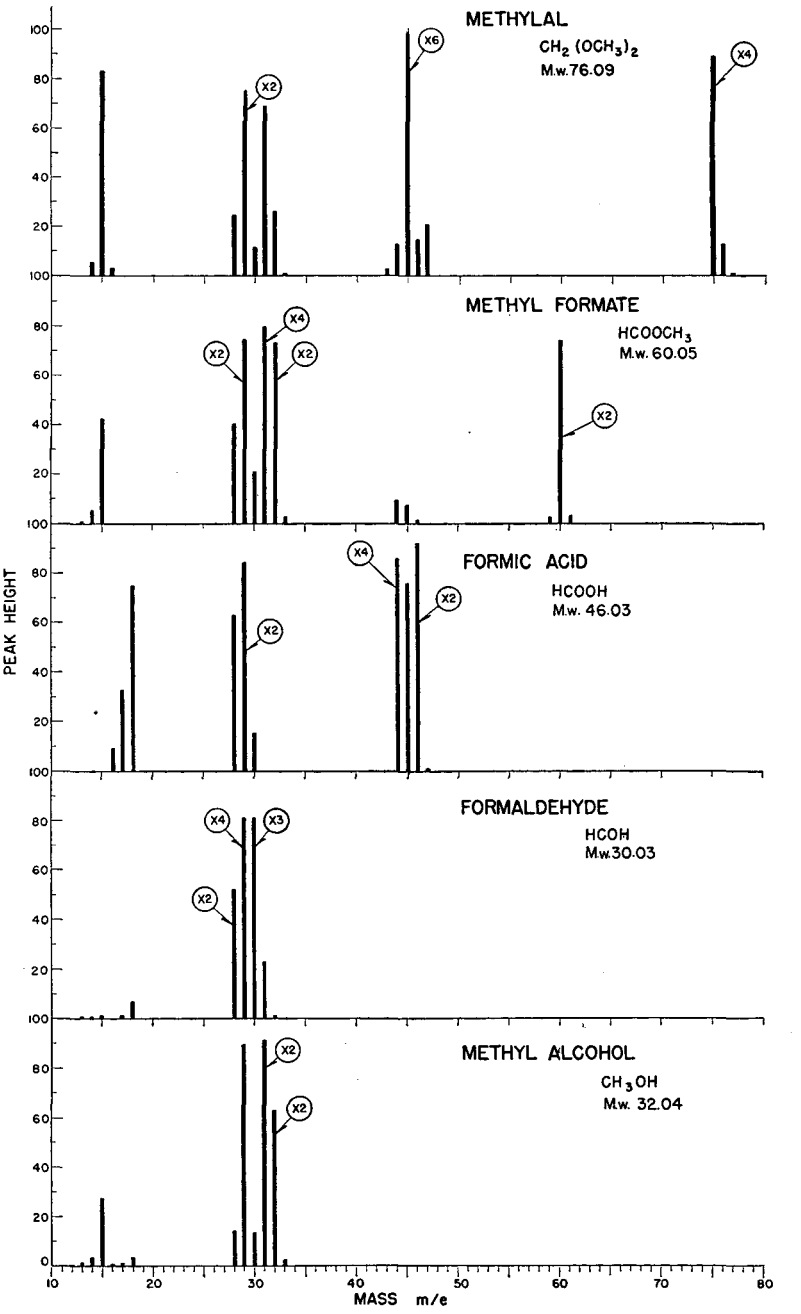

Figure 4. Mass Spectra of Organic Compounds Used for Blending

Peak heights are in arbitrary units and are normalized to a unit introductory pressure. Circled numbers are multiplying factors.

Furthermore, the contribution to the spectra caused by elution of previous substances was almost completely eliminated.

The pure substances were recalibrated frequently. It was decided to perform all the measurements at a tube temperature of 180° ± 5° C. as indicated by the thermocouples. The ionization region, owing to the heat supplied internally from the filament, was somewhat higher. The temperature chosen is probably still low enough not to cause any excess thermal cracking of the substances used.

THE MASS SPECTRA

A mass spectrum is generally characterized as the abundance of the ionic fragment found at a certain electron energy. Spectra represent a cross section of the more general ionization efficiency curves expressing the variation of peak height as a function of the energy of the bombarding electrons. In order to ascertain the best ionization potential, the ionization efficiency curves were taken from 100 e.v. to the appearance potential of the fragments. An electron energy of 70 e.v. was chosen, because in that region the ionization efficiency curves of the compounds are flat. The peak height distribution of the main fragments is shown in Figure 4 for 70 e.v. and a tube temperature of 180° C. with a unit pressure of the compounds behind the leak. The numerical tabulation of these values represents the calibration data for computing a composite spectrum. The peak heights are given in arbitrary units.

COMPUTATION

The distribution of the peaks in Figure 4 also indicates that a mixture of the indicated compounds can be analyzed with relative ease, because there are very large unique peaks for some of the substances. Because some of these peaks are not adjacent to any large neighboring peaks no isotope correction is applied. A very simple procedure, which can be called the successive subtraction method (*1*), can be adopted for calculation. Thus peaks of mass 75 and 60 belonging to methylal and methyl formate can be used for these two substances. Peak 44 can be used for formic acid after subtracting the rather small peaks contributed by methyl formate and methylal. In the same way methanol can be calculated from mass 32. Formaldehyde can be calculated from peak 30, because this peak is small in the other substances, although this peak must also be corrected for the isotope contributions of the other compounds.

Some of the substances are not mutually stable in a mixture. Thus, for example, in a mixture of formic acid and methanol, methyl formate is found in appreciable amounts and the same instability was found in some mixtures with formaldehyde. For this reason binary mixtures were tried at first together with a few three-component blends, as are indicated in Table I. The values given are obtained as the average of two separate runs, and then normalized to 100%.

Table I indicates that the values obtained by means of the mass spectrometer agree within 1 or 2% of those calculated for the blends. Considering the purity of the substances used and the difficulties in making accurate blends and analyzing them, this work seems to indicate a possible application of the mass spectrometer to these types of compounds, even though the results did not attain the same analytical reproducibility as in the case of hydrocarbons. Further improvements in the technique of handling these oxygen-containing substances should improve the analytical results.

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Additivity of Raman Spectra of Dioxane-Benzene Mixtures

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Hanle and Heidenreich have studied the intensities of the 835 cm.$^{-1}$ Raman band of dioxane and the 850 cm.$^{-1}$ band of benzene and have reported a striking deviation from additivity in mixtures of dioxane and benzene containing less than 10% of dioxane. The present report shows that this result may be attributed to the overlapping of the two bands and the presence of a faint dioxane band at 852 cm.$^{-1}$ When correction is made for these effects, the Raman spectra are additive.

THE Raman spectrum of a mixture of nonpolar or slightly polar compounds should be a superposition of the spectra of the pure components, the intensities of the bands being proportional to the concentrations of the substances to which they belong. Daure (*4*, *5*), Dadieu and Kohlrausch (*3*), Crigler (*1*, *2*), Rank, and his co-workers (*9*), and others have shown experimentally that this is generally true.

Hanle and Heidenreich (*7*), however, observed a marked deviation from additivity in the Raman spectra of mixtures of 1,4-dioxane and benzene. If the Raman spectra were truly additive the ratio between intensities I_D and I_B of two bands of dioxane and benzene, respectively, should be proportional to the ratio between concentrations C_D and C_B in volume per cent. Hence, (I_D/I_B) (C_B/C_D) = a constant.

When C_D is very small, C_B varies but little from mixture to mixture, and $(I_D/I_B)/C_D$ should be very nearly constant. Nevertheless, in comparing the 835 cm.$^{-1}$ band of dioxane and the 850 cm.$^{-1}$ band of benzene, Hanle and Heidenreich found that the quantity $(I_{835}/I_{850})/C_D$ increased by a factor of 4 as C_D decreased from 12 to 1 mole %. They observed a similar effect in the

Raman spectra of mixtures with small concentrations of cyclohexane in benzene.

Kohlrausch (*8*) and Goubeau (*6*) have referred to the work of Hanle and Heidenreich without questioning the correctness of their results or offering any explanation of the phenomenon observed. No subsequent publication has substantiated or contradicted their results. Suspecting that the lack of additivity observed by Hanle and Heidenreich might have been caused by erroneous correction for continuous background, the authors repeated their work with some care. The results are reported in the present paper.

EXPERIMENTAL

Merck's benzene and Eastman's 1,4-dioxane were used. Each compound was redistilled, and the middle fraction, consisting of about one third of the distillate, was taken. Nine mixtures were prepared with 1, 2, 3, 4, 5, 6, 7, 10, and 12.5 mole % of dioxane in benzene, respectively. In order that the composition might not change appreciably, each mixture was made up shortly before it was to be used and was frozen in a Pyrex bulb sealed to the Raman tube, and this system was evacuated and sealed off. The sample was distilled into the Raman tube and drained back into the bulb to remove any dust particles from the tube. Then the entire sample was again distilled into the tube and frozen, and the tube was sealed off.

The Raman tubes used had an inside diameter of 10 mm. and an exposed length of 128 mm. The filter jacket contained a 10-mm. layer of a solution of 200 grams of sodium nitrite per liter of distilled water. The filter solution was circulated by a stainless steel pump and was cooled with running water in a heat exchanger. A water-cooled mercury arc operated at 10 amperes was used. The lamp was folded three times, so as to make it equivalent to four lamps evenly spaced around one side of the filter jacket. It was backed by a cylindrical reflector of polished aluminum. The entire apparatus was enclosed and cooled by means of an exhaust blower. The Raman tube had a fairly constant temperature of about 28° C.

The Raman spectra were photographed with a Lane-Wells spectrograph. The slit used was 85 microns by 4 mm. Exposures of 1.5 and 4.5 hours were made.

Eastman's Tri-X Pan cut films were used. The films were calibrated with a four-step sector and a 10-watt tungsten lamp. The light was diffused to the sector through two glass plates, frosted on each side and placed 2 cm. apart. A condensing lens formed an image of the sector on the spectrograph slit with unit magnification. The slit was fairly uniformly illuminated with this arrangement. The films were developed for 25 minutes in Eastman's Microdol developer at 19° C.; the films containing the Raman spectra of one sample and the two films containing the sectored strips were processed together in a plastic holder. Tests showed that reciprocity law failure did not introduce appreciable error.

Microphotometer traces of all the Raman spectra were made with a Leeds & Northrup Knorr-Albers photoelectric microphotometer. Stepwise traces of the sectored strips were also made at appropriate wave lengths. Characteristic curves were drawn for all sets of films. From the microphotometer traces of the pure components and of the mixture containing 10 mole % dioxane, extinctions were determined at several frequencies in the region of interest. The intensities at these points were determined with the aid of the characteristic curves, and intensity distribution curves were drawn (Figure 1). From the microphotometer traces of the other mixtures, only the maximum extinctions and intensities were evaluated. In general, the background extinctions were less than about 0.03. For values so small, the photometric error may be as much as 100% or even larger. Hence, corrections were applied for the background in only a few cases.

RESULTS

It was found at the beginning of this investigation that 1,4-dioxane has a Raman band at 852 cm.$^{-1}$, about $^1/_{20}$ to $^1/_{16}$ as strong as the 835 cm.$^{-1}$ band. This weak band was not observed by Villars (*12*), but was reported by Simon and Feher (*10, 11*) and by Williamson (*13*). It was not mentioned by Hanle and Heidenreich and apparently received no attention in their work. Neither did Hanle and Heidenreich remark that the 850 cm.$^{-1}$ Raman band of benzene is very broad and rather strongly shaded toward the violet. Actually, it contributes appreciably to the total intensity at 835 cm.$^{-1}$ in the spectra of the mixtures. Kohlrausch (*8*) lists a very weak benzene band at 825 cm.$^{-1}$; however, it did not appear as resolved in the spectra of pure benzene, and so it was not considered separately in the present work.

The intensity distribution curve for the spectrum of a mixture containing 10 mole % of dioxane is shown in Figure 1,*a*. This must be regarded as the superposition of the two intensity distribution curves represented by the broken lines. In order to compare the maximum intensities of the 835 cm.$^{-1}$ dioxane band and the 850 cm.$^{-1}$ benzene band, it is necessary to estimate the contributions of each curve to the maxima of the superposition curve.

If, in the spectrum of pure dioxane (Figure 1,*b*), D is the ratio between the intensity at 850 cm.$^{-1}$ and the maximum intensity of the 835 cm.$^{-1}$ band, then in the spectrum of a mixture the contributions of dioxane to the total intensities at 835 and 850 cm.$^{-1}$ may be expressed as I_{835}, the true intensity of the dioxane maximum in the mixture, and $D\,I_{835}$, respectively. Similarly, the contributions of benzene to the 850 and 835 cm.$^{-1}$ maxima are I_{850} and $B\,I_{850}$, where B is the ratio between the intensities at 835 and 850 cm.$^{-1}$ in the spectrum of pure benzene (Figure 1,*c*).

Thus, the observed maximum intensities, I'_{835} and I'_{850}, are related to the correct intensities, I_{835} and I_{850}, by the expressions

$$I'_{835} = I_{835} + B\,I_{850}$$

$$I'_{850} = I_{850} + D\,I_{835}$$

These equations may be solved for the correct intensities, giving

$$I_{835} = (I'_{835} - B\,I'_{850})/(1 - B\,D)$$

$$I_{850} = (I'_{850} - D\,I'_{835})/(1 - B\,D)$$

whence

$$I_{835}/I_{850} = (I'_{835} - B\,I'_{850})/(I'_{850} - D\,I'_{835})$$

D and B could be determined directly from the microphotometer traces with sufficient accuracy for the present work. The distance between the two maxima was measured on each of

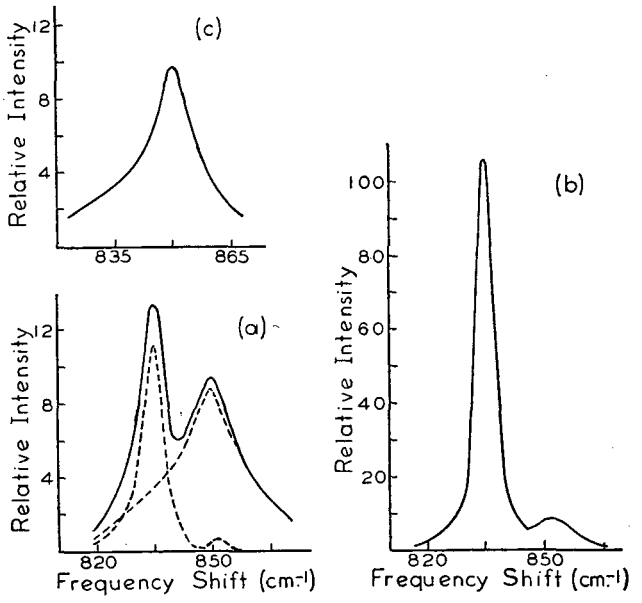

Figure 1. Intensity Distribution in 850 Cm.$^{-1}$ Region of Raman Spectra

a. **Mixture of 10 mole % dioxane in benzene.** ***b.*** **Dioxane.** ***c.*** **Benzene. Exposure time, 1.5 hours**

the traces for the mixtures. This distance varied somewhat on different traces; the average value for all the traces was therefore used.

From each microphotometer trace for pure dioxane, the intensities at the 835 cm.$^{-1}$ maximum and at the appropriate distance to the long wave-length side of this maximum were determined. The ratio between the latter and the former intensities was taken as D. Similarly, from the traces for pure benzene, B was determined as the inverse ratio between the intensity of the 850 cm.$^{-1}$ maximum and that at the correct distance to the short wave-length side from the maximum. Three values of D and of B were determined in this manner from three spectra of the pure components. The average values, 0.057 and 0.31, respectively, were used in determining the correct intensity ratio, I_{835}/I_{850}, for each mixture.

In Figure 2, the quantity $(I'_{835}/I'_{850})/C$, containing the uncorrected maximum intensities, is plotted against C, the mole per cent of dioxane in the mixtures (which is nearly equal to the volume per cent). The curve in the figure is reproduced from the paper of Hanle and Heidenreich. For values of C less than 6 mole %, the curve represents the plotted points almost perfectly. For larger values of C the deviation is fairly small. This indicates that Hanle and Heidenreich must have used the uncorrected maximum intensities in plotting their curve.

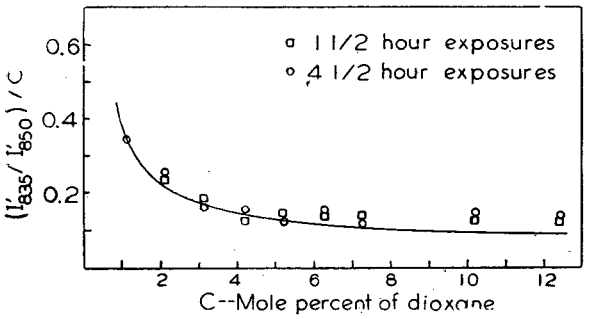

Figure 2. Observed Values of $(I'_{835}/I'_{850})/C$ Plotted against C

Values not corrected for overlapping of benzene and dioxane bands. Curve reproduced from Hanle and Heidenreich's paper

In Figure 3, the quantity $(I_{835}/I_{850})/C$, calculated with the use of the corrected maximum intensities, is plotted against C. This quantity is practically constant over the range of the concentrations studied. The slight rise with increasing concentration of dioxane, which amounts to about twice the experimental error, is just what is to be expected, inasmuch as not $(I_{835}/I_{850})/C$ but $(I_{835}/I_{850})(100 - C)/C$ should be constant. Thus, the principle of superposition is valid for the mixtures investigated.

Probably the most important source of error in this work is the photometric error. This is relatively large, as some of the intensities were so small that the lack of uniformity of the emulsion would have a considerable effect.

The contribution o benzene to the total intensity at 835 cm.$^{-1}$ is more disturbing than that of dioxane at 850 cm.$^{-1}$ The band at 852 cm.$^{-1}$ is so weak that with small concentrations of dioxane it would hardly appear in the absence of the stronger benzene band.

Except for the bands studied, no pair of dioxane and benzene bands could be found in the Raman spectra obtained which might be used in analysis. The dioxane bands which appeared in addition to the 835 cm.$^{-1}$ band were masked by the stronger benzene bands. It is possible that, with considerably longer exposures, the weak 486 cm.$^{-1}$ band of dioxane and the much weaker benzene band listed by Kohlrausch (*8*) at 406 cm.$^{-1}$ might be used for analysis. However, even with a 27-hour exposure with the mixture containing 1 mole % of dioxane, the former band did not appear, while the latter was very weak.

CONCLUSIONS

It appears certain that the results reported by Hanle and Heidenreich for mixtures of dioxane and benzene are erroneous. When the overlapping of the 835 cm.$^{-1}$ dioxane band by the 850 cm.$^{-1}$ benzene band and the contribution of the 852 cm.$^{-1}$ band of dioxane to the intensity at 850 cm.$^{-1}$ are taken into account, the Raman spectra of dioxane and benzene are additive, at least for small concentrations of dioxane in benzene.

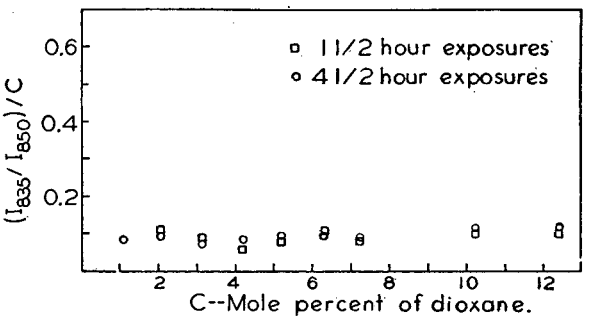

Figure 3. Values of $(I_{835}/I_{850})/C$ Plotted against C

Corrected for overlapping of benzene and dioxane bands

Mixtures of dioxane and benzene represent one of the more unfavorable cases for quantitative analysis, although one of considerable importance. The absence of dioxane bands, which are resolved from close benzene bands and are sufficiently strong to permit accurate comparison, makes the analysis very difficult. In spite of this difficulty, analysis of mixtures of dioxane and benzene with the use of Raman spectra is feasible. As little as 1 or 2 mole % of dioxane can be detected with certainty.

The results of the present work emphasize the necessity of recognizing that Raman spectra consist of bands having definite intensity distributions. From a list of the frequency shifts of the intensity maxima alone, it might have been concluded that the 852 cm.$^{-1}$ band of dioxane was solely responsible for the erroneous results of Hanle and Heidenreich. Only after a study of the intensity distribution over a fairly wide region of the Raman spectra of benzene and dioxane could the actual situation be ascertained.

ACKNOWLEDGMENT

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A Common-Matrix System of Spectrochemical Analysis

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A general method of emission spectrochemical analysis is described which is applicable to any inorganic material. Lithium carbonate, which acts as a flux and diluent, is used to modify extensively the effects of different major constituents present in the samples. The method is rapid, uses only a small amount of sample, and yields results that are usually correct within a factor of 2.

THERE are many instances where a semiquantitative spectrochemical analysis will serve as well and cost less in time and money than an accurate quantitative analysis. This is particularly true when a wide variety of materials must be analyzed and the cost of establishing a spectrochemical system for one or two samples is prohibitive.

The method described yields semiquantitative results usually correct within a factor of 2, is rapid and applicable to practically all inorganic materials, and requires only a small amount of sample.

One of the many factors which dictate the method of analysis to be employed in any given problem is the degree of accuracy required. For the sake of convenience, accuracies may be spoken of in terms of the expressions of Wright (*8*)—i.e., guesses, estimates, and determinations. Spectrochemical methods are capable of any degree of accuracy from purely qualitative results to quantitative determinations with accuracies of ±2% of the amount of the element present. Lockyer (*4–6*) may be credited with the discovery of quantitative spectrochemical analysis in 1873, and since that time there have been many successful methods published for spectrochemical determinations. However, the type of analysis that yields results of intermediate accuracy (estimates) was practically left untouched when the major efforts were turned to the search for refinements that would yield accuracies of ±10% or better. A method described by Harvey (*2*) appears to have been the only serious attempt to generalize semiquantitative analysis which has been published. Thus, there are very few methods described in the literature which yield results of intermediate accuracy and are at the same time simple, rapid, and applicable to almost any material. The method described here is the result of an attempt to fill these requirements.

DISCUSSION OF THE PROBLEM

The effect of one element on the behavior of another in the arc is a determining factor in the approach to any generalized spectrochemical method. Although the effects of small concentrations are usually not serious, variations in the major constituents have severe and easily demonstrable influences on the spectra of each other as well as on the minor constituents. The simplest solution to this problem appears to be to give all samples the same major constituent, thus reducing the concentrations of all elements to the level of minor constituents or traces in the common matrix. At the same time the diluent may be selected for the fluxing action it has on the sample, thus making it possible to reduce a variety of chemical combinations to a common form.

Eighteen substances were tested in the arc to observe their burning characteristics. These substances, with observations on burning behavior, are shown in Table I. Of the seven fluxes that were observed to burn well in the arc, four were not investigated further because of special considerations. Thus, sodium fluoride is toxic and liberates noxious fumes in the arc, potassium carbonate is hygroscopic, and potassium acid sulfate cakes when ground in a mortar. Although anhydrous sodium borate burned well, the spectrograms were badly obscured, apparently by band spectra. The three remaining fluxes, barium, sodium, and lithium carbonates, were tested more thoroughly. Calibration curves were prepared for several elements in these fluxes and precision and accuracy were studied. It was observed that the presence of alkali in samples tended to suppress the spectral lines of other elements; for this reason an alkali flux appeared attractive, as it would overshadow in effect the relatively small variable amounts of alkalies already present in the sample. As the final choice lay between sodium and lithium carbonates, lithium carbonate was chosen because it is frequently necessary to determine sodium, whereas lithium is seldom encountered. The use of lithium carbonate in spectrochemical analysis is not without precedent. DeGramont (*1*) used lithium and sodium carbonates as fluxes in the preparation of samples for spectrographic analysis. Harvey (*2*) recommended the use of lithium carbonate as a flux and buffer under certain circumstances. Wilson (*7*) also employed lithium carbonate as a diluent in a spectrochemical method which depended for its interpretation on the ratios of intensities of selected spectral lines to the intensity of a lithium line without resort to calibration curves—i.e., ratios were obtained but not actual concentrations of elements in the sample.

APPARATUS

An Applied Research Laboratories 1.5-meter grating spectrograph, densitometer, and arc source were used. A special type of electrode (anode), similar in design to that described by Hasler (*3*) was prepared with the aid of a commercially available cutting device. The dimensions of the electrodes are shown in Figure 1.

Table I. Fluxes Tested for Arcing Characteristics

Flux	Observations
$Na_2B_4O_7$	Burns well
$Na_2B_4O_7.5H_2O$	Unsatisfactory. Jumps out of arc
BaF_2	Unsatisfactory. Forms ball that jumps out of arc
$BaCO_3$	Burns well
Pb_3O_4	Unsatisfactory. Forms lead pellets that jump out of arc
$Na(NH_4)_2PO_4$	Unsatisfactory. Blows out of arc
NaF	Burns well
$CaCl_2$	Unsatisfactory. Flutters and sputters
$NaBO_3$	Unsatisfactory. Blows out of arc
$NaBO_3.4H_2O$	Unsatisfactory. Blows out of arc
B_2O_3	Unsatisfactory. Flutters
$NaHCO_3$	Unsatisfactory. Jumps out of arc
$KHCO_3$	Unsatisfactory. Jumps out of arc
K_2CO_3	Burns well
Na_3PO_4	Burns fairly well. Does not wet electrode
$KHSO_4$	Burns well
Na_2CO_3	Burns well
Li_2CO_3	Burns well

METHOD

Purification of Lithium Carbonate. Dissolve 2.2 kg. (5 pounds) of lithium nitrate in water at 40° C. to make 2 liters of solution. Pour part of the solution into a large platinum dish and supercool the contents of the dish, in an ice bath, to 15° C. If crystallization occurs before this temperature is reached, redissolve the crystals and cool again. At 15° C. seed the solution with a lithium nitrate crystal. Quickly stir the crystalline slurry and filter immediately on a Whatman No. 41 filter paper or its equivalent in a Büchner funnel. Crystallize the entire 2 liters in this manner. Save the mother liquor for a subsequent second yield.

Fill the platinum dish with crystals from the first crystallization, add not more than 1 ml. of water for each 100 ml. of crystals, heat to 40° C., precipitate crystals, and filter as before. Process the entire yield of crystals from the first crystallization in this manner. Combine the mother liquor with that from the first crystallization. Recrystallize the twice crystallized crystals a third time as described above. Place the thrice crystallized lithium nitrate in a platinum dish, add not more than 1 ml. of water for each 100 ml. of crystals, and dissolve completely at 40° C. Filter the solution through a No. 41 paper in a Büchner funnel. Prepare a saturated solution of ammonium carbonate in water at room temperature. Filter. Place 3 volumes of ammonium carbonate solution in a platinum dish and add to it 1 volume of solution, at 40° C., of the thrice crystallized lithium nitrate. Stir, cover, and allow to stand overnight. Filter the precipitated lithium carbonate on a No. 41 paper in a Büchner funnel. With the vacuum off, flood the crystals with 200-proof ethanol, stir, and then remove the ethanol by vacuum filtration. Wash the material a total of three times in this manner. Place the crystals in a platinum dish, dry at 105° C. for 30 minutes, and ignite at 450° C. for 30 minutes. Reprocess the mother liquor from the lithium nitrate crystallizations for a second yield.

Calibration. Prepare a mixture of 1% of each element to be determined (carbonates are desirable) in lithium carbonate and grind together thoroughly. Progressively dilute these with more lithium carbonate so that standards containing 0.3, 0.1, 0.03, 0.01, and 0.003% are obtained. Pack the annular spaces about the center posts of three or more electrodes level full with each standard. Adjust the arc gap to 6 mm.; do not adjust it while the arcing is in progress. Arc each electrode with a current of 7.5 amperes until the sample is burned away. The complete burning of the sample is clearly defined by a sudden change in the color and sound of the arc, and an increase in the potential drop across the arc. The average burning time is about 60 seconds. Use a slit width of 50 microns. Process the film (Eastman Spectrum Analysis No. 2) and measure the transmittances of the spectral lines shown in Table II. Adjust the densitometer to read 100% transmittance on the unexposed film; make no background corrections. From an emulsion calibration (gamma) curve previously prepared for the region 3100 to 3400 Å on the emulsion bearing the same emulsion number, determine the relative intensity of each line measured and calculate the ratio of line intensity to the intensity of the chosen lithium line (2475.3 Å). Average the three or more ratios so obtained for each line and plot concentrations *vs.* average intensity ratios as shown in Figure 2.

Samples. Weigh one part (about 10 mg.) of dry, finely pulverized sample into a mortar. Stir into this a small amount (0.5 to 1.0 mg.) of purified gentian violet dye. Add 9 parts of lithium carbonate and grind the materials until they assume a uniform violet color. Fill and arc the electrodes under the same conditions as prevailed during calibration and measure the spectral line transmittances of elements to be determined. If any intensity ratio is beyond the range of the calibration curve, dilute the sample still further with lithium carbonate and repeat the arcing. From the calibration curve read the concentration of the element in the lithium carbonate mixture and multiply this by the dilution factor to obtain the concentration in the original sample.

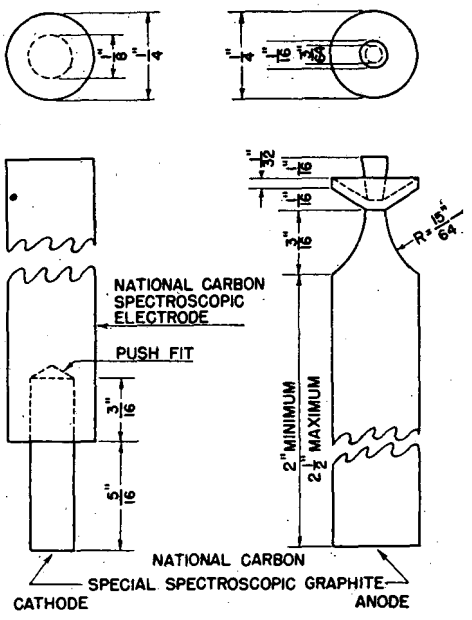

Figure 1. Electrodes

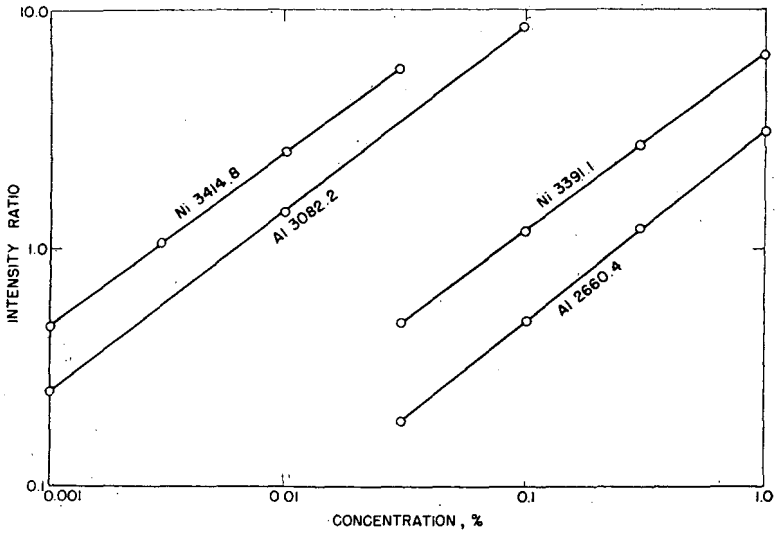

Figure 2. Calibration Curves

Table II. Spectral Lines Used and Ranges Covered

Element	Line, Å.	Range, %	Element	Line, Å.	Range, %
Ag	3382.9	0.0001–0.03	Mo	3132.6	0.001–0.1
Al	3082.2	0.001–0.1		3112.1	0.1–1.0
	2660.4	0.03–1.0	Na	3302.3	0.01–1.0
B	2496.8	0.03–1.0		2852.8	0.2–1.0
	2497.7	0.01–1.0	Ni	3414.8	0.001–0.03
Ba	4554.0	0.0003–0.01		3391.1	0.03–1.0
	3071.6	0.03–1.0	P	2535.7	1.0–3.0
Be	3130.4	0.003–1.0		2553.3	1.0–3.0
Bi	3067.7	0.003–0.1	Pb	2833.1	0.003–0.3
	2898.0	0.1–1.0		2663.2	0.1–1.0
Ca	4302.5	0.001–0.1	Sb	2598.1	0.1–1.0
	4318.7	0.03–0.3	Si	2516.1	0.01–1.0
	3179.3	0.3–1.0		2881.6	0.01–1.0
Cd	3261.1	0.03–1.0		2514.3	0.1–1.0
Co	3453.5	0.003–0.1	Sn	3175.0	0.003–0.1
	3334.1	0.03–1.0		2840.0	0.003–0.3
Cr	4254.3	0.001–0.01		2429.5	0.1–1.0
	4289.7	0.003–0.03	Sr	4077.7	0.0001–0.003
	4351.8	0.01–0.3		4438.0	0.3–1.0
Cu	3274.0	0.001–0.03	Ti	3372.8	0.003–0.3
	2961.2	0.1–1.0		3217.1	0.03–1.0
Fe	3020.6	0.003–0.1	V	3184.0	0.001–0.03
	3021.1	0.01–0.3		3202.4	0.03–1.0
	3008.1	0.03–1.0	W	4008.8	0.03–1.0
K	4044.1	0.03–1.0		2947.0	0.1–1.0
Mg	2795.5	0.001–0.03	Zn	3282.3	0.03–3.0
	2779.8	0.03–1.0	Zr	3392.0	0.01–1.0
Mn	4030.8	0.001–0.01	Li	2475.3	Reference
	2576.1	0.01–1.0			

Table III. Results of Analysis of Bureau of Standards Samples

Sample Name	No.	Element	N.B.S.[a], %	Found, %	Error Factor[b]
Iron ore, magnetite	29a	Si	1.34	1.4	1.04
		Mn	0.023	0.025	1.09
		Mg	0.057	0.057	1.00
		Al	0.24	0.21	1.14
		Ti	0.18	0.21	1.17
Dolomite	88	Si	0.15	0.19	1.26
Opal glass	91	Fe	0.057	0.044	1.30
		Pb	0.090	0.081	1.11
Chrome refractory	103	Si	3.8	5.5	1.45
		Al	11.1	16.	1.44
		Mg	9.8	10.	1.02
		Fe	11.2	17.	1.52
		Ti	0.50	0.52	1.04
		Mn	0.16	0.14	1.14
		Ca	0.56	0.27	2.07
		Cr	25.2	33.	1.31
Flint clay	97	Fe	0.69	0.61	1.13
		Na	0.089	0.060	1.48
		Si	20.0	25.	1.25
		Zr	0.18	0.24	1.33
		Mg	0.157	0.25	1.59
		K	0.49	0.47	1.04
		Al	20.5	23.	1.12
		Ca	0.07	0.023	3.05
		Ti	1.43	2.3	1.61
		Cr	0.054	0.070	1.30
osphate rock	56a	Si	5.15	6.0	1.16
		Al	1.08	1.0	1.08
		P	14.4	20.	1.39
		Mg	0.08	0.11	1.38
		Fe	1.50	1.7	1.13
		Na	0.207	0.17	1.22
		Ti	0.048	0.040	1.20
		Mn	0.14	0.11	1.27
		K	0.23	0.20	1.15
Sheet brass	37[c]	Pb	0.90	0.60	1.50
		Sn	0.99	1.0	1.01
		Fe	0.21	0.16	1.31
		Cu	70.36	75.	1.07
		Zn	27.09	32.	1.18
Lead-base bearing metal	53a[c]	Sb	10.28	11.	1.07
		Sn	10.22	9.7	1.05
		Bi	0.05	0.048	1.04
		Ag	0.006	0.0065	1.08
Phosphor-bronze bearing metal	63[c]	Pb	9.74	7.6	1.28
		Sn	9.91	11.	1.11
		Fe	0.27	0.21	1.28
		Al	0.05	0.026	1.92
Steel, Cr-W-V	50a[c]	Si	0.48	0.85	1.77
		V	0.97	1.3	1.34
		Cu	0.047	0.028	1.68
		Ni	0.045	0.11	2.45
		Mn	0.28	0.25	1.12
		Cr	3.52	3.5	1.01
		W	18.25	18.	1.01
Steel, Ni-Mo (SAE 4620)	111a[c]	Mn	0.74	1.5	2.03
		Mo	0.222	0.20	1.11
		Ni	1.74	1.6	1.09
		Cr	0.243	0.23	1.06
Steel, 18Cr-11Ni (columbium bearing)	123a[c]	Si	0.46	0.70	1.52
		Mo	0.12	0.11	1.09
		Co	0.75	0.26	2.88
Argillaceous limestone	1a	Si	6.6	7.2	1.09
		Al	2.2	2.5	1.14
		Mg	1.32	1.4	1.06
		Fe	1.14	1.6	1.40
		Na	0.29	0.31	1.07
		Ti	0.096	0.090	1.07
		Mn	0.029	0.037	1.28
		K	0.59	0.90	1.52
		Sr	0.10	0.12	1.20
Calcium molybdate	71	Fe	1.92	3.2	1.67
		Mo	35.3	25.	1.41
		Ti	0.06	0.040	1.50
Fluorspar	79	Si	0.88	1.6	1.82
		Pb	0.23	0.17	1.35
		Mg	0.079	0.050	1.58
		Fe	0.105	0.068	1.55
		Al	0.01	0.082	8.20
		Ba	0.063	0.060	1.05
Pb-Ba glass	89	Pb	16.0	12.	1.33
		Mg	0.018	0.075	4.17
		Al	0.096	0.064	1.50
		Na	4.2	2.9	1.45
		Mn	0.068	0.050	1.36
		K	6.97	5.5	1.27
		Ca	0.15	0.085	1.77
		Ba	1.25	0.50	2.50
Borosilicate glass	93	B	3.94	6.5	1.65
		Fe	0.053	0.035	1.52
		Al	1.03	0.60	1.72
		Na	3.08	2.4	1.28
Soda feldspar	99	Al	10.1	9.2	1.10
		Mg	0.032	0.085	2.66
		Fe	0.047	0.037	1.27
		Na	4.0	5.1	1.27
Plastic clay	98	Al	17.9	11.	1.63
		Mg	0.43	0.33	1.30
		Fe	1.43	1.6	1.12
		Na	0.21	0.17	1.23
		Ti	0.86	0.57	1.52
		K	2.63	2.2	1.20
		Ca	0.15	0.060	2.50
Soda-lime glass	128	B	0.47	0.66	1.40
		Mg	2.02	1.9	1.06
		Fe	0.027	0.030	1.11
		Al	0.98	0.73	1.34
		Na	6.2	7.6	1.23
		K	0.82	0.60	1.37
		Ca	3.33	2.2	1.51
		Ba	0.44	0.65	1.48

[a] Calculated from National Bureau of Standards certificate values.
[b] Error factor is defined as factor by which result must be multiplied, if too small, or divided, if too large, to equal correct result.
[c] Metal samples. Metals were converted to salts and Bureau of Standards values converted to concentrations in salts.

ANALYSIS OF NATIONAL BUREAU OF STANDARDS SAMPLES

Twenty National Bureau of Standards samples of different types were analyzed for a total of 111 determinations. Metal samples were weighed, dissolved in acid, ignited, and weighed again to obtain a factor by which the known concentrations of elements in the metals could be multiplied to find the true concentrations in the salt. The samples thus prepared were then diluted with lithium carbonate in the usual manner. The results are shown in Table III. The average error factor was 1.47, and 91% of the results were correct within a factor of 2.0. This indicates that the results obtained by this method are accurate to only one significant figure; the second significant figures reported in Table III are in doubt. Results of analyses are therefore usually reported to two significant figures and the second figure is designated as doubtful.

DISCUSSION

A wide variety of material has been analyzed by the method described and in many cases the accuracy has been verified by subsequent chemical analyses. The method has been found particularly useful in the analysis of lubricating oils containing metallic additions, catalysts, engine deposits, and corrosion products. In these laboratories many materials submitted for chemical analysis are first analyzed in this fashion.

Few serious cases of interference between elements have been observed. When such cases do occur, they are frequently detected by the discrepancy of results obtained from two different spectral lines of the element being determined. The judicious choice of lines for calibration and the use of reliable wave-length tables will practically eliminate interference difficulties.

Samples that contain significant amounts of organic matter or water are ashed prior to the addition of lithium carbonate. Metallic samples are dissolved in acid and ashed as described under Bureau of Standards samples.

The calibration curves may be used indefinitely; the small shifts which are so noticeable in accurate quantitative work are not of sufficient magnitude to have a significant effect on the accuracy of the method. Of course, if the arcing or instrument conditions are changed, the curves should be checked.

Commercially available lithium carbonate was contaminated with undesirable impurities and had to be purified before it could be used. All precipitations were made in platinum dishes and great care was exercised to prevent contamination in all

stages of the process. The resulting lithium carbonate was analyzed spectrographically and found to contain only traces of copper (about 0.0003%), calcium (about 0.001%), and strontium (less than 0.0001%). To correct for the effect on the calibration curves of the residual impurities in the purified lithium carbonate, the concentrations of elements added to the lithium carbonate were plotted rather than the sum of the residual plus the added concentrations. Thereafter, when the curves were used, the concentrations of elements in the lithium carbonate–sample mixture were read from the curves as the amounts in excess of the concentrations present in the lithium carbonate.

The use of gentian violet dye in the sample mixture serves only to show when the components are thoroughly mixed. It has no effect on the arc. Commercially available gentian violet (certified grade) contained a high concentration of sodium and had to be purified. The dye was twice crystallized from ethanol by the addition of water. The crystals were filtered in a Büchner funnel and dried 24 hours in a vacuum desiccator over Drierite.

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Direct Spectrochemical Analysis of Solutions

Using Spark Excitation and the Porous Cup Electrode

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The porous cup electrode consists of a 0.25 × 1.5 inch right cylindrical graphite rod, with a 0.125-inch hole drilled along its axis from one end to within approximately 1.1 mm. of the other end. It is used as the upper electrode, with the open end up. In operation, a long-nosed pipet, containing 0.2 to 0.3 ml. of the solution to be analyzed, is inserted into the cavity in the upper electrode until its tip touches bottom; the solution is then expelled as the pipet is withdrawn. The lower electrode is a solid 0.125-inch graphite rod. A synchronous spark from a Baird spark source or a 220-volt intermittent alternating or direct current arc is first applied for 5 to 10 seconds, using a 2-mm. analytical gap. The heat thus produced helps the liquid to soak through the bottom of the porous cup and reach the sparking surface. After a short rest (~15 seconds), the sparking is resumed and the exposure begun. The liquid feeds through by wick action, constantly renewing the thin surface film of liquid as it is dispersed by the spark. Spattering does not occur, as the spark never strikes the body of the liquid. Almost all the energy of the spark is dissipated in vaporizing and exciting the liquid film, so that the sample does not boil over. A sample of the size mentioned lasts as long as 240 seconds. Successful analyses have been made of acidic, neutral, and slightly alkaline solutions having a wide range of salt concentrations. The technique is also applicable to some solutions in organic solvents. The following approximate sensitivity limits have been attained: 0.01 to 0.1 p.p.m. Be, Mg; 0.1 to 1.0 p.p.m. Ag, Al, B, Li; 1.0 to 10 p.p.m. Bi, Cb, Co, Cr, Cu, Fe, Ga, Ge, Hf, In, La, Mn, Ni, Pb, Re, Ti, V, Zr; 10 to 100 p.p.m. As, Au, Cb, Ce, Cs, Hg, P, Pt, Ru, Sb, Sn, Th, Zn; 100 to 1000 p.p.m. Te, W.

WHEN this laboratory began to receive a large number of solutions for quantitative analysis, a search was made for a spectrochemical method versatile enough to handle the variety of compositions, concentrations, and degrees of radioactivity encountered, yet simple enough to lend itself to routine operations. Because spark methods appeared to offer the prospect of better precision and accuracy than did arc methods, the known techniques for sparking solutions were examined.

The spectrochemical analysis of solutions with spark excitation has usually been performed by a residue method, an impregnation method, or a continuous feed method.

METHODS FOR SPECTROCHEMICAL ANALYSIS

Residue Methods. Rivas (*28*) proposed the sparking of dried residues on graphite, but the most successful method of this type to appear so far seems to be the copper spark technique recently described by Fred, Nachtrieb, and Tomkins (*11*) and extended by Bachelder, Conway, Nachtrieb, and Wildi (*1*). The latter technique gives adequate precision (average deviation ±7.6% for 119 intensity ratios run in quadruplicate) and excellent sensitivity.

In the copper spark method, the spark volatilizes and excites all elements in the residue simultaneously, although possibly not to the same extent. It has been proved possible to use molybdenum as an internal standard for determining many chemically and physically unrelated elements by this technique. The applicability of the technique, however, is somewhat limited; it cannot conveniently be used with solutions that contain high concentrations of salts, reagents that attack the electrodes, or materials that leave fluffy or deliquescent residues.

Impregnation Methods. WITH GEL ELECTRODES. Some 25 years ago, Errera (*7*) proposed the use of agar sticks soaked several hours in the sample solution as electrodes for the spark analysis of solutions. This technique was later simplified by Rohner (*29*), who impregnated thin gelatin disks with the solution to be studied, and sparked them. The use of gels is inconvenient, however, and these techniques have not been widely adopted.

WITH OXIDE LAYERS. When determining boron by the copper spark technique, Fred, Nachtrieb, and Tomkins (*11*) deposited a layer of calcium oxide on copper, and impregnated this with the

solution to be studied. Mann (*24*) proposed the impregnation of aluminum electrodes, rendered porous by anodizing, with the sample solution.

With Filter Paper. Ewles and Curry (*8*) impregnated a tight roll of filter paper with the solution and sparked this.

With Graphite Electrodes. One of the simplest and most widely used techniques available for liquid samples is that in which a flat-topped graphite electrode is impregnated with a drop of the solution, and sparked immediately. This technique was suggested in 1936 by Scheibe and Rivas (*31*). When used with the modifications proposed by Sloviter and Sitkin (*32*), it gives excellent accuracy and precision. Their studies show the elements present in solutions of steels to be volatilized at the same rate, thus justifying the use of the internal standard procedure. The chief limitation of the method is the fact that not all solutions will quickly and spontaneously penetrate such electrodes.

Continuous Feed Methods. Spark-residue methods suffer less from preferential volatility effects than do arc methods, but the composition of the radiating vapor as a whole may change in either method as the sample is consumed. This change and the change in excitation conditions which accompanies it are of major importance for some combinations of elements. Many workers have thought it advisable to avoid these and other difficulties by feeding the solution into the spark in some continuous manner.

Spark-in-Mist Techniques. Bouchetal de la Roche (*3*) extended the Lundegårdh mist-in-flame method by passing a spark through the flame. Uzumasa and Okuno (*36*) merely passed a high voltage spark through the mist, as did Lamb (*21*). Neither of these techniques had the simplicity or versatility desired.

Spark-to-Bulk-Liquid Techniques. The most popular method of this type has been the use of a spark passed between a solid electrode and the free surface of a liquid. Jolibois and Bossuet (*17*) used a constant-level liquid chamber with 5000-volt direct current excitation. Sparking tubes similar to that of Twyman and Hitchen (*34, 35*) are available commercially. Somewhat simpler versions of this method of feeding were described by Gerlach and Schweitzer (*13*). Walti (*37*) introduced the solution into a cup hollowed out in the lower (graphite) electrode through a hole punctured in its side wall. Nedler and Efendiev (*25*) merely struck a spark between a solid upper electrode and the liquid coming up from below through a 2-mm. hole in graphite plaque.

Lundegårdh (*23*) attributes to Necke the suggestion that the solution be slowly fed into the spark from above through a hollow upper electrode. Keirs and Englis (*20*) obtained very good precision with a simple arrangement of this type.

Attempts have been made to have the spark strike between two liquid surfaces. Lomakin's dropping electrode (*22*) is somewhat elaborate, and requires 30 to 40 ml. of solution. De Gramont (*14*) and Duffendack and Thomson (*5*) pass the spark between the liquid issuing from two quartz capillaries.

Spark-to-Liquid-Film-on-Solid Techniques. In all the techniques mentioned above, the spark struck a body or column of liquid, and a certain amount of spattering resulted. In order to minimize this, several techniques have been developed to introduce the liquid into the spark as a thin film supported by a solid electrode.

Rohner (*30*) has suggested that the upper electrode be held vertically and that the lower electrode be held at slightly less than 90° to it. The liquid is then dropped on the free end of the lower electrode, and runs down into the spark in a thin film.

The same effect is achieved in a somewhat smoother manner by Sventitskiĭ (*33*). A copper disk (20 × 1 mm.) is held vertically, and slowly rotated in its own plane. Its lower edge dips into the solution, and the spark is struck between its upper edge and an opposing electrode. (A similar device, using a rapidly revolving graphite disk, had previously been proposed for use with arcs by Pierucci and Barbante-Silva, *27*). Using the copper disk and a low-voltage intermittent (condensed) alternating current arc, Sventitskiĭ and co-workers have been able to determine the halogens (*33*) and sulfur (*2*). The rotating disk apparatus has recently been made available commercially.

Thin film feeding has also been achieved by capillarity; Hartley's device (*15*), the first ever proposed for sparking liquids, used this approach. Pavlovschi and Mavrodineanu (*26*) fashioned their upper electrode from a microchemical filter stick, with one half cut off to expose the fritted-glass surface, and their lower electrode from a sintered quartz rod. The former was filled with solution, and the latter immersed in a reservoir of solution. Excitation was provided by a Tesla coil.

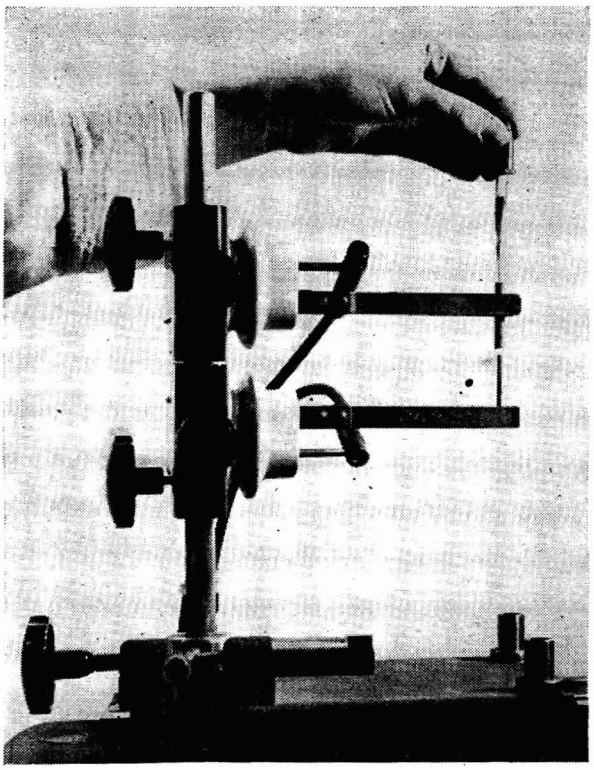

Figure 1. Porous Cup Electrode Being Filled

In a technique described by Fred and Corvin (*10*), the lower electrode consists of a flat-topped piece of 0.6-cm. (0.25-inch) graphite rod firmly seated in a shallow aluminum cup whose inside diameter is larger than that of the electrode. The sample solution is placed in the intervening space and is transported into the spark through the graphite by capillarity.

Each of the techniques mentioned was capable of dealing with some types of solutions, but none had the combination of versatility and simplicity necessary for the work at hand. The residue and impregnation methods placed too many restrictions on the nature or concentration of the solutes. The solid-to-bulk-liquid techniques all used auxiliary apparatus of various degrees of complexity, and gave rise to a troublesome amount of spattering. The thin film devices listed are limited either by the nature of the solutes, where metal parts are involved, or by the viscosity and surface tension of the solution, where fairly thick porous electrodes are concerned.

EXPERIMENTAL

The continuous feed approach offered the promise that the composition of the radiating vapor would always represent that of the solution, and among the continuous feed techniques, the capillarity methods seemed to offer the prospect of greatest simplicity. Effort was therefore directed toward doing away with auxiliary apparatus and circumventing the difficulties caused by high viscosity and surface tension.

A technique was developed which uses no auxiliary apparatus other than a pipet, produces no splashing, and is not limited to solutions of low viscosity.

By means of a long-nosed pipet the solution is inserted into a porous graphite cup (see Figure 1), which is used as the upper electrode. A high-voltage spark is passed between the cup and a graphite lower electrode. After a 5- to 10-second prespark period, followed by a rest, the sparking is resumed. The liquid then feeds through to the sparking surface by wick action, and is there dispersed and excited by the spark. The analytical gap is 2 mm. The

spectrum obtained is measured with a densitometer and the results are interpreted by any procedure desired.

Exposure Technique. PREPARATION OF ELECTRODES. The porous graphite cup is prepared by cutting spectrographic graphite rods 0.6 cm. (0.25 inch) in diameter into 3.75-cm. (1.5-inch) lengths, and drilling a 0.125-inch hole along the axis from one end to within 1.1 ± 0.2 mm. of the other end. A greater floor thickness than this may prevent the passage of some solutions; a lesser thickness increases the danger of puncture during operation. A porous cup electrode as used and a cutaway view of the same electrode are shown in Figures 1 and 2. These electrodes can be produced at the rate of about two per minute, using a drill press with the simple jig illustrated in Figure 3.

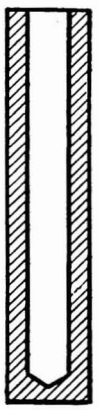

Figure 2. Porous Cup Electrode

The jig is in many respects similar to the electrode clip once used on the Hilger arc-spark stand. It has a vertical well, *A*, 0.28 inch in diameter and 1 inch deep, which holds the electrode. The bottom surface of this well is formed by surface *B*. A 0.25-inch hole, *D*, is drilled horizontally through the block in such a way that it overlaps hole *A* by about 0.03 inch (0.8 mm.). A 0.25-inch metallic cylinder, *C*, is ground flat and grooved in the manner illustrated in Figure 4. This cylinder is provided with a handle, inserted into *D*, and secured there by means of a pin through its end. When the flat surface, *F*, is in a vertical position, *A* is unobstructed. When an electrode is inserted into *A* and the handle of *C* is turned, the ends of the grooves engage the electrode and prevent it from rising or rotating while the drilling is in progress.

The jig is placed on the table of the drill press, and an ordinary 0.125-inch drill is inserted loosely into the jaws of the chuck. A metal plate whose thickness is equal to the desired floor thickness of the porous cup electrode is placed on horizontal surface *B*, and the vertical stop of the drill press is adjusted so that the tip of the drill will be stopped at the surface of this plate when the drilling head is lowered. The chuck jaws are then tightened. The jig is now placed so that the axis of *A* coincides with the axis of the drill, and the jig is clamped in position. A cut electrode is inserted into *A*, clamped in place by rotating the jamming cylinder, *C*, and the cavity is drilled. *C* is then released and the finished electrode extracted.

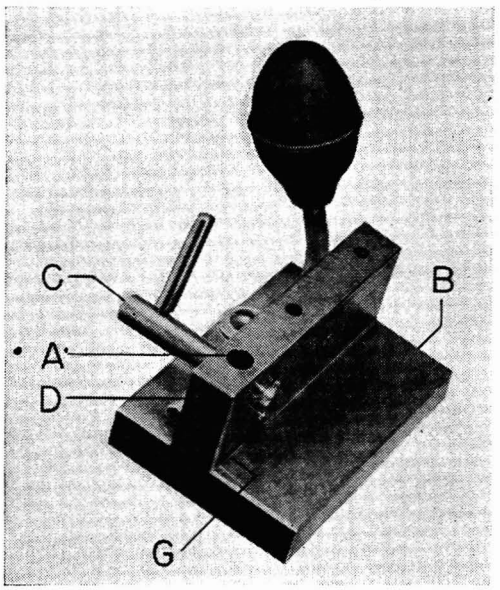

Figure 3. Outside View of Jig

In order to prevent the accumulation of graphite powder within *A*, a clover-leaf pattern of holes has been drilled through the bottom surface of the well. Any graphite powder not falling through these holes can be removed by blowing a blast of air through channel *G*, using the rubber bulb and air tube shown in Figure 3.

The two blocks are made of brass; cylinder *C* is of soft steel and is kept well oiled.

The lower electrode is a graphite rod 0.125 inch in diameter with a flat or pointed end.

TYPES OF SOLUTIONS. All aqueous solutions that wet graphite can be analyzed by this technique. Acidic, neutral, and weakly alkaline solutions ranging in concentration from fractions of 1 p.p.m. to 150% (weight/volume) (saturated ammonium thiocyanate solution) have been run routinely. Solutions alkaline with ammonium hydroxide can be handled, but some solutions of pH 9 and above do not wet graphite well enough to give the desired wick action. The use of the wetting agent Triton has helped in some cases, but the best solution of this difficulty, when permissible, is acidification of the solution. If possible, solutions are usually made 10% in sulfuric acid, which gives them an optimum viscosity and wetting power. This is by no means necessary, however.

Although no systematic effort has been made to detect preferential adsorption of trace elements by the graphite, comparison of intensity ratios of a given line pair in a given solution in exposures of various lengths failed to indicate such adsorption. Working curves (see Figure 5) also showed no evidence of it.

Organic liquids present no special difficulties other than flammability. This hazard can often be eliminated by mixing the liquid with water, adding a third component to homogenize the mixture if necessary. Several hundred analyses have been made in this manner on a highly volatile ketone. At the author's suggestion, Gassman and O'Neill (*12*) used the porous cup electrode technique to determine phosphorus, barium, calcium, and zinc in lubricating oils.

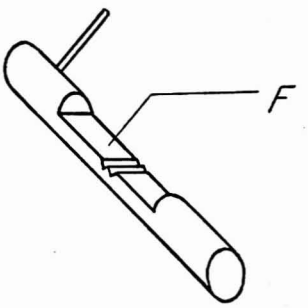

Figure 4. Jamming Cylinder

Radioactive solutions are analyzed by inserting the cup partway into an enclosed chamber. The cup may still be filled from the outside, but the dispersed mist originating within the chamber is conducted away by a suitable ventilating and filtering system. The slow air current used for ventilation passes through the chamber in a direction away from the slit in order to keep the front window from fogging.

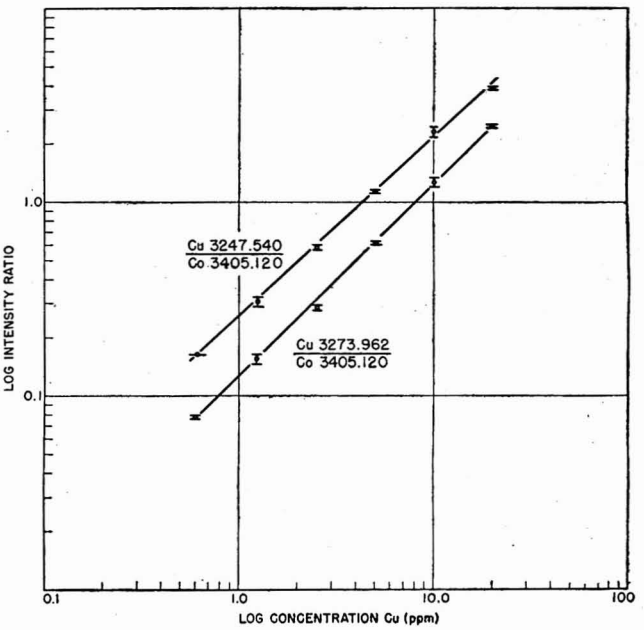

Figure 5. Working Curves for Determination of Copper

Optical Bench Fixtures. The Bausch & Lomb arc-spark stand has been most convenient for use with the porous cup electrode. On rare occasions, the spectrum contained faint lines of the elements present in the electrode clips. This was due to attack of the clips by acidic solutions; the difficulty was eliminated, where necessary, by using platinum foil liners for the clips.

Filling Technique (see Figure 1). The pipet used for filling the porous cup electrode is made by drawing 5-mm. Pyrex tubing in such a fashion as to produce a section at least 1.75 inches long whose outside diameter is less than 0.125 inch. The tip of the dropper should touch the bottom of the cavity as the liquid is expelled, so that bubbles will not be formed inside the cup. (The expansion of such bubbles during sparking would cause the solution to spill over the top.)

Source of Excitation. The low-voltage intermittent arc (alternating or direct current) can be used with the porous cup electrode, but most of the work so far has been done using a Baird spark source with a synchronous interrupter. The power input of this source is 0.45 kv.-amp., but less than 0.140 kv.-amp. is dissipated in the analytical gap. The Bausch & Lomb spark source has also proved satisfactory. Use of the 0.67-kv.-amp. step of the A.R.L. high voltage source seems to cause overheating on exposures of over 1 minute, unless the amount of power dissipated in the analytical gap is reduced by some means such as ohmic resistance or an additional gap in series in the discharge circuit.

On the other hand, the excitation must not be too weak, lest the sensitivity suffer. Pavlovschi and Mavrodineanu (*26*), for instance, using a Tesla coil, worked with solutions of aluminum in the 300 to 4000 p.p.m. range, whereas aluminum can be determined by the present method at a concentration of 1 p.p.m.

Sample Size. The volume of the cup cavity is approximately 0.32 ml. In operation, the cavity is filled completely with the sample solution. Shorter electrodes can easily be made if it is desired to use smaller samples.

Exposure Time. A single filling will permit an exposure of 120 to 240 seconds, depending on the viscosity of the solution. If longer exposures are necessary, the sparking may be interrupted and the cup refilled. As a matter of convenience, exposures are usually made for a standard period (180 seconds).

Optical Conditions. An image of the spark is focused on the grating when the Abney mounting is used, and on the parabolic mirror when the Wadsworth mounting is used.

DISCUSSION

Appearance of the Spark. The spark produced by this method is pink, unless a large amount of some metal having strong visible radiation of some other color is present. The pink color is due to the H_α line, which in turn is due principally to the large amount of water always present. At the beginning of the prespark period, the spark shows the normal blue-white carbon-to-carbon color; the appearance of a pink color is a signal that the solution has begun to feed into the spark. Similarly, a change in the color of the spark toward the end of the run indicates that the feeding has slowed down. Changes in color are usually accompanied by changes in the tone of the spark, and are probably a sign of a change in excitation conditions.

Appearance of Spectra. When photographed on SA1 film, using a 1.5-meter Abney mounting grating, spectra are essentially free of background up to approximately 2700 Å. Beyond the cyanogen bands, background transmission is 80% or greater. These figures are, of course, affected by length of exposure, constituents, and flow rate.

The cyanogen bands are weaker than in dry graphite-to-graphite spark spectra.

Spectra taken with the porous cup electrode are characterized by the presence of weak open bands beginning at 3064 Å., and degraded to the red. These are due to the free hydroxyl radical. These bands were also observed by Jolibois (*16*) when the solution was made the cathode of a 5000-volt (direct current) 40 ma. discharge. They have interfered with only two analytically useful lines in the 3 years the present method has been in use.

Table I. Approximate Detection Limits for Various Elements

Element	Raie Ultime by PCE[a]	Classification	Total Excitation Potential	Approximate Sensitivity, P.P.M. PCE[a]	Solid-to-bulk liquid	Mist-in-flame
Ag	3280.683	(I)	3.8	1	...	5
Al	3961.527	(I)	3.1	1	...	..
As	2780.197	(I)	6.7	100	...	..
Au	2675.95	(I)	4.6	100	6	20
B	2497.733	(I)	4.9	0.5	...	..
Ba	4130.664	(II)	10.9	50[b]	...	100
Be	3131.072	(II)	13.2	0.02	...	..
Bi	3067.716	(I)	4.0	5	...	..
Cb	3094.183	(II)	8.0	5	...	..
Cd	2265.017	(II)	14.4	100	...	200
	3610.510	(I)	7.3	100	...	.
Ce	3942.736	(II)	...	25[b]	...	.
Co	3453.505	(I)	4.0	2	...	1
Cr	2843.252	(II)	12.6	2	100	0.5
Cs	8521.10	(I)	1.4	15	...	50[b]
Cu	3247.540	(I)	3.8	0.6	...	0.5
Fe	2599.396	(II)	...	2.5	100	5
Ga	2943.637	(I)	4.3	10	...	..
	4032.982	(I)	3.1	10	...	...
Ge	3039.064	(I)	4.9	10	...	...
Hf	2820.224	(II)	>9.2	4	...	...
Hg	2536.519	(I)	4.9	50	...	200
In	3256.090	(I)	4.1	10	...	.
K	4044.140	(I)	3.1	200[b]	...	8[b]
La	3949.106	(II)	9.1	5	...	..
Li	6707.844	(I)	1.8	0.1	...	0.1
Mg	2795.53	(II)	12	0.01	5	5
Mn	2593.729	(II)	12.2	2	...	0.3
Na	3302.323	(I)	3.7	35[b]	50	11.5[b], 2 [c]
Ni	3414.765	(I)	3.6	10	...	10
P	2535.65	(I)	7.2	80	...	..
Pb	2833.069	(I)	4.4	10	...	100
Pd	3404.580	(I)	4.4	2	3	20
Pt	2659.454	(I)	4.6	100	...	.
	3064.712	(I)	4.0	100	...	..
Re	3460.47	(I)	3.6	10	...	..
Ru	3498.942	(I)	3.5	100	...	10
Sb	2598.062	(I)	5.8	100	...	.
Sn	2839.989	(I)	4.8	100	...	.
Sr	4077.714	(II)	8.7	0.5[b]	...	0.2
Te	2385.76	(I)	5.8	1000	...	..
Th	3290.59	..	7.31	100	...	..
Ti	3349.035	(II)	11.1	3	...	..
V	3093.108	(II)	11.2	5	...	..
W	4008.753	(I)	3.4	500	...	..
Y	3710.290	(II)	10.0	0.1	...	..
Zn	3282.333	(I)	7.8	25	500	3000
	3345.020	(I)	7.8	25	...	..
Zr	3496.210	(II)	10.5	2	...	..
	3273.047	(II)	...	2	...	..

[a] Porous cup electrode.
[b] Figure not obtained from most sensitive line.

Emission lines are normally as sharp as those obtained by other spark techniques. In the same way that substances having high vapor densities often give broad lines with the ordinary spark, solutions that go through the porous cup electrode too quickly also produce lines which are weak and show pressure broadening. This is sometimes the case with hydrochloric acid solutions; this difficulty can be eliminated, however, by adding some indifferent material such as sulfuric acid or glycerol to the solution to increase its viscosity.

In order to observe the vertical variation of intensity in the source, stigmatic spectra were obtained, using the full slit height of a Wadsworth mounting (Jarrell-Ash) instrument. Inspection of the spectra showed that there was no noticeable tendency for the lines to be more intense near the porous cup electrode; there was little vertical variation in most cases. The same was true of the background. A few of the weaker lines were less intense at the center, but this was not characteristic of all weak lines.

It therefore seemed that ordinary optical devices for increasing the vertical uniformity of spectral lines—i.e., focusing on the grating or prism or use of cylindrical optics—would suffice for quantitative work.

Excitation. The degree of excitation achieved is affected by the type of source used. When the Baird high voltage spark is used, the spectra showed primarily spark excitation. The raies ultimes of an element in a porous cup electrode exposure are not always those which are most prominent in the spark spectra of the element in bulk or as a dried residue on copper, but are

often lines having medium to high excitation potentials. The exceptions to this are usually elements whose spark lines are either very difficult to excite (alkalies, boron) or inconveniently located (nickel) (see Table I).

Despite the fact that the presence of liquid makes it necessary to avoid sustained high temperatures, arclike excitation can still be achieved by using the 220-volt intermittent (condensed) alternating or direct current arc.

As an illustration of the variation possible, a 10% sulfuric acid solution containing 1% aluminum and 0.5 p.p.m. of lithium was analyzed by the porous cup electrode, using a 220-volt intermittent (condensed) alternating current arc. In one set of exposures, conditions were made sparklike: the capacitance was 4 μfd. and the series inductance 20 μh. There was essentially no series resistance. In these exposures, the easily excited Li I 6707.8 was hardly perceptible, but the difficultly excited H I 6562.7–6562.8 was very prominent. However, when a series inductance of 2000 μh. was inserted, the lithium line became very dense and the H line(s) very weak. It is thus possible to use arclike conditions with the porous cup electrode when the atoms of the element being determined have an excitation potential which make this advisable.

Despite the presumed buffering action of the water particles, the presence of a third solute can affect the intensity ratio of two trace lines. Different atomic and ionic species have different tendencies to lose or gain energy during collision with a given particle in a given energy state, so that the identity of the particles constituting the spark "atmosphere" often has an important effect on the intensities of the lines emitted by trace elements. In the present method, particles resulting from the decomposition of water and graphite always constitute a major portion of this atmosphere. Should one introduce any species of particle which is particularly efficient at supplying or removing energy from the term level at which the trace element particle finds itself, this foreign element would not have to be present in large amounts to have a considerable effect on the intensity of the trace element line. Studies (*6*) on the effect of a third solute on line ratios using spark-to-bulk-liquid techniques show that some line ratios are very sensitive to changes in the constitution of the spark atmosphere.

The same is true of the porous cup electrode technique. Foreign elements constituting 1000 p.p.m. (0.1%) of the solution have sometimes been found to affect both the location and slope of working curves. In such cases, of course, it is necessary to have standard solutions resemble sample solutions as closely as possible in gross composition. A separate study is being made of the effect of foreign elements on the intensities of trace element lines in porous cup electrode exposures.

Quantitative Analysis. TECHNIQUE. Quantitative analysis with the porous cup electrode presents nothing new in principle. Standard solutions resembling the samples are prepared, and an internal standard having a line of convenient wave length is added to both sample and standard. If the element to be measured has a sensitive line in the hydroxyl band region, one of the lines of this band may often be used as a reference line, and no internal standard need be added. Only the wave length of the internal standard need be considered—for instance, both Zr 3273 and Na 3302 have been found satisfactory comparison lines for Li 3232.

The exposures are usually taken on SA1 film or plates. The spectra are measured with a Leeds & Northrup densitometer; the intensities of background and line plus background are read directly from the densitometer tracing by using a scale prepared for the purpose from a Seidel calibration curve (*19*). Background intensity is then subtracted from (line plus background) intensity, and the log of the difference is plotted in the usual manner against the log of the concentration. Further details on quantitative porous cup electrode technique will be found in a forthcoming paper on the spectrochemical determination of hafnium-zirconium ratios (*9*).

SENSITIVITY. Sensitivity limits for solutions of the indicated elements in 10% sulfuric acid solution (lead, barium, and strontium were in 3% nitric acid) are given in Table I. The data in the "solid to bulk liquid" column are those of Jolibois and Bossuet (*18*); the "mist in flame" (Lundegårdh) data are from Cholak and Hubbard (*4*). These authors did not necessarily use the lines listed.

As might be expected from the nature of the excitation used, porous cup electrode sensitivities tend to exceed mist-in-flame sensitivities where high energy excitation is required (beryllium, magnesium, phosphorus), and the reverse is true where low energy excitation is more efficient (alkalies).

Jolibois (*16*) observed that when a 5000-volt direct current discharge was passed between a solid electrode and a solution containing copper sulfate, lines of copper were observed in the discharge only when the solution was made the anode. It thus appears that it might be more efficient to use the porous cup electrode with a polarized discharge such as the intermittent (condensed) direct current arc.

PRECISION. Duplicate samples have given intensity ratios agreeing to within 2 to 5% of their mean, depending on the lines used and the amount of foreign material present. The average agreement has been to within $\pm 3.1\%$ of the mean. This figure includes data obtained from lines ranging between 5 and 97% transmission.

Preliminary experiments have shown that precision is improved slightly by using another porous cup electrode, filled with dilute sulfuric acid or sample solution, as the low electrode. The porous cup electrode is placed in the lower electrode clip; the open end is down, and dipping into a reservoir of liquid if necessary. This technique has the effect of diminishing the intensity of carbon lines, background, and cyanogen bands, but reduces sensitivity and gives rise to excitation conditions which may differ from those obtained with the single porous cup electrode arrangement. The principal drawback of the double porous cup electrode arrangement is the fact that two electrodes must be serviced. Where precision is the most important consideration, however, this modification may prove useful.

The working curves obtained have always been straight lines, with no evidence of curvature at either end. Figure 5 shows the working curve obtained with trace amounts (0.60 to 20.0 p.p.m.) of copper in 10% sulfuric acid, using 50 p.p.m. of cobalt as an internal standard.

ACCURACY. Results obtained on spiked samples submitted by outside laboratories have averaged within $\pm 5\%$ of the truth. There has been little opportunity, however, to check analytical accuracy systematically by independent methods, and it is felt that no estimate of accuracy is justified at present.

ACKNOWLEDGMENT

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Apparatus for Quantitative Separation of Butadiene from Its Dimer

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A method is described which permits quantitative removal of 4-vinyl-1-cyclohexene from butadiene without changing the concentration of small amounts of C_5 hydrocarbons in the butadiene fraction. In this procedure the sample is charged into a simple column and is distilled at a rapid rate with slight reflux until vapors no longer come over. The packing is dried by brief standing at room temperature, and the condensed overhead vapors are used for tests requiring a sample that is free from dimer. The residue may be further treated and analyzed chemically for dimer and other relatively nonvolatile materials. Data and graphs showing accuracy and reproducibility of the method are presented. The column is adaptable to other separations of similar nature in which small concentrations of relatively high boiling material are to be removed from volatile samples.

IN THE analysis of butadiene, the dimer, 4-vinyl-1-cyclohexene, is an impurity which not only has been difficult to determine accurately, but also has been objectionable in various analyses in which high boiling impurities interfere. This interference is particularly pronounced in the determination of C_5 hydrocarbons by the Dorell weathering test apparatus (*3*); it causes high results even when present in comparatively low concentrations. The Dorell method, therefore, requires a sample free of dimer. Simple flash distillation methods fail to effect a good separation of dimer. Hobbs and Rector (*2*) present a method which is a marked improvement over unrefluxed distillations.

The apparatus described below was originally developed for quantitative separation of dimer prior to its analysis by chemical methods. It has, however, proved equally satisfactory for obtaining a dimer-free overhead sample in which the C_4 and C_5 components are not altered in concentration. In this way, the bottoms, such as dimer and nonvolatile residue, and overhead vapors may be separated by a single distillation in a manner satisfactory for subsequent analysis of each. The apparatus, moreover, has shown itself to be adaptable to many separations of a similar nature.

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APPARATUS

The complete apparatus is shown in Figure 1. *A* is a 125-ml. flat-bottomed flask with a 24/40 standard-taper joint, serving as boiling kettle for the apparatus. The short vacuum-jacketed column, *B*, consists of a ground-glass joint to fit flask *A*, a 125-mm. section of 0.3-cm. (0.125-inch) glass helix packing which serves as the dimer-stripping section, and a small dry ice reflux condenser, *C*. Vapor outlet *D* is connected to condensing trap *F* by means of vapor line *E*. The trap is maintained in a dry ice–acetone bath, *G*. All glass is Pyrex, and connections to vapor line are made with neoprene tubing. Water bath *W* is a 1000-ml. beaker.

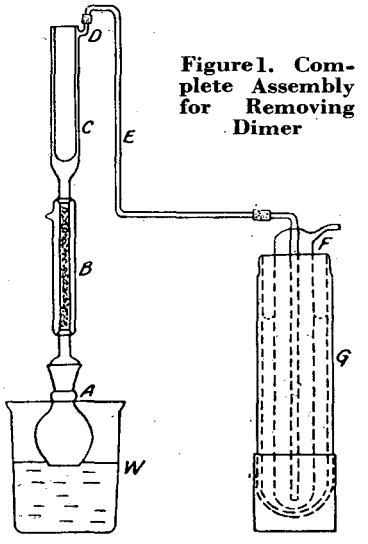

Figure 1. Complete Assembly for Removing Dimer

The column is shown in Figure 2 with complete dimensional data.

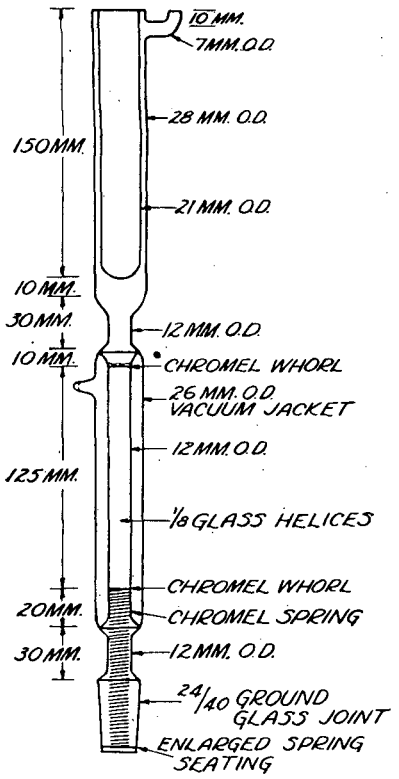

Figure 2. Column for Dimer Removal

The vacuum jacket surrounding the packed section is unsilvered. Columns without jackets have worked satisfactorily, although greater reproducibility is possible with a jacketed column. The packing in the column is retained by two whorls of Chromel wire. The upper whorl is kept in place by glass crossbars sealed into the inner wall. The lower whorl is retained and held firmly against packing by means of a closely wound Chromel wire spring which fits snugly to the wall of the column. At a point within the ground-glass joint the end of the spring is enlarged to form an adjustable ring somewhat larger than the smallest inside diameter of the joint. In this way, the spring, upon insertion into position, seats itself against the wall of the joint and maintains firm, constant pressure against the packing.

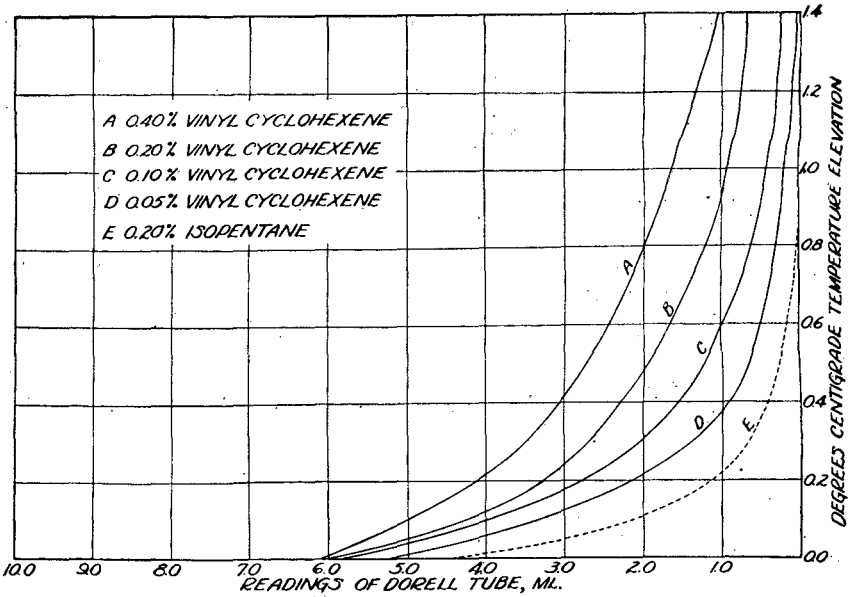

Figure 3. Dorell Curves for Vinyl Cyclohexene

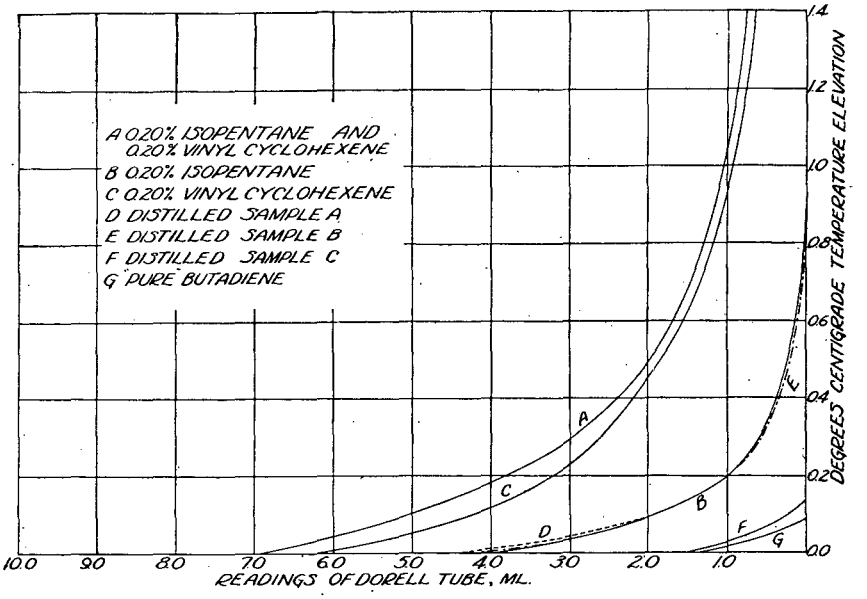

Figure 4. Dorell Curves for Isopentane–Vinyl Cyclohexene Mixtures

OPERATION

The column is mounted on a ring stand, and the vapor line is connected to the glass receiver, *F*, which is kept in a dry ice–acetone bath (below −40° C.). About 1 inch (2.5 cm.) of pulverized dry ice is placed in the column condenser, *C*. The chilled sample (50 or 100 ml.) is measured in a chilled graduate and introduced into flask *A*. The flask is connected to the column (no stopcock grease is necessary). Approximately 500 ml. of warm water (45° ± 5° C.) are added to the water bath and refluxing of sample is started at a rate of about 2 drops a second by momentarily immersing the flask in the water bath at required intervals. Initial vapors should be completely condensed. After 1 minute on "total reflux," the reflux rate is cut down to 1 drop every 4 seconds and about 0.3 cm. (0.125 inch) of the bottom of the sample flask is submerged in the water bath.

The transition from total reflux is readily achieved by agitating the dry ice in the condenser during the first minute and then permitting the ice to go undisturbed. If the reflux rate falls too low, slight agitation of the dry ice or constant pressure by a test tube or glass rod on the ice will bring up the rate. More dry ice is added when necessary. When the volume of the residual butadiene decreases to approximately 10 ml., the flask is submerged in the water bath to the neck and the distillation is permitted to continue to dryness. About 10 minutes are required for the distillation of a 50-ml. sample. After the flask reaches dryness, the residual dry ice in the condenser is removed and the assembly is permitted to stand for 5 to 10 minutes to ensure complete drying of packing. Liquid dimer may be visible in the flask or on the lower portion of the packing if appreciable quantities are present in the sample. The glass trap is disconnected from the flask, the condensate is thoroughly mixed, and the sample is used for the Dorell weathering test or for other tests requiring a dimer-free sample. The complete distillation operation should take less than 30 minutes.

If a dimer analysis is desired on the residue remaining in the column, chloroform is introduced into the column through vapor outlet *D*, a steam-heated water bath is substituted for *W*, the last traces of butadiene are stripped from the column, and the cooled chloroform residue is analyzed for dimer by the bromination method of Hablitzel and Jezl (*1*). As the recovery is quantitative, other tests on the nonvolatile portion may also be made.

RESULTS AND DISCUSSION

In order to test the efficiency of the column for dimer removal, butadiene samples before and after distillation through the

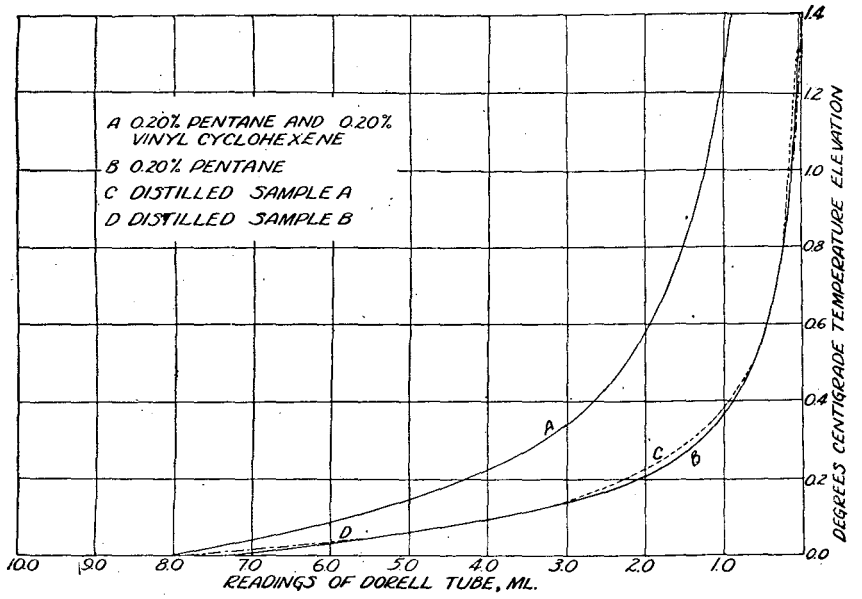

Figure 5. Dorell Curves for Pentane–Vinyl Cyclohexene Mixtures

column were analyzed in a Dorell weathering test apparatus in the specified manner.

The Dorell apparatus is a modification of the Cottrell boiling point apparatus, especially adapted to volatile liquids. It consists of a glass tube graduated from 0 to 40 ml., the first 10 ml. of which are accurately calibrated and enclosed by a removable vacuum jacket. Heat is supplied by a pinpoint heater of copper fused into the bottom of the tube and in contact with a special cartridge heater. A Cottrell pump sprays the vapor-liquid equilibrium mixture formed at the pinpoint heater onto a thermometer having a range from −10° to +10° C. and accurately graduated to 0.1° C. A sample chilled to about −20° C. is charged to the tube to the 40-ml. mark, the thermometer is inserted into place, and the sample is "weathered away" to dryness. Temperature readings are taken at 10.0, 9.0, 8.0, 7.0, 6.0, 5.0, 4.0, 3.0, 2.5, 2.0, 1.5, 1.0, 0.8, 0.6, 0.4, 0.2, 0.1 ml., and at the dry point. Special precautions are taken to keep the pump operating efficiently during the weathering of the last 10 ml.

Because of submersion of the thermometer bulb in the somewhat superheated sample, readings at 10 and 9 ml. were generally high and were disregarded. If no constant boiling temperature was apparent, the 8-ml. value was taken as the constant boiling temperature from which temperature elevations were calculated. The operation of the Dorell apparatus has been described fully (*2*, *3*).

Figure 3 shows the effect of various concentrations of dimer (4-vinyl-1-cyclohexene) on the Dorell weathering test. Only the last 10 ml. are plotted. Temperature elevations were obtained by algebraically subtracting the constant boiling temperature from the observed temperature for a particular volume reading. A curve for 0.20% isopentane (2-methylbutane) is included for comparison. From these curves it is apparent that the effect of dimer on Dorell weathering is many times that of isopentane.

Figure 4 shows the effect of distillation through the column on samples with varying concentrations of dimer and isopentane. So-called "pure" butadiene, which was used in preparing all mixtures, was butadiene of high purity which, when analyzed by a Dorell apparatus, showed an elevation of no more than 0.1° C. from the constant boiling temperature to the dry point.

Curves *D* and *E* coincide with *B*, indicating that the column neither removed measurable isopentane from the overhead sample nor permitted dimer to escape into the condensing trap. The completeness of dimer removal is further indicated by curve *F*, which nearly coincides with curve *G* for pure butadiene.

Figure 5 shows the effect of distillation on samples containing *n*-pentane instead of isopentane. Here, curves *C* and *D* coincide with *B*, indicating again a quantitative removal of dimer without loss of the C_5 hydrocarbon.

Table I summarizes data obtained in this laboratory on most of the C_5 compounds normally present in butadiene. Dimethylacetylene is included because its effect on Dorell weathering is comparable with a C_5 compound. Column 1 gives the composition of the mixture before dimer removal; column 2 shows the Dorell analysis obtained by analyzing the sample before dimer addition and determining the equivalent amount of C_5 calculated as isopentane, by use of the curves in Figure 6; and column 3 shows the analysis after dimer removal, again by use of the curves in Figure 6. The values in columns 2 and 3 should be identical, within limits of the accuracy of the Dorell weathering test apparatus, if the column is giving quantitative separations. This laboratory has found that the Dorell apparatus can repeat to approximately 0.03% of the true value at about 0.2% isopentane. At higher concentrations the inaccuracy increases until at 1.0% isopentane the discrepancy approximates 0.1%. A 10 to 15% relative error should therefore be allowed on either side of the expected value for the Dorell apparatus. Considering this

Table I. Analysis of Synthetic Samples Distilled through Dimer-Removing Column

Mixture Composition	Weight % Isopentane: Theoretical Dorell analysis[a]	Weight % Isopentane: Actual analysis
0.20% dimer (b.p. 129° C.)	0.00	0.02
0.50% dimer	0.00	0.03
1.00% dimer	0.00	0.02
0.24% isopentane (b.p. 27.9° C.)	0.24	0.24
0.00% dimer		0.22
0.24% isopentane	0.24	0.25
0.20% dimer		0.22
0.20% isopentane	0.20	0.20
0.20% dimer		0.20
0.50% isopentane	0.50	0.50
0.20% dimer		0.50
1.00% isopentane	1.0	1.0
0.20% dimer		1.0
0.20% *n*-pentane (b.p. 36.0° C.)	0.50	0.50
0.00% dimer		0.48
0.20% *n*-pentane	0.50	0.50
0.20% dimer		0.48
0.20% dimethylacetylene (b.p. 27.1° C.)	0.65	0.65
0.20% dimer		
0.40% dimethylacetylene	1.2	1.2
0.20% dimer		
0.50% dimethylacetylene	1.5	1.2
0.20% dimer		
0.06% isoprene (b.p. 34.1° C.)	0.23	0.20
0.00% dimer		
0.06% isoprene	0.23	0.25
0.20% dimer		0.22
0.20% isoprene	0.63	0.65
0.20% dimer		0.62
0.20% *cis-trans*-piperylene (b.p. 42.3° C.)	0.83	0.80
0.00% dimer		0.80
0.20% *cis-trans*-piperylene	0.83	0.80
0.20% dimer		0.85
0.05% 1,3-cyclopentadiene (b.p. 41.0° C.)	0.22	0.18
0.00% dimer		0.20
0.05% 1,3-cyclopentadiene	0.22	0.20
0.20% dimer		0.20

[a] Values obtained by comparing Dorell weathering curves of undistilled samples containing stated concentrations of C_5 hydrocarbons (without dimer) with standard isopentane Dorell curves (Figure 6).

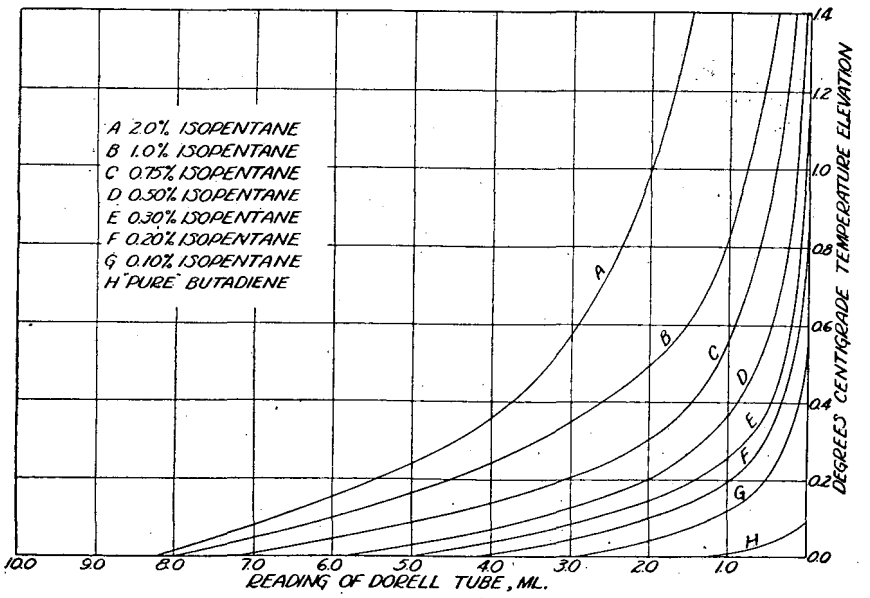

Figure 6. Standard Dorell Curves for Isopentane

inaccuracy of measurement, the results in Table I for the wide range of C_5's are satisfactory.

Although the foregoing data concern only butadiene and its dimer, other C_4 and C_5 compounds have been satisfactorily separated. The method has been adopted generally by both producers and consumers of butadiene for separations in conjunction with the Dorell test for C_5's, the chemical determination of dimer, and the preparation of samples prior to determination of conjugated diene content. Private communications indicate that results are very gratifying.

By a slight modification of conditions, such as reflux rate and temperature of the water bath, dimer in concentrations greater than stated above may be removed. Styrene may likewise be separated from butadiene, as has been demonstrated in this and other laboratories. Other separations of similar nature readily suggest themselves.

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Determination of Butadiene Dimer (4-Vinyl-1-cyclohexene) in 1,3-Butadiene

Bromination Method

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AN ACCURATE determination of dimer (4-vinyl-1-cyclohexene) in 1,3-butadiene is required not only for determining the purity of the butadiene but also for controlling an impurity which is considered detrimental to polymerization grade butadiene. All previous methods used for dimer determination have been mainly physical in nature, making use of the dimer's relative nonvolatility (*8*). Such methods either have been time-consuming or have lacked accuracy and precision. The procedure described in this article overcomes these objections by using the fractionating column of Jezl and Hablitzel (*3*) for removing the butadiene quantitatively and determining the dimer present in the residue chemically by bromination.

The investigations in this laboratory centered around chemical methods for determining dimer in 1,3-butadiene in order to avoid repeated weighings and to decrease the over-all time required by physical methods. The requirements for such a method included a means for the removal of the butadiene without loss of dimer, and a quantitative chemical determination not affected by the presence of *p-tert* butylcatechol (an antioxidant) and other nonvolatile residues usually present in polymerization grade butadiene.

Subsequent to the work described herein several chemical methods for determining dimer or pure 4-vinyl-1-cyclohexene in products other than butadiene were described. Laitinen, O'Brien, and Wawzonek (*5*) in determining 4-vinyl-1-cyclohexene in recycle styrene found iodine chloride addition satisfactory in a dioxane medium; the reaction was 93 to 97% complete, depending upon the purity of the material. These investigators found iodine bromide and bromine in glacial acetic acid unsatisfactory. Warshowsky and Elving (*9*) described the determination of butadiene dimer in tetrahydrophthalic anhydride using a cyclohexane extraction with the unsaturation determined by the bromide-bromate titration method of Mulliken and Wakeman (*6*).

Johnson and Clark (*4*) reported satisfactory bromination of 4-vinyl-1-cyclohexene, purified by fractionation, with a bromine

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A method for the accurate and rapid determination of butadiene dimer (4-vinyl-1-cyclohexene) has been developed. The procedure includes a physical separation of the dimer from the volatile hydrocarbons by a rapid distillation of the butadiene sample, utilizing a small, simply designed fractionating column with chloroform added as a chaser. The dimer is retained in the chloroform residue while the butadiene is completely expelled by the refluxing chaser. The residue is then brominated in a glacial acetic acid medium using a potassium bromide-bromate solution and the excess bromine is determined iodometrically. From these titration data the weight per cent of dimer is calculated. Inhibitor (*p-tert*-butylcatechol) and other nonvolatile material usually found in butadiene do not interfere. Data present show the precision of the method with synthetic mixtures of dimer and butadiene.

number of 297.5, slightly higher than theoretical, using their improved bromination procedure. Previously the present authors had investigated the bromination of dimer using the Johnson and Clark procedure (*1*, *2*) and obtained an average experimental bromine number of 286 on a redistilled center cut of a butadiene dimer fraction from Koppers United Company. This variation was undoubtedly due to the presence of impurities in the dimer fraction of polymerization grade butadiene. The authors believe that the lower bromine number represents the typical "dimer fraction" formed in polymerization grade butadiene.

To secure a quantitative separation of the dimer from the butadiene a simple fractionating column was developed (see Figure 2 of *3*). This column has been described in detail with full operational instructions and information on other valuable laboratory applications (*3*). By employing an intermediate boiling solvent, inert to bromination, as a chaser, the dimer and other relatively nonvolatile material can be quantitatively recovered in a medium suitable for bromination.

The method presented incorporates the bromination procedure of Johnson and Clark (*4*) adapted to the special conditions due to the subsequent treatment and nature of the material to be analyzed, and the procedure of the authors for the quantitative separation of 1,3-butadiene from its dimer. The present method was submitted in 1945 to the Committee on Butadiene Specifications and Methods of Analyses of the Office of Rubber Reserve of the Reconstruction Finance Corporation. It was adopted as the official referee method for polymerization grade butadiene and later formed the basis for test method L.M.2.1.15.

PROCEDURE

Apparatus. A small distillation column similar to the one shown in Figure 2 of (*3*).

Reagents. Chloroform, C.P. grade.

Potassium Bromide-Bromate Solution, 0.5 *N*. Dissolve 49.6 grams of potassium bromide and 13.9 grams of potassium bromate in distilled water, dilute to 1 liter, and standardize.

Glacial acetic acid.

Potassium iodide solution, 15%.

Sodium Thiosulfate Solution, 0.1 *N*. Dissolve 25 grams of reagent grade sodium thiosulfate pentahydrate in 1 liter of freshly distilled water and allow to stand a week before standardization and use.

Starch Indicator Solution. Make a suspension of 6 grams of soluble starch in 25 ml. of water and add cautiously about 10 ml. of 10% sodium hydroxide. Stir until the solution gelatinizes, transfer the mass to a bottle, and dilute to 1 liter.

Carbon tetrachloride.

Dry ice.

Distillation. Set up the column in a well ventilated hood and fill about one half of the condensing chamber at the top of the column with pulverized dry ice. Introduce approximately 10 ml. of chloroform into a 125-ml. Pyrex flask with a standard 24/40 ground-glass joint at the neck. Chill the flask containing the chloroform, a 50-ml. graduated cylinder, and the butadiene sample to be analyzed, to approximately −20° C.; and add 50 ± 0.25 ml. of the sample, measured in the graduate, to the flask. Record volume and temperature and calculate the weight of sample from Table IV.

Connect the flask to the column and half fill a 600-ml. beaker with cold water for the heating bath. Start the butadiene boiling by lowering the flask, into the bath, condensing all of the initial butadiene vapors until the column is completely wetted. Then distill off the butadiene by increasing the temperature of the bath (pass steam into the cold water) and by allowing the amount of dry ice in the cooling chamber to decrease. Remove the butadiene at a rapid rate in order to reduce the distillation time to a minimum. Keep sufficient dry ice in the bottom of the cooling head to maintain a reflux of one drop per second until most of the butadiene is removed. Stop the dry ice cooling when the bottom of the column becomes warm and the temperature of the water bath approaches the boiling point of the chloroform chaser (about 60° C.). Increase the temperature of the water bath to almost its maximum temperature and allow the chloroform vapors to rise gradually in the cooling chamber until they begin to distill off the top of the column.

To ensure complete removal of the butadiene, continue the distillation until about 5 ml. of chloroform residue remain in the flask. Remove the hot water and replace with cold water. Pour 10 ml. of glacial acetic acid down the column through the vapor outlet to remove any dimer on the packing. A normal distillation requires less than 30 minutes.

Bromination. Transfer the residue from the distillation flask to a 500-ml. iodine number flask. Rinse the distillation flask with two 20-ml. portions of glacial acetic acid and transfer the rinsings to the iodine flask. From a buret add 0.5 *N* potassium bromide-bromate solution at the rate of 2 to 3 drops per second until the mixture turns to a definite yellow color due to excess bromine, then add 2 ml. more and shake the flask for several minutes. If the color fades, add more bromide-bromate solution. The total shaking time should be approximately 5 minutes.

Do not confuse the color formation occurring at times after the addition of the first few drops of the bromide-bromate solution with the indication of excess bromine. The inhibitor and other nonvolatiles present may produce a permanent coloration. In certain instances, off-color butadiene samples give such dark chloroform residues that the color of the glacial acetic acid mixture overshadows the appearance of any excess bromine. In these cases, add a calculated excess of bromine-bromate solution, the amount depending on the probable dimer content of the sample. If the estimated dimer content is 0.1% or less, add 4.0 ml. of 0.5 *N* bromide-bromate; if between 0.1 and 0.2%, add 6.0 ml.; and if between 0.2 and 0.4% add 11.0 ml.

Add 5 ml. of 15% potassium iodide solution from the lip of the flask and shake the flask for 1 minute. Dilute the contents of the flask with 100 ml. of distilled water and titrate the liberated iodine with 0.1 *N* sodium thiosulfate solution, using 1 ml. of starch solution as an indicator near the end point.

For the best and most rapid bromination, use no more than 12 ml. of bromide-bromate solution with 50 ml. of glacial acetic acid. In case of higher concentrations of dimer, reduce the size of the sample used to keep the required volume of the bromide-bromate solution below 12 ml. This procedure, however, can be used for any concentration of dimer. For high concentrations (above 0.8%) dilute the chloroform-dimer residue to a definite volume with carbon tetrachloride and brominate an aliquot portion.

Calculations. Calculate the weight per cent of dimer in the sample from the following equation:

$$\text{Wt.\% dimer} = \frac{[(S \times N) - (s \times n)] \times 2.79}{W}$$

where S = volume of bromide-bromate solution used, N = normality of bromide-bromate solution, s = volume of thiosulfate solution used, n = normality of thiosulfate solution, and W = weight of butadiene sample.

Table I. Determination of Butadiene Dimer in Synthetic Mixtures

Weight Per Cent Dimer		Absolute
Synthesis	Bromination	Error
0.000	0.000	0.000
0.050	0.050	0.000
0.111	0.110	−0.001
0.150	0.155	+0.005
0.200	0.201	+0.001
0.200	0.198	−0.002
0.200	0.206	+0.006
0.200	0.206	+0.006
0.200	0.206	+0.006
0.200	0.206	+0.006
0.200	0.206	+0.006
0.200	0.204	+0.004
0.220	0.220	0.000
0.250	0.260	+0.010
0.475	0.460	−0.015
0.591	0.612	+0.021
0.630	0.660	+0.030
1.000	0.982	−0.018

Table II. Repeatability of Results Using Bromination Method for Determination of Butadiene Dimer

	Results		Deviation
	Run 1	Run 2	from Mean
Synthetic mixture (0.220%)	0.224	0.217	±0.003
Synthetic mixture (0.200%)	0.204	0.208	±0.002
Synthetic mixture (0.150%)	0.158	0.152	±0.003
Synthetic mixture (0.111%)	0.110	0.111	±0.0005
Synthetic mixture (0.475%)	0.464	0.456	±0.004
Synthetic mixture (0.050%)	0.049	0.050	0.0005
Stored butadiene sample	0.305	0.305	0.000
Synthetic mixture (1.000%)	0.976	0.988	±0.006

The factor 2.79 is obtained from $\frac{7.992 \times 100}{286}$, in which 7.992 is calculated from the equivalent weight of bromine, 286 is the experimental bromine number of dimer, and 100 converts the results to per cent dimer.

DISCUSSION OF PROCEDURE

Investigations in this laboratory of the bromination of dimer found in polymerization grade butadiene indicated that the reaction was very consistent over a wide range of conditions. For this specific procedure, therefore, the careful controls required for the general procedure for olefinic hydrocarbons (4) were unnecessary. Temperature during titration, the amount of excess reagent, and the time of contact had little effect on the extent of bromination. The Johnson and Clark bromination procedure gave good results on some samples when followed carefully as directed. However, the amount and nature of the residue from the dimer-removing column made a few minor changes in their bromination step necessary to ensure accurate results when adapted to this specific use.

The dimer content of most butadiene samples is of such magnitude that the entire residue from a distillation should be brominated. Many butadiene residues are colored after contact with the bromide-bromate reagent, and this coloration can be mistaken as an indication of excess bromine. Addition of a larger excess of reagent than specified by Johnson and Clark, after the presence of free bromine is suspected, prevents incomplete bromination in cases of colored residues.

Pure 4-vinyl-1-cyclohexene can be brominated completely; therefore, the amount present in butadiene can be accurately calculated by using the theoretical bromine number. However, as it is believed that the dimer fraction of commercial butadiene is not pure 4-vinyl-1-cyclohexene, the average experimental bromine number of 286 should be used if the total dimer content is desired.

DISCUSSION OF RESULTS

Known mixtures of dimer and butadiene were prepared and analyzed by the procedure described above (Tables I and II). This wide range of concentrations was studied over a period of several months by several analysts. A large number of identical mixtures were used (0.200% in Table I) to determine the reproducibility of the method while making minor variations in the distillation conditions. Table II gives the results obtained by duplicate analysis of several synthetic mixtures to show the degree of precision of the method.

If Table I and other data available at this time are used, the average result obtained for concentrations near 0.2% should be within 0.01% of the actual concentration. As for the repeatability of analysts in the same laboratory, results near the 0.2% range should not differ from the mean by more than 0.005%. Reports received from other laboratories using this method on synthetic mixtures show a degree of accuracy similar to that found in the authors' laboratory.

Butadiene and other olefinic hydrocarbons interfere in the bromination step if they are not completely removed from the dimer-chloroform chaser. Because the chloroform chaser may be refluxed for a considerable time without loss of dimer, all hydrocarbons boiling below 50° C. can be readily removed. Inhibitor (*p-tert*-butylcatechol) and other nonvolatile matter in finished butadiene cause little, if any, interference. Acetylenic polymers and wash oil, if present, may appear as dimer. If interference is suspected, correction for these nonvolatile materials can be made by evaporating off a sample of butadiene, heating the residue on a steam bath, simultaneously flushing with a slow stream of nitrogen to drive off the dimer, and brominating the residue in the manner specified above. This bromination value should be subtracted from the total value. However, no interference from these sources has been discovered in butadiene samples analyzed in this laboratory.

OTHER APPLICATIONS OF METHOD

Styrene, if present and not removed, would brominate completely to the dibromo derivative. Dimer can be determined in the presence of styrene by the method described above if the amount of styrene in the chloroform residue is determined by optical methods or other suitable means. Styrene is quantitatively separated from butadiene along with the dimer. Results obtained for the dimer content of various dimer-styrene mixtures, by correcting bromination data for the styrene present, are shown in Table III.

Table III. Bromination of Styrene and Dimer Mixtures after Separation from Butadiene in Column

Weight % by Synthesis		Weight % Dimer by Bromination after
Styrene	Dimer	Correcting for Styrene
0.040	0.20	0.21
0.40	0.20	0.20
1.00	0.20	0.20
1.00	0.30	0.29
1.00	0.40	0.40

Table IV. Liquid Densities of 1,3-Butadiene at Various Temperatures (7)

Temp.	Density	Temp.	Density
° C.	G./ml.	° C.	G./ml.
0	0.645	−16	0.664
−2	0.647	−18	0.666
−4	0.650	−20	0.668
−6	0.652	−22	0.670
−8	0.654	−24	0.673
−10	0.657	−26	0.675
−12	0.659	−28	0.677
−14	0.661	−30	0.679

When the total weight of styrene and dimer can be obtained, as in styrene recycle streams, the concentration of dimer can be determined by brominating a weighed sample of the mixture by the method of Johnson and Clark and applying the equation:

Wt. % dimer = (bromine No. of sample − 153.4) × 0.754

where 153.4 = bromine number of styrene. $0.754 = \frac{100}{286-153.4}$,

in which the denominator is the difference between the experimental bromine number of dimer and the bromine number of styrene.

$$\text{Bromine No.} = \frac{[(S \times N) - (s \times n)] \times 7.992}{W}$$

In this equation, all symbols are the same as before, W being the weight of the sample. The bromination obviously requires the complete absence of butadiene from the bromination mixture to avoid erroneous results.

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Electrolytic Separation of Rhodium from Iridium

At Controlled Cathode Potential

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The electrolytic separation of rhodium from iridium has been successfully accomplished using a controlled cathode potential circuit of the Lingane type. The solution contained 3.5 molar ammonium chloride and the cathode was operated at 0.4 volt more negative than the saturated calomel electrode. Rhodium deposits were found to contain oxide and it was necessary to reduce the electrolytic deposit in hydrogen gas at 450° C. Iridium, although it does not deposit at voltages up to 1.0 volt more negative than the saturated calomel electrode, codeposits strongly at −0.3 volt (*vs.* S.C.E.) in the presence of rhodium. The cause of this induced codeposition of iridium has not been explained but it has been prevented by preliminary destruction of complex ions, oxidation of iridium to the tetravalent state before electrolysis, and addition of sufficient ammonium chloride (3.5 *M*). The adaptation of the electrolysis separation to the Gilchrist-Wichers scheme for the platinum group metals is discussed.

THIS paper describes the quantitative electrolytic separation of rhodium from iridium. The only attempt to separate rhodium from iridium electrolytically that has been reported in the literature was made by Smith (*10*) in 1892. The results were erratic and no quantitative separation was obtained.

The most satisfactory method of separating rhodium from iridium has been a chemical method described in 1935, by Gilchrist and Wichers (*1*), who made use of the fact that titanous chloride will reduce rhodium to the free metal, but leaves iridium in solution. Karpov and Fedorova (*2*) reported a similar selectivity when vanadium dichloride was used as the reducing agent. Consideration of these two reactions suggested the probability that the two metals can be separated by suitable electrolytic reduction of rhodium, which evidently is much more easily reduced to metal than is iridium.

A research was therefore planned to investigate the electrolytic behavior of solutions of the two metals. As a result, a procedure has been developed which provides an alternative separation to that of Gilchrist and Wichers.

APPARATUS

Electrolytic Equipment. The electrolyses were conducted at constant cathode potential automatically controlled with a Lingane (*5*) type of circuit. Current at a constant potential was supplied to the electrode leads of a Sargent-Slomin electrolytic analyzer in which the connections to the rectifier-transformer were removed. In this apparatus platinum gauze electrodes of the Sargent-Slomin type were attached directly by slide-in chucks to the motor provided for rotation of the anode. The electrolysis vessel was a 150-ml. beaker provided with a split watch glass cover.

Hydrogen Reduction Apparatus. During the research it was found necessary to subject the electrolytic deposits of rhodium to reduction in hydrogen. The hydrogen reduction apparatus consisted of a heated glass chamber in which the electrode was hung, and hydrogen and nitrogen purification trains. The reduction chamber was a Pyrex tube about 22.5 cm. (9 inches) in length and 6.25 cm. (2.5 inches) in diameter, mounted vertically. It was fitted at the top with a 71/60 inner member standard-taper joint. At the base it was constricted and sealed to an inlet tube from the gas purification train. The top of the reduction chamber was closed with a cap made from the outer member of a 71/60 joint. The cap was fitted with a glass hook for supporting the electrode and also with an outlet tube for escaping gas. The exit tube was connected through a 12/5 ball and socket joint to a sulfuric acid bubbler which prevented back-diffusion of oxygen into the apparatus. From the bubbler the gas was exhausted to the outdoors. The upper half of the reduction chamber was heated electrically by 20 feet of No. 14 coiled Nichrome wire separated from the glass by asbestos spacers. A Variac transformer was used for control of the temperature. The electrode to be reduced was attached to the hook of the cap by means of a short length of platinum wire wrapped several times around the slightly bent stem of the electrode.

Hydrogen and nitrogen gas were obtained from commercial pressure cylinders. By means of a three-way stopcock, either gas could be admitted to the washing train. The incoming gas was passed first through 150 ml. of potassium pyrogallate solution, 150 ml. of concentrated sulfuric acid, then soda lime, Drierite, and glass wool, and was led into the reduction chamber through a 12/5 ball and socket joint.

Nitrogen gas was used to displace air before filling the apparatus with hydrogen and also at the end of the reduction for displacing hydrogen before opening the apparatus to the air.

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PROCEDURE

The procedure for the electrolytic separation of rhodium and iridium which was finally developed in the research was applied directly to a solution containing 6 to 30 mg. of rhodium and not more than 60 mg. of iridium as chlorides or chloro salts. Organic matter must be absent.

Preparation for Electrolysis. Evaporate the solution to 10 ml. in a 150-ml. beaker covered with a watch glass supported by glass hooks, add 2 ml. of nitric acid, and evaporate to dryness on the steam plate. Rinse down the walls of the beaker, add 2 ml. of hydrochloric acid, and evaporate to dryness again. Repeat the evaporation once more to remove the bulk of the nitrates present. Rinse down the watch glass and supports with 15 ml. of distilled water and add 5 ml. of 1 to 10 hydrochloric acid. Replace the watch glass and pass chlorine gas from a cylinder through the solution for 5 minutes. Allow the glass chlorine delivery tube to remain in the beaker. Heat the solution to 50° C. for 5 minutes, cool to 25° C., and add 18.7 grams of ammonium chloride. Dilute the solution to 50 ml. and heat with occasional stirring to 95° C. Cool quickly to 25° C. with stirring in an ice bath. Again pass chlorine gas into the solution in the covered beaker for 5 minutes. If much iridium is present, a precipitate may be present at this point. Add 1 gram of hydroxylaminehydrochloride to reduce the chlorine in the solution and to serve as anodic depolarizer, to prevent the evolution of chlorine during the electrolysis. Add 1 gram of ammonium sulfate and stir the solution with the chlorine delivery tube to dissolve the salt. Remove and rinse the watch glass and delivery tube and dilute the solution to 100 ml. It is then ready for electrolysis.

Prepare the cathode by first cleaning it with acid or by pyrosulfate fusion, followed by washing and drying. Then place it in the chamber of the reduction apparatus. Pass nitrogen through the apparatus for 15 minutes. Substitute hydrogen for nitrogen and in the course of the next 15 minutes bring the temperature of the tube to 450° C. and hold it at that temperature for 30 minutes. Allow the electrode to cool for 30 minutes in hydrogen and then in nitrogen for 15 minutes. Remove it from the reduction apparatus and allow it to stand 5 minutes near the balance, followed by 5 minutes in the balance case, and then weigh it to the nearest 0.01 mg. An Ainsworth Type TX semimicrobalance was used for the weighings in the work described.

Electrolysis Procedure. Place both electrodes in the appropriate clamps of the analyzer. Place the sample beaker under the electrodes and insert the salt bridge from the saturated calomel electrode (*5*) so that its tip will be near the cathode and at about mid-height. Lower the electrodes into the solution and turn on the rotating anode. Set the automatic potential control apparatus to maintain the cathode potential at −0.25 volt with reference to the saturated calomel electrode. Cover the sample beaker with a split watch glass.

Hold the potential at −0.25 volt for 20 minutes, and then change it to −0.30 volt for 20 minutes. Add another gram of hydroxylamine hydrochloride at the end of the second 20 minutes. Change the potential to −0.35 volt for 25 minutes and finally to −0.40 volt for 25 minutes. Test for completeness of deposition of rhodium with the test described below, if there is any doubt about complete precipitation. Terminate the electrolysis by first stopping the rotating anode and then slowly raising the electrodes while washing them with a jet of water from a wash bottle. After the electrodes are out of the solution, turn off the current potential control device. Wash the cathode by dipping it twice in distilled water and once in 95% ethyl alcohol. Dry at 110° C. for 5 minutes. Reduce the deposit as already described in hydrogen for 30 minutes at 450° C., and cool and weigh as before. The deposit is rhodium metal and is free from oxide and iridium. The rhodium may be removed from the platinum electrode by fusion in pyrosulfate.

Test for Rhodium. The test for rhodium is applied only when it appears certain that practically all the rhodium has been plated out.

IN THE ABSENCE OF IRIDIUM. To a 5-ml. sample of electrolyte in a 25-ml. test tube add 5 ml. of 20% stannous chloride solution in 1 to 1 hydrochloric acid. Prepare a similar tube using 5 ml. of a standard rhodium chloride solution (0.001 mg. of rhodium per ml.) and a third tube containing 5 ml. of distilled water in place of the sample. Place all three tubes in a bath of boiling water for 10 minutes. If deposition of rhodium has been complete, there will not be a detectable difference between the sample and distilled water tubes, both of which should be colorless, while the solution containing 0.005 mg. of rhodium will be distinctly pink. This provides a limiting method for the detection of undeposited rhodium.

IN THE PRESENCE OF IRIDIUM. The interference caused by a yellow color developing from the reaction between stannous tin and solutions of iridium can be sufficiently overcome by modifying the procedure used in the absence of iridium. Remove two 5-ml. test samples of electrolyte and to one of them add 0.005 mg. of rhodium. Treat both tubes with 5 ml. of stannous chloride reagent and place them in boiling water for 10 minutes. If the colors in the two tubes differ appreciably, it follows that the amount of rhodium left in the electrolyzed solution is of an order not exceeding 0.005 mg. If the colors are not appreciably different, it is concluded that the amount of rhodium left in solution is relatively large, for the color is not greatly affected by the addition of another 0.005 mg. of rhodium. Spectrographic tests of the iridium residue left after evaporation of the remaining electrolyte showed that the amount of rhodium left in solution after the application of both the above tests did not exceed about 0.003 mg.

Materials. Rhodium chloride (approximately $RhCl_3.2H_2O$) and iridium chloride (approximately $IrCl_4.2H_2O$) were obtained from the American Platinum Works, Newark, N. J. Spectrographic examination of each showed less than 0.001% of the other metal. Solutions of both salts were prepared and the metal content of aliquots was determined by evaporation and ignition of the residue in hydrogen.

RESULTS AND DISCUSSION

Rhodium and Iridium Complexes. It was observed part-way through this research that the method of preparation of the solution containing rhodium and iridium affects the deposition. If solutions prepared in various ways were first evaporated to dryness with aqua regia and then dissolved in dilute hydrochloric acid, uniform behavior on deposition could be obtained. Presumably the treatment with aqua regia destroys any unusual complex ions present and the final solution contains only chloro complexes.

Little is known about the relative stabilities of these complex chloro ions. For the $RhCl_6^{---}$ ion, Latimer (*4*) gives the value 10^{-12} for the dissociation constant. For the $IrCl_6^{---}$ ion, 10^{-14} is given. These values are at least in favor of a separation. Iridium also forms the $IrCl_6^{--}$ ion, but its dissociation constant is not known. Experiments in which the iridium was intentionally oxidized to the higher valence before electrolysis showed a better separation; this is probably not because of greater stability of the higher valent ion, but rather because of the insolubility of ammonium chloroiridate.

Codeposition of Iridium. Although iridium does not deposit electrolytically at −1.0 volt (*vs.* S.C.E.), it does codeposit badly at −0.3 volt in the presence of rhodium. Codeposition of iridium equal in weight to 50% of the iridium present was observed. A possible explanation of a catalytic effect of rhodium metal on the cathode was tested and eliminated by attempting the deposition of iridum on a rhodium-plated cathode. No deposition of iridium could be obtained at potentials up to −1.0 volt (*vs.* S.C.E.). The codeposition seems to be a peculiar function of the simultaneous deposition of the two metals. The crystal lattices of both rhodium (*7*) and iridium (*8*) are face-centered cubes. For rhodium the length of the cube is 3.820 Å. and for iridium it is 3.805 Å. The closest approach of the rhodium atoms is 2.700 Å., of iridium 2.690 Å.

The temporary precipitation prior to the electrolysis of much of the iridium as ammonium chloroiridate tends to minimize the codeposition. Experiments were done in which solutions containing both rhodium and iridium were treated with chlorine and ammonium chloride before electrolysis. Much of the iridium precipitated as ammonium chloroiridate. As the electrolysis proceeds, the ammonium chloroiridate slowly dissolves, evidently because of the electrolytic reduction of $IrCl_6^{--}$ ion to $IrCl_6^{---}$. Ammonium chloroiridite is much more soluble than ammonium chloroiridate.

The greatest single influence on the codeposition was found to be the addition of the ammonium chloride and the resultant precipitation of ammonium chloroiridate. The optimum concentration of ammonium chloride is 3.5 *M*. With suitable preparation of

the sample solution, the codeposition of iridium is completely eliminated, while the deposition of rhodium is not prevented.

Oxide in Rhodium Deposits. It was discovered early in the research that electrolytic deposits of rhodium were noticeably heavier than corresponded to the theoretical weights obtained by ignition of the salt to metal. Later it was found that the excess weight was lost if the rhodium deposit was reduced in hydrogen at 450° C. The deposit also changed from a dark gray to a light gray color on reduction in hydrogen. Table I shows losses in weight on reduction.

Table I. Effect of Reduction of Rhodium Deposits with Hydrogen

(Theoretical weight of rhodium = 0.03136 ± 0.00003 gram)

Expt.	Weight of Deposit, Gram: Before reduction	After reduction	% Loss
62	0.03168	0.03136	1.0
68	0.03165	0.03142	0.7
69	0.03190	0.03141	1.5
70	0.03228	0.03140	2.7

No mention of oxide in rhodium plates has been found in the literature. However, Smith (*11*) and Langness (*3*) report having obtained rhodium plates which were black in color; is is likely, therefore, that they contained oxide. Neither Smith nor Langness made any comparison of their electrolytic results with results by any other analytical method. Smith assumed the composition of his starting materials to correspond to the formulas. Langness does not state how her theoretical rhodium values were obtained. It is likely, therefore, that the deposits of both Smith and Langness contained oxide. The presence of oxide in the rhodium deposits obtained in the present work is attributed to the rapid discharge of hydrogen ion at the cathode, which increases the pH of the adjacent solution and causes hydrolytic precipitation of the rhodium. Similar oxide deposits have been observed in the electrolysis of nickel and chromium (*6*) and of iron (*9*). This tendency is minimized by use of as low a potential as possible during the early stages of the electrolysis. A stepwise increase in applied potential has been used in the procedure.

Separation of Rhodium from Iridium. After the previously described procedure had been developed, it was applied to the separation of various mixtures of rhodium and iridium. Table II shows typical data.

Table II. Electrolytic Separation of Rhodium from Iridium According to Proposed Procedure

Expt.	Ratio Rh:Ir	Rh Taken, G.	Rh Found, G.	Error, G.
121	1:2	0.01533	0.01535	+0.00002
122	1:4	0.01533	0.01554	+0.00021
123	1:3	0.01533	0.01524	−0.00009
125	1:2	0.01533	0.01534	+0.00001
128	1:4	0.01533	0.01529	−0.00004
129	1:10	0.00612	0.00609	−0.00003
130	1:4	0.01533	0.01532	−0.00001
131	1:1	0.03068	0.03057	−0.00011
132	1:1	0.03068	0.03068	0.00000
133	1:1	0.03068	0.03064	−0.00004

ADAPTATION TO GILCHRIST-WICHERS SCHEME FOR PLATINUM GROUP

In the procedure of Gilchrist and Wichers (*1*), osmium, ruthenium, platinum, and palladium are first separated, leaving iridium and rhodium in solution together with an excess of dimethylglyoxime used in the precipitation of palladium. Before the separation of rhodium from iridium, the organic matter is destroyed by evaporation with sulfuric and nitric acids. The final evaporation is carried to fumes of sulfur trioxide and until the volume of acid left is no more than 10 ml. This solution contains much sulfuric acid, whereas the electrolytic procedure described above was applied to chloride solutions of the elements. This excess sulfuric acid must be eliminated in some manner in order to apply the electrolytic separation. Although it could be neutralized with sodium hydroxide, the amount of sodium sulfate formed interferes in subsequent evaporations.

A satisfactory way of avoiding this difficulty is to precipitate the rhodium and iridium together as hydrated dioxides from boiling solution at pH 8 in the presence of sodium bromate, by the method of Gilchrist and Wichers. The precipitate is filtered, washed free of alkali salts, and dissolved in hydrochloric acid. After evaporation with aqua regia and hydrochloric acid to destroy complexes as previously described, the residue is once more dissolved in 15 ml. of water and 5 ml. of 1 to 10 hydrochloric acid. The solution is then ready to be treated with chlorine as directed in the electrolytic procedure. Experiments 132 and 133 were performed in this manner. Organic matter was first added to the solutions in the form of filter paper, which was destroyed by evaporation with sulfuric and nitric acids. The hydrated dioxides were then precipitated and dissolved and the resulting solution was electrolyzed after the usual treatment.

SUMMARY

The electrolytic separation of rhodium from iridium in 3.5 *M* ammonium chloride solution has been successfully accomplished using a controlled cathode potential circuit of the Lingane type.

Rhodium was completely deposited from a 3.5 *M* solution of ammonium chloride at a cathode potential 0.4 volt more negative than the saturated calomel electrode.

The weights of the electrolytic deposits of rhodium obtained were higher than those of the metal obtained from ignition of rhodium salts in hydrogen. The difference was traced to the presence of oxides, which were deposited chemically with the rhodium under the electrolytic conditions. It was found necessary to subject all electrolytic deposits of rhodium to reduction in hydrogen at 450° C. to ensure a deposit free from oxide.

When rhodium was electrolyzed in the presence of iridium in a chloride solution, free from ammonium chloride, much codeposition of iridium occurred at a cathode potential of −0.30 volt *vs.* S.C.E. The codeposition has not been explained, but it has been eliminated by the destruction of complexes, oxidation of iridium with chlorine, and addition of sufficient ammonium chloride to produce a concentration of 3.5 *M*.

The accuracy of the electrolytic rhodium determination is about ±0.3%. Spectrographic tests showed the actual deposition of rhodium to be better than 0.1% of the rhodium present, while contamination of rhodium with iridium could not be detected from the weights of rhodium obtained.

The procedure has been adapted to the Gilchrist-Wichers scheme for separation of the platinum group metals.

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Chromatographic Properties of Silicic Acid–Celite

Relation between Water Content and Adsorptive Strength

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The relation between water content and chromatographic adsorptive strength has been investigated for different samples of silicic acid mixed with Celite. Greater adsorptive strength after prewashing was found to be associated with greater content of "structural" water—that is, water removable only by ignition. A simple relation was found between the adsorptive strength of a sample of (unprewashed) silicic acid and its content of water removable by prewashing or mild heating, "free" water; greater adsorptive strength was associated with a lower content of free water. It was found, as expected, that the adsorptive strength of a given sample of silicic acid attains a maximum when all the free water has been removed, and that when all the structural water has also been driven off the adsorptive power almost completely disappears. Appropriate prewashing of the column increases the adsorptive power of the adsorbent and goes far toward eliminating the differences in adsorptive properties exhibited by different commercial lots of silicic acid. Some exceptions are noted and discussed.

IN PRECISE chromatography, an understanding of and control over the factors that cause significant variations in the adsorptive strength of the adsorbent are essential. It has long been recognized (*2, 9, 13, 15, 18*) that the adsorptive strength of adsorbents such as alumina and silica gel or silicic acid is markedly dependent on the water content. Because silicic acid is an extremely useful and versatile chromatographic adsorbent, an investigation has been made of the relation between the water contents and the adsorptive strengths of a variety of samples of silicic acid suitable for chromatographic work.

Fells and Firth (*5*) and Kiselev (*8*) have drawn a distinction between "free" water and "fixed" or "structural" water in silica gels. The free water is that which is readily removed essentially reversibly by heating at temperatures of 100° to 200° C.; its removal leads to an increase in the adsorptive strength. On the other hand, the fixed water can be removed completely only by heating to around 1100° with consequent destruction of the gel structure; furthermore, when all the fixed water has been removed the adsorptive properties essentially completely disappear (*9*). The free water can vary widely in amount in a given gel and is considered merely to be enclosed in the meshes or pores of the gel; the fixed water is more nearly constant in amount in a particular gel. It has been suggested (*9*) that the fixed water arises from the elimination of water between adjacent hydroxyl groups attached to the silicon-oxygen skeleton. The process of aging of silica gels has been interpreted (*5*) as the change of fixed to free water.

In order to increase and control more precisely the adsorptive strength of silicic acid, various procedures have been used for its activation; among these are mild heating (*18*), prewashing with suitable solvents (*11, 16*), and evacuating over a drying agent (*7*). The prewashing procedure has been employed most extensively (*12, 16, 17*) and is the most convenient of the methods. It is significant that a common feature of all these procedures is the removal of water from the adsorbent.

The present investigation comprises a study of the variation of the adsorptive strength of a given sample of silicic acid with changes in its water content and a comparison of the properties of seven samples of silicic acid obtained from different commercial sources. From the many different techniques which have been described for the characterization of chromatographic adsorbents (*2, 3, 10, 14, 18, 20*), a method similar to that of LeRosen (*10*) was adopted as the simplest and most practical. Specifically, certain compounds representing a variety of structural types and adsorption affinities were chosen and their rates of development compared on the different samples of adsorbent under standard conditions.

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EXPERIMENTAL

Adsorbents. The seven samples of silicic acid that were used were all of reagent or C.P. grade. They included three samples of Merck silicic acid, which were identified by the code numbers 43243, 40665, and 40446, and one sample each marketed by the General Chemical Company, New York, the City Chemical Corporation, New York, the Mallinckrodt Chemical Works, St. Louis, and the J. T. Baker Chemical Company, Phillipsburg, N. J. [These code numbers are reported to refer to the packing date and presumably, although not necessarily, designate different production lots; because they afford the only convenient means of distinguishing between different shipments of materials, which may have different properties, they are used for that purpose here.] Before chromatography each sample of silicic acid was thoroughly mixed with approximately 0.5 part by weight of Celite 545, a product of the Johns-Manville Corporation.

Solvents. All solvents were distilled in an all-glass still before use. Commercial benzene was dried over calcium chloride before distillation; reagent grade anhydrous ether was used without further drying.

Special Compounds. Samples of *N*-methylisatin (*1*) and 4-nitrotriphenylamine (*6*) were prepared and purified by standard methods; before recrystallization the 4-nitrotriphenylamine was separated from higher nitration products by chromatography on silicic acid–Celite with benzene-ligroin and ether-ligroin development. Commercial samples of 2,4-dinitrodiphenylamine and ethyl centralite (*sym*-diethyldiphenylurea, *N,N'*-diethylcarbanilide) were recrystallized until their melting points became constant.

Chromatography. All chromatograms were carried out in Pyrex tubes of the type described by Zechmeister and Cholnoky (*21*). In most experiments, tubes 200 mm. long with an inside diameter of 19 mm. were used; however, smaller tubes, 9 and 14 mm. in inside diameter, were used in certain experiments. The columns of adsorbent were 150 ± 5 mm. in height, except as otherwise stated.

The prewashing treatment which was used throughout the present work, except as otherwise noted, consisted in washing the column successively with 0.2 *V* ml. of ether, *V* ml. of 1 to 1 acetone-ether, 0.8 *V* ml. of ether, *V* ml. of ligroin (60° to 70°), and finally about 0.2 *V* ml. of the solvent subsequently used as the developer. [The term "*V* ml." has been defined (*16*) as that volume of solvent which is required to wet completely a column of adsorbent; for a 19 × 150 mm. column of 2 to 1 silicic acid–

Celite, V is about 29 ml.]. In addition to increasing the adsorptive strength, the prewash has the effect of decreasing the width of zones and rendering the adsorbent more uniform throughout the column (*16*).

With the exception of ethyl centralite, each of the compounds which were used to test adsorptive strength forms a colored zone on a column of silicic acid–Celite. Zones of centralite were located by means of the red-pink color that they produce when the column is streaked with a 1% solution of ceric sulfate in 85% phosphoric acid.

Zones on silicic acid usually appear symmetrical, with neither the upper nor the lower boundary sharp; consequently the measurement of the position of a zone is a somewhat arbitrary process. Nevertheless, positions measured by different experienced observers generally agree well and the reproducibility of measurements by a single experimenter is usually very good. The authors have adopted the procedure of measuring and recording both the extreme limits of the zone—that is, the position of the first visible material on each boundary of the zone—and the approximate limits of (an arbitrarily chosen) more concentrated portion of the zone. This method of recording the data was chosen in order that the center of gravity of the zone could better be located and a measure of the symmetry of the zone obtained. In recording the positions of zones measurements were made from the top of the column and the convention was used of placing the extreme limits of the zone in parentheses. Thus when the position of a zone is recorded as (70)76–109(116), the extreme limits were 70 and 116 mm. below the top of the column and the limits of the more concentrated portion were 76 and 109 mm. from the top.

The position of each zone was measured in several places on the surface of the column; then the column was sliced in half axially and the position on the axis of the column was measured. For zones which are coned this position is, of course, different from the surface position.

Determination of Water Content. In the determination of the water content of each sample of silicic acid, a 2-gram portion was first heated for about 1 hour in an oven at 200° in order to determine the free water (all the free water is driven off in about 40 minutes) and was then ignited to constant weight over a Meker burner. In the preparation of the samples of intermediate water content for the tests of the effect of variation in water content on the adsorptive strength of a given lot of silicic acid, 50-gram portions of 2 to 1 silicic acid–Celite were heated at 200° for periods of time varying from 5 minutes to about 2 hours. It was shown that Celite 545 does not change in weight during several hours of heating at 200°, so that the entire loss in weight of the samples could be ascribed to the silicic acid.

Activation of silicic acid by prewashing is usually almost identical in its effect on the adsorptive strength with activation by heating at 200°. It was demonstrated that these comparable activating effects are due to the removal of equivalent amounts of water from the adsorbent.

The quantity of water removed during the prewashing process was determined by the use of Karl Fischer reagent; in order to avoid complications caused by the presence of acetone in the Karl Fischer determination, a variation of the usual prewash was used in which V ml. of methanol and V ml. of ether replaced the V ml. of 1 to 1 acetone-ether customarily used. The adsorptive properties were not altered by this substitution. The analysis showed that the amount of water removed by the prewash corresponded to 13.9% of the weight of the silicic acid in the column. Samples of this same lot (Merck 40665) of silicic acid which were activated by heating at 200° lost water corresponding to 13.3% of their weight. Because of the appreciable blank correction in the Karl Fischer analysis, the difference in these results is probably not significant; thus, these two treatments, which produce essentially the same effect on the activation of the adsorbent, apparently also produce essentially the same effect on its water content.

In order that the prewashed columns of the different samples of silicic acid–Celite might be as similar as possible in the tests of comparative adsorptive strengths, an attempt was made to prepare the mixtures in such a manner that after prewashing they would all have the same ratio of (partially dehydrated) silicic acid to Celite. This ratio was arbitrarily chosen as 1.75, which made the proportion of untreated silicic acid to Celite to be taken originally about 2 to 1; the exact proportions for each sample were calculated from the measured water content of the sample, on the assumption that the prewash removed about the same amount of water as did heating at 200° for all the adsorbents, as it had been found to for Merck 40665.

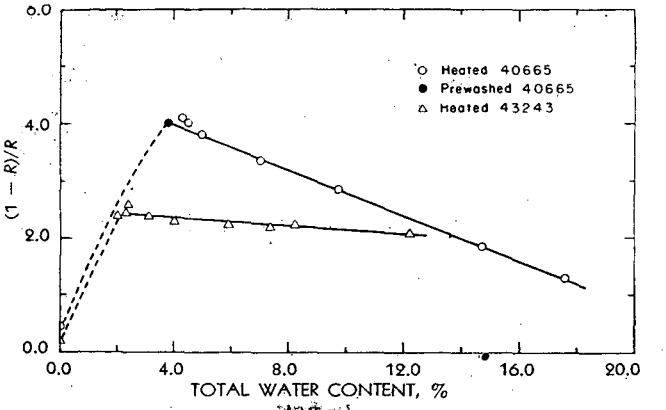

Figure 1. Adsorptive Strength of Silicic Acid–Celite of Varying Water Content

Tested with *N*-methylisatin developed with 10% solution of acetone in ligroin (Test III)

Tests of Adsorptive Strength. In the comparison of the adsorptive strengths of the different samples of silicic acid–Celite, both prewashed and unprewashed adsorbent were tested. The tests were made as follows:

Test I. A 0.7-mg. sample of 4-nitrotriphenylamine was developed with a 3% solution of ether in ligroin (60° to 70°).

Test II. A 1-mg. sample of ethyl centralite was developed with a 4% solution of ether in benzene.

In the tests of the variation of the adsorptive strength of a given lot of adsorbent with variation in water content, the only prewash employed was 0.5 V ml. of the solvent to be used as developer. The following tests were used:

Test III. A 2-mg. sample of *N*-methylisatin was developed with a 10% solution of acetone in ligroin (60° to 70°).

Test IV. A 0.1-mg. sample of 2,4-dinitrodiphenylamine was developed with 1 to 1 benzene-ligroin (60° to 70°).

Test V. A 0.1-mg. sample of 2,4-dinitrodiphenylamine was developed with a 4% solution of ether in ligroin (60° to 70°).

In each test, the ratio of the linear rate of movement of the zone to the linear rate of movement of the solvent in the column, a quantity which was originally considered by LeRosen (*10*) and designated as R, was evaluated without measuring either rate directly by measuring the distance through which the zone moved during the period required for V ml. of developer to flow through the column and dividing this distance by the length of the column.

RESULTS

Reproducibility. In order to demonstrate the sort of reproducibility that can be achieved in successive identical chromatographic experiments on prewashed silicic acid–Celite, there are listed in Table I the observed zone positions in a series of experiments in which every effort was made to maintain strictly identical conditions. These results are typical of those which were obtained throughout this investigation. The degree of downward coning indicated by the data of Table I is typical of most compounds and developers on columns prewashed with acetone-ether. Because neither the direction nor the extent of coning varied appreciably from one sample of prewashed silicic acid–Celite to another, the phenomenon was not of serious concern in the present work.

Relationship of Adsorptive Strengths of Different Lots of Silicic Acid to Their Water Contents. The water contents and

Table I. Reproducibility of Zone Position in Consecutive Identical Experiments[a]

Experiment	Surface Position	Surface Mid-point	Axial Position	Axial Mid-point
1	(72)76–107(111)	92	(88)92–117(122)	105
2	(73)79–110(114)	94	(82)90–110(117)	100
3	(70)75–108(113)	92	(87)95–111(117)	103
4	(71)77–106(110)	91	(84)93–116(121)	104
5	(70)76–109(116)	93	(85)94–115(121)	104

[a] 4-Nitrotriphenylamine (0.8 mg.) developed with 2 *V* ml. of a 3% solution of ether in ligroin (60° to 70°).

Table II. Water Contents and Adsorptive Properties of Silicic Acid

Designation of Silicic Acid Sample	Loss in Weight at 200°, %	Additional Loss in Weight at 1100°, %	Total Water Content, %	Rate of Development[a], *R* Unprewashed column, Test I[b]	Prewashed column, Test I	Prewashed column, Test II
Merck 40446	14.1	4.8	18.9	0.74	0.33	0.150
Mallinckrodt	9.85	4.75	14.6	0.64	0.33	0.157
City Chemical Co.	8.8	4.6	13.4	0.68	0.38	0.155
Merck 40665	13.3	4.3	17.6	0.80	0.41	0.162
General Chemical Co.	9.8	4.0	13.8	0.58	0.37	0.162
J. T. Baker	17.9	3.4	21.3	0.78	0.53	0.26
Merck 43243	9.1	3.4	12.5	0.66	0.60	0.26

[a] Values refer to rate of movement of mid-point of zone.
[b] *V* ml. of ligroin (60° to 70°) used to wet column before sample was introduced.

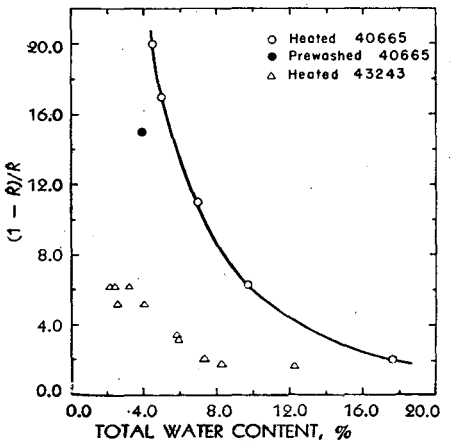

Figure 2. Adsorptive Strength of Silicic Acid–Celite of Varying Water Content

Tested with 2,4-dinitrodiphenylamine developed with 1 to 1 benzene–ligroin (Test IV)

the adsorptive properties of the different samples of silicic acid are summarized for comparison in Table II. The loss in weight of each sample at 200° represents the content of free water; the additional loss in weight at 1100° represents fixed or structural water.

The data in Table II show that the unprewashed adsorbents vary considerably in adsorptive strength and are all relatively weak; in general, those of greater water content tend to be the weaker. On the other hand, after the prewash has been used to remove the free water and increase the adsorptive strength of each of the adsorbents, five of the seven samples are found to possess very similar adsorptive properties. The differences which do exist can be correlated with the content of structural water in each of the adsorbents. Thus, when the adsorbents are arranged as in Table II in the order of decreasing content of structural water, it is found that this is also, within experimental error, the order of decreasing adsorptive strength in both Test II and, with one minor exception, Test I. Although no unequivocal interpretation of these relationships is apparent, it is possible to speculate on the role of the water in the adsorbent. In view of the demonstrated importance of hydrogen bonding in adsorption on silicic acid (*4*, *17*) it is reasonable to suppose that the adsorption of the compounds studied in the present work involves hydrogen bond formation with hydroxyl groups of the silicon-oxygen skeleton. Perhaps an increase in the content of structural water leads to an increase in the number of sites where such adsorption can take place and thus increases the "adsorptive strength" of the prewashed adsorbent or heated adsorbent by shifting the equilibrium between unadsorbed and adsorbed material. On the other hand, an increase in the content of free water may lead to a decrease in the number of such sites available because the free water itself occupies these sites. The possibility that an intensity factor—i.e., a difference in the energy of adsorption—as well as a capacity factor, is responsible for the differences between the adsorbents should not be overlooked, although it seems somewhat less likely, at least according to this simplified picture. All these considerations are speculative and although the generalizations presented here account for most of the facts, some exceptions can be found.

Variation of Adsorptive Strength of a Particular Sample of Silicic Acid with Changes in Its Water Content. From among the five similar adsorbents listed in Table II, Merck 40665 was selected for a detailed study of the relation between water content and adsorptive strength. In addition, a similar study was made of Merck 43243 in the hope that the differences which characterize this adsorbent might be clarified. Tests III, IV, and V were applied to samples of varying water content which had been prepared by heating for different periods of time. The results of these tests are presented in Figures 1, 2, and 3, in which the quantity $(1 - R)/R$ is plotted against the water content of the adsorbent. The quantity $(1 - R)/R$ is a measure of the adsorptive strength of the adsorbent; it is essentially the inverse of the rate of movement of the zone, $1/R$, corrected to $(1/R) - 1$, so that when the test compound is not adsorbed at all ($R = 1$), the adsorptive strength will be zero.

In all tests of the typical lot, Merck 40665, the maximum adsorptive strength occurred when all the free water had been removed from the adsorbent. A sample of ignited adsorbent was found to have almost negligible adsorptive strength (Figure 1), in agreement with earlier observations (*9*). Only one test was made of this material because it was prepared only in small quantities. In Tests III and V (Figures 1 and 3), in which the developers contained oxygenated solvents, Merck 40665 shows a linear relation between the content of free water and the adsorptive strength. In Test IV (Figure 2), in which the developer was a mixture of benzene and ligroin, the relation also follows a smooth curve, although it is no longer linear. Further tests of this adsorbent using

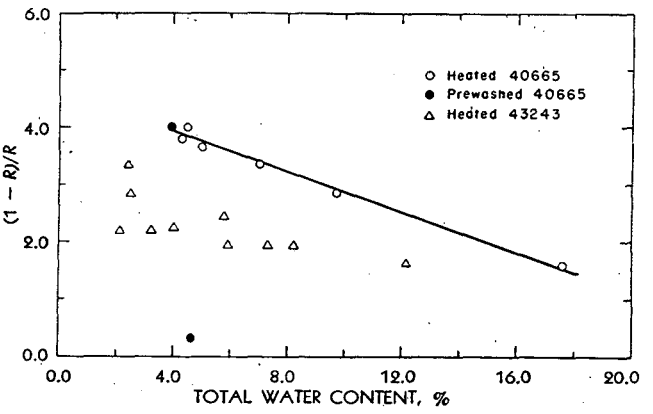

Figure 3. Adsorptive Strength of Silicic Acid–Celite of Varying Water Content

Tested with 2,4-dinitrodiphenylamine developed with 4% solution of ether in ligroin (Test V)

4-nitro-*N*,*N*-diethylaniline with toluene-ligroin development and 2,4-dinitrodiphenylamine with pyridine-ligroin development gave results similar to those shown in Figure 2. The adsorptive strength of prewashed adsorbent was demonstrated to be equivalent to that of heated adsorbent in Tests III and V (Figures 1 and 3). The discrepancy in Test IV (Figure 2), in which benzene-ligroin development was used, can be readily explained when the nature of prewashed adsorbent is considered in detail (*19*).

In contrast to the behavior of the typical lot, Merck 43243 shows considerably less increase in adsorptive strength when the free water is removed (by heating or by prewashing) and, more striking still, the relation between its adsorptive strength and water content is rather erratic according to all the tests, and, at least in Test V (Figure 3), not even single-valued. The explanation of these facts is not apparent. Although the water content of Merck 43243 after prewashing was not measured, the prewashed adsorbent had, as expected, an adsorptive strength similar to that of the samples from which all the free water had been removed. The one sample of ignited adsorbent tested had negligible adsorptive strength (Figure 1).

CONCLUSIONS

Results indicate that columns of similar adsorptive strength may conveniently be produced from several different commercial samples of silicic acid by the use of the prewashing procedure. The differences which do occur in the adsorptive strengths of prewashed silicic acids can be correlated with the contents of structural water in the adsorbents. For a typical lot of silicic acid there is a simple inverse relation between the adsorptive strength, as measured in a variety of different tests, and the content of free water.

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Differential Thermal Analysis of Proteins

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Differential thermal curves for three proteins—wheat gluten, egg albumin, and ashless gelatin—show that all three proteins undergo exothermic reactions when they are heated slowly from room temperature to 225° C. A simple inexpensive apparatus is described.

THE differential thermal method of analysis was first suggested by Le Chatelier (*2*) and widely adapted to the study of clay minerals by Orcel and Caillère (*4*), Norton (*3*), and Grim (*1*). Vold (*5*) has recently published a description of a differential thermal apparatus which she used to determine the heats of fusions of stearic and benzoic acids. It has been possible to show that exothermic effects occur in vacuum-dried proteins during heating up to 225° C. when a differential thermal analysis technique is used. Although 35 to 40 analyses were made of gluten proteins, this report must be of a preliminary nature pending a more exhaustive study of proteins from other sources. Nevertheless, the results thus far obtained may be of importance to protein research.

APPARATUS

The apparatus used in this investigation was essentially the same as that used by the investigators cited above in their work with clay minerals.

It consisted of a copper block 2 inches in diameter and 1.5 inches thick, into which four holes 0.375 inch in diameter and 0.75 inch deep were drilled. These four holes, which hold the samples being analyzed, were evenly spaced around the block 0.375 inch from the edge. Thermocouples passed through porcelain insulators into the sample through 0.25-inch holes drilled at right angles to the sample holes. Calcined aluminum oxide served as reference material, as it does not undergo energy changes when heated up to relatively high temperatures.

The temperature of the block was measured with a Chromel-Alumel thermocouple with the cold junction in ice water and the other in the reference material. Two of the other holes contained the differential thermocouples. One was filled with reference material and the other with the protein being analyzed. The current input to the hot plate used as a source of heat was varied so that a uniform rate of heating of 5.0° C. per minute was maintained. To accomplish this a large Variac, operated manually, was used to regulate the voltage input to the electric hot plate. Several trial runs were necessary before the rate of heating could be maintained constant. The rate-of-heating curves (plotting time against temperature) for gluten and gelatin are presented in Figure 1 (straight lines). Actually, the rate-of-heating curves are not true straight lines but show some minor fluctuations. The average rate of heating for the albumin was 10.36° ± 0.21° C. in 2 minutes, for the gelatin 9.88° ± 0.24° C. in 2 minutes, and for the gluten 9.91° ± 0.14° C. in 2 minutes. Only the temperatures after 4 minutes of heating are included in these averages. About 0.75 gram of sample was required for each run.

The effect of rate of heating upon differential thermal analysis curves of vacuum-dried wheat gluten and flour was investigated slightly, but curves are not included in this paper. The tests

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[2] Deceased December 4, 1947.

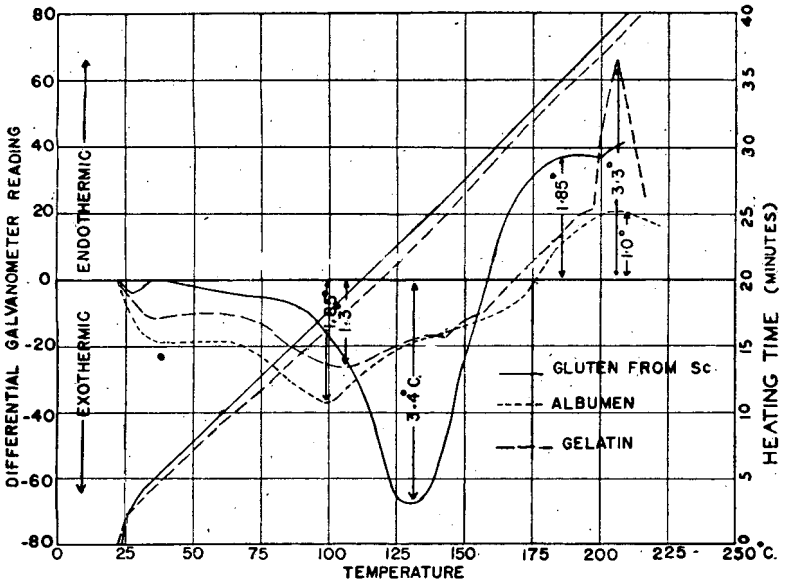

Figure 1. Differential Thermal Curves of Vacuum-Dried Proteins

Straight line shows rate of heating for gluten and gelatin

showed that an increased rate of heating not only increased the magnitude of the dips and peaks but also caused the breaks to occur at higher temperatures. There was a time-temperature interaction—i.e., gluten protein denatured at a lower temperature if heated over a longer period of time.

The protein samples—ashless gelatin, powdered egg albumin, and wheat gluten—were dried in a vacuum oven at 40° C. for 48 hours before being analyzed. The preliminary drying was necessary to prevent the endothermic effects accompanying loss of absorbed water from overshadowing all other effects. The differential thermocouple, connected to a sensitive galvanometer (0.025° per scale), measures differences in the rate of heating in the sample and reference material. Because both materials are in the same small copper block they will heat at the same rate unless energy changes occur in the sample, in which case the sample will heat either faster or slower than the reference material, depending on the nature of the energy change taking place.

Figure 1 shows the rate of heating for the gluten and gelatin samples and the type of curves obtained for each protein studied when the reading of the differential thermal galvanometer was plotted against the temperature of the block. All three proteins studied undergo exothermic effects during heating. The temperature difference between the protein and reference material was greater for gluten than for either gelatin or egg albumin. However, the exothermic effects take place over a wider temperature range for the gelatin and albumin. The maximum temperature difference for albumin occurred at 100° C., for gelatin at about 110° C., and for gluten at about 130° C. The two small inflections on the gelatin curve just below and just above 150° C. are probably not significant and may be due to fluctuations in heating rate.

It was assumed that the thermal curves would not be reversible on cooling, inasmuch as 200° C. is well above the temperature required to denature the proteins. Therefore, no cooling curves were made.

Considerably more work will be required before it will be possible to discuss the exact significance of these differences. However, the results thus far obtained are reported because the method promises to be useful in the study of the heat denaturation of proteins and the influence of moisture content on the temperature at which denaturation occurs.

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Determination of Radiophosphorus in Plant Material by Solution Counting

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IT WAS desirable to determine radiophosphorus in plant material by some means other than counting from a uniformly deposited solid, in order to simplify and speed the analysis without loss of accuracy. MacKenzie and Dean (*9*) developed an accurate method of analysis for P^{31} and P^{32} by precipitating phosphorus as ammonium phosphomolybdate and then reprecipitating as magnesium ammonium phosphate; the latter precipitate was collected as a thin uniform layer on a filter ring under carefully standardized conditions. This procedure gives good results, but it is time-consuming and the uniform layer for counting must be carefully prepared. If the layer becomes too thick, it is necessary to make self-absorption corrections. The area of the deposit must be precisely reproduced and geometry with respect to the Geiger-Müller counter closely maintained.

The method for determing P^{32} in solutions presented here is much more rapid, overcomes most of the disadvantages just mentioned, and is accurate to a standard deviation of 0.7%. Other liquid counters and procedures for counting from solution are described by Olson *et al.* (*10*), Barnes (*2*), Bale *et al.* (*1*), Wang *et al.* (*14*), Barnes and Salley (*3*), Comer and Neller (*5*), and Veall (*13*).

PREPARATION OF SOLUTION FOR COUNTING

Weighed samples of plant material containing from 1 to 30 mg. of phosphorus were heated in 50-ml. Pyrex beakers in an electric muffle at 500° C. for at least 6 hours to destroy organic matter. Nitric acid (8 *N*) was added and evaporated to dryness and the residue was heated at 400° C. for 15 minutes to complete the destruction of organic matter. Silica was dehydrated with concentrated hydrochloric acid. The residue was taken up in 2.5 ml. of 2 *N* nitric acid and transferred with hot water to a 25-ml. volumetric flask if the specific activity of the phosphorus was

A rapid method for the determination of P^{32} in solution is presented, accurate to a standard deviation of a single measure of less than 1%. The Geiger-Müller counter assembly is described, and the preparation of the sample is given along with the counting procedure. The preparation of uranium standards is presented and their use as efficiency standards and for the determination of the resolving time of the counter is given. Factors influencing the count, such as solution volume, K^{40} background, temperature, self-absorption, and adsorption of isotopes on glass, are discussed. The efficiency of the liquid counter is compared with the thin end-window counter.

high. When the specific activity was low, 10 ml. of 2 *N* nitric acid were accurately pipetted into the 50-ml. beaker and carefully stirred and the suspension was transferred without washing to a 15-ml. centrifuge tube. The silica was centrifuged down or, in the case of the volumetric flasks, allowed to settle for 24 hours. These solutions were then counted. If the count exceeded 10,000 counts per minute, the solutions were diluted.

Total phosphorus was determined by the molybdivanadophosphoric acid method (*8*) on an aliquot of the solution used for counting. When the specific activity was high, the colored solution on which the total phosphorus was determined was used for counting.

GEIGER-MÜLLER COUNTER ASSEMBLY

Figure 1 is a cross-sectional view of the Geiger-Müller counter and its surrounding cup. The unfilled counter is the same as that used by Solomon and Estes (*12*).

The shell was filled with 1.5 cm. of absolute ethyl alcohol and 8.5 cm. of argon, giving a self-quenched counter with a plateau 200 volts in width and a slope of 3.5% per 100 volts. The counter was slightly light-sensitive and was covered with a black cloth during the count. The background was 25 counts per minute when the cup was filled with distilled water. Shielding with 5 cm. (2 inches) of lead should reduce the background count by at least a factor of two.

The Geiger-Müller counter was clamped tightly to a rigid ring stand at the top of the tube just below where the tungsten leads emerged and at the top of the filling tube. Rubber served as a cushion between the clamps and the glass, yet held the counter tightly. The cup was centered with the counter and clamped tightly in position. Thus, the geometry of the cup with reference to the Geiger-Müller counter was fixed and it was not necessary to have an elaborate centering mechanism. A pinch clamp on the rubber tubing closed the cup at the bottom.

A small funnel was mounted on a swivel directly below the cup. Washings and solutions which were not saved were drained into this funnel, and the solution was conducted by means of rubber tubing into a 20-liter bottle on the floor. The funnel was swung from under the cup when the solution was to be saved. A 20-liter bottle of distilled water was mounted above and behind the counter, the water being used to rinse the cup. With the spacing between the counter and cup between 1.5 and 2.0 mm. the cup held approximately 9 ml. when filled 1.5 cm. above the top of the silvered portion of the counter.

PROCEDURE FOR COUNTING

Prior to counting a series of samples, the cup was filled with chromic acid cleaning solution and rinsed to assure good drainage. The cup was rinsed with the sample to be counted by filling the cup and draining. If the samples being counted have approximately the same specific activity, it is not necessary to rinse the cup with the next sample. Draining the cup and then taking a count without rinsing revealed that less than 1% of the solution remained. Thus, if two samples have counting rates differing as much as 50%, the error introduced will be less than 0.5%.

Rinsing by filling and draining the cup four times with water removed all traces of 50,000 counts per minute from P^{32}; this indicated there was no adsorption of phosphorus in acid solutions (0.01 *N* nitric acid or stronger) on the glass walls of the counter or cup when working with specific activities of the order found in plant material or fertilizers. When working with extremely high specific activities such as carrier-free phosphorus, there may be adsorption on glass surfaces (*7*), but this might be prevented by complexing the phosphorus.

Uranium nitrate or acetate solutions used as permanent standards did show adsorption on the glass surface. After rinsing the cup thoroughly with water, 200 to 250 counts per minute over the background remained from a uranium nitrate solution giving 12,000 counts per minute. Sodium or ammonium tartrate added in equal molar quantities to the uranium nitrate solution formed a soluble complex which completely prevented the adsorption of uranium.

EFFICIENCY STANDARDS AND RESOLVING TIME MEASUREMENT

Uranium nitrate standards were prepared by heating pure uranyl acetate to constant weight at 700° C. to form U_3O_8, a good primary standard. The oxide was converted to the nitrate by the addition of concentrated nitric acid, after covering the oxide with water to slow the reaction, and subsequent evaporation to dryness. Two standards were prepared, one containing 24 grams of U_3O_8 per liter, the second 12 grams. Twenty and 35 grams of sodium tartrate were added to complex the uranium and prevent its adsorption on glass surfaces. The larger quantity of sodium tartrate was added to the smaller uranium standard in order to give approximately the same solution density and self-absorption of the beta-particles. Thus, the difference in the self-absorption between the two uranium standards is eliminated and need not be considered in the calculation of the resolving time. These two standards were counted at regular intervals to determine any changes that might have occurred in the Geiger-Müller counter, scaling circuit, or register—i.e., efficiency standards to determine if the same count was obtained from day to day. One standard counted approximately 11,000 per minute under the conditions described in this paper. The second counted a little over one half this value.

With these uranium standards, the resolving time can be determined easily by a modification of the combined source method (*4, 11*):

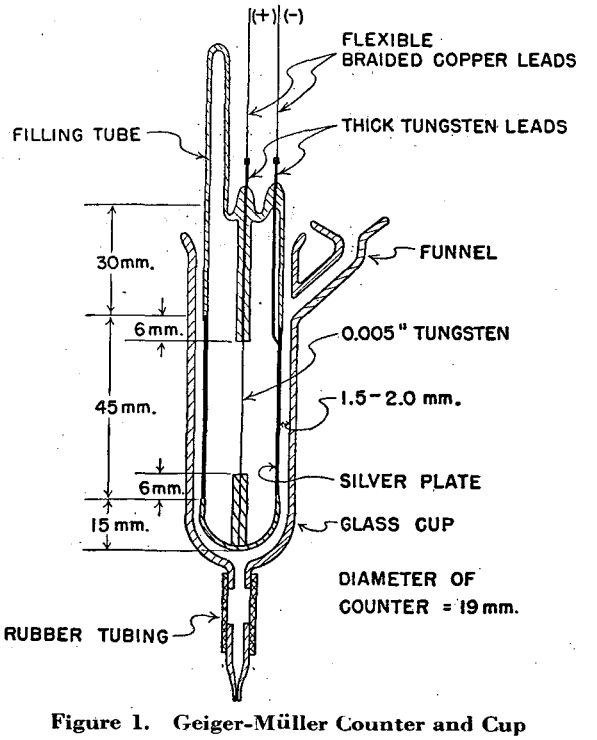

Figure 1. Geiger-Müller Counter and Cup

$$T_d = \frac{2C_1 - (C_2 + B)}{C_1(C_2 + B)}$$

where T_d = resolving time, C_1 = observed counting rate of low uranium standard, C_2 = observed counting rate of high uranium standard, and B = background count. The background count is added to C_2 to compensate for the second background count which appears in the $2C_1$ term. Using this resolving time, the true counting rate is then given by (*4*):

$$C_0 = \frac{C}{1 - CT_d}$$

where C = observed counting rate, C_0 = true counting rate, and T_d = resolving time. To maintain good precision it is wise not to exceed 10,000 counts per minute, because the error in the resolving time correction may become significant above this rate.

ADDITIONAL FACTORS INFLUENCING THE COUNT

Solution Volume. The cup is filled with the sample to a height of at least 0.5 cm. above the silvered portion of the counter. Counts taken at 0.5 and 2 cm. above the silvered portion were the same. Therefore, precautions need only be taken to assure that the silvered area is covered. When working with isotopes from which most of the count is from gamma-rays, this may not be true.

Increased Background from K^{40}. One of the isotopes of potassium, K^{40}, is naturally radioactive and its presence in a solution adds to the count of the sample. Actually potassium has been determined by its radiation (*3, 6*). This K^{40} activity, however, is low and may be disregarded unless the P^{32} sample count is only a few times background. If necessary, K^{40} may be determined by counting a plant sample which has not received P^{32} and used as the counter background. Or, if the ignition temperature of the plant material is increased several hundred degrees, most of the potassium should be lost without losing phosphorus.

Temperature. Variations in temperature have an effect on the characteristics of the Geiger counter, making it necessary to maintain solution temperatures fairly constant. A change from 24° to 28° C. gave an error of less than 1%. The surface area of the solution in the cup is small, and with water at room temperature the error introduced by evaporation is negligible as measured by counting repeatedly a standard over a 2-hour interval.

Self-Absorption Correction. To determine the effect of salt concentration or increased density of solution on the rate of count of P^{32}, a series of solutions containing the same quantity of P^{32} but varying densities was counted. The solutions contained up to 12% sodium chloride at 2% intervals and had densities varying from 0.997 for water to 1.084 for the 12% sodium chloride solution. Over this range the counting rate changed only 2.9%. If the counting loss correction is considered a linear function of density, the density of the solution counted can vary 3.0% before the error becomes greater than 1.0%. In the case of larger variability in density, self-absorption corrections can easily be made.

Adsorption of Isotopes on Glass. There was no adsorption of radiophosphorus on glass but there was adsorption of uranium which could be prevented by complexing with tartrate ion. The behavior of two additional conveniently available radioactive isotopes was investigated.

A solution of ferric chloride in 0.01 *N* hydrochloric acid which gave 15,000 counts per minute and had a specific activity of 7×10^{-6} mc. per mg. of iron revealed no adsorption. F. A. Long of the Department of Chemistry, who furnished the radioiron, investigated radiocobalt. The 0.01 *M* cobaltous sulfate solution having a specific activity of 5×10^{-3} mc. per mg. of cobalt also showed no adsorption.

Thus, it would appear that a number of radioisotopes may not be adsorbed on glass. However, it is possible that some isotopes or "carrier-free" isotopes may show adsorption. Should this occur, a suitable soluble complex may be found which will allow the counting of these isotopes in solution.

SOLUTION COUNTER COMPARED WITH END-WINDOW COUNTER

To determine the relative sensitivity of counting P^{32} from solution as compared with counting from a thin uniform solid using an end-window counter, a quantity of phosphorus was evaporated as a thin uniform film on a metal disk. This disk was placed as close as possible to the end window. An equal quantity of phosphorus diluted to 10 ml. was counted in the liquid counter described here. A comparison of the results revealed that the end-window counter was about twice as efficient as the liquid counter. However, with very low specific activity materials this difference becomes less. With greater mass of phosphorus the self-absorption corrections for counting from the solid increase rapidly, whereas the increase in self-absorption in the solution is slight. Shielding the liquid counter would partially compensate for this factor of 2, because the background count would be less than that of the end-window counter, owing to the smaller diameter and smaller surface area of the sensitive region of the liquid counter.

ACCURACY OF METHOD

The uranium standards counted over a period of several months have given a standard deviation of 0.7% for a single measure compared with the theoretical error of the count, 0.42%. The observed error includes not only that due to probability of count, but also any changes that may have occurred in the counter tube, scaling circuit, or register, as well as effect of geometry of cup with reference to the counter. In the analysis of 180 plant samples, the specific activity was determined to a mean standard deviation of a single measure of 1.9%. The specific activity, of course, involves not only the error of the count but also the error in the determination of the mass of phosphorus. In this case, the mass was determined by a colorimetric procedure; thus, the error of 1.9% is indeed good.

As a further test of this method, as well as a check on the half-life of P^{32} (14.30 days), six samples of radiophosphorus were counted 69 days apart. Observed counting rates varied from 1200 to 6300 counts per minute. After making corrections for resolving time, background, and half-life, the means of the samples counted at these two different dates differed by only 0.6%; excluding one value, the difference between the means was less than 0.1%.

ACKNOWLEDGMENT

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Tracer Techniques Used in Study of Coke Sulfur

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This paper describes the use of radioactive pyrites synthesized from sulfur 35 to trace the pyritic sulfur of coal through a full-scale coking operation into the coke and gases evolved. The synthesis of pyrite from radioactive sulfur is described along with a method for converting the sulfur in cadmium sulfide and barium sulfate to elemental sulfur. The method of preparing sulfur standards containing varying percentages of active sulfur, and the technique of using the Geiger counter are also included.

A STUDY has been completed involving the use of the tagged-atom technique to determine the principal source of sulfur in coke. The details of this study and a complete discussion of the results, as well as other aspects of this investigation of interest to steel men, have been published (*2*). The present paper discusses only the phases of primary interest to analysts. It is generally recognized that sulfur exists in coal as pyritic, organic, and sulfate sulfur, whereas coke contains calcium and ferrous sulfides and sulfur combined with carbon. Because the sulfate sulfur content of most coals is very small, the experiment was designed to indicate how much pyritic and, by difference, how much organic sulfur remain in the coke after carbonization.

Sulfur is a very undesirable impurity in steel and there is a continual effort in the industry to reduce or control the sulfur content of pig iron. By far the largest source of sulfur in the steel process is in the coke charged to the blast furnace. Thus, effort was directed towards studying the role of sulfur in the production of metallurgical coke.

The new "tagged atom" or "tracer" technique is particularly well suited for this type of study. This technique involves th use of radioactive atoms which can be followed through complicated chemical reactions by the use of instruments which detect the radiations emitted. The use of a radioactive element for tracing purposes is based on the fact that for all practical purposes the radioactive form is identical in chemical behavior with the nonradioactive form. The radioactivity of an element is completely unaffected by temperature, pressure, or chemical reaction. Therefore, atoms such as sulfur, from a particular source, may be marked or tagged radioactively and mixed with other untagged sulfur atoms of identical chemical nature, and by the use of detecting instruments the amounts of active sulfur can be quantitatively measured in the mixture. Thus, identifications of particular atoms may be made in this manner which could not be made chemically.

Briefly, the procedure used in this study was to prepare from radioactive sulfur a small amount of iron pyrites, mix it thoroughly with the coal charge to one coke oven, and coke the mixture under normal conditions. The course of the radioactive pyritic sulfur was then traced to the resulting gas and coke. Because it is often difficult to duplicate full-scale conditions experimentally, it was decided, under the sponsorship of the Republic Steel Corporation, to perform the experiment in a full-scale coke oven, rather than in a smaller experimental installation.

Two previous workers have studied this problem in an experimental-scale oven. Thiessen (*8*) found that 62% of the pyritic sulfur and 45% of the organic sulfur of coal remained in the coke, while Lowry (*5*) showed that the ratio of pyritic to organic sulfur in coke remained the same as it had been in the coal.

Two kilograms (4.4 pounds) of radioactive sulfur were obtained from the Atomic Energy Commission. At the time of delivery, this sulfur contained about 80 mc. of sulfur 35 and about 150 mc. of "carrier-free" P^{32} as a minute chemical impurity which was later eliminated in the synthesis of pyrites. Calculations indicated that in order to obtain accurate radioactive readings on the sulfur extracted from samples of coal, gas, and coke it would be necessary to add to the 12 tons of coal, the normal charge to one coke oven, at least 1050 grams (2.3 pounds) of radioactive pyrite (iron disulfide, FeS_2) prepared from part of this radioactive sulfur. This amount would be satisfactory only if the sulfur samples could be obtained for radioactivity assay, as elemental sulfur. The mass dilution of sulfur in heavy precipitates like cadmium sulfide and barium sulfate, sulfur compounds familiar to the analytical chemist, would cause the activity readings and therefore the accuracy to be reduced to a rather low value.

PREPARATION OF THE RADIOACTIVE IRON PYRITE

The first step in the experiment was the preparation of iron pyrite from the radioactive sulfur. With a few minor changes the hydrothermal synthesis as described by Allen and co-workers (*1*) was used.

Stainless steel bombs of approximately 800-ml. capacity, fitted with Teflon gaskets, were each charged with the following solution:

120 grams (0.265 pound) $Fe_2SO_4.7H_2O$
300 grams (0.661 pound) $Na_2S.9H_2O$
30 grams (0.066 pound) elemental radioactive sulfur
350 grams (0.772 pound) water

The sodium sulfide was dissolved in 300 ml. of water. Radioactive sulfur was then dissolved as completely as possible in the sodium sulfide solution, and finally the iron sulfate dissolved in the remaining water was added and the bomb was tightly sealed and heated for 7 days at 250° C. At the end of this reaction period the bomb contents were emptied and washed by decantation with water. A concentrated solution of sodium sulfide was used to wash and remove any unreacted radioactive sulfur. After the material had been washed free from sulfur, the charge was boiled for 15 minutes in 50% hydrochloric acid to remove any remaining ferrous sulfide. The purified pyrite was finally washed in water until free of hydrochloric acid and dried at 100° C. The yield obtained by this method varied from 90 to 100% based on the iron available for reaction. Twenty-seven batches were prepared to obtain sufficient pyrites for the experiment.

The over-all reaction in this synthesis is as follows:

$$FeSO_4.7H_2O + Na_2S.9H_2O + S \longrightarrow FeS_2 + Na_2SO_4 + 16H_2O$$

Actually the reaction is believed to occur in two steps; the immediate reaction of sodium sulfide with iron sulfate to form iron sulfide and sodium sulfate proceeds easily, whereas the bomb reaction of $FeS + S \rightarrow FeS_2$ proceeds much more slowly. Because pyrite forms preferentially at high pH, the excess sodium sulfide serves to give the desired alkaline medium. The sulfur in the iron pyrite does not all originate from the elemental radioactive sulfur added to the charge. Actually only about 43% of the sulfur in the iron pyrite comes from the active sulfur, the remainder being inactive sulfur. Because of the symmetry

of the iron disulfide molecule, both sulfur atoms are identical in chemical behavior.

The prepared pyrite was studied under the microscope and by the x-ray powder picture method and no impurities were detected. A slight trace of marcasite, no trace of free iron, and no amorphous iron disulfide were found. The product gave a good pyrite pattern, and it was concluded that the iron disulfide was largely in the crystalline pyrite form which is believed to be present in coal (*3*). Moisture, sulfur, and iron determinations were also made to determine the purity. For the iron and sulfur determinations, about 0.1 gram of pyrite was covered with 3 ml. of water and treated with 10 ml. of bromine-saturated nitric acid, first at room temperature, then on the steam bath until the sample was dissolved and most of the excess bromine removed. After dilution, the iron was precipitated with ammonia, and the precipitate was ignited and weighed as Fe_2O_3. The filtrate from the ammonia precipitate was acidified with hydrochloric acid and the sulfur precipitated and weighed as barium sulfate. The analysis of the synthesized radioactive pyrite was as follows:

Moisture	0.87%
Iron	45.32%
Sulfur	52.10%

The iron and sulfur are present in almost exactly combining proportions and indicate a pyrites purity of 97.5%. The radioactive phosphorus which had been present as an impurity in the original active sulfur was eliminated by the synthesis of the iron pyrite. This was checked by observing the penetrating power of the beta-rays in the initial sulfur containing the phosphorus impurity and in the prepared pyrite. Like S^{35}, P^{32} emits a beta-ray, the energy of which is 1.72 m.e.v., whereas the beta-ray from S^{35} has an energy of only 0.17 m.e.v. and is considerably less penetrating than that of the P^{32}.

No penetrating beta-radiations were detected in the prepared pyrite. P^{32} has a half-life of 14.3 days as compared with 87 days for S^{35}, so that even if the phosphorus had remained as an impurity in the pyrite, in the several months' time which elapsed only the beta-radiations of S^{35} would be expected in the final readings.

THE COKING RUN

Before coking, the radioactive iron pyrite was thoroughly mixed with the normal charge to one oven in order to reduce the amount of sampling and subsequent analytical work necessary. To facilitate handling, the radioactive iron pyrite was first mixed with 300 pounds of Republic's coking coal. Final mixing with the 12-ton coal charge was accomplished in a cement mixer, from which large coal samples were taken to be used for subsequent analytical work.

The coal which had been sampled and weighed was then charged to a coke oven and coked under normal procedure. The gas line from this oven had previously been adapted for metering and sampling. Because sulfur was of primary interest, gas samples were taken from time to time during the coking operation by passing a known volume of the gas from this oven through an absorption train containing ammoniacal cadmium chloride to adsorb the hydrogen sulfide from the gas. Of the sulfur present in the coke oven gas, 90 to 95% is in the form of hydrogen sulfide (*4*), so that nearly all the gas sulfur was recovered as cadmium sulfide.

Following carbonization the coke was pushed, quenched, and weighed according to the normal practice. A large coke sample was carefully taken, mixed, crushed, and quartered to yield a large average sample which was used for coke analysis.

Table I. Approximate Analysis of Coal and Coke

	Coal Mixture, %			Coke, %		
	Republic	A.D.L.	Bureau of Mines	Republic	A.D.L.	Bureau of Mines
Moisture	3.95	..	..	17.65	..	..
Dry basis						
Volatile matter	28.75	..	..	2.89	..	..
Ash	8.73	..	..	11.71	..	..
Total sulfur	1.09	1.07	1.06	0.90	0.91	0.91

ANALYTICAL WORK

In order to obtain a sulfur and material balance on the run, the coal and coke samples were analyzed at Republic's laboratory for moisture, volatile matter, ash, and sulfur by standard methods generally employed in the steel industry. Because the main interest here is sulfur, the total sulfur determinations were repeated at Arthur D. Little, Inc., and later by the Bureau of Mines. The results of these analyses are shown in Table I.

The coal and coke samples were also analyzed by the Bureau of Mines to determine the forms of sulfur present. Sulfate, and pyritic and organic sulfur in the coal were determined by the method described by Powell and Parr (*6*), the approved method of the Bureau of Mines.

The coke was analyzed for calcium and iron sulfide by a method described by the Chemists' Committee of the U. S. Steel Corporation (*10*), and the sulfur in combination with carbonaceous matter in the coke was obtained by difference. The results of these determinations expressed as per cent sulfur in the dry coal and coke are shown in Table II.

Table II. Analysis of Coal and Coke Sulfur by Types

Coal	%	Coke	%
Sulfate sulfur	0.06	Calcium sulfide sulfur	0.04
Pyritic sulfur	0.45	Iron sulfide sulfur	0.08
Organic sulfur	0.55	Carbonaceous sulfur	0.79
Total	1.06		0.91

The gas samples were also analyzed for total sulfur.

The crude cadmium sulfide obtained in the samples was filtered and placed in an evolution flask, and hydrogen sulfide was evolved with hydrochloric acid into fresh ammoniacal cadmium chloride to obtain a fresh cadmium sulfide precipitate free of tar. In order not to destroy the samples for subsequent counting, the sulfur in each gas sample was obtained by filtering, drying, and weighing the purified cadmium sulfide precipitate.

To obtain all the sulfur in the samples, the primary filtrates from the crude gas samples and the residual acid-soluble sulfur in the evolution flask were oxidized with bromine and the sulfur was precipitated as barium sulfate. The insoluble residues from the evolution flasks were oxidized with bromine and nitric acid, evaporated nearly to dryness, fused with a mixture of sodium carbonate and sodium nitrate, and leached with hot water, and the acidified filtrate was treated with barium chloride to obtain sulfur as barium sulfate. These precipitates were weighed and calculated to sulfur, and this value was added to the sulfur from the cadmium sulfide precipitate to obtain the total sulfur content of each gas sample. This addition was a small one and raised the sulfur values by an average of 5.7%.

PREPARATION OF SAMPLES FOR ACTIVITY MEASUREMENTS

The initial radioactive sulfur obtained from the Atomic Energy Commission had a relatively low specific activity of 40 mc. per gram. Because of the large dilution of this radioactive sulfur with inactive sulfur in the preparation of the pyrites and with the sulfur of the coal, final activity was reduced still further. The time involved in the preparation of the pyrite and in running the experiment also reduced the activity of the final samples. In order to obtain high radioactivity readings, it was desirable to obtain sulfur in the most concentrated form; therefore, a technique was devised to reduce the barium sulfate and cadmium sulfide samples to elemental sulfur. This method involved precipitating the sulfur in iodine solution according to the following reaction:

$$H_2S + I_2 \longrightarrow 2HI + S$$

The cadmium sulfide precipitate was reduced to elemental sulfur by the following procedure:

Apparatus. Johnson sulfur flask, Pyrex Brand, of 275-ml. capacity; 100-ml. separatory funnel, 16 × 150 mm. test tubes, and a cylinder of nitrogen equipped with reducing valves.

Reagents. Concentrated hydrochloric acid.

Hydriodic Acid–Iodine Solution. To 13 grams of iodine in a 250-ml. glass-stoppered Erlenmeyer flask are added 20 ml. of aqueous hydriodic acid, specific gravity 1.7. When iodine is all

dissolved 80 ml. of distilled water are added and mixed thoroughly. This solution must be well stoppered and kept away from sunlight.

Hydriodic Acid–Water Solution. Twenty milliliters of aqueous hydriodic acid (specific gravity 1.7) are added to 80 ml. of distilled water.

Ammoniacal Cadmium Chloride Solution. Twenty grams of cadmium chloride are dissolved in 400 ml. of distilled water, 600 ml. of ammonium hydroxide are added, and the solution is mixed thoroughly.

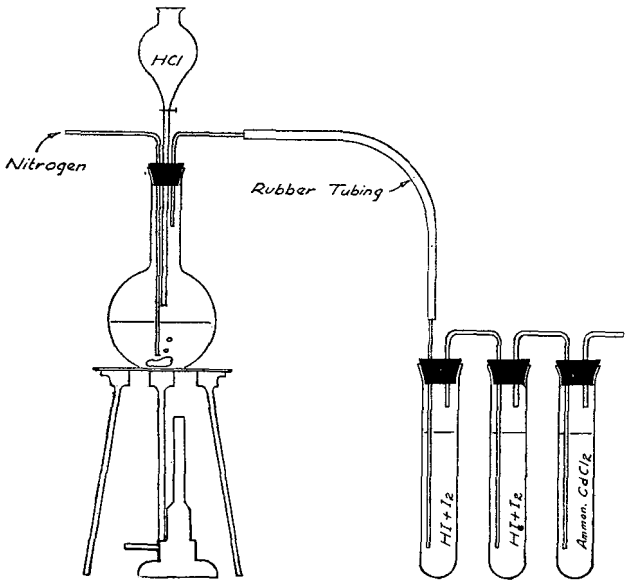

Figure 1. Apparatus for Reducing Cadmium Sulfide to Elemental Sulfur

Procedure. The purified, dried cadmium sulfide precipitate from each gas sample was placed together with the filter paper in the Johnson flask equipped as shown in Figure 1 with a separatory funnel, inlet, and outlet tubes. The inlet tube, which was connected to a nitrogen cylinder, extended to within 0.3 cm. (0.125 inch) of the bottom of the flask. The outlet tube was connected to an absorption train consisting of three test tubes, the first two containing iodine solution and the third ammoniacal cadmium chloride to catch any unabsorbed hydrogen sulfide. This third tube served as a visible check on any sulfur passing the first two absorption tubes.

Knowing the weight of cadmium sulfide added to the flask, a sufficient volume of hydriodic acid–iodine solution was added to each absorption tube to equal about a 10% excess of the iodine necessary to precipitate all of the sulfur evolved from the cadmium sulfide. The volume in each test tube was made up to 20 ml. by adding hydriodic acid water solution. Twenty milliliters of ammoniacal cadmium sulfide solution were added to the third absorption tube. A slow stream of nitrogen was first passed through the apparatus for about 10 minutes to sweep out the air. With the nitrogen flow reduced to very gentle bubbling through the absorption tubes, 50 ml. of concentrated hydrochloric were slowly added to the flask a few milliliters at a time until the entire 50 ml. of hydrochloric acid had been added. Low heat was then applied to the flask until little or no hydrogen sulfide was being evolved. Shaking the flask from time to time was helpful in completing the evolution of hydrogen sulfide. Maintaining a slow flow of nitrogen, the flask was allowed to cool to room temperature.

The contents of the first two tubes were then combined in a 250-ml. beaker containing 100 ml. of hot distilled water and the tubes were rinsed clean with a wash bottle. As soon as the sulfur precipitate had settled completely, the supernatant liquor was decanted, and the sulfur was washed several times with hot distilled water by decantation. Following this, 25 ml. of hydriodic acid water solution were added to the sulfur precipitate, and the beaker was covered with a watch glass placed on a steam bath. This operation removed all but traces of the iodine held by the sulfur. After standing overnight, the sulfur was filtered on a weighed fritted-glass Gooch crucible, washed with distilled water, dried for 1 hour at 95° to 97° C., cooled in a desiccator, and weighed. The sulfur was then ground in an agate mortar and transferred to a stainless steel cup for radioactivity assay

The coal and coke sulfur samples were prepared for activity measurements as follows:

Sulfur was extracted from 5-gram samples of coal and coke by the Eschka fusion method. A total of 40 grams of coal or coke was run in 5-gram lots to obtain enough sulfur for the activity measurements. The fusions were run in small lots in order to prevent loss of sulfur. The barium sulfate obtained by the Eschka method (Scott 906) was then reduced to the sulfide by fusing with a mixture of aluminum, carbon, and sodium bicarbonate (*7*). The total fused mass was pulverized and transferred to an evolution flask containing 2 grams of zinc (20-mesh), the flask was connected to an absorption train, nitrogen was passed through to displace air, 50 ml. of concentrated hydrochloric acid were added through the separatory funnel, and the hydrogen sulfide that evolved was precipitated as cadmium sulfide. Following this, hydrogen sulfide was evolved from the cadmium sulfide into iodine solution to obtain elemental sulfur as described above. It was not possible to evolve the hydrogen sulfide directly into the iodine from the reduced mixture, because the hydrogen which is also evolved tends to react with the free iodine.

Sulfur occurs in coke as calcium and iron sulfide and in combination with carbon. In order to determine whether an exchange of sulfur takes place during coking, large samples of coke were taken and the water-soluble calcium sulfide was extracted, oxidized, and precipitated as barium sulfate, and the barium sulfate was reduced to elemental sulfur, as described above. Using another large sample of coke, the acid-soluble sulfur, calcium, and iron sulfide were evolved into cadmium chloride solution, and the cadmium sulfide was treated as previously described to obtain elemental sulfur for activity measurements.

RADIOACTIVE STANDARDS

In order to compare the observed activity in the coal sulfur samples with the activity as calculated from the dilution of the known amount of radioactive sulfur added to the coal, a calibration curve was made.

A series of sulfur standards was prepared, containing from 0.1 to 1.0% of radioactive sulfur obtained from the same radioactive pyrites used in the coking experiment. To extract the radioactive sulfur, the active iron pyrite was converted to the sulfide with tin according to the method described by Treadwell and Hall (*9*). This sulfide was then evolved with acid into ammoniacal cadmium chloride, and the pure sulfur was prepared by evolving into the iodine solution as described above. Standards were made up by diluting this active sulfur with chemically pure flowers of sulfur. The diluted standards were melted to ensure intimate mixing of the active sulfur with the inactive sulfur, cooled and ground, and their activity was measured. Without melting to obtain molecular mixing of the standards, erratic radioactivity readings were obtained.

RADIOACTIVITY MEASUREMENTS

A portable Instrument Development Laboratories count rate meter, Model 2610, employing a Victoreen bell-type Geiger-Müller counter with a thin mica window was used for all measurements. Its readings indicate the relative specific activity of radioactive samples. Activity is measured in terms of the number of atomic disintegrations per unit time. No correction has to be made in the case of these samples for the decrease in activity with respect to time, because all samples and standards contained active sulfur from the same original batch and thus were of the same age. Therefore, the readings could simply be compared with one another, provided the readings were taken as close together as possible.

The elemental sulfur samples and the standards to be counted were placed in stainless steel cups, the inside dimensions of which were 0.00197 inch (0.5 mm.) deep by 0.707 inch in diameter. The powder surface was "doctored" off level with the cup edges to produce a uniform surface. The cups containing the samples were placed one by one in a larger brass holder, and the counter was brought down on each sample for reading. The counter cups and holder are shown in Figure 2. The sample cup holder served to keep the distance between the sample surface and the counter window always constant at about 0.125 inch.

All the activity measurements were made on "infinitely thick" samples. For every beta-radiating material there is a definite thickness of sample above which the number of radiations emitted per unit area of sample surface is constant. This is because of the internal self-absorption of the material used. Thus, regardless of how thick the sample is, as long as it is equal to or greater than this definite thickness, the same reading will be obtained by a counter held near the sample surface. "Infinite thickness" for the sulfur 35 powder used in this work is less than 0.2 mm., and because the thickness of all samples was 0.5 mm., no correction had to be applied.

In making the activity measurements, all the standards and samples were counted the same day because of the decrease in activity which takes place with time. Readings were made in a constant temperature and humidity room. The standards were counted first, and after the background reading was subtracted, a calibration curve was obtained. The activities of the coal, coke, and gas sulfur samples were measured, and the instrument was checked periodically with a standard. Readings on each sample were obtained by averaging instantaneous readings taken every 5 seconds for a total of 24 readings. The coal and coke samples were emptied from the cups, remixed, replaced, and recounted three times as a precaution, but the readings all checked very closely with the average. The background reading or reading obtained with no sample present was measured frequently and always remained the same. The activity of the total sulfur in the coal premixture before addition of any radioactive pyrites was found to be the same as the background. This value was subtracted from all readings to obtain the activity of the sample itself.

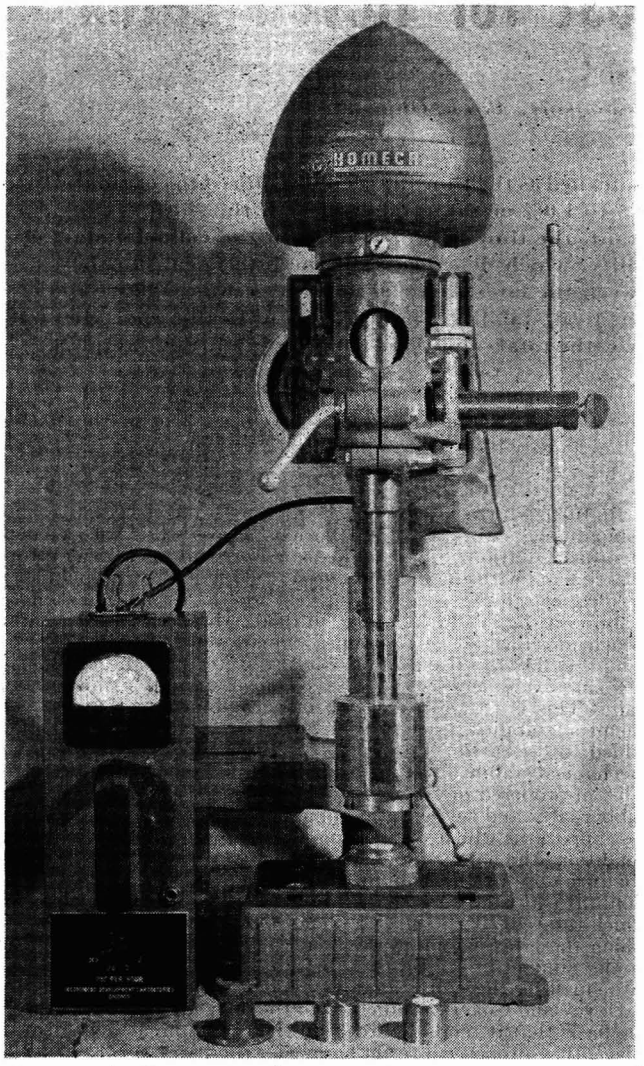

Figure 2. Counting Apparatus

Table III. Distribution of Coal Sulfur in Coke and Gas

Form	Pyritic Sulfur Pounds	Pyritic Sulfur % of coal S	Organic (and Sulfate[a]) Sulfur Pounds	Organic (and Sulfate[a]) Sulfur % of coal S	Total Sulfur Pounds	Total Sulfur % of coal S
Coal	104	100	142	100	246	100
Coke	69.0 ± 3	66	104.0 ± 5	73	173.0	70
Gas	24.2 ± 1	23	36.2 ± 2	26	60.4	25
Total sulfur (coke + gas)	93.2 ± 4		140.2 ± 6		233.4	
% accounted for		89		99		95

[a] Sulfur in coke and gas originating from sulfate sulfur could not accurately be determined without running a similar experiment in which the sulfate sulfur was tagged and traced. The sulfur of both the coke and gas originating from the sulfate and organic sulfur of the coal is obtained together by difference between total and pyritic sulfur and is presented in Table III under organic sulfur.

CALCULATION OF RESULTS

The calibration curve obtained from the standards indicated that the percentage of radioactive sulfur and therefore the percentage of pyritic sulfur in the sulfur of each sample was directly proportional to the activity readings. This is the normal expected behavior at this level of radioactivity. Therefore, the percentage of sulfur which was of pyritic origin in the sulfur samples from the gas and from the coke was calculated from the radioactivity of these gas and coke samples, together with the radioactivity of the coal sulfur samples and the known per cent pyritic sulfur in the coal sulfur as follows: Per cent pyritic sulfur in sample = (activity of sample × 42%)/0.22, where 0.22 = activity reading of coal sulfur and 42% = per cent pyritic sulfur in coal sulfur.

From the quantitative determination of the sulfur in the gas samples, the per cent total sulfur in the gas was calculated for each of the samples of gas taken during the run. An excellent sulfur balance was obtained; 95% of the sulfur charged in the coal was accounted for in the gas and coke. From the average activity measurements of the coal, coke, and gas samples the percentage of pyritic sulfur could be determined, and by difference the percentage of sulfur of organic plus sulfate origin. As the rate of gas evolution and the percentage of pyritic and organic sulfur were known, the rate of evolution of organic and pyritic sulfur in pounds per hour could be determined. Similarly, the amount of sulfur in the coke of pyritic and organic plus sulfate origin could be calculated.

It is also possible by measuring the activity of the calcium sulfide sulfur and the calcium and iron sulfide sulfur of the coke to determine the amount of sulfur from pyritic origin in all three of the forms present in the coke—namely, calcium and iron sulfide and carbonaceous sulfur. It would have been impossible to obtain much of this information by normal analytical procedures.

Table III shows how the two main forms of sulfur in the coal were distributed in the coke and gas, and the amount of each that was accounted for in pounds for the whole run. Where pyritic or organic sulfur content of the coke or gas is listed, it refers to sulfur content of the coke or gas originating from the pyritic or organic sulfur of coal.

It can be seen from Table III that about 66% of the pyritic and 70% of total sulfur remain in the coke. This is an insignificant difference and means that the sulfur is found in the coke without regard to the form originally present in the coal. This confirms the finding of Lowry *et al.* (*5*) based on a statistical study of many different types of coal carbonized in small experimental ovens.

DISCUSSION OF RESULTS

This experiment shows that under typical conditions in a coke oven, pyritic and organic sulfur remain in coke equally well and

indicates to steel men that coal must be low in total sulfur to produce low-sulfur coke by usual methods. Only one coal mixture was tested under one set of conditions and thus one cannot absolutely rule out the possibility that with another coal mixture under the same oven conditions some preferential evolution of one form of sulfur might occur. The data of Lowry *et al.* (*5*) show that this effect does not occur, however, with a wide variety of coals. The main hope for lower sulfur coke from coal entering the oven with a given sulfur content would seem to be in adjustment of coking conditions.

ACKNOWLEDGMENT

The authors are indebted to the late J. H. Slater and Earl C. Smith of the Republic Steel Corporation for their part in sponsoring the study, and to Robley D. Evans and John W. Irvine, Jr., of the Massachusetts Institute of Technology for their valuable guidance.

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Mechanical Stability Test for Hevea Latex

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The mechanical stability test is a rapid, simple method of estimating the colloidal stability or quality of Hevea latex by high speed stirring. Latex particles start to agglomerate as soon as the peripheral speed of the agitator reaches a certain minimum value. Progressive flocculation continues until mechanical coagulation occurs. The end point is defined as the time in seconds required to coagulate 0.5 to 1.0% of the total solids. If the shear is constant, the time is proportional to the colloidal stability, which depends upon the interfacial film between the latex particles and the serum. The mechanical stability time depends critically upon the size, the total solids, and the temperature of sample.

HEVEA latex is a complex, colloidal suspension that is continually changing with age, temperature, agitation due to centrifuging or transportation, preservatives, or addition of compounding ingredients. Ever since latex became an article of commerce, the need has been recognized for a quick, reliable test of the colloidal stability of a given sample.

Three general approaches have been made to this problem of measuring the stability of latex: mechanical, chemical, or heat. The first method is based on the application to the latex particles of a mechanical shear by rapid stirring, slow stirring, shaking, or rubbing. Chemical stability involves the addition of a "sensitizing" agent such as zinc oxide, ammonium sulfate, calcium sulfate, or sodium silicofluoride to the latex, and measurement of its effect by the resultant increase in viscosity or decrease in mechanical stability; while heat involves thermal agitation of the latex particles as well as possible changes in the coating on the particles.

Although many tests have been devised based on one or more of these approaches, the simplest and most efficient in time and sensitivity is the mechanical stability test. This test, as generally run using a high speed stirrer, has been more or less generally accepted as a standard test, but there is some question as to whether it gives a completely accurate index of the stability under conditions that prevail in ordinary handling operations. It has been widely used in various forms for a number of years, and no doubt a number of studies have been made on the effect of different variables, but no publication attempting to summarize such studies has appeared in the literature.

In 1930, Morris (*7*) used a Hamilton Beach drink mixer "to compare the stabilities of the various latices when subjected to vigorous agitation." Several investigators had made exploratory experiments with the method before 1930, but the first published account of a high speed stirring test for the estimation of latex stability was given by Noble (*9*) in 1936. He specified a Hamilton Beach mixer, a 118-ml. (4-ounce) square bottle, and a 50-gram sample at 30% total solids. Noble also stated that the addition of 7% zinc oxide decreases the (mechanical stability) time by one half. This is the first mention of a combined mechanical and chemical stability test where some chemical such as zinc oxide is added to "sensitize" the latex. In 1937, Jordan (*5*) included a mechanical stability test, using a Hamilton Beach drink mixer, in a list of proposed physical testing methods for the examination of rubber latex and rubber latex compounds. Murphy (*8*) devised a test apparatus which was designed to reproduce under controlled conditions the hand rubbing test in which coagulation is brought about mainly by the combined influence of friction and evaporation. It consisted of a molded rubber nose, as a rubbing element, which rotated with a sun and planet motion over a glass plate on which the latex sample was spread. The end point was defined as the number of seconds for the film to commence to break up into small particles of coagulum.

Davey and Coker (*3*) objected to the high speed stirring test on the basis that it tends to break up the coagulum as it is formed; hence, they proposed a tester having a cylindrical (5 × 2 cm., 2 × $^{13}/_{16}$ inch diameter) impeller, with three vertical fins, rotating at

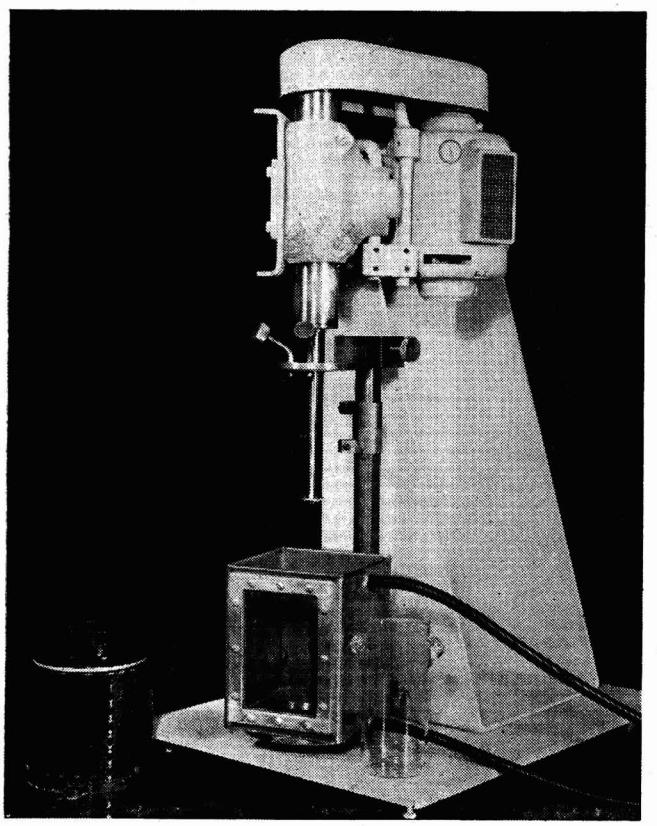

Figure 1. Mechanical Stability Tester

2000 r.p.m. As the total solids were reduced to 30%, which increases the mechanical stability greatly, it was necessary to sensitize the latex by the addition of 50% dry zinc oxide based on the dry rubber content. Martin (*6*) substituted sodium silicofluoride for zinc oxide in the test.

Novotny (*10*) further improved the mechanical stability test by employing a constant speed motor and controlling the solids very carefully. In 1940, the Crude Rubber Committee (*2*) of the Division of Rubber Chemistry, AMERICAN CHEMICAL SOCIETY, proposed a standard procedure for determining the mechanical stability of latex L-13. No further improvements have been made since that time and each user has made his own adaptation of the procedure. All this emphasizes the need for further study of the many variables and rigid standardization of the test.

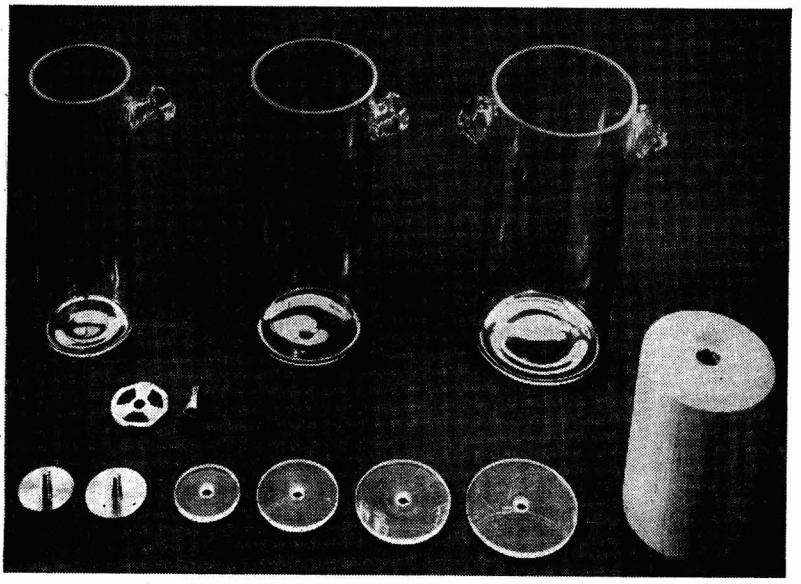

Figure 2. Propellers and Bottles Used in Mechanical Stability Determinations

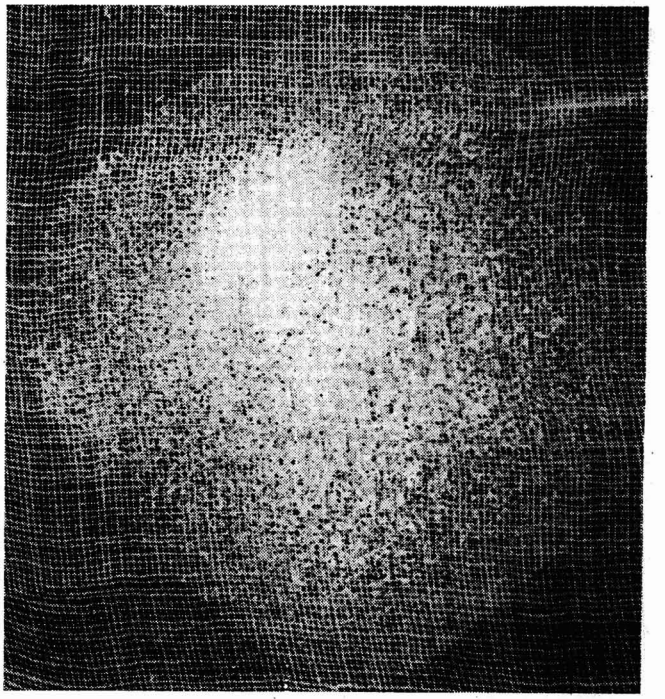

Figure 3. Appearance of Latex Coagulum on Cheesecloth at End Point

APPARATUS

The test apparatus (Figure 1) is essentially a vertical shaft, high speed stirrer capable of maintaining a constant speed of 14,000 r.p.m. for the duration of the test.

The mechanical components consist of a Dumore grinder No. 5 (The Dumore Co., Racine, Wis.) equipped with a special quill designed for vertical rather than horizontal operation. Different speeds were obtained by using a Variac in the universal motor circuit and the speed of the spindle was accurately measured with a Strobotac or a Jaeger portable tachometer. This arrangement was necessary for the study of the test at various speeds, but it is not recommended as standard equipment. For routine testing the equipment should have only one speed. Induction motor drive will deliver constant shaft speed at low load despite minor variations in line voltage.

The stirrer shaft or spindle is 0.6 cm. (0.25 inch) in diameter, although this is apparently not significant, as it rotates in a vortex of air. A Benedict drink mixer ring-type agitator (Figure 2) has been widely used for some 10 years, but its irregular shape makes it difficult to reproduce; hence, a plain polished stainless steel disk 0.844 ($^{27}/_{32}$) inch in diameter and 0.0625 ($^{1}/_{16}$) inch thick is now proposed as a standard agitator. Extensive tests indicate that this simple disk yields results comparable numerically with those obtained with the old standard.

The bottle employed in these tests is a flat-bottomed cylindrical glass container $2^{5}/_{32}$ inches in inside diameter by approximately 5 inches high (Figure 2). These were made directly from Pyrex tubing having an inside diameter of $2^{5}/_{32}$ inches (55 mm.).

The bottle holder is so constructed that the bottle may be conveniently lowered or raised until the agitator is any desired distance from the bottom of the bottle and exactly in the center.

Constant temperature of the sample is maintained by a water tank with a window as shown in Figure 1.

PROCEDURE

Dilute the latex to exactly 51.5% total solids (50% dry rubber content) with distilled water.

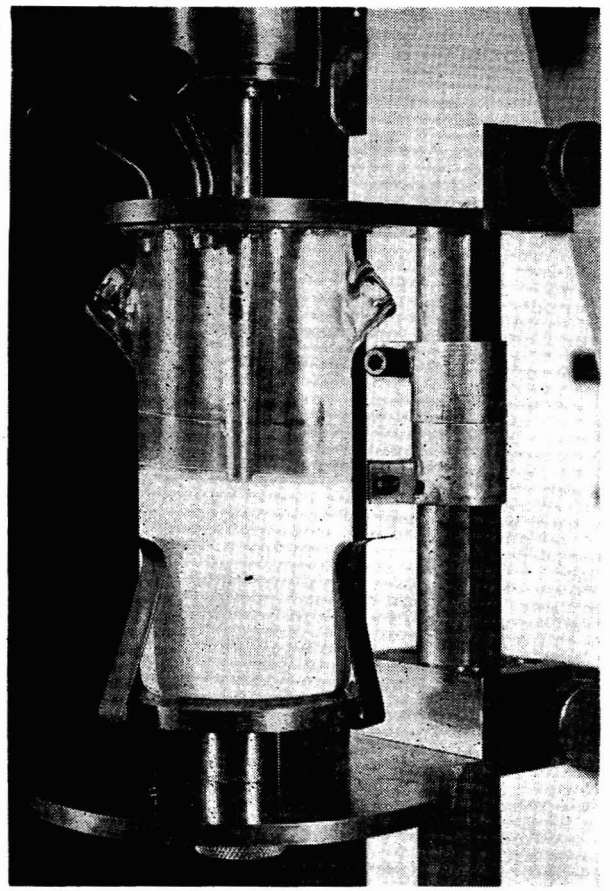

Figure 4. Meniscus Fall Due to Foam Collapse as Test Approaches End Point

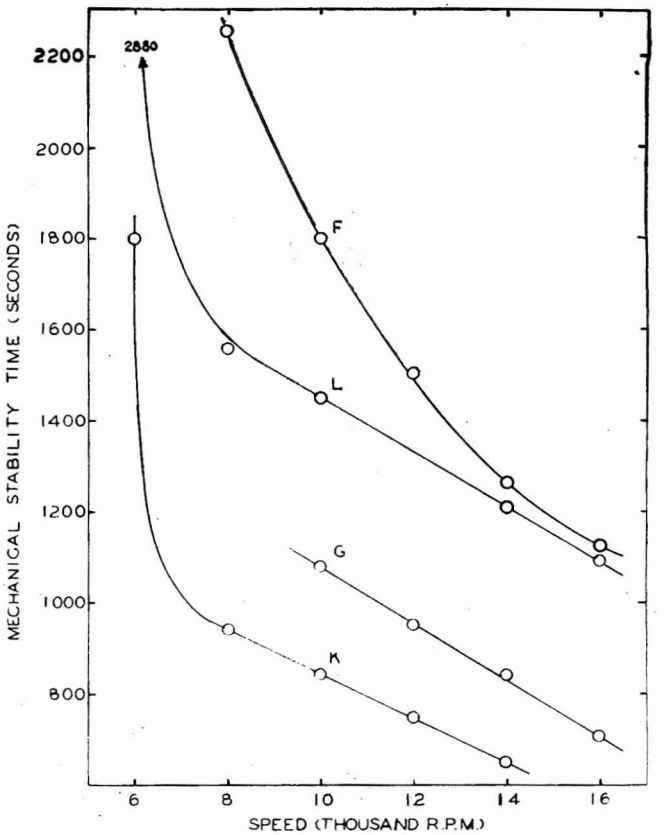

Figure 5. Effect of Speed of Stirring on Mechanical Stability Time

Place 80 ml. of the diluted latex in the test bottle, adjust the temperature of the latex to 35° C., and place in position for stirring at 14,000 r.p.m. The mechanical stability is arbitrarily defined as the time in seconds required to coagulate or agglomerate 0.5 to 1% of the total solids as measured by filtering the latex through fine cheese cloth, washing with distilled water, and observing the quantity of coagulum (Figure 3) by drying and weighing. This determination of the end point is accurate and is an essential part of the procedure, at least until the operator becomes skilled at estimating the end point by the visual method described below.

The approach to the end point can usually be noted visually by a drop of the meniscus of the latex due to foam collapse (Figure 4) and loss of turbulence. Dip a glass rod into the latex and draw it across the palm of the hand (clean and moist); the presence of small flocs of coagulum is evident at the end point, as can be further shown by filtering the latex through cheesecloth. One can also introduce a drop of latex onto a large surface of distilled water in a Petri dish or watch glass. The latex immediately spreads out into a large film which permits easy inspection for minute flocks present at the end point.

FACTORS AFFECTING THE TEST

Speed of Stirring. The greater the speed of stirring the less the time required to reach a given degree of coagulation or the end point (Figure 5). A Benedict ring-type propeller ($^{15}/_{16}$-inch diameter) was used in all these determinations. All latices used were commercial ammoniated concentrates (61 to 62% total solids) designated by letters *A* to *T* in Figures 5 to 13. Abnormally high mechanical stability time values obtained at speeds below 8000 r.p.m. can be explained by assuming that at low speeds the shear has been reduced below the minimum required to overcome the repulsive forces between the latex particles.

Diameter and Thickness of Propeller. A series of stainless steel and plastic disks varying in diameter from 0.75 to 1.47 inches (Figure 2) was used at 14,000 r.p.m. in testing several latices. Mechanical stability time is inversely proportional to the di-

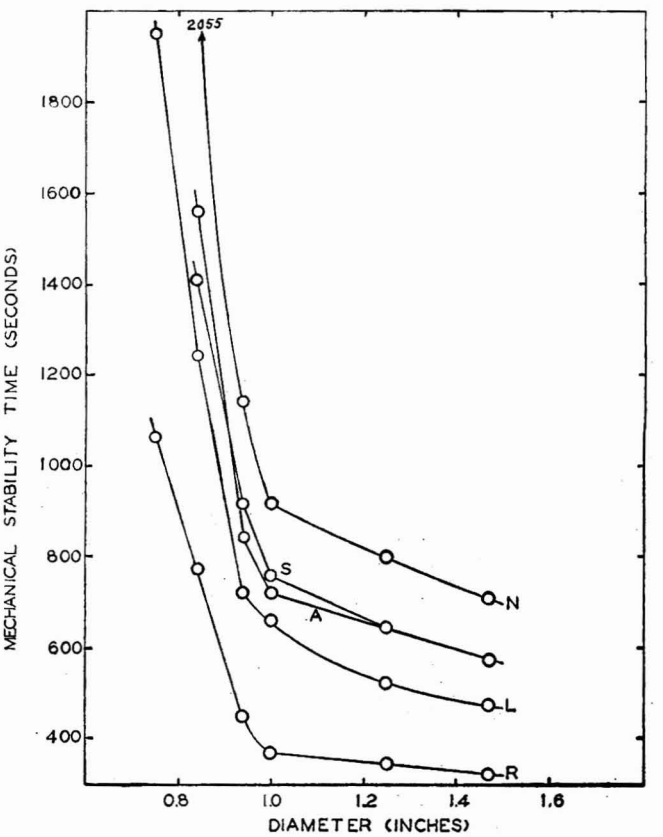

Figure 6. Relation of Propeller Size to Mechanical Stability Time

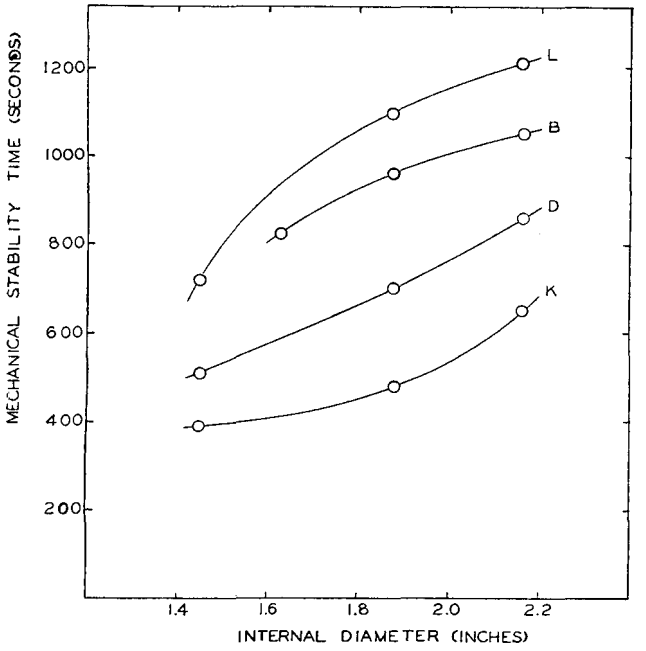

Figure 7. Effect of Test Bottle Size on Mechanical Stability Time

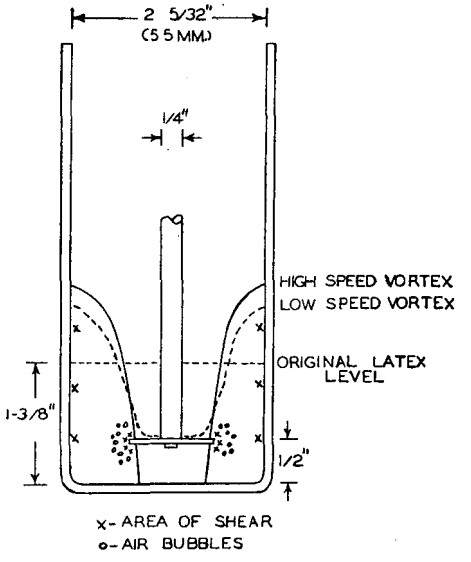

Figure 8. Cross-Section Diagram of Mechanical Stability Test

ameter of the agitator at a given shaft speed (Figure 6). However, as the diameter is decreased below 1 inch the mechanical stability time increases at a far greater rate, giving a region of maximum sensitivity but less reproducibility.

When the thickness of a given agitator was increased 500% the mechanical stability time of latex B decreased only 16.3%; hence, the thickness of the propeller appears to be far less significant than the diameter under these conditions.

Speed *vs.* Diameter of Propeller. Decreasing the shaft speed or the diameter of the propeller (at constant shaft speed) decreases the peripheral speed of the propeller. Decreasing the peripheral speed causes a proportional increase in the mechanical stability time value until a minimum peripheral speed of approximately 50 feet per second is reached. Below this peripheral speed mechanical stability time values become abnormally high. When a 1-inch propeller was run at 10,000 r.p.m., the peripheral

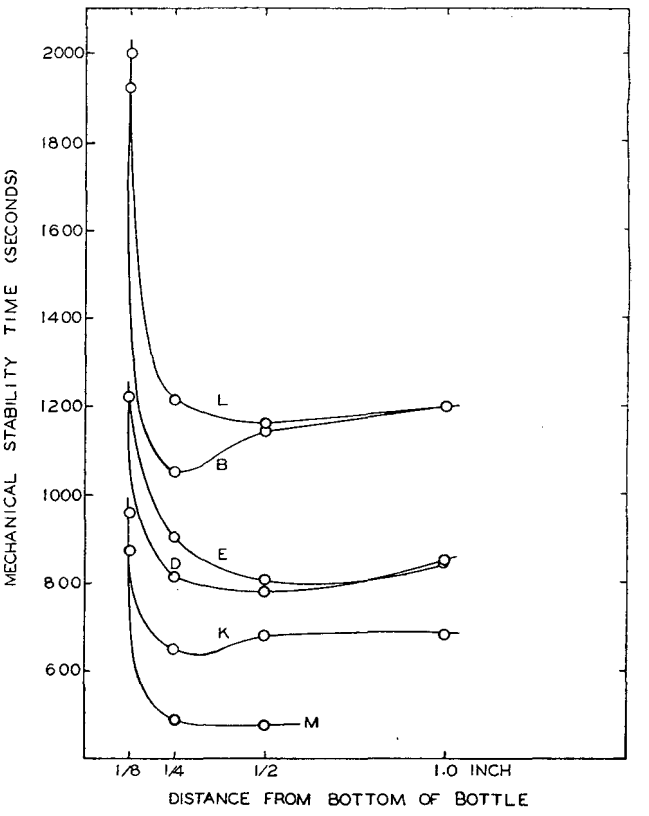

Figure 9. Relation of Mechanical Stability Time to Distance of Propeller from Bottom of Test Bottle

speed was 43.6 feet per second and the mechanical stability time for latex B was 2040 seconds; if the shaft speed was increased to 12,000 r.p.m., the peripheral speed became 52.4 feet per second and the mechanical stability time decreased from 2040 to 825 seconds. If the shaft speed was kept constant at 10,000 r.p.m. but a larger propeller of 1.25-inch diameter was used, the peripheral speed was 54.5 feet per second and the mechanical stability time decreased from 2040 to 720 seconds. Further increases in shaft speed or diameter of propeller caused regular and small decreases in mechanical stability time for this latex. It is probable that this minimum peripheral speed represents the shear required to overcome the electrostatic repulsive forces between the latex particles.

Low Speed–High Shear Stability Test. A wood cylinder 2 inches in diameter by 3 inches high (Figure 2) was mounted on the test shaft in place of the regular propeller. Speeds of 3000 to 4000 r.p.m. gave very high mechanical stability time values, although the shear was sufficient to raise the temperature of the sample from 25° to 50° C. This can be explained by assuming that a minimum peripheral speed of approximately 50 feet per second must be attained. A cylinder 2 inches in diameter would have to rotate at a speed of 6000 r.p.m. to reach this peripheral speed. When the shaft speed was increased to 5000 r.p.m. a normal sequence of meniscus fall followed by a definite end point was obtained in 300 seconds compared to 1285 seconds for the same latex when run under standard conditions. High shear decreases the mechanical stability time markedly if the peripheral speed reaches a minimum of 50 feet per second.

Diameter of Test Bottle. Decreasing the diameter of the test bottle (perfectly round and smooth internally) decreases the mechanical stability time to a small extent (Figure 7), but the rate of decrease becomes greater as the inside diameter of the test bottle approaches the diameter of the propeller. Because these changes are still small compared to the change in mechanical

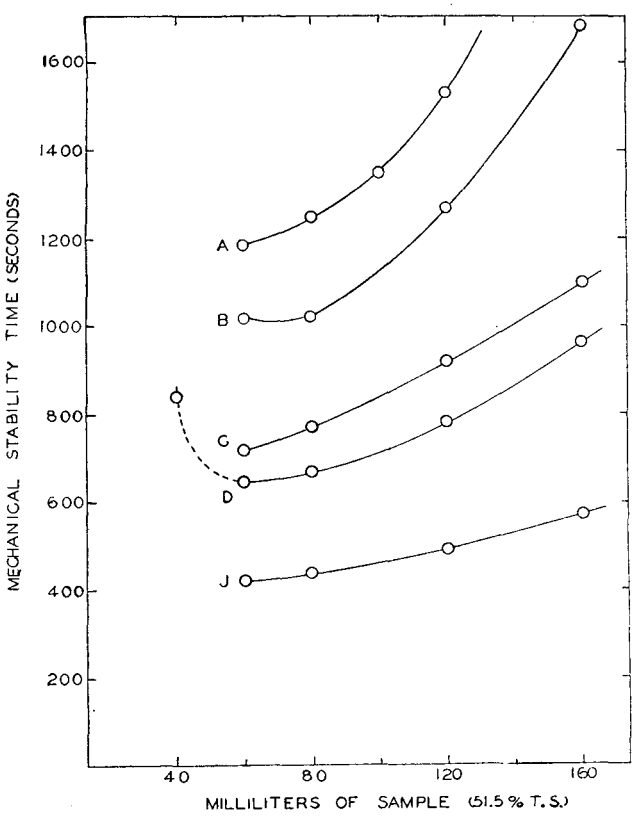

Figure 10. Effect of Latex Sample Size on Mechanical Stability Time

stability time obtained by changing the diameter of the propeller (Figure 6), it is concluded that most of the shear or rub takes place at the edge of the propeller rather than at the bottle wall (Figure 8).

Shape and Inside Surface of Test Bottle. The rub or shear between the latex and the bottle is at a minimum when the bottle is perfectly round and smooth. When the standard test bottle was lined with 0.25-inch mesh hardware cloth the mechanical stability time for a given latex decreased from 1050 to 600 seconds, indicating that the total shear had increased almost 50%. Roughening the inside surface or changing the shape of the bottle increases the shear at the wall and decreases the mechanical stability time for the latex.

Distance of Propeller from Bottom of Test Bottle. The mechanical stability time should decrease as the distance between the propeller and the bottom of the test bottle is decreased if the shear or rub takes place between the bottom of the propeller and the bottom of the test bottle. However, Figure 9 shows that there was little change in mechanical stability time when the propeller was lowered progressively from 1 inch to 0.5 inch, to 0.25 inch from the bottom. Decreasing the distance to 0.125 inch from the bottom of the test bottle gave marked increases in mechanical stability time values, which indicates that the shear had been decreased rather than increased. Consequently, the test should not be run with the propeller less than 0.25 inch from the bottom of the test bottle and the magnitude of any possible errors would be decreased if the distance was increased to 0.5 inch.

Size of Sample. With all other conditions constant, the larger the sample of a given latex the greater the mechanical stability time value (Figure 10). When the sample size was reduced to 40 ml. abnormal results were obtained, which indicated that the rate of shear as well as the sample size had been decreased.

The rate of change of mechanical stability time with an increase in sample size is greater for a high stability latex than one of low

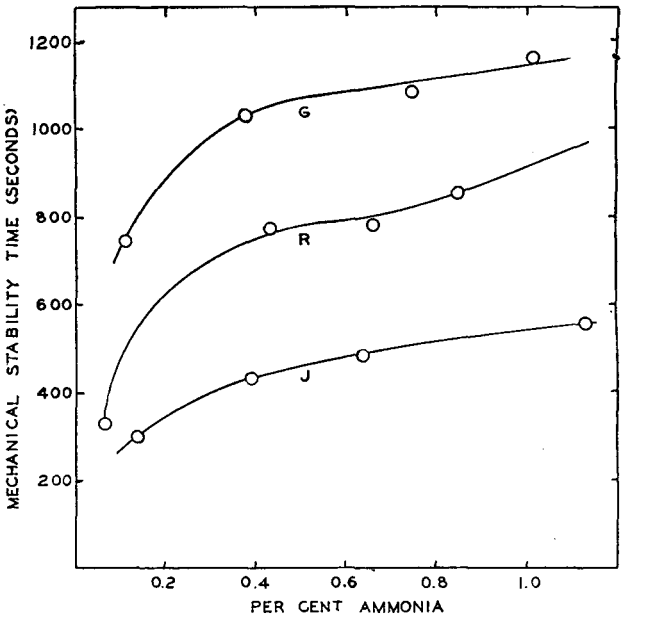

Figure 11. Ammonia Concentration *vs.* Mechanical Stability Time

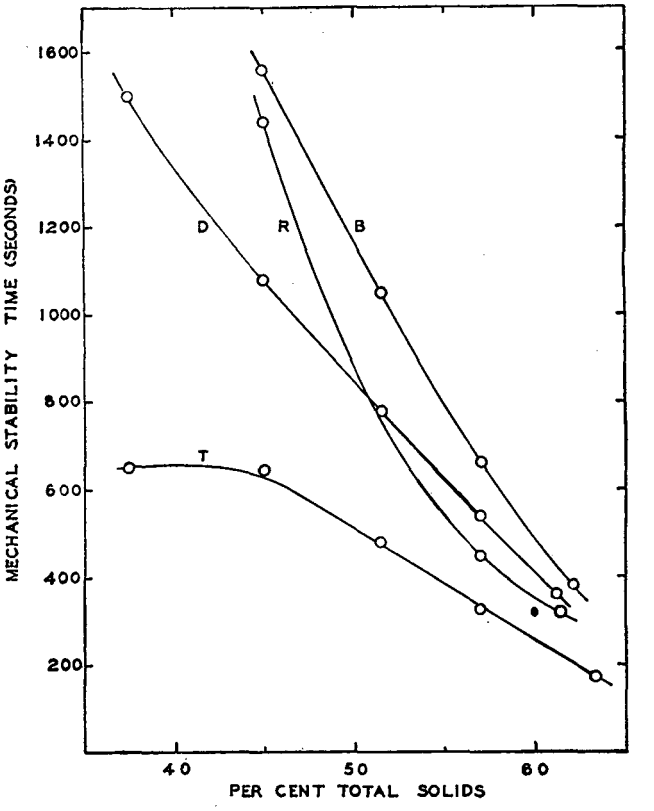

Figure 12. Effect of Dilution of Sample on Mechanical Stability Time

stability. This phenomenon has been noted for several of the other factors studied, and indicates a measure of the probability that two particles will stick together upon collision. Assuming constant shear, the probability depends upon the nature, extent, and degree of hydration of the coating or interfacial film on the individual latex particles.

Ammonia Concentration. Ammonia concentrations greater than 0.4% have little effect on the mechanical stability of latex

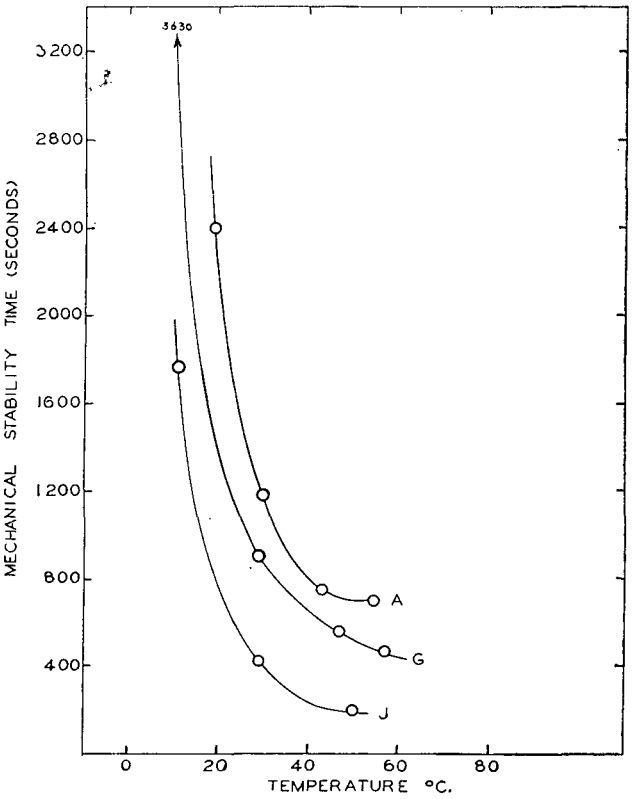

Figure 13. Mechanical Stability Time in Relation to Temperature of Latex Sample

(Figure 11), but concentrations below this figure represent a greater drop in pH and a corresponding more rapid drop in mechanical stability time was observed. Here again, the rate of change of stability time for a high stability latex is much greater than the rate for a low stability latex.

Replacement of Air by Oxygen or Nitrogen. No change in mechanical stability time values could be obtained by running the test in an atmosphere of pure oxygen or nitrogen instead of air. This indicates that the test involves only a physical change rather than a chemical reaction such as oxidation.

Total Solids of Sample. At high total solids concentrations (61 to 62%) all natural latices tend to have low mechanical stability (Figure 12). As the total solids is decreased the stability increases very rapidly, indicating that not only are the latex particles farther apart, but there is probably a solvation effect. Dilution is critical and should be done very carefully. Two latices that had a difference of 270 seconds at 51.5% total solids differed by 480 seconds at 45% total solids. The lower the total solids content of the sample the greater the sensitivity of the test; however, this advantage is offset by longer running time. The present recommended dilution of 51.5% total solids appears to be a satisfactory compromise. Extrapolation of the curves in Figure 12 to zero stability time indicates that the total solids would be 69%—the approximate limit of concentrating latex.

Temperature. The mechanical stability time of a given latex varies inversely with the temperature and the rate increases rapidly as the temperature of the latex is lowered below room temperature (Figure 13). The stability of a poor quality latex can be increased from 400 seconds at room temperature to 1800 seconds by cooling to 10° C. This increase is very significant in the storage, aging, and compounding of latex. Heating latex from 25° to 60° C. causes a decrease in mechanical stability time, but the rate of change decreases rapidly as the temperature approaches 60° C. and the mechanical stability is always low. This is really a combination of two stability tests—heat and mechanical. Therefore, temperature is very critical and close control should be maintained while the test is being run.

MECHANISM OF MECHANICAL STABILITY TEST

High speed stirring appears to agglomerate or cause the single latex particles to gather into minute clumps or flocs. Van Dalfsen (*12*) called this microflocculation. If the mechanical force applied is sufficient to overcome the repulsion of like charges on the latex particles (this corresponds to the shear at minimum peripheral speed of the agitator), then the agglomeration takes place at a rate depending upon the probability of particles' touching where there is no coating. If the mechanical shear is constant, the time required to reach a certain degree of agglomeration or coagulation depends upon the colloidal characteristics of the latex being tested. In turn the colloidal stability of natural latex depends upon the interfacial film (*1*) between the latex particles and the serum in which they are suspended. This coating is made up of lipides (*4*), soaps, and proteins which possess an electric charge (*11*) and a certain degree of hydration. If one of these factors is reduced, the stability of the latex is reduced; if one or more of these factors is increased either by natural reactions in the ammoniated latex during aging (*12*) or by the addition of proteins, soaps, lipides, or water, the stability of the latex is increased.

The test described is entirely mechanical; the shear or rub takes place at the outer edge of the agitator. This shear is directly proportional to the peripheral speed of the agitator. The process of agglomeration or microflocculation of single latex particles starts as soon as the test is started and proceeds as a result of continual mechanical agitation until the flocs become large enough to be visible without magnification. If stirring is continued a single large ball of coagulum is produced which occludes liquid latex. The "end point" is merely an arbitrary stopping point in this progression from billions of single latex particles to one big ball of coagulum. This has been arbitrarily defined as the point where 0.5 to 1.0% of the total solids has been coagulated to a size large enough to be retained by cheesecloth (Figure 3). Experience has shown that this end point is reproducible with an accuracy of 3% or better.

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Determination of Deuterium in Water

Conversion to Methyl Deuteride and Methane and Measurement by Mass Spectrometer

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A mixture of a hydrocarbon of relatively high molecular weight whose hydrogen atoms are of mass one and the same hydrocarbon containing some deuterium atoms should provide a nearly ideal solution suitable for the evaluation of distillation columns operating under reduced pressure. Such mixtures can be analyzed for deuterium by burning the hydrocarbons to water and converting the water to methane and methyl deuteride. This gaseous mixture can then be analyzed with great accuracy by means of the mass spectrometer.

PREPARATION of a binary test mixture suitable for the evaluation of laboratory distillation columns operating at reduced pressure has been under investigation in this laboratory for some time (*2*). A mixture of a hydrocarbon of relatively high molecular weight whose hydrogen atoms are of mass one and the same hydrocarbon containing some deuterium atoms should provide a nearly ideal solution. This idea was first suggested to the authors by M. S. Newman. The use of such a solution requires a method for determining the concentration of deuterated hydrocarbons in the mixture.

Preliminary experiments disclosed that, when the hydrocarbons of high molecular weight were analyzed for deuterium content directly by the mass spectrometer, the results were not sufficiently accurate. A search was then instituted for another analytical method or for a simple but more accurate mass spectrometric method.

Because a precise procedure for burning hydrocarbons to water has been described (*4*), it was thought that the deuterium content of the hydrocarbon mixture could be determined by combustion and analysis of the water for deuterium, either by direct mass spectrometric measurement or by one of the usual methods involving the measurement of physical constants. However, because adsorbed water vapor is always present on the inner glass surfaces of a mass-spectrometer vacuum system, exchange reactions complicate the direct determination of deuterium in water. Furthermore, determination of deuterium in water by the usual physical methods requires a relatively large sample and careful techniques to avoid contamination. The recent paper by Fischer, Potter, and Voskuyl (*3*) describes a method for determining deuterium in water involving equilibration of the water sample with hydrogen gas and subsequent analysis of the equilibrated gas on the spectrometer. The most dilute deuterium solution analyzed contained 26.1 mole % deuterium and the experimentally determined deuterium concentrations deviated 1 to 1.9% on an absolute basis from the actual deuterium concentration in the five analyses presented. Five milliliters of water were used for each analysis. This procedure, however, can probably be extended to small samples of low deuterium content.

The present paper describes a method for the quantitative determination of deuterium in water by the reaction of excess methyl magnesium iodide with the water and subsequent analysis of the methane–methyl deuteride mixture by the mass spectrometer. Pure methyl deuteride for mass spectrometric investigation has been prepared by Evans, Bauer, and Beach (*1*) and by Turkevich, Friedman, Solomon, and Wrightson (*6*) by using pure deuterium oxide with the Grignard reagent. The measurement of the methane generated by the reaction of methyl magnesium iodide with compounds containing active hydrogen is the basis of the Zerewitinoff determination (*7*). The equations for the reaction of methyl magnesium iodide with water may be written:

$$CH_3MgI + H_2O \longrightarrow \overline{CH}_4 + \overset{\displaystyle OH}{\underset{\displaystyle I}{\overset{|}{\underset{|}{Mg}}}} \qquad (1)$$

$$CH_3MgI + \overset{\displaystyle OH}{\underset{\displaystyle I}{\overset{|}{\underset{|}{Mg}}}} \longrightarrow \overline{CH}_4 + MgO.MgI_2 \qquad (2)$$

Theoretically, 2 moles of methane are generated for 1 mole of water, but, in conformity with published results, the authors experienced difficulty in obtaining theoretical yields of gas. This difficulty is due to the formation of a solid hygroscopic product during the reaction; the total added water does not have intimate contact with the Grignard reagent after the first hydrogen has reacted. In the present work, gas samples were taken after the initial vigorous reaction had completely subsided and no attempt was made to secure theoretical yields of methane. The authors assumed that the proportion of deuterium in any unreacted material was the same as in the reacted portion. This method of determining deuterium in water can be successfully employed with as little as 10 to 20 mg. of water, is capable of high accuracy, and can be carried out without any unusual precautions to guard against dust or other inert impurities.

EXPERIMENTAL PROCEDURE AND RESULTS

Methyl deuteride (CH_3D) was prepared using the apparatus and injection technique previously described (*5*). Immediately before use, the apparatus was warmed with a flame in a current of nitrogen and allowed to cool with a strong stream of nitrogen flowing through the apparatus. There were then injected into the methyl magnesium iodide solution 50 to 100 mg. of 99.87% deuterium oxide. After the reaction had subsided, a gas sample

Table I. Mass Spectral Patterns and Sensitivities for Methane and Methyl Deuteride

Mass	CH_4			CH_3D		
	B[a]	E	T	B	E	T
17	...	...	...	100.0	100.0	100.0
16	100.0	100.0	100.0	78.6	76.0	78.2
15	86.5	83.1	83.5	22.9	22.5	22.3
14	17.7	17.1	17.0	9.5	9.4	8.8
13	9.0	8.2	8.3	5.5	5.2	5.0
12	2.9	2.4	2.6	2.8	2.4	2.5
Sensitivity of parent mass	53.9	Equal to CH_3D to 1%	61.8	53.1	Equal to CH_4 to 1%	65.0

[a] B = Bureau of Mines; E = Evans, Bauer, and Beach; T = Turkevich, Friedman, Solomon, and Wrightson.

Table II. Analysis of Deuterium in Water

Gas Sample	% N_2 Present	% Air Present	Experimental, Mole % D (as CH_3D)	Experimental Average, Mole % D	Calculated, Mole % D (as D_2O)
1	32.7	3.3	1.37 1.37	1.32	1.42
2	1.1	0.6	1.27 1.26		
1	6.9	0.2	2.73 2.72	2.70	2.83
2	4.9	0.2	2.77 2.59		
1	20.3	26.3[a]	4.71 4.70	4.75	4.84
2	3.4	0.3	4.80 4.78		
1	22.6	26.9[a]	14.54 14.52	14.63	14.79
2	12.8	25.8[a]	14.60 14.68		

[a] Air presumably leaked into gas sampling bulb after sample had been collected and before analysis was made.

was taken. Without removing the syringe from the neoprene serum stopper, 50 to 100 mg. of water were injected and a second gas sample was taken. Additional samples of gas were taken without removing the syringe. The mass spectral patterns of nine samples of methyl deuteride, prepared in three batches from two different vials of deuterium oxide, did not vary by more than 0.2 for the 16 mass peak and not more than 0.1 for the other mass peaks.

Comparisons of patterns and sensitivities for methane and methyl deuteride with those of Evans *et al.* (*1*) and of Turkevich *et al.* (*6*) are given in Table I. All three sets of data were obtained on Consolidated mass spectrometers at electron-accelerating voltages of 55, and pattern values were corrected for C^{13} content (1.1%). The pattern values compare favorably; the fragmentation values from the present work are slightly higher, possibly because of a higher ionization chamber temperature. The sensitivity difference between methane and methyl deuteride for this work (methyl deuteride lower than methane by 1.5%) compares more favorably with that of Evans *et al.* (sensitivities equal within 1%) than with those of Turkevich *et al.* (methyl deuteride higher than methane by 5%).

A series of water standards of known isotopic composition was prepared by weight dilution methods, using normal distilled water and 99.87% deuterium oxide as the ingredients. Using the same apparatus and technique as in the preparation of methyl deuteride, two successive gas samples were prepared from each water standard. Two analyses of each sample were made, using the mass spectrometer. Table II shows that air or nitrogen or both are present in large amounts in four of the eight samples. From the good results obtained, it is apparent that no special precautions need to be taken to prevent this contamination.

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Determination of Acrylonitrile and Alpha, Beta-Unsaturated Carbonyl Compounds

Using Dodecanethiol

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Methods are described for the determination of from 2 to 200 mg. of acrylonitrile and α,β-unsaturated aldehydes and esters by the addition of excess primary mercaptan and determination of the unreacted mercaptan iodometrically or amperometrically. The salts of the corresponding acids do not react with the mercaptan under the conditions employed and other unsaturated compounds in general do not interfere.

NO CHEMICAL method for determining acrylonitrile in appreciable quantities has been described in the literature. A method for determining small amounts in air by hydrolysis to ammonia was developed by Petersen and Radke (*11*) and a physical method by infrared spectroscopy was described recently by Dinsmore and Smith (*2*).

In studying the disappearance of mercaptan (thiol) in butadiene-acrylonitrile copolymerizations, it was found that primary mercaptans disappeared rapidly in the presence of a base; the primary mercaptan had reacted with the acrylonitrile in what appeared to be a quantitative manner. At about the same time, the reaction of acrylonitrile as well as other open-chain α,β-unsaturated nitriles with certain organic mercaptans was patented by Harman (*4*). Since that time, Hurd and Gershbein (*5*) have reported that acrylonitrile and alkanethiols or thiophenols react practically quantitatively in the presence of a small amount of alkaline condensing agent.

This paper describes the conditions under which acrylonitrile and some α,β-unsaturated esters and aldehydes can be determined accurately by adding an excess of *n*-dodecyl or other pri-

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mary mercaptan and titrating the unreacted mercaptan iodometrically or amperometrically with silver nitrate.

REAGENTS

Alcoholic Mercaptan Solution. Dissolve 25 grams of crude *n*-dodecyl mercaptan (dodecanethiol) in 1 liter of ethanol (Formula 2-B) or 2-propanol. *n*-Hexyl mercaptan (hexanethiol) was also tested with satisfactory results and presumably an equivalent quantity of any primary mercaptan may be used, but the commercially available dodecyl mercaptan is a good compromise in solubility and odor. Dilutions of this solution are used for determination of less than 10 mg. of acrylonitrile or other active compound. No secondary or tertiary mercaptans were included in this study.

Basic Catalyst. Dissolve 1 gram of potassium hydroxide in 20 ml. of alcohol, or use undiluted Triton B (40% aqueous solution of trimethylbenzylammonium hydroxide).

Standard Iodine Solution. Use 0.05 to 0.1 *N* iodine solution in aqueous potassium iodide.

Standard Silver Nitrate Solution. Use 0.005 to 0.025 *N* silver nitrate for amperometric titration.

ANALYTICAL PROCEDURES

Procedure for 20 to 200 Mg. of Reactive Compound. Pipet 10 to 50 ml. of alcoholic mercaptan, depending on the optimum excess of mercaptan to be used (see Table I), into a 125-ml. iodine flask. Weigh into the flask sufficient sample to contain 20 to 200 mg. of acrylonitrile or other active unsaturated compound. If necessary, neutralize any acidity with base. Add 1 ml. of alcoholic potassium hydroxide or 0.25 ml. (5 drops) of Triton B in excess. Stopper the flask and allow it to stand the optimum time for completion of reaction (see Table I). Acidify with 1 to 2 ml. of glacial acetic acid, dilute to about 75 ml. with alcohol, and titrate with iodine solution of appropriate strength to a faint yellow end point permanent for 30 seconds of swirling.

If a reaction time of 2 minutes is used, dilute the same volume of mercaptan solution as was used for the experimental determination to about 75 ml. with alcohol and run a blank by direct titration with iodine.

If a longer reaction time is used, run a blank containing the mercaptan and the basic catalyst through the entire procedure and also run a direct blank titration of mercaptan with no base. The difference between these blank values represents the error due to air oxidation of mercaptan in the basic solution. A part of this error proportional to the amount of excess mercaptan present in the experimental run must be subtracted from the direct titration blank. Because the greater part of the active compound reacts with mercaptan within 2 minutes, this method of blank correction is satisfactory. Alternatively, oxygen may be swept from the alcoholic mercaptan with an inert gas before addition of sample or basic catalyst.

$$\% \text{ active compound} = \frac{(B - A)N \times E \times 100}{W}$$

where A = ml. of iodine required for the unknown, B = ml. of iodine required for the direct mercaptan blank (corrected if necessary), N = normality of iodine, E = milliequivalent weight of reactive compound, and W = weight of sample.

In the above method, the concentration of reactive compound should not be less than 0.5 mg. per ml. of solution during reaction.

Procedure for 2 to 30 Mg. of Reactive Compound. Pipet the proper amount of mercaptan solution, diluted if necessary, and weigh the sample into a beaker, keeping the ratios such that the concentration of reactive compound does not fall below 0.2 mg. per ml., preferably 0.5 mg. per ml., and the requirements of Table I are met. Add the basic catalyst as in the iodometric method and allow the alkaline mixture to stand the required length of time.

Acidify with glacial acetic acid, and add sufficient ammonium hydroxide to neutralize the acid and to make the solution 0.25 *M* in ammonia. Dilute to 100 ml. with alcohol, add about 1 ml. of 0.1 *M* ammonium nitrate, and titrate amperometrically with 0.005 to 0.025 *N* silver nitrate, using the general technique described by Kolthoff and Harris (*7*).

Make blank runs on the mercaptan solution under the same conditions as used in the determination, and correct the blank proportionally as in the iodometric procedure if necessary. Calculate as in the iodometric method, substituting silver nitrate volumes and normality for those of iodine.

Notes. As low as 5 mg. of acrylonitrile have been determined by the iodometric method, but with loss in accuracy.

The amperometric method should be limited to a maximum of 30 mg. because of errors involving fouling of the platinum electrode with silver mercaptide.

Acrylonitrile and acrylates are too volatile to weigh from Hill weighing bottles. Stoppered or screw-cap vials are satisfactory.

The amperometric procedure was used in this investigation primarily to avoid interferences peculiar to the iodometric titration, and for the determination of small quantities of reactive compound. The potentiometric method of Tamele and Ryland (*16*) could probably be used to replace the amperometric method of determining the excess mercaptan, although it was not used in this work.

Table I. Optimum Excess of Mercaptan and Reaction Time

Compound	Optimum Excess of Mercaptan, %	Optimum Reaction Time, Min.
Acrylonitrile, acrylates, maleates	25–100	2
Methacrylates, crotonates, aldehydes	100–150	10–15
Ketones (semiquantitative estimation)	200–400	20–30

DISCUSSION AND RESULTS

Because acrylonitrile was studied most extensively, this discussion of method, errors, and results concerns this compound unless otherwise stated.

The mercaptan method for determining acrylonitrile is accurate and precise if the following errors are kept to a minimum: the error due to air oxidation of mercaptan, the cyanoethylation error due to reaction of acrylonitrile with the alcoholic solvent, and the error due to alkaline hydrolysis of the acrylonitrile.

The oxidation error can be controlled least readily. High results will be obtained if the reaction time is prolonged without blank correction. This error will increase if too large an excess of mercaptan is used or the solution is too concentrated. However, the error from this source is negligible with a 2-minute reaction time and may be minimized for longer reaction intervals by making the proportional blank correction.

The cyanoethylation of alcohol causes a negative error, but this is of importance only if the basic catalyst is in contact with the solution before the mercaptan is added. The reaction with mercaptan is much more rapid than with alcohol, but the latter reaction is favored to some extent by excess basic catalyst and by long reaction times.

Hydrolysis of acrylonitrile in the presence of water and base can be a serious source of error if more than 25% water is present in the reaction mixture, but the error is negligible under normal operating conditions.

The optimum amount of catalyst seems to be about 50 mg. of potassium hydroxide or 250 mg. of Triton B. Somewhat smaller quantities may be advantageous in the amperometric procedure where the solution volume is low and where water may be present. Up to five times these amounts will not cause appreciable error in iodometric acrylonitrile determinations, but may cause errors in running other determinations with longer reaction times because of oxidation and hydrolysis.

The acrylonitrile-mercaptan reaction does not go to completion, or the accuracy is affected by other factors, if the concentration of acrylonitrile in the reaction mixture is less than about 0.5 mg. per ml. However, the error is under 1% for 0.2 mg. per ml., and this figure is used as the lower limit for the amperometric method, as the accuracy of this method is of the same magnitude as this error.

The acrylonitrile used for this study was freshly distilled research grade material. The initial value found by the iodometric method was 99.7% for one such sample. From the reproducibility shown in Table II it is apparent that this material was essentially of this purity and that the maximum error in accuracy of the method is of the order of −0.3%. Upon standing in a stoppered transparent bottle at room temperature, the purity decreased over a period of about one month to 98.5%. The de-

crease was consistent and outside the errors of reproducibility. No polymer formation was observed. Other samples of slightly lower initial purity behaved in a similar manner.

Alcoholic solutions of acrylonitrile are stable for at least 10 days, whereas water solutions decrease in concentration rapidly owing to hydrolysis or volatilization. This is of importance in analysis of gas streams. It has been demonstrated by the authors that methanol is a satisfactory solvent for collecting traces of acrylonitrile from gas streams, whereas water is a very poor solvent because of the high partial pressure of the acrylonitrile above the solution. For collecting larger quantities in a vapor stream, the gases were passed into basic alcoholic mercaptan solution to prevent loss of acrylonitrile due to the sweeping out effect of the gases.

Table II. Reproducibility

Iodometric method		
Sample weights, mg.		20–200
Average of mean deviations, %		±0.22
Number of results		126
Amperometric method		
Sample weights, mg.	30	10
Mean deviation, %	±0.3	±1.0
Mean error[a], %	−0.5	−0.9
Number of results	15	16

[a] Based on iodometric method for larger quantities.

OTHER APPLICATIONS OF THE METHOD

In general, α,β-unsaturated esters and aldehydes react quantitatively in this method, while the salts of the corresponding acids do not react at all under the conditions employed, an important consideration from an analytical standpoint. Ketones react only semiquantitatively.

Table III gives a summary of results on some other compounds studied. The compounds were purified to some extent and analyses by another method are given in several cases. Inasmuch as the most satisfactory method that the authors have found for determining unsaturation of this type of compound is the pyridine sulfate dibromide method of Rosenmund, Kuhnhenn *et al.* (*13*) as modified by Rowe, Furnas, and Bliss (*14*), using mercuric acetate as catalyst, results are given by this method in some cases.

Ethyl acrylate gives results comparable to those for methyl acrylate and the method has been used for control analysis of both compounds.

Cinnamaldehyde and crotonaldehyde, and presumably other α,β-unsaturated aldehydes, differ from the other compounds in their action in that the reaction product with mercaptan is unstable in dilute acetic acid, so that low results are obtained, together with a distinct odor in the case of cinnamaldehyde, if the mixture stands for more than about 2 minutes in the acid solution before iodometric titration.

Table III. Summary of Results

Compound	Purity by Pyridine Sulfate Method, %	Mercaptan Method: Excess mercaptan, %	Reaction time, min.	Purity, %	Mean deviation, %	No. of results
Methyl acrylate, freshly distilled	...	10–25	2	99.8	±0.1	4
Ethyl acrylate, vacuum distilled	...	30	2	98.4	±0.0	2
Diethyl maleate, Eastman Kodak, pure	10–16[a]	35–175	2	99.3	±0.1	4
Ethyl crotonate, vacuum distilled	95.3[a]	65–95	10–30	95.1	±0.2	5
Allyl crotonate, vacuum distilled	98.8[a]	75–250	10–25	98.7	±0.3	11
Methyl methacrylate, vacuum distilled	97.5[a]	30–120	10–30	98.8	±0.2	10
Cinnamaldehyde, vacuum distilled from $NaHCO_3$	...	130–170	2–10	99.4	±0.2	8
Crotonaldehyde, distilled from $NaHCO_3$	...	85	10–20	99.4	±0.1	4
Vinyl acetate, distilled	...	170–250	2–20	75–83	...	9
Mesityl oxide, distilled	97.9[a]	150	2–30	45–49	...	4
		400	25	94.4	...	1
o-Hydroxybenzylideneacetone, recrystallized	100.3[b]	100–400	3–10	26–81	...	8

[a] With mercuric acetate as catalyst.
[b] Without catalyst.

Several compounds listed in Table III do not give quantitative results. Vinyl acetate probably does not react in the same manner as the other compounds, because it is not an α,β-unsaturated carbonyl compound and is known to act as an acetylating agent. The reaction is probably not quantitative, although no attempt was made to prove the purity of the samples examined. The α,β-unsaturated ketones apparently do not react quantitatively, although they may approach complete reaction with a very large excess of mercaptan.

No type of unsaturation other than α,β-carbonyl unsaturation has been found to react under the conditions herein described. Among the other unsaturated and aromatic compounds studied, and found not to react, are acetylene, allyl alcohol, allyl acetate, styrene, butadiene, myrcene, vinyl chloride, 2-vinylpyridine, and benzanilide. In the presence of hydrogen cyanide the solution must be acidified with hydrochloric acid instead of acetic acid before titration.

INTERFERENCES

In addition to the interferences that would normally be encountered with iodometric or argentometric titrations, some substances react with mercaptan or catalyze air oxidation of mercaptan. Elemental sulfur appears to react with mercaptan, causing high results. Benzoyl peroxide and presumably other peroxides interfere, because they are capable of reacting both with mercaptan and with iodides. Hydroquinone, and to even a greater extent, quinone, catalyze air oxidation of mercaptan in basic solution, causing high results. Other phenolic polymerization inhibitors have not been studied.

NATURE OF REACTION BETWEEN α,β-UNSATURATED CARBONYL COMPOUNDS AND PRIMARY MERCAPTAN

The reaction between a mercaptan and an α,β-unsaturated ketone has been considered as a 1,4 addition to a conjugated series of double bonds (*8*):

$$RCH{=}CHC(=O)R'' + R'SH \rightleftharpoons RCH(SR')CH{=}CR''(OH) \rightleftharpoons RCH(SR')CH_2C(=O)R''$$

A number of compounds of the above type have been prepared (*8, 9, 12, 15*).

The reaction between a mercaptan and an α,β-unsaturated ester apparently occurs in the same manner (*5, 6, 9, 10*).

The reaction of acrylonitrile with a primary mercaptan had not been reported, as far as was known, when this study was started. The production of a β-thioalkyl (or aryl) ether of a nitrile had been forecast, however, by the work of Bruson and Riener (*1*) on the cyanoethylation of active hydrogen groups and of Gershbein and Hurd (*3*) on the reaction of hydrogen sulfide with acrylonitrile.

Based on the authors' work (see Tables I and III), the substitution of a methyl group for each hydrogen on the β-unsaturated carbon of an ester progressively slows up the reaction with mercaptan. This is also true of methyl substitution on the α-unsaturated carbon. Again, although the α,β-unsaturated esters react readily with a primary mercaptan, the salts of the acids do not react. These effects are predictable by modern electronic considerations.

CONCLUSIONS

The conditions for determining acrylonitrile accurately by adding an excess of *n*-dodecyl (or any primary) mercaptan in the presence of a basic catalyst were found. Optimum conditions are

a 2-minute reaction interval, small excess of mercaptan, use of a basic catalyst, and mixture of the mercaptan with acrylonitrile before addition of the base.

This method with modification in some cases can be employed for accurately determining α,β-unsaturated esters and aldehydes. The salts of the corresponding acids and other simple or conjugated unsaturated compounds do not react.

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Reduction of Error in Flame Photometry

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In the determination of sodium and potassium with a Perkin-Elmer Model 18 flame photometer, much of the mutual interference of these elements was eliminated by the use of a newly designed amplification circuit. Further reduction in interference, both mutual and from foreign solutes, was effected by working at lower concentration ranges than have previously been recommended. With interferences thus minimized, most biological materials can be analyzed with no more than the instrument error of about 2%, but when the test element is in low ratio to the total ash, it may be necessary to correct the determinations by the use of an empirically derived correction curve.

STUDENTS of flame photometry are agreed that this new analytical art is subject to numerous errors, some of which are understood and can be dealt with, whereas others remain obscure (*1*, *2*, *4–7*). Most of the published work has been done with the instrument described by Barnes, Richardson, Berry, and Hood (*1*) or its commercial counterpart, the Model 18 Perkin-Elmer flame photometer (*8*). This consists essentially of a Meker burner, an atomizer for spraying solutions into the flame, and a photoelectric colorimeter for measuring the characteristic light emitted.

The original work (*1*) was done with solutions of known composition. It brought out the importance of constant conditions in the flame and atomizer, and the fact that foreign substances in the solution decrease or increase the light output. The interference of foreign substances or even of too much sodium or potassium has troubled all subsequent workers, and various means have been proposed to overcome it. Thus Berry, Chappell, and Barnes (*2*) have suggested that, in suitable situations, counterbalancing quantities of the interfering substances be added to the standard solutions with which the photometer is calibrated. Obviously, this device would work only in routine analyses of a particular material. These authors also proposed, for a corrective of more general applicability, the use of lithium as an internal standard in flame photometry. They claimed that the internal standard was highly effective against errors caused by fluctuating gas pressure, and partially effective against errors caused by fluctuating air pressure and the presence of foreign molecules.

The present paper is based on nearly three years of experience with flame photometry, with emphasis on the reduction of errors. The study was made in connection with a survey of the sodium and potassium content of foods and public water supplies (*3*).

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INSTRUMENTAL IMPROVEMENTS

The work was begun with a Model 18 Perkin-Elmer flame photometer. (Production of the Model 18 instrument was discontinued in 1946 in favor of a new model which the manufacturer considers greatly improved.) In this instrument the glass atomizing chamber was held in position around the base of the burner by a coiled spring. The arrangement was such that the chamber was readily displaced, as by touching the intake tube with the beaker holding the test solution. Such displacements occasionally caused large errors in reading, and the defect was corrected by clamping the chamber in a reproducible position.

Less obvious, but more important, were certain defects in the electrical circuit. The instrument when calibrated for the most desirable range, 0 to 10 p.p.m., was operating at practically its limit of sensitivity, and therefore showed considerable drift and instability. Moreover, the amount of interference given by sodium with potassium readings and by potassium with sodium readings was both considerable and inconstant. These shortcomings were substantially reduced by employing a new electrical circuit, for which the authors are indebted to R. L. Schoene of the Schoene Electronics Laboratory, Evansville, Ind.

The original circuit is shown in Figure 1. With this circuit, amplifier drift occurring between the time of setting the zero and taking the reading appears in the dial reading as an error. To obtain higher sensitivity without undue drift a two-stage modified Wynn-Williams bridge circuit was adopted, giving an increase in electrical sensitivity of many times with only about the original amount of drift. To eliminate from the reading any error caused by this drift, a null balance push button was provided. The resulting circuit is shown in Figure 2. In use, the modified instrument is balanced by adjusting the appropriate

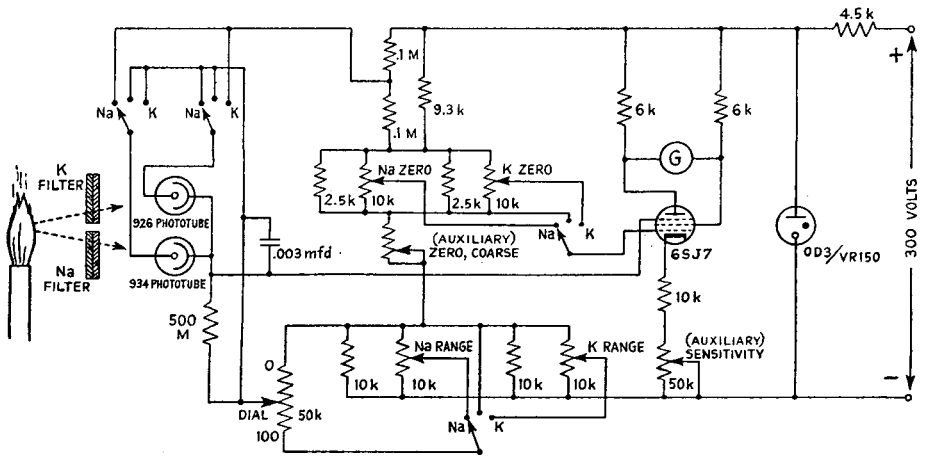

Figure 1. Original Circuit

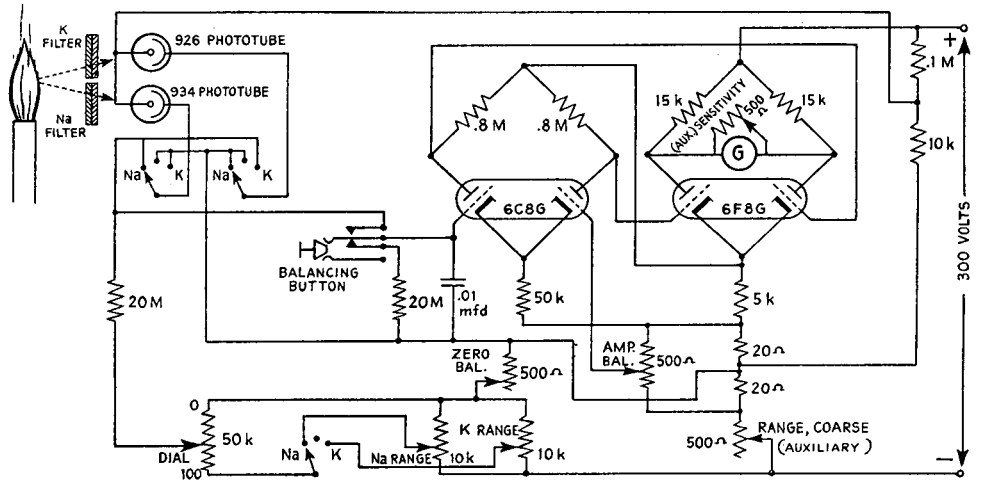

Figure 2. Improved Circuit

control, so that pressing or releasing the button causes no change in the galvanometer deflection. When so adjusted, the dial reading will be proportional only to the amount of light striking the phototube, even though the galvanometer deflection has drifted since the zero balance was made.

The original circuit used a phototube load resistor of 500 megohms, which made it necessary to do the switching from one phototube to the other (for sodium or potassium) in the anode circuit. The phototube not in use still had its cathode connected to the amplifier, giving one possible source of the interference of sodium with potassium and vice versa. With the modified circuit, the large increase in sensitivity allowed a reduction of this resistance to only 20 megohms, which is low enough to permit switching in the cathode circuit, thus completely disconnecting from the amplifier the phototube not in use.

The new switching arrangement substantially reduced the amount of interference. Formerly the interfering action of sodium on potassium determinations was evident at all sodium-potassium ratios. Now the effect is nil at ordinary operating ratios, and only slight at extreme ratios. A typical example of the improvement is found in the effect of 4000 p.p.m. of sodium on the assay of a 4 p.p.m. potassium solution, wherein the error was reduced from 122 to 8%. Again, with 1000 p.p.m. of sodium and 4 p.p.m. of potassium, the error was reduced from 103% to zero. A general picture of the interferences under the new switching arrangement is given in Figures 3 and 4. Thus it appears that interference in flame photometry is not wholly an inherent shortcoming, but is to a considerable extent a function of instrument design.

STANDARDIZATION

For primary standards of sodium and potassium, the spectroscopically pure chlorides made by Johnson, Matthey and Co. were employed (distributed in the United States by Jarrell-Ash Co., Boston, Mass.). The solutions were kept in bottles of No-Sol-Vit glass (T. C. Wheaton Co., Millville, N. J.). Perkin-Elmer standard solutions were used in routine work, after they had been checked against the primary standards.

The fuel used was propane. A pressure regulator at the propane cylinder delivered gas to the line at 2 pounds per square inch (140 grams per square centimeter). A needle valve fitted with a gear drive enabled the operator to obtain the exact working pressure desired. A dial gage reading 0 to 30 ounces per square inch was located at the needle valve. For all sodium determinations the propane was delivered at 20 ounces per square inch, and for potassium at 25 ounces per square inch. It is important that the final pressure reduction be obtained with a needle valve, rather than a regulator of the diaphragm type which is subject to flutter and lag.

The compressed air required for aspirating and atomizing was cleaned by passage through a Selas ceramic filter (Selas Corp. of America, Philadelphia, Pa.). The filtered air flowed to a pressure regulator set for 20 pounds per square inch. The needle valve and dial gage in the photometer itself were used to reduce the pressure further to the 13 pounds per square inch used for all determinations.

The electric current available for operating the amplifier was subject to surges from nearby machinery. Constant voltage was secured by the use of a Raytheon voltage regulator. Even with regulation and the improved amplifier, it was found desirable to turn on the amplifier and the burner for a 15-minute warm-up before use.

The photometer was standardized for the range 0 to 10 p.p.m. of either sodium or potassium—i.e., the range rheostats were adjusted at the time of use so that 10 p.p.m. of either element produced a dial reading of 100, and pure water a reading of zero. In theory, the standardization is permanent, but in practice it should be done before and after each determination.

There are several advantages of standardizing for the 0 to 10 p.p.m. range, rather than for the higher ranges commonly used:

At the 0 to 10 p.p.m. range the relation between concentration and dial reading is linear, instead of curvilinear. Hence no calibration curve is required, and calculations are simplified.

Smaller samples are required, with less preparatory work.

Interferences from foreign solutes and the mutual interference between sodium and potassium are minimized.

Errors due to viscosity effects in the atomizer are minimized.

Pollution of the atomizer chamber with spray residues is minimized.

In water analysis, advantage can be taken of the fact that most water samples naturally fall in the 0 to 10 p.p.m. range, and can therefore be run without concentration.

With the improved amplifier it was possible to standardize for ranges lower than 0 to 10 p.p.m., even as low as 0 to 1 p.p.m., at which level 1 dial unit corresponds to 1 part of sodium or potassium in 100,000,000 parts of water. At this extreme range the problem of contamination becomes serious, and instrumental defects are also magnified. The use of extreme low range standardization therefore appears to be limited to the analysis of minute samples and to research on certain problems in the physics of flame photometry.

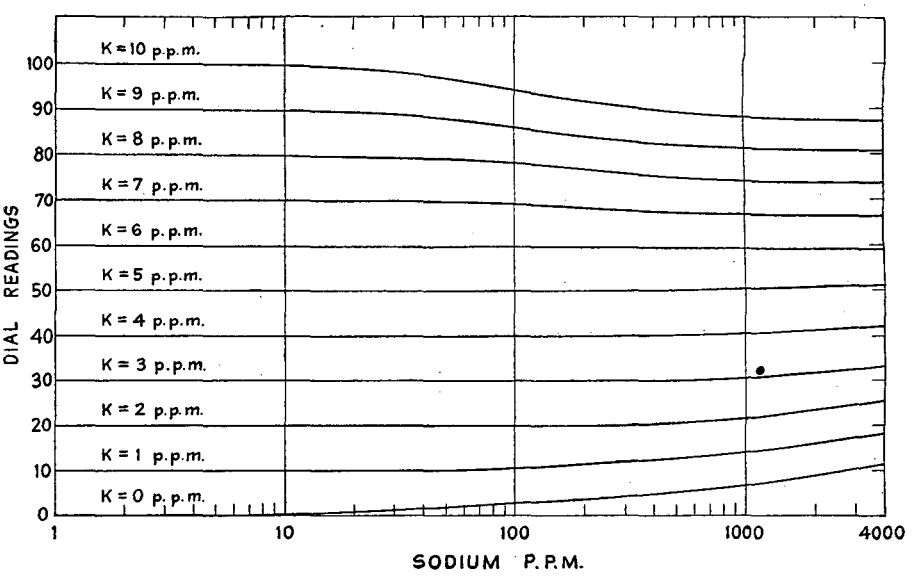

Figure 3. Interference of Sodium with Potassium Determination

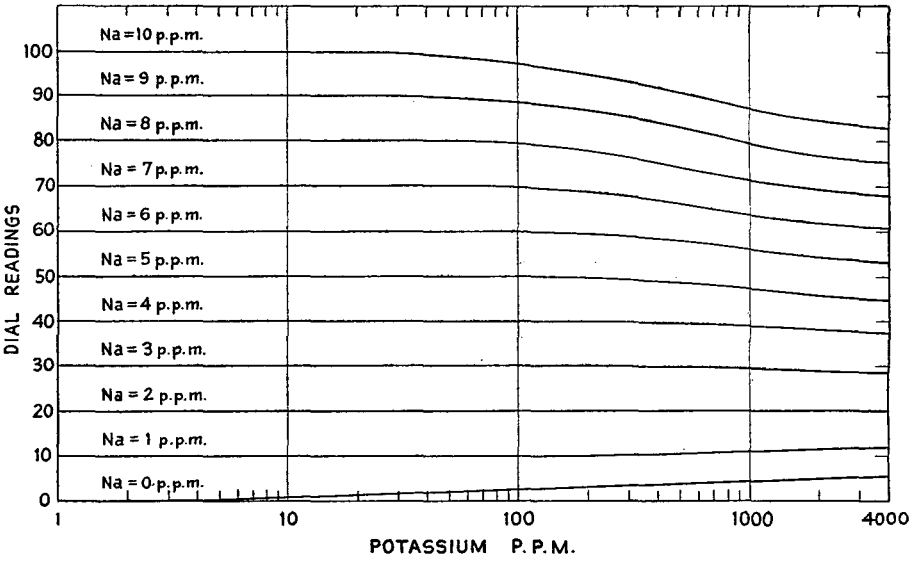

Figure 4. Interference of Potassium with Sodium Determination

PREPARATION OF SAMPLES

Biological fluids rich in sodium and potassium, such as urine, serum, sweat, saliva, whey, and fresh milk can be analyzed without ashing, provided no particulate matter is present which might clog the atomizer. It is merely necessary to dilute with distilled water, so that the sodium or potassium content falls in the range 0 to 10 p.p.m. Buttermilk, and milk which has been soured, evaporated, or dried, should be ashed because of particles present. Fruit juices, soft drinks, and alcoholic liquors usually contain so much interfering material (sugar or alcohol) in proportion to their content of sodium and potassium that ashing is required. Most water samples require no preparation whatever for potassium determination, but dilution with distilled water is sometimes required for sodium. All solid samples must of course be ashed.

The following ashing technique, based on experience with hundreds of diverse samples, is applicable to nearly all organic materials.

The sample, usually 1 to 50 grams according to the expected sodium or potassium content, is weighed in a shallow fused quartz dish. If damp, it is dried in an oven at just below 100° C. It is then placed in a cold muffle furnace, and heated to 550° until the gray ash stage is reached. The furnace is then allowed to cool, and the dish removed. The ash is moistened with distilled constant-boiling hydrochloric acid, and the excess acid is removed by warming. The sample is again heated in the furnace to 550° until the ash is white (generally 1 or 2 hours). If the ash fails to become white after several hours at 550°, it is moistened with a 25% solution of distilled nitric acid, dried, and again heated to 550°. Color still remaining is generally due to iron, and cannot be eliminated. Finally the ash is moistened with a few drops of hydrochloric acid, dissolved in water, and made up to 100 ml. No measurable loss of sodium or potassium occurs in this procedure.

AVOIDANCE OF CONTAMINATION

In practicing flame photometry one encounters contaminative sources of sodium and potassium in many and unexpected forms. For 2 days in January 1947 the atmosphere was hazy from a Texas dust storm, and during that period the photometer could not be operated satisfactorily. The handling of soap powder in a distant part of the laboratory may pollute the air for hours. Tobacco smoke contributes potassium in considerable quantity. It has been said that operation of flame photometers near the seacoast is difficult during ocean winds. The air in a poorly ventilated room may become polluted from the chimney fumes of the photometer. Barnes (*1*) has cautioned against fingerprints on glassware, and the warning should be extended to filter paper folded with the fingers. Common filter paper, even if untouched, may contribute sodium and potassium, while acid-washed papers strongly adsorb these elements from trace solutions. The top lining of the muffle furnace should be inspected for loose particles which might drop into the ashing dishes.

In handling glass-stoppered bottles and volumetric flasks one should cultivate the habit of inserting and withdrawing the stopper without giving it the customary grinding twist, the abrading action of which releases sodium and potassium to the solution. One should especially avoid the use of glass stirring rods in scraping ash from quartz dishes. New glassware may exhibit a surface concentration of soluble alkali, most of which can be removed by soaking the glass for several days in distilled water. This treatment should be applied to glassware in which solutions for flame photometry are to be stored.

INSTRUMENT ERROR

To ascertain the instrument error—i.e., the error in repetitive readings—the photometer was standardized with sodium and potassium for the ranges 0 to 100, 0 to 10, and 0 to 1 p.p.m. Calibration curves were plotted for the 0 to 100 p.p.m. ranges of sodium and potassium. The 0 to 10 and 0 to 1 p.p.m. ranges were found to be so nearly linear that perfect linearity was assumed.

Solutions containing 10, 50, and 90 p.p.m. of sodium and of potassium were assayed on the 0 to 100 p.p.m. range; solutions of 1, 5, and 9 p.p.m. were assayed on the 0 to 10 p.p.m. range; and solutions of 0.1, 0.5, and 0.9 p.p.m. were assayed on the 0 to 1 p.p.m. range. Because the new amplifier was designed for the 0 to 10 p.p.m. range, it was necessary when working at the other ranges to deviate somewhat from the established operating conditions. Thus, for the 0 to 100 p.p.m. range the sensitivity control was turned down and the gas pressure was lowered from 20 for sodium, and 25 for potassium, to 16 and 20 ounces per square inch, respectively. For the 0 to 1 range the sensitivity control was turned up and the gas pressure raised to 30 ounces for both sodium and potassium.

Each determination was made 20 times, and the instrument was standardized before and after each reading.

The mean deviations were calculated and are expressed as per cent error in Table I. It is evident that no claim can be made as to instrument error except under specified conditions. When the photometer is standardized for the 0 to 10 or 0 to 100 p.p.m. range, and readings are made on the upper half of the dial, the error is about 2%, and this can be reduced considerably by taking two or more readings and averaging them. The instrument error does not include error from interferences, which is a matter for separate consideration.

INTERFERENCES

The term "interference" covers various effects of dissolved substances on the light produced by the test substance. Even the test substance, when sufficiently concentrated, may interfere with itself, as witness the curvilinear responses in calibrating a flame photometer at the higher ranges. If the interferant colors the flame, the flame may absorb some of the light of the test substance, or, if the light filter is not perfect, the light of the interferant may register as that of the test substance. In the latter case, the effect may be especially great when, as shown above, a faulty electronic system is also involved. All interfering substances, whether or not they color the flame, tend to alter the rate of aspiration or flow into the atomizer, the nebulosity of the spray, and the rate of propagation of the flame. These effects are related to the physical properties of the interferant in ways that are not understood.

Some of the observations of others (*2*, *7*) on the interferences engendered by foreign solutes have been qualitatively confirmed. Thus it was found that methanol and ethanol enhance the light emitted by sodium, whereas urea and sucrose depress it. Numerous other substances were studied in this connection, but it will suffice here to consider only the extreme examples of *tert*-butyl alcohol, which enhances the light output, and glycerol, which depresses it. Both substances were prepared metal-free by distillation, which makes them better study materials than inorganic salts that might contain traces of sodium or potassium.

It was of special interest to ascertain the effect of dilution on interference. Stock solutions were prepared to contain 50,000 p.p.m. (5% w/v) of the interferant and 50 p.p.m. of sodium or potassium. These were assayed first with the photometer standardized for the range 0 to 100 p.p.m. Then the solutions were diluted 1 to 10 and assayed at the 0 to 10 p.p.m. range. Finally, the diluted solutions were in turn diluted 1 to 10 and assayed at the extreme low range of 0 to 1 p.p.m. The findings are recorded in Table II.

It is significant that the interference at any given concentration was approximately the same for both sodium and potassium. However, when the concentration was decreased, the interference also was decreased, even though the ratio of interferant to metal remained constant. It therefore follows that, for minimum interference from foreign solutes, flame photometers should be operated at the lowest feasible range.

During this experiment observations were made on the rate of aspiration of the solutions containing 5 p.p.m. of sodium with 5000 p.p.m. of glycerol and 5 p.p.m. of sodium with 5000 p.p.m. of *tert*-butyl alcohol, in comparison with a solution containing 5 p.p.m. of sodium in pure water. The glycerol, which depressed the light output 24%, depressed the aspiration rate only 6%. The *tert*-butyl alcohol, which increased the light output 30%, depressed the aspiration rate 18%. The nebulosity of the spray does not lend itself readily to quantitative measurement, but from observation of the atomizing chamber it was evident that the glycerol produced a coarse spray and the alcohol a fine one which probably carried more sodium to the flame. It was noted incidentally that only about 3 to 5% of the atomized solutions entered the burner; the rest collected on the walls of the atomizing chamber and went down the drain. The alcohol appeared to decrease the flame propagation rate, making the flame less "hard" and increasing its area.

The relation of standardization range to the interference of sodium and potassium with each other was determined in an experiment similar to the one with the organic materials. Stock solutions were prepared to contain 40 p.p.m. of sodium with 40,000 p.p.m. of potassium, and 40 p.p.m. of potassium with 40,000 p.p.m. of sodium. Only spectroscopically pure chlorides were used. These solutions were assayed first with the photometer standardized for the range 0 to 100 p.p.m. Then they were diluted 1 to 10 and assayed at the 0 to 10 range. Finally, the diluted solutions were again diluted 1 to 10 and assayed at the 0 to 1 range. Thus in each range the interfering (dominant) element was in a 1000 to 1 ratio to the element being assayed. Concentrations of the interfering element alone were also subjected to assay for the test element, for the purpose of demon-

Table I. Instrument Error with Pure Solutions of Sodium and Potassium at Several Concentrations and Standardization Ranges

Standardization Range, P.P.M.	Concentration, P.P.M.	Sodium Mean Error, %		Potassium Mean Error, %	
		Single readings	Duplicate readings	Single readings	Duplicate readings
0 to 1	0.1	21.3	18.7	36.5	27.0
0 to 1	0.5	5.5	3.2	6.1	3.9
0 to 1	0.9	3.4	2.6	5.2	3.4
0 to 10	1.0	6.3	5.2	15.0	13.5
0 to 10	5.0	1.6	1.0	2.8	2.1
0 to 10	9.0	1.4	0.9	2.1	1.7
0 to 100	10.0	4.1	3.1	7.6	5.4
0 to 100	50.0	2.4	1.9	2.5	1.9
0 to 100	90.0	1.8	1.4	1.3	0.9

Table II. Relation of Standardization Range to Interference by Organic Solutes

Standardization Range, P.P.M.	Sodium, P.P.M.	Potassium, P.P.M.	*tert*-Butyl Alcohol, P.P.M.	Glycerol, P.P.M.	Interference, %
0 to 100	50.0	...	50,000	...	>+100
0 to 100	...	50.0	50,000	...	>+100
0 to 100	50.0	...	...	50,000	−34
0 to 100	...	50.0	...	50,000	−37
0 to 10	5.0	...	5,000	...	+30
0 to 10	...	5.0	5,000	...	+32
0 to 10	5.0	...	...	5,000	−24
0 to 10	...	5.0	...	5,000	−22
0 to 1	0.5	...	500	...	+2[a]
0 to 1	...	0.5	500	...	±0[a]
0 to 1	0.5	...	...	500	−8[a]
0 to 1	...	0.5	...	500	−6[a]

[a] Several readings averaged to lessen instrument error characteristic of this standardization range.

strating the amount of error due to light filter leakage. The findings are recorded in Table III.

At the 0 to 100 p.p.m. range a strong negative interference is evident, amounting to over 50% for both sodium and potassium. The positive error due to filter leakage is relatively small. At the 0 to 10 range the negative interference is declining and becoming offset by the positive filter error. At the 0 to 1 range the negative interference has beome so small that the filter error dominates it. The leakage of sodium light through the potassium filter is especially noticeable. It would seem that if the filters employed to isolate the sodium and potassium radiations performed perfectly, the amount of interference would decline sharply from the highest to the lowest ranges, just as it did in the case of glycerol and *tert*-butyl alcohol. But with filters less than perfect, the practical point is brought out in Table III that interference is minimal when the photometer is standardized for the 0 to 10 p.p.m. range.

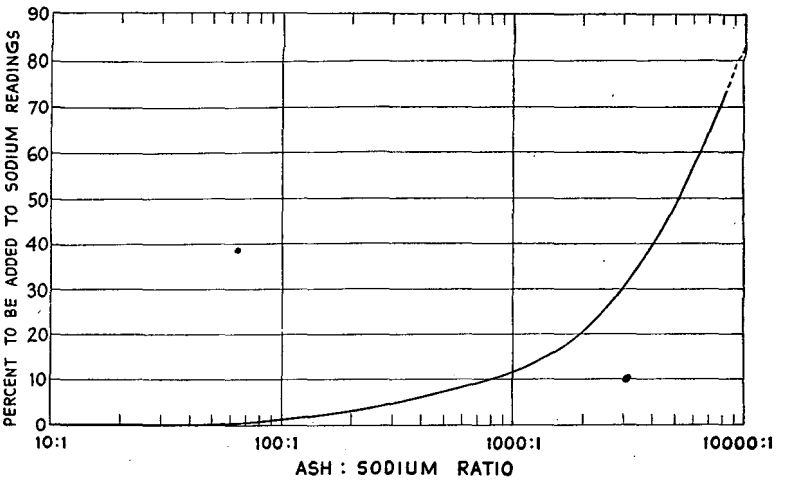

Figure 5. Correction Curve

Based on ratio of ash (including added acid) to amount of sodium found

The sodium-potassium interference study was extended to cover widely different ratios of these elements within the limits of the 0 to 10 p.p.m. range. The findings are charted in Figures 3 and 4, from which it is evident that the amount of interference depends not only on the sodium-potassium ratio, but on the absolute quantities present. For the ratios usually encountered in biological materials the interference is small, if any, although it may be considerable in the case of certain plant products containing much potassium and only a trace of sodium

Table III. Relation of Standardization Range to Mutual Interference of Sodium and Potassium

Standardization Range, P.P.M.	Interfering Element, P.P.M.	Test Element, P.P.M. Present	Test Element, P.P.M. Indicated	Interference, %
0 to 100	K 40,000	Na 40.0	Na 18.6	−54
0 to 100	K 40,000	Na 0.0	Na 0.6	
0 to 10	K 4,000	Na 4.0	Na 3.8	−5
0 to 10	K 4,000	Na 0.0	Na 0.5	
0 to 1	K 400	Na 0.4	Na 0.51[a]	+28[a]
0 to 1	K 400	Na 0.0	Na 0.13[a]	
0 to 100	Na 40,000	K 40.0	K 19.3	−52
0 to 100	Na 40,000	K 0.0	K 2.6	
0 to 10	Na 4,000	K 4.0	K 4.3	+8
0 to 10	Na 4,000	K 0.0	K 2.9	
0 to 1	Na 400	K 0.4	K 0.84[a]	+110[a]
0 to 1	Na 400	K 0.0	K 0.45[a]	

[a] Several readings averaged to lessen instrument error characteristic of this standardization range.

PRACTICAL ERROR

It remains to be shown how much error is involved in practical analyses of biological materials where the interfering substance, ash, is no definite chemical. Because the ashes of both plant and animal products are rich in potassium, the ash-potassium ratio is never large, and serious interference with potassium determinations is unlikely. The ashes of all animal products and some plant products contain sufficient sodium so that serious interference with sodium determinations is unlikely. But in certain plant products, especially fruits, grains, nuts, and legume seeds, sodium is a trace element in the presence of relatively enormous amounts of ash. This is the place to study interference at its worst.

The ash of the bean, *Phaseolus vulgaris*, variety Great Northern, is a typical low-sodium plant ash consisting mainly of potassium, calcium, magnesium, phosphate, and sulfate. To obtain a supply for study, 400 grams of dry beans were ashed as described under Preparation of Samples. The ash, 14.872 grams, was made up to 200 ml. by the addition of 25 ml. of constant-boiling hydrochloric acid and sufficient water. A small amount of insoluble matter settled and was discarded. For purposes of flame photometry, the added hydrogen chloride must be considered part of the ash, for it exhibits a negative interference of about the same magnitude as ash proper. As constant-boiling hydrochloric acid contains approximately 20% w/v of hydrogen chloride, the acid in this case contributed 5.0 grams, making a total of 19.872 grams of ash in 200 ml. Dilutions of this stock solution were used in a series of sodium recovery experiments.

A dilution of 25 ml. of stock solution with water to 50 ml. showed a sodium content of 5.225 p.p.m. A dilution of 25 ml. of stock solution and 5 ml. of a 10 p.p.m. sodium solution with water to 50 ml. showed a sodium content of 5.795 p.p.m. Because of the magnification of instrument error in the analysis of such a small recovery increment, both dilutions were read 20 times and the averages taken. The ash content of the recovery test dilution (ash proper plus added acid plus the added trace of sodium chloride) amounted to 49,683 p.p.m. The ratio of ash to sodium found was therefore 49,683 to 5.795, or 8573 to 1. The added sodium amounted to 1 p.p.m., of which 0.57 p.p.m. was recovered. In short, it was necessary to increase the reading for sodium by a correction factor of 74.4% when the ash-sodium ratio was 8573 to 1.

Following the same general procedure, eight more recovery tests were made, the dilutions and additions being calculated so that each successive solution had an ash-sodium ratio approximately one half as great as the one before it. In this way it was possible to simulate all the ratios encountered in everyday work. The findings are shown as a correction curve in Figure 5. The use of this curve is justifiable only with flame photometers operated at the 0 to 10 p.p.m. range and equipped with the electrical circuit described in this paper. As new models of flame photometers become available, similarly derived correction curves can be used as a measure of their improved performance.

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Conductometric Measurement of Ash in White Sugars

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Conductometric methods provide a simple, rapid, and accurate means of determining ash content of white sugars. The relation for white sugars derived from Hawaiian raws is: % ash (resulfated less 10%) = 0.00047 × specific conductivity (micromhos). The most desirable concentration was 25 grams of sugar in 100 ml. of solution. Only 43% of total conductivity of water should be subtracted in making the determination. Sucrose purity of white sugars may be calculated by conductivity ash measurements, moisture determinations, and known ratios of ash to total dry nonsucrose in sugar products.

THE value of conductometric measurements for determining the ash content of sugar products has been generally recognized in the sugar industry for a number of years. Numerous papers have been published on the subject, and the methods are described in varying detail in a number of handbooks (*1, 2, 5*).

Because of the speed, simplicity, and precision offered by conductometric measurements, these methods were investigated in this laboratory several years ago. This investigation led to the replacement of the time-consuming gravimetric ash procedure with conductometric methods for routine ash determinations on white sugars, raw sugar, char waste water, refinery sirups, and certain other refinery products.

The present paper describes the investigation of conductometric methods for use in determining the ash content of white sugars.

ANALYTICAL METHODS USED

Conductometric Method. The conductometric method of determining ash in sugar products is based on the principle that in a solution of the sugar, the mineral matter that constitutes the ash dissociates, whereas the sucrose, a nonelectrolyte, does not dissociate. A measurement of the conductance of the solution will therefore give a measure of the concentration of the ions present, and thus provide a direct indication of the total mineral or ash content of the product.

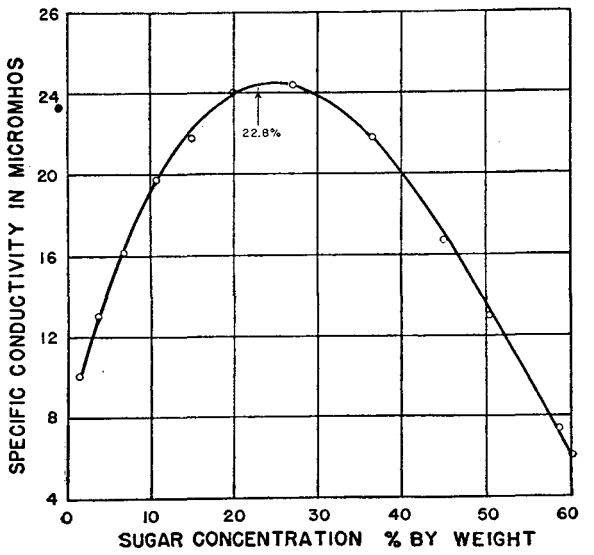

Figure 1. Typical Relation between Conductivity and Concentration of White Sugar Solutions

As conductance, measured in mhos, is the reciprocal of resistance in ohms, a Wheatstone bridge can be used to measure the conductivity of a solution. The instrument employed in recent years is the portable Leeds & Northrup sugar ash bridge (No. 4961). The conductivity cell used with this equipment is a dipping type cell with platinized gold electrodes. The instrument has a range in conductance from 1 to 120,000 micromhos—i.e., mhos × 10^{-6}—and is equipped with means for compensating for the temperature of the solution and for the cell constant of the conductivity cell. This permits obtaining the specific conductivity directly.

In making a determination, the sample of sugar is first dissolved in a low conductance water such as a distilled water of good quality. The concentration of solution selected for white sugars was 25 grams of sugar dissolved in water to give 100 ml. of solution (dry substance = 22.8% solids). This concentration was used because it provided the most accurate basis for conductivity measurements on white sugars.

Figure 1 was obtained by preparing a series of solutions of different concentrations from the same white sugar. The conductivity increases up to about 20% rds, levels off between 20 and 30% rds, and then decreases from 30% rds up to saturation. By using a concentration equivalent to 22.8% rds, approximately the maximum conductivity reading is obtained and the flat part of the curve, where analytical error has minimum effect, is utilized. Numerous tests of this kind have been made with various types of sugar products of different ash content and the same characteristic type of curve has been obtained in all cases. Nees (*4*) reported similar data and also came to the conclusion that a concentration of 25 grams in 100 ml. was optimum.

Because of the presence of small amounts of mineral matter in the dissolving water, it is necessary to apply a correction for the conductivity of the water. However, because of the depressing effect of the sucrose on conductance, only a portion of the water conductivity should be subtracted from the measured conductivity of the solution. In order to determine the amount of correction, a series of solutions was prepared, using the same sugar sample but water samples of increasing conductivity, starting with a specially prepared, triple distilled conductivity water of practically zero conductance. The results of these tests are shown in Figure 2. A straight-line relationship was obtained, and calculation of the water correction from the slope of the line indicates that only 43% of the specific conductivity of the water should be subtracted for sugar solutions of this concentration. [Similar tests have been made in this laboratory with solutions of other concentrations and the water correction factors established. Calton, Weitz, and Calendar (*3*) have used this method to determine the water factor for a concentration of 5 grams in 100 ml.]

The conductometric method used in this investigation on white sugars involved weighing out 25 grams of the white sugar, dissolving it in a distilled water of good quality to give a total of 100 ml. of solution, measuring the conductivity at 20° C. on

the sugar ash bridge, and subtracting 43% of the distilled water conductivity, to give the net specific conductivity in micromhos. Filtration was not found necessary, and no adjustment or correction was made for the alkalinity or acidity of the solution, as tests showed that pH had little effect between the ranges of about pH 5 and 8, which would cover the pH range of practically any normal sugar product.

Gravimetric Method. The resulfated ash method with a 10% deduction was used for the ignition ash determinations in this investigation. Although other methods such as carbonated ash, and sulfated ash without deduction are also widely used, the sulfated ash with 10% deduction is the method officially recognized by the International Commission for Uniform Methods of Sugar Analysis and is the one most generally used in the sugar industry. Obviously, a relationship with conductivity can be easily established for any gravimetric method desired.

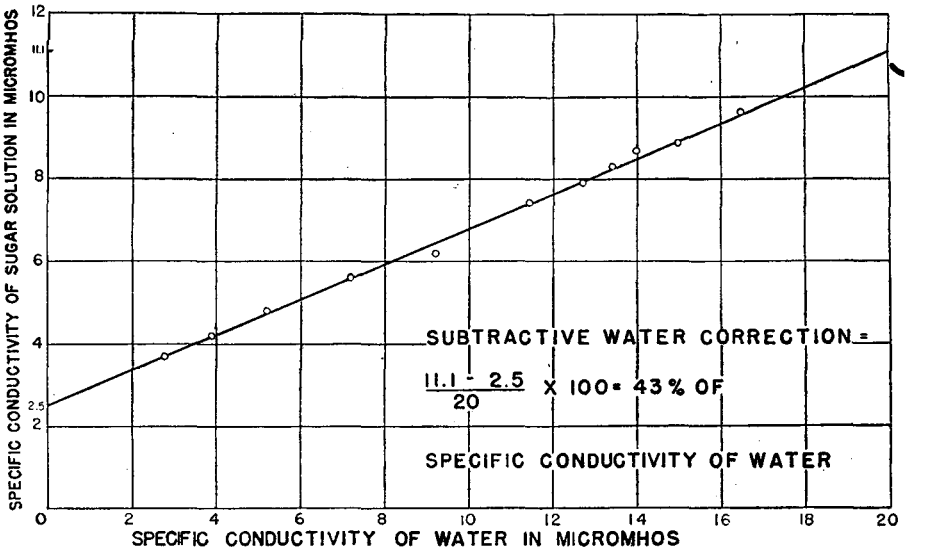

Figure 2. Effect of Conductivity of Water on Conductivity of White Sugar Solution

Concentration, 25 grams of sugar per 100 ml. of solution

The procedure for determining sulfated ash involves weighing a small amount of sample into a dish, adding a few drops of sulfuric acid, igniting the sample in a muffle at about 550° C., and cooling the sample. In this investigation, the additional step of resulfation was employed to assure complete conversion of all ash to sulfate. This requires a second addition of acid, and a second igniting and cooling before the ash can be weighed. The ash is then calculated to a percentage basis and a deduction of 10% of the total ash is applied. This gives the ash content in terms of "per cent sulfated ash less 10%."

DESCRIPTION OF INVESTIGATION

The relationship between the conductance of a solution and the gravimetric or ignition ash of the same product is usually established by a direct comparison of the two determinations. A conversion factor is then developed by taking the average of a large number of such comparative determinations. This method appears satisfactory in many cases where an appreciable amount of ash is present. However, in products such as white sugars which contain extremely small amounts of ash, the comparative method is subject to considerable error, chiefly due to the inaccuracies in the gravimetric procedure.

In the investigation described, a procedure, which is believed to be considerably more accurate, was used in establishing this relationship for white sugars. This procedure involved adding known amounts of sugar ash to white sugar solutions of extremely high purity and then comparing the incremental ash added and the corresponding incremental increase in conductivity. Experimental data are based on products derived from Hawaiian raw sugars.

The addition of ash was accomplished by preparing several stock solutions, each containing a different sugar product of relatively high ash content. Stock solutions of raw sugar, brown sugar, No. 4 liquor, and molasses were thus prepared and added in increasing quantities (5, 10, 15, 20, 30 ml., etc.) to a series of solutions of a given high purity white sugar. The amount of added ash, based on the ash content of the stock solution, could then be easily and accurately calculated. By determining the conductivity of each solution in the series, a ratio was established between the incremental amount of ash added and the incremental increase in conductivity and this formed a basis for establishing an accurate conversion factor, in accordance with the following formula:

$$\text{Factor} = \frac{\text{\% ash increment}}{\text{av. conductivity increment}}$$

Table I indicates the results of one of a series of such tests.

The stock solution added was prepared from a light brown sugar containing 2.168% ash (resulfated less 10%). This stock solution was made up to a concentration of 10 grams of sugar per liter, so that each milliliter contained 0.01 gram of sugar or 0.0002168 gram of ash.

A series of white sugar solutions was then prepared to which increasing quantities of the stock solution were added. The solutions were adjusted in each case for the amount of solids added in the form of stock solution, so that a standard concentration of 25 grams of solids in 100 ml. of solution was maintained. Table I shows the amount of stock solution added, the specific conductivities of the final solutions, and the corresponding conductivity and ash increments for each solution. The ratio of the averages of these two increments indicates a factor of 0.000468 for this particular series of samples.

In the course of this investigation, 28 series of tests were made, involving 224 different samples and determinations. White sugars of high purity were used; the confectioners' sugar had an ash content of about 0.001% and the granulated sugar about 0.01%. Details of each series of tests are not shown. However, a summary of the entire group of tests is presented in Table II. The final average factor obtained was 0.000472. Expressing this as a formula:

Table I. Typical Example of Method of Calculating Conductivity-% Ash Conversion Factor for White Sugar

(Series 2, test 16; stock ash solution = 10 grams of light brown sugar per liter; granulated sugar solution = 25 grams of sugar per 100 ml. of solution)

Stock Solution Added per 200 Ml. of Final Solution	Specific Conductivity of Final Solution	Specific Conductivity Increment per 5 Ml. of Stock Solution	Ash Increment per 5 Ml. of Stock Solution
Ml.	*Micromhos*	*Micromhos*	%
5	32.4	...	.....
10	37.2	4.8	0.002168
15	41.6	4.4	0.002168
20	46.2	4.6	0.002168
30	55.2	4.5	0.002168
40	64.8	4.8	0.002168
50	74.2	4.7	0.002168
60	83.4	4.6	0.002168
		Av. 4.63	0.002168

$$\text{Factor} = \frac{\text{\% ash increment}}{\text{av. conductivity increment}} = \frac{0.002168}{4.63} = 0.000468$$

Table II. Tabulation of Conductivity-Ash Factors for White Sugars

Grade of Sugar	Test No.	Source of Added Ash	Ash in Source (% Resulfated Ash Less 10%)	D, Ash Increment, %	E, Average Conductivity Increment, Micromhos	Factor D/E
Confectioners'	1	Raw sugar	0.4328	0.002229	4.49	0.000496
	2	Raw sugar	0.415	0.002087	4.38	0.000476
	3	4 liquor	1.08	0.002225	5.27	0.000410
	4	4 liquor	1.02	0.002101	4.58	0.000459
	5	Light brown sugar	2.03	0.002091	4.18	0.000500
	6	Light brown sugar	2.105	0.002168	4.66	0.000465
	7	Light brown sugar	1.92	0.001978	4.69	0.000421
	8	Medium brown sugar	2.52	0.002596	5.43	0.000477
	9	Medium brown sugar	2.595	0.002673	5.97	0.000448
	10	Medium brown sugar	2.645	0.002724	6.04	0.000451
	11	Dark brown sugar	3.315	0.003414	7.49	0.000456
	12	Molasses	12.775	0.002555	4.70	0.000543
	13	Molasses	12.915	0.002583	5.40	0.000478
	14	Molasses	13.035	0.002607	5.33	0.000490
Granulated	15	Raw sugar	0.4150	0.002087	4.21	0.000496
	16	Raw sugar	0.4328	0.002229	4.49	0.000496
	17	4 liquor	1.02	0.002101	4.36	0.000482
	18	4 liquor	1.08	0.002225	5.27	0.000422
	19	Light brown sugar	2.105	0.002168	4.63	0.000468
	20	Light brown sugar	1.92	0.001978	4.60	0.000429
	21	Light brown sugar	2.03	0.002091	4.18	0.000500
	22	Medium brown sugar	2.595	0.002673	5.83	0.000459
	23	Medium brown sugar	2.645	0.002724	6.01	0.000453
	24	Medium brown sugar	2.52	0.002596	5.43	0.000477
	25	Dark brown sugar	3.315	0.003414	7.69	0.000444
	26	Molasses	12.915	0.002583	5.40	0.000479
	27	Molasses	13.035	0.002607	5.31	0.000491
	28	Molasses	12.775	0.002555	4.70	0.000543
		Average factor				0.000472
		Probable error				±0.000004

$$\% \text{ resulfated ash less } 10\% = 0.000472 \times \text{specific conductivity in micromhos}$$

$$= 472 \times \text{specific conductivity in mhos}$$

In practice, the last figure is dropped, and a factor of 0.00047 is used, as the instrument reads directly in micromhos.

DISCUSSION OF RESULTS

The stock solutions were prepared from sugar products varying from about 0.5 to 13% ash. The factors shown in Table II represent the average for each series of samples. The average of all factors was 0.000472 and a calculated probable error was about ±0.000004. The results of this study indicate that the type of sugar ash has no appreciable effect on the relationship and that a value of 0.00047 can therefore be used to give a reliable indication of the per cent ash in white sugars derived from Hawaiian raw sugars.

An inspection of the literature shows a record of similar factors by other investigators. A number of years ago Nees (*4*) reported the establishment of a similar relationship for beet sugars, based on a direct comparison between sulfated ash less 10% and conductivity.

$$\% \text{ sulfated ash in beet sugars} = \frac{\text{specific conductance} \times 10^5 \text{ at } 25^\circ \text{ C.}}{231.5}$$

Converting this to the basis employed in the present investigation—i.e., 20° C. and specific conductivity in micromhos—this is equivalent to a factor of 0.00048.

Another relationship for white sugar, including unwashed and washed granulated and also remelt sugars, was established by Zerban and Sattler (*6*), both of whom have done a large amount of investigative work on conductivity measurements on a variety of sugar products. The relationship between conductivity and sulfated ash less 10% reported in this second case, when expressed on the same basis, indicates a factor equivalent to 0.00053. This slightly higher factor may be due to the different type of sugars tested, their generally higher range of ash content, and the slightly different method of conductivity determination employed.

In view of the results of this investigation, the factor of 0.00047 was officially adopted in this laboratory about 10 years ago and has been in regular use since that time. The conductometric method of determining ash in white sugars is used for ash determinations on all routine control samples of all grades of white sugar. It has also found wide use for refinery investigations of centrifugal washing, quality comparisons of white sugars, and calculation of white sugar purities.

CONDUCTIVITY-PURITY DETERMINATIONS

The calculation of purity from conductivity has been found of extreme value because of the difficulty and inaccuracies involved in making polarimetric purity determinations on such high purity products as white sugars. The method of conductometric purity determination is simple, if the moisture is known and the ratio of ash to total dry nonsucrose constituents is established.

For a given type of raw sugar, the ratio of ash to dry nonsucrose is generally about constant. This ratio furthermore is approximately the same for products, such as soft sugars, derived from this raw sugar. It is assumed that this same ratio holds for white sugars also. It has been found by determining the ratio for these products over a long period of years that under the particular conditions in this refinery, the ash content represents approximately 29% of the total dry nonsugars. If this ratio is known and the conductometric ash and moisture are determined, the conductometric purity or sucrose content can be determined as follows:

$$\% \text{ total dry nonsucrose} = \frac{\% \text{ conductometric ash}}{0.29}$$

Then

$$\% \text{ sucrose} = 100 - (\% \text{ total dry nonsucrose} + \% \text{ moisture})$$

Assume that a white sugar has a conductivity of 20 micromhos. This is converted to per cent ash by multiplying by 0.00047, giving an ash content of 0.0094%. The total dry nonsucrose is then calculated as 0.032%. If the sugar has a moisture content of 0.025%,

$$\% \text{ sucrose} = 100 - (0.032 + 0.025) \text{ or } 99.94\%$$

Comparative polarimetric purities of high purity products such as white sugars are very difficult to ascertain with any degree of accuracy. However, comparisons on products of slightly lower purity have shown good agreement. This method of calculating purity has proved of considerable value for rapidly determining the sucrose content of white sugars of various types and grades, particularly in special investigations on refinery processes. One large sugar refinery in England uses this method of purity control altogether—i.e., refining operations are controlled on a conductometric purity basis rather than the usual polarimetric purity basis.

SUMMARY

Conductometric methods provide a simple, rapid, and accurate means of determining the ash content of white sugars. The relationship for white sugars derived from Hawaiian raws is:

$$\% \text{ ash (resulfated less 10\%)} = 0.00047 \times \text{specific conductivity (in micromhos)}$$

The method used in determining this relationship is believed to be much more reliable than the usual direct comparison

method. This factor compares very favorably with those developed by other investigators.

The most desirable concentration for conductivities of white sugars was found to be 25 grams of white sugar in 100 ml. of solution. Only 43% of the total conductivity of the water should be subtracted in making such a determination.

The sucrose purity of white sugars may be calculated by means of conductivity ash measurements, moisture determinations, and known ratios of ash to total dry nonsucrose sugars in sugar products.

Conductometric ash determinations made on the basis described are much more accurate than gravimetric methods, have provided a precise index of white sugar quality, and have proved valuable for control and investigation of refining operations.

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Conductometric Determination of Ash in Raw Sugars

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This paper describes a conductometric method for determining the ash content of Hawaiian raw sugars. A uniform relationship was found to exist between the specific conductance of a solution of raw sugar and the ignition ash content of the sugar. This is a direct relationship, and avoids the special methods used by other investigators, which involve acid addition, multiple readings, etc. The relationship established for a solution of 5 grams of raw sugar per 100 ml. of solution (4.9% solids) is: % resulfated ash = 0.00160 × specific conductance (micromhos). The procedure described has been in very satisfactory use in this laboratory for over 10 years.

CONSIDERABLE investigative work has been done in this laboratory on conductometric methods of determining the ash content of sugar products. The application of these methods for determining the ash content of white sugars has been described (*2*). The present paper discusses another application of conductivity methods—the measurement of the ash content of raw sugars. The samples involved in this study were raw sugars from plantations in the Hawaiian Islands. The relationship described is therefore primarily applicable to raws from this source.

METHODS OF ANALYSIS EMPLOYED

Conductometric Method. The method used for determining the conductivities of raw sugars was similar to that employed for white sugars (*2*). The Leeds & Northrup sugar ash bridge with dipping type conductivity cell was utilized and specific conductivity readings were thus obtained directly. The solutions were made up to a concentration of 5 grams of raw sugar dissolved in a distilled water of good quality to give 100 ml. of solution (4.9% solids). This is the generally accepted concentration for conductivity measurements on raw sugar.

In accordance with the usual practice, a correction for the conductivity of the distilled water was applied. However, tests showed that the extent of correction was dependent upon the concentration of sugars present and that the correction of 43% of the specific conductance of the distilled water that was determined for a 25-gram-per-100-ml. solution did not apply to a 5-gram-per-100-ml. solution. In order to determine the extent of correction, a series of solutions was prepared from the same sample of raw sugar but with various water samples of different conductivities. The results plotted in Figure 1 indicate that 73% of the distilled water specific conductivity should be subtracted from the specific conductivity of the solution to give the specific conductivity of the raw sugar. This factor is apparently independent of the amount of ash normally present in such sugar products.

A similar correction factor was determined by Calton, Weitz, and Calendar (*1*) in their work on beet sugars. They employed a concentration of 5 grams of granulated sugar in 100 ml. of solution and determined a water correction factor of 77% of the distilled water conductivity, although they reported variations in their correction factor from 74.6 to 79.9%. Although this factor of 77% is somewhat higher than the factor determined in the present case, it is in reasonably good agreement, particularly when the difference in the two types of sugars involved is considered. Furthermore, the small difference in factors would have little effect on the conductometric ash of the raw sugar, inasmuch as the total water correction will usually amount to only about 1% of the solution conductivity.

In summary, the conductometric method involves weighing 5 grams of the raw sugar, dissolving it in distilled water to give a total of 100 ml. of solution, and measuring the specific conductivity at 20° C. on the sugar ash bridge. From this reading is subtracted 73% of the specific conductance of the dissolving water to give the net specific conductivity of the raw sugar in micromhos. The sample is not filtered, as there is small difference between the conductometric ash of a filtered and an unfiltered sample. No correction is made for pH, as tests indicate that between about pH 5 and 8 there is little change in conductivity. The pH of normal raw sugars falls well within this range.

Gravimetric Method. In determining the ash chemically, the resulfated ash method, described in standard reference books, was employed, with a 10% deduction. The determinations were made directly on the raw sugars to give the total ash rather than the soluble ash, which would be obtained if the samples had been dissolved and filtered prior to ashing. The former procedure was followed because the total ash is considered to be of primary significance.

DESCRIPTION OF INVESTIGATION

In developing the relationship between the conductivity of a raw sugar solution and its ash content, direct comparisons were made between the specific conductivity and the resulfated ash,

Table I. Typical Relationship between Resulfated Ash and Specific Conductivity of Hawaiian Raw Sugars

(Annual composite samples from various plantations for one typical year)

Plantation	Resulfated Ash, %	Specific Conductivity, Micromhos	Ash-Conductivity Factor
1	0.57	370	0.00154
2	0.47	303	0.00155
3	0.54	336	0.00161
4	0.36	238	0.00151
5	0.47	296	0.00159
6	0.35	228	0.00154
7	0.51	297	0.00172
8	0.61	380	0.00160
9	0.56	349	0.00160
10	0.47	308	0.00153
11	0.55	337	0.00163
12	0.49	310	0.00158
13	0.41	248	0.00165
14	0.54	340	0.00159
15	0.49	300	0.00163
16	0.44	285	0.00154
17	0.50	308	0.00162
18	0.51	319	0.00160
19	0.48	308	0.00156
20	0.56	368	0.00152
21	0.42	263	0.00163
22	0.51	329	0.00155
23	0.52	329	0.00158
24	0.50	290	0.00172
25	0.42	270	0.00156
26	0.45	269	0.00167
27	0.45	275	0.00163
28	0.42	280	0.00150
29	0.49	308	0.00159
		Av.	0.00159

Table II. Conversion Factors for Raw Sugar Ash

(Factor × specific conductivity of 5 grams per 100 ml. of raw sugar solution = % resulfated ash)

Year	No. of Annual Composite Samples	Average Factor
1	26	0.00160
2	26	0.00162
3	31	0.00161
4	30	0.00160
5	31	0.00161
6	32	0.00158
7	32	0.00161
8	28	0.00156
9	29	0.00160
10	30	0.00158
11	29	0.00159
12	29	0.00159
13	23	0.00160
14	26	0.00163
Total	402	Av. 0.00160

less 10%, both of which were determined by the previously described methods. This involved over 400 such comparisons on annual composite samples of raw sugars from each of the 25 to 30 plantations in the Hawaiian Islands, for a period of 14 years.

Table I shows a typical set of comparative data for one group of annual composites and is representative of the results obtained in the other comparisons made. The samples involved in this case represented raw sugar from 29 different plantations. The ash content of these raw sugars varied from 0.35 to 0.61%. (Subsequently, tests have been made with raw sugars having an ash content as high as 1%.) The ash-conductivity factor in the case of this series of samples varied from 0.00150 to 0.00172 and averaged 0.00159.

Table II shows the average factors for the other series of annual composites involved. These factors, covering 14 different years, varied from 0.00156 to 0.00163 and averaged 0.00160. This average of 0.00160 indicates the apparent relationship between the gravimetric ash and the specific conductivity of Hawaiian raw sugars and permitted establishment of the following formula for converting the specific conductivity in micromhos of a raw sugar solution (5 grams per 100 ml. of solution) to per cent resulfated ash less 10%:

$$\% \text{ ash} = 0.00160 \times \text{specific conductivity (micromhos)}$$

In order to indicate the agreement between the conductometric ash calculated with this formula and the gravimetric ash, the conductometric ash was calculated for each of the samples run. Table III shows this relationship for the same group of samples listed in Table I. The agreement is very close, the difference averaging ±0.01, with extremes of +0.02 and −0.03.

DISCUSSION OF RESULTS

The data presented indicate that the agreement between the two methods is very good and entirely satisfactory from a practical standpoint. Some of the slight deviations noted may be due to differences in the composition of the ash in the different samples and to small variations in the proportion of soluble and insoluble ash; they may also result from analytical error, particularly in the determination of gravimetric ash. In fact, a recheck of discrepancies almost invariably indicated that the gravimetric ash rather than the conductivity was in error.

It is believed that the use of a direct comparison method, as opposed to the more involved procedure used for white sugar (*2*), is justified because of the larger amount of ash present in raw sugars. This larger ash content tends to minimize percentage errors in the gravimetric ash determinations on raw sugars and thus gives a more reliable relationship between ash and conductivity.

The use of the direct comparison or C ratio method is possible in this case because raws from only a single locality—i.e., the Hawaiian Islands—were involved. The general composition of ash in these raw sugars was thus not subject to the variation encountered by investigators working with raws from various areas such as Cuba, Puerto Rico, and Louisiana.

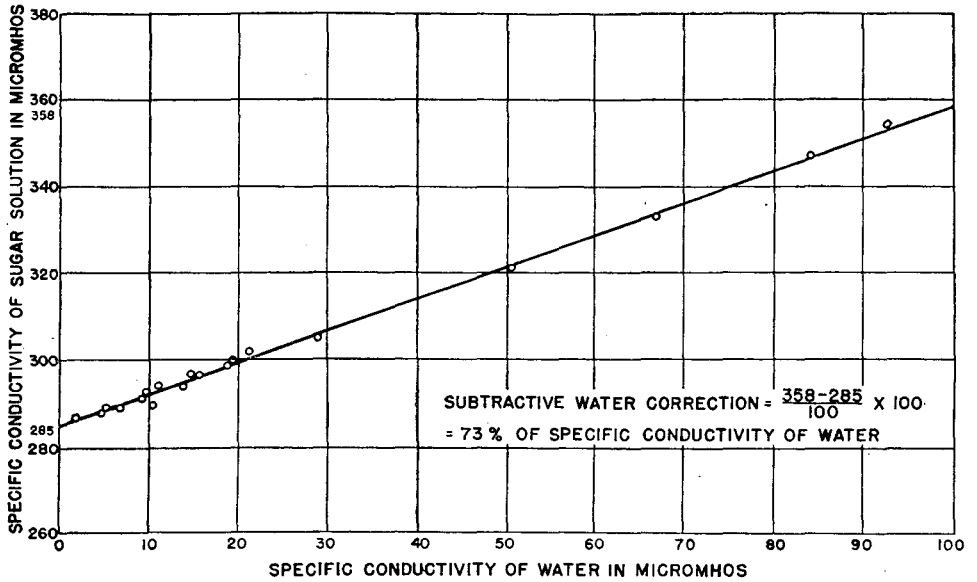

Figure 1. Effect of Conductivity of Water on Conductivity of Raw Sugar Solution

5 grams of sugar per 100 ml. of solution

Table III. Comparison of Gravimetric and Conductometric Ash in Raw Sugar

Plantation	Resulfated Ash, %	Conductometric Ash (0.00160 × Specific Conductivity), %	Difference, %
1	0.57	0.59	+0.02
2	0.47	0.48	+0.01
3	0.54	0.54	0
4	0.36	0.38	+0.02
5	0.47	0.47	0
6	0.35	0.36	+0.01
7	0.51	0.48	−0.03
8	0.61	0.61	0
9	0.56	0.56	0
10	0.47	0.49	+0.02
11	0.55	0.54	−0.01
12	0.49	0.50	+0.01
13	0.41	0.40	−0.01
14	0.54	0.54	0
15	0.49	0.48	−0.01
16	0.44	0.46	+0.02
17	0.50	0.49	−0.01
18	0.51	0.51	0
19	0.48	0.49	+0.01
20	0.56	0.59	+0.03
21	0.42	0.42	0
22	0.51	0.53	+0.02
23	0.52	0.53	+0.01
24	0.50	0.47	−0.03
25	0.42	0.43	+0.01
26	0.45	0.43	−0.02
27	0.45	0.44	−0.01
28	0.42	0.45	+0.03
29	0.49	0.49	0
		Av.	±0.01

Comparison of the results obtained with those of other investigators, primarily Zerban, Sattler, and co-workers (*3–6*), shows good agreement in the case of Hawaiian raw sugars. Zerban and Sattler (*5*) report an average C ratio of 1630 for 31 samples of Hawaiian raws. Their C ratio, which is equivalent to a factor of 0.00163, is in extremely good agreement (within 2%) with the factor of 0.00160 obtained in the present investigation.

Obviously, the development of the relationship described herein is considerably simplified because raws from only one general locality are involved. This permits the use of the direct C ratio and avoids the necessity of using the acid conductivity method (*6*) which might be required if raw sugars from various geographical areas were involved. This latter method is based on the following formula:

$$\% \text{ total ash} = 0.001566\,K - 0.0001954K_1 + 0.4160$$

It employs a rather elaborate technique due to the hydrochloric acid addition, and obviously is more time-consuming because of the necessity of carefully preparing two solutions and making two conductivity determinations for each sample. It is therefore not as suitable for routine ash determinations as the simpler C ratio method.

SUMMARY

An investigation of conductometric methods of determining the ash content of Hawaiian raw sugars has resulted in the development of the following relationship:

Resulfated ash less 10% =
0.00160 × specific conductivity (micromhos)

In view of results obtained during 14 years, this method was officially adopted in this laboratory about 10 years ago and has been in satisfactory use since that time.

In order to detect any possible change in this relationship due to variations in composition of ash, check comparisons have been made between the gravimetric ash and conductometric ash on representative samples of raw sugar each year since adoption of the procedure. The results of such checks have indicated that there has been no change in the relationship originally established.

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Analysis of Three-Component Systems Containing Two Mutually Immiscible Components

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THE principle of this method has been used for many years to analyze binary systems, where the sample is titrated turbidimetrically with a third component which is miscible with only one of the components of the binary. From the phase diagram, if the amount of the third component that was added is known, the composition of the binary can be computed. This method has been extended to apply to ternary systems.

Dahl (*2*) established a mathematical method for determining three-component systems containing a strongly polar component and a strongly nonpolar component (the components that are mutually immiscible); the third component is a solvent for both of the other components. A typical system of this type would be water-methanol-benzene.

Normally, to analyze this system, each component would have to be determined separately, or at least two components would have to be determined, and the third obtained by difference. The weakness of the difference method lies in the fact that the value obtained by difference contains the accumulated errors from the two values that were determined.

The method described below has several advantages over existing methods. The time for the total analysis is considerably shortened and because it is necessary to determine only one component, one can choose the component that can be determined most simply, most precisely, and most accurately.

The ternary phase diagram for the analysis is determined in a very short time according to the system described below, and it is necessary to establish this diagram only once.

Considering a homogeneous ternary system containing components *A*, *B*, and *C*, *A* and *B* are mutually immiscible, and *C* is a solvent for both *A* and *B*. *A* is determined chemically. A separate sample is then titrated with *A* until a turbidity results from *A*'s exceeding its solubility in the mixture or being introduced in such amounts as to force *B* out of solution. For the turbidimetric titration, *B* could be used as well as *A*. For most samples both *A* and *B* will yield turbidimetric end points of the

A method has been devised for the analysis of one-phase ternary systems without determining each component separately. This method can be applied only when two of the three components are mutually immiscible. One component is determined by chemical or physical means. A separate sample is then titrated with one of the components of the system until a turbidity results. The composition of the sample can be computed from the phase diagram for the particular system, using the turbidimetric titration and one chemical analysis. The procedure is especially applicable to control analysis.

same quality. However, in some samples one component will yield a better end point than the other.

The titration to turbidity brings the composition of the sample onto the curve in the phase diagram for the particular system involved. The chemical analysis for A establishes the point on the curve. This point corresponds to the composition of the sample after titration with A. The amount of A which was added is measured, and by subtracting the amount of A which was added from the composition which was read off the curve, the composition of the original sample can be obtained.

PROCEDURE AND RESULTS

To prepare the phase diagram mixtures of two of the miscible components of the system, each component accurately measured, are placed in a water bath at a desired temperature, and sufficient time is allowed for the mixtures to reach the temperature of the bath. Each mixture is titrated with the third compound of the system until there is a faint permanent turbidity. The percentage of each component of the mixtures at this turbidity point is calculated. The points are plotted on a triangular coordinate graph paper and connected with a smooth curve.

Procedure. One of the components of the system is determined by chemical or physical means. For the turbidimetric titration a separate sample is used, large enough to give a turbidimetric titration of about 20 ml., if possible. The sample is placed in a water bath at the same temperature at which the phase diagram was prepared. After it has reached the desired temperature, the sample is titrated with one of the two components which are mutually immiscible until there is a faint permanent turbidity. The composition of the original mixture can then be calculated from the graph.

Sample Calculation. In a system consisting of components A, B, and C where C is miscible with both A and B, and A and B are immiscible, $A = 50\%$ by analysis. A 50-gram sample is used for the turbidimetric titration with B. The titration with $B = 20$ grams. Then the sample plus the titration $= 50 + 20 = 70$ grams. Amount of A in sample $= 0.50 \times 50 = 25$ grams; $25/70 \times 100 = 35.7\%$ A in sample after titration.

From the graph at the point where $A = 35.7$, there is $\frac{30.8\%\ B}{33.5\%\ C}$ in sample after titration.

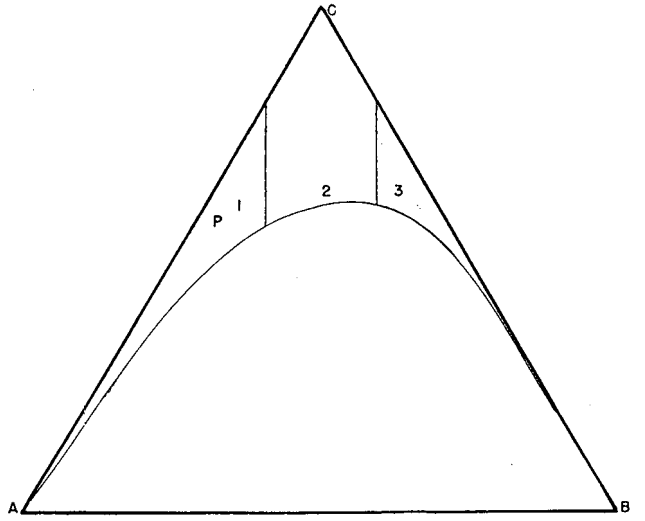

Figure 1. Phase Diagram for Three-Component System
A. Chlorobenzene
B. Water
C. Methyl alcohol

$$\frac{(0.308 \times 70) - 20}{50} \times 100 = 3.12\%\ B \text{ in original sample}$$

$$\frac{0.335 \times 70}{50} \times 100 = 46.9\%\ C \text{ in original sample}$$

In the following systems, to prepare the graphs, different known mixtures of two of the miscible components were titrated with the third component to the turbidity end points. These points were plotted on triangular coordinate graph paper and joined with a smooth curve. All the systems were studied at 25° C.

In Figure 1 and Table I the system used was chlorobenzene–water–methyl alcohol. For the synthetic samples (Table I) water was determined by the Karl Fischer method (*3*).

Table I. Methyl Alcohol–Chlorobenzene–Water

Sample No.	% Methyl Alcohol		% Chlorobenzene		% Water	
	Found	Present	Found	Present	Found	Present
1	70.0	70.17	4.60	4.97	25.68	24.86
2	78.8	80.07	10.7	9.97	10.30	9.96
3	60.5	59.08	34.0	35.90	5.6	5.03
4	52.3	50.99	41.9	44.00	5.7	5.01
5	31.9	30.73	65.9	67.31	2.3	1.96
6	83.2	82.01	1.67	3.00	15.2	14.95
7	75.3	75.46	19.5	19.49	5.2	5.06

Table II. Acetic Acid–Carbon Tetrachloride–Water

Sample No.	% Acetic Acid		% Carbon Tetrachloride		% Water	
	Found	Present	Found	Present	Found	Present
1	86.0	85.49	10.3	9.66	3.46	4.82
2	77.0	76.69	18.7	18.48	4.21	4.94
3	67.8	67.33	30.6	30.69	1.56	1.95
4	52.2	52.27	47.1	47.22	0.72	1.00
5	76.3	75.79	4.04	4.85	19.70	19.44

Table III. Glycerol–Acetic Acid–Benzene

Sample No.	% Glycerol		% Acetic Acid		% Benzene	
	Found	Present	Found	Present	Found	Present
1	45.0	44.06	50.4	51.05	4.63	4.90
2	33.7	34.13	61.5	61.00	4.74	4.88
3	24.0	24.34	67.0	65.93	9.03	9.73
4	9.68	9.77	71.9	70.82	18.5	19.41
5[a]	5.16	5.19	66.6	64.08	28.4	30.73

[a] Turbidity end point was not very sharp.

Because the phase curve (Figure 1) lies close to the 0% chlorobenzene line, the samples can be successfully titrated with water to sharp end points.

The synthetic samples for the system carbon tetrachloride–acetic acid–water (Table II) were also titrated with water. The acetic acid was determined by direct titration with standard base. The curve was very similar to Figure 1, and, for the same reason, the end points were sharp. For the system acetic acid–glycerol–benzene, benzene was used for the titration. The curve (Figure 2) slopes more gradually at each end than Figure 1. Good results were obtained for the first four samples (Table III), as they lay generally to the AC side of the graph and, when titrated with B (benzene), gave good end points. In the case of the fifth sample the end point was poor, as can be shown by drawing a line from the point representing the percentage composition of the sample

to the point at 100% benzene. This line intersects the curve at a point were the curve is falling off rapidly. A sharper end point is indicated for a titration with glycerol for this particular sample. Glycerol was determined using periodic acid (*4*). The monoethanolamine in the system monoethanolamine–pyridine–ethyl ether was determined by direct titration with standard acid. Ethyl ether was used to titrate the samples (Table IV) to the turbidity end point. The curve (Figure 3) slopes gradually toward points *A* (100% ethyl ether) and *B* (100% monoethanolamine). The end points for the samples (Table IV) became sharper as the samples became higher in percentage of monoethanolamine. This is indicated by drawing lines from the point representing the composition for each sample to the point at 100% ethyl ether. As the samples become higher in monoethanolamine, the lines hit the curve progressively more directly. If the lines for these particular samples are drawn to point *B* (100% monoethanolamine) rather than to *A* (100% ethyl ether), they do not intersect the curve. Therefore, if monoethanolamine had been used for the titration, no turbidity end point would have been obtained.

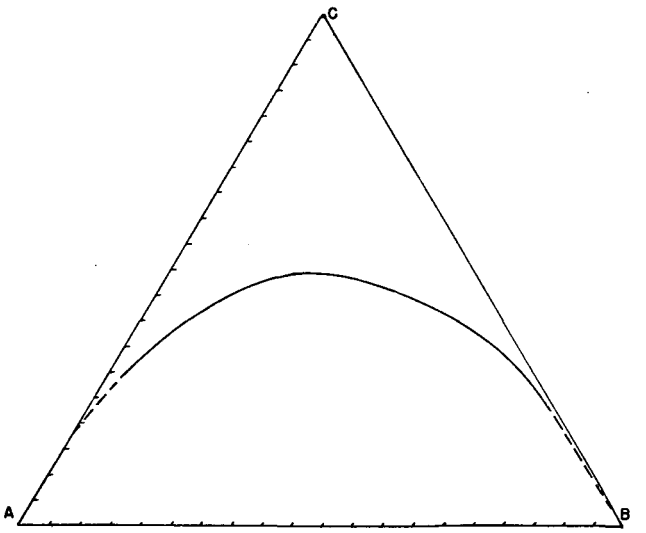

Figure 2. Phase Diagram for Three-Component System
***A.* Glycerol. *B.* Benzene. *C.* Acetic acid**

The benzaldehyde was determined in the system benzaldehyde-dioxane-water, using a hydroxylamine method (*1*). Water was used to titrate to the turbidity end point in samples 1 to 4 (Table V) and benzaldehyde was used to titrate sample 5 (the ternary diagram is similar to Figure 1).

Table IV. Pyridine–Ethyl Ether–Monoethanolamine

Sample No.	% Pyridine		% Ethyl Ether		% Monoethanolamine	
	Found	Present	Found	Present	Found	Present
1	62.6	64.94	12.8	10.00	24.9	25.1
2	62.2	63.1	11.8	10.6	26.0	26.3
3	42.2	41.5	4.4	5.3	53.4	52.2
4	29.1	29.5	9.9	9.1	61.0	61.4
5	62.1	59.9	21.8	24.0	16.2	16.1
6	50.1	49.4	9.1	10.0	40.7	40.5

Table V. Benzaldehyde–Dioxane–Water

Sample No.	% Benzaldehyde		% Dioxane		% Water	
	Found	Present	Found	Present[a]	Found	Present
1	41.0	40.5	54.00	54.66	5.02	4.85
2	30.6	30.40	64.4	64.8	5.0	4.9
3	16.4	14.66	71.8	71.86	11.7	11.70
4	9.96	9.95	84.7	85.11	5.3	4.95
5[a]	4.99	4.94	59.7	60.5	35.4	34.5

[a] Titrated with benzaldehyde.

Table VI. Water-Pyridine-Benzene

Sample No.	% Water		% Pyridine		% Benzene	
	Found	Present	Found	Present	Found	Present
1	5.18	5.08	59.8	59.5	34.9	35.4
2	5.24	5.20	81.7	81.23	13.1	13.1
3	12.28	12.26	75.5	74.8	12.2	12.2
4	15.06	15.31	80.1	79.8	4.93	4.93
5	34.72	35.57	60.5	59.6	4.69	4.69

Table VII. Ethyl Vinyl Ether–Ethanol–Water

Sample No.	% Ethyl Vinyl Ether		% Ethanol		% Water	
	Found	Present	Found	Present	Found	Present
1	9.72	9.95	75.7	80.11	14.3	9.94
2	24.7	25.01	64.1	65.01	10.9	9.97
3	9.96	10.00	61.6	65.08	28.3	24.95
4	22.9	23.00	54.6	55.08	22.5	21.96
5	45.0	45.02	49.66	49.98	5.41	4.98

The water was determined in the system benzene-pyridine-water by the Karl Fischer method (*3*). Samples 1 to 3 (Table VI) were titrated with water, and samples 4 and 5 were titrated with benzene. The curve for the system is similar to Figure 1.

In the system ethyl vinyl ether–water–ethanol, the ethyl vinyl ether was determined by the method of Siggia and Edsberg (*5*) (Table VII). Figure 2 most closely resembles the curve for this system.

DISCUSSION

If *C* is the component determined chemically, two final analyses may result. It can be seen in Figure 1 that, in the case of *C*, for each point on the curve there is a second point on the curve that corresponds to the same amount of *C*. This does not mean that the determination of *C* could not be used for the analysis. It is true that this procedure will yield two possible points on the curve; however, one point could very readily be discarded by one of several ways. A known excess reagent may be added in the turbidimetric titration; this will result in a two-phase system. By the relative size of the upper and lower layers, the true point can usually be selected from the false point. Another method of selecting the true point is to take the refractive index of the sample at the turbidimetric end point. After the turbidity is obtained, a drop or two of *C* is added just to eliminate the turbidity. The refractive index is taken on this clear sample. This procedure can usually eliminate one of the possible points. If the two possible points lie at, or near, the top of the curve, resolving the correct point is difficult.

The best method of choosing the correct point is to run two

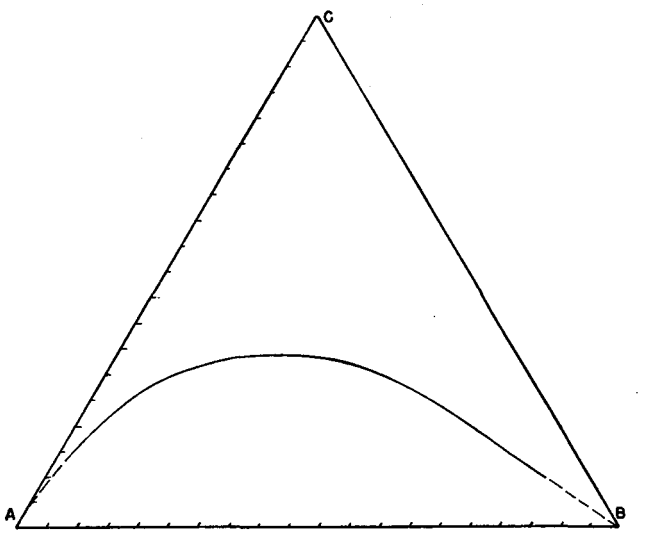

Figure 3. Phase Diagram for Three-Component System
***A.* Ethyl ether. *B.* Monoethanolamine. *C.* Pyridine**

turbidimetric titrations, one with A and one with B on a separate sample. This will accurately indicate the correct point even if the two possible points are near the top of the curve. If C is the component most easily or most accurately determined, the extra turbidimetric titration is worth the extra 15 minutes it takes to run.

The sharpness of the turbidimetric end point depends on the composition of the initial sample and on whether A or B (Figure 1) was used for the turbidimetric titration. The turbidimetric end point can be described as follows:

Starting with a sample of a composition described by a point on the phase diagram and titrating with A, for instance, the composition of the solution being titrated will follow a line between the point representing the original composition of the sample and the point representing 100% A. Where this line intersects the phase curve is the point where the turbidity will first be noted. The more directly this line hits the phase curve, the sharper the turbidity can be detected. From Figure 1 it will be seen that if the original composition of the sample lies somewhere in region 2, the sharpness of the end point will be just about the same whether A or B was used for the turbidimetric titration. However, if the original composition of the sample, represented by point p, is somewhere in region 1, then titrating with A will yield a very poor end point, as the line from p to 100% A approaches the phase curve asymptotically. However, if B is used for the turbidimetric titration of this sample, a sharp end point will be obtained, because the line between p and 100% B intersects the phase curve almost at right angles. Similarly, if the composition of the original sample were somewhere in region 3, titrating with A would yield a much sharper end point than would titrating with B. The turbidimetric end point can usually be obtained within a drop.

A serious difficulty arises when the component thrown out of solution has approximately the same refractive index as the solution; the end point appears as an opalescence if the refractive indexes are close (the end point would be invisible if the refractive indexes were the same).

The accuracy and precision that can be obtained depend on the accuracy and precision of the method used to determine the necessary one component, the care taken in preparing the phase diagram, the component chosen for the turbidimetric titration, and the sharpness of the turbidimetric end point (dependent on refrac-fractive index).

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Analysis of Beryllium-Copper Alloys

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A procedure for the analysis of copper alloys containing small amounts of beryllium has been developed. Copper is first removed and discarded. Beryllium is generally determined first in the remaining solution, except when samples contain phosphorus or zirconium, which must be removed. Nickel and cobalt are determined on the solution resulting from beryllium removal. Special modifications are used for silicon and chromium. Silver is determined gravimetrically as the chloride.

COPPER alloys containing 0.1 to 0.3% beryllium and 1% nickel, cobalt, and silver, and, in some cases, silicon, chromium, zirconium, or phosphorus, have been studied in an effort to attain certain physical properties, especially conductivity and tensile strength. The typical beryllium coppers, well known for their age-hardening properties, contain less than 3% beryllium.

Very little information on the analyses of these alloys could be found, although their properties and uses have been more thoroughly described. A gravimetric procedure (*5*) for the analysis of beryllium coppers containing nickel, aluminum, silicon, and zinc has been published, as well as one (*2*) for copper alloys and white metals containing lead, iron, manganese, zinc, cobalt, tin, and antimony. There are numerous references to the analysis of copper alloys not containing beryllium (*1*, *6*, *7*) and to the determination of a single constituent in them.

However, the combinations of elements found in the more recently developed copper-base alloys containing beryllium are such that some special procedure must be set up. In view of the desirability of having a procedure that fits in well with the demands of the particular alloys in question and of the usual equipment of the industrial laboratory, a study has been made.

One of the major problems has been that of separation and determination of beryllium (*10*) because of its very small percentage. The presence of other elements to be determined, especially zirconium (*9*) and phosphorus (*3*), presents new difficulties.

The following general procedure has been found useful. It consists of first removing and discarding the copper and using the remaining solution for determination of the other constituents. In general, beryllium was determined first, except in samples containing phosphorus or zirconium, where removal of these was necessary. Nickel or cobalt was determined on the prepared solution resulting from beryllium removal. Special modifications were necessary to care for silicon and chromium, if present. Silver was determined gravimetrically as the chloride.

PROCEDURE

Copper Removal. For the determination of all the alloying constituents, except silver, phosphorus, and silicon, by the methods used, copper removal is required. Two methods have been found useful—hydrogen sulfide precipitation and electrolysis. The former procedure is given as a part of the general method. Copper determination itself is not required.

At least a 2-gram sample is weighed into a 400-ml. beaker, moistened with water, dissolved in the minimum amount of nitric acid (about 10 ml.), and taken to dryness on a hot plate. Care must be taken to remove all nitric acid. To the oxides, which have previously been washed down from the beaker with a stream of water, 20 ml. of hydrochloric acid are added. The solution is diluted to 200 ml., then heated to near boiling. At this point complete solution should be obtained except for a white precipitate if silver if present. Usually it is possible to notice whether solution results immediately following the hydrochloric acid addition and heating. Heating to fumes with

sulfuric or perchloric acid may be necessary, especially for samples containing silicon and chromium, which are somewhat difficult to dissolve. Into the hot solution a stream of hydrogen sulfide is passed until precipitation is complete. Filtration with the aid of suction, followed by washing several times with hot water acidulated with hydrochloric acid and saturated with hydrogen sulfide, removes the precipitated sulfide. After boiling free of hydrogen sulfide, the solution is ready for use as a prepared solution for determination of its constituents beryllium, zirconium, cobalt, nickel, phosphorus, and chromium.

Beryllium. For samples not containing iron (as an impurity), phosphorus, or zirconium (which must be removed) beryllium is determined immediately after copper and hydrogen sulfide removal by precipitation as beryllium hydroxide.

Several grams of ammonium chloride are added, prior to precipitation by addition to the hot solution of filtered high quality ammonium hydroxide. After settling, the precipitated hydroxide is filtered and washed with hot ammonium chloride solution. Ignition to beryllium oxide is carried out in platinum crucibles at 1000° C.

Samples containing zirconium and phosphorus must have these elements removed prior to beryllium determination, as indicated in the procedures below.

Zirconium. The prepared solution is adjusted to an acidity of 10% by volume in hydrochloric or sulfuric acid and a little macerated filter pulp is added, and the solution is cooled to 10° C. A cold 6% solution of cupferron is added slowly until a fine white precipitate appears where the reagent meets the solution. After standing a few minutes, filtration is made on Whatman 42 filter paper, followed by washing with cold 5% hydrochloric acid containing 1.5 grams of cupferron per liter. Ignition in a platinum crucible by first slowly charring the paper, then gradually increasing the heat to 1000° C., yields zirconium oxide, from which zirconium can be calculated.

Before using the filtrate for determination of beryllium it is necessary to destroy the cupferron in it by adding 10 ml. of nitric acid and 20 ml. of sulfuric acid and heating to fumes. Upon dilution to 200 ml. this solution is ready for beryllium determination.

Phosphorus. Attention has been called to the precipitation of beryllium and zirconium as the phosphates (*9*). For this reason special precautions are necessary.

Phosphorus may be precipitated with ammonium molybdate either before or after copper removal. In the latter case, 20 ml. of nitric acid are added to the prepared solution, and the solution is boiled to remove free chlorine (due to high hydrochloric acid concentration in contact with nitric acid). Phosphorus may then be precipitated as the ammonium phosphomolybdate as in the procedure given below. Afterward, beryllium may be determined on the filtrate containing ammonium molybdate, but cobalt or zirconium cannot be determined in this solution because of molybdenum interference.

Alternatively, precipitation may be made directly on a sample of the alloy dissolved in nitric acid. One gram of the sample is dissolved in 20 ml. of nitric acid in a 250-ml. Erlenmeyer flask. Dilution to 100 ml. neutralization with ammonium hydroxide, reacidifying with a little nitric acid, and heating to about 70° C. make the solution ready for precipitation. Ammonium molybdate solution (75 ml.) is added and the mixture well shaken for about 5 minutes. After precipitation the mixture is allowed to stand for several hours, filtered on a weighed fritted-glass crucible, washed with cold 1 to 100 nitric acid, dried at 110° C. for one hour, and reweighed.

Nickel. Two methods were used: the cyanide titration method for samples containing no cobalt and the gravimetric method using dimethylglyoxime. The latter is usable for all samples submitted and has the advantage of requiring no standard solutions.

PROCEDURE FOR CYANIDE TITRATION. The solution remaining from removal of beryllium is made ammoniacal, and a slight excess (1 to 5 ml. per 100 ml. of solution) of ammonium hydroxide is added. To this solution are added 2 ml. of 10% potassium iodide solution, followed by exactly 1 ml. of a silver nitrate solution of known strength (0.100 *N*). A precipitate or sol should now be present. A previously standardized solution of potassium cyanide (approximately 0.1 *N*) is used for titration to disappearance of the silver iodide.

PROCEDURE FOR DIMETHYLGLYOXIME METHOD. Before the determination, beryllium and zirconium may be removed by ammonia precipitation or held in solution by citric acid. The acidic solution is heated to about 70° C. and a 1% solution of the precipitant in alcohol is added (0.4 ml. for each milligram of nickel present plus 5 to 10 ml. in excess). The solution is made ammoniacal while stirring and allowed to cool slowly to room temperature over several hours. Filtration is made on a weighed medium fritted-glass crucible, the precipitate is washed with cold water and dried at 120° C. for 2 hours, and the crucible is reweighed.

Nickel and cobalt determinations on separate aliquots are preferred rather than simultaneous determination on the same solution.

Cobalt. Precipitation with 1-nitroso-2-naphthol has been found satisfactory. Copper, beryllium, and zirconium must be removed.

The solution is made free of nitrates by evaporation to fumes with sulfuric acid. After dilution to 200 ml., the solution is neutralized with ammonium hydroxide and 10 ml. of hydrochloric acid are added. It is then heated to 80° C. and 125 ml. of freshly prepared 1-nitroso-2-naphthol (8 grams in 300 ml. of acetic acid and 300 ml. of water) are slowly added. Three hours' standing is usually sufficient for complete precipitation, but if desired a little additional reagent may be added as a test. Suction filtration on a Whatman No. 42 filter paper, washing with cold water, and ignition in a porcelain crucible at 900° C. for an hour yield the oxide. If nickel is present, washing with 1 to 2 hydrochloric acid followed by hot water instead of the cold water wash is necessary.

In an attempt to eliminate the lengthy gravimetric method for cobalt, a colorimetric procedure (*4, 8*) using hydrochloric acid has been tried.

A suitable sample size can be obtained by diluting the hydrogen sulfide–free of filtrate to 500 ml. and taking a 25-ml. aliquot. This is placed in a 100-ml. volumetric flask, 5 ml. of stannous chloride (15% in hydrochloric acid) are added, and the solution is diluted to 100 ml. with cold concentrated hydrochloric acid. A blue color is developed whose transmittancy is measured at 650 mμ and compared with that of a previously prepared curve made from known cobalt concentrations. Cobaltic oxide was used as a source of the element.

In general, this colorimetric method is limited in its usefulness to cases where ammonium salts are entirely absent and to samples of less than 1% cobalt. The method has the disadvantage that the acid fumes cause corrosion of the measuring instrument (in this case, Fisher electrophotometer) if uncovered cylinders are used.

Silver. Silver is most conveniently determined by dissolving a 1-gram sample in the minimum amount of nitric acid (8 ml. of 6 *N*), diluting to 100 ml., and precipitating as silver chloride just below boiling by addition of hydrochloric acid (3 to 100) dropwise. After settling, filtration is made on a weighed fritted-glass crucible, followed by several washes with dilute nitric acid (2 to 100) and finally a cold water wash. The crucible is dried at 110° C. and weighed.

Silicon. A 4-gram sample is dissolved in 20 ml. of nitric acid and 20 ml. of 1 to 1 sulfuric acid with the aid of heat. Twenty milliliters of perchloric acid are added, and the solution is evaporated to fumes. After dilution to 150 ml. and heating, the silica is filtered off, followed by washing alternately with hot water and hot 1 to 10 hydrochloric acid. (Filtrate is reserved for chromium.) The residue is ignited in a platinum crucible at 1000° C. and then weighed. Several drops of sulfuric acid are added to the moistened residue. The crucible is then filled one third full with hydrofluoric acid and heated on a hot plate to remove excess hydrofluoric acid. Ignition at 1000° C. precedes reweighing. Per cent silicon is calculated by multiplying the difference in weight by 0.4672.

Chromium. Perchloric acid as an oxidant brings chromium to the higher oxidation state, after which it can be titrated with ferrous ammonium sulfate.

In samples containing silicon, there may be a residue from silica ignition (presumably chromium silicide). If so, it is fused with sodium carbonate and the melt is dissolved in the smallest amount of 1 to 2 hydrochloric acid. This solution is added to the

Table I. Typical Results of Analyses

Sample	Beryllium		Cobalt		Nickel		Silver		Phosphorus		Chromium		Silicon	
	C[a]	D[b]	C[a]	D[b]	C[a]	D[b]	C[a]	D[b]	C[a]	D[b]	C[a]	D[b]	C[a]	D[b]
131	0.30	0.32	1.6	1.62	...	...	3.3	3.15	...	...	...	...	...	...
134	0.20	0.21	1.2	1.29	...	...	1.1	1.15	...	...	0.50	0.60	0.15	0.09
140	0.20	0.20	...	...	1.3	1.23	3.0	3.17	...	...	...	...	...	...
156	0.23	0.25	1.2	1.24	...	...	3.0	...	...	...	...	...	...	...
157	0.15	0.19	1.2	1.22	...	...	3.0	...	0.10	0.11	...	...	...	...
159	0.27	0.28	1.2	1.25	...	...	3.0	...	...	...	...	...	...	...
164	0.24	0.23	...	...	1.2	1.14	3.0	...	0.04	0.05	...	...	...	...
165	0.24	0.25	...	...	1.2	1.19	2.0	...	0.04	0.04	...	...	...	...

[a] Charged analyses.
[b] Determined analyses.

bulk of that to be used for determination of chromium. Copper is removed in the usual way with hydrogen sulfide.

For alloys containing no silicon the filtrate from copper removal is used directly for chromium determination. If it is desired to remove beryllium prior to chromium determination, chromium is oxidized either by perchloric acid or in alkaline solution by means of bromine water. Beryllium is then precipitated in the presence of hexavalent chromium, which can be determined on the filtrate.

PROCEDURE. Perchloric acid (25 ml. of 70%) is added to the solution for the determination and the mixture is heated to fumes for about 15 minutes. Titration is then made by use of a previously standardized solution of ferrous ammonium sulfate (0.1 *M*) and, for back-titration, standard potassium permanganate.

DISCUSSION

The reported analyses were compared with the charged analyses rather than with synthetic standards. However, duplication of results was within a desirable precision range, duplicates running within 0.01 or at most 0.02% (actual percentages) of each other. More than 250 determinations on 100 samples have been made.

A more extensive use of colorimetric methods would shorten the time of analysis considerably. However, the process of setting up standard curves and the presence of colored interfering ions are not to be neglected in this connection.

Iron, if present as an impurity, would precipitate with zirconium. However, in the samples with which the author has worked, the extent of the iron impurity has been negligibly small, as shown by combining the ignited beryllium oxides from several samples and making photometric comparison. Aluminum, if present, would introduce other difficulties and would need to be removed prior to beryllium determination.

The chief problem incurred was that of a means of separation which would permit use of one sample for all determinations. This problem was solved as each alloy of different composition was presented, and in many cases was met by aliquoting portions of the sample after copper removal. Especially was this true of samples containing both nickel and cobalt.

ACKNOWLEDGMENT

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Determination of Sulfaquinoxaline and Sulfaguanidine in Commercial Feeds

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THE incorporation of such compounds as sulfaguanidine and sulfaquinoxaline in commercial poultry feeds has become common practice. The authors have, for the past 2 years, applied a procedure for the determination of sulfaguanidine in mixed feeds, which is short and accurate, and lends itself to the simultaneous analysis of a number of feed samples. It is a combination of ideas from several adaptations of the technique described by Bratton and Marshall (*1*). The application of the procedure for sulfaguanidine to the accurate determination of sulfaquinoxaline depends essentially on an alkaline extraction instead of the use of dilute acid.

There is also a difference in dilution before the characteristic color is developed.

DETERMINATION OF SULFAQUINOXALINE

Extraction. Weigh 5 grams of feed containing about 0.0125% of sulfaquinoxaline into a 250-ml. beaker, and add 100 ml. of distilled water and 0.5 ml. of 2.5 *N* sodium hydroxide. Bring to a boil cautiously with occasional stirring, boil gently for 1 minute, and cool. Transfer quantitatively to a 250-ml. flask, dilute to volume, shake well, and allow to settle.

Pipet 50 ml. into a 100-ml. volumetric flask, add 18 ml. of 15% trichloroacetic acid, dilute to the mark, and shake well.

Filter through a 9-cm. S. & S. 589 Blue Ribbon filter paper. Reject the first 10 ml. of filtrate.

Color Development. Pipet 5 ml. of the clear filtrate into a 100-ml. beaker containing 2 ml. of 15% trichloroacetic acid and 3 ml. of distilled water. Add 1 ml. of a freshly prepared sodium nitrite solution (0.1 gram dissolved in 100 ml. of distilled water), stir well, and allow to stand.

A procedure for the determination of sulfaguanidine in mixed feeds has been adapted from the technique described by Bratton and Marshall. Its application to the accurate determination of sulfaquinoxaline depends on an alkaline extraction instead of the use of dilute acid. The procedure is short and accurate and may be used for the simultaneous analysis of a number of feed samples.

At the end of 3 minutes, add 1 ml. of a solution of ammonium sulfamate (0.5 gram, practical grade, in 100 ml. of distilled water), mix, and let stand for 2 minutes. Finally add 1 ml. of a solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride and let stand for 10 minutes. Maximum color may develop in about 1 minute at normal temperatures, but the authors recommend 10 minutes to avoid temperature effects. The last reagent is prepared by dissolving 0.1 gram of *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Eastman Kodak) in 100 ml. of distilled water. It should be stored not longer than 7 days in an amber glass bottle in the refrigerator.

At the end of 10 minutes, determine the intensity of the purplish-red color by means of a Cenco-Sheard photelometer using a 525-mμ filter and a cuvette 1 cm. in depth.

Generally, a reagent blank will give 100% transmittance as compared to distilled water, but this should be checked. High corn rations may give extracts with a very faint color. To overcome this difficulty, it is the authors' custom to take 5 ml. of the extract, dilute it to 13 ml., and use this mixture as a blank.

Calibration of Photelometer. A working standard containing sulfaquinoxaline at a concentration equivalent to 0.005 mg. per 1 ml. is prepared by dissolving 0.100 gram in distilled water containing 3 ml. of 5 *N* sodium hydroxide, diluting to 1000 ml. with distilled water, and shaking well. Then 25 ml. of this solution are diluted accurately to 500 ml.

The characteristic color is developed on solutions prepared by measuring from a buret 1, 2, 3, and 4 ml. of the working standard and diluting in each case to 5 ml. with distilled water.

A table giving the percentage values corresponding to photelometer readings may be readily calculated by means of the equation $C = K \log 100/R$, where C is the concentration in milligrams for 1 ml. of the extract and R is the photelometer reading.

The relationship between concentration of sulfaquinoxaline and photelometer readings observed in this laboratory was as follows:

Sulfaquinoxaline, Mg./1 Ml.	Photelometer Reading
0.005	42.5
0.004	51.0
0.003	61.0
0.002	71.0
0.001	85.0

Recovery Tests. Sulfaquinoxaline (1.5625 grams, Merck) was dissolved in about 100 ml. of distilled water containing 0.2085 gram of sodium hydroxide and diluted to 250 ml. (Solution I), and 10 ml. of this solution were diluted to 500 ml. (Solution II). Then 5-gram portions of commercial poultry mixed feed, ground to pass a 1-mm. sieve, were weighed into 250-ml. beakers, 5-ml. aliquots of Solution II were pipetted into each beaker, and after thorough stirring with a glass rod, the beakers and their contents were placed in a constant temperature oven at 100° C. overnight.

Under these conditions, the drug was added at a level of 0.0125%, comparable to that recommended as good feeding practice. Recovery has invariably fallen within ±2%, which is believed to be excellent in view of the small percentage present and the complex nature of the feed from which it is extracted.

A dry mix was also prepared by first mixing, quantitatively, 1 gram of sulfaquinoxaline with 25 grams of a "high energy" poultry feed. This mixture was then thoroughly incorporated with 74 grams of the same feed. Finally, 12.5 grams of the second mixture were added to 987.5 grams of feed in a suitable glass jar and mixed adequately. Theoretically, this final mixture contained sulfaquinoxaline at a level of 0.0125%. Two other samples containing 0.00625 and 0.0250% sulfaquinoxaline were prepared in a similar way. The results of analysis are presented in Table I.

DETERMINATION OF SULFAGUANIDINE

The procedure for the determination of sulfaguanidine in commercial mixed feeds closely resembles the method described above.

Table I. Recovery of Sulfaquinoxaline

Mixture No.	Sulfaquinoxaline Added, %	Sulfaquinoxaline Recovered, %
1	0.0063	0.0066
		0.0069
		0.0068
2	0.0125	0.0124
		0.0122
		0.0128
3	0.0250	0.0256
		0.0250
		0.0246

Extraction and Color Development. Weigh 1 gram of feed into a 250-ml. beaker, and add 100 ml. of distilled water and 1.0 ml. of (1 to 1) hydrochloric acid. Bring the contents of the beaker cautiously to a boil with occasional stirring.

Transfer quantitatively to a 250-ml. flask, dilute to volume, shake well, and allow to settle. Pipet 50 ml. into a 100-ml. volumetric flask, add 18 ml. of 15% trichloroacetic acid, dilute to mark, and shake well.

Filter through a 9-cm. S. & S. 589 Blue Ribbon filter paper. Reject the first 10 ml. of filtrate. Transfer 10 ml. of the subsequent filtrate to a 100-ml. volumetric flask, dilute with distilled water, stopper, and shake well. Develop the color on a 5-ml. aliquot of this dilution, using same procedure as for sulfaquinoxaline.

Table II. Recovery of Sulfaguanidine

Mixture No.	Sulfaguanidine Added, %	Sulfaguanidine Recovered, %
1	1.00	0.98
2	1.00	1.03
3	1.00	0.99
4	1.00	1.02
5	1.00	0.99
6	1.00	1.01

Calibration. The photelometer was calibrated by means of appropriate dilutions of sulfaguanidine containing 0.005 mg. per 1 ml. Data from which the results were calculated are as follows:

Sulfaguanidine, Mg./1 Ml.	Photelometer Reading
0.005	31.4
0.004	38.8
0.003	48.3
0.002	61.0
0.001	77.2

Table II shows excellent recovery values on a series of commercial feeds.

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Determination of Uranium(VI) in Presence of Anions

Ammonium Thioglycolate as a Colorimetric Analytical Reagent

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Hexavalent uranium forms a yellow-orange soluble complex with ammonium thioglycolate in a basic solution. The color intensity remains constant over a wide pH range and is independent of the exact reagent concentration. The complex is fairly stable and unaffected by many common anions. A number of cations interfere either by reacting with the reagent or by precipitating in ammoniacal solutions, but some of these interferences may be eliminated by the use of excess reagent or complexing agents. The method is best suited to the determination of uranium in the concentration range 0.100 to 1.600 mg. in 25 ml. Solutions containing uranium in this range obey Beer's law.

COLORIMETRIC methods for uranium are usually concerned with the estimation of the element in the presence of other cations. Among the reagents reported in the literature are ammonium thiocyanate (*1*), which has been shown to be fairly specific with respect to cations, potassium ferrocyanide (*4*), sodium salicylate (*4*), and others (*4*).

More recently a number of other colorimetric methods have been developed by workers on the Manhattan Project. A comprehensive review of these methods, edited by Rodden and Warf (*3*), has been prepared for the National Nuclear Energy Series. The method used to the greatest extent was based on the uranyl-peroxide complex formed in alkaline medium.

The interference of anions such as oxalate, fluoride, tartrate, and citrate in most colorimetric determinations has been noted. When these anions are present the colorimetric procedure usually involves a preliminary separation such as the destruction or volatilization of the anions by fuming with acid. Because uranium forms strong complexes with these organic anions with a resultant effect on precipitation and separation procedures, a direct colorimetric uranium analysis in the presence of these anions would be useful. In the separation of the rare earths by ion exchange columns (*5*), the rare earths are eluted with citrate ion and precipitated with oxalate ion. The analysis of the eluate for uranium would require a method unaffected by citrate and oxalate ions or a procedure for the removal of these ions. Analysis of uranyl hexafluoride and uranyl sulfate solutions containing excess sulfate ion is subject to the same limitations.

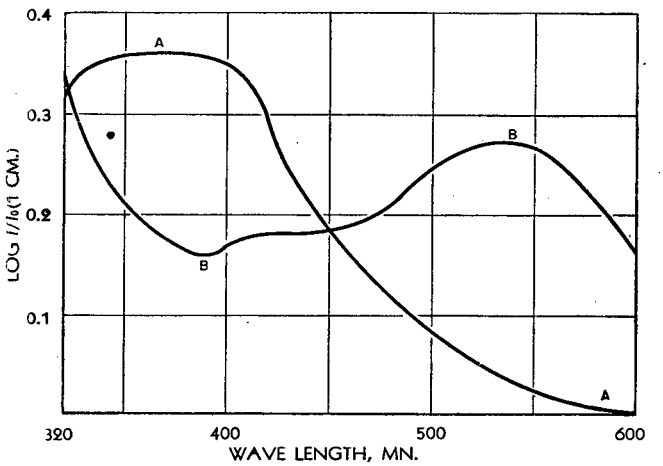

Figure 1. Spectral Absorption of Uranium Thioglycolate and Iron Thioglycolate

A. **1.060 mg. U**
B. **0.100 mg. Fe**
Complexes were developed by adding 2 ml. of a neutralized 10% solution of thioglycolic acid, adjusting to about pH 10.1 with ammonium hydroxide (1 to 1), and diluting to 25 ml.

In an investigation of conditions affecting the determination of iron with ammonium mercaptoacetate (thioglycolate) Swank and Mellon (*6*) reported that uranyl ions formed a strong orange complex with the reagent. Anions had little effect on the iron complex. This suggested the possibility that the reagent might be used for uranium solutions containing anions that would interfere in other colorimetric methods.

The authors have found that the uranium thioglycolate complex may be used as the basis for a fairly sensitive colorimetric method for uranium, and that it is unaffected by relatively large amounts of chloride, nitrate, sulfate, perchlorate, oxalate, tartrate, citrate, acetate, and fluoride ions. Unfortunately, many cations do interfere, but in many cases the interfering cations will not be present in the solutions described above. When the interfering cations, aluminum, thorium, titanium, and zirconium, are present in small amounts it may be feasible to complex them with tartaric acid.

EXPERIMENTAL

Solutions and Methods. AMMONIUM THIOGLYCOLATE. Ten milliliters of thioglycolic acid, obtained from the Eastman Kodak Company, were diluted with about 50 ml. of water, neutralized with 1 to 1 ammonium hydroxide, and made up to a final volume of 100 ml. This concentration of ammonium thioglycolate was used throughout the investigation.

STANDARD URANIUM SOLUTION. A solution of uranium, 10.6 mg. per ml., was prepared from C.P., uranyl nitrate hexahydrate and was standardized by evaporating aliquots to dryness and igniting to the oxide, U_3O_8, at 850° C. An aliquot of the standard solution was diluted to give a solution 0.106 mg. per ml.

AMMONIUM HYDROXIDE. A 1 to 1 solution was prepared from C.P. reagent.

FOREIGN ION SOLUTION. Reagent grade salts were taken for the preparation of all foreign ion solutions. The potassium salts of the nitrate, chlorate, chromate, and bichromate ions were used and the remaining anion solutions were prepared from their ammonium salts. The cation solutions were prepared from chloride salts with the exception of lead nitrate.

Redistilled water was used for all solutions. The complexes were developed in a 25-ml. volume by adding 2 ml. of ammonium thioglycolate and 2 ml. of 1 to 1 ammonium hydroxide in excess of neutrality. Solutions were read against a reagent blank in a Beckman spectrophotometer using 1-cm. cells.

Uranium Thioglycolate Complex. The uranium thioglycolate complex must be developed in a basic medium, but critical adjustment of pH is not necessary. In this investigation the concentration of ammonium hydroxide added gave a pH of about 10, but no change in color intensity resulted on varying the pH over the range 7.6 to 10.7.

The concentration of ammonium thioglycolate is not critical within limits. From 1 to 4 ml. of the neutralized 10% reagent may be added without affecting the color. The color intensity increases slightly as excess reagent is added.

The color is stable for at least 30 minutes in direct light. A decrease of about 4% in color intensity results on standing 2 hours.

The yellow-orange complex does not show a sharp absorption maximum; fairly strong absorption takes place over the range 330 to 400 mμ (Figure 1).

A plot of extinction against concentration for 0.100 to 1.600 mg. of uranium gave a straight-line curve, indicating that the color obeys Beer's Law (Figure 2).

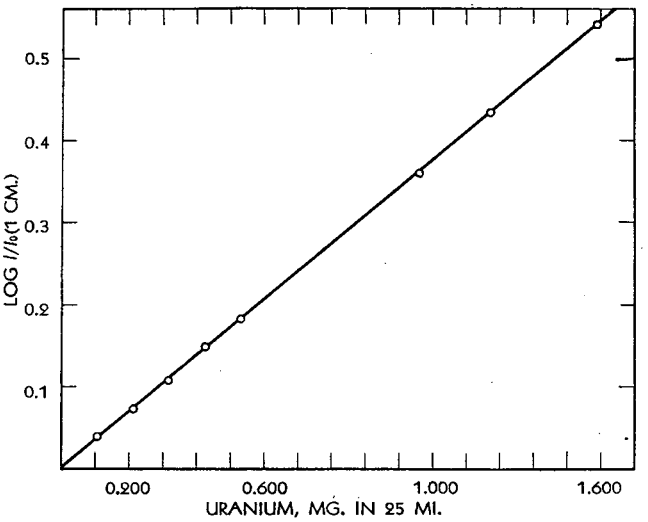

Figure 2. Calibration Curve for Uranium(VI) Using Ammonium Thioglycolate

Effect of Anions. As much as 500 mg. of chloride, chlorate, nitrate, or sulfate ion may be present without affecting the color intensity of 0.530 mg. of uranium. Smaller concentrations of fluoride, tartrate, acetate, citrate, or oxalate are also without effect on the color. Carbonate and phosphate ions must be absent. As little as 1 mg. of either ion causes an appreciable decrease in color intensity. The effect of a number of anions on the color developed with 0.530 mg. of uranium is indicated in Table I. An allowance was made of ±2.5% error for the method. In the case of tartrate ion, 500 mg. may be tolerated if 4 ml. of ammonium thioglycolate are used. More than 8 ml. of reagent are required to restore the color when 500 mg. of fluoride ion are present.

Effect of Cations. Cation interference presents a more serious problem (Table II). Iron, copper, nickel, lead, and cobalt contribute to the color intensity. Aluminum, thorium, chromium, titanium, zirconium, and magnesium precipitate in ammoniacal solution. Cadmium, when present to the extent of 50 mg., causes a 4% decrease in the color intensity of 0.530 mg. of uranium. Many of the interferences may be eliminated partially or completely. Aluminum, thorium, titanium, and zirconium may be kept in solution by adding tartaric acid in the ratio of 3 ml. of 10% reagent to 50 mg. of cation. The latter three may also decrease the color intensity if present in sufficient concentration. Excess reagent restores the color. The precipitation of magnesium may be prevented by adding ammonium chloride in excess. Manganese forms a green complex which fades on standing.

Table I. Effect of Anions on Uranium Thioglycolate Complex

(0.530 mg. of uranium in 25 ml. of solution)

Solution	Anion Added	Anion Concn., Mg./25 Ml.	Change in Color Intensity, %	Approximate Limiting Concn., Mg.
1	Chloride	500	±0	>500
2	Nitrate	500	±0	>500
3	Sulfate	500	±0	>500
4	Fluoride	300	−9	200
5	Tartrate	500	−14	200[a]
6	Nitrite	500	Intense yellow	...
7	Acetate	500	+4	400
8	Citrate	200	Fades rapidly	20
9	Carbonate	10	−30	<1
10	Orthophosphate	1	Destroys color	...
11	Chlorate	500	±0	>500
12	Molybdate	500	Intense orange	...
13	Oxalate	100	−7	50
14	Chromate	100	Intense green	...
15	Bichromate	100	Intense green	...

[a] No change in color intensity in presence of 500 mg. of tartrate ion when 4 ml. of ammonium thioglycolate are used.

Table II. Effect of Cations on Uranium Thioglycolate Complex

(0.530 mg. of uranium in 25 ml. of solution)

Solution	Cation Added	Cation Concn., Mg./25 Ml.	Change in Color Intensity, %
1	Al^{+++}[a]	100	±0
2	Th^{+4}[a, b]	50	±0
3	Zr^{+4}[a, c]	5	±0
4	Fe^{++} or $^{+++}$	0.025	+24
5	Cu^{++}	1	+6
6	Ti^{+++}[a, c]	5	±0
7	Ba^{++}	200	±0
8	Ca^{++}	200	±0
9	Mg^{++}[d]	200	±0
10	Ni^{++}	1	+200
11	Pb^{++}	1	+13
12	Co^{++}	1	Intense red
13	Cd^{++}[b]	50	±0
14	Mn^{++}[e]	50	±0
15	Cr^{+++}[f]	10	Precipitate

[a] Complex with 3 ml. of 10% tartaric acid per 50 mg.
[b] Add 8 ml. of 10% ammonium thioglycolate.
[c] Add 4 ml. of 10% ammonium thioglycolate.
[d] Add excess ammonium chloride.
[e] Allow to stand 10 minutes.
[f] Precipitate forms in ammoniacal solution. Chromium is not complexed by 10 ml. of 10% tartaric acid.

Interference of Iron. At 380 mμ an iron solution absorbs approximately five times as strongly as a solution of uranium of equal concentration. Because iron is a frequent contaminant in many materials, a method whereby it might either be removed or tolerated is desirable. One technique which might be employed is to make use of the mixed colors formed when ammonium thioglycolate is added. The iron thioglycolate complex absorbs fairly strongly at 380 and also at 600 mμ (Figure 1). The uranium complex also absorbs at 380 mμ but not at 600 mμ. The concentration of iron present in a solution may thus be estimated by a spectrophotometric measurement at 600 mμ. The optical density of this concentration of iron at 380 mμ may be calculated from a calibration curve and subtracted from the total density of the iron-uranium solution at 380 mμ. Values for uranium obtained by this method proved to be accurate (Table III).

DISCUSSION

The pH range for optimum color development may extend beyond 7.6 to 10.7, the limits investigated. Adjustment of the solution to this range is easily made and considerable latitude is permitted in the addition of ammonium hydroxide before an appreciable change in pH occurs.

The color will fade if too large an excess of tartaric acid is used to prevent cation precipitation. Satisfactory results are obtained if the tartaric acid added is equal to three or four times the weight of the cation as its oxide. This is an agreement with Hillebrand and Lundell (*2*).

Table III. Estimation of Uranium in Uranium-Iron Solutions Using Mixed Color Method

U Present, Mg.	Fe Added, Mg.	U Found, Mg.	Error, %
0.530	0.025	0.540	+1.9
0.424	0.050	0.420	−0.9
0.318	0.100	0.315	−0.9
1.060	0.100	1.080	+1.9
1.060	0.025	1.060	±0
0.530	0.050	0.530	±0

The mixed color method for solutions containing iron is not recommended where the ratio of iron to uranium is greater than 1 part of iron to 5 parts of uranium. With larger concentration of iron, the compensation for interference becomes greater than 50% of the total value.

SUMMARY

Ammonium thioglycolate may be used for the accurate estimation of small amounts of uranium in the presence of a relatively large number of anions. The method is not specific for cation solutions but, if necessary, certain of the cation interferences may be eliminated.

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Spectrographic Determination of Impurities in Beryllium and Its Compounds

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A method is described for the simultaneous quantitative spectrographic determination of aluminum, calcium, chromium, iron, manganese, magnesium, and silicon in beryllium metal and its compounds. The procedure involves the conversion of the sample to beryllium oxide, the addition of graphite powder, tin oxide, and barium hydroxide to the beryllium oxide, and the excitation of this mixture in a high amperage direct current arc. The mixture that is added to the beryllium serves the dual purpose of stabilizing the arc discharge and providing for good internal standard compensation of excitation variables. The experimental error is about ±5% of the amount present.

BERYLLIUM metal and its oxide are finding increasing uses in various industrial (*12, 18, 28*) and nuclear energy (*25*) applications, but little is known about the effects of impurities on the physical properties of these materials (*9, 14, 18, 28*). No analytical methods that are directly applicable to the determination of the common impurities in beryllium-base materials have been reported in the literature. Because spectrographic methods are particularly useful for the simultaneous determination of a number of impurities, it seemed appropriate to investigate the application of this technique to these analyses.

The carrier distillation technique (*23, 24, 26*) has been applied to the spectrographic analysis of beryllium oxide, but this method is not applicable to the determination of important impurities such as calcium, aluminum, and silicon, whose oxides are too refractory to be vaporized satisfactorily during the carrier distillation. This technique is also encumbered by the influence of the manner of preparation of the oxide matrix on the per cent of the impurity which is volatilized and excited during the carrier distillation (*2*).

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EXPERIMENTAL

Reagents. In any analysis for small amounts of common impurities, contaminations from manipulations and reagents becomes a major problem. All chemical work was therefore carried out in platinum ware and only purified acids and ammonium hydroxide were used. The purified hydrochloric acid and ammonium hydroxide were obtained by passing the respective gases into distilled water in quartz containers. The nitric acid was distilled in a quartz distilling apparatus and was stored in quartz containers.

Apparatus. The following equipment was used in this investigation: a Jarrell-Ash, Wadsworth mounting, 21-foot grating spectrograph (*11*), a water-cooled excitation stand (*22*), a 250-volt direct current arc source of 20-ampere capacity (*8*), an A.R.L.–Dietert developing machine (*21*), and an A.R.L.–Dietert densitometer (*7*). The external optical system used with the spectrograph has been described (*8*).

Preliminary Experiments. The desirability of developing an analytical method applicable to the analysis of both beryllium metal and its compounds required that the samples be excited in a form which could be easily obtained from the metal, oxide, or other compounds. Beryllium oxide best satisfied these requirements.

Table I. Operating Conditions for Analysis of Beryllium Oxide

Composition of sample charge	1.0% tin oxide 10.0% barium hydroxide 33.3% graphite 55.7% beryllium oxide
Weight of charge	15 mg.
Type of electrodes	Anode (lower electrode) 0.25-inch diameter, graphite, 0.75 inch long, drilled to wall depth of 2 mm. with wall thickness of 0.5 mm. Supported on 0.125-inch diameter graphite pedestal[a] Cathodes (upper electrodes) 0.125-inch diameter graphite, 1 inch long and pointed
Analytical gap	4 mm.
Excitation source	250 volt d.c., 16 amperes
Duration of excitation	20 seconds
Wave length photographed	2750–4000 Å.
Order	2nd
Slit	0.05 mm.
Sector apertures	8-step sector pattern
Plate emulsion	SA No. 1
Development	4 minutes at 21° C. in Eastman D-19

[a] Fabricated from National Carbon Company special spectroscopic graphite rods.

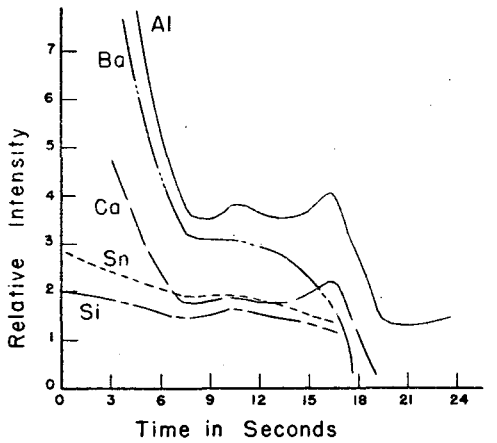

Figure 1. Distillation Rates of Impurities from Beryllium Oxide–Graphite Mixtures

The direct current arc has been the most convenient excitation source for the analysis of inorganic materials, but the poor reproducibility usually associated with this discharge has greatly limited its use for quantitative applications. This poor reproducibility arises chiefly from the large effective excitation temperature variations produced by wandering of the cathode spot and general arc instability, and the fractional distillation effects which institute large variations in the analytical intensity ratio during the excitation unless internal standards with almost identical vaporization characteristics are utilized. The purpose of the preliminary experimental work was to establish the experimental conditions necessary to obtain a stabilized arc of reproducible character and to find suitable internal standards whose vaporization and excitation response were similar to the impurities to be determined.

Direct current arcs of currents as high as 18 amperes between graphite electrodes did not provide an electrode temperature high enough to vaporize an amount of the beryllium oxide from the electrode cavities to stabilize the discharge. Furthermore, the formation of a mobile bead of the oxide caused frequent ejection of the material from the electrode cavity. The admixture of powdered graphite satisfactorily eliminated the formation of mobile beads but still did not achieve the desired stabilized excitation conditions. Moving plate spectrograms (*19*) showed that the impurities under consideration vaporized uniformly during the initial period of the arcing cycle, while the beryllium was vaporized during the entire period the sample was being consumed in the arc. These large differences in the vaporization behavior of the beryllium and the impurities to be determined eliminated the use of beryllium lines for internal standard purposes. This observation led to further studies on arc stability and rates of vaporization of mixtures of beryllium oxide, powdered graphite, and oxides or hydroxides of other metals whose determination in beryllium was not important. The objectives of this study were to find a metallic oxide or hydroxide which, when added to the beryllium oxide–graphite mixtures, would fulfill the internal standard requirements mentioned previously. No individual oxide possessed these qualifications, but a combination of tin oxide and barium hydroxide appeared to offer the best possibilities. Figure 1 illustrates the rates of vaporization of several of the impurities as compared to the barium and the tin. A detailed study was made to determine the optimum experimental conditions for the excitation of a mixture of beryllium oxide, powdered graphite, barium hydroxide, and tin oxide. The operating conditions that were selected are detailed in Table I.

The graphite–beryllium oxide ratio was not so critical as indicated in the table. The preliminary experiments were performed on mixtures in which the graphite was standardized at 33.3%, while the other components accounted for the remainder. The behavior of the 10% barium hydroxide was similar to a distillation carrier (*23*), as the impurities were virtually completely volatilized within the 18- to 20-second

Table II. Line Pairs Used in Analysis of Beryllium Oxide

Line Pair *Å.*	Concentration Range *P.p.m.*	Excitation Potential *Electron volts*
Al I 3961.527 / Ba I 3993.404	50–2500	3.1 / 4.3
Ca II 3179.332 / Sn I 3141.809	100–5000	13.1 / 6.1
Cr II 2843.252 / Sn I 2913.542	50–1000	12.6 / 6.4
Fe I 2966.900 / Sn I 3032.775	90–500	4.4 / 6.2
Fe I 2813.288 / Sn I 2913.542	500–5000	4.2 / 6.4
Mg I 2781.417 / Sn I 2913.542	100–5000	7.2 / 6.4
Mn II 2949.209 / Sn I 2913.542	50–1000	12.8 / 6.4
Mn I 2801.064 / Sn I 2913.542	5–60	4.3 / 6.4
Si I 2881.578 / Sn I 2850.618	50–5000	5.1 / 5.4

Table III. Precision Data on a Typical Sample

Plate No.	Plate Type	Intensity Ratios Al/Ba	Ca/Sn	Cr/Sn	Fe/Sn[a]	Mn/Sn[b]	Mg/Sn	Si/Sn
3606	SA 1	3.32	2.14	0.45	0.72	1.37	1.47	1.77
		3.45	2.31	0.47	0.74	1.40	1.49	1.77
		3.69	2.21	0.43	0.72	1.29	1.43	1.85
3635	SA 2	..	2.29	0.50	0.72	1.40	1.41	1.64
		..	2.27	0.48	0.71	1.39	1.49	1.56
3642	SA 1	3.40	2.42	0.50	0.77	1.38	1.36	1.79
3643	SA 1	3.55	2.27	0.53	0.82	1.33	1.42	1.81
		3.50	2.37	0.51	0.78	1.39	1.47	1.69
3644	SA 1	3.28	2.75	0.51	0.79	1.39	1.56	1.61
		3.85	2.45	0.48	0.74	1.33	1.29	1.83
3649	SA 1	3.40	2.50	0.51	0.79	1.40	1.64	1.79
		3.97	2.45	0.49	0.73	1.40	1.55	2.00
3652	3–0	..	2.48	0.44	0.67	1.38	1.46	1.73
		..	2.66	0.52	0.77	1.37	1.49	1.60
		..	2.71	0.53	0.80	1.41	1.56	1.76
Mean % deviation		5.1	5.8	5.1	4.7	1.7	4.3	4.8

[a] Line pair Fe 2813.3 / Sn 2913.5

[b] Line pair Mn 2801.1 / Sn 2913.5

period during which the barium was vaporizing and stabilizing the arc discharge (Figure 1). The tin oxide was added solely as an internal standard; its concentration was determined by line intensity relationships. No significant variation of the integrated intensity ratios was observed when the arcing current was varied between 14 and 18 amperes, and when the charge weight was varied between 10 and 20 mg.

In selecting the line pairs, consideration was given to their freedom from interfering lines, intensity-concentration relationship, excitation potentials, and proximity to the other lines being measured. A tabulation of the wave lengths (*10*) of the selected line pairs, the concentration range for which they are applicable, and their excitation potentials (*6, 10, 13, 15, 16, 20*) is found in Table II. Several line pairs such as $\frac{\text{Ca II } 3179.332 \text{ Å.}}{\text{Sn I } 3141.809 \text{ Å.}}$, $\frac{\text{Cr II } 2843.252 \text{ Å.}}{\text{Sn I } 2913.542 \text{ Å.}}$, and $\frac{\text{Mn II } 2949.209 \text{ Å.}}{\text{Sn I } 2913.542 \text{ Å.}}$ show unfavorable excitation potential relationships; the choice of these pairs was dictated by the other considerations. The data in Table III show that the excitation fluctuations in the light source were sufficiently reduced so that the reproducibility for the line pairs with unfavorable excitation characteristics was not seriously impaired.

Quantitative Calibrations. The synthetic standards for all the impurities except silicon were prepared by dissolving weighed amounts of distilled beryllium basic acetate (*1, 27*) in 1 to 1 distilled nitric acid and then adding the proper volumes of standard solutions of the various impurities. The solutions were evaporated to dryness and ignited to the oxides in a muffle furnace at 700° C. The silicon standards were prepared by grinding pure beryllia with silica in a boron carbide mortar and making successive dilutions of this mixture. The other mixture components were incorporated with all the standards by dry grinding in a boron carbide mortar. These standards were then excited and photographed under the conditions detailed in Table I.

The per cent transmission data from the densitometric measurements were converted to intensity ratios in the usual manner (*4, 17*). Residual corrections, if necessary, were made by the zero-intercept method of Cholak and Story (*3*). Figure 2 illustrates the working curves obtained by plotting log intensity ratio against log concentration. The aluminum and silicon curves were found to level out at higher concentrations because of self-reversal of the lines, but no suitable alternative lines could be found in the wave-length region covered.

The residual correction mentioned above included the total concentration of the various impurities in the base beryllium basic acetate and in the mixture (hereafter called graphite mixture) of powdered graphite, barium hydroxide, and tin oxide. The small amount of the impurities present in the graphite mixture became significant when lower concentration of the impurities were measured in actual samples. Because the barium hydroxide and the graphite largely characterized arc discharge, it was found possible to make a sufficiently accurate determination of these impurities in the graphite mixture by exciting the latter under the usual experimental conditions and determining the concentration from the extrapolated working curves. These concentrations were then subtracted from the respective values obtained when samples were analyzed.

Table IV. Comparison of Spectrographic and Spectrophotometric Values for Iron and Manganese

Sample	Spectrographic	Spectrophotometric	Difference
	P.p.m.	*P.p.m.*	%
	Iron		
MS-BL	560	600	6.9
MS-BI	2880	2880	0.0
MS-BF	620	640	3.2
MS-BO	240	230	4.2
		Av.	±4.7
	Manganese		
MS-BL	78	79	1.3
MS-BI	97	99	2.0
MS-BF	36	35	2.9
MS-BO	<4	0.9	...
		Av.	±2.1

Procedure for Analyzing Samples. Beryllium metal is converted to the oxide by cautiously dissolving the metal in purified dilute hydrochloric acid, adding an excess of purified ammonium hydroxide, evaporating the slurry to dryness, and igniting it at 700° C. for 1 hour. Samples of the halide salts are readily converted to the oxide by pyrohydrolysis (*5*), while nitrate and hydroxide samples are converted to oxide by direct ignition. The resulting oxide is ground in a boron carbide mortar with the proper amount of previously prepared graphite mixture and charges of 15 ± 1 mg. are weighed into the electrodes and excited as detailed above. The concentrations of the impurities are then determined from the working curves in the usual manner. The correction for the impurities in the graphite mixture is subtracted from the calculated percentage.

DISCUSSION OF RESULTS

Precision data obtained from repeated analysis of the same sample are shown in Table III. Several of the elements which

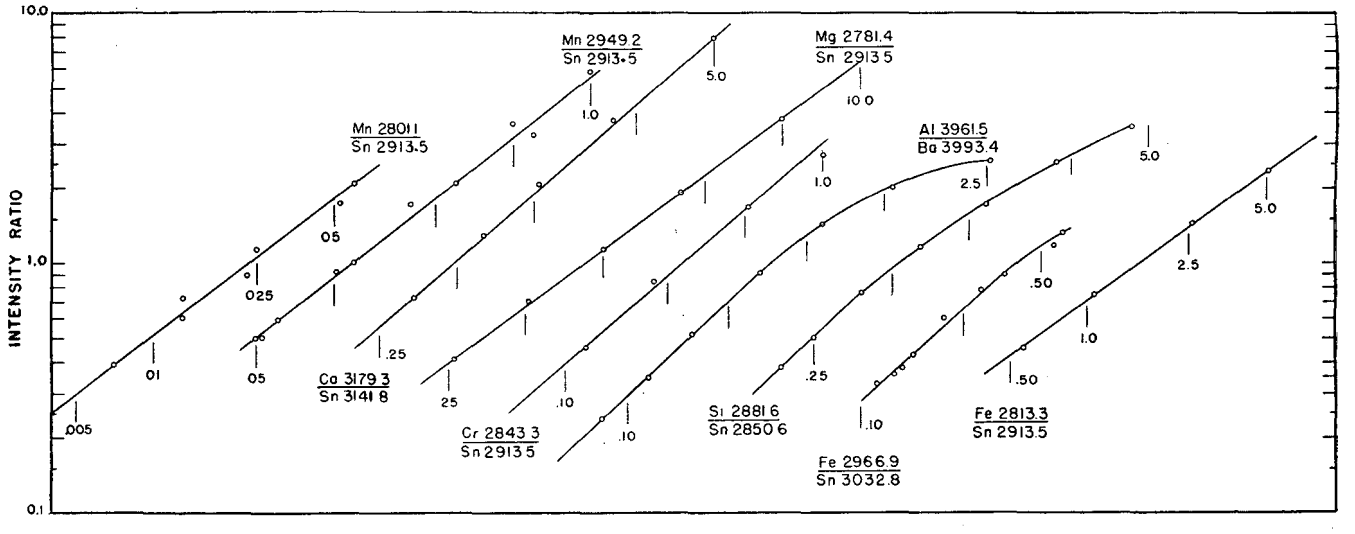

Figure 2. Calibration Curves for Analysis of Beryllium Oxide

Table V. Data from Recovery Experiments

Element	Amount Added	Amount Found	Error
	P.p.m.	*P.p.m.*	%
Cr	200	200	0.0
Fe	400	410	2.5
Mg	300	295	1.7
Mn	80	80	0.0
Si	2720	2730	0.4

could be checked spectrophotometrically were determined by the chemical analytical group, and the results from the two procedures are compared in Table IV. Included in Table V are typical results from recovery experiments in which known amounts of the impurities in question were added to a beryllium oxide base which had been previously analyzed. The results in Tables III, IV, and V indicate that adequate accuracy and precision can be achieved by this method.

Table III shows that the intensity ratios did not change significantly when the emulsion type was varied. It is therefore possible to increase the sensitivity for most elements by using faster plates. The concentration range can also be increased by using a low contrast plate such as Eastman Spectrum Analysis No. 2. The high dispersion of the spectrograph used in this investigation is not an essential requirement for these analyses; the method should therefore be adaptable to any instrument of moderate dispersion. It should also be possible to extend the method to the determination of most of the metallic impurities in beryllium and its compounds.

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Colorimetric Determination of Columbium and Tungsten in High-Temperature Alloys

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THE authors recently had occasion to analyze high-temperature alloys containing both columbium and tungsten. The separation of these elements by classical methods was a difficult and tedious procedure. Because tungsten accompanied columbium in precipitation reactions, it was most difficult to obtain a clean-cut separation of these elements, in one precipitation.

Hillebrand and Lundell (*7*), referring to the separation of the mixed oxides of columbium and tungsten, state, "Extraction of the mixed oxides with ammonium hydroxide or ammonium sulfide, or extraction with water after a fusion with sodium carbonate and sulfur are all unsatisfactory, as are also the boiling of an alkaline solution of the tungstate, columbate, and tantalate with ammonium nitrate or treatment of the alkaline solution with magnesia mixture." Referring to the separation of columbium from tungsten, using cupferron in a tartaric acid–sulfuric acid solution, they maintain, "Tungsten is partially precipitated no matter how much sulfuric and tartaric acids are used."

To avoid such lengthy and tedious separations with their inherent possibilities of error, attention was turned to eliminating separations of columbium from tungsten by determining both elements colorimetrically from the same solution. A solution of columbium and tungsten in concentrated sulfuric acid (*11*, *12*) was found well suited for such colorimetric determinations.

In accordance with the work of Klinger and Koch (*8*), columbium was determined in an aliquot of this solution by means of the yellow percolumbic acid produced by hydrogen peroxide. Titanium interfered by also producing a yellow color with hydrogen peroxide in concentrated sulfuric acid. Thanheiser (*17*) mini-

A method is presented for the colorimetric determination of moderate quantities of columbium and tungsten in high-temperature alloys. Columbium is determined as the yellow percolumbic acid produced by the action of hydrogen peroxide upon solutions of columbium in concentrated sulfuric acid. Tungsten is determined as the yellow thiocyanate resulting from the action of stannous chloride and potassium thiocyanate upon solutions of tungsten. The interferences of other elements found in high-temperature alloys are discussed. The method is rapid and the accuracy is equal to that of gravimetric procedures.

mized this interference by the use of phosphoric acid. In the present work, this interference of titanium was also reduced but without the use of phosphoric acid.

Then, based upon the investigations of Feigl and Krumholz (*2*), a method was developed for determining tungsten from another aliquot of the same solution. This was accomplished by means of stannous chloride and potassium thiocyanate, which produce the yellow tungsten thiocyanate in a lower valence state. Various modifications of the Feigl and Krumholz procedure have been reported (*3, 4, 6, 13, 14–16, 18, 20*). In these investigations potassium thiocyanate was first added to the tungsten solution, then stannous chloride. (Some workers preferred using titanous chloride instead of stannous chloride to hasten the color development.) Gentry and Sherrington (*5*) found that by employing these methods little precision was obtained, as the tungsten was not reduced to a definite valence state by the stannous chloride prior to the formation of the thiocyanate complex. They modified the procedure of Feigl and Krumholz (*2*) by first reducing the tungsten to a definite lower valence state by stannous chloride solution and tin amalgam, and obtained good precision. However, columbium was also reduced and formed an intense yellow color with potassium thiocyanate.

The present authors also observed that when potassium thiocyanate was added to the tungsten solution, followed by stannous chloride, even under exactly identical and rigorously controlled conditions the colors obtained varied from a bright yellow to a yellowish green, with correspondingly varying colorimeter readings. When, however, the tungsten solution was first boiled with stannous chloride for a minute and cooled, and then potassium thiocyanate was added to the cold solution, excellent precision was obtained and the color at all times remained a bright yellow. Here the tungsten was also reduced to a definite valence state. However, unlike the method of Gentry and Sherrington (*5*), the columbium was not reduced and therefore did not interfere with the colorimetric determination of tungsten.

The proposed procedure for the colorimetric determination of columbium and tungsten is comparatively rapid and involves but two separations. First, the oxides of columbium, tungsten, silicon, some molybdenum, and some titanium are separated by fuming with perchloric acid, diluting, boiling with sulfurous acid to hydrolyze the columbium (*1*), and then treating with cinchonine to effect a more complete recovery of tungsten (*1*). Secondly, after solution of the mixed oxides in concentrated sulfuric acid, the solution is diluted with tartaric acid solution and the molybdenum is precipitated with hydrogen sulfide (*19*). The filtrate, containing columbium, tungsten, and some titanium, is taken to fumes of sulfuric acid, first destroying the tartaric acid. Without further separations, tungsten and columbium are then determined colorimetrically in aliquot portions of the resulting sulfuric acid solution.

APPARATUS

A photoelectric colorimeter such as the Klett-Summerson, with a 420 millimicron filter and a test tube cell, was used.

SPECIAL REAGENTS

CINCHONINE SOLUTION. Dissolve 125 grams of cinchonine alkaloid crystals in 200 ml. of dilute hydrochloric acid (1 to 1 by volume).

CINCHONINE WASH. Dilute 30 ml. of cinchonine solution to 1 liter with distilled water.

STANNOUS CHLORIDE REAGENT. Dissolve 50 grams of C.P. stannous chloride crystals in 100 ml. of hydrochloric acid (38%). Warm if necessary. The solution must be clear. Dilute to 500 ml. with hydrochloric acid (38%).

POTASSIUM THIOCYANATE REAGENT. Dissolve 25 grams of C.P. potassium thiocyanate crystals in distilled water to make 500 ml. of total solution.

PROCEDURE

A. Solution of Sample and Separation of Columbium, Tungsten, and Other Acid-Insoluble Constituents. Weigh a 2.5-gram sample of the alloy into a 600-ml. beaker, if the columbium content is under 1.5% and the tungsten content is under 2.0%. (Should the columbium or tungsten content of the metal be over 1.5% or 2.0%, respectively, reduce the sample size accordingly.) Dissolve the sample with 50 ml. of a mixture of 1 volume of hydrochloric acid (38%), 1 volume of nitric acid (70%), and 2 volumes of distilled water. Carry along a reagent blank treated exactly like the sample in this and succeeding operations. Add 35 ml. of perchloric acid (71%) and heat until the perchloric acid begins to reflux. Cover and heat so that the perchloric acid refluxes 20 to 30 minutes. Cool, dissolve with 50 ml. of distilled water, add 50 ml. of saturated sulfurous acid and a small quantity of paper pulp, and dilute to 300 ml. with distilled water. Stir and boil gently 10 to 15 minutes. Add 10 ml. of cinchonine solution, stir, and digest on the steam bath for 1 to 2 hours. Remove from the steam bath and let stand 24 hours. Filter through a No. 40 Whatman filter paper, washing with warm cinchonine wash. Discard the filtrate.

B. Solution of Columbium and Tungsten in Residue. Ignite the precipitate in a platinum dish at 600° C. To remove silica, add 3 drops of sulfuric acid (1 to 1) and 5 ml. of hydrofluoric acid, evaporate, and reignite at 600° C. for 10 minutes. Fuse with 5 grams of sodium bisulfate (fused), cool, add 4 ml. of sulfuric acid (97%), heat to fumes, then cool.

C. Removal of Molybdenum. Pour the cooled solution obtained above into 40 ml. of tartaric acid solution (10%), rinsing the dish with an additional 10 ml. of tartaric acid solution. Stir to effect solution and cool. Add 6 ml. of ammonium hydroxide (29%), dilute to 100 ml. with distilled water and boil for 1 minute. Pass in hydrogen sulfide for 30 minutes. Add 100 ml. of boiling distilled water and pass in hydrogen sulfide for 5 minutes more. Add a little paper pulp and digest on the steam bath for 1 hour, keeping the solution covered. Filter through a No. 40 Whatman filter paper into a 1-liter tall-form beaker. Wash with sulfuric acid (1 to 99) saturated with hydrogen sulfide. Discard the precipitate.

D. Destruction of Tartaric Acid and Preparation of Master Sulfuric Acid Solution. Evaporate the filtrate obtained in Section C to approximately 50 ml., and add 150 ml. of sulfuric acid (97%) and 30 ml. of nitric acid (70%). Stir well. Using moderate heat, evaporate until sulfuric acid fumes appear or the tartaric acid just begins to char. Cool, add 30 ml. of nitric acid (70%), and evaporate again as above. Cool and add 15 ml. of perchloric acid (71%). Rinse down spray with distilled water. Evaporate until fumes of sulfuric acid appear and continue the evaporation with continuous strong fuming (250° C. or higher) until a volume of 125 ml. is reached. Cover with watch glass. Allow to cool at

Table I. Accuracy of Proposed Procedure

Composite Standard	N.B.S. 101a	N.B.S. 50a	N.B.S. 123a	N.B.S. 153	N.B.S. 116a	Columbium Present	Columbium Found	Tungsten Present	Tungsten Found
	Grams	*Gram*	*Grams*	*Grams*	*Gram*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	...	...	2.50	...	...	18.8	19.2	2.8	2.8
2	1.77	0.061	0.67	...	...	5.0	5.0	11.8	12.2
3	1.05	0.120	1.33	...	...	10.0	10.2	23.3	23.3
4	0.32	0.180	2.00	...	...	15.0	15.2	35.0	35.0
5	...	0.240	2.67	...	...	20.0	20.2	46.8	47.0
6	...	0.061	3.33	...	...	25.0	24.5	14.8	14.8
7	...	0.120	4.00	...	...	30.0	30.0	26.3	26.7
8	...	0.180	4.67	...	...	35.0	34.8	38.0	38.0
9	...	...	2.50	2.50	0.20	18.8	18.6	42.3	41.6

Nominal composition of standard samples used:

N.B.S. 101a. 18.33% Cr, 8.99% Ni
N.B.S. 50a. 3.52% Cr, 0.97% V, 18.25% W
N.B.S. 123a. 0.12% Mo, 0.037% V, 0.75% Cb, 0.02% Ta, 0.11% W
N.B.S. 153. 4.14% Cr, 8.36% Mo, 2.03% V, 8.43% Co, 1.58% W
N.B.S. 116a. 0.33% V, 3.25% Al, 25.08% Ti

Where weight of composite standard was over 2.5 grams (weight recommended in procedure), quantities of reagents specified in Section A of procedure were increased proportionately.

room temperature for 0.5 hour and then transfer to a dry 200-ml. volumetric flask, rinsing the beaker with sulfuric acid (97%). Dilute to exactly 200 ml. with sulfuric acid (97%), stopper, and mix. This solution is hereafter referred to as the master solution.

E. Colorimetric Estimation of Columbium. Transfer 100 ml. of the master solution to a dry 100-ml. volumetric flask, add 0.10 ml. of 30% C.P. hydrogen peroxide, and mix well. Let stand 10 minutes at room temperature. Using a 420-mμ filter, compare the color intensity of the test solution against a portion of the same master solution, containing no hydrogen peroxide. Find the columbium content by reference to a graph prepared from similarly processed National Bureau of Standards steels and by application of corrections for the interference due to tungsten and titanium. To correct for the interference of tungsten deduct 0.43 mg. of columbium for each 25 mg. of tungsten in the sample.

To correct for the interference of titanium deduct 1.38 mg. of columbium for each 10 mg. of titanium present in the 100-ml. aliquot for columbium. The titanium present is determined as follows:

Dilute 10 ml. of the master solution to 100 ml. with distilled water, add 0.5 ml. of 30% C.P. hydrogen peroxide, and mix. Compare the test solution of the sample against the reagent blank solution in the photoelectric colorimeter using a 420-mμ filter. Find the weight of titanium in a 100-ml. aliquot of the master solution by reference to a graph based upon known titanium solutions.

F. Colorimetric Estimation of Tungsten. Transfer 5 ml. of the master solution to a dry 100-ml. long-necked volumetric flask which is used to minimize evaporation losses. Add exactly 20 ml. of sulfuric acid (1 to 3) and then exactly 10 ml. of stannous chloride reagent. Cover with a small watch glass and boil gently for 1 minute. Cool in air 2 minutes and then chill to 13° to 17° C., keeping the flask stoppered. Add exactly 10 ml. of potassium thiocyanate reagent and swirl well. Let stand at the above temperature for 15 minutes. Without further dilution, compare the test solution of the sample against the reagent blank in the photoelectric colorimeter, using a 420-mμ filter. Do not allow the temperature of the solution in the colorimeter cell to rise above 20° C. Obtain the per cent of tungsten from a graph based upon similarly processed National Bureau of Standard steels.

DISCUSSION

Mellor (*11*) has shown that columbic oxide is soluble in concentrated sulfuric acid. Noyes (*12*) described the solubility of tungstic oxide in concentrated sulfuric acid. Accordingly, in the proposed procedure the perchloric acid–insoluble compounds of columbium and tungsten are dissolved by means of sulfuric acid.

In the colorimetric determination of columbium, all traces of nitric acid must be absent; if present, it will cause a rapid bleaching of the percolumbic acid color. The nitric acid is effectively removed by fuming the sulfuric acid solution in the presence of perchloric acid.

It was found that 0.1 ml. of 30% hydrogen peroxide is sufficient to develop fully the color of 30 mg. of columbium in 100 ml. of concentrated sulfuric acid. A larger quantity of hydrogen peroxide would increase the titanium interference as described below.

The columbium color develops fully after 10 minutes and once developed is stable up to 1 hour. Temperature variations (10° to 30° C.) do not affect the intensity of the developed color. Contrary to the observations of Thanheiser (*17*), who stated that tungsten does not produce any color with hydrogen peroxide in concentrated sulfuric acid, it was found that tungsten produces a slight yellow color, which is $^1/_{60}$ as intense as that of an equal concentration of columbium. A small correction is therefore applied in the final calculation of the columbium content of the sample. Up to 19 mg. of columbium in 100 ml. of the master solution may be determined by this method.

Titanium also produces a yellow color with hydrogen peroxide in concentrated sulfuric acid (*8*). Where more than 0.10% of titanium is present in the sample, a fraction of it will be precipitated with the columbium and tungsten in the initial separation (*10*) and will interfere in the colorimetric estimation of columbium. The intensity of the titanium color with hydrogen peroxide in concentrated sulfuric acid was found to increase with the concentration of hydrogen peroxide. It was, therefore, advantageous to use as small a quantity of hydrogen peroxide as practicable which would at the same time fully develop the columbium color. The most suitable volume of 30% hydrogen peroxide was found to be 0.10 ml. Here the titanium color is $^1/_{15}$ as intense as that of an equal concentration of columbium. Inasmuch as the intensity of the titanium color with hydrogen peroxide in concentrated sulfuric acid increases also with the amount of water present (*8*), it is imperative that the dehydration of sulfuric acid be accomplished as effectively as possible by strong fuming (250° C. or above). In determining the titanium content of the master solution for the purpose of applying a correction to the columbium content (see Section E), the solution is diluted 10 times with water. Hydrogen peroxide is then added and the titanium is determined colorimetrically. In the concentrations employed columbium and tungsten do not produce a color (*9*), nor do they precipitate.

In the colorimetric determination of tungsten, it was found that the color developed fully within 15 minutes at 15° C. and remained stable at this temperature for 2 hours. At higher temperatures, however, the maximum color was not developed and the yellow color faded slowly, once developed. Columbium and titanium do not interfere. Up to 25 mg. of tungsten in 100 ml. of the master solution may be determined by this method.

Molybdenum is retained to a large extent with the columbium and tungsten in the initial separation (*9*) and would interfere in the determinations of both columbium (*17*) and tungsten (*6*, *15*), if provision were not made for its removal.

Because some tungsten may accompany the molybdenum in the hydrogen sulfide separation previously described, the tungsten may be recovered by a reprecipitation of the molybdenum sulfide, where higher accuracy is desired.

Iron, manganese, phosphorus, chromium, nickel, cobalt, and vanadium are not retained in the initial separation in quantities that would interfere with the colorimetric estimation of columbium or tungsten. Tantalum and zirconium, although precipitated in the initial separation, do not interfere in the determination of either columbium or tungsten (*8*, *17*, *18*).

The above-described method of analysis is limited to high-temperature alloys containing up to 6% of columbium and tungsten. Various National Bureau of Standards samples and synthetic mixtures of these samples were analyzed by this method (Table I).

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Penetration of Sintered Metals by Solutions of Surface-Active Agents

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A method was devised to give an indication of the comparative wetting and penetrating qualities of surface-active agents. A definite volume (30 ml.) of surface-active agent solution is added to the most porous of a series of five stainless steel filtering crucibles with porous, sintered, stainless steel filter elements of varying porosities. A record is made of the time in seconds required to deliver the first drop and the first milliliter of solution. Based on the time for delivery under each category, a rating is given, and the comparative wetting and penetration of each surface-active agent studied are evaluated. The same method could be applied for copper, bronze, medium steel, glass, canvas, cloth, and other textile materials. This technique could also be used in research on metal cleaning or lubrication problems.

ONE of the most convenient and useful methods for determining penetration and wetting power of surface-active agents is that of Draves and Clarkson (*1*), commonly referred to as the Draves sinking time test. This method consists of determining the time required to sink a standard weighted cotton skein to the bottom of a solution contained in a standard cylinder.

Although this is satisfactory for evaluating wetting power and penetration in the textile industry, it is not applicable to metal surfaces. Therefore, a test method was devised, as part of an investigation of materials for the detection of leaks in pipe lines, for evaluating penetration and wetting properties of surface-active agents on metals. This procedure used stainless steel filtering crucibles with porous, sintered, stainless steel filter elements of varying porosities, and recorded the time required to deliver defi-

Table I. Seepage Data on Wetting Power and Penetration

No.	Surface-Active Agents	Concentration, % by Weight, Fresh Water	α-Seepage Value	α-Seepage Rating	β-Seepage Value	β-Seepage Rating	Total Seepage Rating	Order of Effectiveness for Wetting Power and Penetration	Surface Tension Data[a]
1	Sample A	0.10	87	(1)	178	(2)	(3)	(1)	28.8
2	Sample B	0.20	100	(2)	169	(1)	(3)	(2)	31.6
3	Sample C	0.20	106	(3)	195	(3)	(6)	(3)	31.1
4	Sample D	0.20	145	(4)	215	(4)	(8)	(4)	43.0
5	Sample E	0.20	158	(5)	238	(5)	(10)	(5)	31.7
6	Sample F	0.20	210	(6)	287	(6)	(12)	(6)	29.5
7	Sample G	0.10	304	(7)	404	(7)	(14)	(7)	43.1
8	Fresh water	100.00	355	(8)	425	(8)	(16)	(8)	75.0

[a] Dynes per cm. at 25° ± 1° C.

nite volumes of solution. These readings were used to indicate the comparative effectiveness of wetting power and penetration of the test samples. The action of any surface-active agent is specific for each kind of material (*2*), and comparisons of different wetting agents should be made under the conditions of intended use.

PROCEDURE

The order of comparative effectiveness of various surface-active agents, as shown in Table I, was determined by obtaining "seepage values," using in series five Micro Metallic stainless steel filtering crucibles (Emil Greiner Company, New York, N. Y.), 32 mm. in diameter, 48 mm. high, and with a capacity of 30 ml. each, with porous, sintered, stainless steel filter elements welded into the base. The filter elements contained about 35 to 40% voids, and the average pore size of each element in each crucible was: 65 microns (D crucible), 35 microns (E crucible), 20 microns (F crucible), 10 microns (G crucible), and 5 microns (H crucible). The crucibles were nested in a vertical position so that the most porous or D crucible was uppermost, followed in order by the E, F, G, and H crucibles. These were placed on a graduated cylinder of 10-ml. capacity with 0.1-ml. subdivisions and a glass funnel was used to direct the delivery of solution into the cylinder.

Thirty milliliters of the various surface-active agent solutions at room temperature (21° C.) were poured into the D crucible, which was immediately covered with a watch glass, and the time in seconds was recorded when the first drop left the bottom of crucible H, and when the first milliliter of solution was delivered in the graduated cylinder. The time in seconds required to deliver the first drop of solution was designated as "α-seepage value," and the time in seconds to deliver the first milliliter of solution was designated as "β-seepage value." The various surface-active agent solutions were then given a numerical "seepage rating" under each category, so that the one requiring the least time for delivery had a seepage rating of 1 (the most effective), the one requiring the next lower time a seepage rating of 2 (the next most effective), and so forth. The numerical seepage ratings under each category were then totaled, and designated as a "total seepage rating." The surface-active agent solution with the lowest numerical total seepage rating was considered the most effective; the next lowest, the next most effective. In cases where the total seepage ratings were equal, the solution with the most effective α-seepage rating was given preference in effectiveness of wetting power and penetration. With samples A and B as shown in Table I, although the total seepage rating is 3 for both samples, sample A was designated as more effective because it possesses the more effective α-seepage rating.

Between tests, each crucible was thoroughly washed with water at a temperature of approximately 135° F., dried with a towel, thoroughly rinsed with acetone, and finally dried by air free from moisture and grease, using an air line at approximately 25 pounds per square inch pressure. As an alternative method, the crucibles may be dried for 5 to 10 minutes at 105° C., after the acetone rinse. Several samples were tested using the alternative method of washing, and no significant differences in results were obtained.

RESULTS AND DISCUSSIONS

It is believed that the five crucibles used in the above test give a fair indication of the comparative wetting and penetrating qualities of the various surface-active agents on metals, and are representative of the average conditions that could be encountered in the field. The use of the five crucibles in series, as described, increases the time spread and gives a better evaluation for each of the surface-active agents studied. However, the same method could be used with one, two, or any other combination of crucibles, depending upon the conditions of intended use.

Check results under α-seepage value, as shown in Table I, can be obtained with the average surface-active agent within ± 4 seconds, and under β-seepage value within ± 8 seconds. The results shown in Table I are the average of three readings. The filtering crucibles together with the filter elements used were of stainless steel; the values shown in Table I apply to the wetting and penetration of this metal, which was used to eliminate the possibility of inconsistent results due to corrosion. The method could be applied to filter elements of other metals, such as copper, bronze, medium steel, and glass, and disks of canvas, cloth, and other textile materials could be substituted for the metallic porous sintered filtering elements. By conducting the test in an inert atmosphere such as carbon dioxide or nitrogen, the possibility of corrosion of metals could be controlled. The technique described could also be used in research on metal cleaning or lubrication problems. Table I, which shows the comparative order of effectiveness for wetting power and penetration of the various surface-active agents, also shows surface tension values for each agent. It indicates that there is no direct relationship between surface tension values and wetting power (*2*, *3*).

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Determination of Cottonseed Oil on Tin Plate

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TIN plate, at the time of its production, is coated with a thin film of a vegetable oil in order to inhibit oxidation of the plate during storage and to provide some degree of lubrication for subsequent forming operations. The majority of tin plate produced by the continuous electrotinning process is lubricated with edible grade cottonseed oil, either by spraying with an emulsion or by electrostatic precipitation from an atomized mist. The preferred amount of oil is 0.20 ± 0.05 gram per base box. (A base box is that amount of tin plate which covers an area of 31,360 square inches. The total surface area is thus 62,720 square inches.) A perfectly oriented cottonseed oil film of such a weight is equivalent to approximately five molecular layers on each surface of a plate.

The method generally used in the past for oil determinations on tin plate involved extractions of oil from about 500 square inches (3226 sq. cm.) of plate with acetone or carbon tetrachloride in a Soxhlet extractor, evaporation of the solvent, and weighing of the residual oil. The quantity of oil collected in this manner is normally about 3 to 4 mg. Although satisfactory results are obtainable by this method, the elapsed time is usually 3 hours or more. Because electrolytic tin plate is produced at speeds in the range of 600 to 1000 feet per minute, a test requiring 3 hours cannot be expected to permit adequate control of operations. The method described below permits determination of the amount of cottonseed oil on any spot of electrolytic tin plate within 5 minutes after the plate is produced. When desired, a complete pic-

A method is described for the determination of small amounts of cottonseed oil on tin plate. The tray of a hydrophil balance is modified to permit the complete vertical immersion of samples of tin plate in the water filling the tray. The oil film of the tin plate is transferred to the water surface by repeatedly dipping the sample into the water. The amount of oil thus transferred is then measured by the usual surface balance technique.

ture of the oil distribution, edge to edge and top to bottom, may be completed within 0.5 hour.

APPARATUS

The hydrophil balance as obtained from a supply house was modified slightly for use in this method. A transverse slot, 0.5 inch wide and 3.5 inches long, was cut in the bottom of the aluminum tray and a well, 1.75 inches deep, was attached to the tray under the slot (Figure 1). The balance is prepared for use and operated in the usual manner.

STANDARDIZATION OF COTTONSEED OIL

In order to evaluate the oil film on a sample of tin plate, it is first necessary to determine the characteristics of the original cottonseed oil. A convenient standardizing solution is 0.5 gram of edible grade cottonseed oil in 1 liter of redistilled, chemically pure benzene. Using a capillary pipet calibrated to 0.01 ml., 0.05 ml. of the solution is transferred, drop by drop, to the clean water surface of the balance. The benzene evaporates rapidly, leaving a film of oil on the water. If the sliding barrier is at the extreme left end of the tray, the indicator attached to the torsion wire will show only slight, if any, deflection. By sliding the barrier toward the surface balance in 1-cm. steps and applying a countertorque to balance any deflection of the pointer, a curve similar to that of Figure 2 is obtained. The straight, steep portion of the plot gives the coefficient of compression of the film, and the extrapolation of this line to the axis of zero torque gives the length of the film at zero pressure. The width of the tray is fixed, and the weight of oil film per centimeter of tray length may be calculated. The value thus obtained serves as the basis for subsequent determinations of oil films on tin plate, applied from the tested lot of oil. In tests on numerous lots of edible grade cottonseed oil, the standardization procedure has consistently produced results very closely approximating 1.5×10^{-6} gram per cm. This value has been used for oil from many lots received from several suppliers without introducing appreciable error.

DETERMINATION OF OIL ON ELECTROLYTIC TIN PLATE

After the water surface is cleaned, the movable barrier is placed at the extreme left end of the tray. A sample of tin plate of known area—for example, 4.0 square inches—is held with clean tweezers and passed vertically through the water surface into the well and then withdrawn. This operation is repeated five or six times, allowing about 8 to 10 seconds for each complete cycle. Under normal circumstances all removable oil will be transferred from the metal to the water surface by this procedure. If the standard weight of a compressed cottonseed oil film under zero pressure is known, a curve similar to that in Figure 2 can be obtained and the weight of oil transferred from the tin plate determined with considerable accuracy. Referring to Figure 2, however, it will be noted that the value of the intercept of the extrapolated line with the horizontal axis (28 cm.) corresponds to a value of approximately 3 degrees of torque on the real curve. Observation of many such curves indicated that the shape of this portion of the plot is sufficiently reproducible so that the observed film length, when the pressure exerted against the floating barrier is equivalent to 3 degrees of torque, represents the value that would be obtained from the completed curve. In practice this is done by presetting the vernier to 3 degrees, thus deflecting the indicating needle. The oil film is then compressed slowly with the moving barrier until the indicator returns to the center mark.

From the observed film length, the standard weight of oil per centimeter of tray length, the area of the sample, and the area of a base box of tin plate, only a simple calculation is necessary in order to arrive at the final result in grams per base box. Assuming the standard weight of cottonseed oil to be 1.5×10^{-6} gram per cm., it can be shown that when using a sample having an area of 4.7 square inches (2.17 inches square) the final result can be read directly from the centimeter scale on the tray; 1 cm. is equivalent to 0.01 gram per base box. In the case of a sample having an area of 4 square inches, the observed length of the film in centimeters multiplied by 0.0118 gives grams per base box.

Because the methods of applying oil to electrolytic tin plate do not always provide good transverse distribution of the oil film, it is usually necessary to average the results obtained from at least five samples taken across the width of the tin plate as produced. Distribution of the oil between the top and bottom surfaces of the plate may be determined by coating one side of a sample with paraffin or a quick-drying lacquer and comparing the result with that obtained from an uncoated sample adjacent to the first in the direction of plating.

An alternative procedure is possible whereby the oil can be extracted from a sample of tin plate with benzene. Instead of evaporating and weighing, the solution need only be evaporated to a given volume and a known portion transferred to the hydrophil balance. After the solvent evaporates, the residual oil is deter-

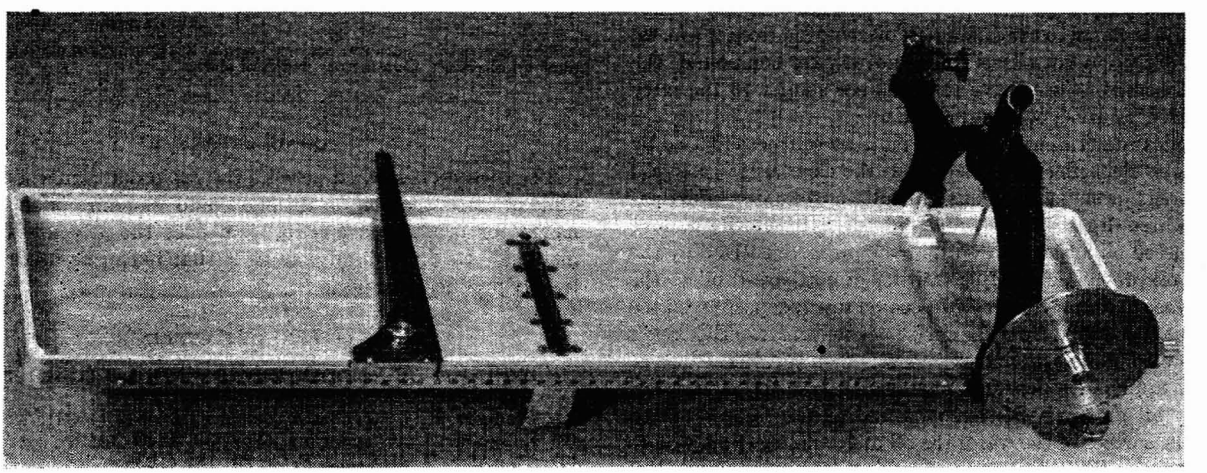

Figure 1. Hydrophil Balance as Modified for Determination of Oil on Tin Plate

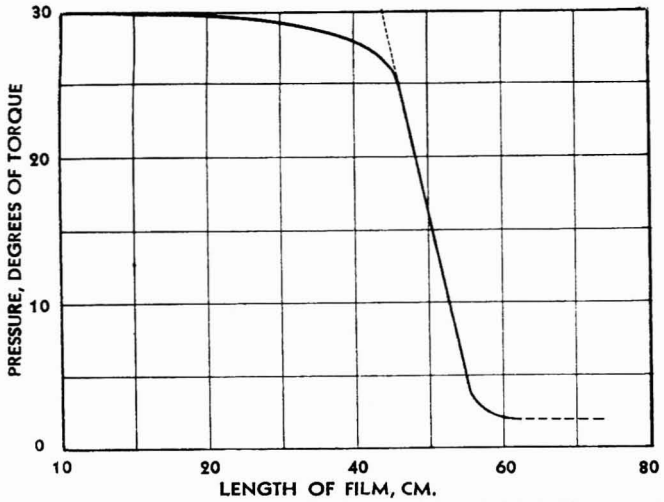

Figure 2. Pressure-Length Curve for 0.000042 Gram of Cottonseed Oil

mined in the usual manner. Satisfactory results have been obtained by this method, but the procedure is less convenient.

RESULTS

Numerous check tests have been made comparing the simplified hydrophil balance method and the usual extraction and weighing procedure. The data in Table I were obtained over a period of 4 days and are typical of the correlation obtained. A sheet was taken from the production units at periodic intervals and samples from the same sheet were used for each comparison. The deviations are reported as departures of the hydrophil values from the extraction values, as the latter method was the established procedure at that time. Consideration of the errors to which the extraction method is subject in the course of a tedious sequence of operations leads to the conclusion that the hydrophil method is fundamentally more accurate.

DISCUSSION

Langmuir (*3*) discussed the properties of monolayers of pure fatty acids and their glyceryl triesters on water surfaces, and Blodgett (*1*) showed that successive oriented monolayers of such materials may be deposited on glass or metal surfaces by repeatedly passing the glass or metal slowly through a water surface covered with a monolayer of the fatty acid under constant pressure. Subsequently, Blodgett (*2*) and Schaeffer (*4*) reported that films of such materials can be removed by passing the coated article through a water surface covered with a heavy, gray film of an "indicator oil." In this procedure the fatty acid flows from the metal to the water surface as a monolayer, pushing back the gray film and leaving a seemingly clear spot, the area of which is directly proportional to the amount of oil removed from the metal surface. Although cottonseed oil is not a pure compound, the individual components behave in a manner similar to the fatty acids studied by Blodgett and Schaeffer.

Attempts at determining oil on tin plate by the method used by Blodgett and Schaeffer were made, but the clear areas produced were of irregular shape and thus difficult to evaluate. Furthermore, repeated dipping of a sample did not satisfactorily remove the cottonseed oil. It appeared that the pressure exerted by the indicator oil prevented the free transfer of cottonseed oil to the water surface and that some cottonseed oil was redeposited on the tin plate as it emerged from the surface of the water. Blodgett's technique for building up multilayers was then reversed—that is, instead of applying a constant pressure against the monolayer, essentially all pressure was removed and as the sample passed through the surface of the water the oil molecules were allowed to spread freely. While this procedure does not remove the last molecular layer of oil from a metal surface, neither does the solvent extraction method. The results tabulated in Table I thus represent only the oil removed from the surface of the tin plate, and a correction factor amounting to approximately 0.05 gram per base box should be added to each value in order to arrive at the true weight of oil originally present.

The hydrophil balance procedure is applicable regardless of the method by which the oil is applied to the tin plate—by emulsion oiling, by electrostatic oiling, or by other means. In so far as could be observed, neither the ease of removal nor the accuracy of the results is affected by the small amount of emulsifying agent (about 1% by weight of the oil) involved in emulsion oiling. The procedure is carried out entirely at room temperatures. Strictly speaking, the pressure exerted by such monomolecular films is a function of temperature, but the error introduced by neglecting the effect of normal fluctuations of room temperature on cottonseed oil is insignificant. There is no marked effect of room temperature variations on the ease with which oil is transferred from the metal surface to the balance.

The usefulness of the hydrophil balance method is limited by the ease with which cottonseed oil oxidizes and polymerizes in air. Edible grade cottonseed oil is composed largely of olein and linolein, and oxidation results in structural changes which tend to produce low results on the hydrophil balance. Extensive oxidation accompanied by polymerization results in hardening of the oil film so that it does not flow from the metal to the water. Oil determinations with the hydrophil balance can be made satisfactorily within 2 hours after the tin plate is produced, but plate retested 4 hours after production has shown oil values 0.05 gram per base box less than when fresh. Aged films may be removed from the metal by solvent extraction, but the polymerized agglomerates of oil are not sufficiently dispersed in the solvent to permit the use of the hydrophil balance for determining the concentration of oil in the solvent.

Table I. Comparison of Hydrophil Balance and Extraction Methods

Test No.	Oil Determination: Hydrophil balance[a]	Oil Determination: Solvent extraction	Deviation[a]
	Gram per Base Box of Tin Plate		
1	0.33	0.38	−0.05
2	0.12	0.10	+0.02
3	0.18	0.16	+0.02
4	0.15	0.15	0.00
5	0.27	0.28	−0.01
6	0.14	0.14	0.00
7	0.11	0.15	−0.04
8	0.13	0.14	−0.01
9	0.15	0.13	+0.02
10	0.11	0.14	−0.03
11	0.16	0.18	−0.02
12	0.07	0.08	−0.01
13	0.13	0.13	0.00
14	0.10	0.07	+0.03
15	0.21	0.16	+0.05
16	0.08	0.08	0.00
		Av. deviation	±0.02

[a] All values for hydrophil balance method are averages of eight determinations on alternate spots across width of sheets.

CONCLUSIONS

The method described permits the rapid determination of thin films of cottonseed oil on tin plate, and therefore offers a better means for production control than does the solvent extraction method. Its chief disadvantage is that it can be used only for freshly produced tin plate.

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Analysis of Gases by Absorption and Combustion

Convenient Apparatus and Procedures

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Convenient, improved methods and apparatus are described for the analysis of fixed and hydrocarbon gases by progressive absorption and combustion. The apparatus is constructed in unit sections which can be assembled in various combinations, thus providing versatility of application. A mercury lift gives definite control of gas displacement and eliminates the tedious use of leveling bulbs. A constant-pressure reservoir confines the gas during complete cycle passages and automatically maintains constant pressure without the use of manually operated leveling bulbs. A specially designed manifold support, along with extensive use of ball and socket joints, gives flexibility and reduces breakage. An innovation described is the use of precipitated copper oxide containing 1% iron oxide for the determination of paraffins by combustion at 700° C. without the addition of air or oxygen. Three methods are described for the analysis of common types of gas mixtures frequently encountered in the petroleum industry, each of which utilizes only one sample portion of gas for the analysis. The first method combines absorption and combustion procedures for determining acid gases, oxygen, nitrogen, hydrogen, carbon monoxide, acetylenes, ethylene, isobutylene, other mono-olefins (propylene plus normal butylenes), and paraffins (methane through butane). The second method covers the determination of alkyl acetylenes, 1,3-butadiene, isobutylene, normal butylenes, and butanes in C_4 hydrocarbon mixtures by selective absorption. The third method gives a procedure for the determination of conjugated diolefins, alkyl acetylenes, tertiary olefins, residual olefins, and pentanes in C_5 hydrocarbon mixtures by selective absorption from the vaporized sample.

A NUMBER of methods are available for the analysis of gases encountered in the petroleum industry. Unfortunately, the published information is widely scattered and, generally, the available textbooks covering this subject do not give complete details of the methods or do not present modern practice (*1, 11, 13, 15, 28, 46*). Perhaps the most useful general treatment of this subject is that made 18 years ago by Shepherd (*42*).

The complete analysis of complex mixtures of gaseous hydrocarbons and fixed gases by chemical methods usually requires a preliminary fractionation to obtain simple mixtures which permit the determination of the individual components. In some instances it is possible to analyze multicomponent mixtures without fractionation, provided it is not necessary to distinguish between all members of homologous series. Needless to say, it is desirable to avoid fractionation because this operation is time-consuming and requires specialized apparatus and techniques. When fractionation is necessary the use of partially selective methods, such as those described below, often reduces the number of fractions that must be prepared.

Gaseous samples not requiring preliminary fractionation, as well as cuts from distillations, are commonly analyzed by progressive absorption and combustion methods. Most of the apparatus described in the literature and commercially available for this purpose is unsatisfactory in one or more of the following respects: Brine is used as a confining liquid, mercury is used as a confining liquid in such a manner that heavy mercury reservoirs must be lifted manually during each operation, or rubber connections are used to increase flexibility.

Brines are not entirely satisfactory as confining liquids because they dissolve appreciable and varying amounts of gases (*24, 26*), temperature changes may cause salt deposits in the burets and connecting lines, and spillage creates an unsightly condition in the laboratory. Mercury is a more satisfactory confining liquid but frequent raising and lowering of heavy mercury reservoirs are a serious drawback to its use. The literature describes a number of devices designed to eliminate the manual effort required to raise heavy mercury reservoirs (*4, 5, 43, 44*) but none of these has simplicity of design and/or ease of operation.

Rubber connections result in errors of two types: A gas may diffuse through the rubber tubing and be lost to the air, producing the effect of a leak, or gas may dissolve in the rubber and later be evolved as a contaminant when a sample of different composition is being analyzed. The work of Branham (*6*) convincingly demonstrates that rubber tubing connections are undesirable in gas analysis and unpublished work by the authors confirms his findings in every detail. A high degree of flexibility can be achieved by the use of ground-glass spherical joints, and the use of such joints eliminates to a considerable extent the apparent practical need for rubber connections.

In a fundamental study of these and other problems of industrial gas analysis over a period of years, the apparatus and methods described below were devised for the analysis of gases by progressive absorption and combustion. Mercury is used as the confining liquid in the buret, and a mercury lift which is an improvement of that described by Blair and Wheeler (*4*) permits the handling of a quantity of mercury without manual effort and tedium. By use of spherical joints, rubber connections are completely eliminated from the apparatus manifold without sacrificing versatility of application.

The methods described are applicable to the analysis of three types of samples: The first method combines absorption and combustion procedures and is applicable to mixtures containing acid gases, oxygen, nitrogen, carbon monoxide, acetylenes, ethylene, isobutylene (2-methylpropene), other mono-olefins (propylene plus normal butylenes), and paraffins (methane

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through butane). In this method, 1,3-butadiene does not interfere when present in concentrations of less than 10% and 1,2-butadiene does not interfere in any concentration; however, both isomers are included in the mono-olefin group. Cyclopropane (*8*), when present, is also included in the mono-olefin value. Acid gases such as carbon dioxide, hydrogen sulfide, and mercaptans (thiols) are determined collectively. The second method covers the analysis of C_4 hydrocarbons and enables the determination of alkyl acetylenes, 1,3-butadiene, isobutylene, normal butylenes, and butanes by progressive absorption using a single portion of the gas. Any 1,2-butadiene present is included in the normal butylene value. The third method is applicable to C_5 hydrocarbons and permits the determination of conjugated diolefins, alkyl acetylenes, tertiary olefins, other C_5 olefins, and pentanes by progressive absorption from the same sample portion. When present, *cis*-piperylene (a conjugated pentadiene) interferes because it is slowly polymerized in the diolefin reagent and is slowly absorbed by the tertiary olefin reagent. In the presence of cyclopentene, a tertiary olefin determination cannot be made because this component is absorbed slowly, but not quantitatively, by the tertiary olefin reagent.

METHODS CONSIDERED

Acetylenes. Of the reagents that have been considered for the determination of acetylenes by absorption, alkaline potassium iodomercurate (*27*) is selective and gives satisfactory results. Ammoniacal cuprous chloride quantitatively absorbs acetylene (*50*) but also reacts with carbon monoxide and oxygen. Ammoniacal silver chloride solution (*53*) and alkaline mercuric cyanide solution (*47*) have been used for acetylene absorption but are considered unsatisfactory for general use, inasmuch as they also absorb appreciable amounts of olefins.

Olefins. A number of reagents are in common use for the determination of olefins by complete absorption. Acid mercuric sulfate solution (*17*) is most suitable for this purpose because it absorbs olefins rapidly and completely, has a high absorption capacity, and requires the use of only one pipet to effect complete removal. Although this reagent reacts slowly with carbon monoxide to form carbon dioxide (*7*), this action is suppressed by saturation of the reagent with magnesium sulfate (*12*); no interference is encountered in the use of this reagent in the methods of analysis described below. Aqueous bromine solutions are used extensively to absorb olefins but they have several disadvantages—namely, it is necessary to shield the solution from light to avoid attacking saturated hydrocarbons (*36*), it requires the subsequent use of an alkaline solution to remove bromine vapor from the residual gas, and the authors' experience indicates that the accuracy obtainable with this reagent is dependent both on the olefins present and on their concentrations. Fuming sulfuric acid is a common reagent for the absorption of olefins, but it also attacks paraffins to some extent (*55*) and it is necessary to use an alkaline solution to remove acid gases left in the residual gas. Concentrated sulfuric acid containing silver salts (*18*) absorbs olefins rapidly and completely but it also reacts with carbon monoxide (*28*) and forms an interfering polymer with propylene and butylenes. Acidic sodium-mercuric nitrate solution (*20*) absorbs olefins quantitatively, but it is not applicable in the presence of carbon monoxide or high concentrations of hydrogen; furthermore, it foams excessively in the presence of isobutylene and a second pipet is necessary to remove the last traces of olefins. A similar reagent containing silver nitrate, mercuric nitrate, and potassium nitrate has the advantages that it absorbs olefins at a fast rate, and is applicable in the presence of isobutylene; objections to its use are that it reacts with carbon monoxide and hydrogen, and requires the use of two pipets.

Although the reagents for determining olefins discussed above are generally useful for the determination of total olefins, they lack selectivity. However, some degree of differentiation is possible by the use of sulfuric acid solutions of different concentrations (*14, 31, 37, 45, 54*). Thus 65% sulfuric acid preferentially absorbs isobutylene admixed with other butylenes and 70% sulfuric acid absorbs tertiary amylenes selectively from C_5 fractions. The 87% sulfuric acid separates ethylene from other olefins, in gas mixtures up to and including C_4 hydrocarbons, inasmuch as it absorbs other olefins at a much faster rate. Because these acids are not completely selective, corrections are sometimes necessary for small coabsorptions.

Several methods, other than absorption in 65% sulfuric acid, are known for the specific determination of isobutylene. McMillan (*29*) describes an accurate method based on the reaction between isobutylene and dry hydrogen chloride; however, this method appears objectionable because it requires a skilled technician, a specialized apparatus, and a separate sample for the determination of this component. Another method for isobutylene involves its reaction with neutral mercuric nitrate solution (*35*); this method is specific for isobutylene, is accurate, and is applicable to very low concentrations of isobutylene, but it requires a separate sample for this determination.

Methods for the determination of olefins by catalytic hydrogenation (*40*) are being used widely. They are both accurate and precise but, used alone, do not offer any means of distinguishing one olefin from another, as is accomplished to a limited extent by selective absorption. In addition, the ever-present possibility of a poisoned catalyst introduces an element of uncertainty and is a deterrent in its general use for service analysis of unknown samples.

Conjugated Diolefins. It has been shown by Tropsch and Mattox (*48*) that 1,3-butadiene can be accurately determined by absorption in molten maleic anhydride. Robey, Morrell, and Vanderbilt (*40*) found that serious errors were encountered in the analysis of samples high in olefin content, particularly samples containing 10% or more of isobutylene, because of polymerization of the isobutylene in the maleic anhydride. They attributed this difficulty to the presence of small amounts of maleic acid as a contaminant in the anhydride and they found that the polymerization was effectively suppressed by the addition of approximately 3% of a high-boiling amine (di-*n*-amylamine) to the maleic anhydride. Work in this laboratory has shown that the maleic

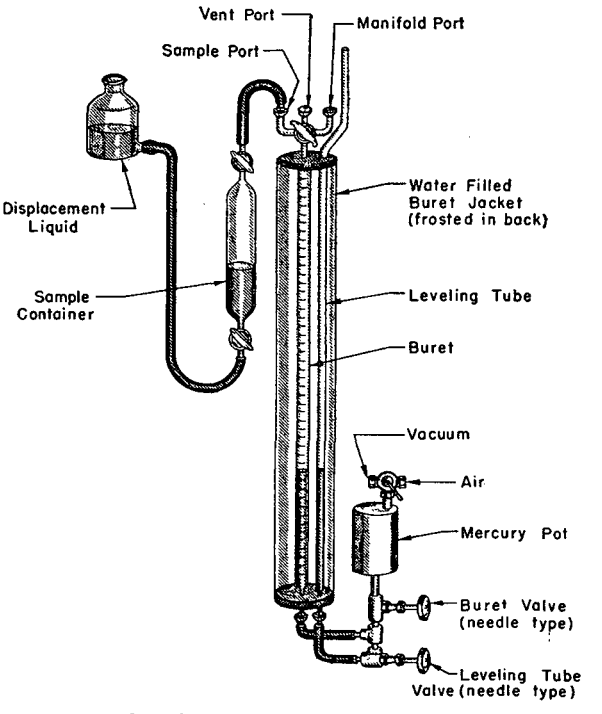

Figure 1. Gas-Sampling and Measuring System

anhydride must contain about 7% of diamylamine to inhibit the polymerization of isobutylene when a water-saturated sample is used. In the development of the methods given below, this method was improved and extended to the determination of conjugated pentadienes in C_5 fractions.

Oxygen. Three reagents are generally used in the determination of oxygen by absorption analysis. Chromous chloride solution (*39*) is the most satisfactory reagent on the basis of speed and minimum solubility of saturated hydrocarbons. The chromous chloride solution absorbs significant amounts of olefins also, but this objection is obviated by removing the olefins prior to the oxygen. Potassium pyrogallate solution (*3*) is in wide use but is somewhat objectionable because it removes oxygen too slowly. Sodium dithionite solution containing 2-anthraquinonesulfonic acid (*16*) absorbs oxygen rapidly enough but foams badly when high concentrations of oxygen are present.

Carbon Monoxide. None of the methods which have been investigated for the determination of carbon monoxide by absorption are entirely satisfactory. Ammoniacal cuprous chloride and acid cuprous chloride solutions (*33*) absorb carbon monoxide rapidly but also absorb saturated hydrocarbons appreciably. The acid solution is used in the methods described below because it absorbs carbon monoxide more completely than the ammoniacal reagent and because it readily reduces itself when oxygen has been absorbed, provided it is kept in contact with metallic copper. The ammoniacal reagent evolves large quantities of ammonia gas which causes considerable difficulty when a wet buret is used because the ammonia is dissolved in the water in the buret and is slowly released during subsequent manipulations, thereby contaminating the residual gas. The solubility of saturated hydrocarbons in the acid solution is minimized by saturating it with sodium chloride. The use of iodine pentoxide in fuming sulfuric acid (*41*) is unsatisfactory in the presence of hydrogen or paraffins. It is possible to determine carbon monoxide by selective oxidation over copper oxide (*22*) but, at the temperature required, significant amounts of ethane and its higher homologs are simultaneously oxidized.

Hydrogen. The determination of hydrogen by direct absorption is not very satisfactory. Palladium black (*19*) and colloidal palladium (*38*) are sometimes used for this purpose, but unfortunately these reagents are easily poisoned. The determination of hydrogen by selective combustion over copper oxide (*21*) is rapid and does not require addition of oxygen to ensure complete combustion. The combustion is best done at 270° ± 10° C., a temperature at which some of the hydrocarbons are oxidized to a slight but generally negligible extent. Selective combustion of hydrogen over platinized silica gel catalyst (*23*) is unsatisfactory because appreciable amounts of saturated hydrocarbons are adsorbed on the catalyst (*25*).

Paraffins. There are no absorbents that are suitable for the determination of saturated hydrocarbons. The only chemical methods known require combustion of the gas in some manner. A number of investigators (*45, 49, 54*) state that complete combustion of methane is achieved by passage over copper oxide wire heated to 800° to 900° C. However, at this temperature, dissociation of the copper oxide occurs (*10, 37*) with the consequent slow evolution of oxygen. In addition, it is necessary to pass the gas repeatedly over the oxide to achieve complete oxidation of methane (*13*). Precipitated copper oxide containing 1% of iron oxide gives rapid and complete combustion of methane and its homologs at a temperature of only 700° C. (*9, 34*). This method is free of explosion hazard and does not require the addition of oxygen for the combustion; therefore, it is used in the methods below for the determination of saturated hydrocarbons. Combustion by explosion (*2*) has found wide application, but the sample must be carefully blended with oxygen to obtain complete combustion and to avoid error due to oxidation of nitrogen (*28*). Slow combustion over a heated platinum filament (*52*) is in common use but is somewhat hazardous, owing to the danger from possible explosive mixtures. In addition, it is difficult to maintain a constant filament temperature and to avoid incomplete combustion without danger of melting the platinum filament. Hydrocarbons are burned rapidly and completely when passed over platinized silica gel at 500° C. (*25*). However, this method is not completely free of explosion hazard and it is necessary to mix a measured quantity of oxygen with the sample prior to combustion.

APPARATUS

The various commercially available apparatus for the analysis of gases by absorption and combustion generally lack features which are necessary and/or desirable for the analysis of special samples of petroleum products. The apparatus described incorporates in the design many worth-while features of standard commercial equipment as well as special parts that facilitate gas analysis operations, particularly in the petroleum industry. This apparatus is being manufactured under license by the Precision Scientific Company.

Gas-Measuring System. The 100-ml. gas-measuring buret is of special design: It is equipped with a 4-way stopcock having a 90° angle bore that permits connection between any two adjacent ports. The graduated volume of the buret includes all the space up to the stopcock barrel but does not include the bore of the stopcock plug. By means of the stopcock, it is possible to purge the sample connection tubing with sample, draw the sample directly into the buret, purge the manifold with nitrogen, and pass the sample from the buret through the manifold. The buret is mounted in a water jacket to prevent large temperature fluctuations. In order to provide a good reading background, the buret jacket is frosted halfway around on the outside. A sketch of the gas-measuring system is shown in Figure 1.

Mercury is used in the buret as the confining liquid. To pass the gas sample in and out of the buret, a mercury lift consisting of a tank and valves connected to air pressure and vacuum lines relieves the analyst of handling heavy leveling bulbs. The mercury lift (Figure 1) is supported on the apparatus to the right of the buret and is connected to the buret and leveling tube, through two steel needle valves, by Tygon tubing. A 3-way metal cock directs either air pressure or vacuum to the tank; it is so constructed that the direction of mercury flow in the buret is indicated by the "down" or "up" position of the valve handle. Two steel needle valves control the flow of mercury; one controls the flow in and out of the buret and the other controls the flow to the leveling tube.

The use of the leveling tube facilitates the adjustment of the pressure in the buret to that of the atmosphere by providing a convenient means of noting differences in pressure between the buret and the atmosphere; the buret and leveling tube are of equal diameter and are adjacent to each other in order to permit quick and accurate comparison of the relative levels of mercury. Although compensator manometers are widely used on gas analysis apparatus, the atmospheric pressure changes are rarely of sufficient magnitude to cause measurable errors in the course of an analysis. For this reason, and to avoid difficulties commonly experienced with the use of a compensator manometer, it is practical to disregard normal atmospheric pressure variations during an analysis and, when necessary, to make corrections for abnormal changes in barometric pressure.

Unit Assembly. The manifolds used are constructed of metal or glass, thus avoiding the errors common to apparatus employing rubber connections. Although such a manifold entails the loss of some flexibility, this disadvantage is largely overcome by using ball and socket joints and by constructing the apparatus in unit sections. Four units, which are used in a number of combinations, meet the requirements of most petroleum laboratories; they are shown diagrammatically in Figure 2. Construction of the apparatus in this manner retains a high degree of versatility.

The glass manifolds are constructed in five-stopcock units, using 3-way stopcocks with a 120° angle bore throughout except for two "in and out" type 4-way stopcocks with two 90° angle bores in the combustion unit. Manifold sections are connected by means of stainless steel hypodermic tubing and metal-to-glass spherical joints.

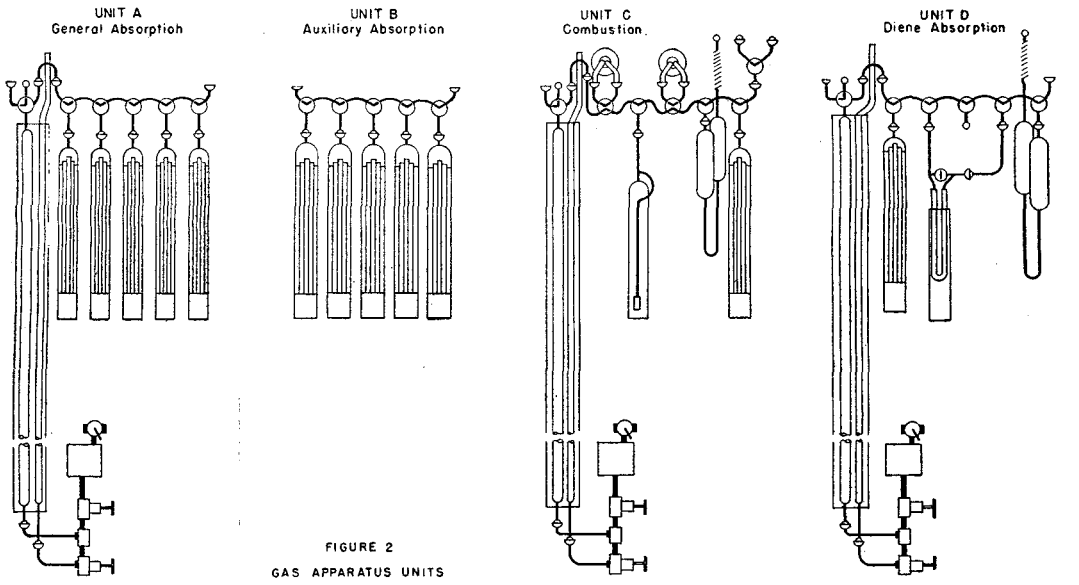

FIGURE 2
GAS APPARATUS UNITS

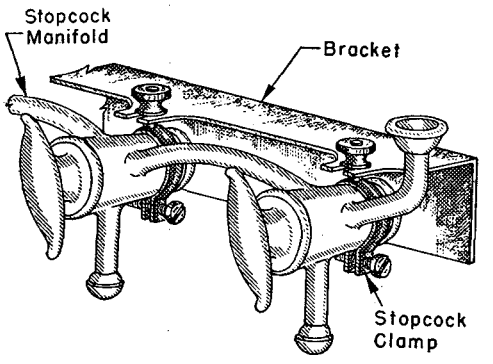

Figure 3. Stopcock Manifold Support

A novel method is used to support the manifold (Figure 3), in which each stopcock in the manifold is clamped to a support frame on the unit panel. The clamps are constructed by soldering a small machine screw stud to a brass hose clamp and a modified hose clamp is placed over the barrel of each stopcock. Notches are milled in a brass or stainless steel angle strip to receive the screw studs and the strip is secured to the unit panel. The manifold is mounted by slipping the machine screw studs of the clamps in the slots on the angle strip and the studs are held in place with small knurled nuts. This method of mounting makes it possible to construct the manifolds on a simple jig and simplifies the glass-blowing operation during its construction. Stopcocks with their band clamps are placed on a jig similar to the mounting strip on the panel and the side arms are sealed to form a continuous manifold. The manifold constructed in this way is easily placed on any apparatus with a minimum amount of strain on the glass parts. This type of support reduces considerably the breakage ordinarily encountered with glass manifolds.

The size of the barrels of the stopcocks used is important, in that the distance between ports in the barrel must be large enough to assure freedom from by-pass leaks. As the manifold is used dry, a hydrocarbon-insoluble lubricant (*32*) is used to lubricate the stopcocks. This is an important though often overlooked detail, because many hydrocarbon gases (particularly olefins) are readily absorbed by most stopcock lubricants, introducing errors similar to those caused by rubber tubing connections.

The pipets, reaction tubes, and reservoirs are connected to the manifold stopcocks by means of spherical joints as shown in Figure 2. The pipet connections are sealed with a water-insoluble grease (Cello-Grease is suitable), and the connections to reservoirs and combustion tubes are sealed with sealing wax.

Combustion Tubes. The combustion tubes, as shown in Figure 4, consist of U-shaped 5-mm. glass or silica tubes filled with copper oxide.

For the combustion of hydrogen, use is made of a Pyrex tube packed with copper oxide wire and maintained at a temperature of 270° ± 10° C. For the determination of saturated hydrocarbons, use is made of a tube packed with precipitated copper oxide and maintained at 700° ± 20° C.; this tube is made of fused silica, Corning glass type No. 172, or other glass capable of withstanding operation at 700° C. For ease of operation, it is preferable to maintain the temperatures required for these combustion tubes by means of electric furnaces equipped with variable transformers or temperature-controlling devices.

Absorption Pipets. Three recommended types of gas pipets (*30*) are shown in Figure 5.

The contact pipet consists of a glass vessel filled with 5-mm. thin-walled glass tubes and located in a jacket which serves as a reservoir for the reagent. In this type of pipet the entering gas displaces the reagent downward, leaving the large surface area of the glass tubes wet with a film of reagent that absorbs the reactive gas. Where applicable, this pipet is preferable to other types because of the simplicity of construction and operation. However, for successful operation it is necessary that the reagent used be sufficiently viscous to maintain a film of reagent on the surfaces of the glass tubes supported in the pipet. In addition, the contact pipet is the only pipet that produces a large, reproducible surface of reagent and, consequently, it is especially useful for making timed passes where it may be necessary to determine accurately a correction for coabsorption.

The bubbler pipet consists of a glass vessel fitted with a sealed-in gas-inlet tube which passes through the shoulder of the pipet and terminates in a sintered-glass dispersion thimble near the bottom of the pipet. In this type of pipet the glass passes through the inlet tube and is dispersed in very small bubbles through the reagent contained in a surrounding jacket.

The contact bubbler pipet is a contact pipet in which the gas is introduced at the bottom of the pipet in a manner similar to that described for the bubbler pipet, the gas inlet tube terminating in an upward bent orifice. This type of pipet is used only when the

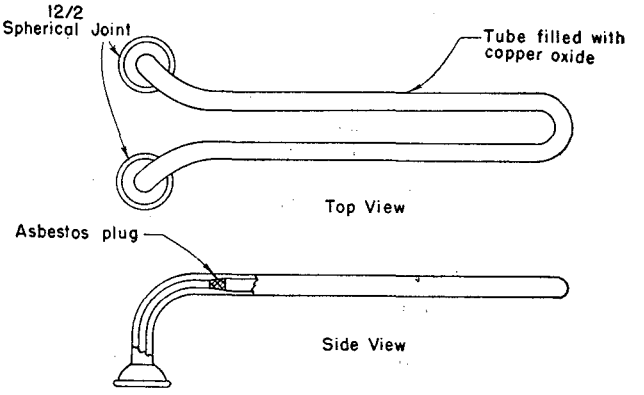

Figure 4. Copper Oxide Combustion Tubes

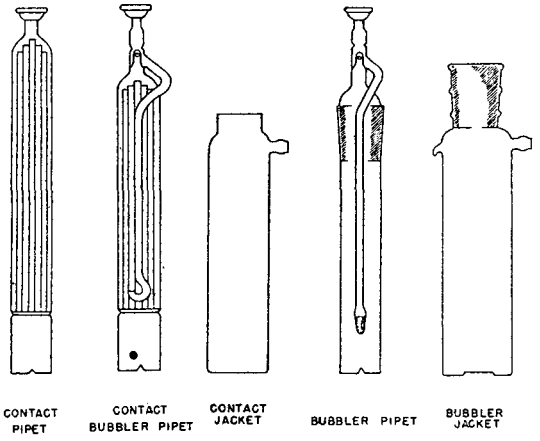

Figure 5. Gas-Absorption Pipets and Jackets

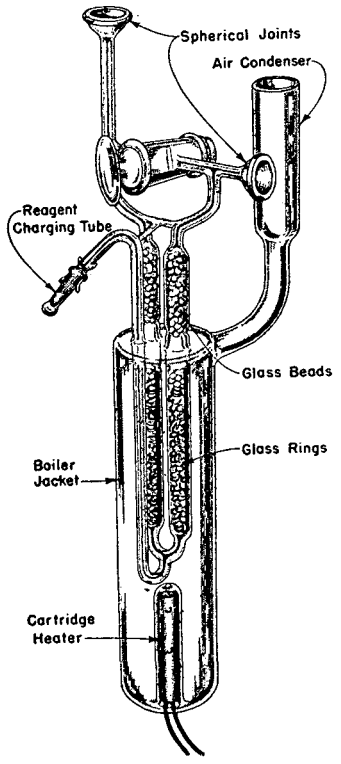

Figure 6. Diene Absorber

reagent forms a flocculent precipitate with the reactive gas. Contact of the oxygen and carbon monoxide reagents with air is prevented by the lubricated ground joint connecting the pipet and jacket and by connecting rubber expansion bags to the vent tubes.

In the determination of 1,3-butadiene and conjugated pentadienes, use is made of a U-tube absorber filled with glass helices and equipped with a siphon tube for convenient filling and cleaning without removal from the manifold. The details of the diene absorber are shown in Figure 6.

The glass helices increase the reactive surface and, at the same time, break the foam that otherwise interferes seriously. The lower portion of the tube is maintained at approximately 100° C. by means of a boiling water jacket which is conveniently heated with a cartridge type heater. The jacket is equipped with a simple air-cooled metal condenser. Small indentations in the heater well create "hot spots" which cause localized boiling and prevent superheating of the water and consequent bumping.

The analysis of C_5 hydrocarbons requires the use of a vaporizing unit, shown in Figure 7, which consists of a Geissler pump and a liquid sample tube.

Constant-Pressure Reservoir. The constant-pressure reservoir, shown in Figure 8, consists of a mercury-filled cylindrical glass bulb connected by rubber tubing to a leveling bulb of similar design. Suspending the leveling bulb is a spring of suitable dimensions and elasticity, that allows the leveling bulb to rise or lower just sufficiently to counteract the weight of mercury withdrawn from it or displaced into it, thus maintaining fairly constant pressure in the reservoir. Although this device was developed independently in this laboratory, it operates on the same principle as that described by Wilson (*51*). The constant-pressure reservoir makes possible the confinement of a gas at nearly constant pressure over mercury during complete cycle passages and automatically maintains this constant pressure. Moreover, it avoids abrupt changes in the solubilities and reaction rates due to pressure variations during passage of the gas, and minimizes leaks in the system which may be promoted by pressure fluctuations.

REAGENTS

Acid Cuprous Chloride Solution (Carbon Monoxide Absorbent). To prepare approximately 1 liter of acid cuprous chloride solution, dissolve 96 grams of sodium chloride in 450 ml. of distilled water. Add 135 grams of cuprous chloride and stir until solution is complete. Add slowly and with constant stirring, 500 ml. of concentrated hydrochloric acid solution. Transfer the solution to a 2-liter Erlenmeyer flask partially filled with copper strips and fitted with a two-hole stopper through which a glass tube extends nearly to the bottom of the flask and a second glass tube extends just into the neck. Flush the flask and contents with nitrogen. Allow to stand, with occasional agitation, until colorless. Use the solution in bubbler pipets which are packed with twisted 0.6-cm. (0.25-inch) strips of 16-gage sheet copper to keep the solution reduced. In filling the pipets, displace the solution with nitrogen to avoid oxidation; also seal the pipets to their jackets with water-insoluble lubricant and exclude oxygen by the use of nitrogen-filled rubber expansion bulbs.

Acid Mercuric Sulfate Solution (Olefin Absorbent). To prepare approximately 1 liter of acid mercuric sulfate solution, dissolve 164 grams of mercuric oxide in 715 ml. of 29% sulfuric acid. Add 480 grams of magnesium sulfate heptahydrate to the solution and mix thoroughly to achieve saturation. Decant the clear solution. Use this reagent in a contact pipet.

Chromous Chloride Solution (Oxygen Absorbent). To prepare approximately 1 liter of chromous chloride solution, mix 90 ml. of concentrated hydrochloric acid with 900 ml. of saturated aqueous chromic chloride solution. Reduce this solution with amalgamated zinc. Protect from the air during the reduction and provide for the escape of evolved hydrogen through a water trap. Store under nitrogen and allow the solution to stand at least 48 hours before use. Use this solution in bubbler pipets; take precautions to exclude oxygen as directed for acid cuprous chloride solution (above).

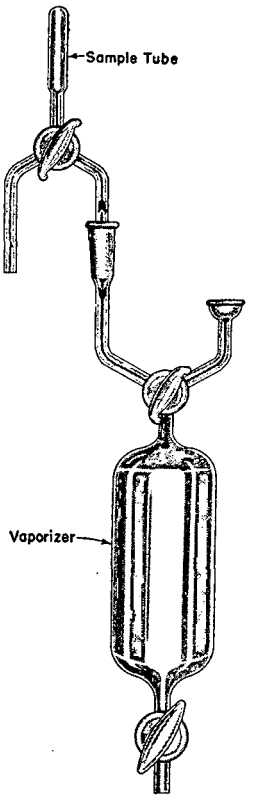

Figure 7. Geissler Pump and Liquid Sample Tube

Copper Oxide Wire (Hydrogen Combustion Agent). Screen C.P. copper oxide wire. Collect that portion which passes through a 10-mesh screen but is retained on a 20-mesh screen, and heat to 900° C. in a muffle furnace for 3 to 4 hours. Cool and store in a clean glass-stoppered bottle.

Precipitated Copper Oxide (Hydrocarbon Combustion Agent). Add a slight excess of 30% sodium hydroxide solution to 305 grams of cupric nitrate trihydrate and 5 grams of ferric nitrate nonahydrate dissolved in 3 liters of water, boil gently for 20 minutes, cool, and filter. Dry the precipitate and crush until the greater portion is approximately 10-mesh size; completely reduce with hydrogen at 400° C. and thoroughly oxidize again at the same temperature. Cool and screen; collect the portion that passes a 10-mesh screen but is retained on a 20-mesh screen. Store in a clean glass-stoppered bottle.

Maleic Anhydride (Diene Absorbent). Use a good grade of maleic anhydride having a minimum melting point of 52° C. To use a product that has a melting point lower than 52° C., purify by distillation. (As maleic anhydride vapors are toxic, the handling of this material in open containers should be carried out under an efficient hood.)

Diamylamine (Polymerization Inhibitor). Purify di-*n*-amyl-

amine or a commercial mixed diamylamine product by distillation over potassium hydroxide pellets.

Potassium Hydroxide Solution (Acid Gas Absorbent). To prepare 1 liter of potassium hydroxide solution, dissolve 700 grams of C.P. potassium hydroxide pellets in 700 grams of water. Use in a contact pipet.

Potassium Iodomercurate Reagent (Acetylene Absorbent). Slowly add, with stirring, 100 ml. of 50% potassium hydroxide solution to 25 grams of mercuric iodide in 100 ml. of distilled water; preferably, prepare the quantity needed each day from these two stock solutions. Use in a contact pipet when analyzing samples that are expected to contain less than 5% acetylenes; for larger concentrations of acetylenes, use a contact-bubbler pipet.

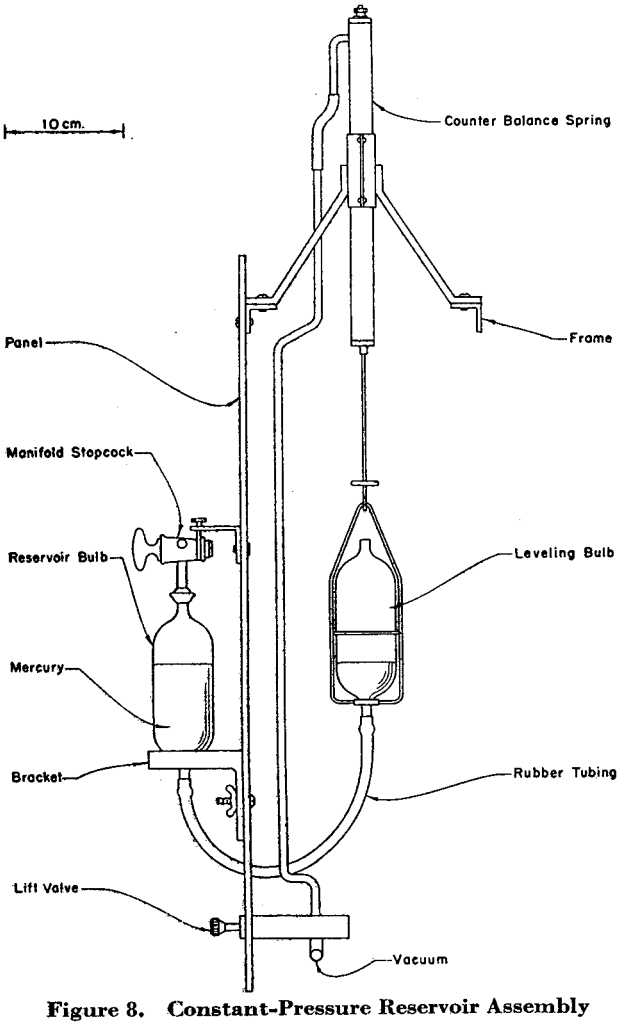

Figure 8. Constant-Pressure Reservoir Assembly

Sulfuric Acid 65, 70, and 87% (Olefin Absorbents). Because the concentrations of these acids are critical, adjust them to the desired concentrations within ±0.5% sulfuric acid as determined by titration with standard sodium hydroxide. Although these acids can be used for 6 to 10 determinations in a single day, use fresh portions each day or whenever there is any visible evidence of polymer formation.

Brine (Displacement Liquid). Prepare a nearly saturated solution of sodium chloride and make slightly acid with hydrochloric acid.

Carbon Dioxide (for Flushing Maleic Anhydride). Carbon dioxide gas should be supplied at an essentially constant pressure of approximately 3 pounds per square inch. Total impurities insoluble in 50% potassium hydroxide solution, as measured by absorption of at least 500 ml. of the gas, must not exceed 0.01%. The gas is best obtained by vaporization of the solid in a suitable pressure vessel rather than from a commercial liquefied-gas cylinder; the latter usually contains fairly large amounts of dissolved impurities and thus requires bleeding off a large portion of carbon dioxide in order to reduce the impurities to the specified limit. For a single apparatus, quantities of carbon dioxide sufficient for 8 hours' operation can usually be obtained by placing the crushed solid in a suitable 1-liter container equipped with a Bunsen or a spring type safety valve. To expel air from the container and connection lines, the gas should be vented for 20 to 30 minutes before use.

Nitrogen. Nitrogen gas should be supplied at a pressure of approximately 3 pounds per square inch. Commercial cylinders equipped with a two-stage reducing valve are satisfactory.

Hydrocarbon-Insoluble Lubricant. Make a paste of 100 grams of anhydrous glycerol, 29 grams of starch, and 14 grams of C.P. D-mannitol; heat slowly to boiling, remove immediately from the flame, and stir frequently until cool.

GENERAL OPERATING TECHNIQUES

Moistening Buret. During an analysis it is important that the water content (relative humidity) of the gas be the same whenever the gas is measured. In order to avoid possible errors from variation in the water content of the gas, it is convenient to keep the gas in the buret saturated with water vapor throughout the analysis by maintaining a film of water on the inside wall of the buret; thus the sample is always in equilibrium with water when its volume is measured. A satisfactory procedure for wetting the buret wall with water is as follows:

Remove the plug of the buret stopcock and, with a capillary eye dropper, introduce about 1 ml. of water into the buret. Insert a small wad of cotton into the stopcock barrel, lower the mercury level to a point just below the 100-ml. mark, then immediately raise the mercury to force all excess water into the cotton wad. Dry the stopcock and replace the plug after lubricating with hydrocarbon-insoluble lubricant.

Whenever the film of water on the buret wall disappears, it must be renewed again (in continuous use this may occur two or three times a day). For accurate measurements, care must be taken to avoid the use of an excessive amount of water over that required to give a film on the buret wall. As the lubricant used is water-soluble, care must be taken to prevent drops of water from coming in contact with the lubricant.

Operation of Buret. Operation of the buret, employing the mercury lift and leveling tube assembly described by Figure 1, is very simple.

To charge the buret with exactly 100 ml. of gas at atmospheric pressure, turn the buret stopcock to communicate the buret with the atmosphere through the sample port, open the leveling tube valve, turn the lift valve to the "up" position, and open the buret valve until the mercury level rises to just below the buret stopcock. Turn the buret stopcock to communicate the sample port with the atmosphere, connect to the sample source by means of the adapter (ball joint with a short piece of rubber tubing clamped to the sample port), and flush the connecting line with gas to the atmosphere. Turn the buret stopcock to communicate the sample port with the buret, turn the lift valve to the "down" position, open the buret valve, allow sample to enter the buret until the mercury level is slightly below the 100-ml. mark, and close the buret valve. Turn the lift valve to the "up" position and carefully open the buret valve until the mercury level in the buret rises to exactly the 100-ml. mark. Turn the stopcocks in the manifold to connect to the open vent and release the excess pressure in the buret by opening the buret stopcock momentarily to the manifold. To avoid small errors possible from expansion of the gas, vent the buret a second time and check the volume of the gas after opening the leveling tube valve and equalizing the levels of the mercury.

If the sample is under reduced pressure, the container is connected to a reservoir filled with displacement liquid (brine or mercury) and the level of the displacement liquid is raised to create a slight pressure in the sample container. No displacement liquid is required when the sample is contained as compressed gas or as liquefied gas in a metal bomb. If the sample is a liquefied gas it is generally sampled from the liquid phase. In either case, the sample is admitted in such a manner that the mercury levels in the buret and leveling tube are maintained approximately the same while the sample enters the buret.

To pass the gas in and out of the reagents during the analysis, turn the buret and manifold stopcocks to connect the buret with the appropriate pipet and raise or lower the mercury in the buret by means of the lift. Whenever it is desired to read the volume of the gas, open the leveling tube valve, raise or lower the mercury level in the buret to equalize exactly the mercury levels in the

buret and leveling tube, and observe the reading of the buret (which is now at atmospheric pressure).

Diluting with Nitrogen. If, in the course of an analysis, it is known that the sample volume will become less than 20 ml., it is necessary to add a carrier gas; nitrogen as an inert gas serves well for this purpose.

To make the dilution, flush the manifold with nitrogen, draw approximately 20 ml. of nitrogen into the buret, and pass the gas into the chromous chloride reagent to remove any oxygen that may be present as a contaminant. Accurately measure the volume of the nitrogen and transfer it to the pipet in which the volume of the gas is expected to fall below 20 ml. during the analysis.

Testing for Leaks. In order to obtain reliable results, it is essential that the operator be constantly on the guard against possible leaks in the apparatus.

To detect and locate leaks in the apparatus, raise the reagent levels in the pipets and the mercury level in the constant-pressure reservoir to their reference marks just below the manifold stopcocks and fill the buret with air. Turn all stopcocks, except the vent stopcock at the end of the manifold, to communicate with the buret. Place the air in the buret under pressure by raising the mercury level several inches and turn the buret stopcock to connect the buret with the manifold. Note the mercury level in the buret and allow to stand for 10 to 15 minutes. At the end of this period, check the buret reading for any change. Also check the reagent levels to determine whether air has entered any of the pipets during the test period. Relubricate or replace any faulty stopcocks or ground-glass connections.

GENERAL METHOD FOR ABSORPTION-COMBUSTION ANALYSIS

This method is intended for the analysis of gas mixtures containing acid gases, oxygen, nitrogen, hydrogen, carbon monoxide, acetylene, ethylene, propylene, butylenes (including 1,3- and 1,2-butadiene), and saturated hydrocarbons up to and including butane. The method is not capable of distinguishing between individual saturated hydrocarbons, but does indicate the average molecular weight (carbon number) of the paraffins present. The assembled apparatus, made from three of the unit types described by Figure 2, is shown diagrammatically in Figure 9.

Preparation of Apparatus. Carefully clean and lubricate all manifold stopcocks with hydrocarbon-insoluble lubricant and lubricate the spherical joints on the manifold with water-insoluble lubricant. Fill the required pipets with their respective reagents and attach them to the manifold in the following order (the pipets must be arranged in the same order as that in which the absorptions are made; otherwise reactive gas will remain in the manifold and not come in contact with reagent):

Position to Right of Absorption-Unit Buret	Type of Pipet	Reagent
1	Contact	50% potassium hydroxide
2	Contact or contact-bubbler	Potassium iodomercurate
3	Contact	65% sulfuric acid
4	Contact	87% sulfuric acid
5	Contact	Acid mercuric sulfate
6	Bubbler	Chromous chloride
7	Bubbler	Cuprous chloride
8	Bubbler	Cuprous chloride
9	Contact	50% potassium hydroxide
Position to Right of Combustion-Unit Buret		
2	Bubbler	Chromous chloride (for storage of nitrogen)
5	Contact	50% potassium hydroxide

Wet the buret walls with water and test the apparatus for leaks as described above.

Oxidize the copper oxide in the hydrogen combustion tube by drawing air through the tube for 45 to 60 minutes while heating the tube at 450° ± 50° C. Reduce the furnace temperature to 270° ± 10° C. and activate the copper oxide by taking approximately 80 ml. of hydrogen and 20 ml. of nitrogen in the buret and passing the mixture through the copper oxide tube into the constant-pressure reservoir. Return the gas through the copper oxide tube to the buret and repeat until a constant residual volume is obtained. Adjust the temperature of the precipitated copper oxide tube to 700° ± 20° C. During the time the furnace is being heated to operating temperature, draw air through the tube in order to oxidize the copper oxide (additional activation is not necessary).

Flush both the absorption and combustion manifolds, and both copper oxide tubes with nitrogen. Allow the nitrogen to remain in the combustion tubes for approximately 1 minute, then establish atmospheric pressure in the manifold by opening momentarily to the atmosphere.

Because it is necessary to correct for the volumes of the absorption and combustion manifolds and for the volume of the hydrogen combustion tube in calculating the results, determine these volumes for each individual apparatus. To do this, draw approximately 50 ml. of oxygen-free nitrogen into the buret and measure accurately. Fill the sections whose volumes are to be measured with pure oxygen and flush into the chromous chloride pipet by means of the nitrogen in the buret, until a constant residual volume is obtained. Record the difference between the measured volume before and after passage into chromous chloride as the manifold volume. In determining the volume of the hydrogen combustion tube, place the chromous chloride pipet in the position usually occupied by the potassium hydroxide pipet. Be sure that the tube is at operating temperature during the calibration, and that it is fully oxidized and at equilibrium with oxygen.

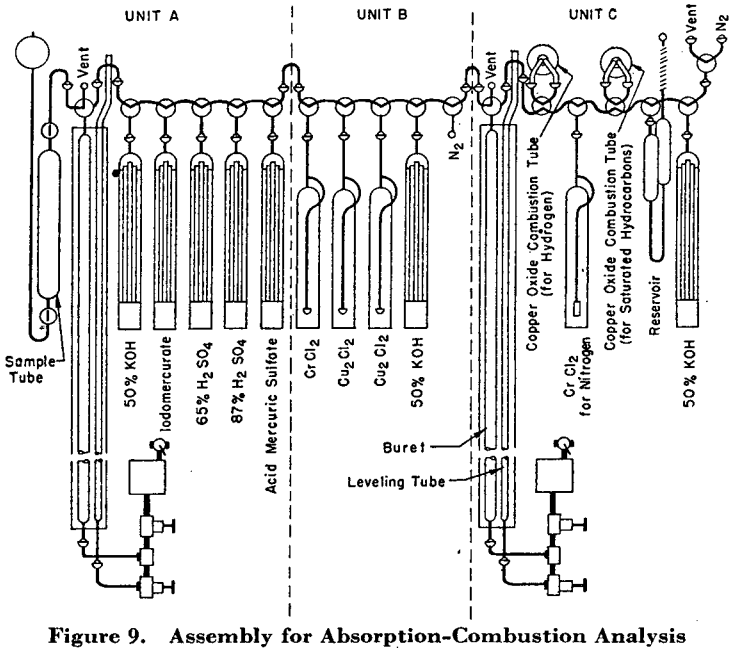

Figure 9. Assembly for Absorption-Combustion Analysis

Saturation of Reagents. As a preliminary step in the analysis, saturate each reagent to be used with the residual gas that will be present in the system after absorption in that particular reagent. Preferably, pass 90 to 100 ml. of the gas into the various reagents as described below for the analysis of the sample, but do not read the volumes or record the data; discard any residual gas from these absorptions. If a series of samples of similar composition is being analyzed, perform the presaturation step only for the first sample—i.e., each analysis serves to presaturate the reagents for the following determination.

Absorption Analysis. Flush the connection between the sample container and the buret with sample. Introduce 100 ml. of sample into the buret, accurately measure the volume, and record the buret reading and the buret temperature. Flush the manifold with nitrogen and pass the gas through the absorption and combustion steps indicated below, recording the residual volume and buret temperature at the end of each step.

For the determination (or removal) of carbon dioxide, sulfur dioxide, hydrogen sulfide, mercaptans, and other acid gases, pass the measured gas into the potassium hydroxide pipet. Allow the gas to remain in the pipet for 5 seconds and withdraw it to the buret. Repeat until a constant residual volume (or a constant absorption per pass if acetylenes are present in high concentrations) is obtained.

To determine the acetylene content pass the residual gas from the acid gas determination into the pipet containing potassium iodomercurate. Allow the gas to remain in contact for 20 seconds and withdraw it to the buret. Make five such passes and read the volume of residual gas. Continue in groups of five passes until a constant residual volume is obtained.

To absorb any isobutylene present, pass the remaining gas into the 65% sulfuric acid. Make a total of ten passes into the acid, allowing the gas to remain in the pipet for 20 ± 2 seconds. Measure the volume of the residual gas. If less than 4 ml. of gas has been absorbed, make ten more uniform, 20-second passes and again measure the volume. If, however, more than 4 ml. of gas has been absorbed in the first ten passes, make 20 more uniform, 20-second passes, measuring and recording the volume and buret temperature at the end of the 10th and 20th passes.

To determine the propylene and residual butylene content collectively, pass the remaining gas into the 87% sulfuric acid, allow to remain 20 ± 2 seconds, and withdraw the residual gas to the buret. Make ten such passes and measure the volume. Continue in groups of five 20-second passes until a constant residual volume (or a constant absorption per group of five passes if ethylene is present) is obtained.

Pass the remaining gas into the acid mercuric sulfate pipet; make ten passes without timing, measure the volume, and continue in groups of ten passes until a constant residual volume is obtained.

Pass the gas remaining after the ethylene determination into the chromous chloride solution until a constant volume is obtained.

Pass the gas remaining after the oxygen determination four times through the first cuprous chloride pipet, measure the volume, and pass through the second cuprous chloride pipet until a constant residual volume is obtained. Then pass the gas through the adjacent potassium hydroxide solution until a constant residual volume is obtained. (Because the acid mercuric sulfate reagent slowly oxidizes carbon monoxide, the absorption in potassium hydroxide following the absorption in cuprous chloride is to be regarded as carbon monoxide.)

Combustion Analysis. Before transferring the residual gas from the absorption analysis to the buret of the combustion unit, flush the combustion unit manifold and hydrogen combustion tube with nitrogen, accurately measure about 20 ml. of oxygen-free nitrogen into the buret, and store the nitrogen in the chromous chloride pipet. Record the buret temperature. Use this portion of nitrogen only as a carrier gas and mix it with the sample if the sample volume falls below 20 ml. at any point in the analysis.

Pass the gas remaining after the absorption analysis several times between the burets of the absorption and combustion units in order to mix it with the nitrogen retained in that portion of the absorption unit manifold that had not previously been traversed by the gas. Measure the gas in the combustion unit buret, and record the volume and buret temperature.

To determine the hydrogen content, establish atmospheric pressure in the manifold and hydrogen combustion tube, pass the gas through the tube (maintained at 270° ± 10° C.) directly in the gas reservoir at a rate of approximately 10 ml. per minute, and return to the buret through the combustion tube at the same rate. Repeat the operation for four cycles, but increase the rate to approximately 30 ml. per minute. Measure the volume and continue in groups of two cycles until a constant residual volume is obtained. Pass the gas through the combustion tube into the potassium hydroxide pipet until no further absorption occurs, and record the final volume and buret temperature. (When a mixture of hydrogen and saturated hydrocarbons is passed over the hot copper oxide wire, the hydrogen is oxidized completely to water and a small amount of the saturated hydrocarbons is sometimes oxidized to carbon dioxide. Methane and ethane are oxidized to a negligible extent, the larger part of the carbon dioxide being produced from propane and butane.)

Retain in the buret such a volume of residual gas that the combustion product can be measured in the buret (methane, 90 to 95 ml.; ethane, 45 to 47 ml.; propane, 30 to 33 ml.; butane, 22 to 24 ml.), transfer any excess residual gas back to the absorption unit, and store in the second potassium hydroxide pipet. Accurately measure the volume retained in the buret for the combustion analysis and record together with the buret temperature. Flush nitrogen through the manifold and hydrocarbon combustion tube to the atmosphere and establish atmospheric pressure.

Pass the gas through the combustion tube (maintained at 700° ± 20° C.) directly into the gas reservoir at a rate of approximately 10 ml. per minute, and return to the buret through the combustion tube at the same rate. Repeat the operation for two cycles, but increase the rate to approximately 30 ml. per minute. Measure the volume and continue in groups of two cycles until a constant residual volume is obtained. Accurately measure the volume and record together with the buret temperature. Pass the gas into the potassium hydroxide pipet to remove the carbon dioxide formed by the combustion; repeat the passes until constant residual volume is obtained. Again pass the gas through the combustion tube until no further change in volume is noted and remove the carbon dioxide as before, making certain that the combustion tube is thoroughly flushed free of carbon dioxide. Record the final volume and the buret temperature.

Calculations. Correct all measured gas volumes to a common temperature; assume the temperature coefficient of expansion of the gas to be 0.5% of the volume per degree over the range 15° to 30° C. If the dilution procedure has been employed, subtract the volume of nitrogen added (corrected to the same common temperature) from the residual volume found in the particular absorption during which the nitrogen was added. If the volumes of nitrogen and sample are kept within the limits specified, no correction is applied for the expansion resulting from dilution with nitrogen.

If a constant volume is not obtained in the removal of acid gases, multiply the absorption for the last pass by the total number of passes and subtract the product from the total absorption in potassium hydroxide solution; use this corrected residual volume as the initial volume of the absorption following.

If a constant absorption per group of five passes is obtained in either the 65 or 87% sulfuric acid reagents, multiply the absorption for the last group of five passes in that acid by the total number of such groups of passes and subtract from the total absorption (this corrects for coabsorption of other hydrocarbons). Use this corrected residual volume as the initial volume of the absorption following.

Calculate the volume per cent absorbed in each step of the absorption analysis by means of the following equation:

$$\text{Component, volume \%} = \frac{(I - R)(100)}{V}$$

where I = initial volume, at the beginning of a given absorption, corrected as described above; R = residual volume at the end of the given absorption, corrected as described above; and V = corrected volume of sample charged to the buret.

Calculate the hydrogen content of the original sample by means of the following equation:

$$\text{Hydrogen content, volume \%} = \frac{(A - B)(C + N_1)(100)}{(A)(V)}$$

where A = volume of sample measured in the buret of the combustion unit; B = residual volume after combustion of hydrogen over copper oxide and subsequent passage into potassium hydroxide solution; C = residual volume after carbon monoxide determination; and N_1 = volume of the manifold between the burets of the absorption and combustion units.

Calculate the volume of saturated hydrocarbons, V_s, in the portion of the sample oxidized over precipitated copper oxide by means of the following equation:

$$V_s = (D + E) - F$$

where D = volume of residual gas used for the determination of saturated hydrocarbons; E = volume of nitrogen added to dilute the sample; and F = residual volume from the determination of saturated hydrocarbons after the removal of carbon dioxide.

Calculate the saturated hydrocarbon content of the original sample by means of the following equation:

$$\text{Saturated hydrocarbon content, volume \%} = \frac{V_s(B + N_2)(C + N_1)(100)}{(V)(D)(A)}$$

where N_2 = volume of the combustion unit manifold between the buret and the stopcock of the potassium hydroxide pipet plus the volume of the first copper oxide tube.

Calculate the nitrogen content of the original sample from the difference between 100 and the sum of all other components. As a check on the calculations, also calculate the nitrogen content by means of the following equation:

$$\text{Nitrogen content, volume \%} = \left[\left(\frac{N_3(B + N_2)}{D} - N_2\right)\left(\frac{C + N_1}{A}\right) - N_1\right]\frac{100}{V}$$

where N_3 = corrected volume of residual nitrogen after all combustions and absorptions.

Calculate the carbon number of the saturated hydrocarbons, if desired, by means of the following equation:

$$\text{Carbon number} = \frac{J - K}{V_s}$$

where J = residual volume after oxidation over precipitated

copper oxide, and K = residual volume after absorption of carbon dioxide liberated in the determination of saturated hydrocarbons.

ANALYSIS OF C_4 HYDROCARBONS BY PROGRESSIVE ABSORPTION

This method is intended for the determination of various components in C_4 hydrocarbon fractions using a single sample portion. The method distinguishes among alkyl acetylenes, 1,3-butadiene, isobutylene, normal butylenes, and butanes; it is not capable of distinguishing between individual *n*-butylenes and butanes. The apparatus required is assembled from two of the unit types described by Figure 3 and is shown diagrammatically in Figure 10.

Preparation of Apparatus. Fill the required pipets with appropriate reagents in the following order (the pipets must be arranged in the same order as that in which the absorptions are made; otherwise reactive gas will remain in the manifold and not come in contact with reagent):

Position to Right of Buret	Type of Pipet	Reagent
1	Contact	50% potassium hydroxide
6	Contact	50% potassium hydroxide
7	Contact or contact-bubbler	Potassium iodomercurate
8	Contact	65% sulfuric acid
9	Contact	Acid mercuric sulfate
10	Bubbler	Chromous chloride

Clean and dry the diene absorber and attach to the manifold in the position shown in Figure 10. Heat the water in the jacket to boiling and maintain gentle boiling throughout the determination. Place approximately 3 grams of maleic anhydride in a scoop and melt over a small flame. Add approximately 0.2 gram of diamylamine and draw the mixture into the absorber; to do this, connect the buret to the diene absorber, insert the tip of the inlet tube of the absorber beneath the surface of the molten maleic anhydride, and lower the mercury in the buret. After all the maleic anhydride is in the absorber, form a plug in the inlet tube by applying a small quantity of the solid powdered anhydride to the tip of the tube while it is still hot. Clean the glass joint of the inlet tube, lubricate with hydrocarbon-insoluble lubricant, and close with the ground-glass cap. Slowly lower the mercury in the buret to withdraw part of the air from the inlet tube and turn the buret stopcock so that the absorber communicates with the atmosphere (this brings the level of the maleic anhydride in the inlet tube to a height sufficient to prevent trapped air from leaking into the sample).

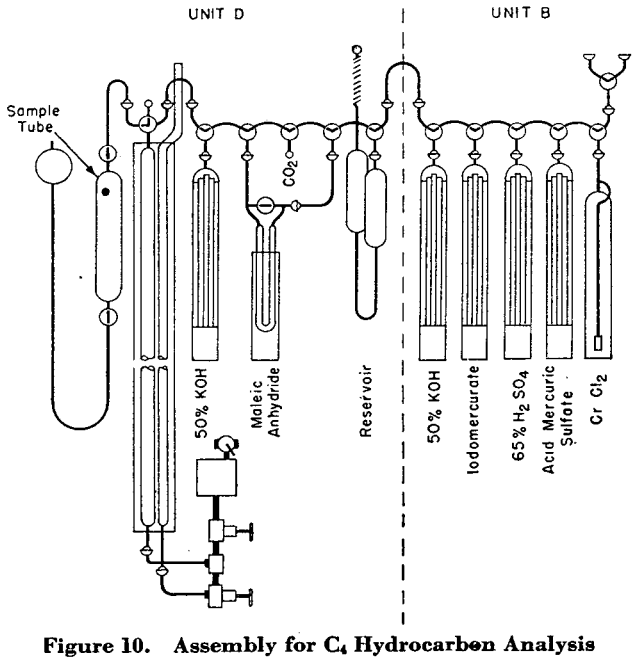

Figure 10. Assembly for C_4 Hydrocarbon Analysis

Wet the buret wall with water and test the apparatus for leaks in the manner previously described.

Saturation of Reagents. As a preliminary step in the analysis, saturate each reagent with the gas that is present in the system after absorption in that particular reagent. If a series of samples of similar composition is being analyzed, perform the presaturation for only the first sample—i.e., each analysis serves to presaturate for the following determination.

Bring the mercury levels in the buret and gas reservoir as well as the reagent levels in the pipets to their respective reference points just below the pipet stopcocks. Flush the diene absorber with carbon dioxide for 1 to 2 minutes to displace all the air and turn the stopcocks on the butadiene absorber to communicate across the manifold. Measure 95 to 100 ml. of sample into the buret and flush the manifold and buret stopcock with nitrogen. Pass the gas into the various reagents as described below for the analysis of the sample, but do not read the volumes or record the data (unless they are desired for purposes of checking the analysis to follow). Discard any residual gas from these absorptions.

Analysis of Sample. Flush the connection between the sample container and buret with sample. Introduce 100 ml. of sample into the buret, accurately measure the volume, and record the buret reading and the buret temperature. Flush the manifold with nitrogen and pass the gas through the absorption steps indicated below, recording the residual volume and buret temperature at the end of each step.

If acid gases are present, pass the measured sample into the potassium hydroxide pipet. Allow the gas to remain in the pipet for 5 seconds and withdraw it to the buret. Repeat until a constant residual volume (or a constant absorption per pass if acetylenes are present in high concentrations) is obtained.

Pass the gas remaining after the acid gas absorption through the diene absorber into the gas reservoir and return the gas through the maleic anhydride to the buret (one cycle). Repeat this operation twice and, with the residual gas in the buret and the reservoir mercury level at the reference mark, establish atmospheric pressure in the buret, in the manifold, and on both sides of the diene absorber. Measure the volume and repeat the diene absorption cycle in groups of three until a constant residual volume is obtained for three consecutive groups (nine cycles). If the residual volume continues to decrease by more than 0.1 ml. per group of three cycles over more than three such groups, discard the gas and test for leaks. If no leaks are found, refill with fresh reagent and repeat as above.

Store the residual gas in the buret. Rapidly, pass a moderate stream of carbon dioxide through the diene absorber into the second potassium hydroxide pipet, until the reagent level reaches the perforated plate at the bottom of the pipet; pass the gas at a fast rate but avoid splashing of maleic anhydride during flushing. Stop the flow of carbon dioxide and immediately transfer the gas in the pipet to the buret without passing through the diene absorber. Pass the gas several times into the potassium hydroxide pipet. Repeat the flushing with carbon dioxide and again remove the carbon dioxide with potassium hydroxide until a constant residual volume or a constant absorption per pass is obtained. Discard the maleic anhydride after about 300 ml. of butadiene have been absorbed. After removing the maleic anhydride by suction through the inlet tube, clean the absorber, if necessary, with water drawn through the hot absorber into a vacuum trap. Dry thoroughly with a stream of air before charging with fresh maleic anhydride. Replace the potassium hydroxide solution after approximately 30 analyses.

Determine the alkyl acetylene, isobutylene, residual olefin, and oxygen contents by passage of the gas into the appropriate reagents as described in the general method (above).

Calculations. Correct all measured gas volumes to a common temperature and correct for any coabsorptions, if necessary, as described in the general method (above).

Calculate the composition in volume per cent by means of the following equation:

$$\text{Component, volume \%} = \frac{(I - R)(100)}{V}$$

where I = initial volume at the beginning of a given absorption, corrected as described above; R = residual volume measured at the end of the given absorption, corrected as described above; and V = corrected volume of sample charged to the buret.

If the oxygen present in the sample is known to be the result of air contamination, results may be calculated on an air-free basis by means of the following equation:

$$\text{Component, volume \% (air-free basis)} = \frac{(21)(C)}{21 - A}$$

where C = volume per cent of the component found, and A = volume per cent of oxygen content.

Table I. Recovery of 2-Butene and Isobutylene from Maleic Anhydride by Carbon Dioxide Flushing

	Volume of Gas, Ml.		
	2-Butene		
	Expt. A	Expt. B	Isobutylene
Initial	100.0	100.0	100.0
After 5 passes	81.4	81.6	81.4
After 10 passes	81.2	80.6	81.2
After 15 passes	81.2	80.4	80.8
After 20 passes	...	80.2	80.8
After 25 passes	...	79.9	...
After 30 passes	...	79.9	...
After CO_2 flushing	100.0	100.0	100.0

ANALYSIS OF C_5 HYDROCARBONS BY PROGRESSIVE ABSORPTION

This method is intended for various components in C_5 hydrocarbon fractions, using a single sample portion. The method distinguishes among alkyl acetylenes, conjugated pentadienes (other than *cis*-piperylene), tertiary amylenes, and residual amylenes; it does not distinguish among individual pentadienes, amylenes, and pentanes. *cis*-Piperylene, if present, interferes because it slowly polymerizes in the diolefin reagent and is slowly absorbed by the olefin reagent.

The apparatus is identical to that used for the analysis of C_4 hydrocarbons, except that a Geissler pump (Figure 7) is attached to the manifold for vaporizing the sample. The assembled apparatus is described diagrammatically by Figure 11.

Preparation of Apparatus. Measure 130 ml. of air, in two portions, into the gas buret and transfer to the Geissler pump; adjust to atmospheric pressure by means of the leveling bulb and mark the Geissler tube at the mercury level. Discard the air in the Geissler pump, then measure 65 ml. of air into the buret, transfer to the pump, and adjust the height of the leveling bulb so that the mercury level in the pump is at the previous mark. (The air is now at approximately 0.5 atmospheric pressure.) Fix the leveling bulb holder in this position so that it can be reproduced at any time.

Dilution with Nitrogen. Attach the C_5 sample tube (Figure 8) to the Geissler pump by means of the ground joint. Displace the air in the capillary of the sample tube and in the connection to the buret by flushing with mercury past the sample tube stopcock and the buret stopcock, respectively; close the upper stopcock on the Geissler pump. Place the leveling bulb at the predetermined level, carefully open the upper stopcock of the Geissler pump as well as the stopcock of the sample tube, and allow sample to vaporize into the chamber of the pump until the mercury level has reached the 130-ml. mark. Close both stopcocks.

Accurately measure approximately 130 ml. of nitrogen, in two portions, and transfer to the chamber of the Geissler pump. Pass the mixture of C_5 hydrocarbons and nitrogen at least five times between the buret and the chamber of the pump to ensure thorough mixing. Store the gas in the chamber of the pump.

Saturation of Reagents. Accurately measure approximately 95 ml. of sample-nitrogen mixture into the measuring buret and saturate each reagent with the gas that will be present in the system after absorption in that particular reagent, as in the preceding method.

Analysis of Sample. Transfer the remaining sample-nitrogen mixture from the Geissler pump to the buret and accurately measure the volume. Proceed with the analysis of the sample in the same manner as described for the analysis of C_4 hydrocarbons (above), omitting the determination of acid gases and oxygen. In addition, flush the diene absorber with carbon dioxide for 12 to 15 minutes between analyses.

Calculations. Correct all measured gas volumes to a common temperature as in the preceding methods.

Calculate the blending factor for converting volumes of the sample-nitrogen mixture to volumes of the original sample by means of the following equation:

$$\text{Blending factor, } F = \frac{(V_1)\ (100)}{(V_1 - V_2)\ (V_3)}$$

where V_1 = corrected total volume of sample plus nitrogen—i.e., the sum of the measured portions of sample-nitrogen mixture, V_2 = corrected volume of nitrogen used for blending, and V_3 = corrected volume of the portion of the blend used for the analysis.

Calculate the composition in volume per cent by means of the following equation:

$$\text{Component, volume \%} = F\ (I - R)$$

where I = initial volume at the beginning of a given absorption, corrected as described above; R = residual volume at the end of the given absorption, corrected as described above; and F = blending factor.

DISCUSSION

The methods described above have been successfully applied for approximately 10 years to a large number of analyses in this laboratory. The sampling and measuring system, employing a mercury lift and leveling tube, has proved to be a definite aid in speeding gas analysis manipulations and in eliminating the tedious handling of heavy leveling bulbs. The use of a water-wetted buret eliminates errors that are otherwise introduced by the changing water content of the gas during analysis. It is recognized that a measurement error is inherent in the use of a wet buret, inasmuch as the measured sample volume is in error by the amount of water used to wet the buret. However, this error is small compared to those which would be caused by the water content of the sample changing during analysis or by solubility effects encountered in the use of water or an aqueous solution as the displacement liquid in the buret. Also contributing to an improved design are other apparatus refinements, such as the constant-pressure reservoir for gas storage and complete cycle passages, the all-glass manifolds, the special stopcock manifold support, and construction of the apparatus in unit sections.

The procedures described have been developed by combining various methods to obtain a maximum amount of information by analyzing a single portion of gas. Use of such comprehensive methods when combined with low temperature distillation, infrared absorption analysis, mass spectrometric analysis, and other physical methods, permits the complete analysis of many of the complex gas mixtures encountered in the petroleum industries.

In the Tropsch and Mattox method for the determination of 1,3-butadiene it is necessary to saturate the maleic anhydride with a portion of the sample in order to eliminate errors caused by the high solubilities of butylenes. This is a great disadvantage from various points of view, particularly with samples of high butadiene concentration. In the latter case, a second saturation is frequently necessary, so that the reagent is rapidly exhausted

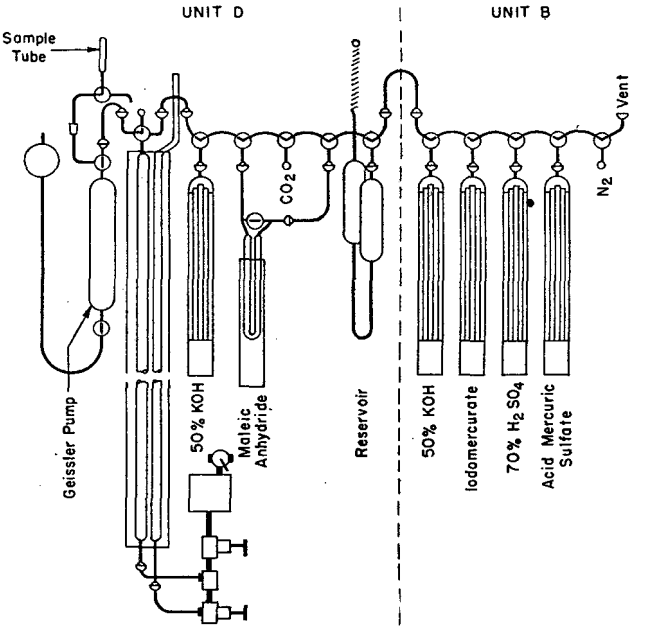

Figure 11. Assembly for C_5 Hydrocarbon Analysis

Table II. Analysis of C_4 Hydrocarbon Mixtures[a]

Components	Sample 15, Volume %				Sample 16, Volume %				Sample 18, Volume %				Sample 19, Volume %		
	Test 1	Test 2	Test 3	Blending data	Test 1	Test 2	Test 3	Blending data	Test 1	Test 2	Test 3	Blending data	Test 1	Test 2	Blending data
Acid gases	0.0	0.0	0.0	...	0.0	0.0	0.0	...	0.0	0.0	0.0	...	0.1	0.1	...
Acetylene	0.1	0.0	0.0	...	0.0	0.0	0.0	...	0.0	0.0	0.0	...	0.0	0.0	...
1,3-Butadiene	18.3	18.2	18.2	18.38	2.1	2.1	2.0	1.80	0.0	0.2	0.1	...	0.3	0.3	...
Isobutylene	7.2	7.3	7.3	7.06	8.2	7.8	7.7	7.98	10.2	10.2	10.4	10.34	47.1	47.1	46.57
n-Butylene	56.4	56.7	56.9	57.44	72.8	73.3	73.5	74.13	11.4	11.3	11.0	11.36	41.7	41.8	43.28
Butane	17.6[b]	17.6[b]	17.5[b]	17.22	16.6[b]	16.6[b]	16.6[b]	16.09	78.2[b]	78.1[b]	78.3[b]	78.30	10.6[b]	10.6[b]	10.15
Oxygen	0.3	0.2	0.1	...	0.3	0.2	0.2	...	0.2	0.2	0.2	...	0.2	0.1	...

[a] Samples obtained from Phillips Petroleum Co.
[b] Calculated by difference.

Table III. Analysis of C_5 Hydrocarbon Mixtures

Composition of Sample, Mole %		Isoprene, Mole %		Tertiary Amylene, Mole %		*n*-Amylene, Mole %		Pentane, Mole %	
		Present	Found	Present	Found	Present	Found	Present	Found
25.0	3-Methyl-1-butene								
29.4	Isoprene	29.4	28.6	...	0.0	25.0	25.2	45.6	46.2
45.6	Isopentane								
50.3	2-Methyl-1-butene	...	0.0	50.3	49.3	...	0.3	49.7	50.4
49.7	Isopentane								
22.8	Isoprene								
43.7	2-Methyl-2-butene	22.8	22.6	43.7	43.8	...	0.3	33.5	33.3
33.5	Isopentane								
51.9	2-Methyl-2-butene	...	0.0	51.9	50.3	...	0.3	48.1	49.4
48.1	Isopentane								
49.6	3-Methyl-1-butene	...	0.2	...	0.0	49.6	49.2	50.4	50.6
50.4	Isopentane								
47.0	Cyclopentene	...	0.1	...	...[a]	47.0	45.9	53.0	54.0
53.0	Isopentane								
23.4	Isoprene								
50.8	2-Methyl-2-butene	23.4	22.8	50.8	51.1	25.8	25.1	...	1.0
25.8	*trans*-2-Pentene								
31.7	Isoprene								
37.6	2-Methyl-1-butene	31.7	31.0	37.6	37.5	30.7	31.2	...	0.2
30.7	3-Methyl-2-butene								
53.6	Isoprene								
34.6	2-Methyl-2-butene	53.6	52.8	34.6	35.4	11.8	11.4	...	0.5
11.8	3-Methyl-1-butene								
34.3	Isoprene								
21.5	2-Methyl-2-butene	34.3	33.6	21.5	21.4	...	0.1	44.2	44.9
44.2	Isopentane								

[a] Tertiary amylene determination omitted.

and requires frequent replacement. In the carbon dioxide flushing method described above for the analysis of C_4 hydrocarbons, thorough presaturation of the maleic anhydride is unnecessary. This is shown by the data in Table I, where 2-butene was passed into maleic anhydride and then completely recovered by flushing with carbon dioxide. The elimination of coabsorption of butylenes in the maleic anhydride avoids any error due to absorption and desorption of these nonreactive gases caused by changes in the temperature of the maleic anhydride and in applied pressure during passage of the gas. In the event that the determination of 1,3-butadiene only is required, it is possible to dispense with the presaturation entirely when using the carbon dioxide flushing technique. This results in a more rapid method of analysis.

The absorption-combustion method has been thoroughly tested with simple gas mixtures of known composition and its reliability with unknown complex mixtures has been confirmed by parallel mass spectrometric analysis. In Table II are shown typical results obtained using the method for analysis of C_4 hydrocarbon mixtures. Although the available samples did not contain any alkyl acetylenes, they did cover a good range of olefin, paraffin, and diene concentrations. In Table III are shown typical results obtained by applying the method for analysis of C_5 hydrocarbon mixtures to samples of known composition. It is believed that these data illustrate the degree of accuracy obtainable with conventional gas analysis techniques. Fundamental errors, such as incomplete specificity of absorbents and deviation of gases from perfect behavior, place limitations on such methods that are difficult to completely surmount.

The time required to analyze gases by the above methods is somewhat dependent on the composition of the samples. Generally, in an 8-hour working day, an experienced operator is able to analyze seven to ten samples by the absorption-combustion method, five to six samples by the method for C_4 hydrocarbon analysis, or six to seven samples by the method for C_5 hydrocarbon analysis. The number of analyses per day is less when the samples vary greatly in composition, for this necessitates presaturation analyses for each sample. On the other hand, a larger number of analyses can be made when certain components are known to be absent and their determination may be omitted.

SUMMARY AND CONCLUSIONS

The analysis of gases encountered in the petroleum industry is accomplished by absorption and combustion methods without the inconvenience generally associated with conventional equipment. A convenient mercury lift eliminates the necessity for using manually operated leveling bulbs to control and direct the flow of gas in the apparatus. A special manifold support, which clamps the barrel of each stopcock to a support frame, reduces the breakage ordinarily encountered with glass manifolds. The extensive use of ball and socket joints achieves flexibility without introducing the errors caused by rubber tubing connections. A constant-pressure reservoir permits convenient and automatic confinement of gases over mercury during complete cycle passes. Construction of the apparatus in unit sections allows the apparatus to be assembled to meet specific requirements.

The methods described in the literature have been subjected to close scrutiny. Those most reliable and most amenable to convenient application have been combined with methods developed in this laboratory to achieve several practical and comprehensive methods. Three methods are described in detail. The first method combines absorption and combustion procedures for the analysis of mixtures containing fixed gases as well as hydrocarbon gases. The second method covers the analysis of C_4 hydrocarbon mixtures by progressive absorption. The third method is for the analysis of C_5 hydrocarbons by progressive absorption of the vaporized sample.

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MULTIPLE EXTRACTION APPARATUS

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A compact and simple extraction apparatus is described whereby any number of analytical samples up to 54 can be simultaneously treated with repeatedly renewed solvent at a temperature near the boiling point. With auxiliary fittings the device can also be used for large scale preparation work and as a liquid-liquid extractor.

IN THE extraction of analytical samples of such tissues as animal bones, seed meals, or dried leaves with organic solvents, much time can be saved if a number of the samples can be treated simultaneously without attempting to keep the extracts separate. The loss of weight of the sample then serves as the measure of the content of fat solvent-soluble material. A device that permits simultaneous continuous extraction of any number of samples up to 18 if the samples are bulky, or of any number up to 54 if they are small enough to be contained in 15-ml. Gooch crucibles, has been in use in this laboratory during the past 3 years with entire success. With a few simple attachments, the apparatus can be adapted for the extraction of moderately large quantities of such materials as seed meals for the preparation of either the oil or the fat-free meal, or for use as a liquid-liquid extractor with a capacity of aqueous solution ranging from 200 to 1500 ml.

The apparatus consists essentially of a tall Pyrex jar provided with a stainless steel cover, to the lower side of which block tin tubing formed into nine vertically disposed loops is permanently attached. This serves as the reflux condenser, and the rest of the apparatus consists of means whereby the solvent which drips from the individual loops is made to extract from one to six separate samples. The assembly as set up with two pairs of holders for the extraction of samples in siphon cups and one pair for extraction in crucibles is shown in Figure 1 to illustrate the relative position of the parts. The jar with solvent in the bottom is heated on an electric hot plate, and after proper adjustment of the heat input and water supply, the apparatus may be left in operation unattended overnight.

CONSTRUCTION

The cylindrical glass jar (Corning Glass Works, Catalog No. 850) is 6 inches in outside diameter and 12 inches (30 cm.) high. The cover is a circular plate of heavy gage stainless steel 6.5 inches in diameter with two 0.25-inch holes 0.75 inch from opposite edges. A collar is made from a strip of thin gage stainless steel 0.875 inch wide and 17.75 inches long rolled into a ring 5.5 inches in diameter. The ring is soldered concentrically to the plate

with pure tin, so that the cover fits loosely inside the top of the jar.

The condenser coil is made from 0.25-inch block tin tubing bent into a succession of close S-shaped loops approximately 2.875 inches in maximum height as shown in Figure 2. When complete, there are nine loops of identical size in one direction and eight symmetrical loops together with the two ends of the tubing in the other. It is important that the length of tubing used to form each loop should be the same. The assembly of loops is bent into a ring that fits closely inside the collar of the cover. One end of the tubing is passed through one of the holes in the cover and the other end is bent at right angles close to the under surface of the cover and carried across to the other hole where it is turned upward and passed through. About 5 inches of tubing should project through the top of the cover and should be bent outward as shown. Somewhat more than 7 feet of tin tubing are required.

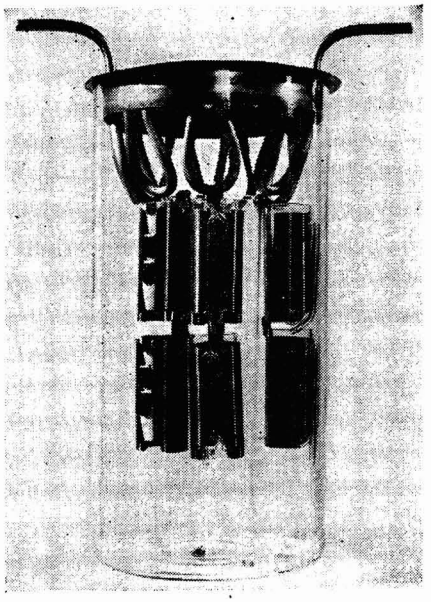

Figure 1. Partially Assembled Extraction Apparatus

2 pairs of holders with glass siphon cups in face and side view, and a pair of holders each with 3 crucibles and stainless steel clips acting as separators. Small clip in top crucibles holds perforated stainless steel disk. In operation, solvent drips from each loop of condenser into siphon cup or crucible beneath.

The coil is soldered inside the cover with pure tin, each upper loop being firmly attached to the steel. Tin is also run to form a small collar around the tubing at each hole to provide a seal. Each separate lower loop is then bent inward through its lower half, so that the lowest points of the loops are equally spaced on a circle 4 inches in diameter concentric with the cover. Any necessary adjustments are made at the same time, so that the suspended loops do not touch each other.

The arrangement provides for maximum cooling effect close to the collar of the cover, thereby preventing loss of volatile solvent and, at the same time, brings the condensed solvent well in from the wall of the jar to drip directly into the siphon cups held below the condenser. For this reason, the accurate spacing of the loops is most important.

The holders for the siphon cups are cut from light gage stainless steel to the dimensions shown in the development in Figure 2, the corners being rounded and the 0.5-inch cuts for the lugs at the bottom being made at the same time. Each holder, eighteen of which are required for the full capacity of the apparatus, is rolled into a cylinder 1.25 inches in diameter so that the opposed free edges are about 0.5 inch apart. The siphon tube of the extraction cup fits into this space. The lugs at the top are formed into hooks as shown and those at the bottom are rolled in to support the A.S.T.M. glass siphon extraction cups (Catalog No. 3920). A slot about 0.5 inch long is cut transversely $^1/_{16}$ inch from the bottom edge of nine of the holders directly under the hook at the top. The other nine holders are cut so that a narrow lug 0.125 inch long (shown in dotted lines in Figure 2) can be bent up and out from the bottom edge in the same relative position as the slots in the first nine holders. The holders can then be assembled in pairs, one suspended by its hook through the slot in the other; the lug on the lower of each pair serves to keep the holders in vertical alignment when hung on their support.

The support for the holders is a 9-inch length of Pyrex tubing 2.5 inches in outside diameter, cut with square ends which are either ground or fire-polished. This stands in the center of the jar and the holders in pairs are hung spaced equally around the upper edge.

A slight modification of nine of the eighteen glass siphon cups is necessary. The straight outflow tube of the siphon is bent inward so as to terminate beneath the bottom of the cup. As is shown in Figure 1, the solvent from the upper cup is thereby discharged into the lower cup when siphoning occurs.

OPERATION

The technique for the preparation of the samples differs according to the nature of the material subjected to extraction.

Chicken tibiae, which are to be extracted in the course of determining the ash weight as part of the assay of vitamin D in poultry feed supplements (*1*), are placed directly in the glass siphon cups, and held in position by a small piece of cheesecloth tucked around the tops of the bones. If carbon tetrachloride is used as the solvent, as suggested by Hubbell, Bliss, and Nolan (*3*), the bones should be previously dried at 110° C. Powdered materials may be weighed in dried paper extraction thimbles (22 × 80 mm. or 25 × 80 mm. cut down to 65 mm.), and held in position by a sufficient quantity of dry solvent-extracted cotton fabric which may either be weighed with the empty thimble or removed after the extraction if the material is such as to permit this. If preferred, Alundum or similar porous extraction thimbles or crucibles can be used. Samples of coarse granular material can be weighed directly in the siphon cups if a small pledget of cotton or glass wool is placed in the bottom to serve as a filter when siphoning occurs. A small piece of cotton fabric should also be placed on top.

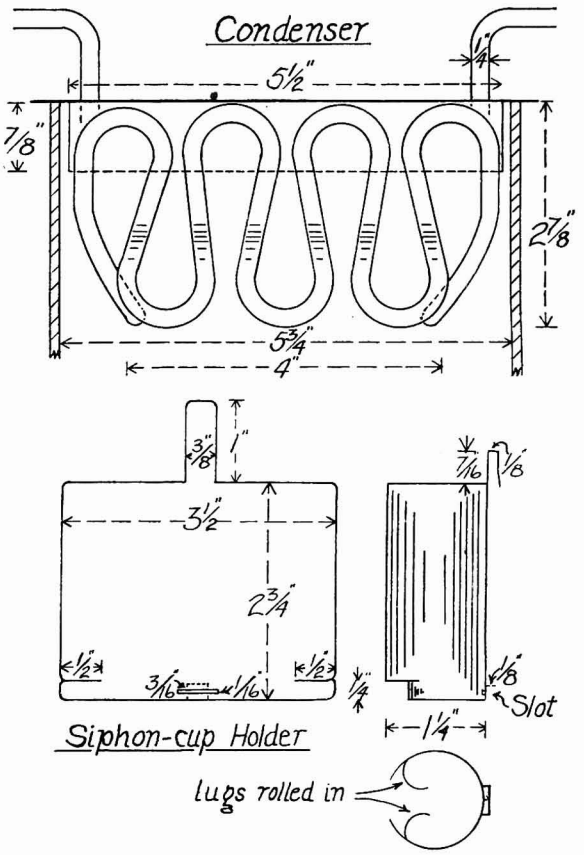

Figure 2. Stainless Steel Cover and Block Tin Condenser Tube Assembly and Stainless Steel Holder

Dimensions in inches

Small samples may also be weighed in 15-ml. Gooch crucibles that contain asbestos mats. Three may be nested together with a small clip of stainless steel plate caught over the upper edge of

each of the lower two to act as a separator (see Figure 1). The top crucible is provided with a perforated disk of stainless steel plate to keep particles of the sample from floating and also to prevent channeling of solvent. This plate is held in place by a lug bent to clip over the edge of the crucible. The nest of three crucibles fits directly in the holder, the solvent dripping down through all three successively.

Samples of dried leaf tissue which are to be extracted with 70% alcohol preliminary to the determination of protein nitrogen may be weighed and placed on small squares of closely woven cotton fabric which are wrapped securely in a loose package and fastened with a metal paper clip which also holds a paper label. Several of these (usually a pair of duplicates) are placed in each of the siphon cups for the extraction.

After the cups or crucibles are suitably charged, they are assembled in the holders and hung on the glass support. Solvent is added to the jar to a depth of a little over an inch, a few angular fragments of quartz are dropped into the annular space as well as the center space in the jar to act as boiling stones, and the condenser is put on with the loops directly over each of the siphon cups. With ether or carbon tetrachloride as solvent, the apparatus can usually be heated adequately at the "low" position of the switch of the hot plate. A shallow constant-level water bath may be used if desired but is not necessary save as an extra precaution against fire when ether is used. The rate of heating is adjusted to obtain siphoning at intervals of a few minutes. With a correctly constructed condenser, the siphoning rate differs insignificantly among the separate cups.

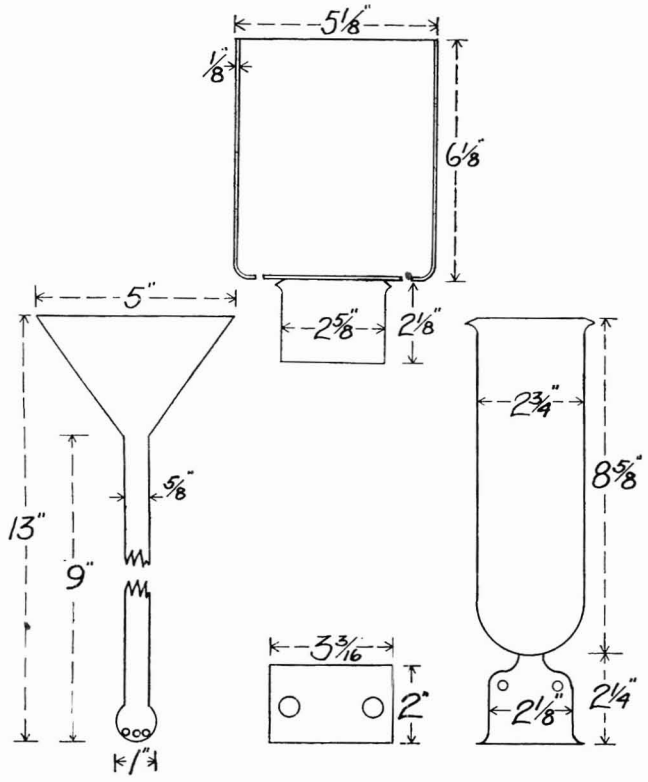

Figure 3. Glass Attachments for Batch Extraction and Liquid-Liquid Extraction

Dimensions in inches

The length of time required for complete extraction must be determined by experience with each material analyzed. It is a function of the frequency of siphoning of solvent.

Any number of holders from one to the full capacity can be used. With small numbers of samples that fill only the top row, a jar 6 inches high (Catalog No. 850) may be substituted.

Strict adherence to the dimensions given is not necessary. Holders can be made to accommodate crucibles or other devices of larger or smaller diameter—for example, glass crucibles with fritted-glass bottoms of medium porosity work extremely well but require a holder of somewhat larger diameter than that shown. Similar equipment of even larger capacity than the present could doubtless be successfully designed.

SUPPLEMENTARY APPARATUS

For the extraction of single large quantities of material such as seed meal, either in preparation for the subsequent extraction of the protein from the fat-free meal or to obtain specimens of the oil, the attachment shown at the top of Figure 3 is used.

This consists of a 2-liter Pyrex beaker (Catalog No. 1000) cut off at a height of about 6 inches, the edge being ground square and smooth. Four or more 0.25-inch holes are pulled in the bottom of the beaker for drainage of the solvent. For a support, a 2.125-inch length of tubing 2.625 inches in outside diameter cut and ground square is used. In order to equalize pressure while the solvent is boiling, several holes may be drilled or pulled in the side, or lips may be pressed in the upper edge as shown in Figure 3.

The specimen of dry ground meal (up to 1000 grams) is enclosed in a bag of closely woven cotton cloth previously extracted with the solvent, the open end being folded close, and is placed in the beaker; the top surface is then flattened. Alternatively, two or more filter papers are fitted tightly into the bottom of the beaker, and the meal is placed on top and covered with several more filter papers closely fitted to the walls of the beaker. The beaker is then rested on the support in the jar, the condenser is adjusted, and the extraction is carried out. The extracted meal is freed from solvent by being dried in the air and the oil can be recovered by distillation of the solvent remaining in the jar. The precaution of using boiling stones must not be overlooked.

For use as a liquid-liquid extractor, a jar 18 inches high is required (Catalog No. 850). The special funnel shown in Figure 3 is constructed from a 5-inch Pyrex funnel (Catalog No. 6100 or 6120), the stem of which is replaced with a 9-inch length of 0.625-inch outside diameter tubing, on the lower end of which a 1-inch heavy-walled bulb has been blown. The bulb is pierced with a number of small holes in the lower hemisphere. It may be found convenient to fasten the funnel to the condenser with fine nickel wire and three small holes may be drilled equally spaced in the top margin of the funnel to permit this.

The fitting shown at the right of Figure 3 is used for aqueous volumes of from 200 to 500 ml. and a similar fitting made from narrower tubing may be constructed for smaller volumes if required. Two lips should be provided for the overflow of solvent as shown in the figure and the support must be pierced with one or more holes. For volumes of 1 liter or more, a Pyrex narrow-mouthed bottle (Catalog No. 1460) of 2-liter capacity can be used. This is supported in the jar on the ring of glass tubing shown in the middle at the bottom of Figure 3.

To operate the apparatus, the aqueous solution is transferred to the tubular fitting or bottle which is placed in the 18-inch jar. Solvent is added to the jar to a depth of about 1 inch and also to the fitting or bottle until it overflows. The funnel is placed in position so that the bulb reaches to the bottom of the aqueous solution. Boiling stones are added and the condenser is put in place. The loops of the condenser serve to center and steady the funnel, but the dimensions of the glass parts must be such that the condenser fits down into place. After extraction is complete (the time required being ascertained by experience) the apparatus is cooled, the funnel is removed and rinsed with water, the fitting is taken out while being rinsed with the solvent, and the extract is transferred from the jar to the apparatus required for the next operation.

DISCUSSION

The main advantages of the apparatus are its flexibility with respect to the number of samples that can be treated simultaneously and the fact that the extraction is carried out at a temperature only a little below the boiling point of the solvent. As in the Underwriters' Laboratory apparatus (*2*), the solvent is thus used at its maximum efficiency.

A wide range of solvents is possible; even solvents of high boiling point could be used, although lagging of the lower part of the jar might then be desirable. Accordingly, the apparatus may find application for the extraction of materials other than dry plant or animal tissues.

The risk of fire is almost negligible if a well-annealed jar is employed and may be practically eliminated by the use of a water bath.

The loss of volatile solvent even on prolonged operation of the apparatus is small, although this is, of course, a function of the temperature of the cooling water and the rate of flow in the condenser. No trouble from solvent loss has been experienced even in warm weather.

Additional advantages are the compactness of the apparatus and the freedom from projecting fragile parts. The jar can be held securely in a frame or by clamps on the hot plate, or by means of the rubber tubing used for the supply of cooling water so that an accidental overturn is impossible.

ACKNOWLEDGMENT

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Coulometric Determination of Halide Ions

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A coulometric method for determination of halide ions is based on measurement of the quantity of electricity required to achieve quantitative reaction of the halide ions according to $Ag + X^- = AgX + e$ when the halide solution is electrolyzed with a silver anode whose potential is controlled carefully. Results of separate determinations of 0.5- to 100-mg. quantities of chloride, bromide, and iodide ions compare very favorably with classical methods. Mixtures of iodide and bromide, or iodide and chloride, can be analyzed accurately by successive electrolysis at the appropriate potentials. The accuracy of the coulometric analysis of such mixtures is better than that obtainable by argentimetric titration. Bromide-chloride mixtures cannot be analyzed very accurately because of the relatively great codeposition (coprecipitation) of silver chloride with the silver bromide.

WHEN a silver electrode is polarized anodically in a solution containing a halide ion X^- the reaction

$$Ag + X^- = AgX + e \qquad (1)$$

can, under proper conditions, be caused to occur quantitatively. Because the silver halide deposits as an adherent layer, which can be washed without loss, this reaction has been used for many years as the basis of an electrogravimetric determination of the halide ions based on the gain in weight of the silver anode. These classical procedures have been reviewed by Böttger (*1*).

In the present study the newly developed technique of coulometric analysis (*3*) has been applied to the determination of the halide ions. The halide solution is electrolyzed in a cell containing a silver anode and a platinum cathode. The nature of the cathode is not critical, and platinum was used simply for convenience. The cathode reaction is $2H^+ + 2e = H_2$. By appropriate adjustment of the total applied e.m.f., the potential of the silver anode is maintained constant at such a value that Reaction 1 proceeds quantitatively and with 100% current efficiency. Under these conditions the current decreases exponentially with time and finally drops to virtually zero when the electrolysis is complete. The quantity of halide ion reacting is then computed by Faraday's law from the quantity of electricity passed, measured by a coulometer in series with the cell.

The total time for an electrolysis is 15 to 20 minutes, and when an automatic potentiostat is used for the potential control the actual operator time absorbed per determination is only a few minutes.

In individual determinations of iodide, bromide, and chloride ions the accuracy compares very favorably with that obtainable by argentimetric titration. The method is applicable on both a macro and semimicro scale. Mixtures of the halide ions can be analyzed by successive electrolysis at the appropriate potentials, iodide ion being deposited first, then bromide ion, and finally chloride ion. The accuracy with iodide-bromide and iodide-chloride mixtures is significantly better than that obtainable by argentimetric titration because coprecipitation errors are smaller.

Inasmuch as Reaction 1 proceeds with thermodynamic reversibility, the optimum potential of the silver anode for a given determination can be computed from the standard potentials of Reaction 1 for the different halide ions. Referred to the saturated calomel reference electrode these standard potentials at 25° C. are chloride −0.024 volt, bromide −0.173 volt, and iodide −0.397 volt, where the negative sign denotes that the silver–silver halide electrode with unit activity of halide ion is negative with respect to the saturated calomel electrode. The potentials of these three couples as a function of the negative logarithm of the halide ion concentration, pX, are shown by the plots in Figure 1. The potential of the silver–silver ion couple as a function of pAg is also shown by the dashed line. The intersections of this "silver ion line" with the silver halide lines, which correspond to the solubilities of the various silver halides in pure water, define the optimum potentials for the individual determinations. At these potentials the total amount of silver oxidized at the silver anode and measured by the coulometer is exactly equivalent to the halide ion present, even though a slight fraction of the silver remains in solution as free silver ion. At more positive potentials the total amount of silver oxidized will be greater than corresponds to the halide present, and high results will be obtained. At more negative potentials a negative error will result. Practically speaking, however, the potential may vary by ±0.1 volt from the theoretically ideal value in the case of iodide, and by ±0.05 volt in the case of bromide, without causing a significant error. In the case of chloride the potential is more critical and must be controlled to about ±0.01 volt.

It is also clear from Figure 1 that the determination of iodide ion should be possible in the presence of 1 *M* or even greater chloride ion. The iodide-bromide separation should be possible up to a bromide ion concentration of about 0.05 *M*. The most difficult separation is that of bromide ion from chloride ion,

which, from the potentials of the two couples, would be expected to be successful only when the chloride ion concentration is smaller than about 0.005 *M*. The limiting concentrations of the second halide ion actually are somewhat smaller than the values predicted from the potentials alone, because of the tendency for codeposition (coprecipitation). As in direct argentimetric titrations of halide ion mixtures, addition of a large amount of an indifferent electrolyte, such as barium nitrate, greatly minimizes coprecipitation.

EXPERIMENTAL TECHNIQUE

For macrodeterminations of 10- to 200-mg. quantities of the halide ions the cell shown in Figure 2 was used. An ordinary 250-ml. beaker served as the electrolysis vessel and the solution volume was approximately 220 ml. Stirring was provided most conveniently by a magnetic stirrer. Cylindrical platinum gauze electrodes were used. The inner, serving as cathode, was 5 cm. high and 2.5 cm. in diameter; the outer, used as the anode, was 5 cm. high and 5 cm. in diameter (effective area ca. 160 sq. cm.). The anode was plated heavily with silver from a cyanide bath, which produced a smooth adherent plate. A silver foil anode would doubtless be equally satisfactory.

Several determinations of the same halide ion may be performed without removing the silver halide deposit on the anode. When the deposit becomes thick it is removed by stripping in a potassium cyanide solution, followed by thorough washing to remove all traces of cyanide ion. Alternatively the deposit may be reduced back to metallic silver by short-circuiting the silver anode with a zinc rod in a dilute sulfuric acid solution, or by electrolysis. When the silver halide previously deposited on the anode is more soluble than the silver salt of the halide to be determined, it must, of course, be removed—e.g., a silver anode previously coated with silver chloride cannot be employed for the determination of bromide or iodide ion, but a silver iodide–coated anode may be used for the determination of bromide or chloride ion.

The potential of the silver anode was controlled against a saturated calomel reference electrode, the salt bridge being filled with a 3% agar gel containing 1 *M* potassium nitrate. The tip of the salt bridge was placed as close as possible to the outer surface of the anode to minimize the amount of *iR* drop included in the apparent anode potential.

The cell used for semimicrodeterminations was similar to that in Figure 2, except that a 30-ml. beaker, and proportionately smaller electrodes, were used.

The electrolysis cell should be protected from white light to avoid photolysis of the silver halide, for this tends to produce a small negative error.

The electrolyses were performed automatically with the potentiostats previously described (*2*).

In macro scale determinations the quantity of electricity passed was measured with the hydrogen-oxygen coulometer described by Lingane (*3*), which was placed in series with the electrolysis cell. For determinations of 0.5- to 5-mg. quantities of the halides on a semimicro scale the hydrogen-oxygen coulometer was not sufficiently precise, and for these determinations a new type of titration coulometer was devised. This consists of a helical silver wire anode (area ca. 80 sq. cm.) and a platinum wire cathode in 75 ml. of an electrolyte composed of 0.2 *M* potassium sulfate (to provide good conductance) and 0.03 *M* potassium bromide. The net coulometer reaction is

$$2Ag + 2Br^- + 2H_2O = 2AgBr + H_2 + 2OH^- \qquad (2)$$

and the hydroxide ion thus produced is titrated with standard 0.01 *N* hydrochloric acid. The end point of the titration is determined most precisely at pH 7 with the aid of a glass electrode. Several determinations may be performed without changing the coulometer electrolyte. A stream of carbon dioxide–free air is bubbled through the electrolyte for stirring, and to exclude atmospheric carbon dioxide. The coulometer is protected from white light to avoid light decomposition of the silver bromide, because this produces hydrogen ion according to the reactions $AgBr + h\nu = Ag + {}^1/_2Br_2$, and $Br_2 + H_2O = H^+ + Br^- + HOBr$. This coulometer can be used to measure as little as 1 coulomb (corresponding to 0.35 mg. of chloride ion) with an accuracy of about 1%, and an accuracy of ±0.1% is obtainable with quantities of electricity greater than about 10 coulombs.

Standard solutions were prepared determinately from pure potassium chloride, potassium bromide, and potassium iodide. The concentrations and volumetric measurement technique were such that the amounts of halide ion present were known to ±0.1% in all cases. All other reagents were of analytical reagent grade, and were tested for halide ion impurities before use.

RESULTS AND DISCUSSION

Chloride. Experiments were made with various supporting electrolytes, and a solution containing approximately 0.1 *M* each of acetic acid and sodium acetate was finally selected as most satisfactory. A neutral unbuffered solution is undesirable because it becomes basic as a result of the hydroxide ion produced by reduction of water at the platinum cathode, and with high concentrations of hydroxide ion there is some probability of the formation of silver oxide on the anode at the relatively positive potential required in the determination of chloride ion. The use of strongly acid solution—e.g., 0.1 *M* perchloric acid—had a tendency to produce slightly low results.

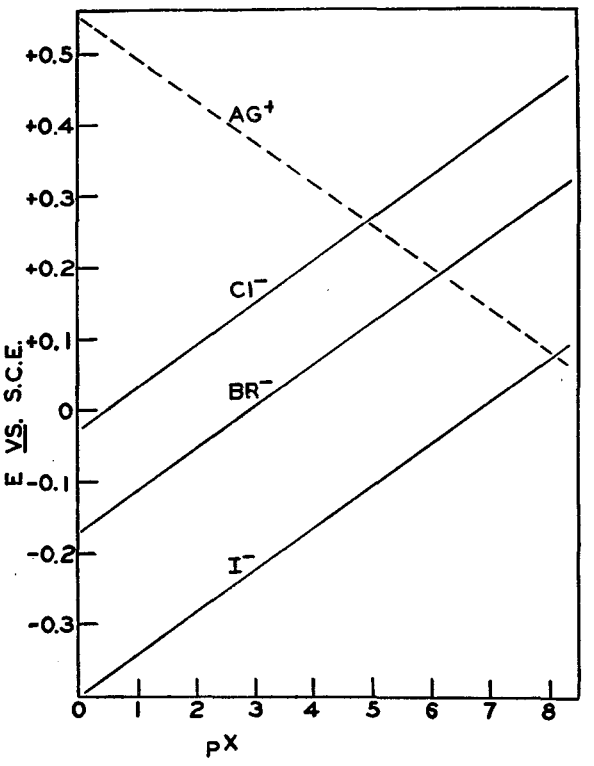

Figure 1. Potentials of Silver–Silver Halide Couples

The potential of the silver anode was controlled at +0.25 volt versus the saturated calomel electrode. Close control of the potential—i.e., to ±0.01 volt—is required. When the potential is less positive than +0.25 volt the deposition of chloride ion is incomplete (see Figure 1). When the potential is more positive than +0.25 volt an appreciable "residual current" is observed. At +0.25 volt the residual current was usually no larger than 1 milliampere, but at +0.30 volt it was about 10 milliamperes and increased very rapidly at still more positive potentials. This residual current is caused by the oxidation of the silver anode to free silver ion. The standard potential of the silver–silver ion half-reaction is +0.558 volt versus the saturated calomel electrode, from which the equilibrium concentration of free silver ion is 1.3×10^{-5} *M* at +0.25 volt, and correspondingly larger at more positive potentials.

The data in Table I demonstrate that the determination of 0.25 to 3 mg. of chloride ion from a volume of 25 ml. on a semimicro scale is precise and accurate to ±0.01 mg., and macro

scale determination of 5- to 80-mg. quantities from a volume of 220 ml. is accurate to ±0.1 mg. In the semimicrodeterminations the solutions contained 5% barium nitrate.

With 80 mg. of chloride ion in a volume of 220 ml. the initial current was of the order of 200 to 300 milliamperes, and it was proportionately smaller with smaller concentrations of chloride ion. In all cases the electrolysis was stopped when the current decreased to 1 milliampere or less, which usually occurred in 15 to 20 minutes. The decrease in current with time obeyed the equation $i_t = i_0\ 10^{-kt}$, where i_0 is the initial current, i_t is the current at time t, and k is a constant which is proportional to the ratio of electrode area to solution volume and to the stirring rate but is independent of concentration. This is the expected relation in a controlled potential electrolysis when diffusion is the current-controlling factor (*3*). The same value for k of 0.20 ± 0.02 min.$^{-1}$ was observed with all concentrations of all three halide ions with the macrocell, from which it follows that the electrolyses were 99.9% complete after 3/0.20 = 15 minutes. This value of k is two to three times larger than that previously observed (*3*) in the reduction of metal ions at mercury and platinum cathodes under otherwise comparable conditions, which reflects the different and more favorable diffusion conditions in the halide determinations. In the reduction of a metal ion the current-controlling diffusion rate is that of the metal ion all the way through the diffusion layer up to the electrode surface. In the halide determinations, silver ion formed at the electrode surface diffuses outward and reacts with the inwardly diffusing halide ion at some distance from the electrode to precipitate the silver halide. This mutual diffusion evidently results in a much shorter diffusion path, and more than compensates for the inhibiting action of the silver halide coating on the electrode.

Bromide. The same acetic acid–sodium acetate supporting electrolyte used for the chloride determination was employed for the determination of bromide ion. Because of the more negative potential of the silver–silver bromide electrode the potential control is not as critical as in the chloride determination, and any value in the range +0.12 to +0.25 volt *vs.* the saturated calomel electrode may be used (see Figure 1). Very satisfactory results were obtained at +0.16 volt, and the residual current at this potential was negligibly small. The electrolysis was stopped when the current decreased to 0.5 milliampere or less, and the total time was 15 to 20 minutes.

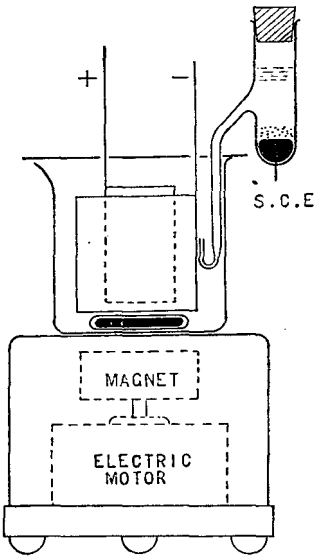

Figure 2. Electrolysis Cell

The data in Table II demonstrate that very satisfactory results are obtainable on both the semimicro (0.5 to 5 mg. in 25 ml.) and macro scale (10 to 200 mg. in 220 ml.).

Iodide. Because the solubility product of silver iodide is the smallest of all the silver halides, the permissible range of potentials for the determination of iodide ion is larger than for the other two halide ions; any value between about −0.1 and +0.25 volt versus the saturated calomel electrode may be used (see Figure 1). This advantage is somewhat offset by the tendency for iodide ion to be air-oxidized, especially in acid medium. The iodine thus produced tends to oxidize the silver anode, $I_2 + 2Ag = 2AgI$, and because the net effect is the formation of silver iodide by a nonelectrolytic process low results are observed. For example, in the determination of 25- to 100-mg. quantities of iodide ion from an acetic acid–sodium acetate supporting electrolyte of pH 5 the results were consistently low by 0.3 to 0.6 mg.

This difficulty was circumvented by two changes in the experimental technique: the use of an ammoniacal supporting electrolyte of pH 9, and removal of dissolved air from the solution by a preliminary electrolysis of about 50 mg. of iodide ion before the iodide solution to be determined is added to the cell. The hydrogen generated at the platinum cathode during the preliminary electrolysis sweeps most of the dissolved air from the solution.

Because of the formation of the silver ammonia complex ion the most positive permissible potential with an ammoniacal supporting electrolyte is considerably less positive than the upper limit (+0.25 volt) in an acetate buffer. The maximum permissible total concentration of all forms of dissolved silver is 10^{-5} *M*. From the dissociation constant (6×10^{-8}) of the $Ag(NH_3)_2^+$ ion, and the standard potential of the silver–silver ion electrode (+0.558 volt *vs.* the saturated calomel electrode), the limiting positive potentials are calculated to be −0.045, −0.081, and −0.163 volt *vs.* the saturated calomel electrode when the concentration of free ammonia is, respectively, 0.1, 0.2, and 1 *M*. Inasmuch as complete electrolysis requires that the potential be at least −0.10 volt, or more positive, it is evident that the concentration of free ammonia must not exceed 0.2 *M*, and preferably should be somewhat smaller. As shown in Table III, very satisfactory results were obtained with solutions containing 0.1 to 0.15 *M* excess ammonia, and with the potential of the silver anode at −0.06 volt *vs.* the saturated calomel electrode. The solutions also contained 5% barium nitrate.

Table I. Coulometric Determination of Chloride

Supporting electrolyte 0.1 *M* sodium acetate–0.1 *M* acetic acid. Silver anode potential at +0.25 volt *vs.* S.C.E. 1 mg. Cl = 0.4743 ml. O_2-H_2 at S.T.P. or 2.82 ml. 0.01 *N* hydrochloric acid)

Cl Taken	H_2-O_2 Coulometer, S.T.P.	Titration Coulometer, 0.01 *N* Acid	Cl Found		Error
Mg.	*Ml.*	*Ml.*	*Mg.*		*Mg.*
0.25	...	0.74	0.26		0.01
0.50	...	1.43	0.51		0.01
1.00	...	2.90	1.03		0.03
2.00	...	5.64	2.00		0.00
3.00	...	8.44	2.99		−0.01
				Av.	±0.01
5.10	2.47	..	5.2		0.1
10.05	4.74	..	10.0		−0.05
25.10	11.83	..	24.95		−0.15
50.05	23.77	..	50.10		0.05
80.2	37.93	..	80.0		0.2
				Av.	±0.1

Table II. Coulometric Determination of Bromide

(Supporting electrolyte 0.1 *M* sodium acetate–0.1 *M* acetic acid. Silver anode potential at +0.16 volt *vs.* S.C.E. 1 mg. Br = 0.2100 ml. O_2-H_2 at S.T.P. or 1.250 ml. 0.01 *N* hydrochlorie acid)

Br Taken	H_2-O_2 Coulometer, S.T.P.	Titration Coulometer, 0.01 *N* Acid	Br Found		Error
Mg.	*Ml.*	*Ml.*	*Mg.*		*Mg.*
0.50	...	0.65	0.52		+0.02
1.00	...	1.26	1.01		+0.01
3.00	...	1.75	3.00		0.00
5.00	...	6.26	5.01		+0.01
				Av.	±0.01
10.0	2.09	..	9.9		−0.1
25.0	5.23	..	24.9		−0.1
50.0	10.50	..	50.0		0.0
100.1	21.00	..	100.0		−0.1
200.2	42.03	..	200.1		−0.1
				Av.	±0.1

Table III. Coulometric Determination of Iodide

(Supporting electrolyte 0.1 *M* ammonium acetate, 0.1 to 0.15 *M* ammonia. Silver anode potential at −0.06 volt *vs.* S.C.E. 1 mg. I = 0.1322 ml. H_2-O_2 at S.T.P. or 0.788 ml. 0.01 *N* hydrochloric acid)

I Taken	H_2-O_2 Coulometer S.T.P.	Titration Coulometer 0.01 *N* Acid	I Found	Error
Mg.	*Ml.*	*Ml.*	*Mg.*	*Mg.*
0.50	...	0.36	0.45	−0.05
1.10	...	0.90	1.15	+0.05
2.00	...	1.57	1.99	−0.01
3.00	...	2.35	2.98	−0.02
5.00	...	3.93	4.98	−0.02
9.98	...	7.78	9.88	−0.10
			Av.	±0.04
10.0	1.35	..	10.2	+0.2
25.0	3.31	..	25.0	+0.0
45.0	5.97	..	45.2	+0.2
80.0	10.51	..	79.5	−0.5
100.1	13.21	..	99.95	−0.15
200.2	26.48	..	200.35	+0.15
			Av.	±0.2

Iodide-Bromide Mixtures. From the foregoing data it is evident that neither bromide ion nor chloride ion reacts at the silver anode under the conditions of the iodide determination. Hence analysis of iodide-bromide or iodide-chloride mixtures is possible by first employing an ammoniacal supporting electrolyte (ammonia and ammonium acetate) for the iodide determination, then acidifying to a pH of about 5 for the bromide or chloride determination.

Table IV. Macroanalysis of Mixtures of Iodide and Bromide

(Supporting electrolyte, 0.1 *M* ammonium acetate, containing ammonia and 5% barium nitrate. Iodide determined at pH 9 and −0.06 volt *vs.* S.C.E.; bromide, after acidifying to pH 5 with acetic acid, at +0.16 volt *vs.* S.C.E.)

Iodide, Mg.			Bromide, Mg.		
Added	Found	Error	Added	Found	Error
10.0	9.8	−0.2	100.0	99.9	−0.1
50.0	49.9	−0.1	100.0	100.4	+0.4
100.0	99.8	−0.2	100.0	100.1	+0.1
100.0	99.65	−0.35	100.0	99.7	−0.3
100.0	99.9	−0.1	100.0	99.9	−0.1
100.0	100.6	+0.6	50.0	50.4	+0.4
100.0	99.7	−0.3	10.0	10.0	±0.0
	Av.	±0.3			±0.2

The typical data obtained on both the macro and semimicro scale shown in Tables IV and V demonstrate that very satisfactory results are obtainable with iodide-bromide mixtures in which the ratio of iodide to bromide varies from 0.1 to 10. These results are considerably better than can be obtained by argentimetric titration of such mixtures, because the tendency for coprecipitation is much smaller under the conditions of the coulometric determinations.

Table V. Semimicroanalysis of Mixtures of Iodide and Bromide

Supporting electrolyte 0.05 *M* ammonium acetate containing ammonia and 5% potassium nitrate. Iodide determined at pH 9.7 and −0.06 volt *vs.* S.C.E., bromide after acidifying to pH 5 with acetic acid, at +0.16 volt *vs.* S.C.E.)

Iodide, Mg.			Bromide, Mg.		
Added	Found	Error	Added	Found	Error
0.50	0.50	0.00	5.00	4.94	−0.06
1.00	0.96	−0.04	2.00	2.01	+0.01
2.00	2.06	+0.06	5.01	4.99	−0.02
2.50	2.45	−0.05	2.51	2.47	−0.04
4.00	3.98	−0.02	4.00	3.94	−0.06
5.08	5.05	−0.03	1.00	1.05	+0.05
5.00	4.93	−0.07	0.50	0.52	+0.02
	Av.	±0.04			±0.04

Iodide-Chloride Mixtures. Iodide-chloride mixtures can be analyzed by the same technique used for iodide-bromide mixtures. The typical results shown in Tables VI and VII demonstrate that the coulometric technique is superior in accuracy to the argentimetric titration of such mixtures.

Because of the great difference in the potentials of the silver–silver iodide and silver–silver chloride couples, and the fact that silver chloride is freely soluble in ammonia, it should be possible to utilize the coulometric technique for the determination of very small amounts of iodide in the presence of very large amounts of chloride.

Table VI. Macroanalysis of Mixtures of Iodide and Chloride

(Supporting electrolytes 0.1 *M* ammonium acetate, 0.1 *M* ammonia, and 5% barium nitrate. Iodide determined at −0.06 volt *vs.* S.C.E. Chloride determined, after acidifying with acetic acid to pH 5, at +0.25 volt *vs.* S.C.E.)

Iodide, Mg.			Chloride, Mg.		
Added	Found	Error	Added	Found	Error
10.0	9.8	−0.2	100.2	100.7	+0.5
25.0	24.55	−0.45	50.0	49.6	−0.4
50.0	49.6	−0.4	50.0	49.8	−0.2
100.0	99.8	−0.2	50.1	50.15	+0.05
200.0	199.5	−0.5	10.0	10.1	+0.1
200.0	199.8	−0.2	10.0	(11.3)	(+1.3)[a]
	Av.	−0.3			±0.25

[a] Omitted from average.

Bromide-Chloride Mixtures. Because the potentials of the two half-reactions involved are close together (see Figure 1), the coulometric analysis of bromide-chloride mixtures presents considerable difficulty. In a series of attempted analyses of mixtures containing 25 to 100 mg. of bromide ion and 25 mg. of chloride ion, from a 0.1 *M* sodium acetate–acetic acid buffer of pH 5 containing 5 to 10% barium nitrate, the results for bromide were 1 to 5 mg. high and the chloride results were correspondingly low.

As far as the potential values only are concerned, these analyses should have been successful, and the fact that they were not is due to the pronounced tendency for chloride ion to coprecipitate with the silver bromide.

Table VII. Semimicroanalysis of Mixtures of Iodide and Chloride

(Supporting electrolyte, 0.05 *M* ammonium acetate, 0.1 to 0.15 *M* ammonia, and 5% barium nitrate. Iodide determined at −0.06 volt *vs.* S.C.E. Chloride determined after acidifying with acetic acid to pH 5, at +0.25 volt *vs.* S.C.E.)

Iodide, Mg.			Chloride, Mg.		
Added	Found	Error	Added	Found	Error
0.30	0.23	−0.07	3.00	2.96	−0.04
0.50	0.51	−0.01	3.00	2.99	−0.01
1.00	0.96	−0.04	2.00	1.98	−0.02
2.00	2.01	+0.01	2.00	1.97	−0.03
2.00	2.00	0.00	1.00	0.99	−0.01
4.00	3.90	−0.10	3.00	2.97	−0.03
5.00	4.85	−0.15	0.50	0.50	0.00
5.00	5.01	0.01	0.50	0.46	−0.04
	Av.	±0.05			±0.02

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Automatic Paper Chromatography

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The preferential elution of a mixture of pigments is conducted in a restricted channel on ordinary filter paper. The progress of the elution is followed automatically as each fraction crosses a patch of monochromatic light. The variations in transmittancy are detected by a photomultiplier tube and recorded by a pen-and-ink recording potentiometer. The separation of microgram quantities is readily attained.

THE use of paper as a chromatographic medium is very old; earlier studies are summarized by Rheinboldt (*7*). Its use has been revived, however, and some of the most striking accomplishments in modern chromatography involve its use, notably in the work on partition chromatography by Martin and his co-workers (*4*).

As part of a general program on this technique, the authors have developed a method for the rapid and automatic examination of paper chromatograms during the process of development (*5*). Great convenience attaches to the use of a confined zone or channel which speeds up the diffusion process and enables one to work with smaller quantities. A typical specimen is shown in Figure 1, in which filter paper is impregnated with a paraffin barrier by a heated die. The process is essentially the one described by Yagoda (*8*) for confined spot tests.

The mixture to be separated is deposited in a small drop at *A* from an appropriate solution and rapidly dried in place by a micro air jet. When the matrix is mounted vertically and a suitable eluent is delivered at point *B*, it spreads rapidly throughout the circular area of this zone and soon begins to rise in the restricted rectangular channel. When the liquid reaches point *A*, it begins to displace the various components of the sample mixture. At some point, *C*, beyond the site of the sample, optical examination of the displaced substances can begin. Some judgment and discretion are required in choosing the distance between *A* and *C*; the optimum value depends upon the ease with which the components of a given system are separated and it is best judged by a preliminary trial. The passage of successive components through zone *C* is detected by a beam of monochromatic light of small area. The light that passes through the paper and components is measured and recorded by the photomultiplier and recorder assembly.

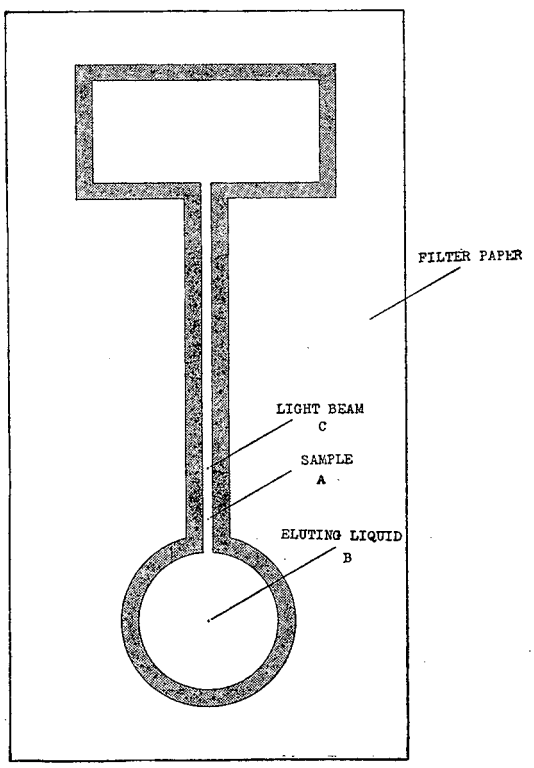

Figure 1. Setup for Analysis of Typical Specimen

EQUIPMENT

The preparation of the confined barriers is a simple matter. An embossing tool was constructed as follows:

A length of wire of rectangular cross section (bus bar) was formed into the contour shown in Figure 1. After forming, it was flattened into a single plane by hammering between two pieces of steel flat stock. One surface was then tinned with a soldering iron and, when cold, was placed upon a somewhat longer sheet of brass which had also been tinned. For convenience in attaching a wooden tool handle, this piece of brass was a short section of right-angle stock. The two pieces were clamped between steel plates by small C-clamps and mounted horizontally in a vise. By heating the entire assembly with a Bunsen burner, the temperature was raised to the melting point of the solder. The wire and supporting plate were thus sweated together. The upper surface of the resulting die was further smoothed and finished by rubbing it over a sheet of fine emery paper. A more elegant and precise technique is to machine the die out of a solid block of brass, by routing out the channels on a milling machine, followed by a surface cut to finish the top.

In preparing the matrices, a piece of filter paper was placed upon a flat layer of filter papers. A piece of paraffined paper was placed on top and the heated die was pressed on the stack. Most commercial forms of wax paper were unsuitable, and it was found necessary to use very fine tissue which had been dipped in melted paraffin and hung up to drain and cool. The die was kept in the inner pot (dry) of a double boiler. If a large number of matrices were to be prepared, the construction of a simple machine for the purpose would be justified. This would contain a steam-heated header in which a variety of dies could be inserted, a simple press arrangement for bringing the die drawn upon the paper, and a simple timer for controlling the time of contact. The authors' apparatus involved no solvents in which paraffin is appreciably soluble, but it is readily apparent why other barriers composed of other impregnants, including low melting point alloys, might be required.

The general arrangement of the equipment is shown in Figure 2.

A concentrated filament lamp, operated from a constant-voltage transformer, served as the light source, and an image of the filament was formed on the entrance slit of a Hilger monochromator. Beyond the exit slit, a low-power microscope objective was mounted which focused a tiny image of the entrance slit on the sample matrix. The Hilger instrument contains quartz optical elements and thus covers the visible and ultraviolet. It was chosen because the authors contemplate an extension of this technique to the ultraviolet. Otherwise a much cheaper instrument is possible; indeed, very useful results can be achieved with selective filters.

The filter paper matrix was supported at one edge between two microscope slides which in turn were mounted on the vertical pedestal of a Reichert mechanical stage. The latter was capable of fine adjustment along three mutually perpendicular axes and readily permitted orientation of the sample in the microscopic monochromatic beam. The light which emerged was conducted by an externally blackened glass rod to the enclosure housing the

photomultiplier tube. This simple light-collecting means could be improved upon by an elaborate optical system, but only if nonreflecting optical elements were used to minimize reflection losses. The cathode, dynodes, and anode of the photomultiplier tube were fed from a stabilized 1000-volt direct current electronic power supply. A two-stage inverse feedback, battery-operated amplifier coupled the photomultiplier tube with a Brown Electronik recorder. This amplifier was operated at low gain and served primarily as an impedance-matching stage for the recorder.

The microcapillary feed for the eluting agent consisted of a fluid reservoir, a stopcock, and bent capillary tube, all mounted on a stand housing vertical and horizontal feedscrew adjustments. By means of these adjustments, the capillary tip could be brought in gentle contact with the filter paper at position *B* (Figure 1). At the desired time, the stopcock could be turned, admitting the eluting liquid to the paper strip. From this point on, recording of the phenomenon was automatic.

TYPICAL RESULTS

The typical results shown in Figure 3 illustrate three cases, the last of which presents an alternative means of separation. For ease of reproduction, these curves have been traced from the original chart records. Pictures of the final samples are not shown here, because ordinary black and white photographs do not appear as striking and definite as the original colored patterns. For the authors' records, each sample has been mounted on black cardboard and filed with the corresponding recorder chart. Greater permanence can be secured by dipping the entire sample in melted paraffin before mounting it. Presumably, any important case could be incorporated in a thermoplastic "sandwich," similar to the practice of preparing identification cards, etc. In some cases, notably *C*, examining the final specimen in ultraviolet light has been useful. The well separated eosin fraction is strongly fluorescent.

A shows the pattern obtained with approximately 1 microgram each of aniline orange and malachite green. From a methanol solution of the mixture, one small drop was deposited at position *A* (Figure 1). The eluting liquid was methanol containing 5% water. In these records, elapsed time is indicated from right to left.

Within 18 seconds after the eluting fluid was admitted, a wave of the fluid had advanced to the optical scanning zone, and the transmittancy increased sharply. An increase in transmittancy arises from the fact that the clear solvent increases the translucency of the paper. Shortly thereafter, the transmittancy decreased rapidly as the malachite green began to diffuse into the scanning zone. It reached a constant value within a few seconds and maintained this value for about 10 seconds. Thereafter a second sharp decrease set in, at which the more strongly light-absorbing aniline orange began to appear. This attained a steady level which persisted for 14 seconds, after which a third unidentified fraction appeared. The aniline orange was known to be impure, as shown later by conventional chromatographic examination, and this record establishes the fact and also that it follows, rather than leads, the aniline orange portion. In this and the remaining examples, the monochromator was set at 550 mμ. Beyond the last decrease in transmittancy, the curve rises slowly, and eventually rises to its original value as every trace of dye is washed out.

B illustrates a somewhat less clear-cut case in the separation of malachite green and Congo red. The second plateau, corresponding to Congo red, is not as well defined, although visual examination of the paper chromatogram would lead one to believe that the separation was just as effective as in the previous case.

C is markedly different. Here a much more dilute solution was employed, at higher recorder sensitivity, and the light scanning zone was located more remotely from the sample site. The methylene blue fraction appeared rapidly, followed by a relatively clear zone which increased the transmittancy almost to its original value. The eosin fraction then appeared. The process required about four times as long as the preceding cases, as indicated by the time axis. In cases of this sort where the degree of separation is fairly complete, it has been found possible to interrupt the process, remove the paper, and after drying it, cut it into two pieces and dissolve away the separate portions. This is not original, but has been employed with success in other investigations (*1*, *2*). The automatically recorded chromatogram gives advance information about the completeness of separation, and it can be verified by spectrophotometric examination of the individual solutions.

The choice of a single wave length is a disadvantage because this cannot be the optimum choice for each constituent. However, because it is monochromatic light, the exact value for the extinction coefficient can be calculated from measurements on large amounts of individual constituents. In some cases, it is actually an advantage, especially if one is examining a material for traces of impurities. If a wave length can be found for which the impurity shows high absorption and the main constituent low absorption, the conditions are most favorable for the detection of very small contamination.

These measurements are semiquantitative and there is good reason to believe that the advantage of the technique lies not merely in its quantitative possibilities, but in the information of other sorts which it can supply. The principal advantage resides in the enormous economy of material. With very little material it is possible to run dozens of tests with various eluting mixtures in order to establish the best eluent. Separation can then be effected in larger amount and the mixture analyzed by more conventional optical methods.

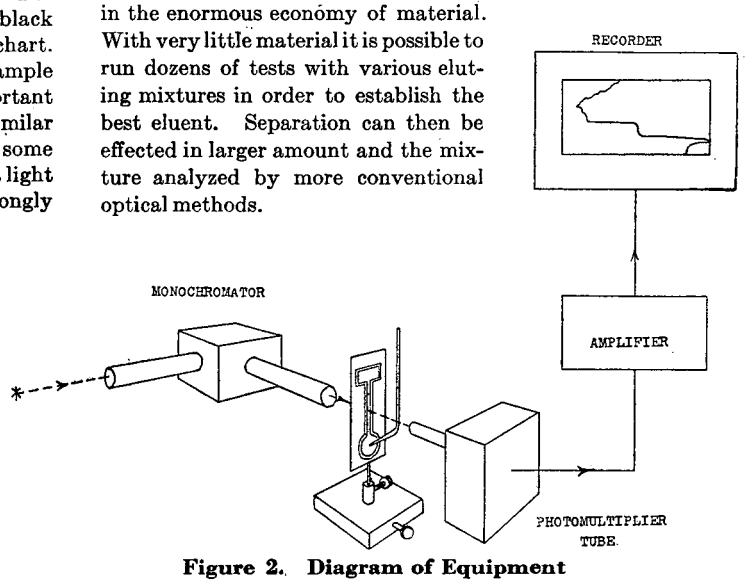

Figure 2. Diagram of Equipment

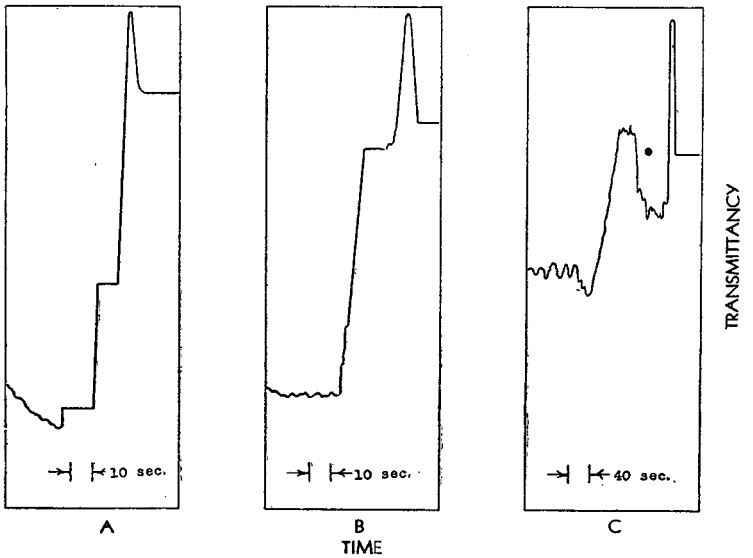

Figure 3. Typical Results

A. **Aniline orange and malachite green**
B. **Congo red and malachite green**
C. **Eosin and methylene blue**

Some advantage would accrue from the use of a logarithmic amplifier (*6*), because the recorded wave heights would then be proportional to the concentration for those substances obeying Beer's law. A modified form of apparatus would permit the measurement of light reflected from the sample to be recorded and the possibility of using the fluorescence of the material, although of restricted application, cannot be ignored any more than it is in conventional chromatography. The extension to the ultraviolet, by either transmittance or reflectance, is even more important because then one is not restricted to colored substances. These, and other possibilities, are under investigation and will be reported later.

The advantages of paper chromatography are not restricted to optical methods. It has been found in this laboratory that conductance measurements are very useful in following the diffusion of ionic species through a paper strip and such measurements are even more easily recorded automatically. Although the use of paper as a chromatographic medium may be considered to have very limited use compared with other adsorbents, the possibility of impregnating it with appropriate soluble or difficultly soluble reagents offers many opportunities to extend its usefulness (*3*).

This instrument, and the several modifications that are under construction, are intended primarily for orientation studies on paper chromatography. Little or nothing has been said here about the type and thickness of the paper, its purity and structure. The instrument has proved very useful in evaluating these factors, the choice of eluting liquids, and almost every factor involved in a proposed separation.

By a minor modification, in which the entire strip could be moved with uniform velocity across the scanning zone, one could obtain a conventional "densitometer" record of a finished chromatogram. Whatever advantages this might possess for quantitative work, it would not provide as complete a picture of the actual dynamics of the process as the present arrangement.

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Filter Paper Chromatography of Penicillin Broths

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The conditions for satisfactory paper-chromatographic separation of penicillins have been examined critically, and a system of operation giving good resolution is described. Two assay methods are indicated, one rapid and of limited application based on maximum diameters of inhibition zones, and the other less simple but of greater accuracy and applicability. In the latter the paper tape is cut, after development, into uniform squares, and the penicillin content of each is determined. The types of penicillin produced by *Penicillium chrysogenum* Q176 on a synthetic medium, and their proportions, are discussed.

THE analysis of mixtures of penicillins by paper chromatography was introduced by Goodall and Levi (*2*, *3*), who described an approximate determination of the different penicillins in a mixture. A qualitative modification of this procedure, more rapid but possessing somewhat lower resolving power, has been reported by Winsten and Spark (*7*). In the present work a number of factors involved in the paper-chromatographic separation of the penicillins have been studied, together with aspects of the assay of the different components. Procedures are outlined which, it is believed, result in improved resolution, reproducibility, and accuracy.

GENERAL PROCEDURE

A filter paper strip, impregnated with buffer solution and then dried, is suspended vertically over the lip of a trough which is later filled with ether. About 0.002 to 0.004 ml. of broth or other aqueous sample, containing 1 to 2 units of penicillin (optimal figures), is spotted at a point on the tape, below the lip, on the outside of the trough. Water-saturated ether, progressing down the tape, is the mobile phase. The stationary phase is water absorbed by the impregnated paper. The entire operation is carried out in a sealed chamber. The distance moved by a given penicillin is a function of the pH of the buffer on the tape, the water content of the system, the distribution coefficient of the penicillin component, and the volume of the ether allowed to flow.

After development, the positions and concentrations of the individual components are determined either by measurement of the zones of inhibition produced when the entire strip is laid on a large agar plate inoculated with *Bacillus subtilis* spores, or by cutting the strip into small uniform squares and measuring the circular inhibition zones produced on *B. subtilis* plates by the individual squares—i.e., the equivalent of a filter-disk assay.

Figure 1 illustrates results obtained by the use of both these methods, and the general chromatographic procedure outlined above. It may be seen that:

The major penicillin components are well separated.

Plating out individual squares gives somewhat better resolution, for there is no loss of resolution due to diffusion of one component into another—which does occur on plating the entire tape.

Measurement of such zones as that of the K types is not easy when the entire strip is plated out. In addition, some doubt was entertained as to the validity of the maximum diameter of a zone as a true criterion of the amount of penicillin present in that zone, irrespective of the shape. (This is the method of assay used by Goodall and Levi, *2*.)

In the "squares" method, a convenient measure of the amount of each penicillin is given by the area under the relevant portion of the curve.

A detailed description of the chromatographic and analytical procedures of the method finally adopted is given below, as well as several factors investigated before establishing this final method. The general procedures adopted during the examination of these factors were those of the experimental part, and only the particular conditions under investigation in each section were varied.

CHROMATOGRAPHIC CONDITIONS

Effect of pH of Impregnating Buffer Solution on Position of Penicillins on Tape. Figure 2 illustrates the results obtained when pure penicillin G was chromatographed on tapes at different pH levels, and developed for 22 hours. It is clear that pH may be used as a tool for controlling the rate of passage of the various penicillins down the tape, a greater rate of movement being possible at lower pH. In addition, tapes at higher pH would require longer development times in order to achieve results equal, in terms of resolution, to those at lower pH.

It emerges that when "20%" potassium phosphate solutions between pH 6.0 and 6.7 are used to impregnate the paper, no change in the distance traveled by penicillin G occurs. This is taken to mean that during the actual experiment, where the small amount of water on the tape is saturated with potassium phosphate, it is saturated with respect to both species—i.e., mono- and dihydrogen phosphates—within the pH range of the impregnating buffer solution mentioned. pH 6.2, falling in the middle of the plateau, would be an extremely suitable choice for the 20% phosphate impregnating solution, for small variations on either side would have minimal effect. This is a satisfactory pH level under the chromatographic conditions specified below, and for maximum stability of penicillin solutions.

The plateau effect is less marked with buffers of lower concentration and is shifted to the acid side. In general, buffer solutions of about 20% concentration have been found more suitable for impregnation purposes than those of lower concentration.

Optimal Time of Flow of Mobile Phase. A close correlation exists between the amount of mobile phase that is permitted to flow down the tape and the pH conditions employed, if a given penicillin is to be brought to a particular point on the tape.

It may be shown that the region of maximum resolution in a chromatogram lies at the middle of its length from the point of application of the substance under investigation to the solvent front. Because penicillin K is the component least likely to be a single entity, and is the most rapidly moving common component reported, it was considered desirable to bring it to the middle of the chromatogram's total length. In order, also, to use the full length of the paper tape, it was desired to bring the K zone to a point just short of the end of the tape, and to make this point about the middle of a theoretical tape twice the length of the actual tape used.

The time required for the ether front to reach the end of such a theoretical tape may be determined by the use of a dye such as Sudan III, which, being soluble in the ether only, clearly demarcates the front. The inclusion of one calibrated tape in each run, from which the progress of the ether front may be followed, allows a time to be set for the development of a particular set of tapes. Figure 3 illustrates the type of curve which may be drawn for such a tape, and the extrapolation to double its length, from which the time of development is determined. A constant rate of drip from the end of the tape is assumed. Slight variations in the time required for development of different sets of tapes do occur, due to temperature changes, use of tapes from different batches of paper, etc. However, the period is usually between 15 and 20 hours for paper of the type of Whatman No. 1.

It has been found that, with a time of flow determined as above, and a tape impregnated with 20% potassium phosphate buffer, pH 6.2, penicillin K does indeed reach a point just short of the end of the actual tape.

Standardization of Humidity Conditions throughout Chromatographic Operation. Although the method of humidifying the tapes for 15 minutes before chromatographing, adopted from Goodall and Levi (*2*), has proved perfectly satisfactory in this laboratory, it was felt that local conditions elsewhere might cause difficulty, because the latter workers have noted the critical nature of this factor. For example, tapes stored in a room of high humidity and then humidified 15 minutes in a saturated atmosphere would conceivably contain more water than those stored in a relatively dry place and then humidified. The present authors have encountered no difficulty over a period of 10 months, covering a wide range of humidity conditions in the laboratory, but it was decided to attempt to define absolute humidity conditions, duplicable anywhere.

In addition, the systems devised by Goodall and Levi, and adopted as routine practice in the present work, are not completely in equilibrium. The ether used is saturated with water, and the atmosphere with water vapor, whereas the paper is still capable of absorbing more water. Changes in the conditions obtaining on the tape therefore occur as the water-saturated ether moves down, and a state of equilibrium is attained eventually only. An attempt was made to introduce conditions such that the process of humidification of the paper would be absolutely defined, so that the amount of water on the paper and in the ether, and the humidity of the atmosphere in the apparatus would be constant and in equilibrium throughout the process of development. The system envisaged was as follows:

A hygroscopic salt, A (with the same action as that of the impregnating buffer solution), was to be included in the buffer solu-

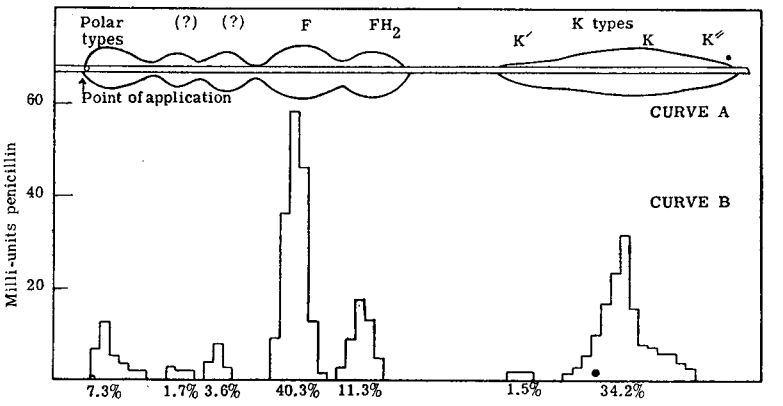

Figure 1. Filter Paper Chromatogram of a Penicillin Broth

Paper tape was cut in half longitudinally after development. The inhibition zones obtained when one of the strips was placed, entire, on a seeded agar plate are shown in scale tracing *A*. The other strip was cut into squares. A determination of the penicillin content of each square gave the data plotted in curve *B*

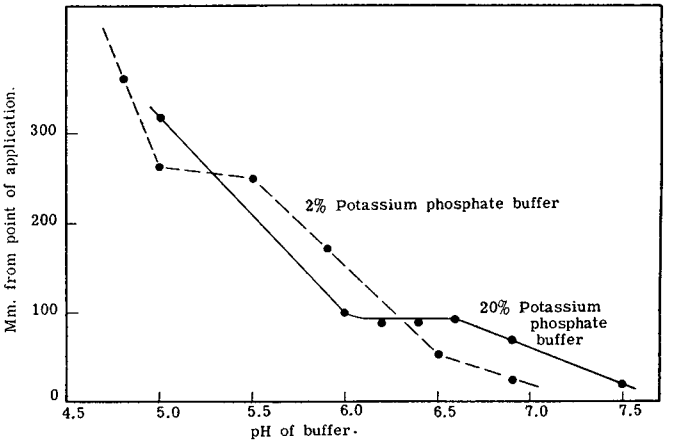

Figure 2. Variation of Movement of Penicillin G with pH of Buffer Solution Used to Impregnate Paper

Mid-point between leading and rear edges of zones plotted

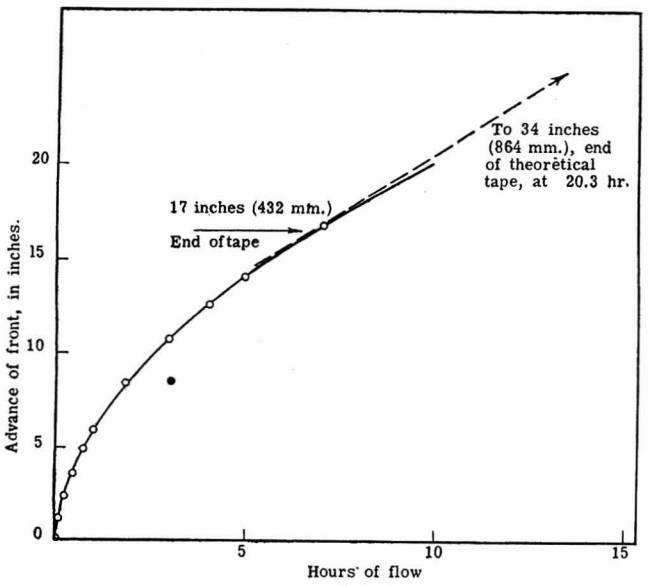

Figure 3. Movement of Ether Front, as Followed with Sudan III
Whatman No. 1 paper

tion with which the paper was impregnated. A series of experiments was to be carried out with tapes stored over saturated solutions of other salts (B, C, D, etc.) of graded constant humidities higher than those of a saturated solution of A. By this means, a series of tapes was to be obtained containing varying amounts of water. The ether to be used in each experiment was to be shaken with the relevant salt solution (B, C, D, etc.), which was also to be placed in the chromatographic apparatus, instead of water. Everything was to be stored in the cold room, at a constant temperature of 4° to 5° C. By this means, transfers of water from the ether to the paper, or from the paper to the atmosphere, etc., during development would be avoided, and a state of complete equilibrium would obtain.

After a considerable number of trials it was found that equilibrium with a saturated solution of a salt of humidity greater than 95% would be required, and because saturated solutions of potassium phosphate yield humidities somewhat below this when in an enclosed space, no additional ingredient A would be necessary in the impregnating buffer. Thus, a system equilibrated against saturated calcium sulfate solution (about 98% humidity) was tried. The tapes were stored over this solution for several days at 4° C. and the ether and apparatus were equilibrated against it. On chromatographing, good separation of penicillins was obtained, and the positions of the components matched those specified below, which were obtained by the ordinary method involving a 15-minute humidification period for the tapes. A system fully equilibrated against saturated calcium sulfate solution at 4° to 5° C. may be regarded as a standard condition. For ordinary routine work, the conditions for humidifying the tapes described in the experimental section have been found to give very satisfactory results in this laboratory.

During the course of the investigation of the above factors, several points of considerable interest were noted:

(*a*) When the ether used was shaken with saturated solutions of salts with humidities up to 66%, and all other conditions as defined in the final method, no movement of the penicillins was obtained. When the ether was treated with saturated salt solutions of approximately 80 and 95% humidity—e.g., ammonium and zinc sulfates, respectively—movement was extremely limited, the limit being, in the second case, a point halfway down the tape. Resolution was extremely poor.

(*b*) Sodium phosphate buffer was found totally unsuitable for impregnating the paper. At pH 6.2 the penicillins moved completely off the tape, and in order to retain them the pH had to be raised to about 8.3. The zones were poorly defined and there was considerable irregularity in the shape of the zones.

(*c*) Tests with sodium and potassium citrate buffers gave moderately good results, which were identical. The anomaly of (*b*) was thought not to be due to the sodium ion per se but rather to the fact that sodium monohydrogen phosphate forms a series of hydrates (mono-, hepta-, dodeca-) whereas the potassium analog does not. This might well disturb the humidity conditions on the tape.

Positions of Individual Penicillin Components on Tape. *It* would be useful if, under defined conditions, figures analogous to the R_f values of Consden *et al.* (*1*) could be assigned to penicillin components. It was thought convenient to express the position of any given penicillin (established by the position of the peak of its inhibition zone) as a percentage of the movement of the fastest moving common crystalline penicillin—i.e., penicillin K. However, in the application of this system to certain broths—e.g., Figures 1 and 5—it was found that several "K type" penicillins may occur, and that the K zone had no real point of maximum diameter, and was extremely long and flat. As a consequence the use of penicillin K as a standard is inconvenient in these cases. Positional data have therefore been based on penicillin dihydro F as standard, its location being arbitrarily designated as 100. Under the conditions of operation, it was found possible to reproduce with reasonable exactness the characteristic positions occupied by definite components. Results of the determination of these characteristic constants for the various common crystalline penicillins are given in Table I, column 2. These data were obtained by plating the entire tape, but similar results may be calculated from the positions of the peaks when the "squares" method is used.

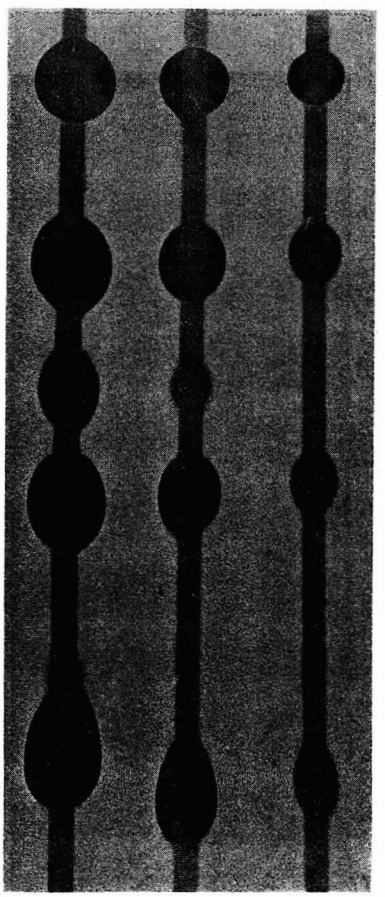

Figure 4. Chromatographic Separation of a Mixture of Crystalline Penicillins

Three tapes shown have equal volumes of a given penicillin solution at three dilutions (1, 1/4, and 1/16, from left to right). The major penicillins from the top of the tape to the bottom are: X, G, F, dihydro F, and K. On the tape with the greatest amount of penicillin the presence of K′ (contaminant of penicillin K) may be noted. Paper tapes were removed before printing

As a result of the application of the chromatographic factors discussed above, and laid down in the final method, resolutions of the type exemplified in Figures 4 and 5 have been consistently obtained.

DETERMINATION OF PROPORTIONS OF DIFFERENT COMPONENTS

"Entire Plating" Method. Some doubt was entertained as to the validity of the maximum diameter of an inhibition zone, irrespective of its shape, as a measure of its penicillin content. Goodall and Levi (*2*) have reported that penicillin G, when moved various distances down the tape, showed an increase in maximum diameter with movement at a level of 30 units, and a decrease at a level of 0.1 unit. At an intermediate level, 3 units, no effect

Table I. Positions of Common Crystalline Penicillins, and Those Produced by *Penicillium chrysogenum* Q176 on a Synthetic Medium[a]

	Crystalline Preparations				Synthetic Broths			
Designation	Position	Av. divergence	No. of Times Encountered Runs	Tapes	Position	Av. divergence	No. of Times Encountered Runs	Tapes
Polar types[b]	...	...	..	..	4.3	± 1.0	7	14
X	8.0	±0.9	12	38	...	...	...	...
?[c]	...	...	..	..	11.0	± 0.4	2	3
?[c]	...	...	..	..	19.5	± 1.7	2	3
?[d]	28.0	±2.7	3	6	...	...	...	..
?[d]	...	...	..	..	32.7	± 1.4	5	8
G	48.0	±1.7	12	38	50.9[e]	± 1.6	7	14
F	77.0	±1.7	11	28	77.5	± 1.8	7	16
FH_2	100[f]	...	12	38	100[f]	...	7	16
K'[g]	136	±4.8	8	13	145	± 7.6	5	10
K	159	±8.2	12	38	168	±12	7	16
K''	...	...	..	..	188	±11	5	9

[a] Components found in two sources whose positions overlap when average divergence figure is taken into account are placed in same category of designation.
[b] Cf. S_1 and S_2 (*1*).
[c] In several broths these, when present in very small amounts, were probably part of tail of pear-shaped "polar type" zone (Figure 5).
[d] Cf. S_3 (*7*).
[e] This component is not definitely penicillin G; its position is the only criterion so far considered.
[f] By definition.
[g] Cf. (*5*).

was noted. These results were obtained by allowing varying amounts of the mobile phase to flow. In the present work a single component of a mixture of penicillins was usually less than 0.4 unit, and diminution of the maximum diameter of its zone of inhibition would be expected, from the above, with increase in zone length. In general, diminution of the diameter of the inhibition zone with increasing elliptical nature of the zone would be expected from the circumstance that the maximum penicillin concentration at any point becomes less as the zone lengthens.

In this investigation penicillin G was moved varying distances by varying the pH of the buffer used to impregnate the tape, maintaining the amount of flow of the solvent constant. From 0.2 to 0.8 unit of penicillin was employed, and comparisons were made with relevant undeveloped standards on paper of the same pH. In most cases no distinguishable effect could be detected. It was, however, not possible to move the penicillin G as far down the tape as was desired without using undue toward conditions of pH or unduly long times of development, which might result in destruction of the penicillin. The use of penicillin K would have allowed a greater range of movement, but was undesirable because of the greater instability of that penicillin.

As a result of the above experience a rapid approximate method for the determination of the components of a mixture has been used, based on the maximum diameter criterion of Goodall and Levi (*2*). The solution under investigation was chromatographed at two and sometimes three dilutions —e.g., 1, 1/2, 1/4 or 1, 1/4, 1/16— and the tapes of each set were laid out on an agar plate of uniform depth, seeded with *B. subtilis*, and then incubated (Figure 4). No standards were necessary, and the assay slope for each plate was readily obtained from the diameters of the components at the different levels. (Assay slope may be defined as the increase of diameter, in millimeters, of an inhibition zone occurring when the concentration of the penicillin is doubled.) The slope used in calculations was the average of the slopes of all components. The composition of the mixture could be obtained in duplicate or in triplicate (though these were not truly independent determinations) by constructing a curve having the relevant assay slope on semilog paper—i.e., diameter *vs.* log relative concentration. The amount of each component could then be read off in arbitrary units from the maximum diameters of the zones, and the composition of the mixture calculated. A typical calculation is exemplified in Table II.

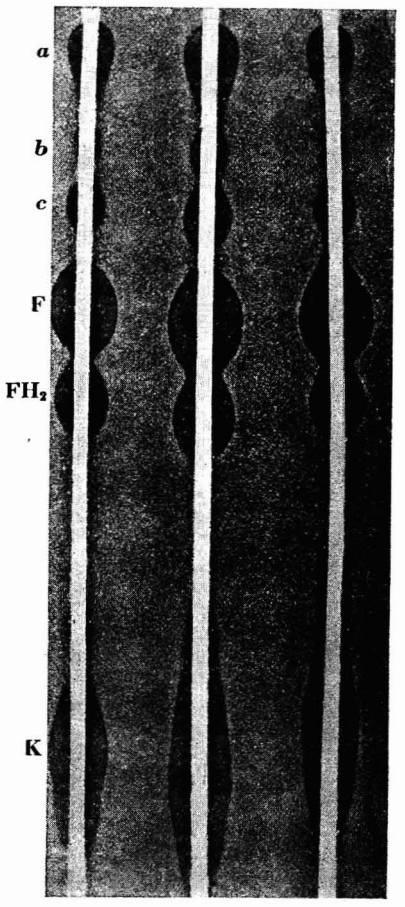

Figure 5. Chromatographic Separation of Penicillins Produced by *Penicillium chrysogenum* Q176 on a Synthetic Medium

Results for three separate broths are shown. The multiple nature of the K zone is evident. Zones designated *a*, *b*, and *c* are described in text

Table III shows the results obtained by the entire plating method on some mixtures of crystalline penicillins. The results are reasonably good. It is in connection with the use of such a method on certain penicillin broths, where extremely elongated zones may be encountered, that difficulties occur. This aspect is discussed in the section on penicillins produced by *Penicillium chrysogenum* Q176 on synthetic medium.

Several additional points of interest were noted:

For components of comparable order of magnitude, the assay slope value tended to increase from the top of the tape to the middle, and then to decrease again to the end—i.e., penicillins X and K tended to have lower assay slope values than G, F, and dihydro F.

The assay slope was not constant, but decreased at very high concentrations of penicillin.

The assay slope of developed penicillins proved, as indicated by Goodall and Levi (*2*), to be greater than that for undeveloped

Table II. Calculation of Components of a Penicillin Mixture by Entire Plating Method

	No Dilution		Fourfold Dilution		
Zone	Diameter, mm.	Penicillin, arbitrary units	Diameter, mm.	Penicillin, arbitrary units	Slope
X	35.9	62	30.0	17	3.0
G	38.1	100	31.8	25	3.2
F	21.0	2.4	Diluted out[a]		
FH_2	36.0	63	30.0	17	3.0
K	36.1	64	30.9	21	2.6
				Av.	3.0

Percentage Composition of Mixture

Penicillin Percentage	X	G	F	FH_2	K
Diln. 1	21.3	34.4	0.8	21.7	22.0
Diln. 1/4	21.3	31.3	...	21.3	26.3

[a] Minor components may be diluted out.

Table III. Analysis of Mixtures of Crystalline Penicillins by Measurement of Maximum Diameters of Inhibition Zones

(Entire plating method)

Mixture No.		Penicillin X, %	G, %	F + FH₂[b] %	F + FH₂[b] %	F + FH₂[b] %	K, %
1.	Theory[a]	39.0	27.0		17.0		17.0
	Found	29.5	26.2	4.8	(26.2)	21.4	18.1
		34.9	27.6	2.9	(20.7)	17.8	16.5
		40.0	27.6	...	(16.8)	16.8	13.8
		24.3	27.1	2.6	(17.3)	14.7	14.3
		47.6	26.2	...	(12.8)	12.8	14.8
		41.0	29.0	3.6	(18.1)	14.5	12.5
		48.0	23.2	2.4	(16.8)	14.4	10.4
2.	Theory	36.8	36.0		12.7		14.6
	Found	40.6	38.1	2.0	(10.1)	8.1	11.4
		46.5	36.2	...	(7.5)	7.5	10.0
3.	Theory	34.1	36.6		14.4		14.7
	Found	26.1	26.1	3.4	(22.2)	18.8	25.6
		36.3	28.6	0.8	(15.3)	14.5	19.7
		37.2	24.3	...	(13.6)	13.6	24.3
		31.8	26.4	4.9	(1.6)	16.7	20.3
		28.3	26.4	2.6	(23.7)	21.1	21.5
		31.8	22.7	...	(18.4)	18.4	26.5
		26.7	...	...	(18.3)	18.3	28.7

Average gross difference from theory[c], 5.3%
Average absolute percentage error[d], 25%
Maximum gross difference observed, 14.7%

[a] Composition of known mixture crystalline penicillin.
[b] Penicillins F and dihydro F were calculated together, because dihydro F used contained about 15% F, and penicillin G used 1.9% F. Penicillin F was not separately added to mixture.
[c] Theory 25%; found 30%; gross difference 5%.
[d] Theory 25%; found 30%; absolute percentage error 20%.

Table IV. Analysis of Mixtures of Crystalline Penicillins by Method of Plating Squares[a]

Mixture No.		Penicillin X, %	G, %	F + FH₂ %	F + FH₂ %	F + FH₂ %	K, %
1.	Theory	12.8	30.4		21.8		35.2
	Found	10.3	31.6	5.9	(16.5)	10.6	42.5
2.	Theory	12.9	26.4		17.4		43.6
	Found	12.1	20.7	3.7	(18.1)	14.4	39.1
3.	Theory	14.3	28.6		17.0		40.3
	Found	15.3	33.0	1.7	(15.9)	14.2	35.7
4.	Theory	24.6	27.5		18.2		29.8
	Found	20.0	33.1	3.0	(19.7)	16.7	27.1
		25.0	28.8	2.2	(20.2)	18.0	25.6
		21.6	35.1	2.0	(19.4)	17.4	24.2
		20.9	31.4	1.8	(17.9)	16.1	29.1
	Theory (check)	24.9	27.6		17.7		29.6
5.[b]	Theory	34.3	31.9		0.0		33.9
	Found	32.6	33.7	...	0.0	...	33.7
		36.5	38.1	...	0.0	...	25.4
6.	Theory	43.1	26.8		0.0		30.2
	Found	52.5	27.5	...	0.0	...	20.0
7.	Theory	42.7	29.4		0.0		27.9
	Found	43.8	26.3	...	0.0	...	31.0
8.	Theory	43.2	26.0		0.0		31.0
	Found	34.4	31.8	...	0.0	...	33.0

Average gross difference from theory, 3.3%
Average absolute percentage error, 15%
Maximum gross difference observed, 10.2%

[a] See footnotes to Table III.
[b] Mixtures 5 to 8 early results; no penicillin F (contaminant of G) detected.

penicillins. The conversion factor (undeveloped assay slope to developed assay slope) of 1.2 quoted by these authors for penicillin G is reasonable.

Method of Squares. In this method, the tape was cut into uniform squares after the development, and these were serially plated. From the diameters of the circular zones formed and a standard curve made with identical squares and penicillin solutions of known concentration, the absolute amounts of the various components could be determined (Figure 1). Table IV gives the results of several analyses carried out by this method on mixtures of crystalline penicillins. The results obtained in the squares assay were somewhat better than those from the entire plating assay.

Because only half the tape is used in each determination (the other may be used for qualitative work, or as a duplicate assay) it was of interest to determine how uniformly the penicillin was distributed across the tape. Figure 6 illustrates a typical tape split down the middle, the two halves being assayed separately. Agreement was good for the larger components, and somewhat less satisfactory for small ones. Even when total recovery of penicillin from the two half-strips has not been identical, it has been found that the proportions of the different components have not been affected.

A considerable advantage offered by the squares method over the entire plating method is the ease with which the recovery of penicillin, in absolute units, may be determined in the former. Because the true amount of penicillin on each square is determined, the total amount recovered on the tape after development may be computed, and referred to the original volume of solution applied and its potency. The potency may be determined with the same test organism and the filter-disk method of assay with squares, as described. Data on the recovery of the various penicillins have been assembled through the use of mixtures of solutions of different crystalline penicillins of known potency. These are reported in Table V. Over-all recovery is satisfactory, and penicillin K is the most susceptible to loss, so that slightly low results might be expected for this component.

In the application of the method to corn steep liquor broths, the few cases studied have shown over-all recoveries never less than 70%, and ranging to 100%, and are regarded as satisfactory. In a study of the penicillins produced by *Penicillium chrysogenum* Q176 on the synthetic medium of Jarvis and Johnson (*4*), however, the situation is disappointing. As may be seen from Table VI, recoveries have been somewhat variable. In any one set of tapes, or "run," the recovery tended to be rather uniform.

Table V. Recovery of Penicillins on Analysis of Crystalline Mixtures by Squares Method

	Total Recovery[a], %	Recovery of Individual Penicillins, % X	G	F + FH₂[b]	K
	93	110	106	...	71
	90	127	107	...	70
	80	78	89	...	72
	100	75	96	...	100
	107	80	102	75	121
	77	85	108	98	83
	86	104	112	91	86
	77	78	113	93	72
	94	89	119	103	102
	90	94	99	84	73
	90	112	79	84	74
	95	107	109	83	81
Av.	90	95	103	88	84

[a] Of original total activity of solution.
[b] See footnote to Table III.

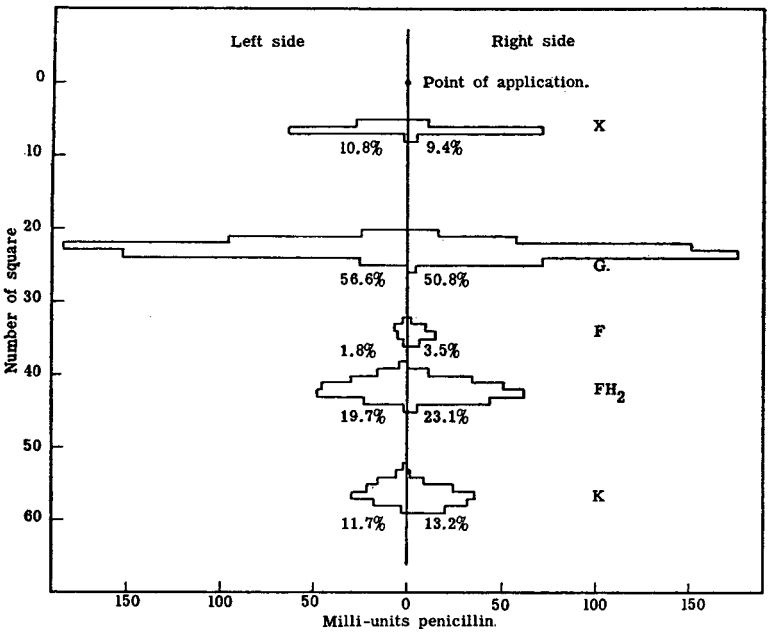

Figure 6. Comparison of Two Halves of a Chromatographic Tape

Squares assay method. Of the total recovered penicillin, 52% was on the left side of the tape, and 48% on the right side. Percentage composition figures calculated from each side are entered under each component

Table VI. Recovery of Penicillin from Synthetic Broths Analyzed by Squares Method

Run No.	Recovery, %	No. of Tapes in Run
1	51	4
2	28	5
3	60	2
4	73	2
5	36	5
6	106	4
7	60	4
8	55	4
9	52	3
10	59	4

Analysis of a number of synthetic broths without precursor has given similar results for percentage composition over several runs in which the recoveries were different and it is therefore probable that no great dislocation of the relative amounts of the different components occurs.

PENICILLINS PRODUCED BY *PENICILLIUM CHRYSOGENUM* Q176 ON SYNTHETIC MEDIUM

A study has been made of the application of the methods described above to the determination of the individual penicillins produced by *Penicillium chrysogenum* Q176 on a synthetic medium (*4*) without the addition of any precursors. Entire plating of tapes after development has shown results exemplified by Figure 5. There occur:

(*a*) A pear-shaped zone around the point of application, tailing downward. More than one component is probably present, and in some cases definite indications of the multiple nature of this zone were obtained, there being apparently three zones overlapping. From the positional figure for this zone of polar compounds, it is clear that at least one large component more polar than penicillin X is present (cf. S_1 and S_2 of Winsten and Spark, *7*).

(*b*) A second, rather small zone.

(*c*) A zone agreeing almost identically in position with penicillin G.

(*d*) Zones that are clearly penicillins F and dihydro F, and whose identity has been established by the inclusion of crystalline penicillin F and dihydro F in the broth.

(*e*) A series of K type penicillins, which form an elongated zone, often showing evidence of at least three components, designated as K′, K, and K″; the middle one is apparently authentic penicillin K (cf. *6*).

As might be expected, methods for the determination of the individual components depending on "maximum diameters" of the zones (*2*) are not satisfactory when applied to mixtures of this type, inasmuch as low values are obtained for the K penicillins, on account of the extreme length of the zone, which has no true point of maximum diameter. The method of squares on the other hand gives higher and, it is believed, more correct results for the K type penicillins in such broths. Comparative data on the application of both methods to synthetic broths (some of which contained various precursors) are given in Table VII.

Positions of Various Components. Table I shows the positions of the components shown in Figure 5, and which are typical of those produced by *Penicillium chrysogenum* Q176 on a synthetic medium without precursor. The penicillins in the K region have, as might be expected, less satisfactorily reproducible positions than that of crystalline penicillin K, owing to the flat nature of the zone.

Average Relative Amounts of Different Penicillins. The following indicates the average amounts of the different components produced by *Penicillium chrysogenum* Q176, on a synthetic medium without precursor. Variations with the time of fermentation, etc., probably do occur, but the general composition indicated appears normal in this laboratory:

Polar types of (*a*) 6.0%; types of (*b*) and (*c*) 3.0%; penicillin F 45.0%; dihydro F 13.0%; K type penicillin 33.0%.

EXPERIMENTAL

Materials. PAPER. Whatman No. 1, available in large sheets, was extremely satisfactory. This was impregnated with buffer and then cut into 1.25-cm. (0.5-inch) ribbons. Whatman No. 4, though affording a more rapid rate of flow, proved inferior to No. 1 in uniformity and resolution obtained. Eaton-Dikeman No. 613 paper, available in rolls of 1000 feet, 0.5 inch wide, was similar in behavior to Whatman No. 1.

ETHER. A good commercial grade of ether was thoroughly washed to remove any alcohol and treated with alkaline pyrogallol several times. Finally it was washed exhaustively with water, and stored in a dark glass bottle at 4° to 5° C.

BUFFER SOLUTIONS. The potassium phosphate buffer referred to throughout as "20% buffer" was prepared as follows. Potassium monohydrogen phosphate was made up at 20% (w/v) and the pH of the solution adjusted to 6.2 (glass electrode) by the addition of 85% phosphoric acid. Phosphate buffers of different pH, where used, were similarly prepared.

CRYSTALLINE PENICILLINS. The following were available: penicillin G, containing 1.9% F, and a trace of a component found between penicillins X and G on the chromatogram; penicillin X, chromatographically pure; penicillin dihydro F, containing about 15% F; and penicillin K, containing a trace of a component referred to in this work as K′. A sample which was mainly penicillin F, with various amounts of other components, was used qualitatively.

AGAR MEDIUM. The nutrient medium used in the assays consisted of 1 gram of glucose, 1.5 grams of meat extract, 6 grams of Bacto-peptone, 3 grams of yeast extract, and 15 grams of agar per liter of water.

B. subtilis SPORE SUSPENSION. A nutrient medium, consisting of 3 grams of peptone and 3 grams of meat extract per liter, was inoculated with *B. subtilis*, Marburg type, and incubated for 6 days on a shaker at 30° C. The suspension was then pasteurized at 80° C. for 10 minutes, and stored in the refrigerator until required. The titer of any spore suspension—i.e., the amount to be added to 1 liter of nutrient agar—was determined by carrying out tests at various levels under the conditions outlined for penicillin standards below, and should be the least amount that yields satisfactory zones.

Apparatus. CHROMATOGRAPHIC CHAMBER. Figure 7 is a diagram of the essential apparatus required for the chromatographic

Table VII. Analysis of Penicillins Produced by *Penicillium chrysogenum* Q176 on a Synthetic Medium (4) by Two Methods of Assay

Method	Polar Types, %	?, %	G Type, %	F, %	FH_2, %	K', %	K Types[a], %
1. Entire	6.6	0.8	3.1	51.3	11.7	...	26.6
Squares	7.9	4.1[b]		49.4	10.0	...	28.9
2. Entire	4.1	0.6	3.0	64.0	12.0	0.9	14.8
	7.6	1.2	4.0	59.6	11.7	1.1	14.6
Squares	6.3	3.5		50.8	10.7	1.7	27.4
	5.5	4.3	3.9	44.8	11.8	1.4	28.4
3. Entire	7.3	1.3	3.8	54.7	14.9	...	18.1
	4.7	0.5	3.1	68.0	10.0	0.8	12.9
Squares	7.5	1.7	3.7	41.1	11.6	1.0	34.8

[a] Where K' was well enough separated, it is reported separately, and K types then refers to the rest of this group. Where resolution was not good enough to allow a separate figure for K', "K types" refers to the entire group.
Figures placed between two components indicate sum of those components.

operation. It was kept permanently in the cold room at a temperature between 4° and 5° C.

A Pyrex jar, *A*, 24 × 12 inches, contained a rectangular stainless steel trough, *B*, supported on a wire frame (not shown) by two glass rods, *C*, attached to the trough by flanges. These rods were fitted directly above the rim of the trough. They served to keep the tape, *D*, clear of the sides of the trough. The tape was maintained taut and in position by two clips, *E*, of suitable weight, and a heavy glass bar, *F*. Several thicknesses of filter paper, *G*, saturated with water, covered the bottom of the jar and extended about 12 inches up the sides of the jar; they were held in position by the wire frame. Two bottles, *H*, equipped with spread filter-paper fans and filled with ether, kept the atmosphere saturated with ether. The jar was sealed with a plate-glass top, *I*, through which passed an inlet, *J*, which could be plugged, or connected to the constant-level bottle, *K*, by a ground-glass joint. (Dotted lines show the bottle twisted into position during development.)

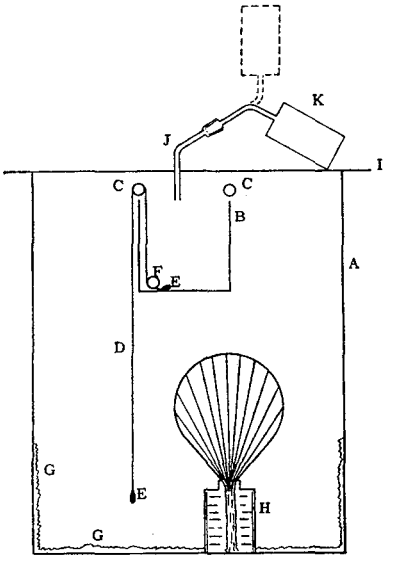

Figure 7. Chromatographic Chamber

Humidification Chamber. A Pyrex jar was kept at room temperature (25° to 30° C.). The top had no inlet and no ether bottles were included. The atmosphere was saturated with water vapor by means of wet filter paper.

Large Assay Plates. These consisted of an aluminum frame (inner dimensions 18 × 10 inches) with a removable plate-glass bottom normally held in place by screws. A glass cover was provided for the plate.

Small Assay Plates. Petri dishes, 3.5-inch internal diameter.

The agar medium was seeded just prior to pouring the plates; 20 ml. of seeded medium were optimal for a Petri dish; an equivalent amount—i.e., one giving the same depth of medium—was used for the large plates. The latter were not sterilized but were cleaned by thorough washing with soap and water, rinsing with distilled water, rubbing down with 50% alcohol, drying off with a piece of sterile gauze, and finally flaming thoroughly. They were then carefully leveled on a leveling table, and poured rather hot (about 70° C.) to ensure uniformity of depth of seeded agar over the entire plate. After pouring all plates were stored in the cold room until needed.

Micropipets. These should deliver 2 to 10 μl.

Procedure. Preparation of Paper. The paper was impregnated with the 20% pH 6.2 phosphate buffer solution described above, laid between two large sheets of blotting paper, thoroughly and uniformly blotted, and hung up to dry.

Each tape was marked at a point that would fall just below the glass bar above the rim of the trough when the tape was clipped and in position. This was to be the point of application of the penicillin solution, and the tape extended at least 17 inches below it. Care was taken to touch the tapes as little as possible with the fingers. As far as was possible forceps were used, but where handling was unavoidable the tapes were touched lightly by the edges only.

Spotting the Paper. The tapes were clipped at each end, and placed in position in the trough, as illustrated. The penicillin solutions, varying in volume with their potency, were then applied at the marked points. A volume of 2 to 4 μl. of solution gave optimal results, but in the case of weak broths up to 16 μl. (applied in two 8-μl. amounts) has been used. In such cases a few minutes were allowed for some of the water to evaporate before proceeding to the next operation. In the case of a large set of tapes, this would in any event occur during the "spotting" of the whole set, and about 3 minutes were allowed after treating the last tape to permit some loss of water from it.

A total of between 1 and 2 units of penicillin, for a take 0.5 inch wide, was optimal. Less than this made the detection of minor components difficult. From 50 to 400 milliunits of any one component could be accurately determined, and the threshold value for detection of a component was about 5 milliunits. Where the "entire" quantitative method was used, solutions under investigation were applied at two or three levels of concentration, or in different volumes, varying by factors of 2 or 4. Where broths were examined, it was found necessary to filter thoroughly through cotton before use.

When complete equilibration against saturated calcium sulfate solution (as previously described) was used, the process of spotting was carried out rapidly in the chromatographic chamber, and not more than 4 μl. of solution was used.

Humidification of Tapes. The trough and pendant tapes were placed on the wire frame in the humidification chamber for exactly 15 minutes (*2*), and then removed rapidly to the chromatographic chamber which was opened as briefly as possible and then sealed again. Care was taken that the tapes hung straight and clear of the trough.

The humidification process was omitted when tapes stored over saturated calcium sulfate solution were used—i.e., in the completely equilibrated experiments.

Development. The tapes were allowed to hang in the chromatographic chamber for one hour. The trough was then filled with the water-saturated ether through the inlet in the glass lid, which had been kept stoppered. When sufficient ether had been added to the trough just to cover the bottom of the inlet tube, the constant-level reservoir was filled, inverted, and clamped in position. The bottom of the inlet tube was about 12 mm. below the top of the glass bars over which the paper passed. The rate of movement of the ether on the paper could be changed by adjusting this distance.

Development was allowed to proceed for a period determined as described above, by the inclusion of a calibrated tape in the run, on to which a spot of Sudan III in ether had been applied instead of penicillin solution. At the end of this period the tapes were lifted from the trough and removed to room temperature, hanging freely from the top clip. They were allowed to dry for 15 minutes.

Plating out and Measurement of Zones. For qualitative results and for the entire quantitative method, either half-tapes (0.25 inch) or the whole tapes (0.5 inch) were used. The plates were removed from the cold room and the tapes immediately laid on the agar as evenly as possible. They were then returned to the cold room for 3 hours, after which they were incubated at 30° C. until the inhibition zones were well defined (about 12 hours).

After incubation, the glass plates were removed from the aluminum frame, and laid on sheets of high contrast photographic paper for preparation of contact prints. It was sometimes desirable to clean off overgrowth of the test organism from the agar with wet soft cotton. Measurements of the zone diameters and positions (at the point of maximum diameter) were made on the print. Calculation of the percentage composition was carried out as illustrated in Table II.

When the method of squares was used, the 0.5-inch tapes were split down the middle into 0.25-inch tapes, one or both of which could be used for the squares assay. The splitting and cutting could be facilitated by marking out the Whatman sheets or the Eaton-Dikeman tapes accurately in pencil before use. They were cut with a steel rule and a sharp blade, care being exercised to handle the squares as little as possible. However, a jig has been routinely used in this laboratory, and has been found most

serviceable. This consisted of a brass bed 0.5 inch wide, into which the entire tape was laid. A bar 0.25 inch wide was then fitted into position on two guide pins, and the tape was split into two 0.25-inch tapes with a sharp blade. The raised sides of the bed were slotted at 0.25-inch intervals with slots 0.01 inch wide, which acted as guides for the blade and the tape could be cut into 0.25-inch squares. Preliminary marking out was eliminated by the use of this jig, and the results were of greater uniformity and precision.

As the tape was used from 1 inch above the point of application to 17 inches below it, a total of 72 squares was obtained. These were handled with forceps and plated seriatim on Petri dishes, each of which accommodated eight squares, two of which, diametrically opposite each other, were standards—i.e., blank squares dipped into a standard penicillin solution (4 units per ml.). Thus twelve plates were required for each tape. It was not necessary to dampen the squares from the experimental tape at all before laying them on the agar, for sufficient water was immediately absorbed.

A set of standards for determination of the slope for the assay was established with two or more replicate blank 0.25-inch squares dipped into penicillin solutions of 1, 4, 16, and 64 units per ml. Where recoveries were to be determined, the solutions under investigation were assayed in the same series.

It has been found necessary, for maximum uniformity of the amount of penicillin solution taken up by each square, when the edge was dipped as above, to touch each one briefly by its edge once or twice against a clean dry filter paper, to remove excess penicillin solution, before laying it on the agar. The volume of penicillin solution absorbed by each square, which is readily determinable by weighing, varies somewhat with the batch of filter paper being used, but is usually close to 4 μl. For purposes of illustration, it will be assumed that the filter paper in use absorbs exactly this volume. The squares dipped in the standard penicillin solutions then contain, respectively, 4, 16, 64, and 256 milliunits of penicillin.

It was found satisfactory to prepare the agar plates up to one week in advance. Where Petri dishes were used, the plates were removed from storage in the cold room, and before the squares were applied, were preincubated for about 30 minutes at 37° C., open and upside down, so that some drying might occur. The length of this preincubation period was such that an applied 16-milliunit standard gave a zone, on incubation at 30° C. for about 10 hours, of about 18-mm. diameter, and a positive indication for a standard with 2 milliunits of penicillin.

The large plates described above could also conveniently be used in this method. Here the squares were plated as above, and sufficient standards included to serve as controls. Each large plate could accommodate the squares from a complete tape, together with standards, and a determination of assay slope in duplicate. It was not usual to preincubate these large plates, but to remove them from storage at 4° C., apply the squares, and then immediately incubate at 30° C. (On account of their size, cooling was slow after pouring them, and preincubation may be regarded as having occurred before storage.)

CALCULATION. The diameters of the inhibition zones on each plate were measured to the nearest 0.2 mm., those on Petri dishes directly after incubation, and those of large plates from contact prints. The diameters of the zones on each plate were corrected to some convenient diameter—e.g., 18 mm.—for a 16-milliunit standard. Thus, if the average diameter of the pair of standard 16-milliunit zones on a given plate was 18.6 mm., 0.6 mm. was subtracted from the diameter of each test zone on that plate. On the large plates little correction was required, as the depth of agar was more uniform than in the set of Petri dishes. A standard curve was drawn (semilog graph paper is convenient), the logarithm of the penicillin unitage being plotted against zone diameter. The curve was found to be a straight line (assay slope constant) below 64 milliunits, but the assay slope usually decreased somewhat at higher penicillin concentrations. The penicillin content of unknown samples could then be read from this curve.

Curves similar to those in Figures 1 and 6 could be drawn for a chromatogram of a penicillin mixture. The amount of any one penicillin present was obtained by summation of the amounts on the squares containing that component, and the percentage composition by reference of the figures for each component to the total amount of penicillin found on the tape. The analytical results obtained were in terms of *B. subtilis* units rather than the usual *S. aureus* units. The results obtained with either organism can be expressed on a weight or molar basis only for penicillins for which the potency (usually expressed in units per milligram) is known. For the more common penicillins, the *B. subtilis* unitage more closely parallels the molar concentration than does the *S. aureus* unitage. It is, of course, feasible to use *S. aureus* as the assay organism. *B. subtilis* was found more suitable, however, because of greater freedom from contamination, greater sensitivity, sharper zones, and the convenience and uniformity of the spore inoculum. Moreover, only one layer of agar need be poured on the plates.

SUMMARY

The conditions necessary for optimal resolution of penicillins on paper chromatograms with an ether–aqueous buffer system and paper of the type of Whatman No. 1 have been investigated.

Impregnation of the paper with approximately 20% phosphate buffer, pH 6.2, a development time of about 20 hours (the time required for the ether front to reach the end of a paper tape twice the length of the tape used), and humidity conditions corresponding to equilibration against saturated calcium sulfate solution gave excellent results. Under such conditions, the fastest moving common crystalline penicillin, penicillin K, moved approximately 350 mm. from the point of application. The calcium sulfate equilibration was intended purely as a standard condition of operation, and in fact gave no better results than the less rigid, but far more practical, humidification of the tapes at room temperature for 15 minutes (*2*), and the use of water-saturated ether. The resolution obtained is, it is believed, superior to that previously reported in the literature (*2, 3, 7*).

For quantitative work, a procedure has been adopted which involves cutting the paper tape into squares and determining the penicillin content of each square by a procedure similar to the paper disk plate assay. This procedure gives improved resolution and greater accuracy than procedures based on measurement of the maximum diameter of the inhibition zones produced when the entire tape is plated. This is particularly marked in the analysis of broths yielding extremely elongated multiple component zones, such as the K type zones of the penicillins produced by *Penicillium chrysogenum* Q176 on a synthetic medium.

Characteristic positional constants (analogous to the R_f values of Consden *et al.*, *1*) have been determined for various penicillins. These constants are of value in chacterizing components of an unknown mixture.

The penicillins produced by *Penicillium chrysogenum* Q176 on a synthetic medium have been studied. At least eight penicillins are formed, penicillins F, K, and dihydro F being the major components. Most, if not all, of the other components appear to be penicillins of unknown structure.

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An Improved Weight Azotometer

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A simplified weight azotometer for micro-Dumas determinations uses the Clarke-Winans principle of weighing displaced mercury for measuring nitrogen volume, but disassembly between determinations is unnecessary. It permits greater precision than the conventional Pregl volumetric azotometer. Calculations have been simplified by introducing a nomogram which gives a single factor for multiplication of mercury weight to get nitrogen weight.

AN AZOTOMETER for micro-Dumas analyses which permits determining nitrogen by weighing an equal volume of mercury has several major advantages over the Pregl volumetric azotometer (*2*, *3*). Errors from caustic adsorption on the surface of the glass and change of calibration from corrosion are completely eliminated. Errors due to the volume estimation are reduced to a minimum because 1 microliter of nitrogen corresponds to approximately 13.5 mg. of mercury. Potassium hydroxide does not come in contact with the stopcock lubricant.

A mercury azotometer has been developed in this laboratory which is similar to that proposed by Clarke and Winans (*1*), but more simple in design. A more important advantage lies in the fact that the portion of the apparatus containing mercury is not disconnected at any time, and so there is no danger of trapping air bubbles in the mercury. In addition to causing a possible error in the amount of mercury weighed, an air bubble also increases the difficulty in adjusting the potassium hydroxide level in the absorption tower.

APPARATUS

The apparatus shown in Figure 1 consists of two units, the carbon dioxide absorption tower and the mercury displacement unit. The absorption tower is constructed of 12-mm. Pyrex tubing and 1-mm. capillary tubing and is about 45 cm. (18 inches) high. A ring, *A*, is etched on the capillary tubing attached to the exit of the absorption tower. The volume of the capillary tubing between etched mark *A* and stopcock *E* should be small, so that any temperature or pressure change during the period of combustion will not affect appreciably the volume of nitrogen gas collected. The mercury displacement unit is attached to the absorption tower by means of a 7/25 ground joint which can be sealed with wax. The sole function of the joint is to facilitate cleaning of the apparatus. The body of the mercury displacement unit is constructed of 8-mm. Pyrex tubing. This is drawn down to join the microstopcocks, *B* and *C*, giving an over-all length of about 12.5 cm. (5 inches). The volume of this part of the unit is approximately 3 ml. The side arm of the unit is made of 5-mm. tubing. A length of 0.5-mm. capillary tubing is attached to *C* and drawn out to give an easily controlled rate of mercury flow.

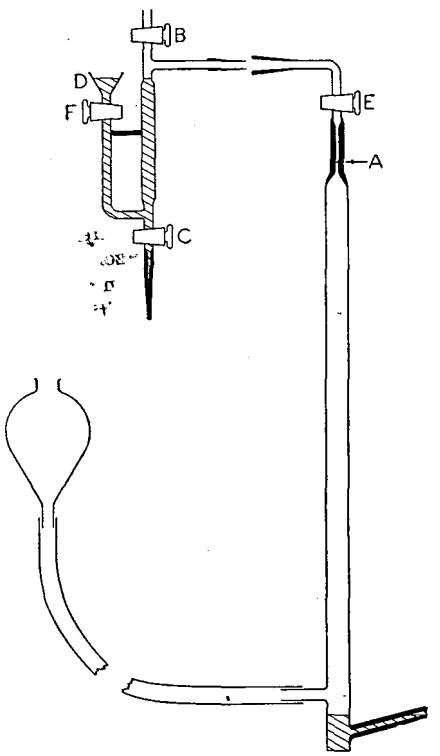

Figure 1. Apparatus

PROCEDURE

Mercury is added to the bottom of the absorption tower to a height approximately halfway between the gas entrance tube and the leveling bulb connection. Sufficient 50% potassium hydroxide solution is added through the leveling bulb to permit the tower to be filled by raising the leveling bulb. The mercury displacement unit is filled through reservoir *D* with clean, dry mercury; care is taken to remove all trapped air bubbles.

The combustion tube is swept out in the usual manner. The level of the potassium hydroxide solution is adjusted to *A* in the capillary portion of the absorption tower with stopcocks *B* and *E* open. This can be done easily by mounting the leveling bulb support on a rack and pinion attached to a ring stand. When the level of the potassium hydroxide solution is adjusted, *E* is closed, and the location of the leveling bulb is marked so that it can be returned to the same position. The bulb is then lowered, and the sample is burned in the usual way.

After the combustion and sweeping procedures have been completed, the leveling bulb is returned to its previous position, and *B* and *F* are closed. *E* is then opened to the mercury displacement unit, and mercury is drained through *C* into a tared container, until the level of the potassium hydroxide solution has just returned to mark *A*. The amount of mercury transferred to the weighing vessel is then weighed to the nearest 5 mg.

CALCULATIONS

The analytical results are calculated from the weight of mercury displaced. The usual calculation is accomplished by determining the volume of mercury from its weight and density at the experimental temperature. The amount of nitrogen may then be determined by reducing the volume to standard conditions, using the experimental temperature and barometric pressure (corrected for the vapor pressure of water over 50% potassium hydroxide). The development of a nomogram for the major part of the work has greatly simplified the calculations.

The data in the International Critical Tables for the density of mercury and the vapor pressure of water over 50% potassium hydroxide as functions of temperature have been fitted by empirical equations within the temperature range 18° to 34° C.:

$$d_{Hg} = 13.576 - 0.00102T$$

$$p_{H_2O}\ (\text{mm.}) = -0.45 + 0.15T$$

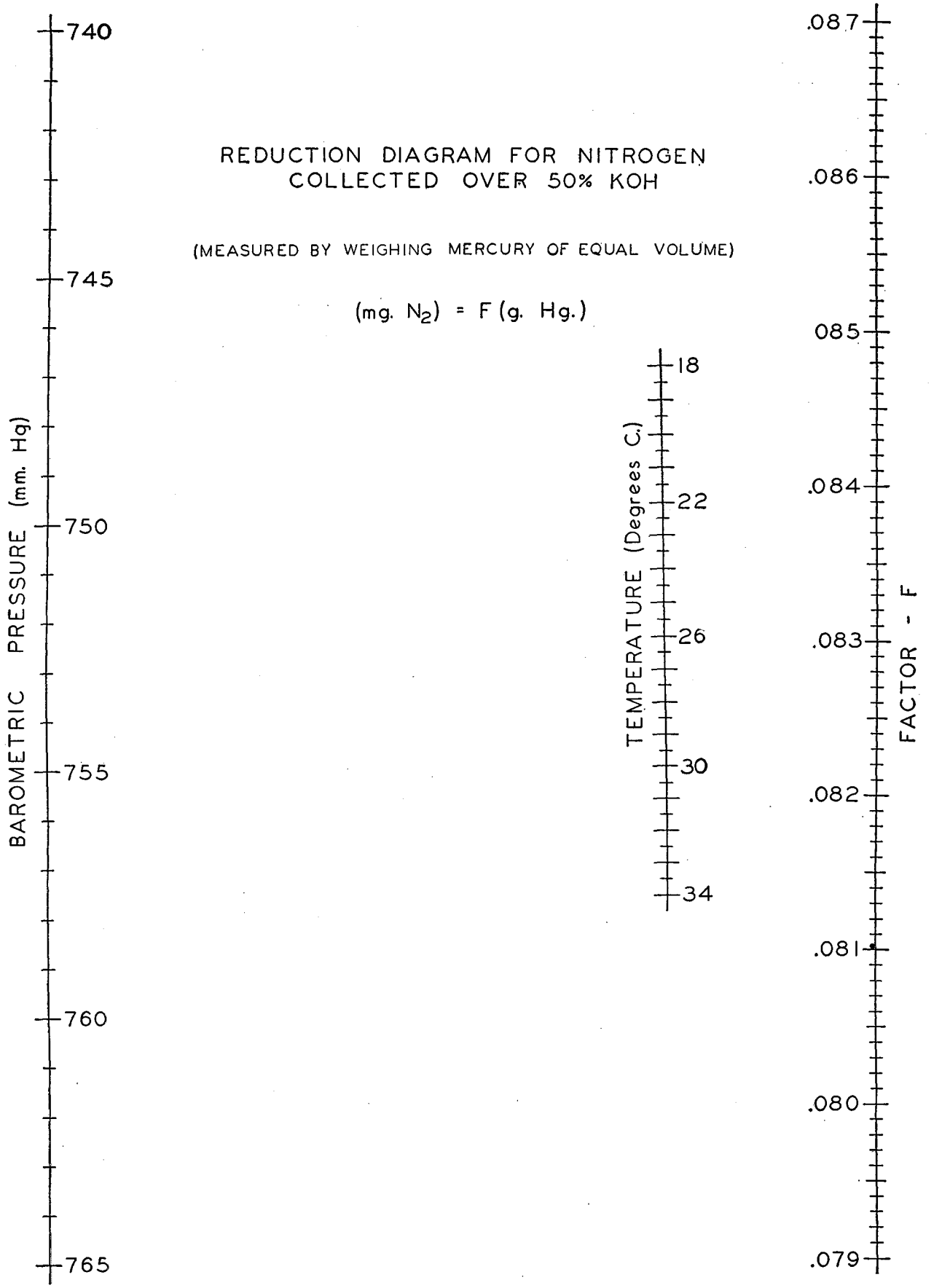
REDUCTION DIAGRAM FOR NITROGEN
COLLECTED OVER 50% KOH
(MEASURED BY WEIGHING MERCURY OF EQUAL VOLUME)
(mg. N2) = F (g. Hg.)
BAROMETRIC PRESSURE (mm. Hg)
740
745
750
755
760
765
TEMPERATURE (Degrees C.)
18
22
26
30
34
FACTOR - F
.087
.086
085
.084
.083
.082
.081
.080
.079

Figure 2

Table I. Factors for Reducing Grams of Mercury to Milligrams of Nitrogen

Temperature, ° C.	Barometric Pressure 740 mm.	750 mm.	765 mm.
18	0.08401	0.08514	0.08685
22	0.08283	0.08395	0.08563
26	0.08168	0.08278	0.08445
30	0.08056	0.08165	0.08329
34	0.07947	0.08055	0.08217

Table II. Typical Analyses

Compound	Sample Weight, Mg.	% N	Theoretical, % N
Dihydrocodeine	4.732	4.63	4.65
	5.688	4.69	
	5.755	4.69	
	6.579	4.68	
Hexamethylenetetramine	2.381	40.31	40.01
	3.186	40.24	
Dinitrophenol	3.958	15.13	15.22
	4.771	15.12	

where T is in degrees centigrade. A factor, F, may be defined by the equation (mg. of N_2) = F (grams of Hg). The empirical equations above, combined with the usual gas law calculations, result in the following expression for F:

$$F = \frac{P + 0.45 - 0.15T}{8250.3\ (1 + 0.003586T + 0.00000028T^2)}$$

P is the barometric pressure in millimeters. This equation for F is the basis for the nomogram shown in Figure 2. Calculation of the weight of nitrogen in a sample is reduced to a single multiplication of the weight of mercury displaced by factor F, nomographically determined.

The percentage of nitrogen in the sample is then calculated as follows:

$$\% \text{ N} = \frac{[F\ (\text{weight of Hg in grams}) - (\text{weight of } N_2 \text{ blank in mg.})] \times 100}{(\text{sample weight in mg.})}$$

The nomogram may be duplicated with the aid of Table I. Lay off parallel, linear scales for barometric pressure and factor F of the desired size and spacing. Locate five points on the temperature scale by the intersection of lines joining corresponding points on the P and F scales. Draw the support for the T scale and subdivide it by linear interpolation.

RESULTS

Analyses for only three compounds are reported in this paper, as no changes were made in the standard combustion procedure. The results shown in Table II are indicative of the usual precision of routine analyses.

A number of benzoic acid and dextrose samples were burned by the standard Dumas method in an effort to determine the consistency of the nitrogen blank. A very limited amount of data is available in the literature on the size of this correction. Only the usual sweeping precautions were observed.

The amount of gas collected when 50 ml. of carbon dioxide were passed through the apparatus corresponded to 0.007 mg. of nitrogen. Ten samples of benzoic acid, varying in size from 3 to 7 mg., were burned, sweeping with the same volume of carbon dioxide. The average nitrogen equivalent was 0.020 mg., with an average deviation of 0.003 mg. Four samples of dextrose (4.8 to 5.2 mg.) were treated in the same way. The blank was slightly lower, averaging 0.018 mg. of nitrogen, with an average deviation of 0.001 mg. The value of the blank used in calculating the analyses of Table II was 0.020 mg. of nitrogen in each case.

These two compounds were chosen for blank estimation because of their great difference in physical and chemical characteristics. The small difference in magnitude of the two blanks perhaps may be explained by the greater occlusion of air in the voluminous benzoic acid.

LITERATURE CITED

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Purification of Di-beta-napthylthiocarbazone

Molecular Extinction Coefficient in Chloroform at 650 Millimicrons

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Di-β-naphthylthiocarbazone, an analog of dithizone, shows promise of becoming a very important reagent for the determination of microquantities of certain metals (*2–4, 6*). For the estimation of mercury, Hubbard (*6*) reports the naphthyl analog is superior to dithizone. Suprunovich (*9*) prepared the compound by a method originally employed by Fischer (*5*) for the synthesis of dithizone and investigated a number of its properties. Hubbard and Scott (*7*) found that the method of Suprunovich gave poor yields and modified the dithizone synthesis of Bamberger, Padova, and Ormerod (*1*) to obtain the naphthyl analog. Both Suprunovich (*9*) and Hubbard (*6*) studied its properties and also those of some of its metal complexes in organic solvents.

The compound is found to react like dithizone with one notable exception: It cannot be stripped from a chloroform solution with dilute aqueous ammonia; thus one is limited in chloroform to a mixed color procedure. More serious, however, is the fact that the general method of purifying dithizone cannot be employed in purifying the naphthyl derivative from chloroform solution.

Because the commercially available material (Eastman Kodak Company product), or that obtained by known methods of preparation, is impure, purification becomes a problem of first importance. A modification of the method of Hubbard and Scott (*7*) eliminates the extraction with water and hastens the precipitation of di-β-naphthylthiocarbazone (DN) through chilling the chloroform–absolute alcohol solution in an acetone–solid carbon dioxide bath. By considering the "molecular extinction coefficient" in chloroform at 650 mμ as a measure of "purity" it was found that the material obtained by the modified procedure was "purer" than that reported by Cholak and Hubbard (*2*). Di-β-naphthylthiocarbazone can be extracted from carbon tetra-

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Eastman practical di-β-naphthylthiocarbazone can be initially purified very conveniently by precipitating from a concentrated chloroform solution on the addition of absolute alcohol and then cooling the mixture in a solid carbon dioxide–acetone bath. The compound is less soluble in carbon tetrachloride than in chloroform and can be extracted from a carbon tetrachloride solution by 1 to 10 aqueous ammonia, leaving behind a yellow material which is possibly an oxidation product of the parent compound. Ammonia extraction of alcohol-precipitated samples from carbon tetrachloride, gives a product with a "molecular extinction coefficient" in chloroform at 650 millimicrons of 55,000 as compared to 46,000 before ammonia extraction. This ammonia-extracted material is still impure. The molecular extinction coefficient of the compound in chloroform at 650 millimicrons has been tentatively established at about 67,000 through complex formation of the compound with mercury (II).

chloride by dilute aqueous ammonia and consequently can be purified by a process similar to that used for dithizone.

EXTINCTION COEFFICIENT THROUGH MERCURY (II) COMPLEX

In chloroform solution, the mercury (II) complex together with impurities, if any, equivalent to 5 mg. of di-β-naphthylthiocarbazone per liter, does not absorb at 650 mμ, the wave length of maximum absorption for di-β-naphthylthiocarbazone. That this is true may be seen by reference to curve *J* of Figure 3. Because the reactions of di-β-naphthylthiocarbazone and dithizone are similar, the method of Sullivan (*8*) for the determination of the molecular extinction coefficient of dithizone may be applied. If a chloroform solution is brought to equilibrium with an aqueous solution of mercury (II) in which there is insufficient mercury to complex all the di-β-naphthylthiocarbazone, it may be assumed that the change in optical density at 650 mμ, brought about by the process, is a measure of the amount of di-β-naphthylthiocarbazone entering the complex. This assumption requires that the impurities, if any, present in a "purified" sample do not absorb at 650 mμ, that the impurities do not react with mercury (II), and that the mercury (II) is quantitatively extracted in the presence of the excess di-β-naphthylthiocarbazone with formation, in the chloroform phase, of a complex with the formula, $Hg(DN)_2$. If these conditions obtain, we have

$$D_o = \epsilon_{650}\, l\, C_o$$

and

$$D_e = \epsilon_{650}\, l\, C_e$$

in which D_o and D_e are the optical densities of di-β-naphthylthiocarbazone at 650 mμ in chloroform before and after complexing with mercury (II) and C_o and C_e are the corresponding concentrations. ϵ_{650} is the molecular extinction coefficient under these conditions and l is the length of the light path through the solution. If a 1-cm. cell is employed, the change in optical density at 650 mμ caused by complexing, is

$$\Delta D = D_o - D_e = \epsilon_{650}\,(C_o - C_e) \quad (1)$$

Assuming the formula $Hg(DN)_2$, the change in concentration of di-β-naphthylthiocarbazone in the chloroform phase is twice the concentration of mercury (II) in that phase after extraction, and we may write,

$$\epsilon_{650} = \frac{\Delta D}{2 \times C_{\mathrm{Hg(II)\ in\ CHCl_3}}} \quad (2)$$

REAGENTS AND APPARATUS

All reagents employed were of analytical grade. These, as well as all water used for solutions, were purified by conventional methods common to this kind of work except as specifically stated.

All glassware, Pyrex brand, was cleaned by treating with a mixture of hot concentrated nitric acid and sulfuric acids, rinsed with distilled water, steamed for 15 minutes, and finally rinsed with redistilled water while still hot.

Spectrophotometric determinations were made with a Cenco-Sheard spectrophotelometer, No. 12,317, carrying 1-cm. Corex type cells. The entrance and exit slits were maintained at 1 mm. and 10 mμ, respectively.

PURIFICATION

By Cooling of Chloroform-Alcohol Solutions. Samples of about 0.5 gram of Eastman practical material were dissolved in 30 ml. of chloroform, filtered by suction on Pyrex sintered glass, and collected in a large Pyrex test tube (25 × 100 mm.). The chloroform was evaporated almost to dryness in a vacuum desiccator equipped with a small heating coil. This residue was dissolved in a minimum of chloroform (approximately 2 ml.), gentle heat being supplied to effect complete solution. To this were added 25 ml. of absolute alcohol and the contents were immediately immersed in a bath of solid carbon dioxide in acetone. After 10 minutes the precipitated di-β-naphthylthiocarbazone was filtered on sintered glass and washed twice with 5-ml. portions of absolute alcohol which had previously been cooled in the same bath. The washed residue was placed in a vacuum desiccator for about 15 minutes to remove residual alcohol. The yield was about 0.3 gram.

The residue from the first precipitation was dissolved in a minimum of chloroform (about 2 ml.), 25 ml. of absolute alcohol were added, and the contents were chilled, filtered, and washed as

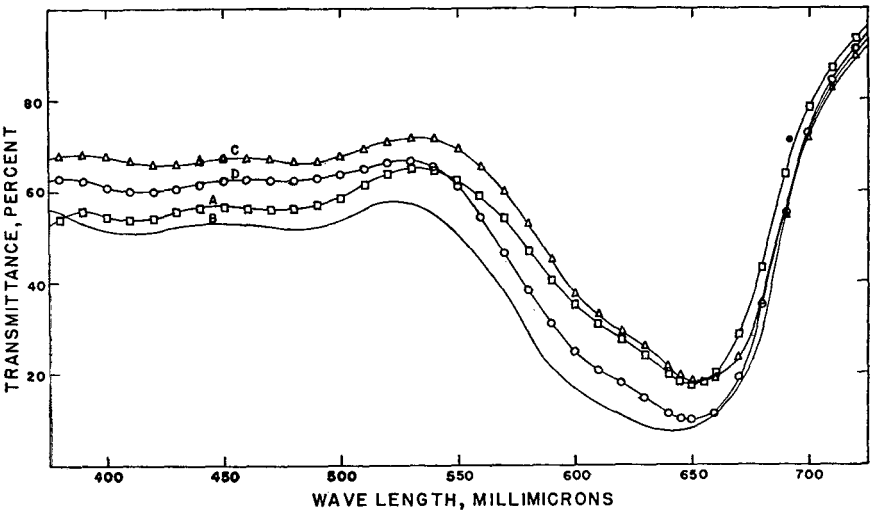

Figure 1. Transmittance Curves for Chloroform Solutions of Di-β-naphthylthiocarbazone

A. Original Eastman product, 20 mg. per liter
B. Cholak and Hubbard (*3*), 10 mg. per liter
C. First precipitation by alcohol, 8 mg. per liter
D. Fourth precipitation by alcohol, 8 mg. per liter

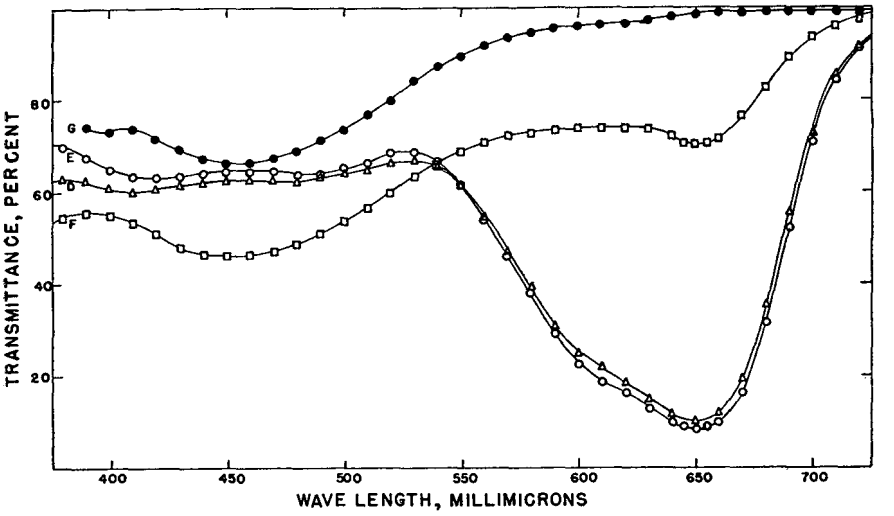

Figure 2. Transmittance Curves for Chloroform Solutions

D. DN, fourth precipitation by alcohol, 8 mg. per liter
E. DN, second purification by ammonia extraction, 7 mg. per liter
F. Product retained by carbon tetrachloride in ammonia extraction, dissolved in chloroform, 1 mg. per 100 ml.
G. Oxidation product (by bromine water) from 8 mg. of DN per liter

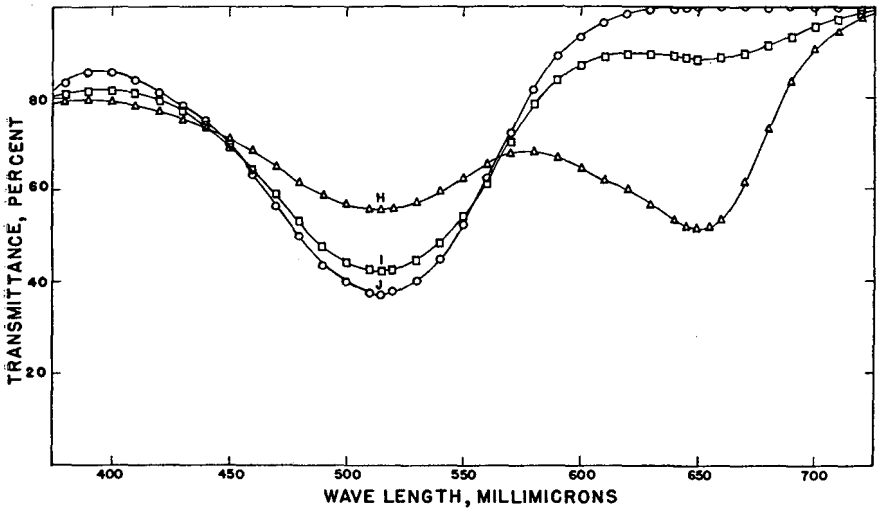

Figure 3. Transmittance Curves for Chloroform Solutions

H. Mixed color, 5 mg. of DN + 0.5 mg. of Hg^{II} per liter
I. Mixed color, 5 mg. of DN + 0.9 mg. of Hg^{II} per liter
J. 5 mg. per liter of DN completely converted to Hg^{II} complex

before. This procedure was repeated twice, using in each case the residue from the previous separation. The weight of the final residue was about 0.1 gram.

Table I records the molecular extinction coefficient in chloroform at 650 mμ of the residues obtained from the first, second, and four separations. In Figure 1 are plotted transmittancy curves for samples obtained in the first (*C*) and fourth (*D*) separations.

By Extraction from Carbon Tetrachloride by Aqueous Ammonia. A 0.1-gram sample of di-β-naphthylthiocarbazone, which had been precipitated from chloroform-alcohol solution, was dissolved in 3 ml. of chloroform. This concentrated solution was diluted with 50 ml. of carbon tetrachloride, transferred to a 500-ml. separatory funnel, and shaken with 300 ml. of 1 to 10 aqueous ammonia; most of the di-β-naphthylthiocarbazone was extracted into the aqueous layer as a red colored solution. The yellowish-green carbon tetrachloride layer was separated, and the aqueous layer was removed and filtered through sintered glass into a clean 500-ml. separatory funnel. Hydrochloric acid (1 to 1) was added to the aqueous solution until the red color changed to blue-green. The released di-β-naphthylthiocarbazone was then extracted with two successive 25-ml. portions of chloroform. The yellowish-green carbon tetrachloride solution was shaken twice again with 300 ml. of 1 to 10 aqueous ammonia and the ammonia extracts were treated as before. The chloroform extracts were combined and evaporated to dryness in a vacuum desiccator equipped with a small heating coil. The yield of the purified material was about 12 mg. and its molecular extinction coefficient in chloroform at 650 mμ was 54,900. After 20 mg. of the once ammonia-purified material had been collected, it was dissolved in carbon tetrachloride and the ammonia purification was repeated.

A transmittancy curve for 7 mg. of the twice ammonia-purified di-β-naphthylthiocarbazone per liter of chloroform is shown as curve *E* in Figure 2. The material gave a molecular extinction coefficient of 55,500. The carbon tetrachloride solution from the second purification was evaporated to dryness to obtain a yellow-brown residue. One milligram of this residue dissolved in 100 ml. of chloroform gave transmittancy curve *F* of Figure 2.

In order to indicate whether the material not extracted from carbon tetrachloride might be an oxidation product, a sample of di-β-naphthylthiocarbazone in chloroform (8 mg. per liter) was shaken with an aqueous solution containing a trace of bromine. After the chloroform layer had turned yellow, sodium thiosulfate was added and the material was further agitated.

The transmittance of the chloroform layer containing "oxidation product" is shown as curve *G* of Figure 2.

MOLECULAR EXTINCTION COEFFICIENT BY FORMATION OF MERCURY(II) COMPLEX

Fifty milliliters of a chloroform solution containing 5 mg. of di-β-naphthylthiocarbazone per liter were shaken with 50 ml. of 1 *N* sulfuric acid containing a large excess of mercury (II) (500 mg. of Hg^{II} per liter) to convert the di-β-naphthylthiocarbazone completely to mercury complex. The material used was from a fourth precipitation from absolute alcohol.

The transmittance curve for this solution in a 1-cm. cell is shown as curve *J* of Figure 3.

Twenty-five milliliter samples of chloroform solutions containing 2.43 mg. per liter (purified by ammonia extraction from carbon tetrachloride) were shaken for 5 minutes with 50 ml. of aqueous

Table I. Molecular Extinction Coefficient of Di-β-naphthylthiocarbazone in Chloroform

(At 650 mμ after successive precipitations from chloroform by absolute alcohol)

Sample	Coefficient after Precipitation		
	First	Second	Fourth
1	37,100	44,000	46,700
2	33,700	42,500	46,300

Table II. Molecular Extinction Coefficients and Optical Densities

(DN at 650 mμ, and mixed mercury complex with DN at 515 mμ in chloroform)

Sample	Concentration DN Mg./liter	Concentration Hg^{II}	Concentration Hg^{II}, moles/liter	Optical Density D_{650}	Optical Density D_{515}	ΔD_{650}, $D_0 - D_e$	Molecular Extinction Coefficient, 650 mμ
3	2.43	..	..	$0.380(D_0/D_e)$	0.072	...	(55,500)
4	2.43	0.20	1.0	0.243	0.124	0.137	68,500
5	2.43	0.20	1.0	0.242	0.123	0.138	69,000
6	2.43	0.20	1.0	0.242	0.123	0.138	69,000
7	2.43	0.40	2.0	0.109	0.188	0.271	67,800
8	2.43	0.40	2.0	0.107	0.184	0.273	68,300
9	2.43	0.40	2.0	0.113	0.184	0.267	66,800
10	8.00	..	..	$0.828(D_0/D_e)$	0.166	...	(36,800)
11	8.00	0.40	2.0	0.560	0.281	0.268	67,000
12	8.00	0.40	2.0	0.562	0.281	0.266	66,500
13	8.00	0.80	4.0	0.300	0.406	0.528	66,000
14	8.00	0.80	4.0	0.300	0.404	0.528	66,000
15	8.00	1.00	5.0	0.168	0.464	0.660	66,000
16	8.00	1.00	5.0	0.149	0.460	0.679	67,900
						Av.	67,400 ± 1000

1 *N* sulfuric acid solutions containing successively 0, 5, and 10 micrograms of mercury (II), corresponding to concentrations of mercury (II) in the chloroform phase of 0, 0.2, and 0.4 mg. per liter, respectively.

Optical densities of the chloroform solutions are recorded in Table II. The optical density at 650 mμ of the solution containing no mercury is the value, D_o, for the original extracting solution and the densities for the solutions containing mercury (II) are values, D_e, for the di-β-naphthylthiocarbazone remaining uncomplexed after extraction (Equation 1). The concentration of mercury (II) in the chloroform phase represents one half the change in concentration of di-β-naphthylthiocarbazone during complexing (Equation 2). The results of this series of experiments are listed as samples 3 to 9 in Table II. Another series of extractions (samples 10 to 16, Table II) was carried out, employing an 8.0 mg. per liter material which had been precipitated from alcohol.

DISCUSSION

In modifying the method of Hubbard and Scott (*7*) for the purification of an Eastman product, the sample was dissolved in chloroform by applying gentle heat, then filtered and evaporated almost to dryness. The solution at this point was reasonably dry and the addition of absolute alcohol to precipitate the di-β-naphthylthiocarbazone allowed the sample to be drastically cooled without separation of ice crystals. This chilling produced a more rapid separation than was obtained at room temperature in the Hubbard and Scott procedure. Beginning with 0.5 gram of original Eastman product, four successive precipitations gave approximately 0.1 gram of final product with a purity greater than was obtained by Cholak and Hubbard (*3*) by either of the purifications employed by them. Results of the first, second, and fourth steps in the purification of two independent samples are shown in Table I.

Cholak and Hubbard obtained a material of "extinction coefficient" 42,000 after two purifications. The present method gives a sample of comparable purity on the second precipitation (Table I) Transmittance curves are shown in Figure 1 for chloroform solutions of the Eastman practical product (*A*), the sample once purified (*C*), and fourth purified (*D*), as well as a reproduction of a transmittance curve obtained by Cholak and Hubbard (*3*) for a twice purified sample (*B*). It will be noted that 8 mg. per ml. of the fourth purified sample show about the same transmittance at 650 mμ and considerably less absorption in the low wave-length regions than does the 10 mg. per ml. sample represented by curve *B*, each of which indicates a greater purity for the former material.

From an inspection of Table I it is apparent that impurities are being slowly eliminated by the alcohol method of purification and a product of higher molecular extinction coefficient can be obtained by a further pursual of the process.

Di-β-naphthylthiocarbazone is so soluble in chloroform that one cannot extract it from chloroform by aqueous ammonia as in the case of purification of dithizone. However, the compound is less soluble in carbon tetrachloride than in chloroform, and can be extracted from carbon tetrachloride with 1 to 10 aqueous ammonia. Although extraction is not complete, as in the case of dithizone with 1 to 100 ammonia, the method may be employed as a means of further purification. Beginning with di-β-naphthylthiocarbazone precipitated from absolute alcohol a 12% recovery was obtained after three extractions of the same carbon tetrachloride solution with 1 to 10 aqueous ammonia. Di-β-naphthylthiocarbazone can be made to revert to carbon tetrachloride from these ammonia solutions by acidifying and shaking with the organic solvent, or can be extracted with chloroform by agitating the ammonia layer with chloroform with or without acidification.

Table III. Ratio of Optical Densities at 650 and 450 mμ and Molecular Extinction Coefficient of Di-β-naphthylthiocarbazone in Chloroform for Various Purifications

Curve	Product	Concentration, Mg. DN per Liter	D_{650}/D_{450}	Molecular Extinction Coefficient at 650 mμ
A	Eastman, practical	20	3.0	13,400
B	Alcohol, 2 precipitations[a]	10	3.8[b]	42,000[c]
C	Alcohol, 1 precipitation, chilled	8	4.2	33,700
	Alcohol, 2 precipitations, chilled	8	4.5	42,500
D	Alcohol, 4 precipitations, chilled	8	5.0	46,300
	1 purification, NH_3 extraction	4.7	5.2	54,900
E	2 purifications, NH_3 extraction	7	5.6	55,500

[a] By method of Hubbard and Scott (*3*, *7*).
[b] Values interpolated from curve of Cholak and Hubbard (*3*).
[c] Value listed by Cholak and Hubbard (*3*).

That the material purified by ammonia extraction contains less impurities than does that precipitated from alcohol may be seen by a comparison of curves *D* and *E* of Figure 2. In extracting a carbon tetrachloride solution with 1 to 10 ammonia, at least two substances are retained by the carbon tetrachloride. The two maximum absorptions shown by curve *F* of Figure 2 indicate that the two materials retained are di-β-naphthylthiocarbazone (compare curves *E* and *F* at 650 mμ) and an oxidation product (compare curves *F* and *G*). The form of curve *G* (oxidation product by bromine oxidation) is the same as that of the material retained by carbon tetrachloride in ammonia separation (curve *F*). This indicates that ammonia extraction has eliminated, at least partly, a yellow "oxidation product" that was present in the alcohol-precipitated sample. If one assumes that the primary substance to be eliminated from an alcohol-precipitated sample is the yellow oxidation product with maximum absorption at 450 mμ, the ratio of optical densities at 650 and 450 mμ should be a guide as to whether this material is being removed in a purification process.

An increasing value of D_{650}/D_{450} as purification proceeds should indicate that the yellow material is being removed (Table III). Noteworthy is the fact that, in general, this ratio increases with increase in molecular extinction coefficient at 650 mμ. If all constituents of the samples obey Beer's law at both wave lengths the ratio should reach a maximum value when a pure material is obtained, provided di-β-naphthylthiocarbazone shows no absorption maximum in the region of 450 mμ. Even with the purest sample prepared (curve *E*, Figure 2) there is no indication that this is the case. It will be noted from Table III, that, although two precipitations from absolute alcohol by the method of Hubbard and

Scott (*3*, *7*) gave a material of approximately the same molecular extinction coefficient as a sample obtained after two precipitations from alcohol by chilling, the latter contained less oxidation product as interpreted from the ratio of optical densities. That the product obtained by ammonia extraction is purer than any of the others is shown clearly by both a high ratio of optical densities and a high molecular extinction coefficient: 54,900 after one and 55,500 after two purifications. The material after the second purification was still impure, as shown by the fact that a carbon tetrachloride solution remained light yellow on extracting with 1 to 10 ammonia.

Extraction of a carbon tetrachloride solution of the Eastman product was not very successful; however, the method may give good results. It is recommended that purification by ammonia extraction begin with alcohol-precipitated material. To obtain a chloroform solution of exceptional purity, one can begin with an alcohol-precipitated sample and carry out several purifications by ammonia extraction from carbon tetrachloride. The di-β-naphthylthiocarbazole in the final ammonia solution can then be extracted with chloroform; the optical density at 650 mμ serves as a guide when one has a solution of serviceable strength.

The molecular extinction coefficient (67,000) as obtained through the mercury (II) complex indicates that all the samples obtained by purification in this work and elsewhere (*3*) are still impure.

Curve *J* of Figure 3 represents a chloroform solution in which 5 mg. per liter of di-β-naphthylthiocarbazone are completely converted to mercury (II) complex. No absorption is shown at 650 mμ; therefore the impurities in the purified material cannot absorb at this wave length unless they react with mercury (II) to form products that also do not absorb at 650 mμ. In Table II the molecular extinction coefficients listed were obtained by the use of two stock solutions which differed widely in purity; samples 3 to 9 from a material having extinction coefficient of 55,500 and samples 10 to 16 from a material having a coefficient of 36,800. If impurities had reacted with mercury (II) the coefficients obtained through the use of the two solutions would have been different. That the change in optical density at 650 mμ is a measure of the amount of di-β-naphthylthiocarbazone which reacts with mercury (II) to form a colored complex having no absorption at this wave length, for concentrations employed, is shown by the data presented in Figure 3 and Table II. The formula, $Hg(DN)_2$, is assumed by analogy to the mercury (II) keto complex of dithizone. If the formulas are HgDN, $Hg(DN)_2$, and $Hg(DN)_3$ molecular extinction coefficients, employing the data from Table II, would be 134,000, 67,000, and 44,500, respectively. The purest sample prepared gave a coefficient of 55,500 and was shown still to contain some material other than di-β-naphthylthiocarbazone. Therefore the values of the molecular extinction coefficient given in Table II, based on the formula $Hg(DN)_2$, are the only reasonable ones under the conditions prevailing.

Some values employing the zinc complex have been obtained, which, although they are somewhat more variable than those in Table II, support the value of 67,000 obtained through the mercury (II) complex. Work on mercury (II) and zinc as well as other complexes of di-β-naphthylthiocarbazone is continuing and the value of 67,000 for the molecular extinction coefficient in chloroform at 650 mμ is to be considered as tentative.

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Microdetermination of Acetyl Groups

Modification of Elek and Harte's Method

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An investigation has been made of the various stages in the microdetermination of acetyl, in which the acetyl group is hydrolyzed by aqueous *p*-toluenesulfonic acid and the acetic acid is distilled under reduced pressure and titrated iodometrically. The method used substitutes alkalimetric for iodometric titration, omits the unsatisfactory correction for sulfur dioxide, and directly avoids error due to carbon dioxide. It was successfully applied to acetylated alkaloids.

THE method of Friedrich, Rapoport, and Sternberg (*2*, *3*) modified by Elek and Harte (*1*) involves hydrolysis by 25% aqueous *p*-toluenesulfonic acid at 100° C., and distillation of the acetic acid at 50 to 60 mm. into an ice-cold receiver containing 0.01 *N* iodine solution and potassium iodide. A correction for sulfur dioxide is applied by deducting the amount of 0.01 *N* thiosulfate used to titrate the iodine in the receiver from a blank using the same quantity of iodine solution. Iodine liberated by adding excess potassium iodate to the titrated distillate is assumed to be equivalent to the acetic acid together with twice the equivalent of the oxidized sulfurous acid. After standing for 20 minutes at 35° C. the solution is again titrated with thiosulfate The difference between the total titer and double the equivalent of the iodine used in the first titration presumably gives the correct percentage of acetyl by the usual calculation.

Investigation of this method showed that a true correction for acid decomposition products from *p*-toluenesulfonic acid cannot be obtained by reaction with iodine. Friedrich and Rapoport (*2*), who first suggested this correction, later discarded it (*3*). Suzuki (*13*) using a similar procedure made no such correction. Hurka and Lieb (*6*), after alkalimetric titration, attempted to determine sulfur dioxide by reaction with iodine,

although it may have been previously removed by boiling (*10*).

In the writer's experience, although *p*-toluenesulfonic acid alone gives insignificant amounts of acid decomposition products, yet both SO_3^{--} and SO_4^{--} are formed in the presence of an acetylated compound. Sulfate, sometimes detected in the Kuhn-Roth method (*10*), also is disregarded in Elek and Harte's method.

Iodometric titration of acetic acid is not free from objection. By using alkalimetric titration, the larger number of reagents required, necessitating their absolute purity, and also the time required for nearly quantitative liberation of iodine by acetic acid, are avoided. While Elek and Harte (*1*) allowed only 20 minutes at 35° C. for the liberation of iodine, Suzuki (*13*) found that at least 2 hours at room temperature was necessary. Kolthoff and Sandell (*7, 9*) consider that acids having a dissociation constant greater than 1×10^{-6} can be titrated iodometrically—for acetic acid $K_a = 1.8 \times 10^{-5}$.

Side reactions in the titration of iodine by thiosulfate are prevented when the pH is not greater than 6.5 and 5 for a 0.01 *N* and 0.001 *N* solution, respectively (*8, 9*).

Pickering (*12*) found that the alternative reaction:

$$4I_2 + Na_2S_2O_3 + 5H_2O = 2NaHSO_4 + 8HI \quad (1)$$

occurring in neutral solution, was favored by a rise in temperature, causing errors ranging from 1.84% at 0° C. to 3.9% at 52° C.

In a comparison of iodometric, acidimetric, and potentiometric titration of acetic acid, Hurka (*5*) obtained values of 623, 640, and 658 micrograms per ml., respectively, on the same solution. For a titer of 5 ml. the difference between the iodometric value, which he assumed correct, and the potentiometric, would amount to 0.28 ml. (5.6%). The end point of the potentiometric titration was taken as 8.18.

The change in the hydrogen ion concentration, according to the equation

$$5I^- + IO_3^- + 6H^+ = 3H_2O + 3I_2 \quad (2)$$

was followed potentiometrically at 25° and 35° C. There was little difference in reaction rate for a 10° C. rise in temperature, the pH of the solution at 20 minutes being between 7.0 and 7.1 in each case. However, when excess thiosulfate was added after 20 minutes' standing, the pH rose more sharply, reaching a higher value than in the first two experiments, and was still rising when the experiment was discontinued. Despite the continued rise in pH (curves 1 and 2) it may be calculated that at a pH of 7.3 attained at 45 minutes, the reaction is approximately 99.7% complete, provided that no side reactions occur.

In both iodometric and alkalimetric methods the effect of carbon dioxide is significant. Carbon dioxide has long been known to liberate iodine from an iodide-iodate solution (*11*), and this has been amply confirmed by the writer, using Elek and Harte's method. Either the carbon dioxide must be removed by boiling the acid distillate (*2, 6, 10*) or, preferably, it must be strictly excluded from the apparatus. In the former case the results achieved are obviously dependent on the concentrations of the acetic acid and carbon dioxide, and on the time of boiling. The ideal of removing all the carbon dioxide without loss of acetic acid is translated in practice into the compensatory removal of the bulk of the carbon dioxide together with a small amount of acetic acid. The loss of acetic acid for various boiling times had been studied by Friedrich *et al.* (*2*) and Hurka (*4*), who arrived at an optimum boiling time of 5 to 8 seconds. Although Friedrich *et al.* pointed out the significance of carbon dioxide in their iodometric procedure, Elek and Harte found its effect to be negligible.

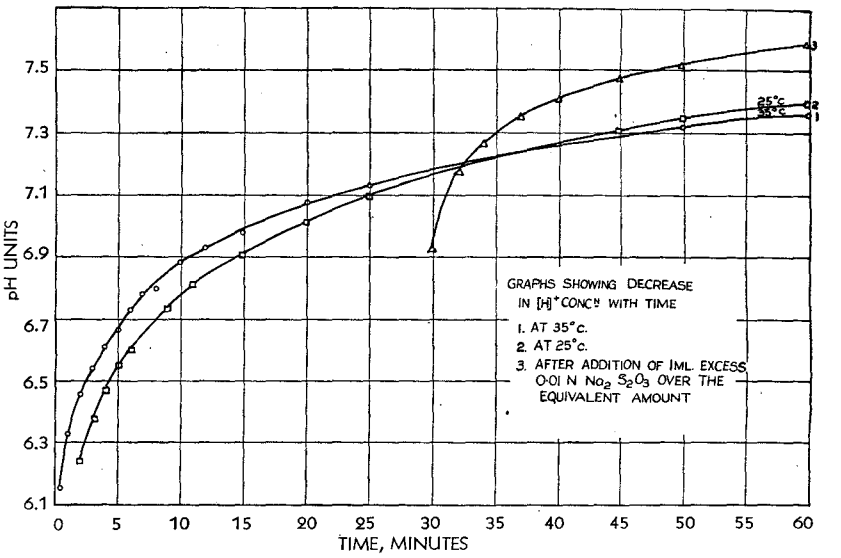

Figure 1. Change in pH with Time

Table I. Effect of Decomposition of Toluenesulfonic Acid

	Mannitol Hexaacetate, 30 Mg.	No Sample
Weight of *p*-toluenesulfonic acid	4.887	4.883
Sulfate		
Weight of $BaSO_4$ equivalent to SO_4^{--}, gram	0.001556	0.001196
Vol. of 0.01 *N* NaOH equal to acidity derived from 0.5 gram of toluenesulfonic acid. ml.	0.14	0.10
Sulfite		
Weight of $BaSO_4$ equivalent to SO_3^{--}, gram	0.001173	0.000816
Vol. of 0.01 *N* NaOH equal to acidity derived from 0.5 gram of toluenesulfonic acid, ml.	0.10	0.07
Total acidity, 0.01 *N* NaOH, ml.	0.24	0.17

Table II. Effect of Carbon Dioxide

	Flask 1, Titer *Ml.*	Guard Flasks, Titer *Ml.*
1. Distillate titrated without boiling	4.79	0.11
2. Distillate boiled for 5 seconds, then titrated	4.57	Nil
3. Blank titrated immediately without boiling	4.78	..
4. Blank boiled for 5 seconds, then titrated	4.79	..
5. Blank stood for 3 hours, then titrated without boiling	5.10	..
6. Blank stood for 3 hours, then boiled for 5 seconds	4.79	..
7. Distillate heated just to incipient boiling, then titrated	4.73	0.07

The apparatus used by Elek and Harte was satisfactory, provided a constant pressure was maintained during distillation. Some acetic acid (0.05 to 0.19 ml., 0.01 *N*) always remained undistilled because of the large surface area provided by the glass rod in the distillation flask.

Variation in the concentration of *p*-toluenesulfonic acid from 12.5 to 50% or use of hydrolysis times greater than 3 hours had no effect.

Sodium thiosulfate, 0.01 *N*, even when preserved with amyl alcohol, required daily checking.

EXPERIMENTAL

Change in Hydrogen Ion Concentration during Liberation of Iodine. A solution containing 1.5 grams of potassium iodide, 5.00 ml. of 0.01 *N* acetic acid, and 45 ml. of boiled distilled water

had a pH of 4.2 (glass electrode). Because the potassium iodide contained some alkali (1.5 grams requiring 0.19 ml. of 0.01 *N* hydrochloric acid for neutralization) this value was somewhat higher than that of a standard 0.001 *N* acetic acid solution (3.87). On addition of 2 ml. of 4% aqueous potassium iodate the pH rose within a few seconds to 6.1. Thereafter the rise in pH with time was followed at 25° and 35° C. (Figure 1, curves 1 and 2). After 20 minutes at 35° C., the conditions which Elek and Harte employed, the pH is between 7.0 and 7.1. Although the rate of removal by hydrogen ions is somewhat increased initially by a 10° C. rise in temperature, it becomes about equal in both cases at 30 to 40 minutes.

After 7 to 10 minutes the reaction is only 99.1% complete (pH 6.8); virtual completion (99.9%) is not attained until after approximately 2 hours (pH 7.7).

Where excess thiosulfate was added after 20 minutes' standing at 35° C. (Figure 1, curve 3), the pH rose to a higher value than in curves and 2.

Decomposition of *p*-Toluenesulfonic Acid. When 0.5 gram samples of microanalytical reagent *p*-toluenesulfonic acid were submitted to distillation either directly or after 3 hours in a boiling water bath, and the distillate was titrated, 0.15 to 0.16 ml. of 0.01 *N* sodium hydroxide was required for neutralization to phenolphthalein. A volume of boiled distilled water equal to that in the receiver required 0.10 to 0.11 ml. of 0.01 *N* sodium hydroxide. Thus, acid distillation products from 0.5 gram of *p*-toluenesulfonic acid in the absence of sample required approximately 0.05 ml. of 0.01 *N* sodium hydroxide. Carbon dioxide was completely excluded from the apparatus in these experiments. On distilling a known amount of acetic acid in the presence of 0.5 gram of *p*-toluenesulfonic acid no extra decomposition could be detected.

In order to determine the extent of decomposition in the presence of sample, the combined distillates from 32 determinations after titration of the acetic acid were analyzed for sulfate and sulfite: sulfate as barium sulfate, 0.0034 gram, sulfite as barium sulfate, 0.0032 gram.

Assuming a constant decomposition for every sample, the total barium sulfate (0.0066 gram) is equivalent to 0.18 ml. of 0.01 *N* sodium hydroxide per determination.

In order to confirm the fact that oxidation of sulfite to sulfate was not occurring during collection of the residues, two experiments were carried out. Approximately equal quantities of *p*-toluenesulfonic acid were heated with 2 ml. of distilled water for 3 hours in the presence and absence of mannitol hexaacetate, and distilled, and the distillates were analyzed for sulfate and sulfite as before. The precipitation of the sulfate as barium sulfate, however, was carried out in an oxygen-free atmosphere. Table I lists the results obtained.

These results show that somewhat more sulfate than sulfite is produced by decomposition of *p*-toluenesulfonic acid.

Effect of Carbon Dioxide. By distilling acetic acid in the presence of *p*-toluenesulfonic acid after 3 hours' heating at 100° C. and taking no special precautions to exclude carbon dioxide, variations between titrations of up to 0.5 ml. of 0.01 *N* sodium thiosulfate were obtained. On allowing acetic acid to stand for varying periods of time in flasks covered with watch glasses, similar differences were observed. Several experiments were carried out to check the boiling procedure recommended by some authors.

Table II summarizes the results when 5.00 ml.

Table III. Analytical Results

No.	Compound	Formula	Weight	Vol. of 0.0100 *N* NaOH (Corr.)	Acetyl Found	Acetyl Calcd.
			Mg.	*Ml.*	%	%
1	Acetanilide	C_8H_9ON	4.290	3.23	32.4	31.85
			5.897	4.46	32.5	
			7.209	5.34	31.8	
			6.370	4.78	32.3	
			8.178	6.09	32.0	
			7.000	5.20	31.95	
			6.375	4.70	31.70	
2	Phenacetin	$C_{10}H_{13}O_2N$	7.557	4.33	24.6	24.0
			7.744	4.41	24.5	
			8.406	4.81	24.6	
			8.783	4.92	24.1	
			6.861	3.93	24.7	
3	Mannitol hexa-acetate	$C_{18}H_{26}O_{12}$	5.299	7.37	59.9	59.4
			7.315	10.18	59.85	
			4.252	5.87	59.4	
			5.230	7.21	59.3	
4	*N,N'*-Diacetyl-ethylenediamine	$C_6H_{12}O_2N_2$	4.598	6.36	59.45	59.7
			4.489	6.22	59.6	
5	1,2-Methylenedi-oxy-3-methoxy-4-acetoxy-10-methylacridone	$C_{16}H_{12}O_5N(CH_3CO)$	9.046	2.67	12.7	12.6
			9.142	2.71	12.7	
6	1-Methoxy-2,3-methylenedioxy-4-acetoxy-10-methylacridone	$C_{16}H_{12}O_5N(CH_3CO)$	8.539	2.59	13.05	12.6
			8.631	2.63	13.1	
7	1,2,3-Trimethoxy-4-acetoxy-10-methylacridone	$C_{17}H_{16}O_5N(CH_3CO)$	8.281	2.40	12.4	12.05
			9.254	2.68	12.4	
8	1,4-Dimethoxy-2-ethoxy-3-acetoxy-10-methylacridone	$C_{18}H_{18}O_5N(CH_3CO)$	9.139	2.55	12.0	11.6
9	1-Methoxy-2-ethoxy-3-acetoxy-4-hydroxy-10-methylacridone	$C_{17}H_{16}O_5N(CH_3CO)$	8.528	2.50	12.6	12.05
			8.554	2.49	12.5	
10	1,4-Diacetoxy-2-ethoxy-3-methoxy-10-methylacridone	$C_{17}H_{15}O_5N(CH_3CO)_2$	8.508	4.36	22.0	21.55
11	1-Methoxy-2-ethoxy-3,4-diacetoxy-10-methylacridone	$C_{17}H_{16}O_5N(CH_3CO)_2$	8.127	4.05	21.45	21.55
			7.628	3.85	21.7	
			8.915	4.53	21.9	
			8.728	4.41	21.75	
			9.008	4.56	21.8	
12	1-Acetoxy-2,3-dimethoxy-4-hydroxy-10-methylacridone	$C_{16}H_{14}O_5N(CH_3CO)$	8.985	2.73	13.1	12.5
13	1,2-Dimethoxy-3-acetoxy-4-hydroxy-10-methylacridone	$C_{16}H_{14}O_5N(CH_3CO)$	8.270	2.42	12.6	12.5
			8.641	2.57	12.8	
14	1,4-Diacetoxy-2,3-dimethoxy-10-methylacridone	$C_{16}H_{13}O_5N(CH_3CO)_2$	7.541	3.91	22.3	22.35
			7.202	3.75	22.4	
15	1-Acetoxy-2-methoxy-10-methylacridone-3,4-quinone	$C_{15}H_{10}O_5N(CH_3CO)$	8.742	2.82	13.9	13.2
			8.636	2.73	13.6	
			8.493	2.71	13.5	
16	1-Acetoxy-2-methoxy-10-methylacridone-3,4-quinone azine	$C_{21}H_{14}O_3N_3(CH_3CO)$	7.805	2.07	11.4	10.8
			9.424	2.45	11.2	
17	1,3-Diacetoxy-2-methoxy-4-hydroxy-10-methylacridone	$C_{15}H_{11}O_5N(CH_3CO)_2$	8.796	4.84	23.7	23.2
			7.892	4.33	23.6	
			9.150	5.01	23.55	
18	1,3,4-Triacetoxy-2-methoxy-10-methylacridone	$C_{15}H_{10}O_5N(CH_3CO)_3$	9.333	6.75	31.0	31.2
			8.366	6.02	30.9	
19	2-Acetoxy-3-methoxy-10-methylacridone-1,4-quinone	$C_{15}H_{10}O_5N(CH_3CO)$	9.224	2.91	13.6	13.2
			9.399	2.96	13.5	
20	1,2-Diacetoxy-3-methoxy-4-hydroxy-10-methylacridone	$C_{15}H_{11}O_5N(CH_3CO)_2$	8.536	4.73	23.5	23.2
			6.645	3.69	23.9	
			8.826	4.82	23.5	
			5.913	3.26	23.7	
21	1-Methoxy-2,3-diacetoxy-4-hydroxy-10-methylacridone	$C_{15}H_{11}O_5N(CH_3CO)_2$	6.878	3.71	23.2	23.2
			6.554	3.55	23.3	

of 0.01 *N* acetic acid and *p*-toluenesulfonic acid were heated for 3 hours and distilled and the distillate was titrated.

DISCUSSION

In view of the above investigations, Elek and Harte's method was modified as follows:

Attempts to determine sulfur dioxide were omitted, because an estimate of the sulfur trioxide simultaneously produced could not be obtained. Under the conditions used by the writer, the amount of acid produced by the *p*-toluenesulfonic acid was approximately the same as the holdup of acetic acid in the distillation flask, together with the amount of sodium hydroxide required to give the phenolphthalein end point in the volume of distillate obtained. Although these two errors compensated one another within narrow limits in this case, other workers would be wise to determine the holdup of acetic acid in their apparatus and the approximate acidity due to decomposition of their sample of *p*-toluenesulfonic acid.

Iodometric titration of acetic acid was replaced by alkalimetric.

Though it is conceded that carbon dioxide can be removed from the distillate by boiling, it is considered more satisfactory to exclude it from the apparatus by hydrolysis in a carbon dioxide–free atmosphere, and by using freshly boiled distilled water for the distillation and washing of the condenser tube, etc.

During distillation at constant pressure the procedure was timed with a stop watch to avoid overheating when the contents of the flask were nearly dry.

Instead of using a 25% aqueous stock solution of *p*-toluenesulfonic acid, 0.5 gram of solid was weighed into the distillation flask for each determination.

Some of the results obtained by this modified procedure are indicated in Table III.

ACKNOWLEDGMENT

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NOTES ON ANALYTICAL PROCEDURES . . .

Technique in Semimicrodetermination of Uronic Acids

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THE procedure of Lefevre and Tollens (*12*) has undergone several changes in the design of apparatus used and in the procedures followed, especially in the macromethods (*2, 6–9, 13–15, 17, 18, 20, 21*). A modification of the macrotechnique which nearly reaches the semimicroscope has been devised (*10*) and several modifications of a micromethod also exist (*3, 4*). In all these a general bulkiness in apparatus is objectionable and the manipulations are not so easy as they can be. The micromethod of Burkhardt *et al.* (*3*) requires an apparatus made by formidable glass blowing and involves a difficult titration technique. Its range of applicability is also questionable.

A micromethod based on the absorption character of the product of reaction between uronic acid and naphthoresorcinol is available but unfortunately is applicable only to uronic acid solutions free of sugars whose reaction products absorb in the same region (*11*). A method involving the gasometric measurement of carbon dioxide evolved (*19*) is very time-consuming and gives too divergent results on such simple uronic acid–rich materials as pectins (*10*). Therefore the apparatus as described below has been devised and used on a semimicro scale. It is less elaborate than some of the preceding and is very simple to operate.

APPARATUS AND REAGENTS

The apparatus is schematized in Figure 1. Absorption tower *A*, water condenser *B*, and scrubbing tower *C* are fashioned from ordinary Liebig air condensers of about 750-mm. length. Tower *A* is slightly pinched at the lower end to hold the 0.6-cm. (0.25-inch) glass helices which pack it to a height of about 250 mm. Tower *C* is similarly packed. Absorption flask *D* and reaction flask *E* are each of about 100-ml. capacity and preferably round-bottomed. Tube *F* is an ordinary drying tube with a piece of rubber tubing and a pinch clamp on the end.

All glass tubing, except for those pieces on the safety bottle, is of 2-mm. bore. Rubber stoppers serve at all points of attachment to the towers and condenser. Fitting the stoppers, glass tubing, and towers together as shown in the drawing eliminates the possibility of any dead circulation spaces in the system. The glass tube running through the condenser terminates several inches below the tip of the condenser but at a point above the surface of the liquid in the reaction flask. Safety bottle *S* is of several liters' capacity and is connected to a water pump. The bottle is fitted with a stopcock leak, *L*, and is connected to the rest of the system through a three-way, T-bore stopcock fitted with an Ascarite drying tube. The only rubber tubing joint is at the junction between this T-tube and the outlet tube from the absorption tower.

Heat can be applied through any desired means. A Glass-col heating mantle for a 250-ml. round-bottomed flask and a Type 200-C Variac voltage regulator were used by the author. This permitted placing a thermometer bulb near the bottom of exterior of the reaction flask to facilitate reaction temperature control. The entire apparatus is mounted on a single ring stand. Other equipment includes a dispensing bottle, fitted with Ascarite and Anhydrone tubes, for storing barium hydroxide solution; a wash bottle fitted with a pressure bulb to which is attached an Ascarite tube; a delivery pipet of convenient size (the author used a 5-ml. automatic pipet with a side-arm filler tube and an overflow chamber); and a microburet of appropriate size.

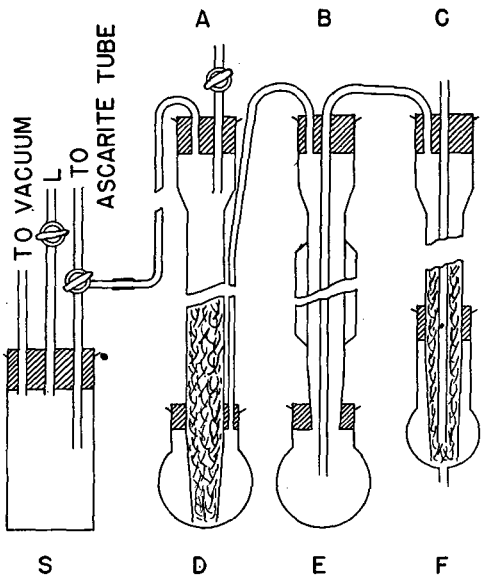

Figure 1. Apparatus

Reagents needed are approximately 0.04 *N* barium hydroxide solution; standardized 0.02 *N* hydrochloric acid solution; 1% phenolphthalein solution; 50 to 60% potassium hydroxide solution; 12.5% hydrochloric acid solution; and distilled water free from carbon dioxide.

METHOD

After assembling all the apparatus, except the reaction flask, add potassium hydroxide solution through the air tube of tower *C* until the level of liquid in the tower is at the top of the packing. With all stopcocks open to the atmosphere, attach reaction flask containing a sample and 50 ml. of 12.5% hydrochloric acid solution, making sure that the 2-mm. glass tube projecting below the condenser is about 1.25 cm. (0.5 inch) above the liquid surface. Fill the stopcocked inlet tube atop *A* with carbon dioxide–free distilled water from the wash bottle and close stopcock. A small piece of rubber tubing on the inlet tube will permit coupling during additions of liquids to *A*. Turn the water pump vacuum on full force and adjust the leak stopcock and the three-way stopcock on the safety bottle, so that air bubbles traveling up the scrubbing tower are separated from each other by at least 2.5 cm. of liquid. At this point in adjusting the three-way stopcock from its wide open position turn it counterclockwise. Then when it is opened at the end of the determination, rotation in a clockwise direction prevents undue application of suction to the absorption tower. Twenty minutes' run at this established rate will free the system of carbon dioxide.

Fill the delivery pipet with barium hydroxide solution from the dispensing bottle and introduce the solution through the inlet tube into *A*, closing the stopcock when the last solution has just entered the tube. Add enough water through the tube to ensure a tower liquid level a trifle below the packing level and again close the stopcock with the tube full of water. Apply heat to bring the temperature of the reaction flask to 150° C. A setting of 60 on the Variac accomplished this in the author's case. At the end of the run, 3 hours for pure uronic acid and 4 hours for materials lower in uronic acid content, turn the three-way stopcock clockwise and let carbon dioxide–free air enter the system slowly through the Ascarite tube. Rinse the contents of *A* into the absorption flask with water added through the inlet tube and immediately titrate the entire contents. These precautions reduce contamination with carbon dioxide from the air to a minimum, obtained in a blank, and used in calculations.

RESULTS

Five milliliters of barium hydroxide solution from the automatic pipet required 8.87 ml. of 0.0225 *N* hydrochloric acid solution for neutralization. In five blank runs a range of 8.16 to 8.26 ml. of hydrochloric acid was needed for titration, with an average of 8.22 ml. This blank of 0.65 ml. of 0.0225 *N* hydrochloric acid is equivalent to 0.0003 gram of carbon dioxide. Samples, ranging in weight from about 0.0125 to 0.0500 gram, respectively, for materials high and low in uronic acid content, were analyzed at varying reaction times with the results given in Table I. These results were obtained with 12.5% hydrochloric acid as the decarboxylating agent. Nineteen per cent acid, as recommended by McCready *et al.* (*13*) was not used, because acid of that strength gave somewhat divergent results on various monosaccharides and produced very much darker hydrolyzates as compared to the 12.5% acid.

DISCUSSION

Applicability to the semimicro analysis of materials as low as 10% in uronic acid content, acceptable accuracy and precision, and simplicity in construction and manipulation constitute the advantages claimed for this design. No special pieces of equipment are needed. Once assembled, dismantling involves only the removal of reaction and absorption flasks, each involving a single rubber stopper connection. The scrubbing and absorption towers can be cleaned by letting dilute hydrochloric acid flow through the inlet tubes and rinsing well afterwards. The scrubbing tower can be recharged without dismantling. The longer and narrower condenser eliminates the need for installing a device for preventing the carry-over of hydrochloric acid; and the purification traps others have used are also unnecessary, at least for the substances listed in Table I. No bubble counter is needed, as the scrubbing tower serves in that capacity. Ground-glass joints, with one exception, offer no advantage over the rubber stoppers, which need not be disturbed once the apparatus is assembled; application of a little grease will take care of any leaks. In laboratories where the apparatus may be in constant use a standard-taper ground-glass joint between condenser and reaction flask is advisable, for the rubber stopper at that point will survive only several dozen determinations. The apparatus requires a minimum of laboratory space and is readily portable.

Table I. Determination of Uronic Acid

Material	Sample	Time	$C_6H_{10}O_7H_2O$	$C_6H_{10}O_7$	Check by Other Methods
	Gram	*Min.*	%	%	%
Galacturonic acid monohydrate	0.0112	120	80.11	...	97.01[a]
	0.0180	150	91.17	...	...
	0.0238	...	89.87	...	...
	0.0187	180	97.86	...	...
	0.0198	...	97.42	...	...
	0.0215	240	98.60	...	...
	0.0212	...	98.68	...	...
Citrus pectin, N.F.	0.0414	60	...	36.87	77.27[b]
	0.0381	120	...	63.69	...
	0.0371	150	...	72.02	...
	0.0359	180	...	75.33	...
	0.0331	210	...	76.43	...
	0.0348	265	...	79.56	...
	0.0397	315	...	78.96	...
Flaxseed mucilage, deashed	0.0444	240	...	29.05	30.33[c]
	0.0448	300	...	29.53	...
Arabic acid[d]	0.0420	270	...	15.05	15.56[e]
Flaxseed carbohydrate, residue I	0.0277	120	...	4.88	None
	0.0216	180	...	7.99	...
	0.0207	270	...	11.26	...
	0.0265	450	...	11.39	...

[a] By titration in water solution.
[b] By National Formulary method (*16*).
[c] Anderson and Lowe (*1*).
[d] Pfanstiehl Chemical Co. product.
[e] Butler and Cretcher (*5*).

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Direct Determination of Chromate Ion with Standard Arsenite and Diphenylamine as Indicator

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IN qualitative analysis it is known that arsenious acid reduces the chromate ion to chromic ion. Jellinek and Kühn (*1*), however, were unable to work out a quantitative method on this basis. An indirect determination of the chromate ion with arsenite has been suggested by Spitalsky (*3*) and by Kolthoff and Sandell (*2*). Zintl and Zaimis (*4*) described a direct potentiometric titration. The authors' aim was to work out on the basis of this reaction a direct and accurate method with a reversibly working indicator.

As the standard oxidation potentials of the systems $Cr_2O_7^{--}/Cr^{+++}$ and AsO_4^{---}/AsO_3^{---} are +1.30 and +0.58 volt, respectively, the potential at the equivalent point is about +0.94 volt. Among the most used oxidation-reduction indicators the oxidation potential of diphenylamine—+0.76 volt in 2 *N* sulfuric acid—corresponds best to this value.

When diphenylamine is added to a chromate solution in the presence of sulfuric acid, the color changes initially from dirty yellow to brownish red and after addition of arsenious acid it varies from red to violet-red, deep blue, or violet. This final color does not change on addition of arsenious acid even in excess, so that this reducing process cannot immediately be indicated by diphenylamine.

The violet-blue color of the diphenylamine-chromate mixture changes instantly to green, if sulfurous acid or ferrous sulfate is added. However, no color change is caused by arsenite, even if a small amount of ferrous sulfate is employed as a transfer agent, although the reduction of chromate has been completed. The color change of the diphenylamine that takes place on the action of arsenite is reversible, if a trace of potassium iodide is present. The probable explanation is that the iodide ion catalyzes the reduction of the blue colored diphenylbenzidine violet to colorless diphenylbenzidine. This conclusion is suggested by the fact that the necessary amount of potassium iodide is proportional to the added diphenylamine.

In itself the reaction between arsenite and chromate proceeds too slowly to base a direct measurement upon this process. Manganese ion has proved to be the most satisfactory catalyst for the reaction. The catalytic effect of the manganese ion has been discussed by Zintl and Zaimis. Fortunately the catalyst exhibits its maximal effect in the same concentration range of sulfuric acid that is necessary for the reduction of a chromate-arsenite system. On the other hand, the potential of the equivalent point is independent of the concentration rates of the system. Thus the determination of chromate can be made in a wide range. Between 1.0 and 0.01 gram every chromate quantity can be estimated.

Owing to the different aquo complexes of the chromic ion the color of the solution can differ at the end point of the titration. The end point, however, is easy to observe. The formation of aquo complexes is impaired by the presence of neutral salts and by cooling. The presence of neutral salts causes the reappearance of the blue color after the titration. Therefore it is expedient to fix the color of aquo complexes through standardization of the temperature.

The method was iodometrically controlled.

SOLUTIONS

Decinormal Arsenite. The pure, dry substance (4.948 grams) is dissolved in a solution of 5 grams of sodium hydroxide, which is prepared in a small amount of water, and, if necessary, boiled. The solution is transferred into a 1-liter flask, diluted with water, and acidified by 10 ml. of concentrated sulfuric acid, and then the flask is filled up to the mark with water. Thus the acidified arsenite solution is compared with alkaline arsenite, very constant in strength, especially after removal of catalyzing impurities—iron, sulfur dioxide, etc.

Catalyst Solution. It is expedient to employ the catalyzing ions in a common solution. When 0.021 gram of potassium iodide and 0.005 gram of manganese sulfate are dissolved in 250 ml. of water, 5 ml. of this solution contain the quantity of catalyst necessary for one determination.

Indicator Solution. One gram of diphenylamine is dissolved in 100 ml. of concentrated sulfuric acid. One drop of this solution indicates the titration end point with great sharpness.

PROCEDURE

The chromate solution sample is acidified with 5 to 6 ml. of concentrated sulfuric acid and distilled water is added to make 50

Table I. Determination of Chromate

$K_2Cr_2O_7$ Taken	$K_2Cr_2O_7$ Found	Difference	
Gram	*Gram*	*Gram*	*%*
0.7088	0.7091	+0.0003	0.04
0.7088	0.7088	0.0000	0.0
0.7088	0.7092	+0.0004	0.05
0.7088	0.7088	0.0000	0.0
0.7088	0.7085	−0.0003	0.04
0.4359	0.4363	+0.0004	0.09
0.4359	0.4359	0.0000	0.0
0.4359	0.4356	−0.0003	0.07
0.4359	0.4359	0.0000	0.0
0.1856	0.1855	−0.0003	0.03
0.1856	0.1855	−0.0001	0.05
0.1856	0.1854	−0.0002	0.1
0.1856	0.1855	−0.0001	0.05
0.11818	0.11813	−0.00005	0.04
0.11818	0.11820	+0.00002	0.01
0.11818	0.11813	−0.00005	0.04
0.11818	0.11818	0.0000	0.0
0.05534	0.05540	+0.00006	0.1
0.05534	0.05529	−0.00005	0.09
0.01073	0.01072	−0.00001	0.1
0.01073	0.01074	+0.00001	0.1

Table II. Determination of Chromium in Steel

Steel Taken *Grams*	Chromium Found *Gram*	Chromium Found %
1.0457	0.00843	0.81
1.0478	0.00804	0.77
0.5393	0.00425	0.79
0.5293	0.00418	0.79
0.3814	0.00261	0.68
1.4848	0.01126	0.75
0.4326	0.00408	0.941
0.2982	0.00293	0.983
0.2738	0.00257	0.940
0.2339	0.00230	0.985

to 60 ml. To the cooled sample are added 5 ml. of catalyst solution and 1 drop of indicator solution. The arsenious acid is allowed to run into the chromate solution from a buret just until the blue color changes to light green. If the blue color reappears within 5 minutes, one more drop of arsenite is added..

The indicator error can be estimated experimentally. This error originates from the oxidation of diphenylamine to diphenylbenzidine through chromate, which is the only compound to show color later and is reformed again after disappearance of the blue. This correction amounts to +0.05 ml. of a 0.1 *N* arsenite solution.

Table I shows that the proposed method gives satisfactory results.

Mercury salts and thiocyanate ion disturb the determination. The former slacken the development of the diphenylamine color, and the rhodanide gives with the indicator a dirty green precipitate.

In the determination of chromium in steel according to this method care must be taken to remove the excess of iron, which would disturb the reaction with diphenylamine. This can be achieved easily with alkaline or ammonium fluoride. To mask 1 gram of ferric sulfate, 0.5 gram of fluoride is enough.

A 0.3- to 1.0-gram sample of steel is taken in an Erlenmeyer flask, and 30 to 35 ml. of water and then 5 to 6 ml. of concentrated sulfuric acid are added. The liquid is boiled over a small flame until complete solution. After evolution of hydrogen has stopped, potassium permanganate solution is added dropwise to oxidize the chromic ion to the chromate stage, which is shown by the dark brown color of the precipitated manganese dioxide. The excess of permanganate is decomposed by boiling. After cooling to room temperature the solution is filtered until the filtrate is clear. The filter is washed with 20 ml. of hot water, 0.5 or 1 gram of pure fluoride salt is then added, and after 1 minute 5 ml. of catalyst solution, 1 drop of indicator, and 0.05 *N* arsenious acid are run into the solution until the color changes to light green.

The arsenite solution is standardized against potassium dichromate in the presence of the amount of fluoride salt indicated above.

If a part of the chromium in steel is present as carbide, this does not dissolve in sulfuric acid. In this case 1 to 2 ml. of concentrated hydrochloric acid are added to the sulfuric acid and the solution is evaporated to dryness to expel the hydrochloric acid. Otherwise the procedure is the same as above.

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Analysis of Barytes

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THE evaluation of barytes involves the determination of barium sulfate, usually after removal of acid-soluble barium salts. Frequently, silica, iron, alumina, strontium, calcium, and magnesium must also be determined.

For the determination of barium sulfate, the acid-insoluble residue (or the original sample, if a separate determination of the soluble barium salts is preferred) is fused with sodium and/or potassium carbonate. The melt is extracted with hot water to dissolve out the alkali sulfate.

According to the method of the New Jersey Zinc Company (*4*), the barium carbonate, together with the other insoluble carbonates, is dissolved in hot dilute hydrochloric acid. If the sample is free from or low in strontium, the barium is then directly precipitated by the very slow addition of a hot ammonium sulfate solution. This method requires prior information as the amount of strontium present, but such information frequently can be obtained only by actual analysis of the sample. Tests recorded in Table I indicate that strontium is largely coprecipitated with the barium sulfate, no matter whether the reagent is added slowly or all at once. Calcium is also coprecipitated, although its interference is less serious than that caused by strontium.

For larger amounts of strontium (*4*), the insoluble carbonates are dissolved in nitric acid and neutralized with ammonium hydroxide, and barium is precipitated as the chromate in a buffered acetic acid–acetate solution. The proper conditions for this precipitation were recently described by Beyer and Rieman (*1*). After reprecipitation, the barium chromate is dissolved in hydrochloric acid and hydrogen peroxide and the barium is finally precipitated as the sulfate.

The filtrate from the barium chromate is not well suited for the determination of strontium, as no prior separation from calcium and other elements was carried out. Strontium and other impurities of the sample must therefore be determined in separate portions.

Kallmann (*2*) recently suggested a 20% solution of hydrogen chloride in *n*-butyl alcohol for the separation of barium and strontium from calcium, and a 4 to 1 mixture of 11.0 *N* hydrochloric acid and *n*-butyl alcohol for the separation of barium from strontium. This method is intended for mixtures of the chlorides of the three alkaline earths not exceeding 1 gram, and containing not more than 500 mg. of combined barium and strontium chlorides and not more than 250 mg. of strontium chloride. As barytes essentially consists of barium sulfate with only minor quantities of calcium and strontium, some difficulty would be encountered in applying this method in its original form to the analysis of barytes. Hence, certain changes in the manipulations have been worked out which simplify considerably the analysis of this mineral.

Table I. Precipitation of Barium Sulfate in Presence of Strontium and Calcium

(Barium sulfate precipitated from hot weakly hydrochloric acid solution of barium, strontium, and calcium by slow addition of hot ammonium sulfate solution)

$BaCl_2$ Taken *Gram*	$SrCl_2$ Taken *Gram*	$CaCl_2$ Taken *Gram*	$BaSO_4$ Found Equivalent to $BaCl_2$ *Gram*	Error *Gram*
0.9000	..	..	0.9002	+0.0002
0.9000	..	..	0.8997	−0.0003
0.9000	0.0052	..	0.9026	+0.0026
0.9000	0.0104	..	0.9056	+0.0056
0.9000	0.0104	..	0.9064	+0.0064
0.9000	0.0208	..	0.9112	+0.0112
0.9000	0.0416	..	0.9198	+0.0198
0.9000	..	0.0120	0.9015	+0.0015
0.9000	..	0.0240	0.9045	+0.0045

Table II. Precipitation of Barium Chloride with Hydrochloric Acid and *n*-Butyl Alcohol in Presence of Other Elements

$BaCl_2$ Taken	$BaCl_2$[a] Found	Compound Used	Amount Taken
Gram	*Gram*		*Gram*
0.9000	0.8996	$AlCl_3$	0.045
0.9000	0.9001		0.180
0.9000	0.9004	$CaCl_2$	0.050
0.9000	0.8992		0.100
0.9000	0.9003	$MgCl_2$	0.02
0.9000	0.8997		0.08
0.9000	0.8996	$MnCl_2$	0.03
0.9000	0.9003	$FeCl_3$	0.02
0.9000	0.8993		0.08

[a] Corrected for solubility (*2*).

PRINCIPLE OF PROPOSED METHOD

In the earlier method (*2*) a 20% solution of hydrogen chloride in *n*-butyl alcohol (Willard and Smith reagent) is used first for the separation of barium and strontium from calcium, followed by the application of a 4 to 1 mixture of 11.0 *N* hydrochloric acid and *n*-butyl alcohol for the separation of barium from strontium.

The method described below involves, as a first step, the separation of barium from all other elements, including strontium, with the aid of the 4 to 1 acid-alcohol mixture. This change greatly simplifies the determination of barium, which thus can be carried out in a very short time without interfering with the determination of other elements. In addition, this change obviates the necessity of precipitating the barium with the Willard and Smith reagent, as filtration of large quantities of barium chloride is rather slow from a 10% solution of hydrogen chloride in *n*-butyl alcohol. Data presented in Table II indicate that precipitation of barium chloride with the 4 to 1 mixture of 11.0 *N* hydrochloric acid and butyl alcohol provides a very satisfactory separation from calcium, magnesium, aluminum, manganese, and iron. The separation from strontium has been discussed (*2*).

PROCEDURE

Use the reagents described in the earlier publication (*2*).

Fuse 1.0000 gram of the original sample with about 8 grams of potassium carbonate in a platinum crucible. Determine acid-soluble barium salts in a separate portion of the sample. Cool and leach the melt with 200 ml. of hot water in a 400-ml. beaker. If a silica determination is required, carry out the leaching process in a platinum or nickel vessel. Filter through an 11-cm. paper (White Ribbon S. & S. or No. 40 Whatman) containing some filter pulp and wash ten times with a hot 2% solution of potassium carbonate and twice with cold water. Hold the filtrate (filtrate I) if a silica and/or alumina determination is required.

Wash the precipitate back into the original beaker, dissolve in hot dilute hydrochloric acid, heat to boiling, then filter through the original paper into a 250-ml. beaker. Wash with hot dilute hydrochloric acid. Hold the precipitate (precipitate I) if a silica determination is required.

Evaporate the hydrochloric acid solution containing all the barium, strontium, calcium, iron, and magnesium and part of the silica and alumina. Dehydrate the silica, take up with dilute hydrochloric acid, boil, and filter. Hold the precipitate if a silica determination is required (precipitate II).

If a silica determination is not required, the dehydration and filtration may be omitted; the final barium chloride, however, must then be tested for presence of silica, by dissolving it off the weighed Gooch crucible with hot water and weighing the crucible after drying.

Determination of Barium. Evaporate the solution of the mixed chlorides to dryness on a steam bath, dissolve the residue in 5 ml. of warm water (60° to 70° C.), then add with constant stirring 55 ml. of concentrated (12.0 *N*) hydrochloric acid. Warm to about 75° C., add 15 ml. of *n*-butyl alcohol, then cool to below 20° C.

Decant the supernatant solution through a tared Gooch crucible, receiving the filtrate directly in a 250-ml. beaker. Transfer the barium chloride into the crucible, policing the beaker and washing the crucible 6 to 8 times with 1- to 2-ml. portions of a cold mixture (4 to 1) of 11.0 *N* hydrochloric acid and *n*-butyl alcohol. Place the crucible on a small cover glass, and dry for 1 hour at 110° C. and finally for 15 minutes in a muffle at 350° C. Cool in a desiccator, then weigh as anhydrous barium chloride.

Correct this weight for the solubility of barium chloride in 75 ml. of the hydrochloric-butyl alcohol mixture, by adding 2.0 mg. to the weight of the barium chloride (*2*). Because barytes rarely contains more than a few per cent of strontium, coprecipitation of strontium chloride with barium chloride need not be feared.

Determination of Strontium. 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 at a temperature not over 180° C. Dissolve the mixed perchlorates of strontium, calcium, magnesium, iron, and aluminum by boiling with 5 ml. of *n*-butyl alcohol and precipitate the strontium by adding 5 ml. of the 20% solution of hydrogen chloride in butyl alcohol. When cool, filter on a dry untared Gooch crucible, policing the beaker and washing the crucible 6 times with 1-ml. portions of 10% hydrogen chloride in *n*-butyl alcohol. Hold the filtrate (filtrate II) if a determination of calcium, iron, magnesium, or aluminum is required.

Inasmuch as the strontium chloride invariably will be contaminated by a little potassium (introduced by the original fusion of the sample with potassium carbonate and only incompletely removed by the wash solution), dissolve the precipitate on the Gooch crucible in hot water and precipitate the strontium either as the sulfate by the addition of sulfuric acid and alcohol or as the carbonate with ammonium carbonate. Correct the final result for the solubility of barium chloride in the 4 to 1 mixture of hydrochloric acid and *n*-butyl alcohol, by subtracting 2.0 mg. from the weight of the strontium chloride.

Determination of Silica. Acidify filtrate I with hydrochloric acid, evaporate to dryness, dehydrate the silica, take up with dilute hydrochloric acid, heat to boiling, and filter. Wash the paper 10 to 12 times with hot water (precipitate III). Hold the filtrate only if an alumina determination is required (filtrate III).

Combine precipitates I, II, and III, ignite in a platinum crucible, cool in a desiccator, and weigh. Determine silica in the usual way by expulsion with hydrofluoric acid.

Determination of Iron, Alumina, Calcium, and Magnesium. Dilute the filtrate from the strontium determination (filtrate II) with one third its volume of water and evaporate on the steam bath in such a way as to avoid condensation on the upper part of the beaker (*3*). When completely dry, add 5 ml. of water, 2 ml. of nitric acid, and 5 ml. of sulfuric acid, and heat on a hot plate until heavy fumes of sulfuric acid escape and finally to complete dryness. Dissolve in dilute hydrochloric acid, and add filtrate III if an alumina determination is required. Precipitate iron and alumina by rendering the solution ammoniacal. Separate iron and alumina by standard methods. Precipitate calcium in the ammoniacal filtrate in the usual way as the oxalate, and magnesium in the filtrate from the calcium oxalate as the magnesium ammonium phosphate.

Table III. Analysis of Known Mixtures

	Used	Found	Used	Found	Used	Found
	Gram	*Gram*	*Gram*	*Grams*	*Gram*	*Gram*
$BaSO_4$	1.0000	0.9996	1.0000	1.0003	1.0000	0.9994
$SrSO_4$	0.0122	0.0131	0.0244	0.0237	0.0366	0.0356
SiO_2	0.0200	0.0192	0.0400	0.0389	0.0600	0.0602
Al_2O_3	0.0150	0.0140	0.0150	0.0143	0.0200	0.0198
Fe_2O_3	0.0100	0.0106	0.0200	0.0198	0.0300	0.0307
$CaCO_3$	0.0150	0.0154	0.0300	0.0309	0.0450	0.0439
$MgCO_3$	0.0104	0.0110	0.0208	0.0214	0.0312	0.0319

CORROBORATION AND VERIFICATION

Because no standard samples of barytes were available it was necessary to prepare synthetic standards from reagents of known purity and suited for fusion with potassium carbonate (Table III).

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Determination of Nitrogen in Difficultly Combustible Ring Compounds

Modification of the Micro-Dumas Procedure

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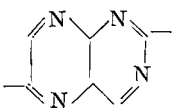
IN THE course of research on pteroyl glutamic acid (*L. casei* factors), its intermediates, and its derivatives, the conventional micro-Dumas procedure yielded very erratic results for nitrogen. That the pteridin heterocyclic ring structure was the cause of the difficulty was substantiated by a search of the literature (*5*). The pteridin ring system, which contains condensed pyrazine and pyrimidine structures, yielded analytical figures for



nitrogen which usually varied within 2% of the theoretical values, though an occasional analysis was even further removed from the actual value. The fact that the values were generally low seemed to indicate incomplete combustion of the compounds analyzed. Consequently, the first attempt to improve the analyses involved the use of oxidizing agents—copper acetate (*1*) and potassium chlorate (*3*)—in the belief that these substances would liberate enough oxygen to aid the combustion of the ring compounds. No improvement was noted.

Table I. Nonpterin Standards Burned at 900° C.

Compounds	% N, Theory	% N, Found
Dimethylglyoxime	24.14	24.53, 23.98, 24.63, 24.23, 24.08
Sulfathiazole	16.46	16.85, 16.51, 16.24
Sulfadiazine	22.37	22.35, 22.74
Azobenzene	15.38	15.67, 15.64

As it had been noticed during carbon-hydrogen combustions that the pterin sample appeared inert until the burner flame was directly under the platinum boat, it was decided to use a higher temperature in the Dumas determination than the conventional 700° C. To see whether the higher temperature would affect the carbon dioxide-carbon monoxide equilibrium, several compounds which gave theoretical values at 700° C. were burned at 900° C. (Table I).

These results indicated that the higher temperature did not affect the equilibrium; accordingly, the movable furnace only was adjusted to a temperature of 900° C. A series of analyses for nitrogen run on a group of analytically pure pterins is shown in Table II.

A semimicronitrometer was used for the semimicroanalyses because the sample size ordinarily taken for the micro-Dumas determination releases enough nitrogen gas in the case of these compounds to overflow the graduated capacity of the micronitrometer. It is impossible to take a 3- or 4-mg. sample and still remain within the range recommended by Pregl (*2*), yet to diminish the sample taken in order to conveniently fit the nitrogen evolved into the 1.5-cc. microburet, increases the demands on the microanalytical weighing. The sample sizes represented in Table II averaged about 5.2 mg., with extremes of 4.1 and 6.1 mg.

In the microanalyses of Table II the samples averaged 2.7 mg. and a micronitrometer was used. The extremes were 2.5 to 2.9 mg. These small samples gave volumes of nitrogen of roughly 1 cc. The usual correction of 1.1% of the total volume of nitrogen (*4*) for the adhesion of liquid to the walls of the nitrometer, for the vapor pressure of the potassium hydroxide solution, and for the temperature reduction of the barometric reading from room temperature to 0° C. was employed.

For the sake of comparison a series of values was obtained by analyzing a typical pterin (2-amino-4-hydroxyl-6-methylpteridin) at 700° C. Per cent nitrogen, theory, was 39.53. Per cent nitrogen found was 40.26, 37.82, 38.04, and 42.47. The same erratic results experienced with the pteridins at conventional Dumas temperatures were later encountered in the combustion of two other compounds, a quinoxaline and a quinoline.

Compound	% N, Theory	% Nitrogen Found 700° C.	% Nitrogen Found 900° C.
Quinoxaline-2-aldehyde	17.88	17.05	17.72
Quinoline-2-acrylic acid	7.03	5.01, 5.14	7.11

The microcombustion furnace used in this work was of the Flaschentrager type manufactured by the American Platinum Works. It contained heating elements above, below, and behind the combustion tube, but not in front. The best analytical results were obtained when the reduced copper was placed exactly in the middle of the stationary furnace. The combustion tubes employed were of quartz.

Table II. Pterins Burned at 900° C.

Compounds	% N, Theory	% N, Found
Semimicroanalysis		
2-Amino-4-hydroxyl-6-methylpteridin	39.53	39.45, 39.92, 39.34, 39.66, 39.21, 39.64
2-Amino-4-hydroxyl-6,7-dimethylpteridin	36.65	36.49, 37.14
2-Amino-4-hydroxylpteridin-7-carboxylic acid	33.82	33.51, 33.80
2-Amino-4-hydroxylpteridin-7-carboxylic acid hydrate	31.11	31.60, 31.17
2-Amino-4-hydroxyl-7-methylpteridin	39.53	39.83, 39.44, 39.33
2-Amino-4-hydroxylpteridin-6-acetic acid	31.65	31.99, 32.03, 31.90
2-Amino-4-hydroxylpteridin	42.94	42.43, 42.88, 42.59, 42.75, 42.56, 42.62
2-Amino-4-hydroxylpteridin-6-carboxylic acid	33.82	33.49, 33.30
Microanalysis		
2-Amino-4-hydroxylpteridin	42.94	43.21, 42.58, 42.54, 42.61, 42.57, 42.78
2-Amino-4-hydroxyl-7-methylpteridin	39.53	39.68, 39.41, 39.62, 39.46, 39.91

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Molybdenum Determination in Plant Material

Modification of Thiocyanate Stannous Chloride Method

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THE thiocyanate–stannous chloride method for determination of molybdenum is based on the formation of an amber to orange-red colored compound when a reducing agent such as stannous chloride is added to an acid solution of a molybdate salt in the presence of thiocyanate. The colored compound was identified by Hiskey and Meloche (*3*) as a thiocyanate complex of quinquevalent molybdenum. Hurd and Allen (*5*) found that unless certain conditions are maintained in the molybdate solution during production of the colored compound, neither the yield nor the stability of the compound will be at a maximum.

During an investigation of the molybdenum content of plants (*1*), the author found that, although the conditions specified by Hurd and Allen were maintained, the addition of ferric and nitrate ion may further increase the yield and stability of the colored compound. Although several investigators (*2, 4, 9*) have noted that the presence of ferric ion in a molybdate solution intensifies the molybdenum thiocyanate color, no attempt has been made to study the quantitative aspects of the intensification, and no mention has apparently been made of the similar effect of nitrate.

The results of the present investigation, summarized in Table I, show that when ferric or nitrate ion is present in molybdate solutions, and the conditions specified by Hurd and Allen are maintained during production of the molybdenum thiocyanate color, the intensity of the color is equal to the intensity obtained in molybdate solutions of twice the concentration. The presence of nitrate stabilizes the color so that practically no fading takes place for at least 30 minutes. If, however, the amount of stannous ion is much smaller than the nitrate, rapid fading indicates the complete oxidation of stannous to stannic ion by the excess nitrate; further addition of stannous ion stops the fading and reintensifies the color. The presence of small amounts of stannous ion as compared with nitrate may possibly account for the increased fading with nitrate reported by Grimaldi and Wells (*2*). The intensification of the color with ferric ion also held true when the molybdenum thiocyanate color was extracted with ether (Table II). The intensity of the color for equal concentrations is the same in aqueous as in ether solution (Table III), and the intensification of the color by ferric ion is proportional to the amount of ferric ion, provided the amount added is less than the equivalent of the molybdenum present, and reaches its greatest intensity when ferric ion and molybdenum are present in equivalent amounts. Much larger amounts of ferric ion caused no further change in the intensity of the color (Table IV).

The intensification of the quinquevalent molybdenum thiocyanate colored compound by ferric ion or nitrate may be explained by the following considerations and experimental evidence.

A comparison of the potentials (*6*) of the $Fe^{++} - Fe^{+++}$ ($E° = -0.771$), $NO - NO_3^-$ ($E° = -0.94$) couples, and the potentials of the $Mo - Mo^{+++}$ ($E°$ = ca. 0.2), $Mo^{+++} - MoO_2^+$ ($E°$ = ca. 0.0), $MoO_2^+ - H_2MoO_4$ ($E°$ = ca. − 0.4) couples indicates that ferric ion and nitrate in acid solutions will oxidize molybdenum in a reduced state to the hexavalent state. The reaction between trivalent molybdenum and iron is the basis of a method for determining molybdenum (*11*), and the oxidation reaction of nitric acid is utilized in bringing molybdenite (MoS_2) into solution (*11*).

A comparison of the potentials of the $Sn^{++} - Sn^{++++}$ ($E° = -0.15$) couple and the molybdenum couples indicates that stannous ion may reduce hexavalent molybdenum to a lower valency than quinquevalent molybdenum if present in considerably larger amounts than hexavalent molybdenum. That such a reduction takes place was indicated by the intensity of the molybdenum thiocyanate color in relation to the amount of stannous chloride for a given concentration of molybdenum.

Table IV shows that the intensity is at a maximum when stannous ion is about equal or in a slight excess to the molybdenum, and at a minimum when the concentration of stannous ion is ten or more times the concentration of molybdenum; also that the difference between the maximum and minimum intensities is approximately equal to the intensity of a solution one fourth the concentration. It is believed that nitrate or ferrous ion, possibly as a complex ion with thiocyanate, when present in the solution prevents stannous ion from reducing the quinquevalent molybdenum to a lower state of valency by virtue of their oxidation potentials in relation to the potential of molybdenum in a reduced state. This oxidizing power of nitrate ferrous ion, possibly as a complex ion, may account for the intensification of the molybdenum thiocyanate color brought about when these ions were added to an ammonium molybdate solution in which the molybdenum thiocyanate was produced by an excess of stannous ion (Table V).

The additional 25% increase in intensity when nitrate or ferric ion is added prior to the stannous is believed to be brought about by an oxidation reaction whereby these ions oxidize reduced molybdenum which seems to be present in molybdate solutions of hydrochloric or sulfuric acid. That reduced molybdenum, possibly in the trivalent state, is present in such solutions was indicated by the following experimental evidence.

The intensity of molybdenum thiocyanate color produced by small amounts of stannous ion in a solution of ammonium molybdate in sulfuric acid in which nitric acid was added and then removed by evaporation was 25% higher than in a solution to which

Table I. Comparison of Molybdenum Thiocyanate Color Intensity of Equal Concentrations of Molybdenum in Absence and Presence of Ferric Chloride, Sodium Nitrate, or Both

Concentration of Molybdenum, P.P.M.	Sensitivity, Range of Colorimeter[a]	Size of Absorption Cell, Cm.	Per Cent Absorption Using Green Filter							
			Fe^{+++} or NO_3^- Absent		Fe^{+++}[b] Present		NO_3^-[c] Present		Fe^{+++} and NO_3^- Present	
			Immediate	30 min. later	Immediate	30 min. later	Immediate	30 min. later	Immediate	30 min. later
0.05	4	5	12.0 ± 0.5[d]	10.0 ± 0.5	24.0 ± 0.5	19.0 ± 0.5	24.0 ± 0.5	23.0 ± 0.5	24.0 ± 0.5	22.0 ± 0.5
0.10	4	5	24.0 ± 0.5	22.0 ± 0.5	46.0 ± 1.0	38.5 ± 1.0	46.0 ± 1.0	44.0 ± 1.0	46.0 ± 1.0	40.0 ± 1.0
0.50	3	1	13.0 ± 1.0	12.0 ± 1.0	25.0 ± 1.0	22.0 ± 1.0	25.0 ± 1.0	24.0 ± 1.0	25.0 ± 1.0	23.0 ± 1.0
1.00	3	1	25.0 ± 1.0	23.0 ± 1.0	44.5 ± 1.0	38.5 ± 1.0	44.5 ± 1.0	44.0 ± 1.0	44.5 ± 1.0	41.5 ± 1.0
2.00	3	1	44.5 ± 1.0	40.0 ± 1.0	85.0 ± 2.0	75.0 ± 2.0	85.0 ± 2.0	81.0 ± 2.0	85.0 ± 2.0	80.0 ± 2.0
4.00	3	1	85.0 ± 2.0	78.0 ± 2.0	.....	.....	.....	.....	.....	.....

[a] No. 7-089 Fisher electrophotometer.
[b] Concentration of Fe^{+++} equal or greater than concentration of Mo.
[c] 1 ml. of 5 *N* $NaNO_3$ in 100 ml. of solution.
[d] Average deviations.

Table II. Molybdenum Thiocyanate Color

(Developed in HCl, extracted with ether, and read immediately using No. 3 range of colorimeter)

Concentration of Molybdenum, P.P.M.	Per Cent Absorption	
	Fe^{+++} absent	Fe^{+++} present
0.5	12.0	24.5
1.0	24.0	45.5
1.5	38.0	67.0
2.0	44.5	85.0
3.0	70.0	..

Table III. Intensity of Color in Aqueous Medium and in Ether

(Equal concentrations of molybdenum in presence of $FeCl_3$, using No. 3 range of colorimeter)

Concentration of Molybdenum, P.P.M.	Per Cent Absorption	
	Aqueous (1 cm.)	Ether (1 cm.)
0.25	12.0	12.0
0.50	24.0	24.5
1.00	45.0	45.5
1.50	67.0	67.0

Table IV. Intensity of Molybdenum Thiocyanate Color

(No. 3 range of colorimeter used)

Fe^{+++}[a] in Proportion to Molybdenum	% Absorption (1 Cm.)	Sn^{++}[b] in Proportion to Molybdenum	% Absorption (1 Cm.)
0.0	38.0	3/4	38.0
1/4	45.5	1	64.5
1/2	52.0	2	60.0
1	67.0	10	45.5
4	67.0	>100	45.5
10	67.0		
>100	67.0		

[a] Concentration of molybdenum in solution 1.50 p.p.m.
[b] $FeCl_3$ absent. Concentration of molybdenum in solution 2.0 p.p.m., reading immediate.

nitrate was never added, but equal to the intensity of molybdenum thiocyanate produced in the presence of nitrate or ferric ion. The addition of a large excess of stannous ion reduced the intensity of the molybdenum thiocyanate color in the solutions in which nitrate was absent but not in the solutions in which nitrate was present (Tables I and VI).

The actual reduction of nitrate when added to hydrochloric or sulfuric acid solution of ammonium molybdate in the absence of stannous ion and potassium thiocyanate was demonstrated by the gradual production of nitrite as shown by the sulfanilic and α-naphthylamine test for nitrite (*8*). A similar reaction took place when nitrate was added to an acid solution of trivalent molybdenum but not in an acid solution in the absence of molybdate.

The conclusion may be drawn that the increase in intensity of the molybdenum thiocyanate color by nitrate and ferric ion is possibly brought about partly by preventing the reduction of quinquevalent molybdenum to a lower state of valency by stannous ion and partly by the oxidation of reduced molybdenum which apparently is present in acid solution of molybdates. However, because conditions in such solutions are rather complex, other reasons may account for the intensification of the molybdenum thiocyanate color by iron or nitrate salts.

A method for determining molybdenum in plant material was developed which eliminates the use of a solvent for extracting the molybdenum thiocyanate compound, as is the case in some of the recommended procedures (*7, 9*). If the colorimeter in use contains an absorption cell with a depth of 5 cm., a concentration as low as 0.01 p.p.m. may be measured. If the concentration falls below this limit, the solution must be concentrated by extraction with a very small volume (5 ml.) of ether or butyl acetate.

PROCEDURE

Ashing. To prevent the smoke, emitted during the early stages of burning plant material, from charring the muffle and smoking the room, add about 1 ml. of ethyl alcohol to the oven-dried (100° C.) plant material (between 0.5 and 25 grams) and ignite in a porcelain dish. After flaming ceases, heat over a low flame until the greater part of the material is oxidized, then place the dish in a muffle furnace at 450° to 500° C. for 2 to 5 minutes. The foregoing steps take about 10 to 15 minutes. If the ash contains carbonates (ash from leguminous plants), neutralize with dilute nitric acid; if ash is siliceous (ash from grasses), wet with a 1 *N* sodium nitrate solution. Dry the treated ash first on a steam bath and then in a Hillebrand-Willard crucible radiator over a Bunsen flame; then return to the muffle for a minute or two to ensure complete destruction of all organic carbon. The steps described require about 1 hour. Wet cooled ash with 1 to 1 hydrochloric acid, and then dry on a steam bath. To the dried ash add 28 ml. of 1 to 1 hydrochloric acid and let stand on steam bath for a few minutes before filtering through a No. 40 Whatman filter paper. Wash with 1 to 100 hydrochloric acid solution until the total volume of the filtrate is about 80 ml.; this solution is now ready for the determination of molybdenum.

To solutions that are not distinctly colored with ferric chloride, add 3 or 4 drops of 0.01 *N* ferric chloride. This was necessary only occasionally with solutions obtained from the ash of certain grasses.

Developing Molybdenum Thiocyanate Color. To the sample solution add 1.0 ml. of 5 *N* sodium nitrate and shake thoroughly; then add 6.0 ml. of 10% potassium thiocyanate, and shake again; add 1.0 to 6.0 ml. of stannous chloride dihydrate solution made up in 1 to 9 hydrochloric acid (amount to be added depends on the amount of ferric ion present judged by the disappearance of the $Fe(SCN)^{---}$ color and the absence of fading on further addition of stannous chloride). Finally, make up to volume (100 ml.) with distilled water and shake thoroughly. Determine intensity of color immediately in a colorimeter using a green filter. Before each reading, adjust colorimeter to zero with distilled water. Correct the results for the molybdenum content of the chemicals used. Read the concentration of molybdenum in the sample from standard curves prepared from known molybdenum solutions which contained both ferric chloride and sodium nitrate.

The proposed modifications may possibly increase the precision at the lower range of concentration (0.01 to 0.50 p.p.m.) by stabilizing the color, for in this range even a small degree of fading introduces a large percentage error. As ferric iron is always present in solutions of plant ash, if it is absent from the standard solution used for comparison with the unknown, an error of nearly 50% may result. The elimination of extraction of the molybdenum thiocyanate color with a small volume of solvent not only simplifies and speeds up the procedure but also increases the precision, par-

Table V. Effect of Ferrous and Ferric Iron[a] on Intensity of Molybdenum Thiocyanate Color in Presence of Large Excess of Stannous Chloride

(Concentration of molybdenum in solution 1.50 p.p.m., No. 3 range of colorimeter)

Treatment of Molybdate Solution	% Absorption (1 Cm.)
Fe^{++} or Fe^{+++} absent	38.0
Fe^{++} added after color developed with SCN^- and Sn^{++}	51.0
Fe^{++} added before color developed with SCN^- and Sn^{++}	51.0
Fe^{+++} added after color developed with SCN^- and Sn^{++}	51.0
Fe^{+++} added before color developed with SCN^- and Sn^{++}	67.0

[a] Fe^{+++} or Fe^{+++} present in larger amounts than molybdenum.

Table VI. Intensity of Molybdenum Thiocyanate Color in Solution of Ammonium Molybdate

($FeCl_3$ absent. Concentration of Mo in solution 2.0 p.p.m. No. 3 range of colorimeter)

Sn^{++} in Proportion to Molybdenum	Treatment of Ammonium Molybdate Solution	% Absorption (1 Cm.)	
		Immediate	30 min. later
1	[a]	87.0	..
200	[a]	76.5	50.0
1	HNO_3 not added	64.5	..
200	HNO_3 not added	44.5	40.0

[a] HNO_3 added and then removed by repeated evaporation to near dryness in presence of H_2SO_4.

Table VII. Determination of Molybdenum in Plant Material by Thiocyanate–Stannous Chloride Method without Ether Extraction

Sample	Molybdenum in Original Sample, P.P.M.	Molybdenum Added, P.P.M.	Molybdenum Found, P.P.M.	Molybdenum Recovered, P.P.M.	Molybdenum Recovered, %
Alfalfa	18.4	16.2	34.6	17.0	105
	4.2	15.4	19.6	15.7	102
	7.1	15.4	22.5	15.8	103
	7.1	31.4	38.5	31.9	102
	7.1	48.4	55.5	48.1	99
Barley	18.8	16.5	35.3	17.0	103

ticularly in plant samples high in molybdenum where the possibility of incomplete extraction exists. The absence of the interfering ions, chromium, vanadium, tungsten, and rhenium (*4, 10*), in solutions of plant ash also justifies the elimination of extraction.

The concentration of molybdenum in solutions of plant ash encountered ranges approximately between 0.02 and 2.0 p.p.m. The sensitivity in the range between 0.02 and 0.20 p.p.m. is approximately 0.001 p.p.m. per 0.5% absorption and between 0.1 and 2.0 p.p.m. is approximately 0.01 p.p.m. per 0.5% absorption.

The foregoing method differs from that proposed by Marmoy (*7*) in that the filter paper and residue after filtration are not ignited and resulting ash fused with sodium carbonate, because spectrographic analysis and fusion revealed that the residue contained negligible amounts of molybdenum even with material high in silica; the molybdenum thiocyanate complex is determined directly in the hydrochloric acid solution without extraction; and nitrate and ferric ion are added to the unknown solution and to the standards.

The method was tested by the addition of known amounts of molybdenum to plant samples before ashing. Table VII shows the molybdenum content obtained by the proposed method of the original samples as well as of samples to which molybdenum was added. The amounts recovered were nearly equal to those added.

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Use of Tributyl Phosphate for Separating Acetic Acid from Hydrochloric Acid

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IN AN earlier paper (*1*) it was shown that the fraction of acetic acid extracted from aqueous solution by means of *n*-tributyl phosphate is essentially independent of the acid concentration. Hydrochloric acid, however, showed a pronounced increase of extraction with increased concentration; hence a reasonably complete separation of acetic acid from hydrochloric by this method appeared questionable if the concentration of the latter were fairly high. Work recently completed shows, however, that the fraction of the hydrochloric acid extracted by the ester phase can be expressed by the equation:

$$\frac{[C_{HCl}]_{ester}}{[C_{H^+}C_{Cl^-}]_{water}} = K$$

This relation holds for mixtures of hydrochloric acid and sodium chloride as well as for hydrochloric acid alone, as shown in Tables I and II. Therefore, if the hydrochloric acid in a mixture of the two acids is partially neutralized to a pH approximately the same as that of a comparable concentration of acetic acid alone, a satisfactory separation should take place. This was found to be true.

PROCEDURE

In the work reported earlier, the amount of hydrochloric acid extracted into the ester phase was determined by draining the aqueous layer (lower) from a separatory funnel and then determining the acid in the ester by titrating with standard base. Because the hydrochloric acid concentration in the aqueous phase is always very high compared to that in the ester, significant errors resulted from the aqueous film left in the separatory funnel. The procedure was modified as follows:

Large volumes (80 to 100 ml.) of ester and of aqueous solution were put into a 250-ml. glass-stoppered flask, which was placed in a constant temperature bath (25.00° ± 0.05° C.) and shaken vigorously for about 1 minute at 15-minute intervals for several hours, without being removed from the bath. Complete phase separation took place after about 10 hours at constant temperature, after which pipetted portions of each phase were analyzed. The hydrochloric acid was removed from the ester sample by extracting three times with about 10 ml. of water each time, followed with a fourth portion containing a small amount of base. The hydrochloric acid was then determined gravimetrically as silver chloride. The acid in the aqueous samples was determined by titration with carbonate-free sodium hydroxide. Where mixtures of hydrochloric acid and sodium chloride were

Table I. Distribution of Hydrochloric Acid in Two-Phase System Water–*n*-Tributyl Phosphate

Hydrochloric Acid, *M*		$\frac{(C_{HCl})_{ester}}{(C_{H^+}C_{Cl^-})_{water}} = K$
Water phase	Ester phase	
0.187	0.00124	0.035
0 334	0.00390	0.035
0.631	0.0134	0.034
0.930	0.0292	0.034
1.211	0.0518	0.035
1.445	0.0771	0.037
1.808	0.1257	0.038

Table II. Distribution of Hydrochloric Acid in Two-Phase System Water–*n*-Tributyl Phosphate, in Presence of Sodium Chloride

Water Phase		Ester Phase,	$\frac{(C_{HCl})_{ester}}{(C_{H^+}C_{Cl^-})_{water}} = K$
(H^+), *M*	(Cl^-), *M*	(HCl), *M*	
0.0822	1.078	0.00288	0.033
0.0970	1.105	0.00348	0.033
0.442	0.688	0.00992	0.033
0.445	1.460	0.0203	0.031
0.874	1.877	0.0577	0.035
0.879	1.126	0.0331	0.034
0.884	1.918	0.0596	0.035
0.886	1.398	0.0418	0.034
0.890	1.017	0.0301	0.033

used, the total chloride was likewise determined gravimetrically. Selas porous porcelain filtering crucibles were used.

RESULTS

The data in Table I were used to calculate the distribution coefficient. All equilibrium concentrations are calculated to moles per liter. Complete ionization of the hydrochloric acid in the water phase was assumed for calculating the constant in Table I

The chloride concentrations in Table II are total chloride, and it is assumed that both the acid and salt are completely ionized in the water phase.

The constants calculated for "ion products" ranging from about 0.035 to 3.2 in Tables I and II are in reasonably good agreement.

In order to test the completeness of separating acetic acid from hydrochloric acid by controlling the hydrogen and chloride ion products, the following experiments were performed:

A mixture of aqueous acetic acid and hydrochloric acid was neutralized to pH = 1, using a Fisher titrimeter. The ester was then added and the regular procedure was followed. Analyses of 20-ml. pipetted portions showed 0.351 M total chloride in the aqueous phase and 8.7×10^{-4} M hydrochloric acid in the ester phase. By calculation, the amount of hydrochloric acid in the ester phase is 1.12×10^{-3} if the value $K = 3.4 \times 10^{-2}$ is used; hence the agreement is satisfactory. A second similar mixture was neutralized to pH 2 before extracting with the ester. Here the amount of hydrochloric acid from the ester sample gave only a faint qualitative test as silver chloride and was too small to be determined gravimetrically. Finally a mixture of 0.5 M acetic acid and 0.5 M sodium chloride was extracted. In this case a positive test for chloride from the ester sample was questionable.

It is logical to assume that other similar weak organic acids can be separated from hydrochloric acid by this method.

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RECEIVED August 4, 1948.

CRYSTALLOGRAPHIC DATA

Contributed by Armour Research Foundation of Illinois Institute of Technology

22. Uranyl Nitrate Hexahydrate, $UO_2(NO_3)_2.6H_2O$

Excellent crystals of uranyl nitrate hexahydrate can be obtained from either water or alcohol. Care must be taken to avoid crystallization of the anhydrous salt, because the hydrate is stable only at low temperatures. Either of these two phases may be obtained on a microscope slide; the hydrate only in the presence of excess water.

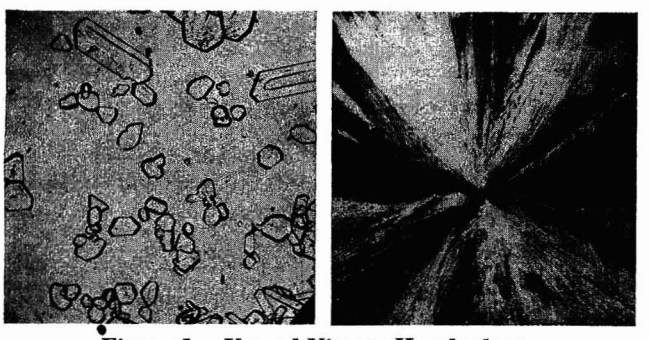

Figure 1. Uranyl Nitrate Hexahydrate

Left. **Crystals from water on a microscope slide**
Right. **Crystals from fusion, crossed Nicols**

CRYSTAL MORPHOLOGY (determined by W. C. McCrone).

Crystal System. Orthorhombic.

Form and Habit. Plates and tablets usually elongated parallel to c. The crystals show brachypinacoid {010}; macropinacoid {100}; macrodome {011}; and bipyramid {111}.

Axial Ratio. $a:b:c$ = 0.877:1:0.609; 0.874:1:0.609 (*4*); 0.8737:1:0.6088 (*2*).

Interfacial Angles (polar). $101 \wedge \bar{1}01 = 59° 30'$; $011 \wedge 0\bar{1}1 = 62° 40'$.

X-RAY DIFFRACTION DATA (determined by W. C. McCrone).

Space Group. V_h^{17} (*3*).

Cell Dimensions. a = 11.58 Å., b = 13.20 Å., c = 8.04 Å. a = 13.15 Å., b = 8.02 Å., c = 11.42 Å. (*3*). a = 7.93 Å., b = 11.45 Å., c = 13.01 Å. (*1*).

Formula Weights per Cell. 4.

Formula Weight. 502.18.

Density. 2.73 (x-ray); 2.807 (*1*); 2.742 (*3*).

Principal Lines

d		I/I_1	d		I/I_1
Armour	Hanawalt		Armour	Hanawalt	
6.72	6.6	Weak (W)	2.84	2.85	W
6.48		Moderate (M)	2.75		W
5.81	5.7	M	2.70		VW
5.66		M	2.63	2.62	VW
4.34	4.33	M	2.60		VW
4.20		M	2.53	2.54	W
3.80		WM	2.47	2.46	W
3.74		WM	2.43		W
3.61	3.63	W	2.37	2.37	
3.56		VW(very weak)	2.32		VW
3.32	3.29	M	2.29	2.28	WM
3.27		M	2.11	2.11	W
2.92	2.93	W	2.09		VW

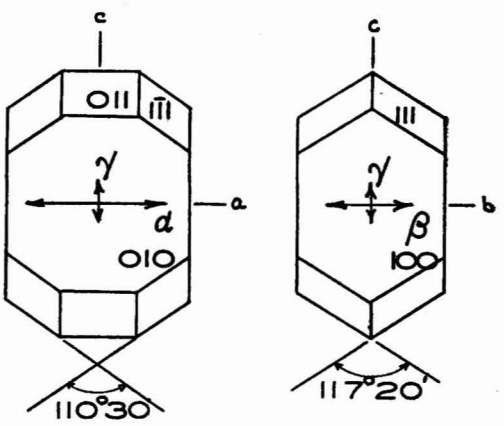

Figure 2. Orthographic Projection of Typical Crystal of Uranyl Nitrate Hexahydrate

OPTICAL PROPERTIES (determined by W. C. McCrone).

Refractive Indexes (5893 Å.; 25° C.). α = 1.482 ± 0.002. β = 1.494 ± 0.002. γ = 1.572 ± 0.002. β = 1.497(*4*).

Optic Axial Angles (5893 A.; 25° C.). $2V = 46°$. 44° (4); $2E = 72°$.

Dispersion. $v > r$ (weak).

Optic Axial Plane. 010.

Sign of Double Refraction. Positive.

Acute Bisectrix. c.

Molecular Refraction (R) (5893 Å.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.515$. R (calcd.) = 55.9 (if R for the uranium atom is assumed to be 11.8). R (obsd.) = 55.9.

Color. Yellow.

Pleochroism. X = light yellow. Y = yellow. Z = yellow-green.

FUSION DATA (determined by W. C. McCrone).

Uranyl nitrate hexahydrate melts at 60° C. and crystallizes readily on cooling as rods elongated parallel to c. All orientations perpendicular to c are shown; therefore all views show a positive sign of elongation. Only very rarely is it possible to find an optic axis interference figure, as $2V$ is small and $Bx_a = c$.

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(1) Clark, G. L., *J. Am. Chem. Soc.*, 46, 379 (1924).
(2) Groth, "Chemische Kristallographie," Vol. 2, p. 142, Leipzig, Engelmann, 1908.
(3) Pauling, Linus, and Dickinson, R. G., *J. Am. Chem. Soc.*, 46, 1615 (1924).
(4) Winchell, A. N., "Microscopic Characters of Artificial Minerals," p. 211, New York, John Wiley & Sons, 1931.

BOOK REVIEWS

Analytical Methods for a Textile Laboratory, 1949. *American Association of Textile Chemists and Colorists, Committee on Analytical Methods for a Textile Laboratory*, compilers. v + 287 pages. Association of Textile Chemists and Colorists, Lowell Textile Institute, Lowell, Mass., 1949. Price, $6.

This handbook is a revision and expansion of material which last appeared as a supplement to the 1945 A.A.T.C.C. yearbook. New sections on methods of sampling, determination of resins, identification of dyestuffs on fibers, and a bibliography of reference texts on analytical chemistry have been added. Methods given for analysis of fiber content, and photomicrographs of various textile fibers, are those which appeared in the 1946 and 1947–48 yearbooks.

A group of "useful tables and calculations" has been considerably enlarged and now contains tables of equivalents for yarn counts and Twaddell-Baumé–specific gravity–percentage concentration, a tabulation of fatty acid composition and characteristics of natural oils, fats and waxes, etc. Additions have been made to the sections on general analytical technique, preparation of standard solutions and reagents, and methods of analysis of chemicals.

Analytical methods selected for this compilation have been chosen for their utility in a textile mill laboratory. The book constitutes an excellent addition to the textile laboratory and should be very useful to the textile chemists and students. Unfortunately, the index omits specific entries for topics in the sections on determination of finishing materials, resins, dyestuffs, and fiber content. Paper and binding are serviceable.

MILTON HARRIS

Radioactive Tracer Techniques. *Geo. K. Schweitzer* and *Ira B. Whitney*. vi + 241 pages. D. Van Nostrand Co., 250 Fourth Ave., New York 10, N. Y., 1949. Price, $3.25.

This book is a laboratory manual designed for use in college courses on the applications of radiochemistry. A manual of experiments for instructional purposes has been badly needed for a number of years, and the authors are to be congratulated on having gathered together and made available with specific directions a wide variety of experiments illustrating most of the major applications of the tracer method. However, the book as a whole falls considerably short of being satisfactory.

The first third of the book is devoted to a discussion of radioactive hazards and the design and functioning of the radiochemical laboratory. The authors have failed, however, to draw a distinction between the low-level academic tracer laboratories and high-level industrial laboratories. The result is a distorted picture. The prospective teacher of a course in tracer chemistry is given the impression that in order to perform safely the experiments described in this book, the student should be provided with a special laboratory (preferably a separate building) into which he enters through a special chamber where he changes all his clothes, that the student must do all his work in surgical gloves under constant monitoring by survey instruments, that after finishing he must bathe before returning to his street clothes, and that he must have periodic blood examinations. The strict regimen laid down by the authors is not followed by the vast majority of radiochemists because it is unnecessary at the levels of activity normally used. The requirements which the authors have set up are such as thoroughly to discourage anyone contemplating the teaching of radiochemistry in a university. Although there is much useful information in the first four chapters, it is virtually impossible to distinguish it without considerable prior knowledge of sound radiochemical laboratory practice, and the reviewer therefore suggests that the reader unfamiliar with this simply omit the first third of the book.

Most of the remainder of the book is devoted to a collection of detailed experiments on the measurement and applications of radioactive tracers. The authors have made an intelligent selection, on the whole, of the 28 experiments which illustrate the tracer principle as applied to chemistry, physics, and biology. Each experiment includes a brief theoretical discussion, a list of apparatus and materials, a detailed procedure, and questions to aid in writing the report. At the end of each section there is appended a list of "other suggested experiments," but these suggestions are usually so general as to be of little practical value. Many of the experiments are, in principle, excellent and should prove stimulating to the student. They have evidently proved satisfactory in the authors' teaching experience and do not demand unusual facilities or equipment. The directions are as a rule clear and detailed. There are occasional rather serious omissions, an example of which is the absence of any description of or specifications for the standard sample holder or mounting card, although there are frequent explicit references to the use of both. Although the experiments are in general well chosen, there are occasional lapses, as in the illustration of radiometric analysis by isotope dilution. Here the student is asked to evaporate 10- to 50-mg. samples of iron in an ether solution onto watch glasses for evaporation and weighing as a precise method of determining iron. The intelligent student will immediately realize that there are a number of ways of determining small quantities of iron with ten times the accuracy in half the time, and an experiment like this is hardly likely to convince him that the tracer method is any boon to analytical chemistry.

These deficiencies are not so serious, however, as the authors' failure to give a clear and sound explanation of the basic prin-

ciples and practical limitations of beta and gamma counting with Geiger tubes. The treatment is sketchy and some important factors are omitted completely. Instead of explaining clearly to the student the nature of self-absorption, for example, and how it is affected by beta-energy, self-scattering, back-scattering, sample density, geometry, and window thickness (all important factors), the authors mention that it exists and "must be taken into consideration." The student is given no hint of the very large errors involved in preparing samples by evaporation, and he is not likely to find out easily for himself, for the directions do not specify that the determinations should be done in duplicate.

The last chapter is an interesting and very useful compilation of the methods used in preparing the special materials used in the experiments. This, together with the experiments, can be recommended as good source material for anyone preparing a course in radiochemistry. DAVID N. HUME

Quantitative Organic Analysis via Functional Groups. *Sidney Siggia.* 152 pages. John Wiley & Sons, 440 Fourth Ave., New York 16, N. Y., 1949. Price, $3.

The present book is a welcome addition to the rather small volume of literature, especially in English, on the determination of functionality in organic compounds. The standard works on organic microanalysis, such as Niederl and Niederl and Pregl, usually contain procedures for the determination of six to ten types of functional groupings. The "Standard Methods" of the Association of Official Agricultural Chemists contains additional procedures for the determination of functional groups. For a comprehensive survey of the topic, recourse has to be had to Meyer's "Analyze und Konstitutionsermittlung organischer Verbindungen." Siggia's book, in a way, bridges the gap between Meyer and the other works. It contains procedures for twenty types of functional groups, including the following: hydroxyl, carbonyl, carboxyl (acids, amides, esters, salts), alkoxyl and alkimide, unsaturation, active hydrogen and reactivity to the Grignard reagent, acetylenic hydrogen, acetal and related types, amino, hydrazines, diazonium salts, groups reducible by titanous chloride (azo, nitro, and hydrazo), mercaptan, alkyl and dialkyl sulfides, sulfonic acids and salts, peroxides, isocyanates and isothiocyanates, vinyl ethers, and oxirane (epoxide) oxygen. Other subjects discussed are the determination of water in organic compounds, miscellaneous topics (bromination, coupling, and hydrogenation), separation techniques, and the use of physical properties for quantitative analysis.

The procedures have all apparently been tested in the author's laboratory and modified as necessary. In many cases the compounds used to test the method are listed. The procedures given cover micro-, semimicro-, and macro-scale operation; gaseous, liquid, and solid samples; and various types of measurement techniques including gravimetric, titrimetric, volumetric, and photometric.

In most cases several different or modified methods are given for the determination of each functional moiety with a statement as to the comparative merits of each. The directions are clear; especially noteworthy are the concise descriptions of reagent preparation. All special apparatus used is illustrated by excellent line drawings. If the method of calculating the experimental data is not obvious, the correct method is clearly described.

There are several statements with which the reviewer is not entirely inclined to agree, but this is a minor item compared to the value which the reviewer believes this book will be to all engaged in work with organic compounds. The reviewer's only regrets are that the book was not available six years ago when he was working in the aliphatic organic chemical industry, and that the book does not include more procedures. In particular, the reviewer would have liked more procedures involving spectrophotometric and polarographic techniques which have definite advantage in handling complex mixtures of organic compounds. However, the book should be judged on the basis of what it is, rather than of what the reviewer would want it to be.

The volume will be a welcome and valuable addition to the working library of the analytical chemist; it will be of service to all engaged in working with organic compounds.

PHILIP J. ELVING

The Analyst's Calendar

Third Summer Symposium

The Third Annual Summer Symposium cosponsored by the Division of Analytical and Micro Chemistry and ANALYTICAL CHEMISTRY is to be held on the campus of the Ohio State University, Columbus, Ohio, June 16 and 17, 1950. The topic of the symposium will be analytical separations.

General chairman of the symposium is H. A. Laitinen, University of Illinois; chairman of the Committee on Local Arrangements is William M. MacNevin of Ohio State University.

International Congress on Analytical Chemistry

A representative gathering of chemists and others interested in modern developments in analytical chemistry met recently in England under the chairmanship of Sir Robert Robinson to consider the desirability of holding an International Congress on Analytical Chemistry in Great Britain. A general committee and an executive committee have been formed to proceed with the organization of such a congress to be held during the summer of 1952.

A grant toward preliminary expenses has been made by the Society of Public Analysts and Other Analytical Chemists, and the organizers hope to have the support of the many other bodies to whom the development of analytical chemistry is vital.

The honorary secretary of the congress is R. C. Chirnside, Research Laboratories, General Electric Co., Ltd., Wembley, England.

Analytical Group, North Jersey Section

The first meeting of the Analytical Group, North Jersey Section, for the 1950 season is to be held October 4 in the Public Service Auditorium, Newark, N. J., at 8 P.M. G. Frederick Smith, University of Illinois, will speak on "Perchloric and Periodic Acids and the Determination of Organic Compounds."

American Society for Testing Materials. Fairmont Hotel, San Francisco, Calif., October 10 to 14

Optical Society of America. Hotel Statler, Buffalo, N. Y., October 27 to 29

Conference on X-Ray and Electron Diffraction. Mellon Institute, Pittsburgh, Pa., November 7 and 8

American Council of Commercial Laboratories. Miami, Fla., December 5 to 7

Third Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 30 to February 2, 1950

Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. William Penn Hotel, Pittsburgh, Pa., February 15 to 17, 1950

Third Annual Summer Symposium. Ohio State University, Columbus, Ohio, June 16 to 17, 1950

AIDS FOR THE ANALYST . . . . .

A Laboratory Flowmeter. George S. Haines, Westvaco Chemical Division, Food Machinery and Chemical Corporation, South Charleston, W. Va.

THE laboratory flowmeter shown in Figure 1, featuring interchangeable precision-bore capillaries and a check valve, has proved very useful and convenient in this laboratory. This flowmeter and its capillaries are rugged and readily constructed. The interchangeable capillaries are attached to the manometer by means of 12/5 spherical joints, using A. H. Thomas spherical joint clamps. The slight flexibility of the semicircularly shaped capillaries and of the manometer permits satisfactory fitting of the two in spite of small dimensional variations. The funnellike shape of the outer spherical joints on the manometer makes for ease of cleaning and filling. For installation in a rigid system the side arms may be equipped with spherical joints for connection to corresponding metal or glass joints in the system. The manometer is easily removed or inserted if the added joints have their axes vertical.

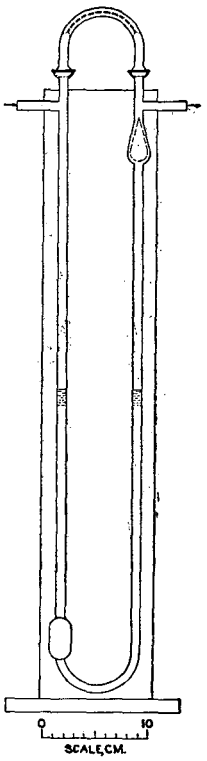

Figure 1. Fluorometer

CAPILLARIES

The use of precision-bore capillary tubing permits the construction of capillaries closely conforming to the desired range of flow rates, and eliminates the trial and error method of obtaining capillaries with the desired capacity.

The capillaries whose calibrations are shown in Figure 2 were constructed from varying lengths of precision-bore capillary (Fischer and Porter Company). They were calibrated by aspirating air through the flowmeter at atmospheric pressure and measuring the time and the volume of water siphoned from the aspirator. The limited data shown in Figure 2 indicate that at 200-mm. water displacement (400-mm. water pressure) the corresponding flow rates vary less than 2% per 1-mm. length of capillary. Thus, a calibrated capillary can be closely duplicated by using tubing of the same length and diameter in the capillary. For accurate use, however, each capillary should be calibrated individually. The data for 74 mm. of capillary tubing 0.74 mm. in inside diameter agree very well with Croxton's data for 50 mm. of capillary 0.676 mm. in inside diameter [IND. ENG. CHEM., ANAL. ED., 14, 69 (1942)].

One of the chief objections to the use of liquid-filled flowmeters, particularly in conjunction with combustion furnaces, is the tendency to exceed the capacity of the flowmeter accidentally and displace liquid into the system being used. This is prevented in the flowmeter described by the check valve in the down-stream side of the manometer.

CHECK VALVE

In constructing the check valve, the valve seat and float are formed to fit each other approximately, with fairly thick walls. The float, of such volume as to permit it to float on the liquid used in the manometer, is formed on the end of a tube. It is then ground into the valve seat using the tube as a handle and Carborundum No. 200, 400, and 600 powders, successively, as abrasives. After the float is ground to a good fit with the valve seat, the float tube is sealed off, and the valve assembly is sealed into the top of the manometer. A bulb of approximately the same volume as the valve assembly is inserted near the bottom of the opposite side of the manometer, to ensure sufficient liquid to buoy the glass float.

The check valve adequately prevents accidental displacement of the manometer liquid during adjustment of the flow rate and permits use of the upper range of the flowmeter without the danger that a gradual increase in flow may introduce liquid into the adjacent system. It also permits sweeping a system with gas at a rate greatly in excess of the capacity of the flowmeter. This feature has proved valuable in metering mixtures of air and chlorine through a photoelectric colorimeter.

ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of L. A. Bedwell in performing the necessary glass working and B. R. Thacker (deceased) in determining the flowmeter calibrations.

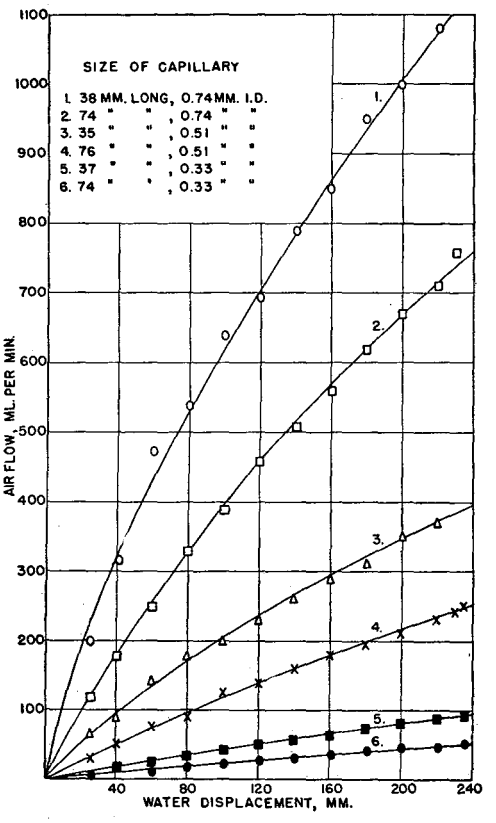

Figure 2. Calibration of Precision-Bore Capillaries