ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editorial Director

Your Journal in 1955

WITH this issue we conclude still another milestone (the twenty-seventh) in the history of ANALYTICAL CHEMISTRY. The year 1955 has been an eventful one, with many new services and innovations added—all for the purpose of serving you, the reader, more effectively.

More than 2100 pages have been required this year to publish the accepted papers dealing with many varied facets of the broad field of analysis, and to provide additional services of practical value to the subscriber. This figure is in marked contrast with 238—the total number contained in Volume I (1929). Surely the field has grown tremendously.

There is no point in enumerating the many additional services that have been introduced in the journal down through the years. While much has been accomplished, we hope that we never become complacent. There are opportunities for further improvement every year.

In glancing at Volume I we were very much interested in the first article—"Quantitative Analysis with the Spectrograph," by Charles C. Nitchie of the New Jersey Zinc Co. We quote all of paragraph one and part of paragraph two:

> In order to satisfy the requirements of modern industrial operations, a laboratory must be in a position to turn out analytical results in the shortest possible time, consistent with reasonable accuracy. Too often the result of a perfectly good analysis is of value only as an explanation of defects and difficulties, rather than as a guide in the production or selection of the material involved. This is particularly true with analyses for those minute amounts of impurities, or of necessary constituents, which often modify to a remarkable extent the chemical or physical properties of materials.
>
> This being the case, it is surprising that so little attention has been paid to the spectrograph as a means for carrying out quantitative analyses. It has long been recognized as an instrument for qualitative work, but even in this field its usefulness has not met with the general recognition which it deserves, particularly in industrial laboratories. . . .

Could any words of ours more dramatically demonstrate the progress that has been made in analysis and quality control since 1929? We think not. However, we cannot forego the opportunity of saying what we have said previously on many occasions, that modern continuous manufacturing processes, so characteristic of the chemical process industries, would not be possible if these tremendous strides in instrumentation had not been made.

Are You Going to Give a Paper?

ANALYSTS will have no dearth of meetings in 1956 where they can present papers. Indeed, one addition to the usual list was commented on briefly several months ago—the XVth International Congress of Pure and Applied Chemistry, to be held in Lisbon, Portugal, September 9 to 16. This congress will be devoted exclusively to the field of analytical chemistry.

Papers by U. S. authors should be submitted by December 31, 1955, to H. A. Laitinen, Department of Chemistry, University of Illinois, Urbana, Ill., who is chairman of the National Research Council Committee to Receive and Review Papers. Each manuscript should be accompanied by a 200- to 300-word abstract.

We hope the U. S. will be well represented at Lisbon. It will at least be 1959 before another analytical congress can be held at the same time as a general IUPAC meeting.

We also would like to report that the February issue of ANALYTICAL CHEMISTRY (out late in January) will be an "exposition" issue. It will contain complete details of the Seventh Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy to be held in the William Penn Hotel, February 27 to March 2.

An exposition will be held again in conjunction with the conference. The opportunity to view new developments made in instruments during the past year and to establish personal contacts with a large number of manufacturers and distributors of laboratory supplies of all kinds, certainly is a very compelling reason for attending the Pittsburgh conference.

Each year the conference has established a new attendance record. We anticipate another new high in 1956. The ACS Pittsburgh Section analysts and the members of the Spectroscopy Society of Pittsburgh are to be congratulated on the continued success of a venture that started only a few short years ago as a modest one-day meeting.

X-Ray Diffraction Patterns of Phenols

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X-ray diffraction analysis is proposed for the positive identification of crystalline phenols. If the phenols have a reasonably high melting point, conversion to the phenyl isocyanate derivative for the purpose of making a diffraction pattern is unnecessary. It is also unnecessary to recrystallize the specimen until the literature melting point is attained. The diffraction patterns of isomers and closely related compounds are distinctly different.

X-RAY diffraction analysis is proposed for the positive identification of crystalline phenols. X-ray powder-diffraction patterns of 51 solid phenols have been made, using characteristic filtered FeK$_\alpha$ radiation. Many phenols are susceptible to oxidation on contact with air; but such oxidation, although it lowers the melting point, does not appreciably modify the diffraction pattern.

In the course of x-ray diffraction studies of coal, coal-related materials, and coal-hydrogenation products, it was desirable to establish the diffraction patterns of possible constituents such as aromatic hydrocarbons (*10*), hydrocarbon derivatives (*9*), and phenols. Such compilations are of interest in the coal tar, dye, plastics, fuel, pharmaceutical, and other industries.

A preceding study by McKinley, Nickels, and Sidhu (*14*) was confined to the phenyl thiocyanate derivatives of simple liquid phenols. The present study is confined to phenols of high enough melting point to permit the preparation of extruded specimens.

PROCEDURE

The specimens were very finely ground in an agate mor-

Table I. Melting Points and Three Most Intense Diffraction Lines of Solid Phenols[a]

Pattern No.	Three Strongest Lines			Melting Point, ° C.		Compound
	1st	2nd	3rd	Found	Literature	
24	12.35	4.69	5.3	49.5	51.5 (*8*)	3-Methyl-6-isopropylphenol (thymol)
4	12.3	3.52	3.23	127.5–131.0	132.5–133.5 (*8*)	Pyrogallol
47	10.7	5.9	4.76 4.34	77.8– 78.2	77.8– 78.6 (*18*)	2-Isobornylphenol
36	6.9	3.86	3.41	153.0–154.0	148.0–149.0 (*8*)	9-Phenanthrol
49	6.6	11.5	4.29	82.4– 83.4	81.6– 81.9 (*13*)	2,4-Dimethyl-6-isobornylphenol
38	6.4	15.1	5.6 4.84	79.5– 80.5	85 (*20*)	4-Octylphenol
[b]	6.30	4.34	5.20	.....	43.5 (*14*)	2,6-Di-*tert*-butyl-4-ethyl phenol
21	6.2	10.7	5.3	69.0– 69.8	.....	3-Methyl-2-isopropylphenol
20	6.2 4.53	4.05	3.375	56.8– 57.8	61.5– 62.5 (*8*)	5,6,7,8-Tetrahydro-2-hydroxynaphthalene
42	6.1	4.29	8.65	69.4– 70.6	70 (*20*)	2,6-Di-*tert*-butyl-4-methylphenol
[b]	5.85	3.28	5.31	.....	.....	*p*-Nitrobenzene azoresorcinol
15	5.8	3.795	6.5	92.2– 93.8	95 – 96 (*8*)	2,3,5-Trimethylphenol
6	5.6	3.675	4.50 3.305	73.2– 74.6	75 (*8*)	2,5-Dimethylphenol
37	5.5	4.46	4.93	67.8– 73.2	70 (*8*)	4,α-Cumylphenol
40	5.5	3.88	4.48	72.0– 72.5	73 (*21*)	4-(α,α-Dimethylbenzyl)phenol
25	5.4	11.5	3.91	53.5– 54.5	.....	2-(α-Ethylpropyl)phenol
18	5.4	5.1	11.9	99.5–100.0	99 (*8*)	4-*tert*-Butylphenol
44	5.4	4.48	7.25	50.0– 51.8	49.5 (*16*)	2-Methyl-4-diisobutylphenol
13	5.4	4.26	6.4	57.5– 59.5	61 (*8*)	4-Isopropylphenol
12	5.4	3.52	3.87	52.4– 53.8	55 (*8*)	5-Indanol
23	5.3	10.7	4.12	49.0– 49.8	54 (*8*)	3-Methyl-5-isopropylphenol
33	5.2	4.68	4.39 4.20	54.0– 55.0	.....	4-(α-Methylbenzyl)phenol
31	5.2	4.18	3.27	121.4–122.6	.....	1-Hydroxyfluorene
19	5.2	3.92	3.68	66.2– 68.4	67.8– 69.0 (*15*)	1,2,3,4-Tetrahydro-5-hydroxynaphthalene
26	5.2	3.91	6.0	94.0– 94.3	92.0– 93.0 (*8*)	4-*tert*-Amylphenol
39	5.0	6.3	11.7 10.6	56.0– 56.8	55.0– 56.0 (*7*)	2,4-Di-*tert*-butylphenol
8	5.0	4.57	3.75	63.0– 65.0	62.5 (*8*)	3,4-Dimethylphenol
30	5.0	4.33	3.87	130.0–131.6	130 (*17*)	4-Cyclohexylphenol
50	4.97	4.50	8.0	124.0–126.5	131.0–131.2 (*18*)	2,4,6-Tri-*tert*-butylphenol
29	4.88	3.66	7.9	46.8– 52.8	55.0– 56.0 (*12*)	2-Cyclohexylphenol
35	4.88	3.37	3.98	169.5–170.5	168 (*8*)	2-Phenanthrol
3	4.84	4.605 4.36	3.00	109.8–110.8	110 (*8*)	Resorcinol
[b]	4.78	7.50	4.36	.....	78 (*14*)	2,6-Di-*tert*-butyl-4-chlorophenol
43	4.74	5.8	8.2	79.5– 80.5	62.1 (*20*)	4,6-Di-*tert*-butyl-3-methylphenol
[b]	4.69	10.0	4.2	.....	170 (*6*)	*o*-Aminophenol
10	4.655	5.9	3.88	147.2–149.0	148.0–149.0 (*8*)	Dimethyldihydroresorcinol
[b]	4.59	5.10	4.18	.....	115.5 (*14*)	2,6-Di-*tert*-butyl-4-cyclohexylphenol
32	4.57	4.72	3.38	170.6–171.0	171 (*8*)	2-Hydroxyfluorene
17	4.54	7.9	4.07	122.6–123.8	123 (*8*)	2-Naphthol
28	4.53	4.20	3.86	163.0–165.0	163.0–165.0 (*8*)	*p*-Phenylphenol
45	4.50	6.1	8.0	48.5– 50.8	51.6– 52.6 (*18*)	2-Methyl-4,6-di-*tert*-butylphenol
[b]	4.50	4.10	7.45	.....	261 (*6*)	Phenolphthalein
[b]	4.46	3.66	5.25	.....	183 (*6*)	*p*-Benzylidene aminophenol
[b]	4.40	4.75	4.23	.....	68 – 70 (*6*)	1-*n*-Hexyl-2,4-dihydroxybenzene
27	4.39	5.2	3.58	55.6– 57.6	56 (*8*)	*o*-Phenylphenol
51	4.14	3.78	4.55	48.2– 49.6	50.5– 51.0 (*8*)	3-Pentadecylphenol
2	4.14	3.22	9.8	172.8–174.4	170.0–173.0 (*8*)	Hydroquinone
34	4.05	6.6 5.7	3.33	155	156 (*8*)	1-Phenanthrol
9	4.05	3.52	6.1 4.68	59.2– 62.2	68 (64) (*8*)	3,5-Dimethylphenol
22	3.99	6.6	5.5	106.5–111.5		3-Methyl-4-isopropylphenol
7	3.98	3.65 3.37	6.1	44.8– 45.8	49 (*8*)	2,6-Dimethylphenol
[b]	3.85	5.47	3.73	.....	121.8 (*6*)	2,4,6-Trinitrophenol
41	3.82	5.3 5.1	4.64	116.0–117.0	.....	4-(α-Methylbenzyl)-3-methylphenol
[b]	3.81	1.98	3.13	.....	191 (*6*)	Pentachlorophenol
14	3.795	5.1	4.74	50.6– 51.8	50.6– 51.8 (*22*)	3-Methyl-5-ethylphenol
46	3.67	6.6 3.83	5.9	208.0–210.0	206 –207 (*19*)	1-Pyrenol
16	3.63	5.85	3.20	94.8– 96.2	94 (*8*)	1-Naphthol
11	3.60	5.6	4.80 3.91	47.0– 48.0	47.0– 51.0 (*8*)	4-Indanol
48	3.55	5.8	10.05	248.0–250.0	248.0–250.0 (*4*)	2-Hydroxychrysene
1	3.43	4.72	8.8	104.2–104.8	105 (*8*)	Catechol
[b]	3.39	3.82	6.70	.....	109 (*14*)	2,6-Diacetyl-3,5-dimethylpheno
[b]	3.38	3.10	7.73	.....	110 (*6*)	1-Nitroso-2-naphthol
[b]	3.35	3.59	2.74	.....	144 (*6*)	2,6-Dibromo-4-nitrophenol
[b]	3.31	3.93	2.63	.....	68 (*6*)	2,4,6-Trichlorophenol
[b]	3.29	4.35	4.68	.....	147 –148 (*6*)	4,6-Dinitroresorcinol
[b]	3.27	4.00	2.83	.....	(anhyd.) 219 (*6*)	Phloroglucinol dihydrate
[b]	3.25	3.51	4.20	.....	114 (*6*)	2,4-Dinitrophenol
[b]	3.25	5.90	7.50	.....	45 (*6*)	*o*-Nitrophenol
[b]	3.22	5.10	7.20	.....	43 (*6*)	*o*-Iodophenol
5	3.20	6.2	4.61 3.57	56.6– 57.4	58 (*6*)	Orcinol
[b]	3.15	3.59	3.47	.....	.....	2,4,5-Trichlorophenol

[a] Arranged in order of first lines. Pattern numbers from Table II.

[b] Phenol pattern from reference (*1*), included for comparison and completeness.

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tar. They were then partly extruded from a section of 19-gage stainless steel tube with an inside diameter of 0.7 mm. (*2*). The specimens were prepared just before use. No binder of any sort was employed. Details of sample preparation have been described (*11*). Binders are particularly troublesome in organic samples because their contribution to the diffraction pattern is comparable to and interferes with that of the specimen proper.

Each specimen was exposed in a Debye-Scherrer camera 114.6 mm. in diameter for 6 hours to obtain the long spacings up to 19 A. in the small-angle region; it was overexposed in a Debye-Scherrer camera 57.3 mm. in diameter for 2 hours to bring out the shorter spacings in the large-angle region. Iron K_α radiation (λ = 1.937 A.) was obtained from a commercial sealed-off x-ray tube equipped with an iron anode, beryllium windows, and manganese dioxide filters.

The intensities were estimated by means of a calibrated strip similar to that of Hanawalt, Rinn, and Frevel (*5*). The strip was prepared with iron K_α radiation and exposures (using a decimal stop watch) of 0.025, 0.050, 0.100, 0.200, 0.400, 0.800, 1.600, and 3.200 minutes on Eastman No Screen film of the same emulsion number used in photographing the patterns. The strip was developed with the same developer and under the same conditions of time and temperature as was used for developing the patterns.

DISCUSSION

Table I lists the phenols studied in the order of the interplanar spacing of the most intense reflection along with the second and third most intense reflections, with the melting points, and with a code number to the complete diffraction pattern in Table II.

Seven of the 51 phenols whose diffraction patterns are presented have been previously analyzed, and the corresponding data have been published (*1*). These phenols are catechol, 2,6-di-*tert*-butyl-4-methylphenol, 1-naphthol, 2-naphthol, *o*-phenylphenol,

Table II. Powder-Diffraction Data[a]

d/n	*I/I*$_1$	*d/n*	*I/I*$_1$	*d/n*	*I/I*$_1$	*d/n*	*I/I*$_1$	*d/n*	*I/I*$_1$	*d/n*	*I/I*$_1$	*d/n*	*I/I*$_1$	*d/n*	*I/I*$_1$
1. Catechol		**2. Hydroquinone**		**3. Resorcinol**		**4. Pyrogallol**		**5. Orcinol**		**6. 2,5-Dimethylphenol**		**7. 2,6-Dimethylphenol**		**8. 3,4-Dimethylphenol**	
8.8	(3) 0.75	16.9	0.50	5.4	0.25	12.3	(1) 1.00	2.86	0.30	2.52	0.30	2.85	0.40	3.03	0.08
5.4	0.60	9.8	(3) 0.75	4.84	(1) 1.00	8.7	0.30	2.66	0.25	2.40	0.30	2.70	0.10	2.92	0.08
4.72	(2) 0.90	7.4	0.20	4.605	(2) 0.98	6.8	0.10	2.56	0.25	2.21	0.25	2.57	0.20	2.745	0.15[b]
4.46	0.30	6.5	0.10	4.36	(2) 0.98	6.1	0.60	2.49	0.25	2.15	0.50	2.42	0.20	2.65	0.10
3.85	0.50	5.6	0.60	3.82	0.20	5.5	0.50	2.34	0.05	2.07	0.06	2.17	0.08	2.54	0.10[b]
3.72	0.50	5.4	0.60	3.52	0.85	4.08	0.20	2.28	0.20	2.03	0.06	2.10	0.20	2.46	0.08
3.43	(1) 1.00	4.84	0.60	3.44	0.85	3.91	0.20	2.21	0.30	2.00	0.06	1.92	0.05	2.395	0.08
2.99	0.30	4.39	0.70[b]	3.305	0.20	3.67	0.30	2.04	0.20	1.91	0.03	1.82	0.25	2.275	0.08[b]
2.91	0.20	4.14	(1) 1.00	3.00	(3) 0.95	3.52	(2) 0.98	1.99	0.15	1.88	0.12	1.78	0.05	2.23	0.08
2.72	0.20	3.92	0.65	2.83	0.30	3.35	0.20	1.92	0.15	1.83	0.12	1.72	0.05	2.18	0.15
2.65	0.50	3.78	0.60	2.78	0.10	3.23	(3) 0.75	1.83	0.12	1.76	0.25			2.12	0.10
2.55	0.04	3.58	0.50	2.68	0.20	3.01	0.50	1.76	0.12	1.65	0.04			2.08	0.08
2.44	0.20	3.42	0.30	2.625	0.20	2.85	0.40	1.67	0.12	1.61	0.04			2.02	0.08
2.39	0.15	3.22	(2) 0.98	2.525	0.25	2.72	0.15	1.63	0.15	1.19	0.02			1.97	0.05
2.31	0.20	3.10	0.30	2.45	0.25	2.59	0.15	1.60	0.05	1.15	0.01			1.95	0.05
2.23	0.04	2.92	0.40	2.41	0.20	2.45	0.20	1.57	0.05	1.12	0.01			1.88	0.05
2.15	0.05	2.75	0.40	2.35	0.20	2.39	0.20	1.55	0.05	1.07	0.01			1.835	0.05
2.07	0.20	2.56	0.20	2.30	0.20	2.28	0.20	1.53	0.05					1.76	0.05
2.03	0.10	2.41	0.30	2.17	0.25	2.22	0.15	1.50	0.12					1.74	0.05
1.99	0.05	2.34	0.10	2.14	0.25	2.19	0.15	1.47	0.05					1.19	0.05
1.94	0.05	2.26	0.10	2.07	0.20	2.15	0.15	1.43	0.06						
1.90	0.05	2.19	0.30	1.96	0.07	1.90	0.08	1.41	0.06						
1.85	0.05	2.10	0.20	1.925	0.15	1.835	0.10	1.34	0.12						
1.80	0.30	2.05	0.05	1.86	0.15	1.79	0.10	1.30	0.04						
1.72	0.15	2.00	0.05	1.81	0.25	1.745	0.10	1.25	0.04						
1.69	0.15	1.91	0.15	1.725	0.20	1.64	0.08	1.23	0.03						
1.66	0.15	1.82	0.30	1.67	0.20	1.60	0.04	1.22	0.03						
1.64	0.15	1.74	0.10	1.62	0.07	1.56	0.04	1.19	0.12						
1.58	0.15	1.68	0.10	1.51	0.07	1.50	0.04	1.16	0.12						
1.48	0.05	1.63	0.10	1.36	0.07	1.44	0.03	1.14	0.05						
1.41	0.05	1.60	0.15	1.28	0.07	1.35	0.03	1.13	0.05						
1.34	0.04	1.55	0.10	1.26	0.04	1.32	0.05	1.10	0.08						
1.32	0.04	1.37	0.03	1.235	0.04	1.16	0.03	1.09	0.06						
1.24	0.04	1.295	0.03	1.21	0.04	1.14	0.03	1.08	0.08						
1.21	0.05	1.23	0.03	1.18	0.04	1.04	0.04	1.02	0.03						
1.17	0.05	1.21	0.03	1.16	0.04										
1.16	0.05	1.16	0.03	1.13	0.04										
1.12	0.05	1.08	0.03	1.12	0.03										
1.10	0.04	1.06	0.03	1.11	0.03										
1.095	0.04	1.04	0.02	1.09	0.03										
1.01	0.04	1.03	0.02	1.08	0.03										
		1.01	0.02	1.074	0.03										
				1.07	0.03										
				1.05	0.03										
				1.045	0.03										
				1.04	0.03										
				0.986	0.03										
				0.983	0.03										

5. Orcinol		**6. 2,5-Dimethylphenol**		**7. 2,6-Dimethylphenol**		**8. 3,4-Dimethylphenol**	
9.9	0.85	11.6	0.50	10.2	0.30	10.0	0.15
6.2	(2) 0.98	6.1	0.12	7.9	0.65	8.0	0.20
5.4	0.10	5.6	(1) 1.00	6.1	(3) 0.70	6.5	0.30
5.0	0.40	4.95	0.50	5.1	0.65	5.9	0.40
4.61	(3) 0.90	4.50	(3) 0.75	4.76	0.05	5.6	0.15
4.21	0.30	4.06	0.12	4.33	0.50	5.0	(1) 1.00
3.95	0.85	3.675	(2) 0.95[b]	4.12	0.50	4.57	(2) 0.60
3.72	0.30	3.48	0.12	3.98	(1) 1.00	4.20	0.30
3.57	(3) 0.90	3.305	(3) 0.75	3.65	(2) 0.90	3.75	(3) 0.45
3.39	0.40	3.02	0.40	3.37	(2) 0.90	3.35	0.40[b]
3.20	(1) 1.00	2.93	0.50	3.31	0.40	3.31	0.30
2.975	0.10	2.83	0.20	3.10	0.20	3.13	0.15

9. 3,5-Dimethylphenol		**10. Dimethyldihydroresorcinol**		**11. 4-Indanol**		**12. 5-Indanol**	
12.3	0.20	9.0	0.50	12.1	0.30	18.0	0.10
9.3	0.85	5.9	(2) 0.80	9.9	0.40	8.9	0.10
7.1	0.60	5.5	0.40	8.3	0.30	6.7	0.50
6.1	(3) 0.90	4.655	(1) 1.00	7.2	0.30	6.1	0.30
5.5	0.90	4.51	0.30	6.5	0.50	5.8	0.30
5.0	0.80	4.03	0.50	5.6	(2) 0.60	5.4	(1) 1.00
4.68	(3) 0.90	3.88	(3) 0.75	4.80	(3) 0.50	4.99	0.40
4.39	0.20	3.76	0.15	4.51	0.40	4.68	0.20
4.05	(1) 1.00	3.67	0.15	4.12	0.20	4.43	0.50
3.88	0.50	3.53	0.15	3.91	(3) 0.50	3.87	(3) 0.70
3.72	0.10	3.34	0.20	3.76	0.45	3.52	(2) 0.75
3.52	(2) 0.95	3.20	0.10	3.60	(1) 1.00	3.315	0.30
3.38	0.80	3.01	0.35	3.51	0.30	3.20	0.15
3.26	0.25	2.92	0.10	3.34	0.20	3.095	0.08
3.15	0.25	2.83	0.30	3.12	0.25	2.98	0.08
3.02	0.60	2.74	0.25	2.99	0.08	2.83	0.15
2.945	0.05	2.58	0.05	2.93	0.08	2.715	0.20
2.87	0.10	2.47	0.25	2.82	0.35	2.64	0.04
2.805	0.10	2.315	0.25	2.70	0.08	2.51	0.15
2.73	0.05	2.16	0.15	2.54	0.15	2.44	0.10
2.65	0.10	2.09	0.10	2.41	0.08	2.39	0.08
2.60	0.25	2.00	0.10	2.24	0.08	2.30	0.06
2.54	0.10	1.965	0.05	2.17	0.08	2.23	0.05
2.43	0.30	1.84	0.10	2.08	0.05	2.13	0.04

[a] Patterns arranged in order of empirical formula (*Chem. Abs.*). *d/n* = interplanar spacing in Angstroms; *I/I*$_1$ = estimated relative intensity; (1) strongest line, (2) second strongest line, (3) third strongest line.

[b] Broad line probably due to partial coincidence of two or more lines.

Table II. Powder-Diffraction Data[a] (*Continued*)

d/n	I/I_1	*d/n*	I/I_1	*d/n*	I/I_2	*d/n*	I/I_1	*d/n*	I/I_1	*d/n*	I/I_1	*d/n*	I/I_1	*d/n*	I/I_1
9. 3,5-Dimethylphenol		**10. Dimethyldihydroresorcinol**		**11. 4-Indanol**		**12. 5-Indanol**		**17. 2-Naphthol**		**18. 4-*tert*-Butylphenol**		**19. 1,2,3,4-Tetrahydro-5-hydroxynaphthalene**		**20. 5,6,7,8-Tetrahydro-2-hydroxynaphthalene**	
2.33	0.05	1.76	0.03	2.04	0.05	2.08	0.08			1.95	0.05			1.82	0.08
2.26	0.10	1.45	0.03	1.90	0.20	2.05	0.06			1.88	0.05			1.75	0.12
2.21	0.25	1.21	0.03	1.81	0.05	2.01	0.08			1.17	0.03			1.67	0.04
2.115	0.25			1.725	0.10	1.97	0.05							1.64	0.04
2.02	0.25			1.65	0.03	1.92	0.08							1.53	0.04
1.94	0.08					1.86	0.06							1.29	0.03
1.885	0.08					1.83	0.05							1.27	0.03
1.82	0.08					1.79	0.20							1.13	0.02
1.79	0.05					1.75	0.05							1.12	0.02
1.75	0.05					1.53	0.05								
1.73	0.05														
1.69	0.04														
1.56	0.04														
1.20	0.02														

13. 4-Isopropylphenol		**14. 3-Methyl-5-ethylphenol**		**15. 2,3,5-Trimethylphenol**		**16. 1-Naphthol**		**21. 3-Methyl-2-isopropylphenol**		**22. 3-Methyl-4-isopropylphenol**		**23. 3-Methyl-5-isopropylphenol**		**24. 3-Methyl-6-isopropylphenol**	
10.1	0.30	11.6	0.70	10.2	0.50	11.75	0.40	10.7	(2) 0.90	10.05	0.45	16.7	0.60	12.35	(1) 1.00
7.0	0.30	8.3	0.60	7.7	0.50	6.6	0.30	7.4	0.30	7.2	0.40	14.5	0.08	8.5	0.30
6.4	(3) 0.50	7.5	0.70	6.5	(3) 0.75	5.85	(2) 0.90	6.7	0.60	6.6	(2) 0.98	12.1	0.10	7.3	0.40
5.4	(1) 1.00	7.0	0.70	5.8	(1) 1.00	5.6	0.35	6.2	(1) 1.00	5.5	(3) 0.90	10.7	(2) 0.98	6.15	0.30
4.95	0.10	5.9	0.20	5.1	0.50	4.82	0.08	5.7	0.50	4.38	0.90	8.3	0.30	5.8	0.10
4.26	(2) 0.55	5.6	0.30	4.76	0.50	4.38	0.45	5.3	(3) 0.90	3.99	(1) 1.00	7.1	0.60	5.5	0.20
3.91	0.50	5.1	(2) 0.80	4.38	0.50	4.04	0.18	4.72	0.50	3.76	0.60	6.5	0.30	5.3	(3) 0.70
3.83	0.50	4.74	(3) 0.75	4.18	0.40	3.86	0.15	4.38	0.50	3.57	0.20	5.9	0.30	4.69	(2) 0.90
3.58	0.45	4.245	0.60	3.95	0.25	3.63	(1) 1.00	3.96	0.70	3.31	0.60	5.3	(1) 1.00	4.44	0.50
3.23	0.15	3.99	0.60	3.795	(2) 0.90	3.43	0.06	3.78	0.70	3.14	0.30	5.1	0.15	4.25	0.50
3.04	0.30	3.795	(1) 1.00	3.57	0.50	3.20	(3) 0.60	3.59	0.30	2.98	0.30	4.88	0.40	4.06	0.08
2.93	0.15	3.55	0.30	3.42	0.70	2.945	0.40	3.37	0.75	2.84	0.10	4.53	0.10	3.84	0.08
2.75	0.10	3.41	0.20	3.30	0.50	2.70	0.40	3.23	0.30	2.78	0.10	4.31	0.40	3.67	0.30
2.67	0.08	3.29	0.10	3.14	0.15	2.395	0.04	3.09	0.10	2.72	0.10	4.12	(3) 0.90	3.49	0.60
2.61	0.15	3.16	0.30	3.00	0.20	2.33	0.04	3.01	0.10	2.65	0.25	3.81	0.50[b]	3.32	0.30
2.50	0.20	2.89	0.30[b]	2.82	0.30	2.25	0.06	2.87	0.10	2.58	0.35	3.57	0.50[b]	3.14	0.20
2.42	0.15	2.65	0.10	2.715	0.05	2.18	0.10	2.74	0.10	2.47	0.30	3.43	0.10	2.995	0.20
2.35	0.08	2.53	0.20	2.56	0.20	2.09	0.06	2.59	0.10	2.34	0.20	3.325	0.40	2.91	0.10
2.31	0.12	2.44	0.05	2.47	0.15	2.02	0.15	2.52	0.12	2.28	0.20	3.22	0.40	2.78	0.15
2.08	0.12	2.35	0.05	2.38	0.25	1.925	0.25	2.29	0.08	2.17	0.20	3.05	0.40	2.60	0.10
2.04	0.08	2.30	0.10	2.29	0.15	1.81	0.15	2.09	0.08	2.12	0.10	2.87	0.40	2.52	0.12
1.99	0.08	2.18	0.05	2.23	0.15	1.75	0.20	1.90	0.08	2.08	0.05	2.785	0.08	2.39	0.05
1.95	0.12	2.08	0.05[b]	2.17	0.12	1.73	0.03	1.845	0.08	1.99	0.15[b]	2.65	0.15	2.33	0.05
1.88	0.10	2.04	0.05	2.13	0.25	1.65	0.03			1.91	0.08	2.60	0.15	2.27	0.12
1.79	0.10	1.99	0.05	2.00	0.05	1.61	0.03			1.88	0.15	2.50	0.10	2.23	0.15
1.73	0.05	1.88	0.08	1.92	0.05	1.59	0.03			1.83	0.15	2.46	0.08	1.97	0.08
1.66	0.05	1.825	0.08	1.84	0.15	1.55	0.02			1.785	0.08	2.37	0.10	1.93	0.08
		1.68	0.04	1.74	0.08	1.26	0.08			1.74	0.08	2.31	0.05	1.79	0.05
				1.68	0.04	1.21	0.03			1.72	0.08	2.27	0.05	1.73	0.05
				1.58	0.04	1.17	0.02			1.645	0.15	2.24	0.08		
				1.24	0.02	1.14	0.02			1.57	0.05	2.205	0.05		
				1.06	0.02	1.08	0.02			1.49	0.05	2.16	0.05		
						1.02	0.02			1.42	0.04	2.13	0.08		
										1.40	0.04	2.03	0.12		
										1.35	0.04	1.99	0.12		
										1.26	0.04	1.94	0.10		
										1.17	0.06	1.79	0.08		
										1.15	0.04	1.71	0.05		
										1.13	0.04	1.62	0.05		
										1.11	0.03	1.58	0.04		
										1.06	0.03	1.55	0.04		
										1.05	0.03	1.52	0.04		
										0.997	0.03	1.33	0.03		
												1.14	0.03		

17. 2-Naphthol		**18. 4-*tert*-Butylphenol**		**19. 1,2,3,4-Tetrahydro-5-hydroxynaphthalene**		**20. 5,6,7,8-Tetrahydro-2-hydroxynaphthalene**		**25. 2-(α-Ethylpropyl)phenol**		**26. 4-*tert*-Amylphenol**		**27. *o*-Phenylphenol**		**28. *p*-Phenylphenol**	
15.6	0.06	18.6	0.20	12.1	0.60	16.7	0.50	11.5	(2) 0.70	11.75	0.50	9.5	0.08	20.4	0.30
7.9	(2) 0.30	11.9	(3) 0.60	8.3	0.30	9.2	0.60	7.3	0.50	10.5	0.20	7.9	0.10	10.05	0.30
5.9	0.04	9.4	0.25	6.8	0.15	8.5	0.10	6.2	0.10	6.9	0.50	6.6	0.12	7.0	0.08
5.3	0.04	7.9	0.20	6.0	0.80	6.7	0.50	5.7	0.40	6.0	(3) 0.75	6.0	0.50	6.0	0.08
5.0	0.10	6.9	0.30	5.7	0.25	6.2	(1) 1.00	5.4	(1) 1.00	5.5	0.60	5.7	0.50	4.97	0.25
4.54	(1) 1.00	6.2	0.30	5.2	(1) 1.00	5.6	0.40	5.1	0.40	5.2	(1) 1.00	5.2	(2) 0.90	4.53	(1) 1.00
4.34	0.03	5.9	0.30	4.82	0.15	5.0	0.40	4.46	0.50	4.84	0.20	4.88	0.25	4.20	(2) 0.95
4.07	(3) 0.25	5.6	0.30	4.59	0.15	4.53	(1) 1.00	4.04	0.40	4.61	0.50	4.39	(1) 1.00	4.06	0.15
3.90	0.03	5.4	(1) 1.00	4.31	0.15	4.38	0.15	3.91	(3) 0.65	4.41	0.40	3.95	0.12	3.86	(3) 0.90
3.71	0.10	5.1	(2) 0.80	4.12	0.50	4.21	0.15	3.795	0.40	4.22	0.40	3.76	0.10	3.65	0.08
3.53	0.20	4.68	0.50	3.92	(2) 0.90	4.05	(2) 0.95	3.60	0.55	3.91	(2) 0.95	3.58	(3) 0.80	3.46	0.15
3.33	0.20	4.18	0.50	3.68	(3) 0.85	3.73	0.40	3.51	0.15	3.77	0.25	3.41	0.40	3.26	0.10
3.15	0.10	3.87	0.50	3.41	0.10	3.375	(3) 0.85	3.30	0.15	3.62	0.25	3.18	0.40	3.16	0.90
2.91	0.10	3.76	0.10	3.31	0.25	3.29	0.20	3.14	0.15	3.34	0.40	3.11	0.12	3.02	0.25
2.78	0.08	3.65	0.20	3.03	0.03	3.21	0.40	2.99	0.50	3.31	0.45	2.99	0.12	2.81	0.08
2.68	0.08	3.57	0.20	2.91	0.03	3.05	0.40	2.76	0.35	3.19	0.15	2.88	0.40	2.75	0.08
2.53	0.02	3.43	0.35	2.75	0.03	2.97	0.10	2.69	0.08	3.07	0.08	2.81	0.25	2.65	0.10
2.47	0.02	3.28	0.35	2.62	0.25	2.83	0.25	2.51	0.08	2.98	0.40	2.61	0.12	2.52	0.08
2.42	0.10	3.12	0.35	2.57	0.25	2.77	0.25	2.39	0.30	2.87	0.15	2.50	0.08	2.40	0.25
2.24	0.03[b]	2.93	0.20	2.51	0.06	2.66	0.10	2.29	0.08	2.75	0.12	2.43	0.25	2.31	0.08
2.14	0.02	2.73	0.15	2.43	0.03	2.59	0.10	2.21	0.08	2.67	0.12	2.38	0.10	2.24	0.15
2.07	0.03	2.64	0.05	2.275	0.10	2.54	0.15	2.12	0.08	2.59	0.08	2.25	0.10	2.19	0.15
1.94	0.04	2.58	0.05	2.05	0.06	2.42	0.15	2.03	0.15	2.48	0.08	2.19	0.12	2.13	0.15
1.795	0.04	2.55	0.05	1.98	0.06	2.34	0.10	1.99	0.15	2.39	0.15	2.13	0.10	2.03	0.10
1.72	0.04	2.49	0.10	1.92	0.03	2.275	0.30	1.94	0.15	2.27	0.08	2.02	0.12	1.91	0.08
1.62	0.03	2.39	0.05	1.84	0.06	2.15	0.15	1.83	0.05	2.22	0.15	1.94	0.10	1.86	0.05
1.57	0.01	2.25	0.10	1.78	0.05	2.09	0.30	1.26	0.05	2.12	0.08	1.91	0.10	1.82	0.08
		2.16	0.10			2.03	0.05	1.17	0.05	2.04	0.08	1.84	0.12	1.66	0.10
		2.10	0.10			1.94	0.10[b]	1.08	0.05	1.96	0.10	1.78	0.12	1.58	0.10
		2.04	0.03			1.905	0.05								
		1.99	0.03			1.86	0.05								

[a] Patterns arranged in order of empirical formula (*Chem. Abs.*). *d/n* = interplanar spacing in Angstroms; I/I_1 = estimated relative intensity; (1) strongest line, (2) second strongest line, (3) third strongest line.

[b] Broad line probably due to partial coincidence of two or more lines.

Table II. Powder-Diffraction Data[a] (*Continued*)

d/n	I/I_1	d/n	I/I_1	d/n	I/I_1	d/n	I/I_1
25. 2-(α-Ethylpropyl)phenol		26. 4-*tert*-Amylphenol		27. *o*-Phenylphenol		28. *p*-Phenylphenol	
1.04	0.05	1.93	0.08	1.74	0.10	1.23	0.03
		1.87	0.08	1.60	0.05	1.19	0.03
		1.825	0.05	1.56	0.05		
		1.71	0.05	1.525	0.05		
		1.67	0.05	1.30	0.05		
				1.21	0.03		
				1.14	0.02		
				1.13	0.02		

29. 2-Cyclohexylphenol		30. 4-Cyclohexylphenol		31. 1-Hydroxyfluorene		32. 2-Hydroxyfluorene	
13.6	0.50	13.9	0.85	18.8	0.25	16.9	0.10
7.9	(3) 0.60	7.0	0.30	16.8	0.75	10.05	0.40
7.15	0.40	6.3	0.30	9.6	0.25	5.6	0.10
6.8	0.40	5.6	0.30	8.3	0.50	5.2	0.10
5.7	0.40	5.0	(1) 1.00	6.2	0.20	5.0	0.10
5.4	0.30	4.33	(2) 0.98	5.5	0.80	4.72	(2) 0.90
5.15	0.30	3.87	(3) 0.90	5.2	(1) 1.00	4.57	(1) 1.00
4.88	(1) 1.00	3.53	0.50[b]	4.65	0.50	4.18	0.40
4.53	0.40	3.39	0.30	4.18	(2) 0.95	4.11	0.40
4.36	0.15	3.28	0.15	3.89	0.10	3.81	0.05
3.66	(2) 0.80[b]	3.05	0.60	3.79	0.10	3.64	0.10
3.56	0.10	2.87	0.25	3.67	0.10	3.38	(3) 0.85
3.42	0.40	2.77	0.25	3.55	0.80	3.07	0.05
3.23	0.30	2.49	0.25	3.45	0.08	2.96	0.05
3.06	0.30	2.43	0.20	3.27	(3) 0.90	2.87	0.03
2.96	0.30	2.35	0.10	3.14	0.10	2.73	0.05
2.715	0.30	2.31	0.10	2.965	0.20	2.62	0.05
2.56	0.30	2.28	0.10	2.85	0.15	2.53	0.20
2.49	0.15	2.21	0.15	2.73	0.08	2.46	0.03
2.34	0.08	2.07	0.15	2.65	0.04	2.395	0.16
2.275	0.08	2.02	0.15	2.54	0.02	2.29	0.03
2.21	0.08	1.98	0.10	2.45	0.02	2.04	0.03
2.17	0.08	1.92	0.05	2.39	0.40	1.99	0.08
2.07	0.25	1.86	0.10	2.30	0.04	1.95	0.02
2.01	0.08	1.83	0.05	2.26	0.04	1.81	0.04
1.97	0.10	1.76	0.10	2.22	0.06	1.68	0.04
1.79	0.08	1.71	0.10	2.13	0.10	1.26	0.02
1.74	0.08	1.58	0.10	2.04	0.10	1.17	0.02
1.70	0.08	1.47	0.04	1.92	0.08		
1.58	0.06	1.40	0.04	1.90	0.08		
1.47	0.03	1.38	0.04	1.84	0.06		
1.38	0.03	1.32	0.04	1.77	0.06		
1.23	0.03	1.23	0.04	1.715	0.15		
1.20	0.03	1.17	0.04	1.64	0.06		
		1.16	0.04	1.58	0.02		
		1.14	0.04	1.54	0.02		
		1.13	0.04				
		1.12	0.04				
		1.10	0.04				
		1.08	0.04				
		1.05	0.04				

33. 4-(α-Methyl benzyl)phenol		34. 1-Phenanthrol		35. 2-Phenanthrol		36. 9-Phenanthrol	
11.75	0.30	14.9	0.60	10.9	0.30	10.1	0.30
7.9	0.25	8.85	0.03	9.5	0.40	9.1	0.05
7.25	0.40	7.4	0.40	6.6	0.05	7.7	0.10
6.8	0.40	6.6	(2) 0.95	5.7	0.30	6.9	(1) 1.00
6.1	0.40	5.7	(2) 0.95	5.4	0.30	6.1	0.60
5.8	0.35	4.72	0.20	5.15	0.30	4.97	0.50
5.2	(1) 1.00	4.53	0.20	4.88	(1) 1.00	4.26	0.50
4.68	(2) 1.00	4.05	(1) 1.00	4.68	0.35	3.86	(2) 0.98
4.39	(3) 0.50	3.72	0.60	4.50	0.35	3.64	0.30[b]
4.20	(3) 0.50	3.46	0.70	3.98	(3) 0.50[b]	3.41	(3) 0.95
3.88	0.30	3.33	(3) 0.80	3.78	0.45	3.11	0.60
3.77	0.30	2.92	0.50	3.59	0.45	3.04	0.30
3.62	0.40	2.72	0.10	3.37	(2) 0.55	2.90	0.03
3.48	0.25	2.64	0.15	3.23	0.25	2.80	0.05
3.38	0.30	2.515	0.08[b]	3.09	0.20	2.70	0.30
3.28	0.40	2.42	0.15	2.93	0.30	2.57	0.20
3.11	0.40	2.30	0.10	2.77	0.05	2.34	0.20
2.975	0.25	2.25	0.10	2.67	0.05	2.25	0.20
2.87	0.25	2.17	0.04	2.55	0.06	2.17	0.20
2.77	0.35	2.04	0.12	2.50	0.15	2.115	0.20
2.67	0.25	1.97	0.12	2.395	0.12	2.02	0.25[b]
2.61	0.25	1.89	0.12	2.33	0.04	1.98	0.04
2.53	0.25	1.75	0.12	2.27	0.25	1.90	0.04[b]
2.38	0.10	1.66	0.12	2.19	0.05	1.835	0.30
2.29	0.15	1.59	0.03	2.11	0.06	1.74	0.08
2.24	0.15	1.29	0.03	2.07	0.06	1.65	0.03
2.19	0.15	1.27	0.04	2.02	0.08	1.63	0.03
2.15	0.20	1.24	0.02	1.90	0.04	1.60	0.03
2.03	0.10	1.21	0.02	1.83	0.04	1.55	0.03
1.98	0.20	1.13	0.02	1.71	0.04	1.525	0.02
1.93	0.10	1.08	0.02	1.65	0.04	1.49	0.02
1.86	0.10					1.30	0.02
1.77	0.10					1.25	0.02
						1.03	0.02

37. 4,α-Cumylphenol		38. 4-Octylphenol		39. 2,4-Di-*tert*-butylphenol		40. 4-(α,α-Dimethylbenzyl)-phenol	
9.1	0.10	15.1	(2) 0.90	11.7	(3) 0.75	9.1	0.30
6.4	0.30	11.75	0.15	10.6	(3) 0.75	7.8	0.10
5.9	0.30	7.9	0.20	8.2	0.60	6.4	0.50
5.5	(1) 1.00	7.4	0.25	7.2	0.60	5.9	0.40
4.93	(3) 0.70	6.7	0.50	6.3	(2) 0.90	5.5	(1) 1.00
4.46	(2) 0.80	6.4	(1) 1.00	5.9	0.50	4.90	0.90
4.31	0.65	5.85	0.50	5.6	0.15	4.48	(3) 0.95
3.88	0.65	5.6	(3) 0.55	5.3	0.60	4.31	0.90
3.64	0.30	5.0	0.50	5.0	(1) 1.00	3.88	(2) 1.00
3.27	0.20[b]	4.84	(3) 0.55	4.66	0.70	3.70	0.30
3.00	0.20[b]	4.515	0.50	4.35	0.40	3.59	0.10
2.715	0.20[b]	4.29	0.25	4.10	0.40	3.42	0.15
2.60	0.25	4.11	0.25	3.73	0.25	3.33	0.20
2.46	0.08	3.99	0.40	3.52	0.25	3.27	0.15
2.39	0.10	3.86	0.10	3.30	0.15	3.08	0.20
2.25	0.05	3.71	0.15	3.14	0.25	3.01	0.20
2.16	0.05	3.58	0.18	3.03	0.15	2.71	0.20[b]
2.04	0.10	3.45	0.05	2.97	0.15	2.60	0.35
1.96	0.10	3.32	0.25	2.86	0.25	2.47	0.08
1.90	0.05	3.205	0.20	2.725	0.20	2.38	0.15
1.86	0.05	3.13	0.15	2.64	0.25	2.32	0.10
1.83	0.05	2.99	0.08	2.53	0.15	2.25	0.08
1.75	0.10	2.90	0.08	2.32	0.15	2.16	0.08
1.67	0.10	2.76	0.15	2.21	0.20	2.10	0.05
1.60	0.03	2.67	0.12	2.17	0.08	2.05	0.15
1.55	0.03	2.49	0.05	2.12	0.08	1.98	0.15
		2.44	0.08	2.04	0.25	1.91	0.10
		2.395	0.08	2.00	0.05	1.87	0.10
		2.31	0.05	1.94	0.05	1.835	0.10
		2.25	0.03	1.89	0.05	1.80	0.05
		2.13	0.03	1.81	0.05	1.75	0.10
		2.02	0.03	1.68	0.05	1.67	0.10
		1.95	0.04	1.62	0.05	1.64	0.05
		1.75	0.04			1.60	0.05
						1.55	0.04
						1.40	0.04
						1.38	0.04
						1.28	0.02
						1.16	0.02

41. 4-(α-Methylbenzyl)-3-methylphenol		42. 2,6-Di-*tert*-butyl-4-methylphenol		43. 4,6-Di-*tert*-butyl-3-methylphenol		44. 2-Methyl-4-diisobutylphenol	
16.7	0.40	8.65	(3) 0.60	10.8	0.25	17.7	0.65
13.9	0.40	7.7	0.55	9.1	0.10	15.3	0.65
9.1	0.15	6.8	0.25	8.2	(3) 0.85	12.7	0.65
7.6	0.20	6.1	(1) 1.00	7.0	0.70	11.3	0.25
7.0	0.15	5.1	0.55	6.6	0.70	9.2	0.25
6.1	0.50	4.64	0.20	5.8	(2) 0.90	8.1	0.10
5.6	0.60	4.46	0.20	5.5	0.10	7.25	(3) 0.70
5.3	(2) 0.90	4.29	(2) 0.90	4.99	0.50	6.8	0.40
5.1	(2) 0.90	4.09	0.30	4.74	(1) 1.00	6.5	0.15
4.82	0.50	3.85	0.30	4.55	0.25	6.0	0.40
4.64	(3) 0.80	3.62	0.30	4.12	0.25	5.4	(1) 1.00
4.20	0.50	3.36	0.25	3.92	0.15	5.15	0.65
3.965	0.15	3.27	0.15	3.745	0.15	4.90	0.25
3.82	(1) 1.00	3.20	0.15	3.60	0.25	4.48	(2) 0.80
3.57	0.30	3.10	0.15	3.38	0.30	4.23	0.40
3.38	0.05	2.91	0.15	3.13	0.20	4.05	0.20
3.29	0.50	2.81	0.20	3.02	0.08	3.82	0.40
3.16	0.50	2.73	0.08	2.92	0.25	3.68	0.10
3.04	0.15	2.66	0.08	2.85	0.10	3.56	0.50
2.83	0.15	2.53	0.12	2.71	0.35	3.45	0.25
2.76	0.08	2.46	0.12	2.51	0.05	3.25	0.25
2.54	0.25	2.28	0.06	2.34	0.06	3.15	0.15
2.46	0.10	2.23	0.25	2.29	0.08	3.07	0.15
2.36	0.25	2.15	0.06	2.19	0.08	2.965	0.25
2.23	0.10	2.09	0.04	2.15	0.08	2.80	0.30
2.11	0.15	2.04	0.25	2.11	0.05	2.71	0.20
1.93	0.08	1.95	0.10	2.07	0.15	2.59	0.20
1.86	0.08	1.81	0.10	1.97	0.15	2.50	0.08
1.80	0.08	1.73	0.03	1.90	0.08	2.38	0.08
1.64	0.08	1.69	0.03	1.77	0.05	2.27	0.20
1.22	0.08	1.59	0.03	1.74	0.05	2.21	0.20
		1.51	0.03	1.71	0.05	2.11	0.05
				1.58	0.03	2.05	0.10
				1.56	0.03	2.00	0.10
				1.25	0.03	1.96	0.15
				1.19	0.03	1.92	0.08
						1.81	0.08
						1.71	0.06
						1.58	0.05
						1.26	0.03

[a] Patterns arranged in order of empirical formula (*Chem. Abs.*). d/n = interplanar spacing in Angstroms; I/I_1 = estimated relative intensity; (1) strongest line, (2) second strongest line, (3) third strongest line.

[b] Broad line probably due to partial coincidence of two or more lines.

Table II. Powder-Diffraction Data[a] (*Continued*)

d/n	I/I_1	d/n	I/I_1	d/n	I/I_1	d/n	I/I_1	d/n	I/I^6	d/d	I/I_1	d/n	I/I_1
45. 2-Methyl-4,6-di-*tert*-butylphenol		**46. 1-Pyrenol**		**47. 2-Isobornylphenol**		**48. 2-Hydroxychrysene**		**49. 2,4-Dimethyl-6-isobornylphenol**		**50. 2,4,6-Tri-*tert*-butylphenol**		**51. 3-Pentadecylphenol**	
10.9	0.40	16.0	0.25	12.3	0.25	20.0	0.25	13.3	0.20	8.8	0.50	13.3	0.35
9.0	0.10	8.1	0.50	10.7	(1) 1.00	11.2	0.40	11.5	(2) 0.98	8.0	(3) 0.90	9.0	0.15
8.0	(3) 0.50	6.6	(2) 0.80	8.7	0.20	10.05	(3) 0.50	9.8	0.40	6.6	0.40	7.4	0.40
7.0	0.30	5.9	(3) 0.75	7.1	0.20	7.25	0.25	7.3	0.20	5.9	0.15	6.6	0.40
6.5	0.10	5.6	0.02	6.7	0.30	6.8	0.12	6.6	(1) 1.00	5.6	0.30	5.8	0.40
6.1	(2) 0.55	5.3	0.04	5.9	(2) 1.00	6.2	0.15	5.7	0.20	5.3	0.40	5.4	0.20
5.5	0.40	5.0	0.01	5.4	0.40	5.8	(2) 0.98	5.1	0.30	4.97	(1) 1.00	5.0	0.30
5.2	0.25	4.82	0.05	5.3	0.15	5.5	0.06	4.72	0.40	4.50	(2) 0.98	4.84	0.30
4.50	(1) 1.00	4.57	0.20	5.05	0.10	5.1	0.40	4.50	0.10	3.64	0.30	4.55	(3) 0.45
4.12	0.20	4.38	0.20	4.76	(3) 0.98	4.76	0.15	4.29	(3) 0.60	3.27	0.30	4.14	(1) 1.00
3.96	0.20	4.21	0.10	4.34	(3) 0.98	4.29	0.30	4.18	0.35	3.11	0.08	3.98	0.35
3.82	0.25	4.05	0.10	4.245	0.25	4.09	0.15	4.06	0.40	2.92	0.30	3.78	(2) 0.90
3.59	0.08	3.83	(2) 0.80	4.11	0.25	3.91	0.40	3.91	0.40	2.78	0.25	3.63	0.30
3.48	0.15	3.67	(1) 1.00	3.91	0.10	3.68	0.25	3.60	0.40	2.70	0.25	3.40	0.30
3.23	0.20	3.40	0.10	3.73	0.40	3.55	(1) 1.00	3.19	0.15	2.45	0.50	3.29	0.35
3.14	0.05	3.28	0.60	3.55	0.40	3.45	0.40	3.11	0.15	2.405	0.08	3.02	0.40
3.05	0.08	3.02	0.25	3.33	0.20	3.30	0.40	3.00	0.05	2.26	0.05	2.87	0.03
2.96	0.15	2.75	0.25	3.15	0.20	3.05	0.25	2.85	0.12	2.16	0.05	2.75	0.03[b]
2.83	0.10	2.67	0.03	3.03	0.20	2.95	0.25	2.715	0.06	2.00	0.40	2.48	0.40
2.77	0.15	2.55	0.03	2.89	0.10	2.87	0.12	2.61	0.03	1.925	0.15	2.41	0.03
2.68	0.08	2.48	0.03	2.77	0.25	2.625	0.06	2.52	0.03	1.79	0.05	2.36	0.12
2.61	0.10	2.31	0.10	2.70	0.10	2.57	0.06	2.405	0.20	1.67	0.04	2.26	0.30
2.53	0.05	2.13	0.08	2.60	0.20	2.49	0.10	2.31	0.20	1.57	0.04	2.225	0.30
2.34	0.10	2.04	0.08[b]	2.45	0.15	2.41	0.03	2.14	0.06	1.25	0.03	2.15	0.15
2.25	0.20	2.00	0.08[b]	2.37	0.15	2.36	0.06	2.10	0.06	1.16	0.03	2.08	0.30
2.10	0.10	1.93	0.10	2.32	0.20	2.29	0.06	2.02	0.06			1.98	0.15
2.04	0.05	1.84	0.10	2.15	0.10	2.13	0.15	1.93	0.05			1.87	0.05
1.90	0.05	1.76	0.10	2.10	0.05	2.06	0.06	1.85	0.05			1.77	0.03
1.75	0.03	1.64	0.03	2.04	0.10	2.00	0.03	1.81	0.03			1.71	0.15
1.63	0.03	1.60	0.08	1.95	0.10	1.95	0.02	1.69	0.03			1.67	0.03
1.23	0.03	1.45	0.02	1.90	0.04	1.89	0.10	1.67	0.03			1.51	0.05
		1.16	0.01	1.87	0.04	1.79	0.03	1.26	0.02				
		1.14	0.01	1.825	0.10	1.76	0.03	1.17	0.02				
				1.76	0.05	1.64	0.02	1.08	0.02				
				1.60	0.04	1.59	0.02	1.03	0.02				
				1.31	0.02								
				1.15	0.02								

[a] Patterns arranged in order of empirical formula (*Chem. Abs.*). d/n = interplanar spacing in Angstroms; I/I_1 = estimated relative intensity; (1) strongest line, (2) second strongest line, (3) third strongest line.

[b] Broad line probably due to partial coincidence of two or more lines.

p-phenylphenol, and thymol (3-methyl-6-isopropylphenol). With the exception of the thymol case, the patterns are in rather good agreement. Since the present data were taken with the iron radiation of relatively long wave length, the reasonably large 114.6-mm. camera, and the absence of any binder or enfolding holder, a high degree of resolution was obtained. This is illustrated by the fact that the present patterns contain more reflections than the comparable patterns in the index (*1*). The method of sample preparation as here employed may induce some preferred orientation, although little or no evidence of this has been observed.

Table III. Impure Compounds with Diffraction Patterns Identical to Those of Recrystallized Compounds

Compound	Melting Point, ° C. Obtained	Melting Point, ° C. Recrystallized
4-(α-Methylbenzyl)-3-methylphenol	116.0–117.0	124.5–125.2
4-(α-Methylbenzyl)-phenol	54.0– 55.0	56.5– 58.0
4-Isopropylphenol	57.5– 59.5	58.5– 59.0
3-Methyl-4-isopropylphenol	106.5–111.5	111.0–112.2

For purposes of comparison and completeness, the 20 phenols listed in the index are included in Table I.

In order to check the effect of impurities on the diffraction pattern, phenols which had been oxidized through aging were subjected to x-ray powder diffraction analysis before and after recrystallization. The results are shown in Table III. In no case was there an appreciable modification of the pattern as a result of the presence of impurities.

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Chemical Analysis of Thin Films by X-Ray Emission Spectrography

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X-ray emission spectrography has been applied to the quantitative analysis of thin (approximately 100 A.) evaporated films of iron, nickel, chromium, and Types 304, 316, and 347 stainless steels deposited on Mylar polyester backing. Oxide films associated with passivity and atmospheric oxidation, isolated from Types 304, 316, and 347 stainless steels, were also studied. The corresponding surface densities of the sample films varied from 1 to 100 × 10^{-6} gram per sq. cm. Analytical sensitivities for the three metals corresponding to a counting rate of 1 count per second ±5% are 0.037, 0.061, and 0.175 × 10^{-6} gram per sq. cm. for nickel, iron, and chromium, respectively. Comparison of the metallic compositional data obtained by x-ray methods for metal and oxide films 300 A. in thickness or less to those obtained independently by microcolorimetric methods indicates excellent agreement. The conclusion is made that accuracies within 2% or better can be obtained for the chemical analysis of such highly dispersed samples by x-rays without the application of corrections for adsorption deviations.

CHEMICAL analysis of surface films, surface residues, and corrosion products on metals and alloys is often essential to an understanding of mechanisms associated with surface reactions. Difficulties in handling samples on the microgram scale by classical microchemical analytical techniques have limited this approach to problems in surface metallurgy (*11*).

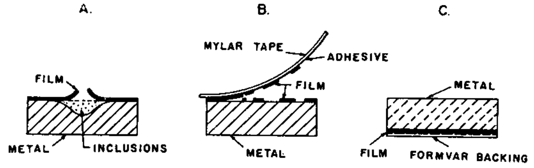

Figure 1. Comparison of surface-sampling methods

A. Bromine-methanol stripping, total solids collected
B. Mylar tape stripping, surface fragments collected
C. Bromine-methanol stripping, sections of film collected

Reliable sampling, in common with microanalytical schemes, is particularly difficult in the isolation of surface layers. Microanalysis of polycomponent samples such as oxide films from polycomponent alloys by wet chemical means is further complicated by requirements of chemical conversion and separation of microgram quantities. Furthermore, prior knowledge of approximate sample composition is generally required for precision microchemistry.

Some effective surface sampling and microchemical schemes for the analysis of thin films and surface residues have been reported (*7, 12*). One such scheme consists of a series of consecutive microchemical operations in which the isolated film or residue is washed, ignited, weighed, fused, leached, and finally analyzed spectrophotometrically (*10*). Although the reliability of the microchemical procedure is established, the method is time-consuming and limited to those elements for which reliable microcolorimetric procedures have been established. A physical technique yielding complete quantitative data on all metallic components by a single evaluation with a minimum amount of manipulation would be extremely valuable in the characterization of thin films and microgram residues.

In spite of the very small fluorescent x-radiation excited in microgram samples of metals, alloys, and related compounds, the sensitivity and relative simplicity of this method recommend it strongly as a tool in metallurgical research (*2, 4, 5*). Some interesting nonroutine metallurgical applications of x-ray chemical analysis have already been surveyed by Koh and Caugherty (*5*). Preliminary calculations by the author indicated that reliable measurements might be achieved utilizing specially prepared samples and low background counting conditions. An extension of the approach of Koh and Caugherty to the analysis of ultrathin films (30 to 150 A.) of metals and oxides by x-ray emission spectroscopy was undertaken. Results were very gratifying, once the difficulties of isolating and mounting film samples and measuring accurately very low counting rates were resolved.

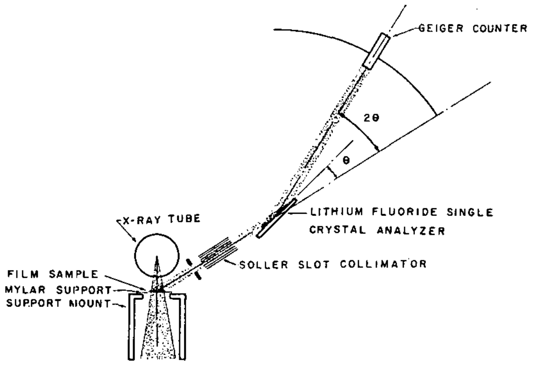

Figure 2. X-ray optics–flat crystal, reflection method

The experimental survey was made on films of pure metals and austenitic stainless steels deposited by evaporation onto Mylar polyester substrates. Oxide films isolated from austenitic stainless steels were also studied. Effort was directed to clarification of the influence of the highly dispersed nature of the samples (1.0 to 100 γ per sq. cm.) on deviations from absorption and secondary emission interactions, characteristic of x-ray analysis of normal size samples (*1, 2, 6*).

EXPERIMENTAL PROCEDURE

Preparation of Film Samples. Isolation and mounting of the sample were most critical in obtaining good precision and low background intensity. Many plastic and metallic film materials were investigated as sample supports. Mylar polyester film was chosen because of the high strength of thin sections (0.00025 inch) and its very low x-ray scattering power. The design of the Mylar support and sample holder contributed considerably toward obtaining a low stable background. In view of the transparency of the samples to both the incident and emitted x-rays, care was taken to ensure that radiation from the interior of the sample holder itself did not get back to the detector.

The metal and alloy films were deposited by vaporization of high purity material in a vacuum (1 × 10^{-5} mm. of mercury) directly onto the Mylar substrate. The oxide films were formed by floating metal films reinforced with Formvar, onto a solution of 5.0% nitric acid–0.5% potassium dichromate at 60° C. for periods from 30 to 100 minutes. The reinforced films were then scooped onto Mylar, and the metal and then the Formvar were dissolved away. The immersion time in the oxidizing acid determined the thickness of the oxide film formed. The oxide film was isolated from the metal by chemical-stripping techniques using anhydrous 1% bromine-methanol in an inert atmosphere. Film samples were also obtained from metal coupons using similar techniques. The area over which the sample was dispersed varied, but was approximately 4 to 6 sq. cm.

Three different variations of this technique (Figure 1) determined whether the sample consisted of film and inclusions, fragmented oxide film, or large sections of film. Both the metal and oxide films were weighed on an analytical microbalance. From these data and accurate measurement of geometric area, the surface film densities were calculated. Approximate film thickness was also calculated from measured weights and areas, and estimated film densities. Microcolorimetric techniques were used for establishing film standards in the calibration of the x-ray method. Chemical microanalysis was also used to evaluate x-ray compositional data obtained on films of *unknown* composition. Sources of error from sampling and chemical microanalysis have been discussed and shown to contribute a maximum standard deviation of approximately 5% of the elements determined (*10*).

Measurement of X-Ray Intensities. The x-ray data were obtained on a North American Philips plane crystal spectrograph with a Machlett OEG-50 tungsten target tube. The x-ray tube was operated at 50 kv. and 35 ma. unless otherwise specified. In addition, several x-ray tubes were checked to get one with not only a low dispersed background, but also a low background at wave lengths corresponding to the metals of analytical interest.

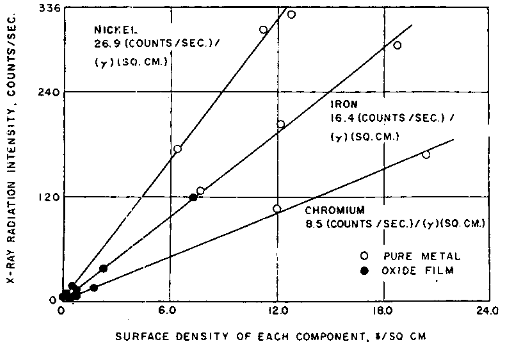

Figure 3. Calibration of x-ray radiation intensities for pure metal and for oxide isolated from Type 304 stainless steel

The basic method has been described in the literature (*3*). The arrangement of the x-ray optics in Figure 2 illustrates the lithium fluoride analyzing crystal and the Soller blade collimator. The collimator was 6 inches long and had a collimation spacing of 0.005 inch. Except for scouting experiments, fixed-angle counting was done at the proper Bragg setting using a North American Philips A62019 argon-filled Geiger tube with a bias voltage of 1500 volts.

A general indication of composition was obtained by scanning over a range of diffraction angles corresponding to elements of interest at a chart speed of 0.5° per minute. The first-order $K\alpha$-lines were scaled for all the elements except molybdenum and niobium for which the $L\alpha$-lines were also measured.

UNCERTAINTIES AND CORRECTIONS

Although precisions one tenth of this limit or less are characteristic of macroanalysis, an over-all uncertainty of 5% is not unreasonable for microanalysis when one considers sampling and contamination errors associated with manipulations in the microgram range. Most of the uncertainty in the analysis of films results from the sampling procedure. This is indicated by the improved analytical precision obtained on dispersed samples of synthetic microgram mixtures of pure metallic oxides. Microcolorimetric techniques on these materials yielded standard deviations one tenth as large or approximately 0.5%.

Scaling was done for a fixed time to yield a probable counting error of 1.0%. Where counting times exceeded 2 minutes the background count was remeasured every 2 minutes to correct for drifting of the counting circuits. Actual counting rates were less accurate than corrected counting rates because of uncertainties from scattering, background, and blank determinations. By extending counting times up to 30 minutes and by making repeated measurements, net-counting rates of 1.0 ± 5% counts per second corrected for background, could be measured. The accuracy of the net-counting measurement was attributed in part to the low stable background of approximately 2 counts per second for the scaling measurements.

Table I. Effect of Film Thickness of Pure Metals on Specific Radiation Intensity

Metal	Surface Concn.,[a] γ/Sq. Cm.	Approx. Film Thickness, A.	Total Intensity, Counts/Sec.	Specific Intensity, (Counts/Sec.)/(γ) (Sq. Cm.)
Chromium	...	...	...	...
Iron	7.7	97	128	16.6
Nickel	6.3	70	170	26.9
Chromium	12.0	169	102	8.5
Iron	12.1	153	200	16.5
Nickel	11.1	125	298	26.8
Chromium	20.5	289	174	8.5
Iron	19.0	240	314	16.5
Nickel	17.8	200	455	26.7
Chromium	31.0	437	264	8.5
Iron	26.2	332	430	16.4
Nickel	...	...	...	...

[a] Obtained by dividing weight of film by its area.
[b] Estimated from surface concn. by using bulk density.

Table II. Effect of Film Thickness of Type 304 Stainless Steel on Specific Radiation Intensity

Component Metal	Surface Concn.[a], γ/Sq. Cm.	Approx. Film Thickness, A.[b]	Total Intensity, Counts/Sec.	Specific Intensity, (Counts/Sec.)/(γ) (Sq. Cm.)
Pure Metal				
Chromium	31.0	437	264.0	8.5
Iron	26.2	332	430.0	16.4
Nickel	17.8	200	455.0	26.7
Type 304	...	104	...	...
Chromium	1.5	...	12.8	8.5
Iron	7.1	...	115.7	16.3
Nickel	0.7	...	18.7	26.8
Type 304	...	410	...	...
Chromium	6.4	...	55.3	8.5
Iron	23.0	...	372.6	16.2
Nickel	2.6	...	68.6	26.6
Type 304	...	808	...	...
Chromium	12.1	...	104.1	8.6
Iron	53.8	...	866.2	16.1
Nickel	6.2	...	161.2	26.5
Type 304	...	1300	...	...
Chromium	18.5	...	160.9	8.7
Iron	75.7	...	1211.2	16.0
Nickel	8.5	...	216.8	26.4

[a] Obtained by dividing weight of film by its area.
[b] Estimated from surface concn. by using bulk density.

Scattering and blank errors were corrected by measurements on untreated Mylar and on Mylar treated identically to that supporting the film sample, except that the sample was absent. The latter measurement provided for possible contamination of the Mylar by the procedures used to deposit the sample.

No corrections were made in the x-ray data for the carbon, manganese, and other minor elements known to be present. The uncertainty introduced by this omission is negligible except for the ultrathin oxide films which contained substantial amounts of silicon. In this case, refinements in the x-ray instrumentation, required to yield data on silicon, were not available.

RESULTS AND DISCUSSION

Radiation Characteristics Specific to Thin Films. Two effects become important when x-ray emission samples are scaled down in thickness. The minimum effective thickness for maximum fluorescent yield is not satisfied, and deviations from adsorption and secondary emission decrease. For nickel, chromium, and iron the critical thickness associated with maximum yield has been experimentally established by Koh and Caugherty (*5*) to be 0.0003 cm. This is in good agreement with the calculated value of Brissey, Liebhafsky, and Pfeiffer (*2*). For the highly dispersed samples of this study, total fluorescent intensities were accordingly lower than for massive samples of

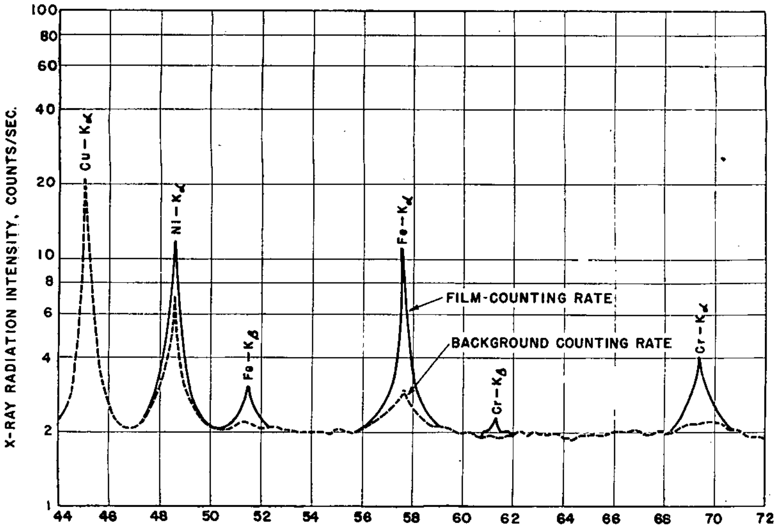

Figure 4. Typical x-ray emission spectrogram for passive film isolated from Type 304 stainless steel

Sample wt. 6.6 γ, x-ray tube operated at 50 kv. and 45 ma.

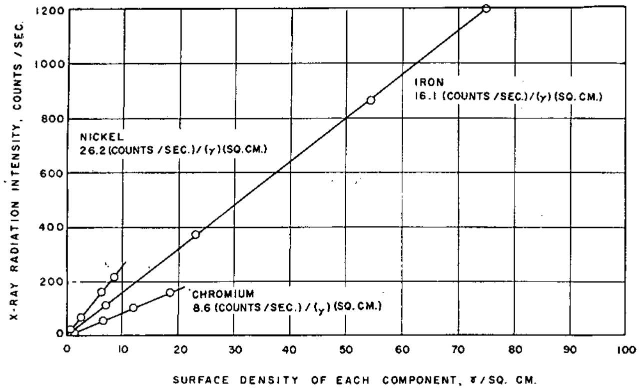

Figure 5. Calibration of x-ray radiation intensities for Type 304 stainless steel

equivalent area. To make a corresponding comparison, measurements are expressed in units of specific radiation intensity, (counts per second)/(γ) (sq. cm.). Although the gross excitation intensity is greater in massive samples, thin films are actually exposed to a higher average x-ray flux per unit thickness.

The fact that deviations decrease considerably with the state of dispersion of the sample has already been reported by Pfeiffer and Zemany (*9*) for a large number of metals. Deviations are defined according to Liebhafsky (*6*) as the difference between the percentage of an element actually present in an alloy and the percentage that one would calculate using the pure element as an x-ray emission standard. It is evident that ultrathin films of metals or oxides with surface densities in the range from 1 to 100 γ per sq. cm. are equivalent to highly dispersed samples. With decreasing sample thickness for films of pure nickel and iron, the specific radiation intensity increases, because of the higher average excitation flux per unit thickness, as well as the lower over-all absorption of the excited radiation (Table I).

Effect of Film Thickness for Metals and Alloys. In the thickness range studied (1 to 31 γ per sq. cm.), nickel, iron, and chromium indicate some variation in specific radiation intensity for the pure metal. The average specific radiation intensities can be interpolated from the slopes in Figure 3 to be 26.9, 16.4, and 8.50 (counts per second)/(γ per sq. cm.) for nickel, iron, and chromium, respectively, for surface densities from 1.0 to 31.0 γ per sq. cm. The corresponding maximum percentage deviation from these values in this dispersion range is approximately 1%. The analytical sensitivities for the three metals, subject to a net-counting error of 5%, are 0.037, 0.061, and 0.175 γ per sq. cm., for nickel, iron, and chromium, respectively.

The effect of film thickness on specific radiation intensity was determined for the same metals in thin films of Types 304, 316, and 347 stainless steels. Data for Type 304 stainless steel in the film thickness range from 100 to 1300 A. are summarized in Table II. A typical x-ray spectrogram of passive film isolated from Type 304 stainless steel is illustrated in Figure 4. The oxide film was gelatinous with a lack of any apparent crystallinity. It was approximately 30 A. thick and had a surface density of 1.65 γ per sq. cm.

The data are in good agreement with deviations calculated by Noakes (*8*) for a similar system. For the compositions and film thicknesses studied, the nickel *Kα*- and iron *Kα*-radiations have negative over-all coefficients and the chromium *Kα*-radiation has a positive over-all coefficient. The maximum percentage deviation as a function of alloy film thickness for iron was −2.6% for the conditions studied. Alternatively, the iron content from uncorrected x-ray data for a film of Type 304 stainless steel, 1300 A. thick, would be too low by this percentage.

A distinction is made between deviations for different thicknesses of the same material and deviations for the same corresponding thickness of a given component in different materials (example, pure iron *vs.* iron in stainless steel). Most of the other deviations were substantially less for the relatively thick stainless steel deposits. The average deviations calculated from the calibration plots in Figure 5 would yield average specific intensities of 26.2, 16.1, and 8.6 (counts per second)/(γ per sq. cm.) for nickel, iron, and chromium, respectively, in stainless steel films. These values differ from those for corresponding thicknesses of pure metals by −2.6, −1.8, and +1.2%, in the same order.

Effect of Film Thickness for Oxides. The effect of film thickness on specific radiation intensity for oxide films up to 300 A. thick isolated from Type 304 stainless steel was also determined. Data are summarized in Table III. The data, plotted in Figure 4, are observed to yield average specific radiation intensities identical with those of thin films of the corre-

Table III. Effect of Film Thickness of Oxides on Specific Radiation Intensity

Component Metal	Surface Concn., γ/Sq. Cm.[a]	Approx. Film Thickness, A.[b]	Total Intensity, Counts/Sec.	Specific Intensity, (Counts/Sec.)/(γ) (Sq. Cm.)
Oxide No. 1	1.65	30	...	...
Chromium	0.24	...	2.0	8.5
Iron	0.80	...	13.8	16.6
Nickel	0.08	...	2.2	27.0
Oxide No. 2	4.95	100	...	...
Chromium	0.72	...	6.1	8.5
Iron	2.50	...	41.3	16.5
Nickel	0.24	...	6.5	27.0
Oxide No. 3	15.00	300	...	...
Chromium	2.25	...	19.1	8.5
Iron	7.45	...	122.9	16.5
Nickel	0.75	...	18.2	26.9

[a] Obtained by dividing weight of film by its area.
[b] Estimated from surface concentration by using bulk density.

sponding pure metals. Oxide films from stainless steels and related alloys apparently can be accurately analyzed by x-ray emission within the net-counting error itself, without making empirical or theoretical corrections for absorption deviations.

The dependence of specific radiation intensity on film thickness for films of nickel, iron, chromium, Type 304 stainless steel, and corresponding oxide films is plotted in Figure 6 to illustrate the influence of sample dispersion on absorption deviations. The critical thickness for the occurrence of significant x-ray deviations is observed to depend on film thickness and film material as one would predict (*8*).

A criterion of ±5% error may be assumed as reasonable for sampling errors characteristic of microanalysis. All errors from x-ray deviations observed for dispersed samples of pure metals, stainless alloys, and oxide films, for surface film densities up to 100 γ per sq. cm., fall within this value. For sufficiently dispersed metal, alloy, or oxide films, characteristic of this study, typical error from x-ray analysis is usually less than 2%.

Comparison of X-Ray and Chemical Data. A comparison of x-ray and chemical analytical data for thin films of austenitic stainless alloys, and for ultrathin oxide films isolated from their surfaces, was made using the calibration data obtained. The data, grouped in Table IV for comparison under the headings "x-ray" and "chemical," were determined independently of each other for at least two samples in each case. Although the x-ray data were determined on the basis of pure metal calibration, there is no significant correlation of the standard deviations with predictable absorption effects. This is attributed to the high state of dispersion of the samples (typical dispersion for any one component, 1 to 10 γ per sq. cm.). The uncertainty is actually greater for the chemical than for the x-ray data. This indicates the analytical advantage gained by the simpler sample preparation procedure characteristic of the x-ray measurements.

Comparison of x-ray to microcolorimetric data in general indicates excellent agreement. It is observed that the alloy compositions of the evaporated metal films are typical of austenitic stainless steels except that the silicon and chromium contents are somewhat on the high side. The data marked "oxide-1" are characteristic of oxide film associated with passivity of these steels in acids under oxidizing conditions. They are characterized by a definite depletion in iron and enrichment in the minor alloying elements, silicon, niobium, and molybdenum. Only microcolorimetric values are available for silicon. At the time this work was done, instrumentation did not permit the microdetermination of silicon by x-ray emission spectrography. The x-ray data for niobium and molybdenum are poor, because the excitation radiation used did not give sufficient intensity in the best wave-length range for these elements. In addition, use of a krypton-filled Geiger tube instead of an argon-filled Geiger tube would have been more appropriate for measurement of the $K\alpha$-wave lengths of niobium and molybdenum (*3*).

The compositional data for the thicker oxide films marked "oxide-2" show the compositional character of these films to be distinctly different from that characteristic of the ultrathin oxide films. Depletion in iron is associated with a marked enrichment in chromium. In addition, nickel is depleted and enrichment in the minor alloying elements is much less marked than for the oxide-1 films. A more complete discussion of the properties of oxide films on stainless steels is covered elsewhere (*11*). The data summarized in Table IV are presented to illustrate how x-ray analysis can be applied directly to the characterization of protective films on corrosion-resistant alloys. Distinctions between the two methods for the data in Table IV are masked by the over-all uncertainty in sampling, characteristic of both methods. For microgram samples, the observed typical standard deviation of 1 to 5% is considered reasonable. The calibration used in calculating the x-ray compositional data was

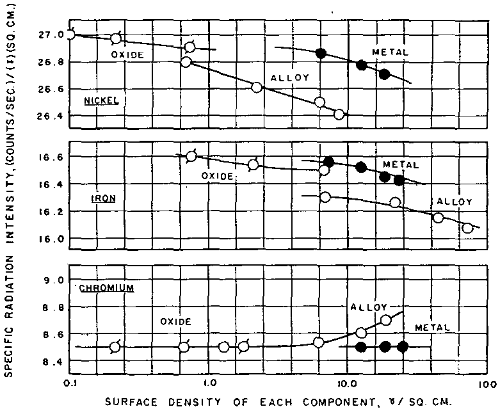

Figure 6. Dependence of specific radiation intensity on film thickness for nickel, iron, chromium, Type 304 stainless steel, and isolated oxide films

Table IV. Comparison of X-Ray and Chemical Analytical Data for Films of Austenitic Stainless Steels and for Oxide Films Isolated from Their Surfaces

Sample size, 25 to 100 γ

Nature of Film Sample	Analytical Method	Fe		Cr		Ni		Si		Nb		Mo	
		%[a]	Std. dev.	%	Std. dev.	%	Std. dev.	%	Std. dev.	%	Std. dev.	%	Std. dev
Type 304 alloy thickness, 300 A.	X-ray	70.5	0.5	20.2	0.2	8.7	0.1	...	...	...	...	...	...
	Chemical	69.6	1.2	20.6	0.7	9.0	0.1	1.0	0.1	...	...	...	...
Type 316 alloy thickness, 300 A.	X-ray	69.5	1.0	19.4	0.2	11.3	0.1	...	...	...	...	7.2	0.4
	Chemical	68.5	1.8	19.6	0.3	10.9	0.2	1.2	0.1	...	...	0.4	0.7
Type 347 alloy thickness, 300 A.	X-ray	70.6	2.0	18.2	0.2	9.8	0.1	...	...	...	...	...	...
	Chemical	69.4	2.1	18.8	0.6	10.2	0.2	0.9	0.1	0.2	...	0.5	0.2
Type 304 oxide—1 thickness, 30 A.	X-ray	37.2	4.0	13.4	2.0	9.4	1.2	...	...	...	...	...	...
	Chemical	34.0	3.1	14.0	3.4	10.7	4.0	9.9	2.0	...	...	...	...
Type 316 oxide—1 thickness, 30 A.	X-ray	42.1	3.2	12.6	0.7	4.9	0.6	...	...	...	...	(1.2)	...
	Chemical	45.0	4.2	13.0	2.0	5.4	2.1	14.5	3.2	...	...	4.2	1.1
Type 347 oxide—1 thickness, 30 A.	X-ray	45.4	4.2	17.7	3.0	7.7	0.8	...	...	(3.0)	...	(0.1)	...
	Chemical	44.5	5.0	20.7	3.2	8.2	2.6	12.9	3.2	5.7	1.0	0.4	0.1
Type 304 oxide—2 thickness, 300 A.	X-ray	15.0	2.0	4.55	3.7	3.8	0.2	...	...	...	...	...	...
	Chemical	15.1	2.6	46.6	5.0	4.0	0.5	3.3	0.1	...	...	...	...
Type 316 oxide—2 thickness, 300 A.	X-ray	25.0	3.2	36.8	3.1	5.0	0.3	...	...	...	...	(0.2)	...
	Chemical	25.2	4.0	36.1	4.3	5.2	0.8	4.0	0.3	...	...	1.6	0.3
Type 347 oxide—2 thickness, 300 A.	X-ray	24.8	2.8	36.1	2.1	2.2	0.1	...	...	...	...	...	...
	Chemical	25.0	3.2	35.2	4.1	2.2	0.1	2.0	0.2	1.2	...	0.3	...

[a] Per cent by weight.

based on the specific radiation intensities obtained for pure metals. For microanalytical systems with smaller sampling errors, use of specific radiation intensities calculated from the corresponding alloy or oxide standards would be preferred.

APPLICATIONS TO MICROANALYSIS

Application of this technique is not limited to microanalysis of corrosion films and residues, but may be applied to routine analysis of experimental alloys or other metallic materials using suitably dispersed samples. An evaluation of the relationship between absorption deviations and the state of sample dispersion for each new system would determine the minimum sample dispersion required for the achievement of a given analytical uncertainty. Once this is done, need for carefully tailored standards may be eliminated in many cases.

In addition to the counting uncertainties characteristic of low intensity x-ray measurements, special care is required for adequate correction of blank and background errors peculiar to highly dispersed samples. Improvement in x-ray tubes and radiation detectors could extend microanalytical applications considerably beyond that described here (*4*). Finally, this particular application is most suitable for the analysis of dispersed samples where errors from absorption deviations are to be minimized. For trace analysis of large samples, a completely different instrumental approach is indicated (*4*).

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Flame Spectra of Twenty Metals Using a Recording Flame Spectrophotometer

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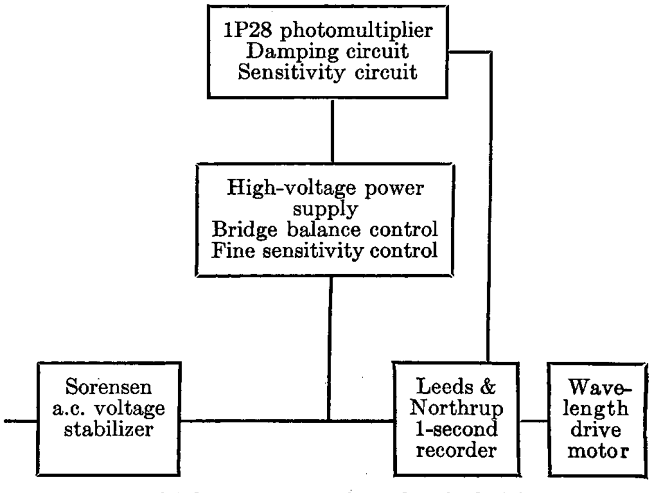
One phase of the Bureau of Mines study of distillate-fuel storage stability required determination of a large number of metals present in trace quantities in these fuels. A Beckman Model DU flame spectrophotometer was modified for this work by installing an automatic wave length drive and a recorder, which reduced the time needed for analysis to about 10 minutes and the sample requirements to 10 ml. These modifications are described, and spectrograms of 20 metals are given with tables of the principal flame lines and an estimated detection limit for each of the metals. The data presented can be used in planning the analysis for any particular metal or combination of metals, as well as aiding the identification of lines in flame spectra. A method of analysis for metals in distillate fuels is briefly outlined.

THE need for determining metals in trace quantities, both qualitatively and quantitatively, in Diesel fuels was the motive for investigating a flame spectrophotometric method in this laboratory. Most metals present in these oils were present in concentrations of less than 1 p.p.m., and it was necessary to concentrate them to within the detection limit of the instrument. A dry-ashing technique was used as a means of concentration, but often the amount of ash obtained provided only a few milliliters of aqueous solution in sufficient concentration for analysis. The use of a recording technique was especially advantageous as the flame spectrum of a sample could be scanned in a few minutes, using about 10 ml. of solution, and providing a permanent record suitable for qualitative analysis. Study of these records frequently revealed unexpected elements that might have been overlooked using a visual scanning technique. An estimate of the quantity of each element present was made, which saved time if subsequent quantitative measurements were necessary.

INSTRUMENT MODIFICATION

The instrument used in this work was a modified Beckman DU flame spectrophotometer. The electrical modifications consisted of the replacement of the Beckman phototube with a photomultiplier phototube and its associated power supply; the incorporation of a Sorensen voltage stabilizer; a Leeds & Northrup adjustable zero adjustable range Speedomax having a 1-second full-scale pen travel; and a wave-length drive. The following block diagram shows the arrangement of these components in the circuits:



The photomultiplier power supply and cathode follower circuit are shown in Figure 1. This circuit is essentially that described by King and Priestly (*5*), with modifications. The circuit changes consisted of the addition of 5000-ohm series resistors (R_{47}, R_{48}, Figure 1) in the high-voltage section and the omission of two condensers in the cathode follower circuit. Two additional sensitivity ranges were obtained by adding a 1.0- and a 1.5-megohm resistor, R_{45} and R_{46}. To obtain more stable operation, a 6074 voltage-regulator tube was substituted for the VR105 and a 5692 tube for the 6SN7. It was unnecessary to change the bridge balance (R_{21}, R_{29}), or sensitivity control (R_{28}) of the photomultiplier power-supply circuit after the initial settings were made. It was more convenient to use the calibrated zero and the 1- to 20-mv. range adjustment of the recorder. The wave-length drive–synchronous motor was wired in parallel with the recorder chart motor, so that the wave-length drive and chart started simultaneously; in addition, a marking pen on the recorder, operated by a manually controlled microswitch, provided an index point. A wave-length scale was prepared by operating the microswitch at definite wave-length intervals, with the recorder and wave-length drive in operation and the reference marks on the chart numbered with the appropriate wave lengths. The scales were sprayed with plastic and trimmed to give strips 1 inch wide, which were placed along sample spectra to identify wave lengths of the principal peaks.

The equipment is shown in Figure 2. Just below the recorder is the photomultiplier power supply with its cables to the photomultiplier housing on the spectrophotometer which replaces the conventional phototube housing. The panel just below the photomultiplier supply is not associated with the flame spectrophotometer. Below this unit is the Sorensen voltage stabilizer.

The mechanical modification consisted of addition of an automatic wave-length drive powered with a 117-volt, 60-cycle, 20-r.p.m. synchronous motor, in combination with a 100 to 1 worm-gear reducer, as shown in Figure 3. This rotated the wave-length shaft at 0.2 r.p.m., whereas the recorder paper was being driven 2.00 inches per minute. The spring tension on the wave-length dial–cam follower was decreased so that the dial rotated readily. The worm gear was installed on the wave-length drive shaft and driven by the worm, which was mounted on an extension of the motor shaft. The worm and worm gear were engaged or disengaged by pivoting the motor and shaft, and a friction catch held the motor in either position. The Gleason-Avery synchronous motor can be readily replaced using a different speed unit when desired.

The burner was a standard bore 4020 hydrogen atomizer-burner. Pressures were set to obtain optimum operating conditions. These pressures were 10 pounds per square inch of oxy-

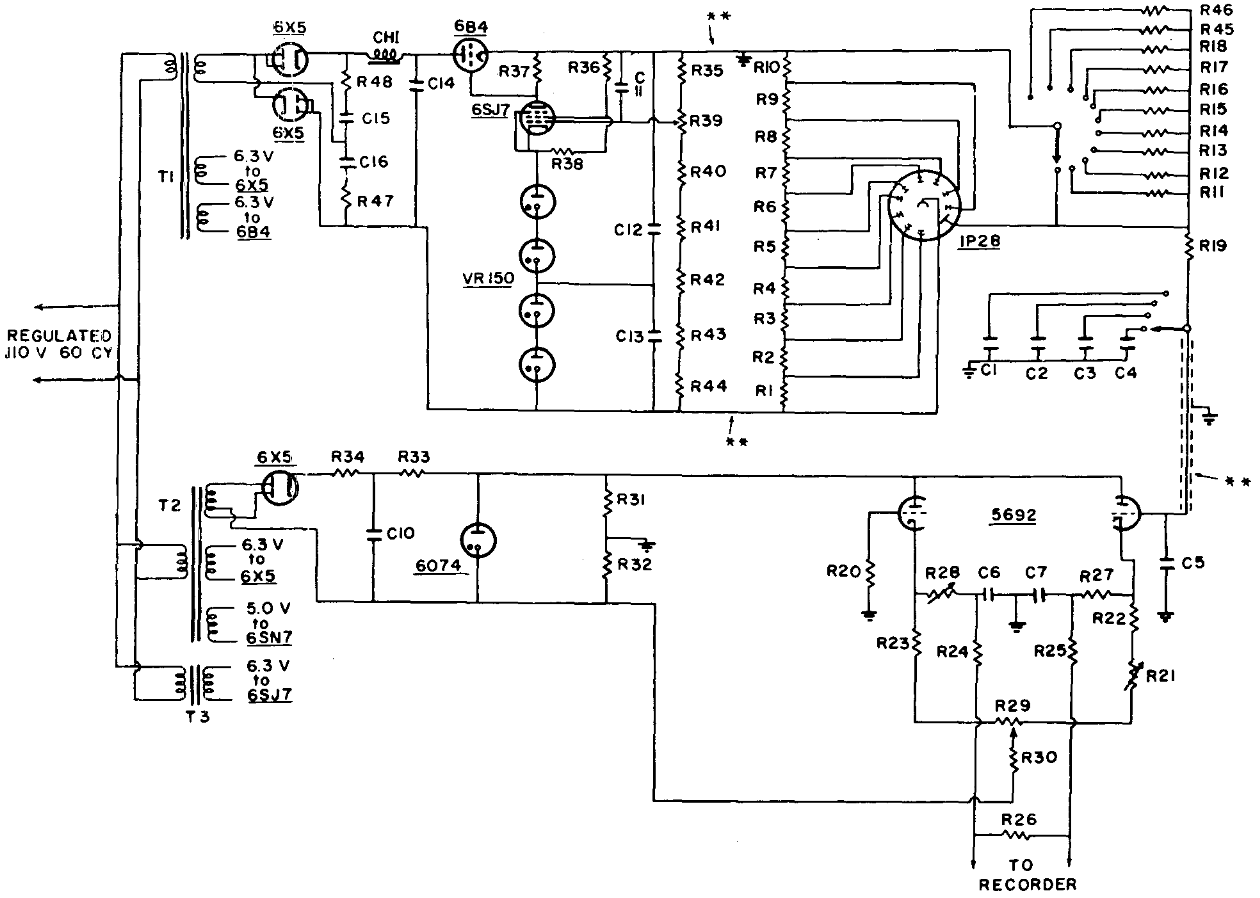


Figure 1. Electronic circuit for recording flame spectrophotometer

C_1	0.2 mfd. 600 volts
C_2	0.1 mfd. 600 volts
C_3, C_{11}	0.05 mfd. 600 volts
C_4	0.01 mfd. 600 volts
C_5, C_{12}, C_{13}	0.01 mfd. mica
C_6, C_7	50 mfd. 50 volts electrolytic
C_{10}	4 mfd. 300 volts electrolytic
C_{14}	4 mfd. 1500 volts oil
C_{15}, C_{16}	4 mfd. 1000 volts oil
R_1 to R_{10}	22,000 ohms, 1 watt
R_{11}	75,000 ohms, 1/2 watt
R_{12}	100,000 ohms, 1/2 watt
R_{13}	150,000 ohms, 1/2 watt
R_{14}	200,000 ohms, 1/2 watt
R_{15}	300,000 ohms, 1/2 watt
R_{16}	400,000 ohms, 1/2 watt
R_{17}	600,000 ohms, 1/2 watt
R_{18}	800,000 ohms, 1/2 watt
R_{19}, R_{20}	3.3 megohms
R_{21}	5000 ohms potentiometer (coarse balance)
R_{22}	2700 ohms, 1 watt
R_{23}	6800 ohms, 1 watt
R_{24}, R_{25}, R_{27}	5000 ohms, 1 watt
R_{26}	500 ohms, 1 watt (recorder connection)
R_{28}	5000 ohms (fine sensitivity potentiometer)
R_{29}	1000 ohms (fine balance potentiometer)
R_{30}	50,000 ohms, 1 watt
R_{31}	15,000 ohms, 1 watt
R_{32}	22,000 ohms, 1 watt
R_{33}, R_{34}	5000 ohms, 4 watts
R_{35}, R_{38}	20,000 ohms, 2 watts
R_{36}	30,000 ohms, 1 watt
R_{37}	500,000 ohms, 1 watt
R_{39}	20,000 ohms, high-voltage adjustment potentiometer
R_{40} to R_{44}	10,000 ohms, 1 watt
R_{45}	1.0 megohm, 1/2 watt
R_{46}	1.5 megohms, 1/2 watt
R_{47}, R_{48}	5000 ohms, 10 watts
T_1, T_2	Stancor P6010 (PC 8406 substituted)
T_3	6.3-volt filament transformer
CHI	15-henry, 75 ma.
**	Cables to photomultiplier housing

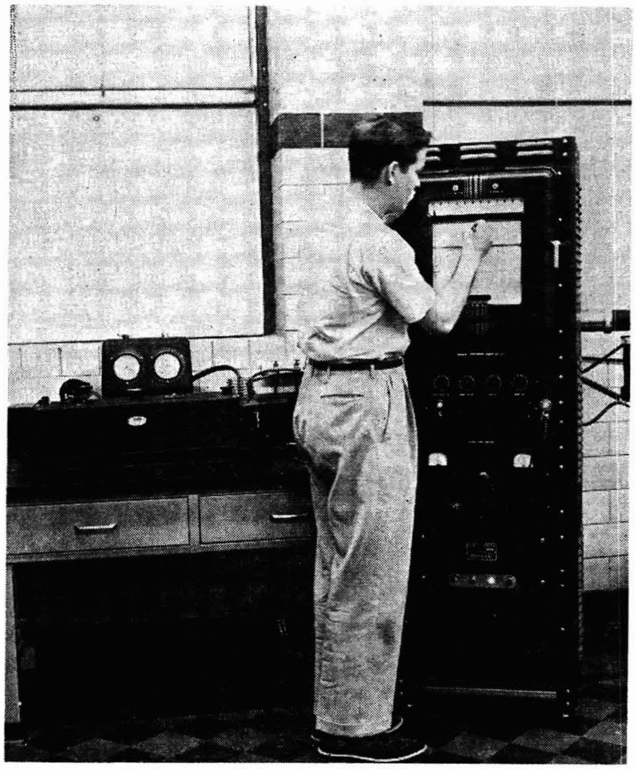


Figure 2. Recorder, power supply, and wave-length drive attached to Beckman DU flame spectrophotometer

gen and 1 pound per square inch of hydrogen, which correspond to 3 liters per minute of oxygen and 12 liters per minute of hydrogen.

SENSITIVITY AND RESOLUTION

No direct comparison of the modified flame spectrophotometer sensitivity with the instrument was made before modification, but the detection limits as determined with the modification are in fair agreement with those published by Beckman (*1*) for the DU flame spectrophotometer with the photomultiplier attachment. Stability with the described modification is good, as

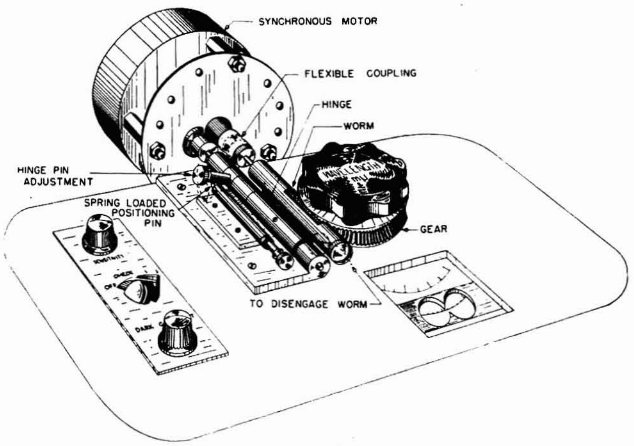


Figure 3. Wave-length drive

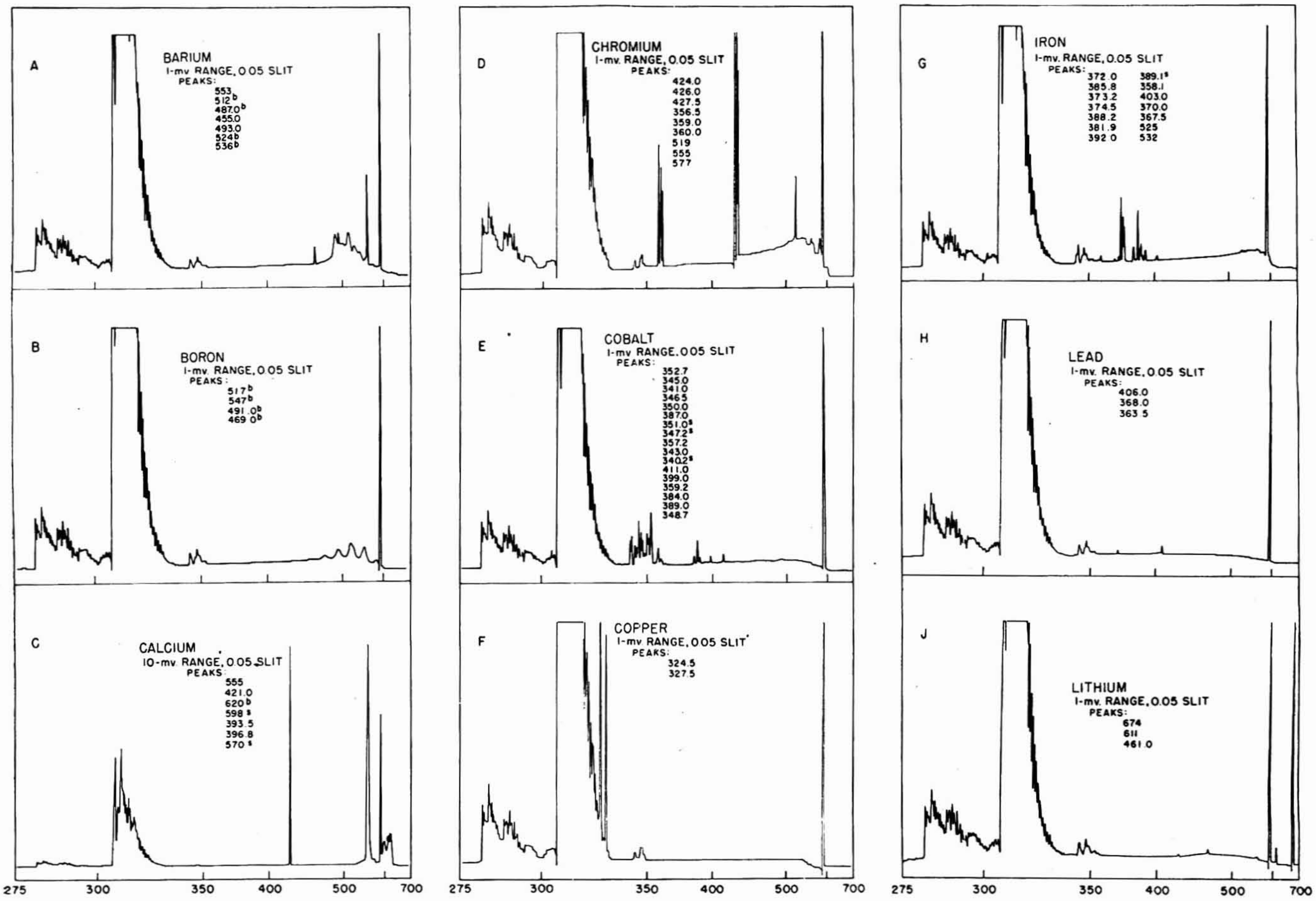


Figure 4. Flame spectra

Table I. Flame Spectra of Aqueous Solutions of Metal Salts

(Metal concn. approximately 100 p.p.m. Beckman flame spectrophotometer with recording attachment)

Peak Wave Length, mμ	Element	Intensity[a] Number	Peak Wave Length, mμ	Element	Intensity[a] Number	Peak Wave Length, mμ	Element	Intensity[a] Number	Peak Wave Length, mμ	Element	Intensity[a] Number
279.5	Mn	[b]	389.4	Co	13	356.6	Ni	8	518.0	Ti	1[c]
279.8	Mn	4	389.6	Fe	8	357.5	Co	7	518.0	B	1[c]
285.2	Mg	1	392.0	Fe	7	357.9	Cr	4	519.3	Mn	5[c]
300.2	Ni	10	393.4	Ca	5	358.1	Fe	9	520.4	Cr	7
305.1	Ni	11	396.8	Ca	6	359.3	Cr	5	524.1	Ba	6[c]
324.8	Cu	1	399.5	Co	10	359.5	Co	11	527.0	Fe	13
327.4	Cu	2	402.8	Pb	4	359.8	Ni	14	528.0	V	3[c]
328.1	Ag	1	403[d]	Fe	10	360.5	Cr	6	532.8	Fe	14
330.2	Na	2	403.1	Mn	1	361.0	Ni	[b]	534.7	Ba	7[c]
337.0	Ni	9	404.4	K	1	361.9	Ni	5	535.0	Tl	2
338.1	Ni	13	405.8	Pb	1	364.0	Pb	3	539.2	Mn	3[c]
338.3	Ag	2	407.8	Sr	3	368.2	Fe	12	545.0	B	2[c]
339.3	Ni	6	411.1	Co	9	368.3	Pb	2	546.9	V	1[c]
340.5	Co	[b]	420.2	Rb	1	370.6	Fe	11	553[d]	Sr	5
341.2	Co	3	421.6	Sr	4	371.0	Mg	3[c]	553.6	Ba	1
341.5	Ni	2	421.6	Rb	2	372.0	Fe	1	554.4	Ca	1[c]
343.2	Co	8	422.7	Ca	2	373.7	Fe	3	555[d]	Cr	8
344.6	Ni	7	425.4	Cr	1	374.6	Fe	4	561.0	Mn	2[c]
345.4	Co	2	427.5	Cr	2	377.6	Tl	1	570[d]	Ca	7[b]
346.2	Ni	3	429.0	Cr	3	380.7	Ni	15	573.7	V	2[c]
346.6	Co	4	455.4	Ba	4	381.6	Fe	6	577	Cr	9
347.4	Co	[b]	460.3	Li	3	383.0	Mg	2[c]	589.0	Na	1
348.8	Co	14	460.7	Sr	1	384.5	Co	12	598[d]	Ca	4[b]
349.3	Ni	4	471.0	B	4[c]	385.8	Ni	12	606.0	Sr	2[c]
350.6	Co	5	485.1	Ba	3[c]	386.0	Fe	2	610.4	Li	2
351.5	Ni	[b]	493.0	B	3[c]	387.3	Co	6	620.3	Ca	3[c]
351.3	Co	[b]	493.4	Ba	5	388.6	Fe	5	662.8	Sr	6[c]
352.5	Ni	1	500[d]	Mn	6[c]				670.8	Li	1
353.0	Co	1	513.9	Ba	2[c]						

[a] No. 1 denotes most intense or persistent line for that metal.
[b] Shoulder on another peak.
[c] Band.
[d] Measured values, corresponding values were not found in tables.

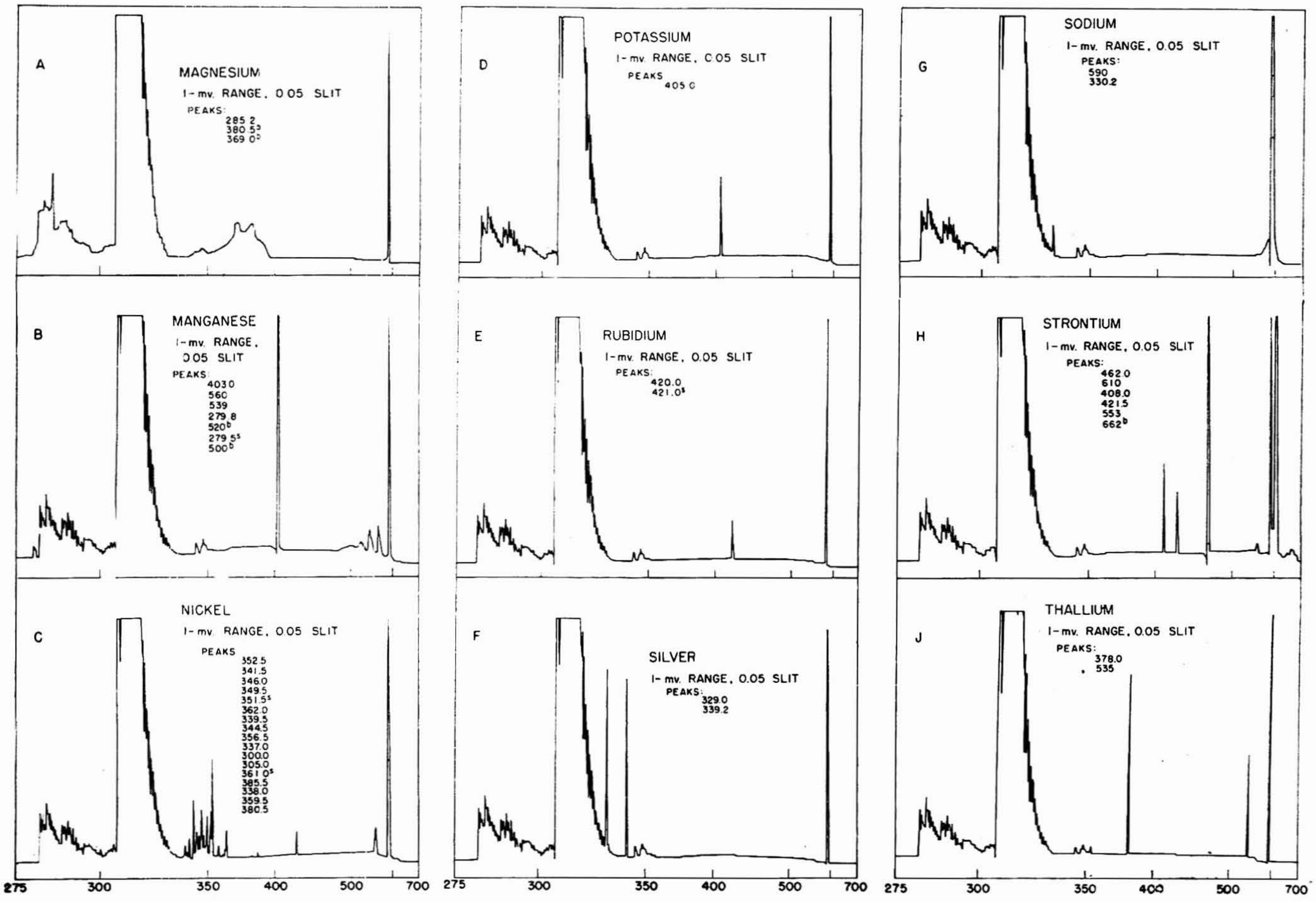


Figure 5. Flame spectra

indicated by zero drift. The resolution is good at 0.05-mm. slit width and permits detection of a smaller quantity of most elements than at 0.1-mm. slit width. Part of this improvement results from the decreased background at narrower slit widths.

FLAME SPECTRA OF METALS

For preliminary investigation 27 metals were used. These elements were selected because they have been reported among those occurring naturally in crude oils. Where possible, the chloride salts of these metals were selected for standard solutions; 20 compounds were chlorides and the remainder were nitrates, sulfates, and acetates. Using distilled water, a standard solution containing 500 to 2000 p.p.m. of the metal was made, and from these stock solutions weighed dilutions were made to 100, 50, and 10 p.p.m. of the metal.

The solutions containing 100 p.p.m. metal were burned and scanned from 700 to 275 mμ, using a 20 r.p.m. wave-length drive motor and a slit width of 0.05 mm. The spectrum of each element was compared with that of distilled water, and the wave length of the lines and bands characteristic of the element or molecule were measured in millimicrons. The heights of the more persistent peaks were recorded in millivolts. Figures 4, 5, and spectrograms *A* and *B* of Figure 6 present reproductions of these charts for the 20 metals that yielded flame spectra in this range and at these concentrations.

Table I is a tabulation of lines and bands found for the 27 elements in order of increasing wave length. The number in column 3 opposite the element indicates the strength of that line compared to other lines found for the same element—No. 1 being the strongest line. Unless otherwise indicated the wave-length values are literature values (*4*, *6*) which for the most part are in good agreement with experimental values of this laboratory, as shown in Table II.

Dilutions containing 10 p.p.m. of metal were tested, using a slit width of 0.10 mm. Although the intensity of lines for some elements was increased, a more irregular background made qualitative identification for many of the metals less certain. Because of this, and to facilitate comparison, all measurements reported in this paper were made at 0.05-mm. slit width.

The intensities of the principal lines for each element were measured, using both a rapid scanning and a manual technique. These measurements were made for three dilutions of each standard solution containing 100 to 10 p.p.m. of the metal. However, for sodium it was necessary to reduce these concentrations to 1.0, 0.5, and 0.1 p.p.m. because of the high intensity of the 589.0 sodium peak. To measure the heights of the lines and narrow bands, a base-line measuring technique was used. This involved averaging the background height on either side of the peak base and measuring from this point to the peak crest. For the wide bands of vanadium, boron, and titanium a measurement was made from the background on the high wave-length side (685 mμ) to the top of the band heads. These data are presented in Table II along with detection limits for the most persistent line or band of each metal. An empirical approach to calculating minimum detectable concentrations for each metal was attempted. The principal peak for each element tested was examined carefully

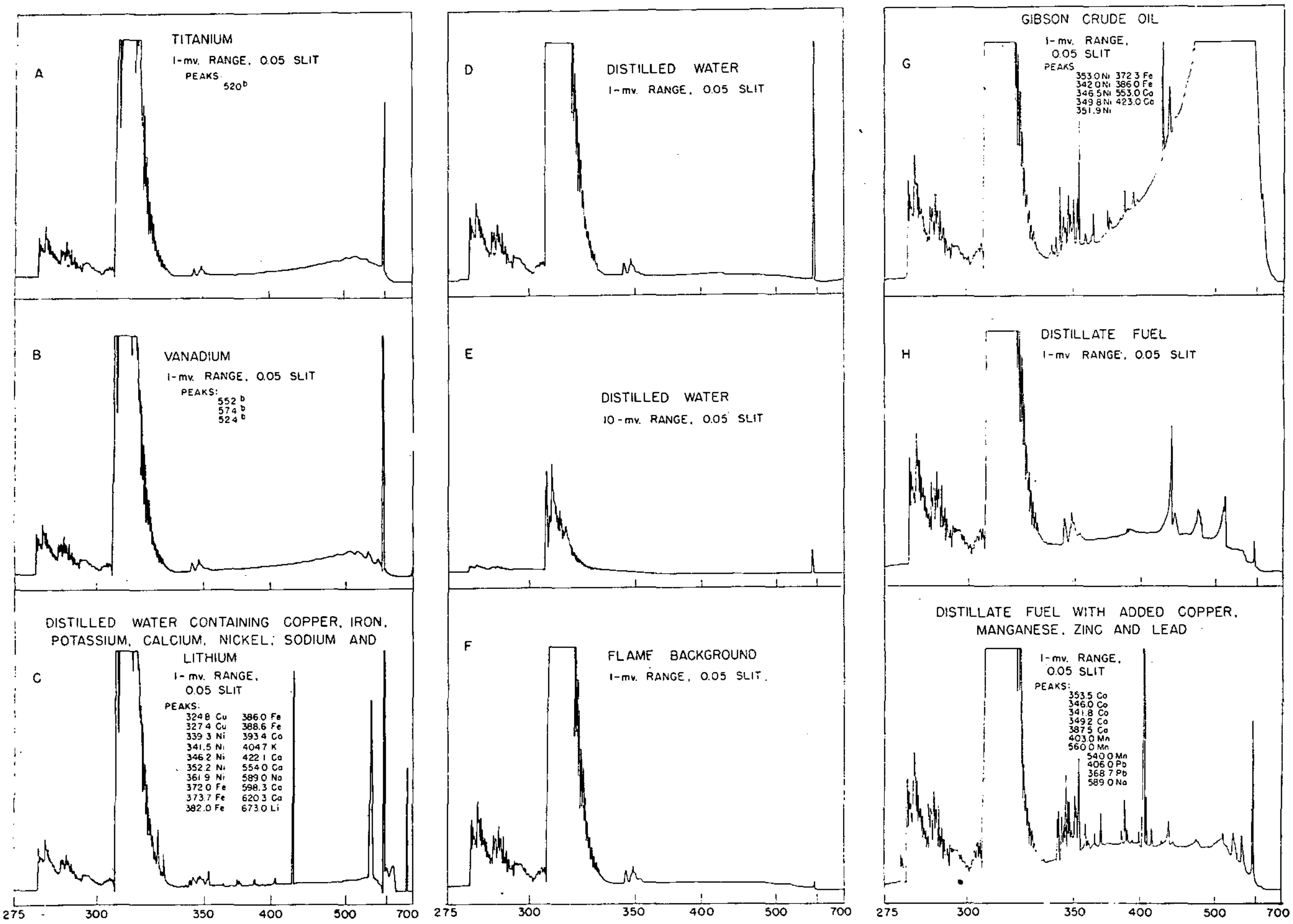


Figure 6. Flame spectra

Table II. Wave Lengths and Detection Limits for Various Elements Using Beckman Flame Spectrophotometer with Recording Attachment

Element	Compound Used	Wave Length of Observed Peak, mμ		Nearest Emission Line from Tables (4)	Element Concn., P.P.M.	Peak Height, Mv.		Element Concn., P.P.M.	Peak Height, Mv.		Element Concn., P.P.M.	Peak Height, Mv.		Detection Limits, P.P.M.	
		Rapid scanning	Manual			Rapid scanning	Manual		Rapid scanning	Manual		Rapid scanning	Manual	Rapid scanning	Manual
Aluminum	$AlCl_3.6H_2O$	None			103.2										
Antimony	$SbCl_3$	None			104.6										
Barium	$BaCl_2.2H_2O$	553	553.6	553.6	100.2	0.611	0.734	50.0	0.320	0.379	10.1	0.079	0.093	1.3	1.1
		512^a		513.9^a (6)			0.102		0.066			0.011			
		487.0^a		485.1^a (6)											
		455.0		455.4											
		493.0		493.4											
		524^a		524.1^a (6)											
		536^a		534.7^a (3)											
Bismuth	$Bi(NO_3)_3 5H_2O$	None			103.0										
Boron	H_3BO_3	517^a	518^a	518.0^a (6)	98.2	0.186	0.175	49.7	0.103	0.102	9.8	0.042	0.042	25.0	10.
		547^a		545.0^a (6)		0.163			0.089			0.038			
		491.0^a		493.0^a (6)		0.135			0.083						
		469.0^a		471.0^a (6)		0.097			0.061						
Calcium	$CaCl_2$	555	555	554.4^a (3)	98.4	10.54	12.20	49.5	5.08	6.11	10.5	1.254	1.420	0.25	0.22
		421.0	422.5	422.7		7.60	9.68		4.17	5.48		1.026	1.356	0.10	0.08
		620^a		620.3^a (3)		1.68			0.900			0.196			
		598^b													
		393.5		393.4		0.098			0.090						
		396.8		396.8		0.050			0.042						
		570^b													
Chromium	$CrCl_3.6H_2O$	424.0	425.1	425.4	102.9	0.714	0.943	50.2	0.366	0.495	10.3	0.067	0.100	1.5	1.0
		426.0		427.5		0.604			0.312			0.057			
		427.5		429.0		0.404			0.208			0.036			
		356.5	357.9	357.9		0.296	0.388		0.152	0.203		0.021	0.041	4.9	2.5
		359.0		359.3		0.223			0.112			0.011			
		360.0		360.5		0.166			0.083			0.005			
		519		520.4											
		555													
		577													
Cobalt	$CoCl_2.6H_2O$	352.7	352.8	353.0	99.2	0.316	0.403	49.5	0.157	0.202	10.3	0.030	0.049	6.9	4.2
		345.0	345.2	345.4		0.229	0.336		0.105	0.162		0.021	0.022	14.7	14.0
		341.0		341.2		0.182			0.087						
		346.5		346.6		0.139			0.061						
		350.0		350.6											
		387.0		387.3											
		351.0^b		351.3											
		347.2^b		347.4											
		357.2		357.5											
		343.0		343.2											
		340.2^b		340.5											
		411.0		411.1											
		399.0		399.5											
		359.2		359.5											
		384.0		384.5											
		389.0		389.4											
		348.7		348.8											
Copper	$CuCl_2.2H_2O$	324.5	324.8	324.8	99.9	1.054	1.340	50.2	0.740	0.944	10.9	0.221	0.300	1.5	1.1
		327.5	327.5	327.4		0.946	1.162		0.520	0.719		0.147	0.207	2.0	1.4
Iron	$FeCl_3.6H_2O$	372.0	372.0	372.0	204.8	0.962	1.146	101.3	0.500	0.617	74.6	0.376	0.472		
		385.8	385.8	386.0		0.830	1.050		0.427	0.554		0.319	0.429		
		373.2		373.7		0.774			0.397			0.300			
		374.5		374.6		0.590			0.299			0.225			
		388.2		388.6											
		381.9		381.6											
		392.0		392.0											
		389.1^b		389.6											
		358.1		358.1											
		403.0													
		370.0		370.6											
		367.5		368.2											
		525		527.0											
		532		532.8											
		372.0	372.0	372.0	50.4	0.260	0.321	25.1	0.116	0.169				2.2	1.5

Iron (*Continued*)	$FeCl_3.6H_2O$	385.8 373.2 374.5	385.8	386.0 373.7 374.6		0.216 0.200 0.148	0.289		0.110 0.098 0.075	0.149				2.3	1.7
Lead	$PbCl_2$	406.0 368.0 363.5	406.0	405.8 368.3 364.0	99.4	0.054 0.035 0.008	0.079	51.5	0.023 0.018	0.038	11.7	None	0.008	23	14.
Lithium	LiCl	674 611 461.0	674	670.8 610.4 460.3	99.8	1.564 0.066 0.028	2.028	28.3		1.219	17.1		0.872	0.24	0.18
			674	670.8	11.4	0.466	0.618	5.8		0.367					
Magnesium	$MgCl_2.6H_2O$	285.2	285.2	285.2	99.5	0.236	0.307	50.0	0.170	0.222	10.1	0.079	0.104	2.6	1.9
		380.5^a		383.0^a (*1*)											
		369.0^a	371.0^a	371.0^a (*1*)		0.435			0.205			0.035			
Manganese	$MnCl_2.4H_2O$	403.0 460 539 279.8 520^a 279.5^b 500^a	403.2	403.1 561.0^a (*6*) 539.2 (*3*) 279.8 519.3^a (*3*) 279.5	98.7	8.79 0.267 0.179 0.061	10.887	50.3	4.82 0.124 0.090 0.040	5.98	10.5	1.054 0.020 0.013 0.005	1.318	0.10	0.08
Molybdenum	MoO_3	None			108.5										
Nickel	$NiCl_2.6H_2O$	352.5 341.5 346.0 349.5 351.5^b 362.0 339.5 344.5 356.5 337.0 300.0 305.0 361.0^b 385.5 338.0 359.5 380.5	352.5 341.5	352.5 341.5 346.2 349.3 351.5 361.9 339.3 344.6 356.6 337.0 300.2 305.1 361.0 385.8 338.1 359.8 380.7	212.2	1.306 0.812 0.624 0.540	1.686 1.026	94.1	0.747 0.451 0.325 0.277	0.898 0.554	72.2	0.585 0.351 0.248 0.209	0.781 0.473	2.2	1.6
		352.5 341.5 346.0 349.5	352.5 341.5	352.5 341.5 346.2 349.5	48.6	0.409 0.248 0.177 0.146	0.552 0.335	24.3	0.221 0.125 0.088 0.073	0.300 0.178					
Potassium	KCl	405.0	404.5	404.4	100.5	0.644	0.865	50.3	0.305	0.384	11.4	0.048	0.069	2.4	1.7
Rubidium	RbCl	420.0 421.0^b	420.0 421.5^b	420.2 421.6	100.2	0.400	0.547 0.166	50.3	0.174	0.224 0.062	11.5	0.020	0.028	5.8	4.1
Silver	$AgNO_3$	329.0	328.1	328.1	100.9	0.818		50.5	0.571		11.2	0.222		1.0	
		339.2	338.3	338.3		0.960	1.220		0.602	0.792		0.192	0.246	0.58	0.46
Sodium	NaCl	590 330.2	589	589.0 330.2	0.98	7.77	10.49	0.50	4.15	5.05	0.11	1.03	1.19	0.0011	0.0009
Strontium	$SrCl_2.6H_2O$	462.0 610 408.0 421.5 553 662^a	462.0	460.7 606.0^a (*3*) 407.8 421.6 662.8^a (*3*)	99.4	10.06 2.76 0.375 0.245 0.060	13.90	12.5		1.913	10.0	1.084 0.260 0.056 0.040	1.450	0.09	0.07
		462.0	462.0	460.7	5.1		0.776	1.96		0.292	1.00		0.118		
Thallium	TlCl	378.0 535	377.8	377.6 535.0	99.6	1.216 0.663	1.608	49.2	0.580 0.322	0.796	10.9	0.128 0.062	0.175	0.85	0.62
Tin	$SnCl_2.2H_2O$	None			99.3										
Titanium	$TiCl_4$	520^a	518^a	518^a (*1*)	101.1	0.233	0.222	50.1	0.122	0.122	10.1	0.043	0.043	50.	10.
Uranium	$UO_2(NO_3)_2.6H_2O$	None			133.5										
Vanadium	$VOSO_4.2H_2O$	552^a 574^a 524^a	548^a	546.9^a (*6*) 573.7^a (*6*) 528^a (*1*)	100.0	0.185 0.140 0.200	0.177	50.4	0.100 0.072 0.107	0.105	10.6	0.048	0.055	10.	10.
Zinc	$Zn(CH_3COO)_2.2H_2O$	None			111.0										

^{a} Band.
^{b} Shoulder.

and the nature of the background in that particular portion of the spectrum noted. Because of an irregular background in the wave-length region of cobalt, nickel, copper, magnesium, and silver, it was doubtful that these elements could be identified qualitatively in an unknown sample unless peak heights of 0.02 to 0.03 mv. were obtained. For such elements as boron, titanium, and vanadium, where only broad bands were present in the recorded spectrum, even greater concentrations were necessary before a distinction could be made among the three elements. Other metals, such as calcium, chromium, iron, lead, lithium, manganese, potassium, rubidium, sodium, strontium, thallium, and barium, could be identified if peaks in the order of 0.01 mv. were obtained. Using this method, detection limits were calculated for 20 metals by assuming a straightline relationship among concentrations from 0 to 10 p.p.m. metal and peak height, as measured by rapid scanning. The final column is detection limits, calculated to show the advantage of a manual measuring technique. These values were calculated using the same minimum detectable peak heights as above and the height of the principal peaks at 10 p.p.m., as measured manually.

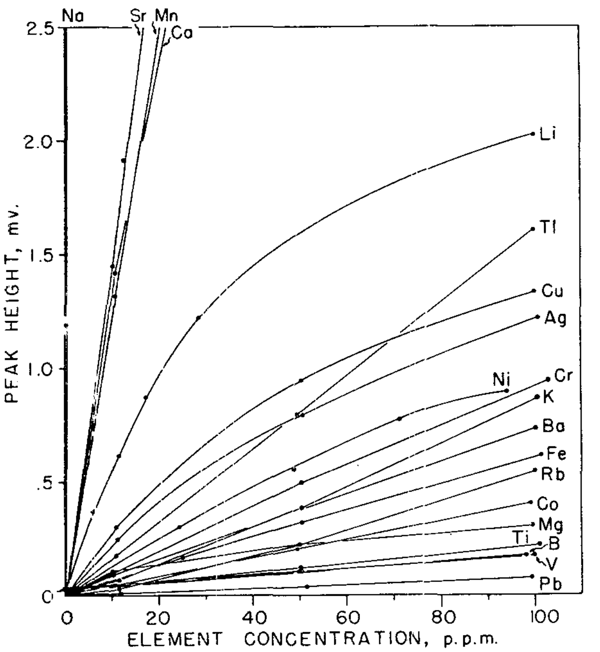


Figure 7. Concentration *vs.* intensity data for 20 metals

The data reported were obtained, using a IP28 phototube and the coarse sensitivity setting on position R_{18} (800,000 ohms). The phototube has now been replaced, and the new one has a sensitivity 2.5 times that of the initial tube. Using this phototube and the sensitivity on R_{46} (1.5 megohms), a fourfold increase in over-all sensitivity can be obtained. Because of the above, it can safely be assumed that the detection limits tabulated in Table II can be lowered by a factor of approximately 4. These detection limits may also be lowered even more by selection of the optimum slit widths for each element. However, the factor of enhancement or interference by other compounds will certainly need to be considered in applying these detection limits to an actual analysis.

In Figure 7 the relationship between concentration and intensity of principal lines for 20 metals is shown graphically. Here it will be noted that intensity is essentially a linear function of concentration for sodium, strontium, manganese, thallium, chromium, barium, iron, cobalt, titanium, vanadium, boron, and lead. Principal peaks of other metals such as lithium, copper, calcium, silver, nickel, and magnesium, tend to lose intensity as concentration increased up to 100 p.p.m. Still others, such as potassium and rubidium, show an opposite tendency of increased sensitivity with increased concentration.

An attempt was made to incorporate lithium or strontium as an internal standard in obtaining these data. Known quantities of the internal standard were added to aqueous samples of other metals, and a calibration curve was obtained by plotting the ratio of the peak height of the standard to the peak height of the metal versus concentration. The internal standard method proved unsatisfactory, because of nonlinearity of curves, interferences, and inconvenience of handling. An alternative technique was adopted for burning an aqueous solution containing 12 p.p.m. of strontium two or three times each day. The peak at 460.7 mμ was used for checking the operation of the burner and the electrical circuits. A constant peak height was maintained by cleaning the burner or, when necessary, by varying the high-voltage adjustment in the electronic circuit. By making such adjustments to maintain constant operating conditions, the analysis of a standard solution was duplicated over a period of several weeks, with an average deviation from the known value of ±2.0%.

Spectrograms *G*, *H*, and *J* of Figure 6 show examples of spectrograms obtained when hydrocarbons are burned. Spectrogram *G* of this figure was obtained by burning a crude oil diluted with an equal volume of chloroform. A spectrographic analysis previously made on this crude-oil sample showed that it contained about 80 p.p.m. of nickel, 25 p.p.m. of iron, and 23 p.p.m. of vanadium. Spectrogram *H* is that obtained when a thermally cracked distillate fuel is burned. The peaks in this curve are caused by the burning hydrocarbon. These are the Swan bands of carbon (C_2) at approximately 516.5, 513, 473.7, 471.5, 469.8, 488.5, 436.5, and 553. The prominent peaks at 431.5, 387, and 389 are very likely methine bands. The bands at 343 and 347 are probably hydroxyl and aldehyde bands, whereas those in the range 328 to 306 and 290 to 280 can be carbon monoxide, carbon dioxide, oxygen, ozone, nitric oxide, nitrogen, and hydroxyl. Spectrogram *J* of Figure 6 shows the same thermally cracked distillate fuel containing added 2-ethylhexoates in approximately the following amounts: 95 p.p.m. of cobalt, 100 p.p.m. of manganese, 65 p.p.m. of zinc, and 240 p.p.m. of lead.

Several samples of organometallic compounds were analyzed, using the flame spectrophotometer. These compounds were 2-ethylhexoates and naphthenates in hydrocarbon solvents and, except for their high concentration of a single metal, presented a similar problem to crude oils in their analysis. To determine the metal content of these compounds, acid digestion with sulfuric and nitric acids was used to break the hydrocarbons down to water-soluble sulfates. The residue of this digestion was diluted with a weighed quantity of distilled water and the resultant solution burned in the flame spectrophotometer. A slow speed (5 r.p.m.) motor was used to scan through the areas of the spectra that were of interest. The heights of the peaks were measured and compared to calibration data, which were established by acid digesting, in an identical manner, a known quantity of a metal sulfate and diluting to several different concentrations before burning in the spectrophotometer.

A similar analysis has been used for semiquantitative analysis of metals in some distillate fuels and crude oils. A wet-ashing procedure (*2*) was used to concentrate the metals in these oils. Equal weights of sample and sulfuric acid were combined in a Vycor beaker and cooked on a hot plate until the oil was completely charred. A muffle furnace was used to complete the ashing, and flame analysis was carried out on a water solution of this ash.

This information should be of value in planning the analysis for any particular element or combination of elements. The data given on hydrocarbons represent the start toward the analysis of a limited group of metals as they occur in distillate fuels.

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Spectrographic Determination of Nickel and Vanadium in Petroleum Products by Catalytic Ashing

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Amounts of nickel and vanadium at the level of 0.1 to 1000 p.p.m. can be rapidly determined in petroleum products by cracking the materials in the liquid phase on ground active silica-alumina catalyst. Nickel and vanadium sorbed on the catalyst by this procedure have the same spectro-emission characteristics as artificial standards prepared by the impregnation of the same support with dilute nickel nitrate and ammonium vanadate solutions. The spectrographic determination is made by blending the ignited support with a chromium oxide–graphite mixture, pelleting the blend, and sparking under controlled conditions. The repeatability of duplicated results is generally better than ±5%, and the average deviation between catalytic and conventional photometric results, determined on eight different kinds of oils, is within ±15%. For concentrations in the range of 10 p.p.m. only 2 to 5 grams of sample are necessary; the ashing process requires approximately 3 hours, and the spectrographic determination of the two elements an additional 1.25 hours.

THERE is still a need in the petroleum industry for rapid and reliable methods for the determination of traces of nickel and vanadium in petroleum products.

Accurate methods require, as a first step, the concentration of metallic compounds, and their transformation into inorganic products. This is done either by slow combustion (*1*, *3*, *4*, *7*) or by sulfuric acid charring (*5*, *6*) of the oil, followed by careful ignition of the coke. More reliable results are obtained by the sulfuric acid procedure. However, this concentration process is a slow operation, which can require many days when the sample has a low metal and a high paraffinic content.

The ashing process can be accelerated without the danger of losing metal by volatilization or mechanical entrainment, if the oil is heated with a cracking catalyst of the silica-alumina type which manifests a powerful adsorbing affinity for nickel and vanadium. Another advantage of this type of ashing is that such a catalyst can also act as an ash collector and a spectrographic buffer for subsequent analysis.

Murray and Plagge (*7*) have already advocated the addition of a small amount of inert material to the oil as an ash collector and as a spectrographic aid in the determination of nickel and vanadium in petroleum products. These authors obtained semiquantitative results by ashing the oil by dry oxidation after the addition of 0.02 weight % of silica or silica-alumina and by analyzing the ash by the rotating sector method.

This paper shows that trace amounts of nickel and vanadium in different types of oil can be rapidly determined with reasonable accuracy by ashing the oil with 7 to 50 weight % of silica-alumina which decomposes the sample catalytically, and by analyzing the ignited product spectrographically. This conclusion is obtained from a comparison of the nickel and vanadium content of a series of petroleum products determined simultaneously by spectrographic analysis after catalytic ashing and by photometric analysis after sulfuric acid ashing of the sample.

EQUIPMENT

Spectrograph, 1.5-meter ARL grating spectrograph.

Excitation source, ARL Multisource unit with a spark-ignited direct current discharge.

Densitometer, ARL projection microphotometer.

Developing, ARL thermostatic developing machine, and ARL film dryer.

Calculator, Dunn-Lowry calculation board.

Spectrophotometer, Beckman, Model DU.

REAGENTS AND STANDARDS

Reagents are all of analytical grade, conforming to the specifications of the Committee on Analytical Reagents of the AMERICAN CHEMICAL SOCIETY.

References to water concerns distilled water, demineralized by ion exchange, to a residual salt content of less than 0.2 p.p.m.

Silica-Alumina, very pure synthetic silica-alumina catalyst containing approximately 87% of silica and 13% of alumina, having an internal surface of approximately 200 square meters per gram. This material is ground in an agate mortar to a fineness comparable to talc. No quantitative amounts of nickel or vanadium were detectable by spectrographic or chemical analysis in this material.

Chromic Oxide, prepared by thermal decomposition of chromium nitrate. The nitrate is first fused and dehydrated in a borosilicate glass beaker by slow heating on hot plate. The dry product is then ignited with a blast burner in a porcelain dish for approximately 3 hours. The oxide is cooled in a desiccator and kept in a dry bottle.

Graphite Powder, spectrographically pure, suitable for briqueting. Can be prepared by finely grinding spectrographic graphite electrodes with a pencil sharpener reserved for that purpose.

Graphite–Internal Standard Mixture. A mixture of 5.000 grams of chromic oxide and 1.000 gram of spectrographic graphite powder is milled in a ball mill with hard ceramic balls for at least 48 hours.

Standard Stock Solutions. NICKEL SOLUTION, 0.4955 gram of nickel sesquihydrated nitrate dissolved in 1000 ml. of water. The nickel concentration of this solution is theoretically 100.0 mg. per liter. This concentration, checked photometrically by the dimethyl glyoxime method, was found equal to the theoretical value, within the limit of precision of the determination.

VANADIUM SOLUTION, 0.2296 gram of ammonium vanadate dissolved in 1000 ml. of water. The vanadium concentration of this solution is theoretically 100.0 mg. per liter. This concentration, checked photometrically by the phosphotungstic method, was found equal to the theoretical value, within the limit of precision of this determination.

Standard Working Solutions. These solutions are prepared by diluting the standard stock solution with water, just before preparation of the catalyst standards.

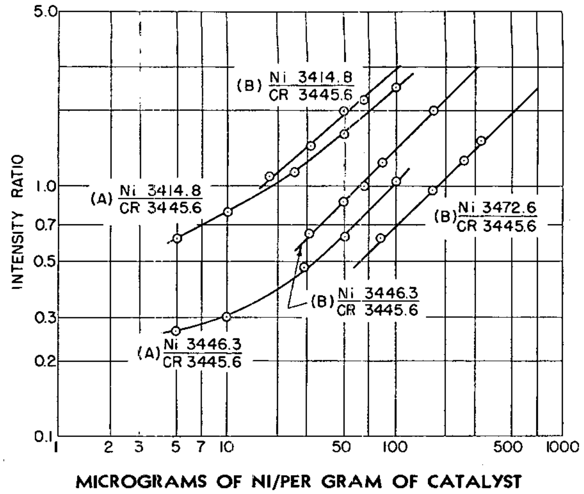

Figure 1. Analytical curves for nickel

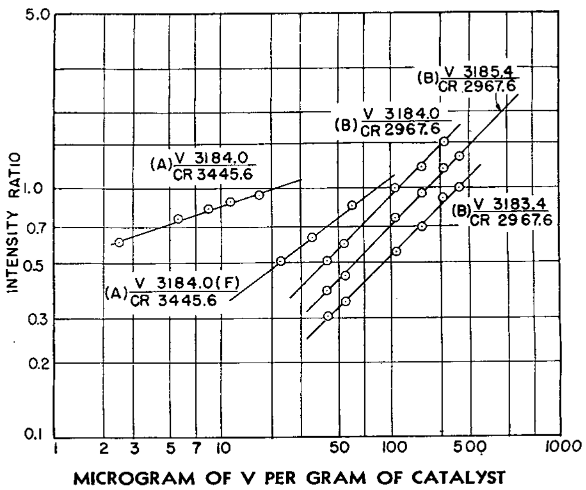

Figure 2. Analytical curves for vanadium

Catalyst Standards. The spectrographic standards are prepared by adding to 10.00 grams of active silica-alumina a known amount of nickel and vanadium from a dilute nickel nitrate and ammonium vanadate solution. The working solutions are adjusted so that 5 to 10 ml. of solution are added at one time. The slurry is stirred gently, and evaporated to dryness on a steam bath. Standards of higher concentration are prepared by repeating the impregnation process.

After the appropriate amount of metal has been added, the material adhering to the wall of the dish is washed with a small amount of water, and the slurry is stirred and again evaporated to dryness. This washing procedure is repeated three times, to ensure even distribution of the elements throughout the standard.

After drying the standard is thoroughly ground in an agate mortar and redried at 105° C. for 2 hours.

Pellets for Arcing. The ignited, metal-impregnated catalyst and the mixture of graphite–internal standard are weighed into a 1-ounce wide-mouthed screw-cap bottle in the proportions of 1 to 2. Eight $^1/_8$-inch stainless steel ball bearings are added to the mixture and the bottle is shaken for 1 minute to obtain a uniform distribution. The mix is pelleted in a small press. The die is $^3/_{16}$ inch in diameter, and $^7/_8$ inch deep. It is filled with the mix, and pelleted at a pressure of 3000 pounds per square inch gage.

The pellet is inserted in the crater of the lower electrode. It is cut flat at the rim of the crater with a steel razor blade.

SPECTROGRAPHIC TECHNIQUE

Excitation and Exposure

Type of sparking	High amperage, Type A	Low amperage, Type B
Capacitance, microfarads	60	60
Inductance, microhenries	480	480
Discharge as charge	0°	0°
Discharge point control	90°	90°
Initiator	Low power	Low power
Resistance, ohms	18	300
Output current, amperes	11.5	2.2
Voltage, volts	330	940
Voltage control	Off	On
Upper electrode, 0.242 inch	15° cone	15° cone
Lower electrode, 0.242 inch	$^3/_{16}$ × $^3/_{16}$ inch crater	$^3/_{16}$ × $^3/_{16}$ inch crater
Gap, mm.	6	4
Slit width, microns	30	40
Prespark, seconds	0	0
Exposure, seconds	20	60
Filter, % transmittance	2 for nickel 2 for vanadium	12 for nickel 4 for vanadium

Photography. Emulsion, Eastman Spectrum Analysis No. 1, 35-mm. film.

Development. Kodak D-19, rocked for 3 minutes. Short stop, 5% acetic acid for 10 seconds. Fixing, Eastman rapid x-ray fixer with hardener for 3 minutes. Washing, running water in large reservoir, for 5 minutes. Drying, blower and lamp heater, 1.5 minutes.

Calibration. With pure iron spectrum, photographed through a split field, using the two-line method of Churchill (*2*).

Densitometry. Transmittance measurements for analytical line pairs are converted into intensity ratios using the calculating board. These ratios are then converted into percentages of nickel or vanadium using analytical curves established from silica-alumina standards containing known amounts of these metals.

Analytical Curves. The analytical curves, represented by Figures 1 and 2, give the relation between the intensity ratios of the nickel or the vanadium line to the reference chromium line, and the amount of metal added to the catalyst, for the two types of excitation conditions.

Each experimental point of the curve is the average value of 10 arcings. The repeatability of the intensity ratio of the analytical line to the chromium-reference line is approximately the same with the standard catalysts and with the oil ash impregnated catalyst. The spread of successive ratio determinations of the same sample can be evaluated from Table I, which gives the average deviation of six arcings.

The wave length of the analytical and reference line, and the arcing conditions are given with the analytical curves. These curves cover a range of concentrations from 3 to 700 γ of metal per gram of catalyst, and intensity ratios from 0.3 to 1.3. The intensity of the vanadium line 3184.0 A. of Figure 2, marked *F*, is compared to the chromium line 3445.6 A. by adding a 50 to 100% split-field filter.

PROCEDURE

Sampling. The sample is kept in a glass bottle not more than two thirds filled.

In handling heavy oils, a representative sample is obtained by raising the temperature of the oil to 70° C., by shaking the bottle vigorously, and by transferring to a 400-ml. beaker an amount of oil large enough for performing a series of catalytic ashing determinations. Duplicate samples are prepared by warming the beaker and stirring the oil thoroughly with a glass rod before the oil is added to the catalyst.

Spectrographic Determination. Two procedures are used to coke the oil on the catalyst, the choice being determined by the metallic content and the volatility of the sample.

Samples with a nickel or vanadium content greater than 1 p.p.m., or nonvolatile samples with a metal content less than 1 p.p.m., are directly blended with the ground catalyst and a small amount of concentrated nitric acid (Blend Procedure). Volatile samples with a nickel or vanadium content below 1 p.p.m. are added dropwise to the hot catalyst (Drop Procedure).

Blend Procedure. Two to 30 grams of oil are blended, by means of a glass stirring rod, with 2 grams of catalyst in a No. 4 to 6 porcelain dish, and a volume of concentrated nitric acid equivalent to 0.1 the volume of the oil is added to the blend. The mixture is carefully heated, with stirring, on an air bath, until foaming ceases. If any liquid remains after this treatment, successive 1-ml. portions of concentrated nitric acid are added (not to exceed one fourth of the volume of the sample), while the temperature is progressively raised until all the oil is charred. The temperature is further increased until fuming is completed.

Table I. Repeatability and Accuracy of Blend Procedure

(Mid-continent 15% bottom)

Date and Operator	HNO_3, Ml./Gram Cat.	Weight Ratio, Cat./Oil	Nickel: Intensity ratio, Ni 3646.3/ Cr 3445.6[a]	Nickel: P.p.m.	Vanadium: Intensity ratio, V 3184.0/ Cr 2967.6[a]	Vanadium: P.p.m.
5/24/55 A	0.0	0.153	0.85 ± 0.04	12.3	0.85 ± 0.06	9.1
5/24/55 A	0.0	0.171	0.77 ± 0.04	12.1	0.85 ± 0.06	9.4
2/7/55 B	0.0	0.189	1.14 ± 0.07	14.1	0.38 ± 0.05	5.1
1/4/55 C	1.0	0.247	0.90 ± 0.05	13.1	0.37 ± 0.04	6.2
1/17/55 C	1.0	0.303	0.75 ± 0.05	12.1	0.39 ± 0.02	8.2
1/4/55 C	2.0	0.119	1.54 ± 0.05	13.6	0.69 ± 0.05	7.3
1/17/55 C	2.0	0.154	1.20 ± 0.06	12.5	0.58 ± 0.04	7.7
12/20/54 C	2.5	0.232	0.95 ± 0.06	13.5	0.38 ± 0.02	6.0
12/20/54 C	2.5	0.244	1.00 ± 0.06	14.9	0.41 ± 0.02	7.2
Average value of spectrographic determinations[b]				13.1 ± 1.0		7.3 ± 1.4
Average value of photometric determinations				11.7 ± 0.5		6.0 ± 0.6

[a] Deviations of ratios are average deviations, measured from six arcings.
[b] Deviations of average values are calculated from nine determinations.

Table II. Accelerated Blend Procedure

(Mid-continent 15% bottom. Weight ratio catalyst/oil = 0.10)

Acid Added, Ml./Gram Catalyst	Ni, P.P.M.	V, P.P.M.
HNO_3, 3.0	(27.4)	6.3
HNO_3, 3.0	12.2	7.1
HNO_3, 3.0 + H_2SO_4, 0.3	11.1	6.0
H_2SO_4, 3.0	12.8	6.6
H_2SO_4, 10.00	12.5	7.0
Average value, accelerated procedure	12.1 ± 0.8	6.6 ± 0.5
Average value, regular procedure (from Table I)	13.1 ± 1.0	7.3 ± 1.4

Table III. Nickel and Vanadium Determination by Blend and Drop Procedures

Nature of Sample	Weight Ratio, Oil/Cat.	Ni, P.P.M. Blend	Ni, P.P.M. Drop	V, P.P.M. Blend	V, P.P.M. Drop
Wyoming tar separator overhead	10	0.71	0.67 ± 0.12 0.67 ± 0.04	1.4	1.6 ± 0.7 1.6 ± 0.1
San Ordo gas oil	15	0.47 0.43 ± 0.04[a]	0.63	0.45 0.35[a]	0.35

[a] Without acid added to slurry.

The blend procedure can be accelerated by combining the acid-catalyst ashing with a dry oxidation, the blend of the sample with catalyst and concentrated acid is cautiously heated in a Vycor dish, while stirring, until foaming ceases and the sludge begins to thicken. It is then rapidly heated on a hot plate to a temperature at which the hydrocarbon vapors can be ignited. The temperature is slowly increased to maintain slow combustion. The dry coke is finally oxidized at 600° C. as in the regular procedure. The accelerated procedure can be successfully performed with concentrated nitric acid, sulfuric acid, or a 1 to 10 mixture of sulfuric and nitric acid. The time devoted to the coking can be reduced with the accelerated procedure from 1.5 hours to 25 minutes.

Drop Procedure. Two grams of catalyst, moistened with a few drops of concentrated nitric acid, are heated to approximately 250° C. in a No. 2 porcelain dish. A volume of 30 to 100 ml. of oil is then added dropwise, at the rate of approximately 2 drops per second, to the hot catalyst. The temperature is then adjusted, low enough to allow sorption of the oil on the catalyst, and high enough to crack and vaporize it within half a second. Intermittent stirring is an aid to uniform cracking.

After coking, the catalyst is placed in an oven at 600° C. for about 2 hours to oxidize the carbonaceous residue and to convert the nickel and the vanadium to their oxides. The catalyst is then finely ground in an agate mortar, blended with the graphite–chromic oxide mixture as quickly as possible to avoid water absorption, pelleted, and finally arced under the conditions given in the spectrographic section.

The weight ratio of oil to catalyst is not too critical. However, this ratio should not be below 1 and above 15 in the blend procedure, nor below 10 and above 50 in the drop procedure. With samples of very low metal content, accurate results can be obtained by repeated additions of oil, after ignition of the coke products at 600° C.

Photometric Determination. The sample is charred with concentrated sulfuric acid according to a procedure similar to that recommended by Milner and others (*6*). Briefly, the ashing proceeds in three steps: The oil is charred with concentrated sulfuric acid in a large porcelain dish, the coke is reduced to a small volume by slow burning in a Vycor dish, and the reduced product is ignited to ash in a platinum dish.

Five hundred grams of oil are blended with 100 ml. of concentrated sulfuric acid in a 1.5-liter porcelain dish, covered with another dish of the same size. The mixture is slowly heated with intermittent stirring to a temperature at which heavy white fumes are evolved. This heating process requires 4 to 5 hours. Two hundred and fifty milliliters of concentrated sulfuric acid are added in 50-ml. portions, at 1-hour intervals. The mixture is kept at the same temperature as long as the oil is not completely charred. If complete coking is not achieved after 24 hours, 50 ml. more of concentrated sulfuric are added. When the oil is completely coked, the temperature is gradually raised to get rid of the excess sulfuric acid. The dry coke is transferred to a 350-ml. Vycor dish, and reduced to a small volume by slow burning on an electric heater at about 500° C. The remaining ash is finally transferred to a 100-ml. platinum dish and ignited for 2 hours at 600° C. After cooling, 2 ml. of hydrofluoric acid and 1 ml. of concentrated sulfuric acid are added to the ash. The liquid is evaporated on a hot plate, to get rid of the silica.

The residual product is fused with sodium carbonate. The vanadium is leached out of the insoluble carbonates with boiling water, and the extract is reduced to 50 ml. after being acidified with diluted hydrochloric acid. The leached carbonates are dissolved in hydrochloric acid. Traces of remaining nickel are extracted from the platinum dish by a pyrosulfate fusion, and added to the nickel solution. The nickel solution is also adjusted to 50 ml.

Convenient aliquots of the vanadium and the nickel solution are treated according to conventional procedures given by Sandell (*8*), the nickel being determined as nickelic dimethylglyoxime, and the vanadium as phosphotungstic complex. Absorbances are measured with the spectrophotometer, using a 1-cm. cell.

EXPERIMENTAL RESULTS

Table I gives the results of repeated analysis of a mid-continent 15% bottom obtained from three operators over a period of 6 months, by using the catalytic ashing method. The experiments were performed with different ratios of catalyst to oil, from 0.1 to 0.3, and with different amounts of nitric acid, from 0 to 2.5 ml. per gram of catalyst. Each intensity ratio is the average value of six arcings. The ratios and the average values of the nickel and vanadium determination are given with their standard deviations.

Table II gives the nickel and vanadium content of the same sample determined by the accelerated blend procedure. In this table each value corresponds to a single determination. The average values of the nickel and the vanadium are given with their standard deviations.

Table III gives the nickel and vanadium concentration of two kinds of oil with a low metal content, determined by the blend and the drop procedure. Figures mentioned with a deviation value are the average value of duplicate determinations run simultaneously.

Table IV gives the nickel and vanadium content of different types of petroleum products determined spectrographically after catalytic ashing, and photometrically after sulfuric acid ashing. The samples are classified according to their decreasing nickel content. Each result represents the average value of duplicate analyses run simultaneously.

DISCUSSION

The reliability of the catalytic cracking method for determining the nickel and vanadium content of petroleum products cannot be directly evaluated from the analysis of synthetic samples, as knowledge of the organometallic components of petroleum products is limited.

The reliability can be indirectly evaluated by checking the reproducibility of the results obtained with different types of oil under different ashing conditions, and by comparing the spectrographic results with the photometric determination after sulfuric acid ashing. The latter analysis is generally accepted as reliable (*4*, *6*).

Tables I and II show that the nickel and vanadium concentration determined by catalytic ashing is not appreciably affected by the ratio of catalyst to oil, or by the amount or the nature of the acid added to the slurry. Table III shows furthermore that approximately the same results are obtained by the blend and the drop procedures.

Table I shows, that with the mid-continent 15% bottom there is an excellent agreement between the results obtained by the spectrographic-catalytic ashing procedure and the conventional photometric-sulfuric ashing procedure. The difference between the results obtained by the two methods, equal to 1.4 p.p.m. for nickel and 1.3 p.p.m. for vanadium, is just at the maximum limit that can be expected from the fluctuation of individual determinations. Because of the great retentive capacity of the catalyst for metallic compounds, slightly higher results obtained by the catalytic ashing method may be a more accurate value of the metal content of the oil.

Table IV. Nickel and Vanadium Determination by Spectrographic and Photometric Procedures

Nature of Sample	Weight Ratio, Oil/Cat. in Spectrographic Procedure	Ni, P.P.M. Spectrographic	Ni, P.P.M. Photometric	V, P.P.M. Spectrographic	V, P.P.M. Photometric
Boscan full crude	1	123 ± 10	107 ± 2	1120 ± 45	1160 ± 3
South California residuum	1	66.5 ± 0.4	66 ± 1	28 ± 7	32.2 ± 0.3
Tia Juana residuum	2.5	16.0 ± 0.4	28 ± 5	71.0 ± 1.5	78 ± 0
Kuwait 65% bottoms	2	17 ± 1	12.5 ± 0.5	35 ± 2	30 ± 1
Mid-continent 14% bottoms (from blend)	4	9.5 ± 0.0 9.0 ± 1.0	12 ± 0	6.7 ± 0.0	7.0 ± 0.3
Mid-continent 15% bottoms	2.5	12.2 ± 0.4	11.5 ± 0.5 ^{a}11.8	4.5 ± 0.6	5.9 ± 0.3 ^{a}6.0 ± 0.3
West Texas heavy gas oil	6	3.3 ± 0.1	4.2 ± 0.1	0.80 ± 0.06	1.1 ± 0.1
West Texas deasphalted gas oil	5	1.70 ± 0.05	1.6 ± 0.1	1.8 ± 0.2	2.2 ± 0.2
Kuwait tar separator overhead	10	0.40 ± 0.05	0.30 ± 0.05	0.85 ± 0.03	1.1 ± 0.1

a Dry oxidation ashing.

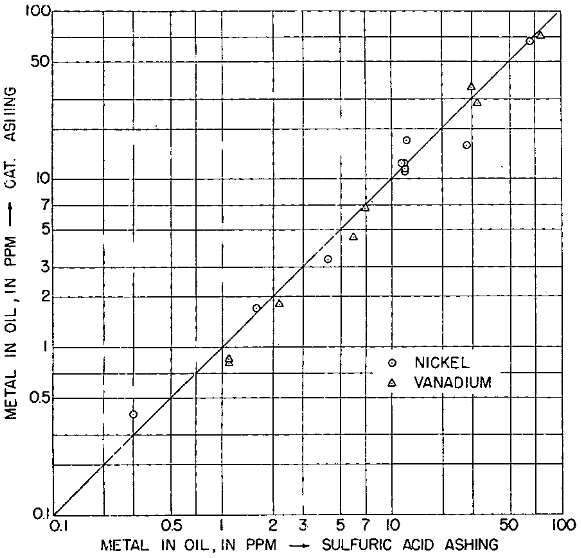

Figure 3. Relation between nickel and vanadium determinations by spectrographic and photometric procedures

Table IV shows that the agreement between the spectrographic and photometric determination appears also with other kinds of samples, from full crude to tar separator overhead. The results for nickel determined by both methods are in fairly good agreement, with the exception of the Tia Juana Residuum sample. The repeatability of the sulfuric acid ashing analysis of this sample is rather poor. This fact would cast some doubt on the homogeneity of that sample. The vanadium results, obtained by both methods, are also in good agreement. At a low concentration level, below 5 p.p.m., there is a tendency to get slightly lower results with the catalytic ashing procedure. It is possible that the results obtained by sulfuric acid ashing procedure are slightly too high, because at low concentrations the photometric determination of vanadium can easily be affected by the slightest turbidity of the solution.

The agreement between the spectrographic and the photometric procedures appears even more clearly in Figure 3, which gives the relationship between the experimental points corresponding to these two procedures for the eight petroleum samples of Table IV. The straight line indicates the position that the points should occupy in a perfect agreement of the two procedures.

Statistical computations show that the ratios of the variance of the results obtained by the spectrographic and the photometric methods fluctuate between larger limits than the theoretical expected value, when the variance is calculated from the range between duplicate determinations of the same sample obtained simultaneously with the same method (*9*). This discrepancy can be due to some random error depending on the nature of the oil, or perhaps to the choice of working curve. Further investigations are being conducted to clarify this point.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the contribution of S. Gerald Hindin to the establishment of the technique for determining trace amounts of nickel and vanadium in silica-alumina using chromic oxide as an internal standard; Charles R. MacIntyre in the establishment of the dropping procedure and for the spectrographic work; James A. Crielly and Walter J. Savournin in the chemical analyses, and Jack Grider in the coordination of the analytical work and the review of this paper. The authors also express their thanks to the Houdry Process Corp. for release for publication of the present paper.

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Quantitative Spectrographic Determination of Vanadium in Petroleum Products by Logarithmic Sector Method

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Vanadium present in minute quantities in certain oils poses a serious problem to the petroleum industry, as it causes catalyst poisoning and boiler corrosion. To keep equipment and catalyst replacement at a minimum, it is necessary to know the vanadium concentration in crude oil, charge stocks, and residual fuels. This paper presents a rapid logarithmic-sector spectrographic method where a sample is ashed by a controlled procedure and diluted with a buffer of graphite and silica to which 1% titanium has been added as an internal standard. A portion of this mixture is packed into a cratered graphite electrode and arced under prescribed conditions. The titanium and vanadium line lengths are determined with a modified Bausch & Lomb optical magnifier. The vanadium concentration is determined by referring to calibration curves, plotting line length difference versus concentration.

RECENTLY the petroleum industry has become increasingly aware of the deleterious nature of vanadium in petroleum processes. Generally, the vanadium is present as a porphyrin complex (*5, 6*) and is oxidized during cracking processes, yielding a low melting pentoxide or vanadate. These products deposit readily and contribute to catalyst poisoning, corrosion, and combustion deposits. Although the vanadium concentration in the sample is relatively low, the large throughput of crude and fuel oils causes the deposits to grow rapidly. Because of the corrosive effect on "crackers" and the large expense involved in replacing or regenerating poisoned catalyst, it is worth while to know the vanadium content of the crude oils and charge stocks, so that adequate precautions may be taken.

Prior to the use of the spectrograph, colorimetric and polarographic procedures, which were extremely time-consuming and subject to various interferences, were used. The phosphotungstate colorimetric method especially is hindered by chromium, and requires prior separation of this element. Thus, because of the inherent difficulties of polarographic extraction or fusion, coupled with colorimetric interferences, the use of the spectrograph is a logical alternative.

Spectrographic determination of vanadium is not new, as Murray and Plagge (*4*), Carlson and Gunn (*3*), and Anderson and Hughes (*2*) have all used the spectrograph to attack this problem. Carlson and Gunn's technique of quenched electrodes gives only fair agreement with chemical results. Murray and Plagge's step sector method compares samples with known standards to estimate the vanadium concentration.

Anderson and Hughes introduced an internal standard and used a densitometer to determine vanadium quantitatively. This method was used in this laboratory for some time, but because it required 2 to 3 man-hours to perform, a method using essentially the Anderson procedure with the exception of substituting a logarithmic sector disk for an expensive densitometer was adopted.

The logarithmic sector, which can be considered as a step sector with an infinite number of steps, is a disk whose periphery is cut to a logarithmic curve. As the sector rotates in front of the entrance slit, the image produced on the photographic plate is a tapered line whose length is proportional to the concentration. Any variation of the vanadium line length caused by differences in emulsions or by changes in the arc during the exposure period is compensated for by the addition of an internal standard, titanium. No plate calibration is required with the logarithmic sector method.

The chief advantages of this method are that a vanadium determination can be performed in about 1 man-hour, which is half the time required by the densitometric procedure, and that no expensive densitometer is necessary.

EQUIPMENT

A Bausch & Lomb large Littrow spectrograph with the camera positioned to photograph the 2500 to 3500 A. range is used. The exposure is controlled automatically with a timer switch.

All electrodes are of high purity spectrographic grade graphite with the anode 2 inches long and $^{1}/_{8}$ inch in diameter, flat cut, and the cathode 1.5 inches long and 0.25 inch in diameter. The end of the cathode is a 30° cone containing an axial crater $^{1}/_{16}$ inch in diameter, $^{1}/_{16}$ inch deep.

A standard 5000 r.p.m. motor with a $5^{3}/_{16}$-inch logarithmic sector disk obtained from the Jarrell Ash Co. is placed in the optical path.

A direct current motor generator, 115-volt, 60-ampere, is used as a source. The arc is initiated by a radio-frequency spark.

The fixed slit used is 20 microns wide, 13 mm. high, and modified with a human hair about 3 mm. below the point of maximum radius of the logarithmic sector disk.

Photographic equipment consists of Eastman spectrum analysis plates No. 1, Eastman D-19 developer, a 3% acetic acid short stop, and Eastman rapid liquid fixer with hardener. Circulating cold water for wash and a drying oven with circulating warm air are also required.

The plates are placed on a special viewing box and read with a Bausch & Lomb optical magnifier, which is modified as follows. Use a razor blade to cut out a typical line (*7*) from a plate exposed under test conditions (after softening emulsion in water). Float this bit of emulsion containing the line on top of water in a large evaporating dish. Slowly bring the removed bottom glass from the magnifier up under the floating piece of emulsion. Take care that the glass surface is clean and no bubbles are trapped under the bit of emulsion. Dry in a dust-free location. When thoroughly dry, brush a thin coating of transparent lacquer or collodion over the emulsion and glass, and when dry, reassemble the magnifier.

METHOD FOR INTERNAL AND BUFFER STANDARDS

Prepare synthetic standards of various vanadium concentrations by using C.P. vanadium pentoxide and spectrographic graphite as diluent. Vanadium concentrations of 1, 3, 9, 30, 56% have been found adequate. Mix these samples with the buffer internal standard mixture in a ratio of 1 part of sample to 9 parts of buffer–internal standard mixture.

Prepare the buffer–internal standard mixture as follows. Prepare a mixture of 1% titanium (as the oxide) in C.P. silicon dioxide and mix the titanium–silicon dioxide powder with spectrographic graphite in a ratio of 1 part of titanium–silicon dioxide with 2 parts of graphite.

All the above chemicals are ground so as to go through 200-mesh screens.

Set the spectrograph as indicated in Table I and arc the samples.

Table I. Excitation Conditions

Type of excitation	D.c. arc with r.f. initiating spark
Circuit constants	
Voltage	60 volts across the electrodes
Current	7.5 amperes
Capacitance	0.4 μfd.
Resistance	20 ohms
Arc gap	5 mm.
Spectral region	2500 to 3500 A.
Exposure time	75 seconds
Log sector disk and motor	In place

No condensing lenses, diaphragms, or filters were used.

Develop the photographic plate for 2.5 minutes at 68° F., short stop for 10 seconds, and fix for 5 minutes. Wash the plate in cold running water for 10 minutes and dry in a drying oven maintained at 95° F.

Draw calibration curves by plotting difference in the line length of the vanadium line at 2977.5 A. and titanium line at 2956.1 A., against the log of the vanadium concentration. A typical calibration curve with 95% confidence limits is shown in Figure 1.

ANALYSIS OF SAMPLES

Thoroughly mix the sample in its original container by vigorous shaking. In addition to shaking viscous samples require heating and dry samples must be ground in a mortar. If samples do not require ash treatment they are run directly after mixing.

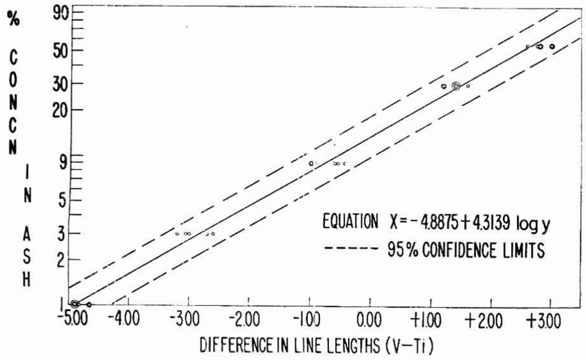

Figure 1. Vanadium 2977.5 A. calibration curve

Ignite 50 ± 0.1 grams of sample in a porcelain crucible (Coors No. 3) tared to the nearest 0.1 mg. Allow sample to burn gently, supplying heat only when necessary and cautiously as the heavy ends have a tendency to mushroom out of the crucible. If water is present, spattering (*1*) can be minimized by adding 2 or 3 ml. of absolute alcohol. When a carbon ash remains, put sample into a muffle maintained at 1000° ± 50° F. until all the carbon is consumed. Cool in a desiccator and weigh crucible against the same tare to 0.1 mg.

Thoroughly mix the ash, weigh 10 mg. into a screw cap vial, and add nine times the sample weight of buffer–internal standard mixture. After thoroughly mixing the sample-buffer–internal standard in a mortar, store in original vial.

Pack the mixture into a cratered electrode by pushing the hollowed end of the electrode into some of the sample contained in the cap of the vial, thus packing the sample level with the top of the electrode.

Next arc the samples and develop the plate as previously described.

Determine the length of lines by moving the modified eyepiece along the line being measured until the rate of extinction of the two lines (element line and the line attached to the face of the eyepiece) is the same. Then by using the scale in the magnifier, estimate the line length to the nearest 0.05 mm. Use the image of the slit hairline as the base from which to measure all lengths. A typical plate is shown in Figure 2.

Table II. Comparison of Log Sector and Densitometric Results

Sample No.	Type	% Vanadium in Original Samples	
		Log sector	Densitometer
1	Italian crude	0.0020	0.0024
2	No. 6 fuel oil	0.0140	0.0152
3	Shedgum residuum	0.0043	0.0056
4	Bunker C	0.0067	0.0063
5	Krasnoor residuum	0.0012	0.0007
6	Ocan Residuum	0.0175	0.0207
7	No. 6 fuel oil	0.0132	0.0172
8	Unidentified residuum	0.0177	0.0251
9	Unidentified residuum	0.0125	0.0106
10	Unidentified residuum	0.0074	0.0054

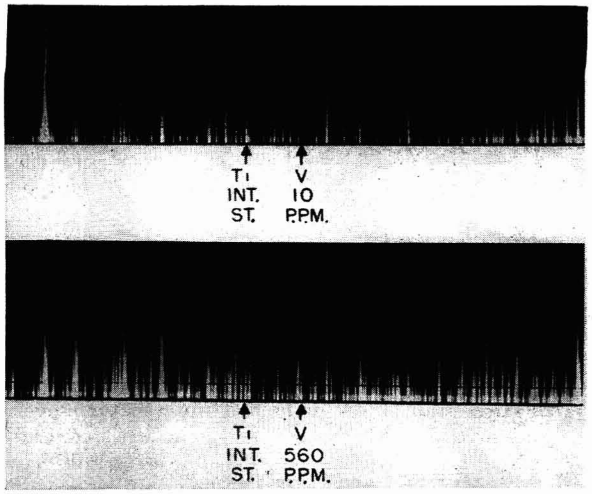

Figure 2. Spectrograms showing relationship between line length and concentration

Subtract the length of the titanium 2956.1 A. line from the length of the vanadium 2977.1 A line.

CALCULATION

If reporting composition of ash or inorganic sample, report elemental concentrations directly in per cent, but if reporting on original sample basis, calculate as per cent or parts per million by the formulas:

$$\% = \%\text{ element in ash} \times \%\text{ ash} \times 0.01$$

$$\text{P.P.M.} = \%\text{ element in ash} \times \%\text{ ash} \times 100$$

DISCUSSION AND RESULTS

The complete method, including ashing the sample, requires 1 man-hour to perform and has been successfully applied to crude oil, charge stocks, residual fuel, and deposit analyses.

Correlation between experimental data and known elemental concentrations is very high. The average correlation coefficient for all concentrations equals 0.99. Perfect correlation would equal 1.00.

Based on the data obtained from repeated tests, the repeatability in terms of 95% confidence limits averages 35%. The 95% confidence limits are a stringent measurement of precision in that they predict only one result in 20 to exceed these limits. That means, if a sample of 10 p.p.m. vanadium was run 20 times only one result would be expected to exceed 14 p.p.m. or be less than 6 p.p.m. A comparison of logarithmic sector data compared to densitometric answers is shown in Table II. In all cases the agreement is excellent and well within the precision of the method.

ACKNOWLEDGMENT

The author wishes to thank Owen J. Black for aid in the statistical analyses of the data.

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Quantitative Determination of Thorium and Uranium in Solutions by Fluorescent X-Ray Spectrometry

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The determination by chemical methods of thorium and uranium in the aqueous and organic solutions from solvent extraction investigations was slow and inadequate. A rapid and accurate x-ray fluorescent spectrometric method has been developed in which the aqueous and organic solutions could be analyzed directly. Sample preparation required only the addition of a suitable internal standard added as a solution. Internal standards of strontium solutions were used for the aqueous samples and bromobenzene solutions for the organic samples. Working curves obtained by plotting intensity ratios of the element to the internal standard versus the concentration of that element gave straight lines. In all the ranges studied the effect of matrix impurities was negligible. The accuracy of the final results is as good as present chemical methods. A sample can be analyzed for both thorium and uranium in 0.5 hour or less.

THE extraction of thorium and uranium from raw materials and their partitioning by use of organic solvent techniques can be accomplished by countercurrent extraction methods. The establishment of the proper conditions for extraction and partitioning of thorium and uranium required numerous reliable analyses for these two elements. A number of variables are involved in the development of any solvent extraction process; therefore, numerous chemical analyses are needed for design purposes, as well as for the determination of the best operational conditions.

A single experiment resulted in many aqueous and organic solutions which required immediate analysis, but weeks were needed to obtain suitable analyses by chemical means. Some of the analyses were unreliable, because of the presence of certain impurities which are difficult to remove. The preparation of solid samples from solutions was difficult and at times resulted in some loss of thorium and/or uranium. Therefore x-ray fluorescent methods (*1*, *3*) applicable to solids resulted in doubtful data attributed to dubious sampling techniques. X-ray fluorescent analysis of liquids (*2*, *7*) appeared to be feasible, since the materials are in solution throughout the various processes.

A rapid method for the quantitative determination of thorium and uranium directly from solutions by use of fluorescent x-ray spectrometry, in which the characteristic spectra of the *K* and *L* series (*6*) are used, is described. The accuracy and reliability of the method, as well as the time required for analysis, are considered.

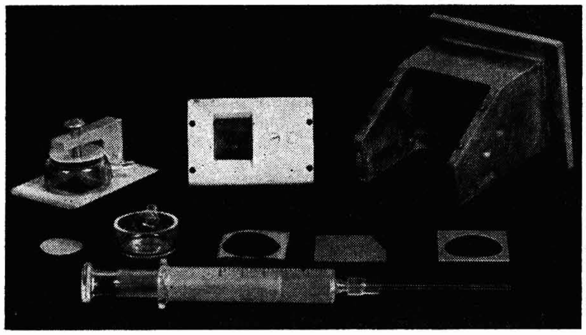

Figure 1. Completed cell, accessory parts, loading syringe

Table I. X-Ray Spectrometer Positions for Working Curves

Spectra	$UL\alpha_1$	$ThL\alpha_1$	$SrK\alpha_1$	$BrK\alpha_1$	Background
Spectrometer setting (2θ deg.)	35.27	37.10	33.87	40.47	38.75
Aqueous feed	×	×	×	—	×
Raffinate	—	×	×	—	×
Organic phase	×	×	—	×	×

APPARATUS

The fluorescent x-ray apparatus in this laboratory consists of a General Electric X-ray Model XRD-3 unit with a No. 1 SPG detector unit, a No. 1 SPG mica spectrometer, and a Victoreen Type 1B89 counting tube. The argon-filled counting tube was found to be satisfactory as no serious attempt was made to determine elements below 0.01% concentration. Detailed directions (*4*, *5*) are given by the manufacturer for the successful operation and maintenance of the equipment. The smallest spectrometer defining slit consistent with rapid counting must be used to avoid overlapping of possible adjacent spectra.

Additional cooling of the sample chamber was provided to minimize the expansion in volume of the liquid sample. A small 10-inch heat exchanger of the Graham condenser type was constructed of brass. It was connected with hose fittings between the water input of the Machlett AEG-50, tungsten-target, x-ray tube, and the water output of the cooler.

The original General Electric X-ray sample holder for solids was modified. Figure 1 gives the completed cell and its component parts. The screw heads on the sample holder were turned down to permit manipulation of the cell assembly, and the angle bracket was made of $^3/_8$-inch hard aluminum. The borosilicate glass cell is 32 mm. in outside diameter and 15 mm. in over-all thickness, and a 5-mm. filling tube is sealed to the side. The open end of the cell is made flat by grinding. In assembling the cell a 0.004-inch Teflon washer is centered over the aperture of the sample plate, a mica window (1.5 to 3 mg. per sq. cm.) is placed upon the Teflon washer, and this is followed by a 0.030-inch fluorothene washer. The glass cell is centered onto the assembly, cushioned by a plastic button, and tightened by hand.

The transfer of liquids is best handled by the use of hypodermic syringes as shown in Figure 1. A piece of 1-mm. bore capillary tubing joined to the syringe by a short length of plastic tube is superior to commercially available metal needles.

EXPERIMENTAL

For most of the studies the x-ray apparatus was operated at ratings up to 50 kvp. and 45 ma. The raw materials and processed sludges used in the present investigation contained up to 60% thorium and 0 to 2% uranium. Therefore, greater emphasis was placed on the thorium analysis. Figure 2 gives the region and x-ray spectral positions which were used, obtained by a rapid scan. The spectra shown were free of interfering element and absorption edge effects. The analyses were arranged into three classifications:

Aqueous Feed Solutions. These include nitric acid solutions of new minerals, processed sludges, feed stock for solvent extraction development experiments, and product solutions containing both thorium and uranium.

Aqueous Raffinate Phase. These represent nitric acid solutions containing 0 to 10 mg. per ml. of thorium and the impurities which were present in the original material.

Organic Phase. Organic solutions (40% tributyl phosphate and 60% aliphatic diluent solvent) are included. These solutions contain essentially thorium, uranium, and small amounts of impurities. Table I gives the spectra criteria and spectrometer positions needed in the determination of working curves.

Known aqueous standard solutions were prepared from high purity thorium metal, thorium nitrate, pure uranium oxide (U_3O_8), mixed phosphates, various rare earths, and various heavy metals. The latter three groups of materials were included in the preparations to determine the effect of the matrix upon the working curves. Solutions were made by adding thorium, uranium, and matrix materials in predetermined, weighed amounts to 10- or 100-ml. volumetric flasks, dissolving in a small quantity of nitric acid, and diluting to the mark. The solutions in the 10-ml. volumetric flasks were used directly, while those in the 100-ml. volumetric flasks were divided into aliquot portions for further dilution. The solutions for the internal standard were prepared from reagent grade, anhydrous strontium nitrate dissolved in dilute nitric acid: 300.0 mg. per ml. for aqueous feed samples, and 36.0 mg. per ml. for raffinate samples.

In the preparation of the organic phase standards, nitric acid solutions containing known amounts of thorium and of uranium were extracted with 40% tributyl phosphate in 60% aliphatic diluent. All the organic phase extracts were carefully analyzed chemically and optically before use. As with the aqueous standards, some were used directly, while others were divided into aliquot portions and further diluted with the mixed solvent. The organic internal standard was prepared to contain approximately 80 mg. per ml. of bromobenzene in the mixed organic solvent, and then it was carefully analyzed chemically for bromine. All subsequent standards were adjusted to correspond to the bromine content of the initial standard.

The analytical sample was prepared by mixing 1.00 ml. of the appropriate internal standard with 10.00 ml. of sample solutions both being at the same temperature. Each solution was transferred as needed to the liquid sample holder which was placed in the fluorescent x-ray unit. Each sample was set manually at the positions indicated in Table I for the sample type and counted.

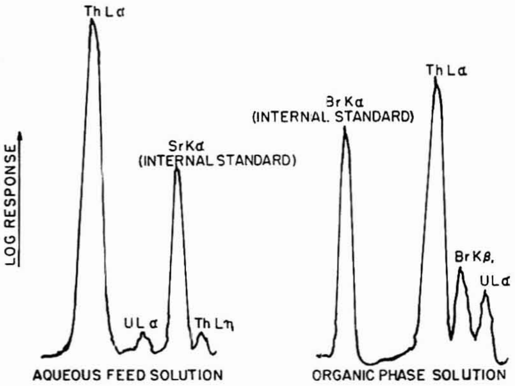

Figure 2. X-ray fluorescent spectra for thorium and uranium determination

An average of three sets of readings was obtained for every sample counted. The scaler measured the elapsed time for a predetermined number of counts; hence, all data were reduced to counts per second. For any element counted a background correction was made:

$$I(A) = A - B \text{ and } I(S) = S - B$$

where

$I(A)$ = intensity for element sought

and

$I(S)$ = intensity for internal standard
A = counting rate in counts per second for element sought
B = counting rate in counds per second for background
S = counting rate in counts per second for internal standard

Then the intensity ratios, $I(A)/I(S)$, versus concentrations of A in milligrams per milliliter for the same sample series were plotted to give the working curves in Figures 3 to 5 for both thorium and uranium. These curves represent single examples of several curves made using varying amounts of different matrix blends.

As the curves were closely linear, the concentrations of thorium and uranium were calculated more conveniently:

$$[A] = D \times R$$

where

$[A]$ = concentration of A in milligrams per milliliter
R = intensity ratio $I(A)/I(S)$

and

$$D = \Delta[A]/\Delta R$$

DISCUSSION

The raw minerals and sludges used in the studies varied widely in composition. One sludge used in the studies contained 45% thorium oxide, 15% rare earth oxides, 4.4% silicon dioxide, 7.0% titanium dioxide, 4.3% ferric oxide, 1.4% zirconium dioxide, 5.3% phosphorus pentoxide, and 1.0% uranium oxide (U_3O_8).

The effect of the above elements on the aqueous feed curves (Figure 3) was examined. Solutions were made in which only thorium and uranium were used, and other solutions were prepared in which matrix blends were added in varying quantities.

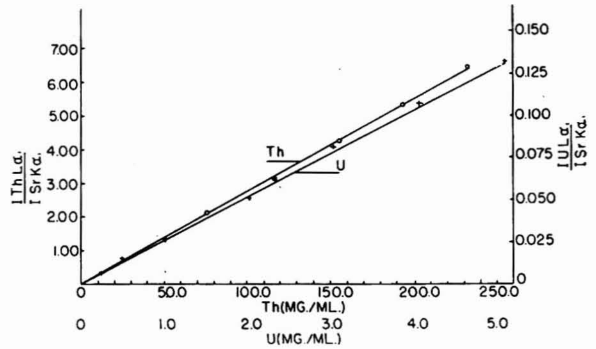

Figure 3. Curves for thorium and uranium aqueous feed solutions

For each point plotted to give the curve in Figure 3 the total solid content was about 340 mg. per ml. A comparison of the data available for thorium indicated that the average values were reproducible with a precision within ±0.5% and an accuracy within 1% in the range between 10 and 240 mg. per ml. of thorium. Above 240 mg. per ml. of thorium, the graph deviated from the 1% linear portion of the curve. As it was undesirable for plant use to correct for dead time losses in the counting tube, counting rates were adjusted to stay within the 1% linearity limit. For the range of 1 to 10 mg. per ml. of thorium the accuracy dropped to about 5%. The uranium analysis gave the same result in the 1- to 5-mg. per ml. range as the thorium analysis in the 1- to 10-mg. per ml. range.

The raffinate analysis resolved itself into one in which a small amount of thorium was present in 97 to 100% matrix. The total solids were unusually high. A curve plotted from data using only thorium in 2 to 4*M* nitric acid reproduced the high matrix curve remarkably well; an accuracy of about 1% of the amount present was observed in the concentration range of 1 to 10 mg. per ml.

Thorium and uranium in the organic phase curves in Figure 5 were almost free of any impurities. Checks between prepared known samples agreed to better than 1% of the amount present. The thorium and uranium curves are linear to 1% throughout the concentrations studied.

From the above analyses it appeared that the effect of the impurities of the absorption of x-rays and on the emission fluorescent spectra was unimportant for the materials studied. This would

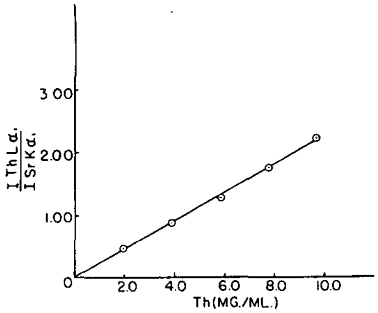

Figure 4. Curve for thorium in aqueous phase raffinates

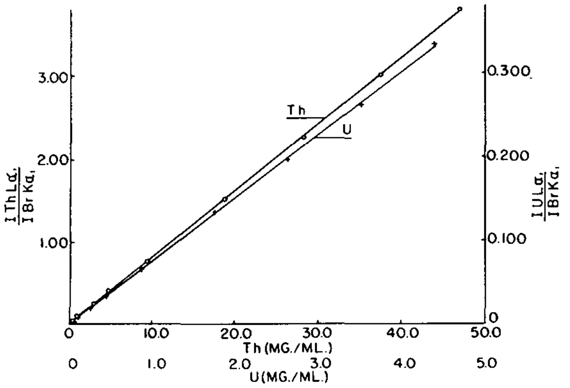

Figure 5. Curves for thorium and uranium in organic phase solutions

not be true at very low concentrations or in a very precise analysis. The homogeneity of the solutions tends to minimize the errors normally present when solid samples are used. Further, use of ratios of the corrected intensities of the element sought to that of the internal standard compensated to a large extent for gross absorption effects.

Some examples of analyses of feed materials, aqueous phases, and organic phases by the x-ray fluorescent method are given in Table II. The x-ray data are based upon single samples on each of which an average of three counts was determined. For the chemical and optical analysis two or more samples were used and the results expressed as the average. Approximately 90% of the samples showed agreement between comparative methods to 1% or better. About 10% of the samples showed differences to 5%, and isolated samples gave considerably higher errors. Further examination of the samples, which gave high deviations, indicated a high phosphate and rare earth content. Known samples were prepared containing weighed quantities of thorium, uranium, and a matrix with a high phosphate and rare earth content. Analysis for thorium and uranium by the x-ray fluorescent method was accurate to 1% or better, but the chemical methods still gave errors to 5%. Further work is in progress critically to evaluate these results.

When determining low concentrations of elements accurately, the presence of impurities in the internal standard may contribute to small errors in the final result. The curve passed through the origin of the graph only if all conflicting impurities are absent and all background effects are fully accounted for. Usually a family of straight-line curves of approximately the same slope are obtained when interfering impurities are present. To correct for this effect it is necessary to determine the intensity ratio at zero concentration of the element in question which then is subtracted from the samples analyzed. If the reagents cannot be purified readily, the use of a different internal standard with new spectral positions might remedy the disturbing effect.

Some of the solutions containing a high solid concentration salted out after the internal standard was added. In such cases the samples were diluted 1 to 1 to solve this difficulty. No significant error was introduced by dilution.

Volumetric flasks are to be preferred over pipets, as thorium-bearing solutions increase considerably in viscosity as the amount of that element increases. Good volumetric practice is imperative for best results.

Each sample preparation required only a few minutes. Using the average of three sets of readings for each element, the determination of thorium and uranium required altogether 0.5 hour or less. With some sacrifice in accuracy, single readings on each element corrected for a predetermined average background value would permit analysis of more than 60 samples for uranium and for thorium in an 8-hour working period.

CONCLUSION

The x-ray fluorescent method using solutions directly is employed routinely to provide development data on materials which are difficult or time-consuming to analyze chemically. It is not limited to thorium and uranium and is being used successfully for a number of elements above atomic number 22. Usually the data are reported to ±0.01% or other equivalent units, although the sensitivity of detection is about 0.004% for a number of elements. For uranium and thorium the limit of detection is better than 1 count per second above the corresponding background at 0.004%.

Table II. Thorium and Uranium Analysis

		X-Ray Fluorescence, Mg./Ml.		Chemical and/or Optical, Mg./Ml.	
No.	Sample No.	Th	U	Th	U
1	Feed 6-A/O	154.00	4.62	154	...
2	MUR-1	56.90	1.78	56	1.7
3	MUR-2	71.00	2.53	69	2.5
4	MUR-3	89.90	2.42	92	2.5
5	HAU-1	48.18	0.25	50	<0.10
6	11-A/O-Raff-11-27	0.22	..	<0.1	...
7	7-A/O-Raff-6-17	13.2	..	12.8	...
8	11-A/O	182.24	5.74	182	...
9	3-PP-Feed	265.93	5.89	253	5.9
10	3-PP-2-O-A	55.02	1.22	54.1	1.4
11	8-P-A-1E-73	65.21	0.07	...	0.072
12	6-P-A-1E	142.74	0.06	...	0.065
13	8-P-OK Liq	53.62	0.00	...	0.000
14	Feed 18-A/O	199.05	3.99	200	4.0

The cost of hundreds of chemical analyses is usually prohibitive for a systematic study of winning elements from complex ores and processed materials. Good chemical analyses are either time-consuming or are unavailable for a number of materials.

At Mound Laboratory experiments are in progress to study and to improve further the precision and accuracy of the x-ray fluorescent method using solutions especially at the lower levels of concentration. Probabilities for improved results are indicated for thorium, uranium, and other elements above atomic number 22. These results are to be published as soon as the programs are completed.

The method using a compensated fixed standard lends itself to continuous control analysis. It could be applied directly to production streams piped from the equipment to the x-ray fluorescent apparatus, with monitored results recorded on strip charts.

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New Brunswick Laboratory of the Atomic Energy Commission chemically analyzed the different samples.

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Determination of Beta,Beta'-Oxydipropionitrile and Ethylene Cyanohydrin with Acrylonitrile by Infrared Absorption

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This paper presents an analytical method for the determination of β,β'-oxydipropionitrile and ethylene cyanohydrin in admixture with acrylonitrile. In several reactions involving acrylonitrile the efficiency is decreased by the formation of by-products, among which are β,β'-oxydipropionitrile and ethylene cyanohydrin. Studies of the effect of catalyst, temperature, etc., to reduce the formation of these undesirable by-products require a method for their analysis in admixture with acrylonitrile. A simple multicomponent analysis by means of infrared absorption spectra is described, which permits a simultaneous determination of the three compounds. This method, which is being used in connection with studies on the cyanoethylation of cotton, has been tested by the analysis of known mixtures and found to be satisfactory.

A MOST important problem in the cyanoethylation of cotton and in other reactions involving the use of acrylonitrile is the loss of acrylonitrile through the formation of the by-products, among which β,β'-oxydipropionitrile and ethylene cyanohydrin are prominent. Because these losses are a major element in the chemical costs of the process, studies of the effects of the variations of catalyst and temperature on this reaction have been made. Before a quantitative study of the formation of β,β'-oxydipropionitrile and/or ethylene cyanohydrin could be undertaken, a satisfactory analytical method for the measurement of these three compounds in admixture was essential. No chemical methods for this analysis were available. This paper describes a method of multicomponent analysis by means of infrared absorption spectra.

EXPERIMENTAL

Infrared absorption measurements were made with a Beckman IR-2T automatic recording infrared spectrophotometer, using a single 0.4-mm. sodium chloride cell for both standardization and "playback."

Acrylonitrile was prepared from commercial grade material by drying over sodium sulfate and filtering: $n_{25^\circ}^{\mathrm{Na}} = 1.38886$.

Ethylene cyanohydrin was also prepared from commercial grade material by redistillation: boiling point 82° per 4 mm. of mercury; $n_{25^\circ}^{\mathrm{Na}} = 1.43737$.

β,β'-Oxydipropionitrile was synthesized by the method described by Bruson (*1*): boiling point 155° per 4 mm. of mercury; $n_{25^\circ}^{\mathrm{Na}} = 1.43914$.

INFRARED ABSORPTION SPECTRA

Complete infrared absorption spectra in the rock salt region from 2 to 12 microns were obtained for the pure compounds in chloroform solution. A detailed study was made of these spectra to select bands which would be suitable for quantitative analysis. In the 8- to 11-micron region (Figure 1) each compound exhibited a strong band at a wave length at which each of the other two components was very transparent. These maxima, which were used for the quantitative analyses, are: acrylonitrile, 10.36 microns; ethylene cyanohydrin, 9.42 microns; and β,β'-oxydipropionitrile, 8.85 microns.

Spectra were scanned over this 3-micron region after balancing the instrument with chloroform in the absorption cell. Thus, the effect of solvent was compensated for in making all measurements. The spectra of the pure compounds and analytical samples were then obtained by playback from the tape recorder immediately after standardization. Entrance slits at the three selected wave lengths were: 10.36 microns, 0.35 mm.; 9.42 microns, 0.44 mm.; and 8.85 microns, 0.28 mm. Applicability of the Beer-Lambert law was checked for each of the three compounds at the selected wave lengths (Figure 2). From the slopes of these types of Beer-Lambert curves, the average values of absorptivities for both maxima and background for each of the three compounds at 10.36, 9.42, and 8.85 microns were obtained. Absorptivities at the maxima of the selected absorption bands for each of the three

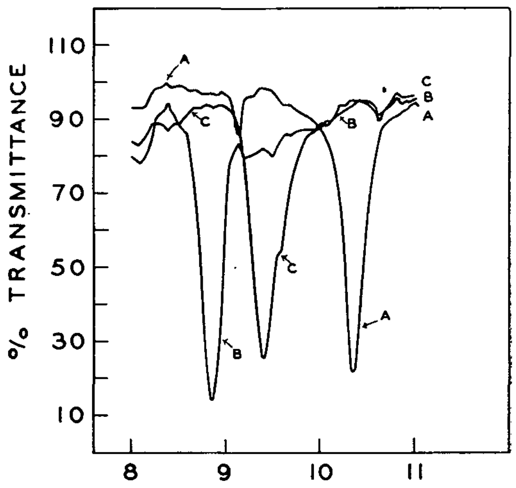

Figure 1. Infrared spectra in chloroform solution, 8 to 11 microns

A. Acrylonitrile
B. β,β'-Oxydipropionitrile
C. Ethylene cyanohydrin

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Table I. Recovery Tests on Mixtures

Mixture No.	Acrylonitrile, %		β,β'-Oxydipropionitrile, %		Ethylene Cyanohydrin, %		Total, %	
	Added	Found	Added	Found	Added	Found	Added	Found
I	76.60	75.08	12.70	12.76	10.70	11.61	100.00	99.45
II	78.71	80.38	14.64	14.41	6.65	7.40	100.00	102.19
III	78.92	80.68	21.08	20.13	0.00	0.00	100.00	100.81
IV	87.00	89.06	0.00	0.00	13.00	13.45	100.00	102.51
V	80.97	79.36	12.12	12.03	6.91	7.52	100.00	98.91
VI	68.13	65.89	17.96	16.78	13.91	15.84	100.00	98.51

Table II. Analyses of Cyanoethylation Mixtures

Run	Per Cent				
	Acrylonitrile	β,β'-Oxydipropionitrile	Ethylene cyanohydrin	Water	Total
1	95.54	0.00	0.00	1.5	97.04
2	95.58	0.00	0.00	1.6	97.18
3	94.59	0.00	2.14	1.5	98.23
4	93.43	1.64	0.00	2.4	97.47
5	95.34	2.20	0.00	3.5	101.04
6	96.65	0.69	0.72	1.6	99.66
7	96.47	0.05	3.22	3.0	102.74
8	94.88	0.00	0.55	3.4	98.83
9	93.86	0.00	1.84	4.3	100.00
10	93.60	1.37	0.00	3.5	98.47
11	91.82	1.22	1.45	3.9	98.39
12	96.31	0.89	0.97	3.1	101.27
Pilot plant	85.55	8.34	0.64	3.5	98.03

pure compounds are: acrylonitrile 10.36 microns, 2.14; ethylene cyanohydrin 9.42 microns, 2.08; and β,β'-oxydipropionitrile 8.85 microns, 2.70. By a simultaneous solution of expressions for the total absorption contributed by each of the three compounds at each of the three selected wave lengths, the following tricomponent equations were obtained:

$$x = (0.4671C - 0.0119A - 0.0199B) \times 100$$
$$y = (0.3715A - 0.0158B - 0.0073C) \times 100$$
$$z = (0.4821B - 0.0364A - 0.0028C) \times 100$$

where x, y, and z are percentages of acrylonitrile, β,β'-oxydipropionitrile, and ethylene cyanohydrin, respectively, in an admixture, and A, B, and C are absorptivities of the sample at 8.85, 9.42, and 10.36 microns, respectively.

The equations were tested by the analysis of mixtures of known ratios of the three components. Results are shown in Table I.

Mixtures from cyanoethylation of cotton in several laboratory experiments and in a single pilot plant experiment were analyzed according to the method described. The results are reported in Table II.

DISCUSSION

In the 8- to 11-micron range, selected for the multicomponent infrared analysis, a strong maximum for each compound, at wave lengths where the other components are almost completely transparent, permits satisfactory simultaneous tricomponent analysis. The selected maxima, completely resolved in the spectra of an admixture containing all three components, yet included in only a 3-micron range, facilitate automatic recording measurements. The method could easily be adapted to null-type instruments using cell-in–cell-out techniques. Figure 2 illustrates that the Beer-Lambert law is followed by all three compounds if the concentration is not more than 10 grams per liter in chloroform. At higher concentrations a departure from linearity was found, increased concentration resulting in less than the expected increase in absorbance. Chloroform solutions of samples containing approximately 10 grams per liter were found to give satisfactory absorbances for the quantitative measurements involved in studies of the kinetics of the formation of by-products from acrylonitrile.

The absorptivities given above are, like all infrared quantitative measurements, strictly applicable only to the instrument on which they were obtained. Consequently, the multicomponent equations are, in the strictest quantitative sense, applicable only to this instrument. They should, however, be expected to be reasonably satisfactory for measurements with any Beckman IR-2 or IR-2T spectrophotometer, operated under conditions which permit use of the above suggested slit widths. For analysis under other settings or with other types of instruments the absorptivities must be redetermined.

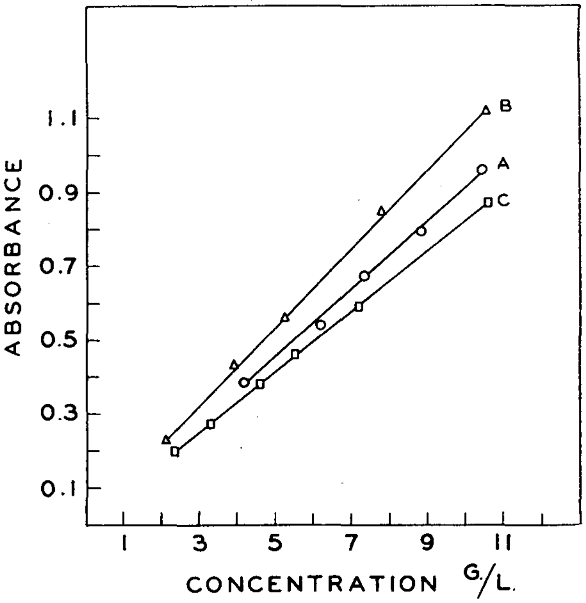

Figure 2. Beer's law in chloroform solution

A. Acrylonitrile at 10.36 microns
B. β,β'-Oxydipropionitrile at 8.85 microns
C. Ethylene cyanohydrin at 9.42 microns

Absorptivities at the three selected wave lengths for the three pure compounds have been measured on a Perkin-Elmer Model 21 split-beam spectrophotometer and multicomponent equations calculated. The instrument settings were: resolution, 915; response, 2; gain, 7; speed, 4; and suppression, 2. At these settings entrance slit widths and calculated absorptivities were found to be:

Acrylonitrile	10.36 μ, slit width 0.11 mm., α = 1.80
Ethylene cyanohydrin	9.42 μ, slit width 0.092 mm., α = 1.73
β,β'-Oxydipropionitrile	8.85 μ, slit width 0.08 mm., α = 2.45

From these data the following simultaneous equations were obtained:

$$x = (0.5565C - 0.0071A - 0.0162B) \times 100$$
$$y = (0.4095A - 0.0173B - 0.0056C) \times 100$$
$$z = (0.5788B - 0.0323A - 0.0065C) \times 100$$

These equations should give reasonably satisfactory results from data obtained with the Perkin-Elmer Model 21 spectrophotometer operated under the conditions and at the slit widths cited.

Data in Table I illustrate that satisfactory results have been obtained by the proposed method. The method is being used to study the effects of variation of catalyst on the formation of β,β'-oxydipropionitrile and ethylene cyanohydrin from acrylonitrile during the cyanoethylation of cotton.

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X-Ray Spectrographic Method for the Determination of Vanadium and Nickel in Residual Fuels and Charging Stocks

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An x-ray spectrographic method is described for the determination of nickel and vanadium in trace quantities. The method is fast, requires in most cases no more than 20 grams of sample, and is comparable with other methods in accuracy. A sample to be analyzed is first ashed and the ash put in solution. A portion of this solution is evaporated on a sample plate and rotated under the x-ray beam. The intensity of the fluorescent radiation from the sample, which is a function of the metal concentration, is determined. Procedures are described for handling interference problems which arise from other elements.

THE occurrence of trace quantities of vanadium and nickel in petroleum is of considerable significance to the refiner. Fluid cracking catalysts are easily poisoned by these elements, with a resultant loss in catalyst activity and poor product yields. The refiner, therefore, must know the concentration of nickel and vanadium in stocks being charged to fluid cracking units. The user of residual fuels finds that vanadium causes corrosion of turbine blades and decomposition of fire brick. As a result of these effects, much effort is expended in analyzing charging stocks and residual fuels for nickel, vanadium, and other elements.

A number of approaches to this analytical problem have been described in the literature. Gamble and Jones (*6*), Key and Hoggan (*9*), and Dyroff, Hansen, and Hodgkins (*3*) describe emission spectrographic procedures for a number of elements in various petroleum fractions. Wrightson (*12*), Dyroff, Hansen, and Hodgkins (*3*), and Glass and others (*7*) describe colorimetric methods for vanadium, nickel, copper, and iron in crudes and other petroleum oils. A polarographic method (unpublished) has been used in these laboratories for a number of years. These methods are all reported by their authors to yield satisfactory results.

The purpose of this work was to investigate still another approach to the quantitative determination of nickel and vanadium by use of the x-ray spectrograph with the hope of reducing both the time and quantity of sample necessary for an analysis. X-ray spectrography, although a relatively new analytical technique, has been described rather thoroughly in the literature (*1, 5*). Dyroff and Skiba (*4*) have applied the x-ray spectrograph to the determination of trace amounts of nickel and vanadium on fluid cracking catalysts.

The method finally developed requires ashing of the oil sample, with the aid of sulfuric acid, and subsequent solution of the ash. An aliquot of the ash solution is evaporated on a glass disk and analyzed with the x-ray spectrograph for vanadium, nickel, and iron. If the iron concentration is more than 10 times that of the nickel, another aliquot is extracted to remove interfering iron and then evaporated on a second glass disk and analyzed for nickel. A sample rotator improves the precision and accuracy of the determination. The method is judged to be equal in speed and accuracy to emission spectrographic methods and somewhat faster than chemical methods. The elapsed time for one determination is about 5 hours, of which 1 hour is analyst working time.

APPARATUS

The apparatus used in this work was a Norelco (North American Philips Co.) x-ray spectrograph. Several modifications of the instrument used in this laboratory have been made to facilitate the analysis of the metallic components of lubricating oils and gasolines. These have been described in an earlier paper (*2*) and have been found to be generally helpful in this procedure.

Models of the Norelco x-ray spectrograph supplied before about March 1953 were equipped with a collimating device made by packing a bundle of nickel tubes in an aluminum holder. This has since been replaced with a collimator made of flat nickel foil plates 4 inches long, spaced 0.010 inch apart. This collimator is necessary to obtain sufficient x-ray intensities for this type of work. The half width of emission lines with this system is about 0.4°. This is sufficient resolution to separate the nickel *K* alpha line from the tungsten *L*1, which is the closest line combination that will be encountered in this work.

The wave lengths of the *K* x-ray emission lines of vanadium are 2.502 A. for the alpha and 2.280 A. for the beta. X-rays of this order are absorbed heavily in the air path between the sample and the Geiger tube. To circumvent this difficulty, a helium path arrangement has been used. The constructional details of such an assembly are described by Davis and Van Nordstrand (*2*). A commercial helium path has recently been made available by the instrument manufacturer.

Table I. Wave Lengths and Spectrometer Angles for Vanadium, Nickel, and Iron Lines Using Lithium Fluoride Crystal

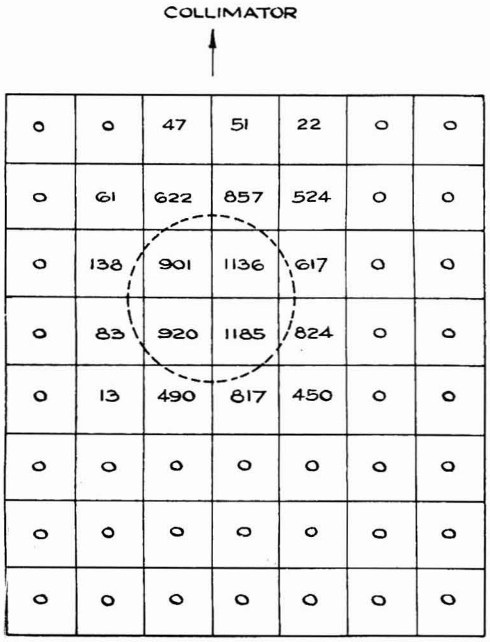
Element	λ, A.	Spectrometer Angle Using LiF Crystal, Degrees
Vanadium *Kα*	2.502	76.92
Nickel *Kα*	1.659	48.65
Iron *Kα*	1.937	57.49

One of the limitations to trace element work with the spectrograph is the x-ray tube. Most tubes currently available emit a spectrum which, in addition to the lines of the target material, shows appreciable amounts of other elements, principally nickel, copper, and iron. If this background intensity is too high, the measurement of the radiation from a very small amount of the element in the sample becomes rather difficult. There is no firm criterion as to when a tube is suitable for use in this type of work, but Friedman and Birks (*5*) state that it is inadvisable to attempt to detect a line intensity of less than one tenth the background. If one is willing to spend longer periods of time counting, this ratio might be somewhat lower. X-ray tubes vary considerably in the amounts of contamination present and it is possible to select or have selected a tube with a low level of contamination. The age of a tube is also a factor, with the general level of contamination increasing with tube life. One x-ray tube manufacturer has recently announced the availability of an x-ray tube of high spectral purity. One of these, an FA60 tungsten target tube, was made available to the authors for this work. A molybdenum tube should also be satisfactory.

Crystals for x-ray spectrography are available in several different materials. Lithium fluoride gives slightly better intensities than any other currently available, and hence was used in this work.

EXPERIMENTAL

In considering the determination of nickel and vanadium by means of the x-ray spectrograph, it seemed possible that sufficient intensities might be obtained from these elements to permit direct determination on the oil sample. The nickel K alpha line (Table I) falls in the most sensitive region for x-ray spectrography. The vanadium line is somewhat less sensitive, because of its longer wave length, but it was thought that its intensity might be raised to a sufficient level by use of the helium path. The examination of charging stocks and residual fuels by this procedure failed to yield sensitivities of a high enough order to be useful in a quantitative determination. The possibility of making a direct determination has not been abandoned, as an improvement in x-ray tube characteristics, and the use of a thin-windowed Geiger tube, may still make such a determination possible.



0	0	47	51	22	0	0
0	61	622	857	524	0	0
0	138	901	1136	617	0	0
0	83	920	1185	824	0	0
0	13	490	817	450	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0

Figure 1. Plot of intensity distribution over sample area

As the direct determination seemed impractical with the present equipment, work on a concentrated sample was next considered. The most convenient method of concentrating the nonhydrocarbon portion of the sample is through ashing. The ashing of petroleum oils is generally accomplished by one of the following three methods.

Dry Ashing. The oil is ignited and burns freely until only a carbonaceous residue remains. Further ignition is done either over a burner or in a muffle furnace until only the ash remains. The possibility of loss of metallic components is greatest with this method.

Wet Ashing. The oil is coked with sulfuric acid and an oxidizing agent, such as nitric acid, is used to oxidize the organic material. When oxidation is complete, the acid is evaporated, leaving the ash residue. This method is time-consuming, but no loss of metallic constituents should occur.

Coking-Ignition. The sample is heated with sulfuric acid to form a coke, excess acid is evaporated, and the carbon residue is oxidized by ignition. This method should also retain all of the ash and is faster than the wet oxidation procedure.

The details and relative merits of these procedures are reported in the literature by various investigators (*6–9, 12*). The coking-ignition procedure has been shown by Gamble and Jones (*6*) to yield satisfactory results. Such a procedure has been used in these laboratories for a number of years and has also been applied to this work.

Quantitative transfer of the ash from the ashing vessel to the x-ray sample holder is virtually impossible; hence, solution of the ash must be effected with subsequent evaporation on the sample holder. This, in early work, was nothing more than a flat aluminum plate. The evaporation of aqueous standards on a flat plate showed that reproducibility was difficult to achieve. The distribution of the sample on the holder seemed to be overly critical, yet the holder was smaller than and completely contained in, the area supposedly being viewed by the collimating system. The assumption had been made that the x-ray photons detected by the Geiger tube were coming from an area illuminated by the x-ray tube and bounded by the dimensions of the collimating system. In view of this nonreproducibility, an investigation was made of the effective radiating area of the sample.

To do this an aluminum plate, made to fit in a sample tray, was ruled in 0.25-inch squares. A count was then taken on this plate at the wave length of the nickel K alpha line. A film of nickel was then evaporated on a piece of aluminum foil and from this a 0.25-inch square was cut. The foil square was moved to cover successively each square of the plate and a count was taken in each position.

These data give an intensity pattern as shown in Figure 1. From this it can be seen that the radiation being detected by the Geiger tube does not come uniformly from the area being viewed by the collimator. Thus, it is evident that to work with small quantities of a solid sample and obtain reproducible results, a good distribution of the sample is necessary. Furthermore, to obtain the best intensity, the sample must be contained in an area not more than 0.5 inch square.

A uniform distribution of the ash on the sample plate was not easily achieved. Evaporation on an untreated surface left the residue in a series of concentration rings which varied in size and spacing with the speed of evaporation and the salt concentration. A number of procedures and conditions were tried to improve this, such as evaporation under controlled conditions, the addition of gelatin, poly(vinyl alcohol), wetting agents, and nonwetting agents, and spraying of the sample onto a hot sample plate.

Spraying the sample onto a hot disk was unsatisfactory because of loss of sample in the spray mist. Gelatin and poly(vinyl alcohol) gave good dispersions when used with the aqueous standard solutions with no acid present. The acid necessarily present in the ash solution caused a charring of the gelatin or alcohol and made evaporation to complete dryness difficult.

The use of water-repellent coating (Desicote) eliminated the formation of concentration rings and gave a satisfactory distribution.

The aluminum plates used in the earlier work were satisfactory when used with the aqueous solutions, but were easily attacked by the acid present in the ash solutions, yielding salts which interfered with the determinations. Platinum, although satisfactory in this respect, gave erratic background counts due to its crystalline structure. Annealing of the platinum improved, but did not eliminate, these diffraction effects.

As glass gives a uniform background and is unaffected by the acid, it proved to be the most satisfactory material for the sample holder. The final work was done using a 15-mm. microscope cover glass which had been treated with Desicote.

To minimize further the effect of the lack of uniformity in the sample radiating area, it was found desirable to rotate the sample during the counting period. A device was constructed (**Figure 2**) to hold and rotate the sample disk at about 30 r.p.m. The post supporting the sample was located so that the sample disk rotates in the area shown by the dotted lines in Figure 1. Table II shows the result of sample rotation. The data shown under "Without

Table II. Effect of Sample Rotation

Without Rotation during Counting, C.P.S.	With Rotation during Counting, C.P.S.
137.3	124.8
143.1	124.7
140.4	125.0
135.8	124.8

Rotation" were obtained by rotating the disk 0.25 turn following each count. The "With Rotation" data were obtained by successive counts on the same disk as it revolved on the rotator at 30 r.p.m. The data represent a fixed 12,800 count on the vanadium line.

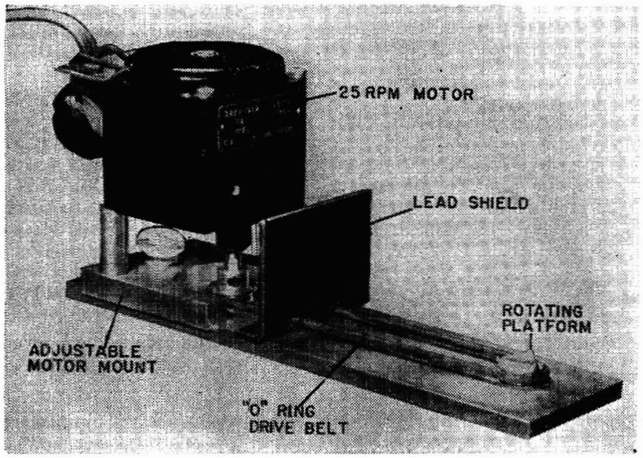


Figure 2. Device for holding and rotating sample

The fluorescent or secondary radiation emitted from the sought elements in the sample is subject to absorption effects from other elements which may be present. An examination of a table of mass absorption coefficients (*11*) shows that, of those elements normally found in charging stocks or residual fuels, iron is the only element which should give any appreciable interference due to absorption. This was found to be the case and in some instances the iron was present in a high enough concentration to absorb all of the nickel radiation from the sample. A generally useful method for handling interference problems such as this is through the construction of a family of calibration curves (*2*) varying the iron concentration through a range of nickel concentrations. This procedure is used when the iron concentration is not more than 10 times that of the nickel. Such a procedure is not practical when the iron concentration is much greater than this ratio because of the extreme reduction in nickel radiation by the iron. When the iron concentration is high, the most convenient method of handling the problem is by the removal of the iron from the nickel and vanadium. Any reagent used in the separation must not remain in the final sample, as any salt deposit on the disk will interfere with the determination.

A number of methods were evaluated, but none completely met the above requirements. The most satisfactory was the thiocyanate method (*10*), which gave a good separation of the iron from the nickel, but also removed part of the vanadium. The excess thiocyanate was easily destroyed with acid. When it was necessary to use the separation procedure, it was found best to determine the vanadium directly on part of the sample ash solution and to remove the iron from another aliquot before determining the nickel.

CALIBRATION CURVES

Calibration curves were constructed for the determination of vanadium, nickel, and iron. In order to take into account the reduction of nickel radiation by the iron present and the enhancement of the iron radiation by the nickel, it was necessary to construct families of curves for these two elements. The following curves or groups of curves were made.

A single curve for vanadium, in which counts per second were plotted against quantities of vanadium on cover glasses ranging from 0 to 15 γ.

A family of curves for nickel, in which counts per second were plotted against quantities of nickel on cover glasses ranging from 0 to 15 γ. Each curve of the family represented a different level of iron content of 0, 5, 25, and 50 γ.

A family of curves for iron, in which the counts per second were plotted against quantities of iron on cover glasses varying from 0 to 50 γ. Each curve of the family represented a different level of nickel content from 0 to 10 γ.

The various metals were deposited on the cover glasses by evaporation of 0.5 ml. of aqueous standard containing the proper amount of each metal.

Typical calibration curves are shown in Figures 3, 4, and 5. Background intensities from sample to sample were found reproducible within the statistical error. The determination of background on each sample was, therefore, abandoned and all of the counts per second values shown include background.

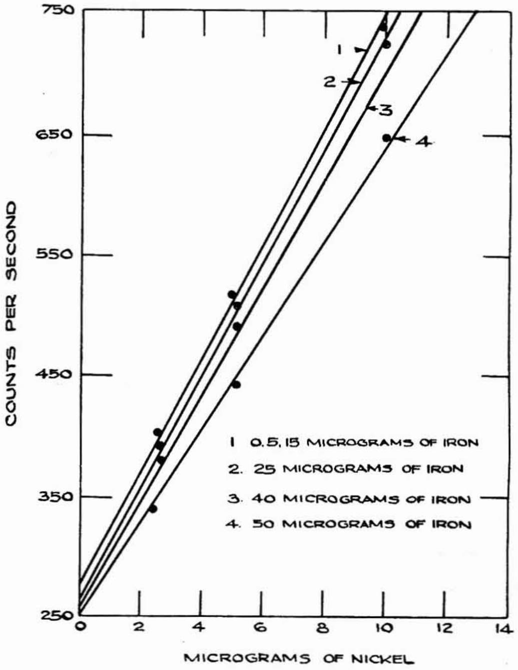


Figure 3. Nickel calibration

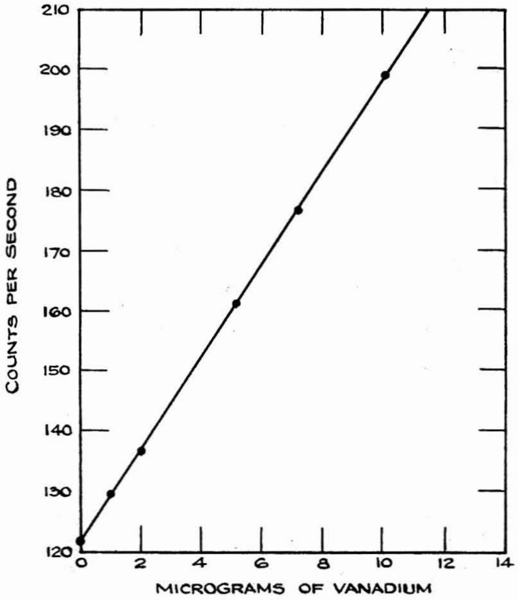


Figure 4. Vanadium calibration

Table III. X-Ray Operating Conditions

Tube	Tungsten
Kilovolts	50
Milliamperes	45
Crystal	LiF
Helium flow	1 liter/minute
Sample rotator speed	30 r.p.m.
Total counts	
Nickel	25,600
Vanadium	12,800
Iron	25,600

PROCEDURE

A sample of the appropriate size is weighed into a 250-ml. Vycor beaker. (Sample size varies with the type of material to be analyzed and the volume to which the solution of the ash is made. The 15-mm. cover glass, when treated with Desicote, will hold about 0.5 ml. of solution. So that only one evaporation will be required, it is desirable to have a minimum metal concentration of 0.5 γ each of vanadium and nickel per milliliter of ash solution. A 20-gram sample is adequate for most charging stocks and a 5- or 10-gram sample is generally sufficient for residual fuels.) An equal volume of concentrated sulfuric acid is added and carefully heated on a hot plate until a solid coke is formed. Additional heat is applied to the sample until the excess acid has been evaporated and no further fumes are evolved. The resulting coke is placed in a muffle furnace at 500° C. until all carbon is removed. After cooling, a drop of 1 to 1 nitric acid and 2 to 3 ml. of water are added and solution of the ash is completed with gentle heating. The solution is transferred to a 5- or 10-ml. volumetric flask and made to volume with distilled water.

A 0.5-ml. portion of the solution of the ash is pipetted onto a 15-mm. cover glass which has been treated with Desicote. The cover glass must be level and should be supported above the table by a disk or a washer which is slightly smaller in diameter than the glass. A 250-watt infrared lamp is mounted about 6 inches above the cover glass and the sample is evaporated to dryness. The glass disk is then placed on the sample rotator and a count is taken on the vanadium, nickel, and iron *K* alpha lines.

If in the above determination the indicated iron-nickel ratio is greater than 10, the following procedure should be used to determine nickel. A 0.5-ml. portion of the solution of the ash is pipetted into a 20-ml. separatory funnel and diluted to about 5 ml. Then 0.5 ml. of 10% ammonium thiocyanate solution is added and shaken well. The iron thiocyanate complexes are extracted with successive isoamyl alcohol washes until the red color is no longer present in the raffinate. The extract is washed with water containing a few drops of the ammonium thiocyanate solution and the wash is added to the raffinate. The raffinate is transferred to a beaker and evaporated to dryness. A drop of concentrated sulfuric acid and 2 ml. of 1 to 1 nitric acid are added to destroy the remaining ammonium thiocyanate, and the solution is again evaporated to dryness. The residue is redissolved in a drop of 1 to 5 nitric acid and 0.5 ml. of water with the aid of gentle heat. After solution is completed, the sample is quantitatively transferred to a cover glass and the evaporation on the disk is carried out as for the vanadium determination. The disk is placed on the rotator and a count is taken on the nickel *K* alpha line. X-ray operating conditions are shown in Table III. Each day a count is taken, on the nickel and vanadium lines, on one of the disks used in the preparation of the calibration curves. If the values so obtained differ from the original, a factor is derived to adjust back to the calibration curves. This factor is applied to all values obtained during the day.

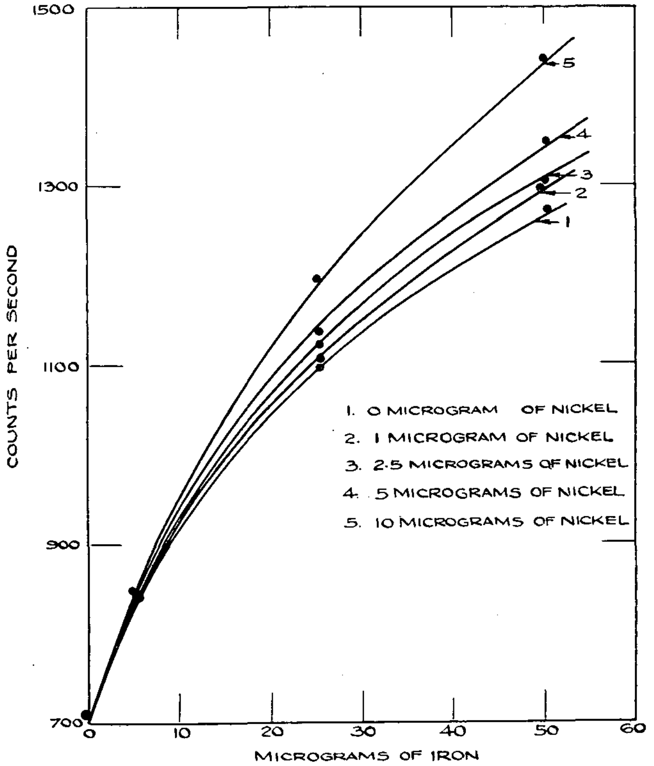


Figure 5. Iron calibration

Table IV. Comparative Results

Sample Description	Nickel, P.P.M.		Vanadium, P.P.M.	
	Polarograph	X-Ray	Polarograph	X-Ray
Synthetic blend, 5 p.p.m. V and Ni	6.10- 6.0	5.50- 5.70	4.8 -4.9	5.30- 5.40
Synthetic blend, 10 p.p.m. V and Ni	11.2 -10.8	10.6 -10.6	9.50-9.40	10.1 -10.1
Feed stock				
1	1.61	1.38	1.54	1.38
2	0.59	0.82	0.50	0.52
3	0.43	0.50	0.11	0.10
4	1.10	1.18	1.76	1.52
5	0.90	0.93	0.34	0.37
6	0.90	1.11	0.34	0.43
7	0.59	0.50	0.64	0.80
8	0.59	0.72	0.64	0.75

Table V. Determination of Precision

	Ni, P.P.M.	V, P.P.M.
	1.30	0.40
	1.25	0.40
	1.25	0.40
	1.30	0.35
	1.30	0.30
Average	1.28	0.37
Average deviation	0.03	0.04
Precision	±2.3%	±10%

CALCULATIONS

The elapsed time required to obtain the fixed counts is converted to counts per second by:

$$\text{Counts/second} = \frac{\text{total fixed count}}{\text{elapsed time in seconds}}$$

and the quantity of nickel and vanadium on the cover glasses is obtained from the appropriate calibration curve. Total of micrograms of metal in the total sample is obtained by:

$$\text{Total micrograms of V or Ni} = \frac{\text{micrograms in aliquot} \times \text{ml. of ash solution}}{\text{ml. of aliquot}}$$

Parts per million of metal in the oil sample are obtained by:

$$\text{P.p.m. in sample} = \frac{\text{total micrograms of metal in sample}}{\text{wt. of sample in grams}}$$

Table IV shows a comparative study made on a number of charging stocks and two synthetic blends between the x-ray method and a polarographic method. In the analysis of feed stocks, it is felt that the agreement is good. To obtain an estimation of the precision of the method, replicate analyses were run on one sample at intervals covering a week's time (Table V).

The indicated precision is ±10% for the vanadium and ±2.3% for the nickel.

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Combined Radiometric and Fluorescent X-Ray Spectrographic Method of Analyzing for Uranium and Thorium

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Employing radioactivity measurements and fluorescent x-ray spectroscopy, a rapid method of analyzing ores for uranium and thorium has been developed. The total radioactivity of uranium and thorium is determined and expressed as per cent equivalent uranium. From the line intensity ratio U$L\alpha$/Th$L\alpha$ measured by fluorescent x-ray spectroscopy the weight ratio of uranium to thorium is calculated. As the relative radioactivity of uranium to thorium is known, the weight per cent of uranium and thorium can be calculated. The time required is approximately 20 minutes per analysis for both elements. The accuracy is ±10% of the amount present in samples containing more than 0.5% of the elements and the lower limit of detection is 0.01 to 0.03% of either element.

As a public service, the Bureau of Mines, College Park, Md., examines a large number of mineralogical samples submitted for identification and for determining possible commercial value. This service includes approximately 2000 tests for radioactivity per year. Samples with measurable activity are studied further to determine the approximate concentration of uranium and thorium.

For various reasons, principally the time and cost of analyses, chemical methods are not satisfactory. Optical spectroscopic techniques have only limited application.

Preliminary investigations indicated that a combined technique for determining total radioactivity, with a subsequent uranium-thorium ratio measurement by fluorescent x-ray spectrography, offered excellent possibilities.

INSTRUMENTATION

Radioactivity is measured with standard commercial Geiger-counter equipment, with provisions for accurately positioning powdered samples.

The x-ray spectrograph used is a modified Norelco 90° spectrometer employing commercially available parts. Any one of the commercial units of the North American Philips Co., the General Electric Co., X-Ray Department, or the Applied Research Laboratories, should be satisfactory.

RADIOACTIVITY MEASUREMENTS

The samples, ground to −325 mesh, are placed in suitable holders and packed to a constant level with the aid of a spatula. The sample is positioned in a holder at a constant distance from the Geiger tube window. The radioactivity is counted for a fixed time, usually 8 minutes, and the intensity is recorded as counts per minute above background. These values are compared directly with calibration curves prepared from radioactivity standards. A series of calibration curves is prepared for the various distances from the sample to the detector window. By choosing a suitable distance, it is possible to remain within the linear response range of the Geiger tube. Regardless of the uranium-thorium ratio, the radioactivity is expressed as "per cent equivalent uranium," that amount of uranium in pitchblende in radioactive equilibrium necessary to give an equal activity.

DETERMINATION OF THORIUM-URANIUM RATIO

After the radioactivity measurements are completed, the samples are placed in the fluorescent x-ray spectrograph to determine the thorium-uranium ratio. These elements have atomic numbers 90 and 92, so that as a first approximation the line-intensity ratio of Th$L\alpha$ to U$L\alpha$ equals the weight–per cent

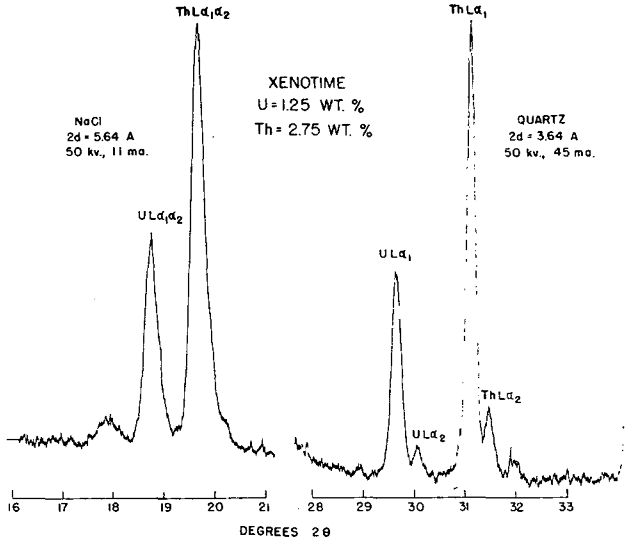

Figure 1. Uranium $L\alpha$ and thorium $L\alpha$ spectra from xenotime

Showing relationship between line intensities and concentrations of respective elements ratio of thorium to uranium, as shown in Figure 1. A more exact value is obtained from the following relationship:

$$\text{Th}L\alpha/\text{U}L\alpha = C\,(\text{wt. \% Th/wt. \% U}) \qquad (1)$$

where C is determined for a particular set of instrumental and operating conditions from samples of known thorium and uranium content.

Details in instrumentation and sample preparation have been described in the literature (*2–4*).

CALCULATION OF URANIUM AND THORIUM CONTENT

The uranium and thorium content is calculated from the following equations.

$$x + y\frac{x}{k} = \%\ \text{equiv. uranium} \qquad (2)$$

where

x = wt. % uranium
y = wt. % thorium/wt. % uranium
k = relative radioactivity of uranium to thorium

(There may be some variation in this constant between minerals. For this work and the equipment employed, a value of 5.0 was used.)

Equation 2 can be solved for x, as the terms y and percentage of equivalent uranium have been determined. The corresponding thorium content is obtained from Equation 3.

$$\text{Wt. \% thorium} = k\,(\%\ \text{equiv. U} - \text{wt. \% U}) \qquad (3)$$

ACCURACY AND SENSITIVITY

To check this method a number of chemically analyzed samples were run as unknowns; the results are shown in Table I. They indicate the method is accurate to ±10% of the amount present except for samples containing less than about 0.5% uranium or thorium. The technique is independent of the mineral type or matrix of the sample. As shown in Figure 2, the lower limit of detection is 0.01 to 0.03% of uranium or thorium. If increased sensitivity is necessary, the x-ray line intensity can be increased by using a more efficient detector and a sodium chloride analyzing crystal.

Table I. Comparison of Uranium and Thorium Analyses by Chemical versus Physical Methods

Sample	% Equiv. U	Th$L\alpha$/ U$L\alpha$	Uranium Wt. % Chemical	Uranium Wt. % Physical	Thorium Wt. % Chemical	Thorium Wt. % Physical
Monazite						
1	1.32	45.8	0.16	0.13	5.71	5.95
2	1.42	12.8	0.37	0.40	4.79	5.10
3	1.98	34.6	0.27	0.25	8.37	8.65
4	1.32	8.75	0.45	0.48	3.90	4.20
5	1.15	35.7	0.093	0.14	4.97	5.03
6	1.38	39.0	0.10	0.15	5.75	6.10
7	2.37	24.3	...	0.40	10.3	9.85
Xenotime	1.80	2.27	1.28	1.25	2.87	2.75
Aeschynite	4.39	1.79	...	3.23	5.79	5.80
Thorianite						
1	1.49	2.75	...	0.96	2.90	2.65
2	0.64	5.49	...	0.30	1.63	1.66
Phosphate rock	0.028	No Th$L\alpha$	0.025	0.028	...	..
Pitchblende	2.90	No Th$L\alpha$	2.85	2.90	...	..
Carnotite						
1	0.078	No Th$L\alpha$	0.093	0.078	...	..
2	0.13	No Th$L\alpha$	0.15	0.13	...	..
Thorite	0.48	6.82	....	0.20	1.70	1.39
Apatite	0.070	5.74	0.031	0.033	0.17	0.18

DISCUSSION

This physical method of analysis is based on the assumption that the samples are in radioactive equilibrium. The majority of mineral samples received by this laboratory are in equilibrium, but this may not be true for chemical separation products. An alternative method has been suggested by Frank Grimaldi of the U. S. Geological Survey in which the uranium content is rapidly determined fluorimetrically (*8*). Radioactive equilibrium is not necessary when using this combined chemical and x-ray spectrographic procedure.

Table II. Interfering Emission Lines and Absorption Edges

Comparison lines
Th$L\alpha_1$ = 12.966 k.e.v.
U$L\alpha_1$ = 13.613 k.e.v.

Comparison edges
ThL_{III} = 16.296 k.e.v.
UL_{III} = 17.163 k.e.v.

Emission Lines	K.E.V.	Absorption Edge	K.E.V.
Y $K\beta_2$	17.011	Br K	13.475
Y $K\beta_1$	16.736	Ir L_I	13.413
Nb $K\alpha_1$	16.614	Pt L_{II}	13.268
Nb $K\alpha_2$	16.520	Pb L_{III}	13.044
Pa $L\beta_1$	16.700	Bi L_{III}	13.424
U $L\beta_2$	16.425		

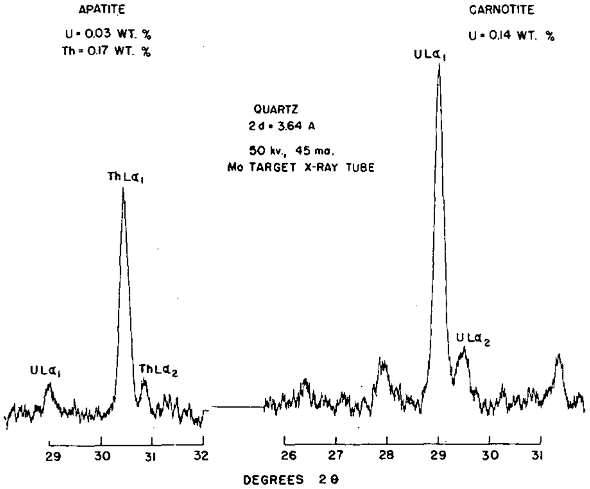

Figure 2. Uranium $L\alpha$ and thorium $L\alpha$ spectra from low concentrations of respective elements

No corrections are applied for density of sample or for absorption of radiation in the sample. They were not found necessary, as the samples of higher density have a greater number of uranium and thorium atoms for a given weight per cent of these elements. This compensation of higher absorption but greater activity per unit volume was verified by the results obtained in the study on samples of widely varying densities.

Several factors must be considered in order to obtain accurate uranium-thorium ratios. An obvious error would be the presence of overlapping lines; these can be detected by obtaining a chart record over the required spectral range. Also, the possible interfering lines can be found in tables similar to those prepared by Campbell and Parker (*6*). Any absorption edge occurring between the two lines, or emission line between the corresponding L_{III} edges causes a variation in the Th$L\alpha$/U$L\alpha$ ratio. Both cases result in an increased Th$L\alpha$/U$L\alpha$ ratio. Possible interferences were tabulated from the paper by Fine and Hendee, as listed in Table II (*7*).

Possible interferences are from preferential excitation by Nb$K\alpha$ and preferential absorption by the PbL_{III} and BiL_{III} edges. A suitable correction can be applied by the use of samples containing known amounts of these elements. Investigations have indicated that, unless these interfering elements are present in quantity greater than 5 to 10%, the effect can be neglected (*1,5*).

Except when an absorption edge occurs between U*Lα* and Th*Lα* lines, the line of longer wave length, Th*Lα*, is absorbed to a slightly greater extent. The fluorescence yield factors of uranium and thorium are essentially equal. The excitation voltage for the thorium *L* series is less than for the uranium *L* series, so that for a given applied voltage the excitation voltage of thorium is exceeded by a larger value. These various effects are compensating, so that no correction was found necessary within the limits of these analyses.

The method is based on a linear relationship between line intensities and concentrations of the elements. When the relative amounts of these elements vary greatly this will be only approximately correct. However, up to about 10% uranium or thorium, this relationship is linear except for matrices of low x-ray density.

The x-ray intensities are measured in the linear range of the Geiger tube or a dead time correction is applied. The scintillation counter with its low counting loss is linear up to 3000 to 4000 counts per second and is sensitive to U*Lα* and Th*Lα*. This study indicates that the scintillation counter is the superior detector for this analysis.

ACKNOWLEDGMENT

A number of chemically analyzed samples were supplied by Frank Grimaldi of the U. S. Geological Survey; his cooperation is gratefully appreciated.

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Quantitative Infrared Analysis of Alkyl Phenol Mixtures

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A method is described for determining the quantitative distribution of phenol, the three isomeric cresols, the six isomeric xylenols, and several isomeric trimethylphenols in coal tar or tar acid oil. The phenolic compounds are extracted from the other organic compounds present with aqueous sodium hydroxide. After springing the phenols with sulfuric acid, they are dried using benzene in a Dean-Stark apparatus. The dried mixture of phenols is fractionally distilled at 50 mm. of mercury to yield five fractions. The cut points of initial boiling point to 113° C., 113° to 121°, 121° to 127°, 127° to 134°, and 134° to 144° C.—all column pressures being 50 mm. of mercury—were selected to remove interfering pairs of isomers from samples subjected to infrared analysis. Interferences and analytical wave lengths, as well as details of the procedure, are given.

DURING research on the low temperature carbonization of bituminous coal, the need arose for a rapid and accurate method for determining the isomer distribution of phenolic compounds in the tar fraction boiling below 230° C. Because of the similar properties of many of the isomers, they cannot be quantitatively separated by distillation, crystallization, or other physical means. Although several methods (*3, 8, 12*) have been developed based on chromatography, they are applicable only to a small number of isomers and require rigid control of conditions.

Chemical methods of analysis are usually limited to the determination of total phenolic compounds. Stevens (*11*) has published a method for the separation of *m*-cresol from *p*-cresol and 2,4-xylenol from 2,5-xylenol by butylation and debutylation; however, this method does not permit the differentiation of all the tar acids.

The limitations of the chemical methods also exist for most instrumental techniques. The very close oxidation potentials prevent the application of electrical methods (*6, 7, 9*). Emission spectroscopy is, of course, useless, and very little has been reported on the use of a mass spectrometer as applied to this type of problem.

Several authors (*1, 2*) have used ultraviolet and visible absorption spectroscopy for the analysis of a few mixtures, but the small number of broad absorption bands confines these methods to rather simple mixtures. Ando (*1*), Friedel (*5*), and Whiffen (*13*) have shown that infrared spectroscopy is suitable for analysis,

Table I. Preparation of Pure Compounds for Calibration

Compound	Original Source	Treatment
Benzene	Thiophene-free ACS grade	Azeotropically dried
Phenol	Mallinckrodt c.p.	Redistilled
o-Cresol	Reilly Co., 98%	Redistilled, heart cut
m-Cresol	Reilly Co., 98%	Butylated, distilled
p-Cresol	Hercules Co., synthetic	Redistilled, heart cut
2,6-Xylenol	Pgh. Cons. Coal Co., R & D	Synthesized
2,4-Xylenol	Reilly Co.	Butylated, distilled
2,5-Xylenol	Reilly Co.	Butylated, distilled
3,4-Xylenol	Reilly Co.	Butylated, distilled
3,5-Xylenol	Reilly Co.	Distilled, recrystallized
2,3-Xylenol	Pgh. Cons. Coal Co., R & D	Synthesized
o-Ethylphenol	U. S. Bureau of Mines	None
m-Ethylphenol	Reilly Co.	Redistilled, heart cut
p-Ethylphenol	U. S. Bureau of Mines	None
2,4,6-Trimethylphenol	Shell Development	None
2,3,6-Trimethylphenol	Shell Development	None

Table II. Boiling Point and Absorption Bands of Methyl Substituted Phenols

Compound	Distillation Fraction	Boiling Point ° C. at 50 Mm. of Hg	Analytical Absorption Band, Microns
Benzene			14.89
Phenol	1	109	9.34
o-Cresol		113	9.04
2,6-Xylenol		115	13.09
m-Cresol	2	121	12.85
p-Cresol		121	12.22
o-Ethylphenol		123	13.30
2,4-Xylenol	3	127	12.27
2,5-Xylenol		127	10.04
2,3-Xylenol		132	9.37
2,4,6-Trimethylphenol	4	132	11.70
m-Ethylphenol		134	11.03
p-Ethylphenol		134	12.07
3,5-Xylenol		137	14.68
3,4-Xylenol	5	142	9.95
2,3,6-Trimethylphenol		144	9.22

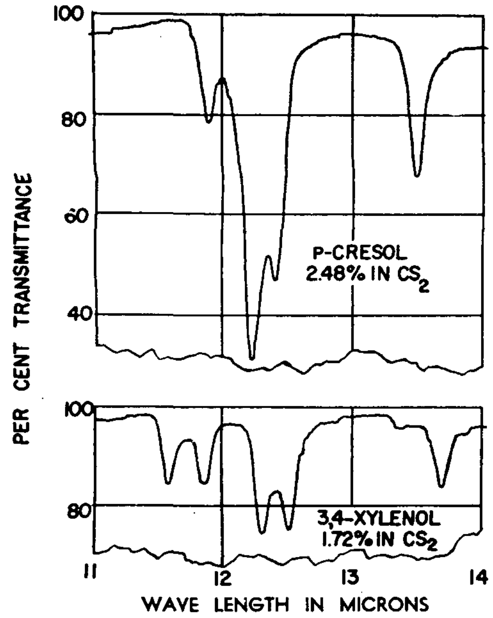

Figure 1. Comparison of spectra of *p*-cresol and 3,4-xylenol

and Woolfolk, Golumbic, Friedel, Orchin, and Storch (*14*) have applied their data to the analysis of many of the isomers found in coal tar. Essentially an extension of Friedel's work, the method detailed here includes all of the isomers boiling below 230° C.

EXPERIMENTAL

The methods shown in Table I were used to prepare high purity compounds for calibration. The distillations were made on a column, 1 inch by 4 feet (about 30 theoretical plates), which was fitted with Cannon packing and operated at a reflux ratio of 5 to 1 with a boilup of 8 ml. per minute. The butylations were carried out as described by Stevens (*11*).

The infrared absorption spectra of the pure materials were obtained using a Baird double beam recording infrared spectrophotometer equipped with sodium chloride optics. All work described in this paper was carried out using approximately 2% (by weight) solutions of the phenols in carbon disulfide with 0.1-mm. cells. The reference cell was filled with carbon disulfide. Bakers Analyzed C.P. carbon disulfide is suitable without any treatment. Comparison of the spectra with those listed in the literature cited showed no extraneous bands.

The individual isomers were run at several concentrations (1.5 to 2.5%), and the wave lengths listed in Table II were selected for analytical use. All of the bands selected are free from interferences of near-boiling compounds, are strong absorption bands, and, with the exception of the 9.04-micron band of *o*-cresol, the absorbance is proportional to concentration. *o*-Cresol exhibited no absorption which obeyed Beer's law; however, experience has shown that for the concentrations of *o*-cresol usually found, the deviation from linearity at 9.04 microns is not a limiting factor in the precision of the analysis.

Figure 1 illustrates the spectra of *p*-cresol and 3,4-xylenol and points out the necessity of separating these two compounds before analysis, as both compounds have strong absorption bands in the 12.2- to 12.4-micron region. As shown in Figure 2, 3,4-xylenol must not be present with 2,4- and 2.5-xylenol. The spectra of the ethyl phenols are very similar to those of their corresponding cresol analogs, and these pairs should not be present in the same cut.

Examination of the boiling points at 50 mm. of mercury (see Table II) indicates that a precise distillation prevents the mentioned interferences, especially if an appreciable amount of *m*-ethylphenol is present to provide a "flat" between 2,4-xylenol and 3,4-xylenol. The distillation cuts selected were initial boiling point to 113° C., 113° to 121°, 121° to 127°, 127° to 134°, and

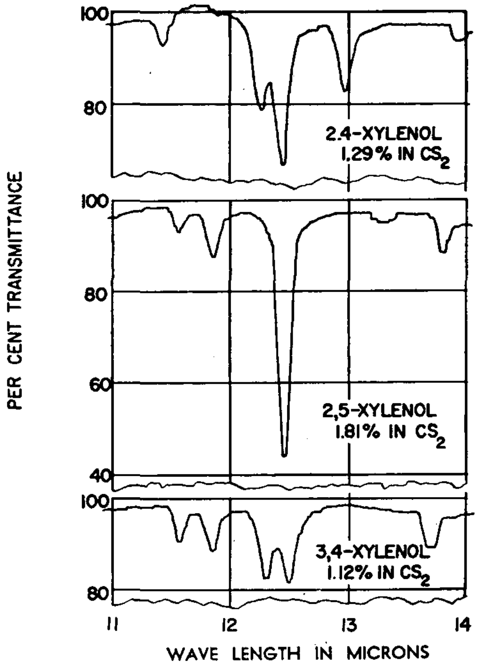

Figure 2. Comparison of spectra of 2,4-xylenol, 2,5-xylenol, and 3,4-xylenol

Table III. Analyses of Synthetic Mixtures

	Mixture Number 1			2			3			4			5		
	Act., %	Anal., %	Dev., %	Act., %	Anal., %	Dev., %	Act., %	Anal., %	Dev., %	Act., %	Anal., %	Dev., %	Act., %	Anal., %	Dev., %
Benzene	48.7	49.3	+0.6												
Phenol	25.7	25.5	−0.2	17.1	18.0	+0.9									
o-Cresol	25.6	25.2	−0.4	33.6	34.3	+0.7									
2,6-Xylenol				8.3	8.2	−0.1									
m-Cresol				18.6	17.6	−1.0	9.9	10.3	+0.4						
p-Cresol				22.5	21.9	−0.6	17.2	18.0	+0.8						
o-Ethylphenol							23.7	24.0	+0.3						
2,4-Xylenol							26.9	25.0	−1.9	13.6	14.4	+0.8			
2,5-Xylenol							22.3	22.5	−0.2	10.1	9.2	−0.9			
2,3-Xylenol										19.8	20.5	+0.7			
2,4,6-Trimethylphenol										16.1	15.1	−1.0			
m-Ethylphenol										25.4	25.4	0.0	50.3	51.0	+0.7
p-Ethylphenol										15.1	15.4	+0.3	13.8	14.7	+0.9
3,5-Xylenol													11.4	11.4	0.0
3,4-Xylenol													16.3	15.1	−1.2
2,3,6-Trimethylphenol													8.1	7.8	−0.3

134° to 144° C.—all column pressures being 50 mm. of mercury. These cuts result in the distribution of isomers as shown in Table II. Synthetic samples were made up to contain the materials predicted in each fraction. These samples were analyzed by the method described here. The results given in Table III indicate an accuracy of one absolute per cent.

ANALYTICAL METHOD

Sufficient tar or tar acid oil to give at least 50 grams of tar acids is weighed into a separatory funnel and extracted four times with an equal volume of a 10% aqueous solution of sodium hydroxide. The combined aqueous phases are acidified with 30% sulfuric acid, and the sprung phenolic compounds separated. The aqueous phase is extracted four times with C.P. benzene, and the benzene phase added to the wet tar acids. The resulting mixture is dried using a Dean-Stark azeotropic drying apparatus and most of the benzene is stripped off. If the sample contains less than 5% *m*-ethylphenol, 5 grams of this compound are added to the weighed dry tar acids. Ten grams of acenaphthene (1,2-dihydroacenaphthalene) are added to act as a "backer" to ensure the distillation of all the phenolic compounds. The mixture is then distilled at a pressure of 50 mm. of mercury on an efficient column having at least 25 theoretical plates, and the mentioned fractions are collected, weighed, and set aside for infrared analysis.

Using an analytical balance, samples of the distillation cuts are weighed in 2-ounce vials, and sufficient carbon disulfide is added to give approximately a 2% solution of the tar acids. Usually 0.2 gram of sample and 10 grams of carbon disulfide are used. Aluminum foil liners in the caps of the vials prevent contamination and evaporation of the solution. The absorption spectrum of the solution from 9 to 15 microns is obtained using 0.1-mm. cells with carbon disulfide in the reference cell. Transmittance values are read from the chart at the wave lengths tabulated in Table II. After converting transmittance to absorbance, the concentration of each component is determined using matrices (*4, 10*) prepared from the spectra of the pure compounds. The sum of the components in each cut may not total 100%; however, if no extraneous absorptions are noted, the analysis is normalized to place it on a 100% basis. Experience of the authors has shown that when the unnormalized totals consistently fall below 95%, the analysis is not trustworthy, and recalibration of the cell is necessary. The calibration may be checked at any time by the use of a synthetic sample made up from the pure isomers, with recalibration usually being necessary every 2 to 4 months. Calculation of the isomer distribution in the original sample is straightforward once the analysis and weight of each distillation fraction is known.

The vials used for weighing may be reused after washing with a detergent and then acetone, but the caps and liners should be discarded after one use since *they cannot be easily cleaned.*

Although the method described seems to be time-consuming, a complete analysis can be obtained in 24 man-hours. If desired the method can be applied to tar acid mixtures containing only two or three isomers with a resulting increase of precision to about 0.5% absolute. The method has been in use for a period of 4 years in this laboratory with satisfactory results.

ACKNOWLEDGMENT

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Automatic Ultraviolet Spectral Scanning of Chromatographic Effluents

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Chromatography has been widely employed for separating complex mixtures to facilitate analysis, which makes it an ideal supplement to ultraviolet spectrophotometry. Hand collection of cuts and subsequent determination of their ultraviolet spectra are time-consuming and costly. An attachment to a Cary spectrophotometer is described with which the ultraviolet absorption spectrum can be obtained automatically on each of 200 or more chromatographic cuts. This record is displayed on one chart after a 16-hour overnight run, from which qualitative identification and quantitative determination of the compounds in the mixture can be made. Gradient elution is used to increase the versatility of the technique. Cuts to be scanned are determined on a volume basis for better quantitative precision, thus avoiding the problem of constant flow rate. With this apparatus, as many as 20 different compounds have been detected in one sample. A quantitative analysis is presented for a synthetic mixture of eight aromatic compounds. The results agree within ±3% of the amounts present.

IN THE application of ultraviolet absorption spectra to the analysis of mixtures of absorbing substances, the analyst is often plagued by the interferences due to overlapping absorption bands. It is often possible to simplify mixtures by separating them into fractions, which may reduce interference enough to allow successful analysis. One very powerful tool for such separations is adsorption chromatography. The determination of the solute concentrations in the eluate from a chromatographic column by ultraviolet absorption, where applicable, offers certain advantages. Both qualitative and quantitative analyses can be made in very dilute solutions, which avoids the necessity for evaporating the solvent, or other tedious handling procedures which may often involve the danger of losses. Secondly, the fact that chromatography can be carried out on very small amounts of sample with consequent very dilute solutions reduces some of the problems in the chromatographic process itself. For example, better resolution is obtained by avoiding column overloading.

In order to make chromatography applicable to the separation of mixtures of many classes of compounds, it is necessary to increase the eluting power of the eluent during a run. For this purpose gradient elution is a convenient and desirable device.

This technique, of gradually increasing the eluting power of the solvent, as opposed to stepwise addition of more polar eluents, has been advocated by several investigators (*1–3, 5–8, 10, 11, 14, 16, 17*). It has two major advantages, it requires no attention from an operator and it reduces the tailing of compounds having nonlinear adsorption isotherms.

In applying these techniques, however, the collection of large numbers of fractions and the determination of their ultraviolet absorption spectra were found to be very time-consuming. Several papers have been published on the use of devices for scanning paper chromatograms at one wave length in the ultraviolet region (*4, 12, 13*). Two papers describe the passing of the eluate from a chromatographic column continuously through an absorption cell in an infrared spectrophotometer and determining a single component at one wave length (*9, 15*).

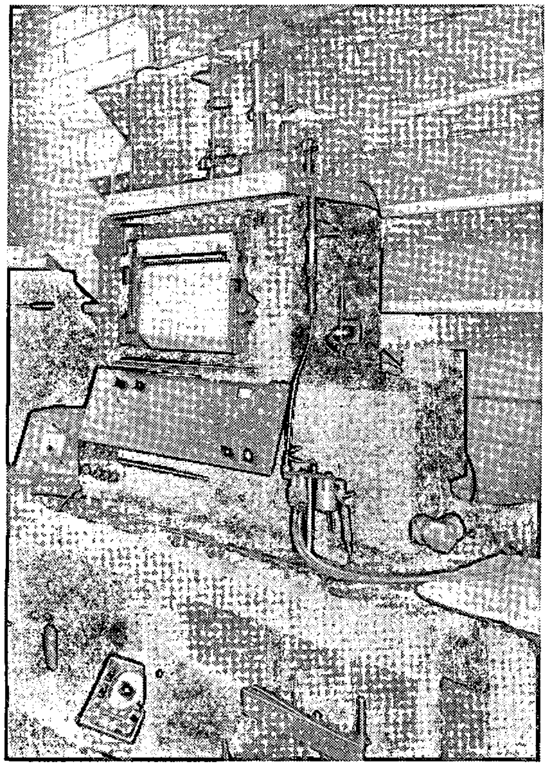

Figure 1. Scanalyzer assembled

This paper describes the "Scanalyzer"—a device for automatically scanning the complete near ultraviolet absorption spectrum, from 400 to 200 mμ, of small volume fractions of the eluate from a gradient-eluted chromatographic column. The fractions scanned are determined on a volume basis instead of on a time basis, since experience has shown that flow rates are difficult to maintain constant. The Scanalyzer requires no attention from an operator after the start of the run. Over 200 scans are made during the course of one night (16 hours).

DESCRIPTION OF INSTRUMENT

The complete Scanalyzer is shown in Figure 1. The basic instrument is a Cary Model 11-M recording spectrophotometer. The array of bottles mounted on top of the instrument constitutes the gradient elution system whose function is to supply to the chromatographic column a flow of solvent whose polarity constantly increases during a run. The pair of solenoid valves at the lower right of the instrument operates in conjunction with the photoelectric level detector (mounted on the cell compartment) to divide the eluate from the column into equal volume increments. The control unit, shown at the left of the instrument, operates the spectrophotometer so that it automatically records the absorption spectrum of each increment of eluate. The fraction collector, on the shelf below the instrument, collects the eluate increments in the order in which they are scanned by the spectrophotometer.

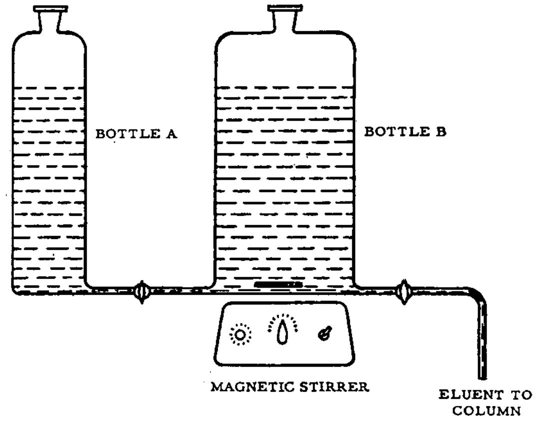

Figure 2. Gradient elution system

Gradient Elution System. The gradient elution system consists of two bottles and a pair of stopcocks. One of the stopcocks is used to connect the bottles together and the other is simply an on-off valve in the eluent supply line as shown in Figure 2.

With both stopcocks closed, bottle *B* is filled with the less polar of the two solvents to be used and bottle *A* is filled with the more polar solvent. If the two solvents have appreciably different densities, the liquid levels in the two bottles should be adjusted so that there is no difference in hydraulic head across the connecting stopcock. When the connecting stopcock is opened no flow of solvent occurs between the two bottles until some of the solvent has been drawn from bottle *B* through the eluent supply line. As solvent is drawn from the system and supplied to the chromatographic column, the levels in the two bottles fall together and the polarity of the solvent in bottle *B* is constantly increased by the flow of polar solvent from bottle *A*.

The type of polarity gradient that this system gives depends upon the geometry of the system—i.e., the sizes and shapes of the bottles. For a system of two cylindrical bottles with solvents of equal density (assuming complete mixing in bottle *B*) the fraction of polar solvent in bottle *B*, and therefore in the eluent, is given by

$$X_B = X_A - X_A\left[1 - \frac{V_c}{V_A^\circ + V_B^\circ}\right]^{\frac{V_A^\circ}{V_B^\circ}} \qquad (1)$$

where X_A = fraction of polar solvent in bottle *A*
X_B = fraction of polar solvent in bottle *B*
V_A° = initial volume of solvent in bottle *A*
V_B° = initial volume of solvent in bottle *B*
V_c = volume of solvent withdrawn from the system

This equation is essentially the same as the one given by Lakshmanan and Lieberman (*10*) for a somewhat similar gradient elution system. Although both systems give the same types of gradients, the advantage of the present system over the one described by Lakshmanan and Lieberman is that the gradient is determined by the geometry of the system rather than by independent adjustments of the flow rates.

A recent paper by Bock and Ling (*3*) describes a two-bottle gravity flow elution system very similar to this one which was independently developed by Parr (*14*).

Chromatographic Column. The solvent from the gradient elution system is fed to the chromatographic column through a length of 13-gage stainless steel hypodermic needle tubing (Superior Tube Co., Norristown, Pa.). The glass to metal connections are facilitated by $\frac{10}{18}$ ₸ stainless steel joints which were fabricated in the company's shops.

The chromatographic column is 9 mm. in diameter (Scientific Glass Apparatus Co., Inc., Bloomfield, N. J., No. 1) and has a perforated base. A $\frac{10}{18}$ ₸ female joint was added to the top of the column in order to connect it to the gradient elution system.

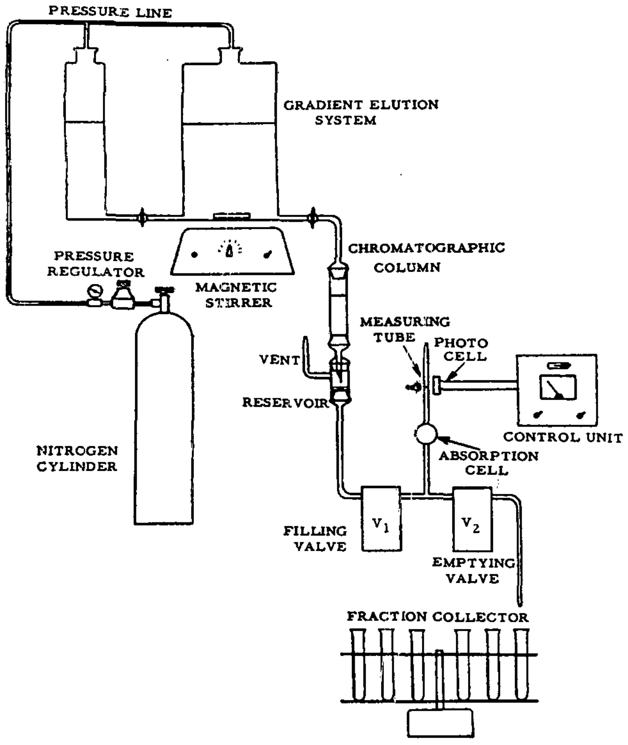

Figure 3. Scanalyzer flow system

When the column is packed, it has an appreciable resistance to the solvent flow, so the system must be kept under pressure in order to maintain an eluate flow of 1 ml. per minute. With a No. 1 column this is generally between 1 and 8 pounds per square inch.

Absorption Cell. A demountable type absorption cell with quartz windows and a 1-cm. stainless steel spacer is used. Polyethylene gaskets are used between the spacer and the windows. The windows are clamped tightly in place by means of stainless steel plates and Allen capscrews. The cell is rigidly fastened to the cover of the spectrophotometer cell compartment, so that it can be put into operation quickly and easily.

Flow Quantizer. The Scanalyzer flow system is shown schematically in Figure 3.

The solvent flows from the gradient elution system, through the chromatographic column, and into the reservoir. At the beginning of each cycle, valve V_1 is open and valve V_2 is closed so that the eluate flows from the reservoir into the absorption cell and the measuring tube. When the liquid rises high enough in the measuring tube to come between the lamp and the photocell, it acts as a cylindrical lens and focuses the light on the photocell. This causes an increase in photocell current which operates a 50-μa. meter-type relay. The meter relay in turn operates a secondary relay in the control unit (shown at the left of the instrument in Figure 1). The secondary relay closes valve V_1 (thus isolating a cell volume of eluate from the reservoir) and starts the spectrophotometer scanning. When the spectrophotometer has recorded the absorption spectrum of the cell full of eluate, the control system opens valve V_2 which empties the eluate from the absorption cell into the fraction collector. When the cell is empty, V_2 closes and V_1 opens. The spectrophotometer then returns to its initial wave length and the cycle is complete.

Fifteen determinations were made of the volume between the two valves V_1 and V_2. (Although this is greater than the volume of the absorption cell, it is referred to as the cell volume.) The data were statistically evaluated and the cell volume was found to be 3.85 ± 0.06 ml. (95% confidence level).

Control Units. Two different control circuits have been used for the Scanalyzer. The first system operated on a time cycle and required a minimum of modification of the spectrophotometer. The present system operates on a wave length basis and eliminates the necessity of scanning unnecessary wave lengths. This saves both time and chart paper, but it requires the installation of adjustable limit switches in the spectrophotometer.

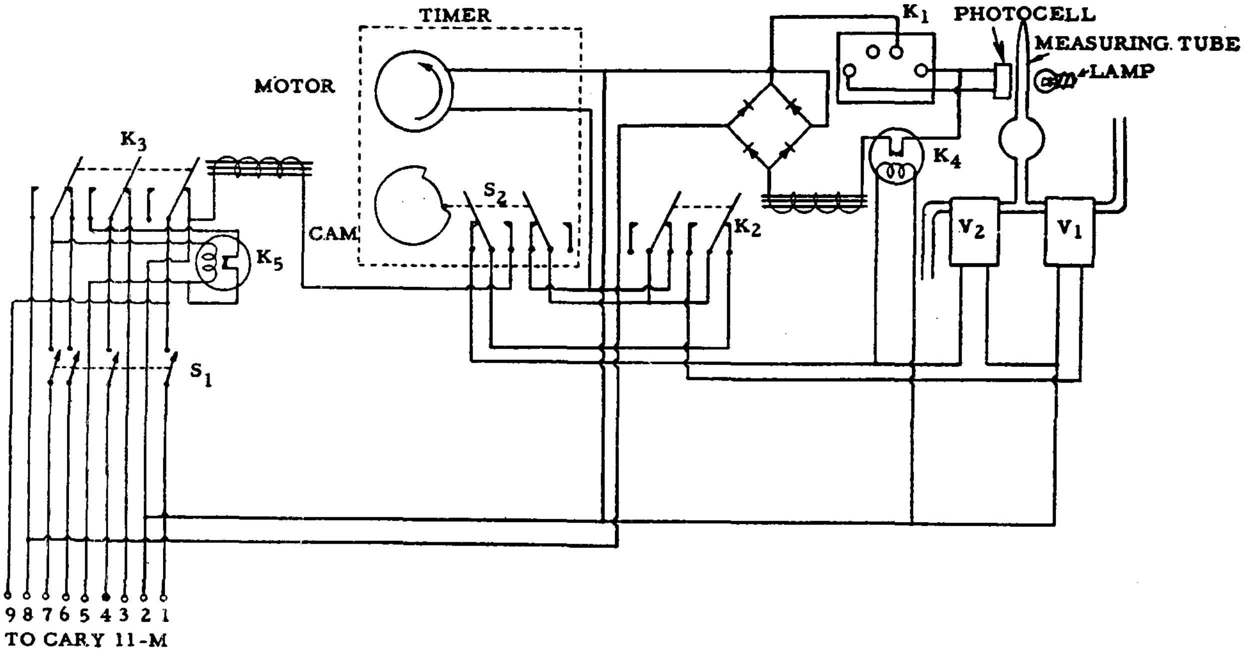

Figure 4. Timer-type control unit

Although the control circuits given here were designed specifically for the Cary Model 11-M, the basic principles are applicable to almost any recording spectrophotometer.

Timer Control Unit. The circuit diagram of the timer-type control unit is shown in Figure 4.

When the eluate in the measuring tube rises to the desired level, the lens effect of the liquid focuses light upon the photocell causing an increase in photocurrent. This photocurrent actuates the meter relay, K_1, which applies a direct current voltage from the bridge rectifier to the secondary relay, K_2. The current drawn by K_2 flows through a holding coil in K_1 so that K_1 remains actuated until the circuit is opened.

The actuation of the secondary relay, K_2, removes the line voltage from solenoid valve, V_1, and applies it to the timer motor and to the scan relay, K_3, whose contacts are put in parallel with the scan, pen, and chart switches of the Cary by switch, S_1. With S_1 in the "auto" position and K_3 actuated, the spectrophotometer scans from 400 mμ (its initial wave length) toward shorter wave lengths and the pen and chart operate to record the absorption spectrum of the eluate in the absorption cell.

The instrument continues to scan until the timer motor has rotated its cam to the position where it actuates the timer switch, S_2, which removes the voltage from the scan relay, K_3, thus stopping the scan.

The scanning time, and therefore the wave-length band scanned, may be varied by adjusting the timer cam. This, however, changes only the short wave-length limit of the spectrum, since the record always starts at 400 mμ.

In addition to stopping the scan, the timer switch, S_2, also applies line voltage to solenoid valve, V_2, to a thermal delay relay, K_4, and to the timer motor. After a 20-second delay, relay K_4 operates, opening the circuit to relay K_2. This resets the meter relay, K_1, and returns relay K_2, to its normal position which closes valve V_2 and opens valve V_1.

The timer motor continues to run until the cam reaches its original position. This returns the timer switch to its normal position which removes the voltage from the motor. Thus, after each scan the timer resets itself to its initial position so that it cannot get out of synchronization with the rest of the system.

In the meantime, another thermal delay relay, K_5, has actuated and started the spectrophotometer to scan back to 400 mμ. Since relay K_3, is now in the unactuated position, the pen and chart circuits are inoperative so that the reverse scan is not recorded.

When the spectrophotometer reaches its 400-mμ limit switch, it stops and waits for the refilling of the absorption cell to initiate another scan.

Wave-Length Control Unit. The circuit of the present control unit is shown in Figure 5. It is similar to that of the timer-operated control system. The only major difference is that the timer has been replaced by a pair of adjustable limit switches which are mounted on the cover of the Cary slide-wire compartment. These limit switches are operated by the same mechanism that rotates the Littrow arm of the monochromator.

When the meter relay, K_1, is actuated by the eluate in the measuring tube, it operates the secondary relay, K_2, and locks itself in by means of a holding coil just as it did in the previous system. The secondary relay, K_2, closes valve V_1, by removing its voltage, and applies voltage to the scan relay, K_3, which starts the scan. The instrument scans until it reaches the short wave-length limit switch which removes the voltage from the scan motor and applies it to the thermal delay relay, K_4, to relay K_5, and to valve V_2. Relay K_5 opens the pen and chart circuits so that the record stops when the scan does. Valve V_2 opens, emptying the cell, and remains open until the delay relay operates 20 seconds later. When the delay relay opens, it resets the meter relay, K_1, and returns K_2 to its normal position. This closes valve V_2, opens V_1, reverses the scan, and opens relay K_6. The instrument scans in the reverse direction until it reaches the long wave-length limit switch which removes the voltage from the scan motor and closes relay K_6. This completes the cycle and the system remains at rest until the cell refills.

Relay K_6 is an additional refinement of the present system over the previous one. Since the scan relay, K_3, is operated through the contacts of K_6, and K_6 is operated by the long wave-length limit switch, the instrument cannot start a forward scan until it has completed the reverse scan. Thus, if the cell fills before the reverse scan is finished it will not result in a shortened forward scan.

Modification of Spectrophotometer. The modifications which were made in the panel switch circuit of the Cary Model 11-M are shown in Figure 6. The heavy lines indicate the additional

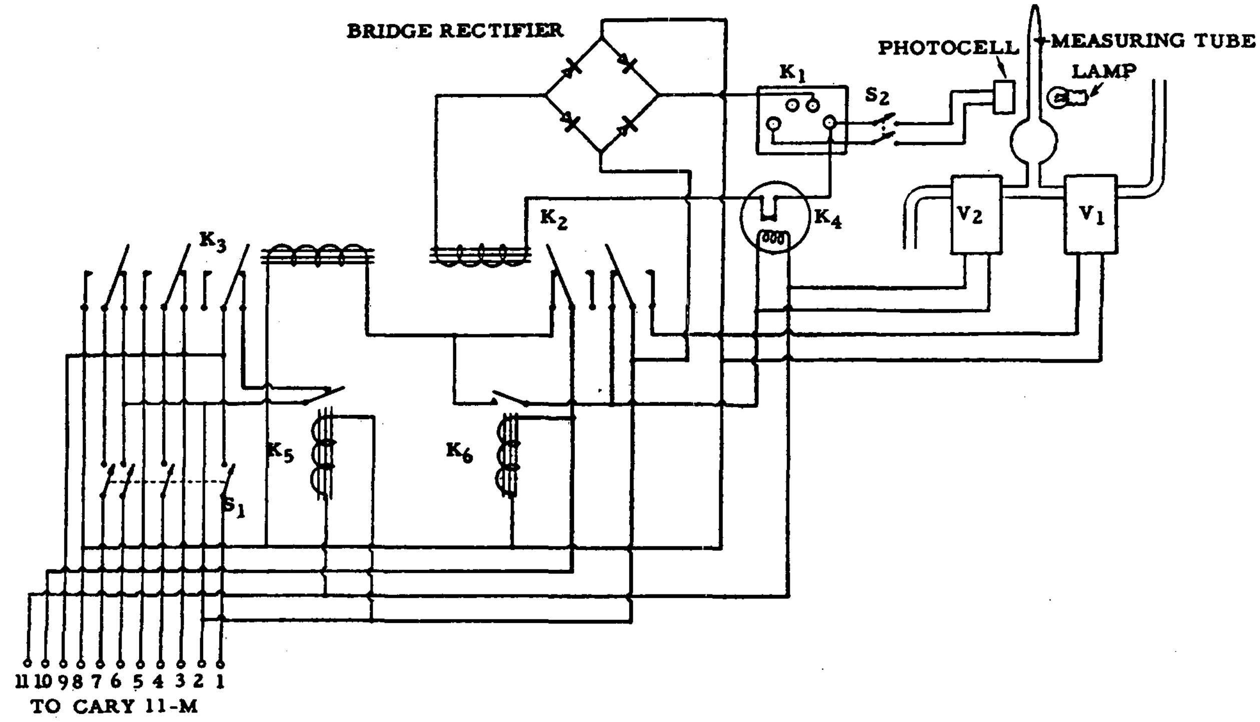

Figure 5. Wave-length type control unit

circuitry required for the timer-type control unit. The wavelength control unit requires the circuitry shown in heavy lines plus the adjustable limit switch circuitry shown in dotted lines. The limit bypass switch shorts out the adjustable switches so that in manual operation the fixed limit switches at 400 and 200 mμ may be used.

The modifications do not alter the manual operation of the spectrophotometer in any way.

Fraction Collector. The fraction collector is a Misco Model 6500 (Microchemical Specialties Co., Berkeley, Calif.). Although the fractions are now collected on a time basis, it is planned to incorporate into the Scanalyzer control circuit a collector actuator which will rotate the collector table after every *n* cuts, where *n* is a number from 1 to 6 that can be chosen at will by a selector switch.

EXPERIMENTAL SECTION

The reagents and components of the test mixture were as follows:

	Grade	Supplier
Silicic acid	Chromatographic grade	Mallinckrodt
Ether	Anhydrous	Mallinckrodt
Iso-octane	ASTM	Enjay Co., Inc.
Benzene	ACS reagent	Baker and Adamson
Styrene	N-99	Dow Chemical Co.
Anisole		Matheson Co., Inc.
Benzaldehyde	NF	Merck & Co., Inc.
Acetophenone		Carbide and Carbon
Phenol	USP	Merck & Co., Inc.
Benzoic acid	CP	J. T. Baker Chemical Co.
Benzyl alcohol	Chlorine-free	Matheson Co., Inc.

Iso-octane was purified according to ASTM D-1017 for the preparation of spectrophotometric grade iso-octane. All other reagents were used without further purification.

Calibration. With the exception of phenol and benzoic acid, the absorptivity, *a*, of each compound in the test mixture was determined in iso-octane. The spectra of phenol and benzoic acid were found to be very sensitive to the presence of low concentrations of polar solvents. Consequently, their absorptivities were determined in mixtures of iso-octane and varying concentrations of ether. In each case a wave length was found where the absorptivity appeared to be independent of polar solvent concentration (isoabsorptive point). The range of ether concentrations was read from the gradient elution curve (Figure 7) after deter-

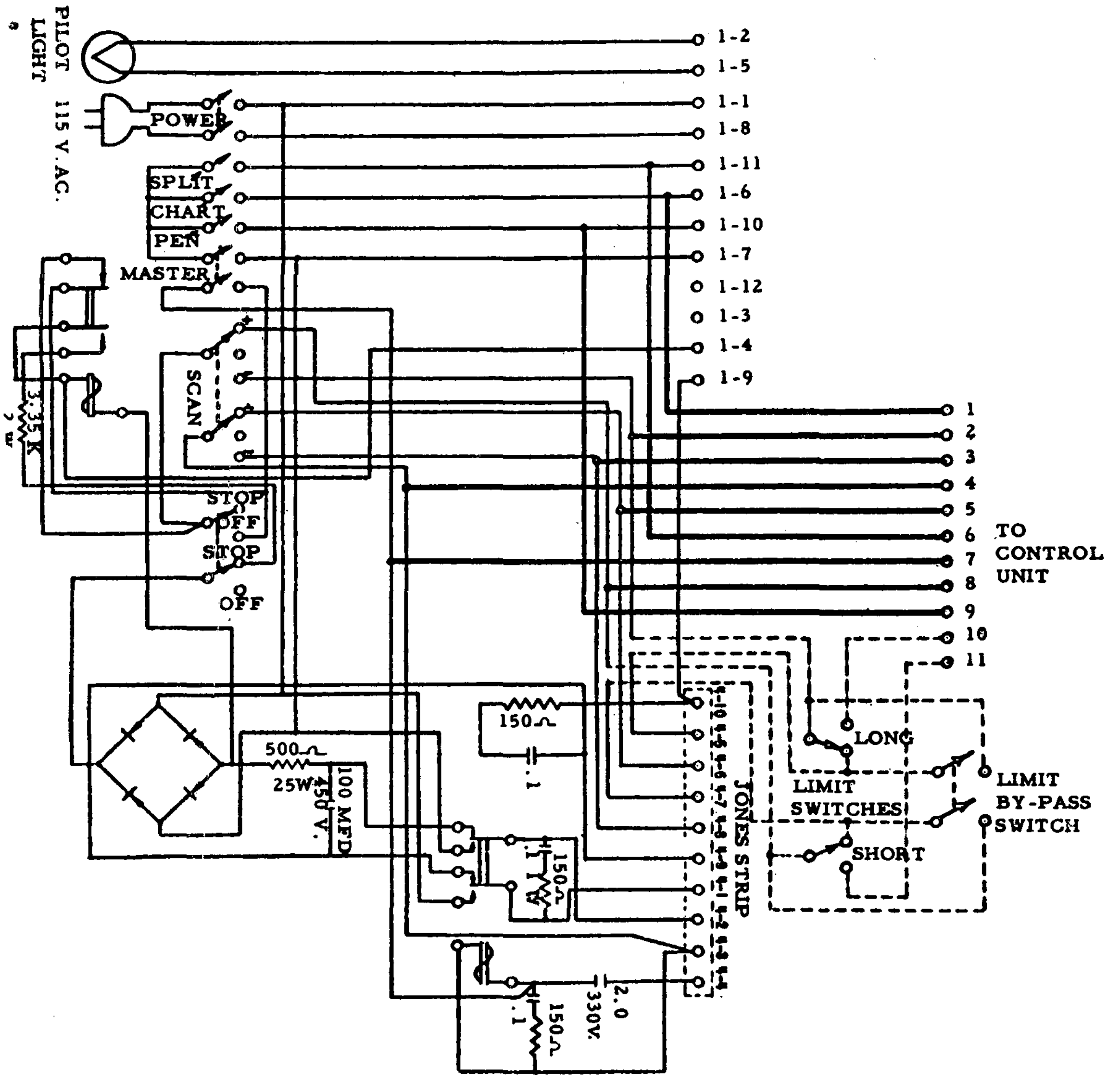

Figure 6. Modified Cary spectrophotometer panel switch circuit

Heavy lines. Additional circuitry required for timer-type control unit
Heavy and dotted lines. Wave-length type control unit.

mining the volume of eluent consumed when the compound in question first appears in the effluent and again when it could no longer be detected. Three different ether concentrations were used in finding the isoabsorptive points.

To eliminate sources of error, the proper concentration of each compound was run in the same cell and at the same scanning speed as for the Scanalyzer analysis. All absorbances were in the range of 0.5 to 1.2. The absorptivity of each compound was determined by averaging the results of three separate determinations. This procedure assumes that any impurity present in the reference compounds is either negligible or transparent.

The absorptivities, *a*, wave lengths, and solvents are listed in Table I.

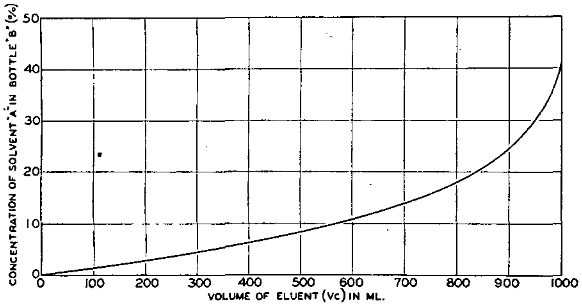

Figure 7. Elution gradient used for synthetic test mixture

$X_A = 0.5$; $V_A^\circ = 110$ ml.; $V_B^\circ = 900$ ml.; Equation 1

The volume of the system was determined by placing a calibrated pipet in place of the column and measuring the total volume required for ten complete scans. This volume was found to be 3.85 ml.

Procedure. The chromatographic column, Scientific Glass Apparatus Co., No. 1, 9-mm. diameter, was packed by suction with the Fisher filtrator. Approximately 10 grams of silicic acid were slurried in a separatory funnel and small increments were added until the column was 7 cm. in height. Air bubbles were removed by tapping the column with a wooden tamper during the packing.

Table I. Spectral Absorptivities of Test Mixture Components

	a	Wave Length, mμ	Solvent
Benzene	2.672	254.5 ± 0.5	Iso-octane
Styrene	144.0	248.0	Iso-octane
Anisole	19.20	278.0	Iso-octane
Benzaldehyde	8.500	289.0	Iso-octane
Acetophenone	7.080	279.0	Iso-octane
Phenol	19.40	283.0	Iso-octane + 2.5–3.0% ether
Benzoic acid	96.30	226.5	Iso-octane + 3.3–4.5% ether
Benzyl alcohol	1.667	258.5	Iso-octane

The top of the column was leveled with the flat end of the tamper. Suction was released when the solvent level was less than 1 cm. above the adsorbent. At no time was the column allowed to run dry.

With suction off, 1 ml. of a 8-mg. per ml. solution of the test mixture in iso-octane was added to the column using a pipet. Suction was then turned on and the sample allowed to penetrate the column using 2 ml. of iso-octane for washing the walls of the

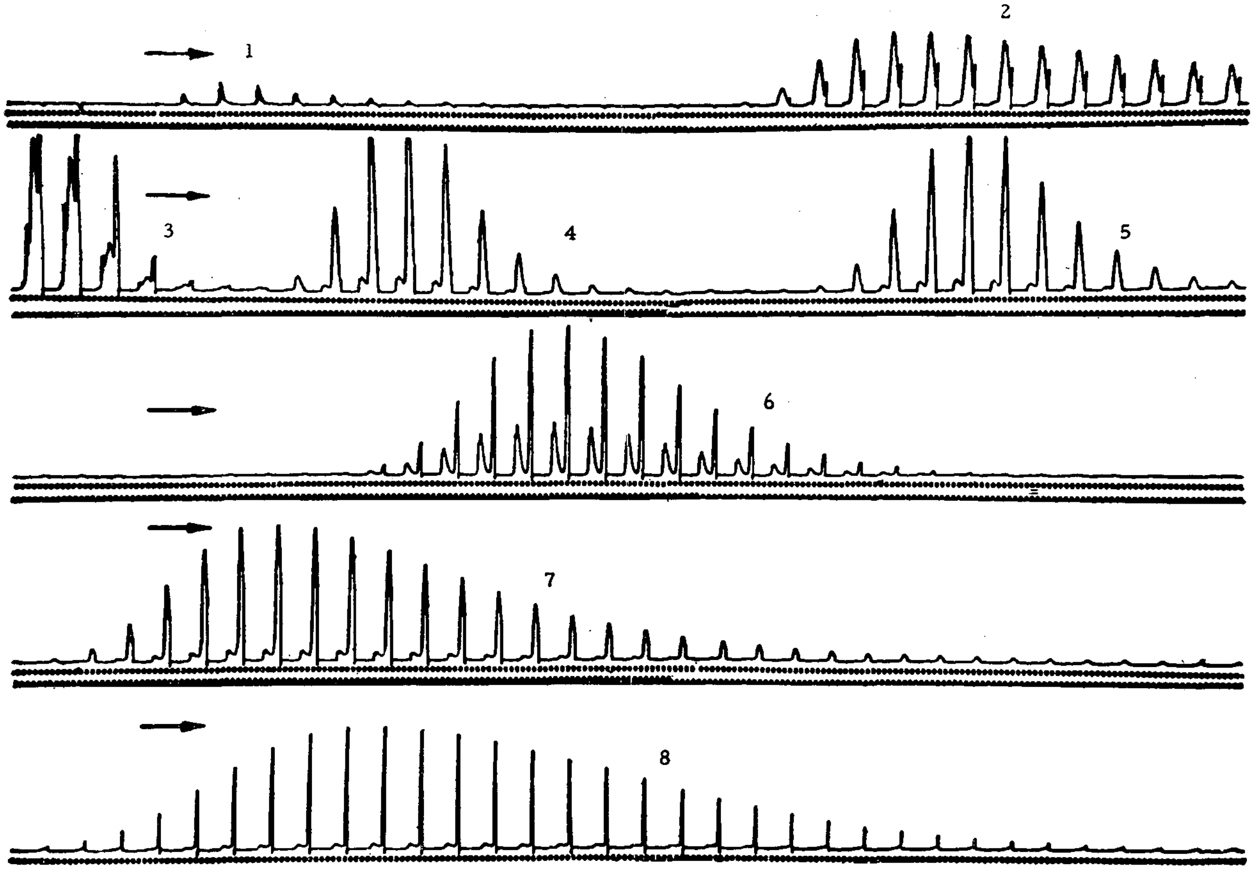

Figure 8. Scanalyzer record of synthetic mixture

1. Benzene
2. Styrene
3. Anisole-styrene
4. Benzaldehyde
5. Acetophenone
6. Phenol
7. Benzoic acid
8. Benzyl alcohol

column. Suction was turned off and the column filled with iso-octane (approximately 4 ml.). The prepared column was removed from the filtrator and placed in position on the Scanalyzer.

The large bottle, *B*, was filled with 900 ml. of iso-octane while 110 ml. of a 1 to 1 mixture of ether and iso-octane was placed in the small bottle, *A*. The 1 to 1 mixture was known to be sufficiently polar to elute all compounds in the test mixture.

Instrument settings were as follows:

Scanning speed	2 mμ/second
Scanning range	361–216 mμ
Air pressure	7 lb./sq. inch
Fraction collector	9 min./fraction

Time required to prepare sample and set up column for overnight run was approximately 1 man-hour.

DISCUSSION OF RESULTS

The Scanalyzer record of the synthetic mixture is reproduced in Figure 8. The synthetic mixture is not an actual sample but includes eight constituents chosen as representatives of several classes of aromatic compounds, which were available in this laboratory.

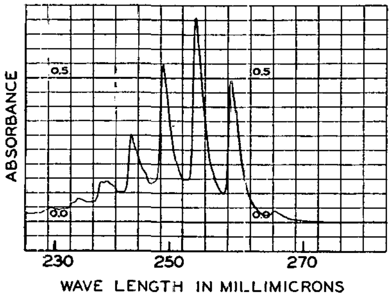

Figure 9. Ultraviolet spectrum of cut 3 (benzene)

Figure 8 is an actual reproduction of the original chart paper record and not an elution graph drawn from the data. The record reads from left to right and from top to bottom with the first scan in the upper left corner.

The record shows that the first compound to be eluted under the conditions of the experiment was benzene, 1, which was completely separated from styrene, 2. The next section of the record, 3, shows that styrene was not separated from anisole. The sharp bands were the anisole bands while the broad band at 248 mμ belonged to styrene. The other constituents were all separated and follow in this order: benzaldehyde, 4, acetophenone, 5, phenol, 6, benzoic acid, 7, and benzyl alcohol, 8.

The separation of styrene and anisole was accomplished easily on other occasions when using less polar solvent in bottle *A*. Section 3 is a good example of displacement chromatography. Although styrene has a maximum concentration in scan 25, it is even more concentrated in scan 36, where the anisole also has a maximum. The second styrene maximum occurs because the styrene remaining on the column is displaced by the anisole. In the absence of anisole, and with no ether in the eluent, styrene would stretch out over almost 100 scans.

The Scanalyzer record also presents vividly the advantages of gradient elution chromatography. Theoretically the ideal chromatographic separation is represented by a graph—plotting concentration versus volume of eluent—which resembles closely the Gaussian distribution. This is to say that the experiment is performed on the linear portion of the Langmuir isotherm and that, therefore, the column is not overloaded. Also, for an ideal separation, the completely separated components of the mixture should have as few blank scans as possible since each blank scan can be considered as time wasted.

Since the ordinate (absorbance) of the record presented in Figure 8 is directly proportional to concentration and the abscissa is the volume eluted, an examination of the record indicates how close to ideality this separation has been. Take acetophenone, for example. Here, four scans are seen on the leading side of the maximum and nine scans on the trailing side with the last three scans representing only approximately 3% of the total acetophenone present in the sample. Therefore, this is a fairly close approximation to a symmetrical curve. Also, only one blank scan separates acetophenone from benzaldehyde, while 10 blank scans separate the acetophenone from the phenol. This again is a fair approximation to this ideal condition. Hence, the conditions chosen for this experiment are well-suited for the separation and analysis of acetophenone in this mixture.

Consider styrene. Four scans are found on the leading side of the maximum and 16 scans on the trailing side. This is far from representing an ideal separation, especially since the styrene was not separated from anisole (scans 35 to 41), which displaced some of the styrene and prevented further tailing (see above). In this case, the conditions were not the best suited for this separation. The other constituents of the mixture fall somewhere between the two extremes as represented by acetophenone and styrene.

Flow rates may have an appreciable effect on the symmetry of the curve. The flow rate was approximately 1.2 ml. per minute for this experiment.

The spectra of phenol and benzoic acid are not the same as the spectra of the reference material in iso-octane but show the effect of having small concentrations of ether in the eluent. The spectra of phenol, 6, have fewer sharp bands than phenol in iso-octane, while the spectra of benzoic acid, 7, show sharper bands than when run in iso-octane.

Although only eight constituents were used in the synthetic mixture, the instrument can be used to analyze more complex mixtures. As many as 20 components have been detected in actual samples submitted for analysis in this laboratory. Since the total sample was eluted after 169 scans and the complete record had 247 scans (the last 80 blank scans are not shown to conserve space), a test mixture containing more than the eight compounds could have been analyzed.

Quantitative Results. Results are listed in Table II.

U is the volume of eluent consumed when the concentration of the solute in the eluate is at a maximum. In the case of styrene it is the first maximum and not the maximum brought about because of displacement by anisole. This value, *U*, is included to indicate the relative speed with which the components were eluted. These values of *U* are applicable only to the particular conditions of this experiment. A change in the elution conditions would bring about a change in the *U* values.

The following formula was used in the quantitative calculations.

$$\frac{\Sigma AV(100)}{aw} = \%\ \text{compound}$$

where

A = absorbance
a = absorptivity of compound
V = cell volume = 3.85 ml.
w = weight of sample placed on column = 8.0 mg.
V and a were determined as indicated under calibration.

Simultaneous equations were required to determine the absorbances of styrene and anisole in the scans where these compounds were not separated. Approximately 2 man-hours were needed to analyze the data and compute the results listed in Table II.

The concentration of each component can also be determined by measuring the volume of each cut and rerunning them in the spectrophotometer. Although this procedure was not followed in this case, Figure 9 is an example of what can be done. It is

Table II. Analysis of Test Mixture

	Per Cent			U,
	Found	Known	Error[a]	Ml.
Benzene	26.3	25.95	+1.35	23.1
Styrene	5.02	5.12	−1.95	96.2
Anisole	6.73	6.65	+1.20	138.6
Benzaldehyde	4.52	4.43	+2.03	173.2
Acetophenone	5.76	5.79	−0.52	231.0
Phenol	13.8	13.59	+1.55	323.4
Benzoic acid	10.3	10.44	−1.34	423.5
Benzyl alcohol	27.8	28.03	−0.82	565.9
Total	100.23	100.00		
Standard deviation			±1.51%	

[a] Expressed as per cent of amount present.

the spectrum of cut 3 (scans 6 and 7), run at scanning rate of 0.4 mμ per second on chart paper supplied by Applied Physics Corp. This procedure is time-consuming. A more desirable procedure would be to combine all of the cuts containing a certain compound, measure the volume, and run a spectrum of the combined cuts.

APPLICATION TO OTHER SYSTEMS

The principles described in this paper have also been applied in a preliminary way to infrared scanning of chromatographic column effluents. An arrangement was devised whereby a Perkin Elmer Model 112 infrared spectrophotometer could be used in a similar manner. In this case also the limit switches were adjustable for any given wave-length interval. In the preliminary investigations, however, only the C—H stretching region around 3.4 microns has been employed. Two major difficulties are apparent: (1) Gradient elution is difficult, since no suitable polar solvent is available which does not have a C—H absorption itself; (2) because of the solvents available, such thin cells are necessary that infrared absorption does not have the sensitivity of detection that ultraviolet absorption does.

The principles described should be applicable to detection of materials separated by other means such as partition and ion exchange chromatography, liquid-liquid extraction, and fractional distillation, provided that the proper concentrations of absorbing substances in nonabsorbing media can be achieved.

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Chromatographic Separation and Quantitative Estimation of Iodine-131-Labeled Derivatives of Sterols, Amines, Acids, and Aldehydes

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Mixtures of compounds labeled with iodine-131 have been chromatographed. Apparatus has been developed for following the course of the development at all stages by automatically scanning the column and recording the distribution of the associated gamma activity. The method has been applied to the separation and quantitative estimation of the components of selected pairs of derivatives of the following types: sterols, amines, acids, and aldehydes.

THE present authors' use of radioactivity as a means of following the development of a chromatographic separation and estimating the quantities of the various components, as well as their purity, was found to offer so many advantages in the separation of certain sterol mixtures (*6, 7*) that they were interested in extending the method to systems characterized by other functional groups and improving the convenience and accuracy of the method. *p*-Iodobenzoic acid-iodine-131 derivatives of sterols and amines, *p*-iodoaniline-iodine-131 derivatives of acids, and *p*-iodophenylhydrazine-iodine-131 derivatives of aldehydes have been separated. The degree of purity of certain of the separated fractions has been measured by th use of carbon-14–labeled components. The first separation were followed by manual spot scanning, then automatic scannin and photographic recording were used, and finally simultaneou automatic scanning and graphic recording were develope

METHOD AND APPARATUS

Preparation of Column. Chromatographic tubes from 1.2 t 4.5 cm. in diameter and from 60 to 120 cm. in length were pr pared from standard borosilicate glass tubing with a constrictio at the lower end. The tubes were coated with General Electr SR-53 silicone resin, and a plug of glass wool and a layer of clea sand were introduced to support the adsorbent.

The adsorbent, two-thirds silicic acid and one-third Celit was prepared as previously described (*6*) with the followi changes. Celite 503 was found to increase the flow of develop and was therefore substituted for the 535 previously used. T Celite was calcined at 500° C. to remove any traces of organ matter. The components were mixed in a ball mill and heate for 12 hours at 200° C. To eliminate inhomogeneities due to di ferent rates of fall of the components down the length of t column during packing, the adsorbent was mixed within t tube by revolving a notched rubber stopper, attached to a gla rod, through the adsorbent before the application of vacuum.

Apparatus. In early runs the tube was attached to a filter flask and the pressure reduced to 20 cm. of mercury to accelerate the flow of solvent. Recently a turret arrangement has been employed to allow two columns to develop simultaneously yet permit either to be brought into position for scanning without the loss of vacuum. The first survey method consisted in moving the counter (sliding on a steel shaft) manually, taking 1- to 5-minute counts at each centimeter down the length of the column. Subsequently an electric drive was added.

The apparatus, Figure 1, consists of the chromatographic assembly made up of the developer reservoir, *A*, chromatographic tube, *B*, and filter flask, *C*, all supported by clamps from a vertical rod (not shown in the diagram), which is firmly attached to the main supporting shaft, *E*.

Table I. Experimental Results

Component	Added to Column Mg.	Added to Column %	Analysis from Chromatographic radioactivity trace, %	Analysis from Radioactive assay of eluates Mg.	Analysis from Radioactive assay of eluates %	Melting Points of Recovered Compounds Found	Melting Points of Recovered Compounds Literature
Cholestanyl *p*-iodobenzoate	>47.2	>50.9	52.1			186	186 (*6*)
Cholesteryl *p*-iodobenzoate	<45.6	<49.1	47.9			184.5	184.5 (*6*)
Δ^7-Cholestenyl *p*-iodobenzoate	<32.4	<50.5	45.8			179 (see text)	
Cholesteryl *p*-iodobenzoate	>31.7	>49.5	54.2			184.5	184.5 (*6*)
N-*p*-Iodobenzoyl-*m*-toluidine	26.1	34.7	33.2			148.0	
N-*p*-Iodobenzoyl-*o*-toluidine	49.1	65.3	66.8			A 177.4 B 177.4	
Acet-*p*-iodoanilide	79.0	57.7	54.1	73.4	51.1	185.0	184 (*3*)
Proprion-*p*-iodoanilide	57.8	42.3	39.3	60.8	42.4	168.0	
Diacet-*p* iodoanilide			6.6	9.4	6.5	161–165	
o-Nitrobenzaldehyde *p*-iodophenylhydrazone	99.9	49.2	47.5			194–195 (dec.)	196 (*3*)
m-Nitrobenzaldehyde *p*-iodophenylhydrazone	103.3	50.8	52.4			146 (dec.)	148 (*3*)

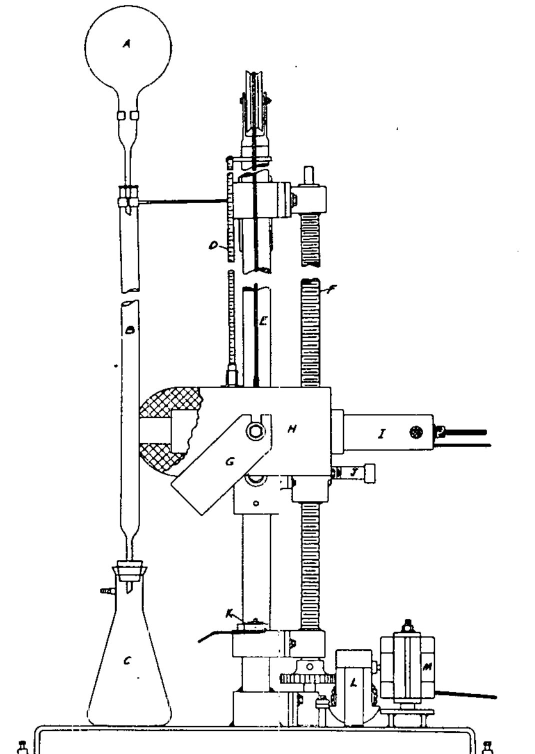

Figure 1. Apparatus for automatic radioactive survey of chromatographic column by scintillation counter

The lead shield, *H*, for the scintillation counter, *I*, is supported by a semicircular yoke, *G*, which permits the counter and shield o be rotated to the vertical position when it is desired to make use of the well-type crystal to count active samples contained in est tubes. The radiation reaching the crystal is collimated by emovable lead plugs with $^1/_8$- and $^1/_4$-inch slit widths. The maller slit width was used in the early stages of column development when the activity was concentrated at the top of the column. The counter, shield, and yoke slide on the vertical steel shaft, *E*, mounted on a firm steel base. The sliding assembly is counterbalanced by a lead weight attached to a cable running over a pulley at the top of the column. A spring-retracting steel centimeter tape, *D*, mounted on top of the shield, indicates the distance the counter has traveled downward from the top of the column.

Parallel to the supporting column and offset to the side is the driving screw, *F*, which is driven by a synchronous motor, *M*, through a double set of worms and gears, *L*. In the diagram, the gear on the driving screw, *F*, has been displaced upward for clarity. The driving screw passes through a bronze bearing attached to the sliding counter assembly. A spring-loaded, retractable ratchet, *J*, engages the groove in the driving screw and draws the yoke, shield, and counter downward at approximately 1 cm. per minute. Withdrawal of the ratchet pin permits the manual return of the counter assembly to the top of the column. A microswitch, *K*, in series with the driving motor, limits the motion of the counter.

When the counter was moved manually, a scaler was used to record the activity. Along with the addition of an electric drive, a motion picture camera was used to record the total scaler count at each minute while the counter scanned the length of the column at constant speed. The standard 16-mm. camera was provided with a solenoid-operated plunger, which depressed the trigger button with just sufficient force and duration to allow one frame to be exposed at a time. The solenoid was actuated at minute intervals by the discharge of a condenser through a timing motor switch. At the expiration of a run the exposed film was cut from the roll, developed, and read in a microfilm reader. The film record gave, for each minute interval, the total count accumulated from the start. Each count was subtracted from the succeeding one in order to obtain the counts per minute for that particular time interval and its corresponding section of the column length. While this method was satisfactory for slow-developing systems, such as the sterols studied, it imposed serious delays in more rapid systems as well as tedious reading, recording, and computation.

Finally, a count-rate-meter incorporating a pulse amplifier and discriminator, as well as a highly stabilized power supply, was constructed. The output of the count-rate-meter was fed into a Brown recorder. Thus, a continuous plot of the activity at each level of the column was produced simultaneously with the scanning of the column. Since the descent of the counter head along the length of the column and the rate of travel of the recording pen were constant, the abscissa of the plot was proportional to the distance from the starting point on the column, whereas the ordinate was proportional to the gamma ray activity at each point.

Unlike conventional chromatography, where only the surface coloration is apparent during development, in radiochromatography the counter responds to the total activity across the column section subject to the geometry of the counting arrangement and the minor limitations for self-absorption, scattering, decay, and background fluctuation; hence, quantitative interpretation is feasible. In practice the shielding surrounding the counter permits the penetration of a small fraction of the radiation originating in sections of the column adjacent to the portion in front of the slit, and the packing and glass scatter some of the rays into the slit. Preliminary experiments showed these effects to be small but the background does rise somewhat as a region of high activity in the column is approached. Within this limitation and that of the precision of the counting instrumentation, the areas under the curves are proportional to the activity contained in the corresponding zones of the column as indicated by the correspondence within an average of 2.5% between the percentage composition of the original mixture and the results of analysis. This conclusion was confirmed by total recovery, in one instance, within 5% (Table I). For unifunctional compounds these are also proportional to the mole fraction present. The somewhat arbitrary choice of a background is the most serious limitation to the quantitative estimation of the various components of the system.

SYSTEMS ANALYZED

Cholestanyl and Cholesteryl *p*-Iodobenzoates-Iodine-131. These were prepared as described by a published method (*6*). The cholestanol-carbon-14 was prepared by the biological reduction of cholestenone-carbon-14 (*7*) by rat liver.

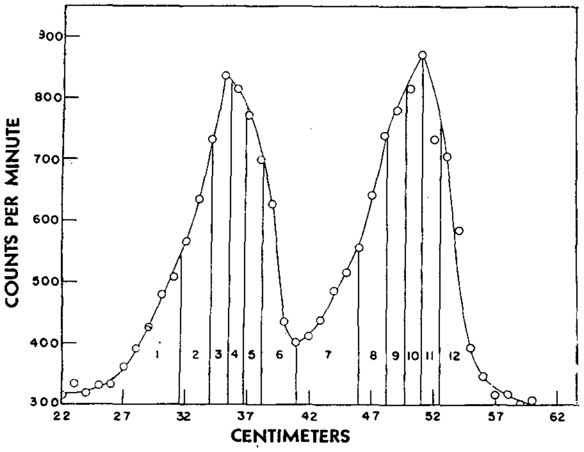

Figure 2. Results of manual survey of developed chromatographic column

Sections 1 to 6, cholesteryl *p*-iodobenzoate-iodine-131
Sections 7 to 12, cholestanyl *p*-iodobenzoate-iodine-131
Counts per minute at each centimeter are plotted against distance down the column

A chromatographic column 1.8 by 60 cm. was prepared as described and pretreated with one holdup volume of 1 to 10 benzene–Skellysolve C. Without allowing the column to run dry, the activity was applied in Skellysolve C and the same solvent was used for development. After about 12 hours the activity front had traveled 58 cm., and preliminary survey indicated that the zones were well separated. The column was allowed to run dry and counts were made at each centimeter down the length of the column. Figure 2 shows the results. From a calculation of the areas under each curve, the column was sectioned at such intervals that each component was divided into six approximately equal portions. Each portion was eluted with benzene-alcohol, the dry residues were hydrolyzed, the hydrolyzates extracted with ether, the dry ether extracts taken up in 90% alcohol, and the sterols precipitated with digitonin. The carbon-14 specific activity of sections 1 to 5 of the cholesterol zone was 5.1 ± 2.1 counts per minute per milligram; that of sections 8 to 12 of the cholestanol zone was 363 ± 6 counts per minute per milligram, indicating that the zones were practically homogeneous (*7*) and that the cholesterol was contaminated with less than 1.4% of cholestanol. From the composition of the original mixture, 49.1% was liver sterol, containing at least 95% cholesterol, and 50.9% was added cholestanol. From the areas under the curves (Figure 2) it was calculated that the cholesterol fraction comprised 47.9% of the total.

Δ^7-Cholestenyl and Cholesteryl *p*-Iodobenzoates-Iodine-131. The esters were worked up and chromatographed as described, except that a column 90 cm. long was employed. During development, numerous surveys were made by the photographic recording technique. When development was complete, the column was allowed to run dry, and a final survey was made. When the data were plotted (Figure 3) an average background was chosen.

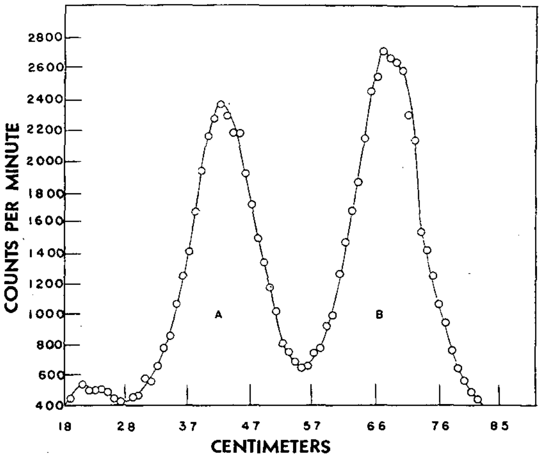

Figure 3. Plot of photographic recording of automatic survey on developed chromatographic column

Zone A, Δ^7-cholestenyl *p*-iodobenzoate-iodine-131
Zone B, cholesteryl *p*-iodobenzoate-iodine-131

The content of each zone was obtained from the expression $(N_{t_2} - N_{t_1}) - B(t_2 - t_1)$ where N_{t_2} and N_{t_1} are the scaler readings at the limits of the particular curve, B is the average background in counts per minute, and t_2 and t_1 are the times, in minutes, when the counter slit passes the limits of the zone. The results are given in Table I. The low results for Δ^7-cholestenol were to be expected, because the original Δ^7-cholestenol was known to contain cholesterol as an impurity. The Δ^7-cholestenyl *p*-iodobenzoate softened at 179° C., and shrank to a light brown mass between 180° and 185° C., but failed to give a clear melt up to 225° C.

***N*-*p*-Iodobenzoyl-*o*-toluidine-Iodine-131 and *N*-*p*-Iodobenzoyl-*m*-toluidine-Iodine-131.** These compounds were prepared according to the method of Cheronis (*4*), using *p*-iodobenzoyl chloride-iodine-131 (*6*).

A chromatographic tube, 1.4 cm. in diameter and 45 cm. long, was filled and packed in the usual way. It was prewashed with one holdup volume of 1% acetic acid in benzene, and the mixed *N*-*p*-iodobenzoyl-*o*-toluidine and *N*-*p*-iodobenzoyl-*m*-toluidine were added, as a suspension, in the same solvent, which was used also for development. In 5 hours the separation was practically complete, as shown in Figure 4.

Since the zone corresponding to *N*-*p*-iodobenzoyl-*o*-toluidine exhibited an unusual convexity along the trailing edge, this zone was divided into two portions, *A* and *B* in Table I and Figure 4, as a test for homogeneity. The three sections were eluted with methanol and the resulting solutions evaporated to dryness. The solutes were taken up in Skellysolve C, filtered, evaporated to incipient turbidity at the boiling point, and allowed to crystallize. The crystals were collected on small filter paper disks. As indicated by the data in Table I the two portions of the ortho zone had the same melting points.

***p*-Iodoaniline-Iodine-131.** This was prepared according to the method of Brewster (*1*) on 1 to 6% scales. The sodium iodide-131, 1 to 10 mc. in aqueous solution, was added with the first portion of iodine-127. It was found that steam distillation yielded the purest product with the least difficulty.

Proprion- and Acet-*p*-iodoanilide-Iodine-131. These were prepared by a published method (*5*).

A chromatographic tube, 1.8 cm. in diameter and 75 cm. long, was prepared in the usual way and prewashed with one holdup volume of a 2.5% solution of 1-propanol in Skellysolve C. The mixed anilides were applied to the column in 5% 1-propanol in Skellysolve C. The same solvent was used as developer. After 18 hours the zones were well separated, were symmetrical, and the radioactivity between them had fallen to background. The smoothed recorder trace is shown in Figure 5. However, unexpectedly, a third zone appeared which traveled faster than the two main zones. Iodine analysis indicated that this component was diacet-*p*-iodoanilide-iodine-131. The areas under the curves for the three zones were determined and the percentage of each component was calculated (Table I).

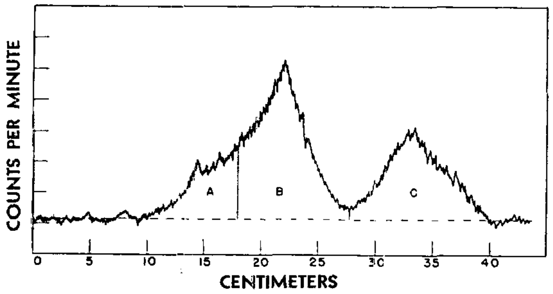

Figure 4. Recorder trace of developed chromatographic column

Radioactivity in arbitrary units is plotted against distance from top of column
Zone A and B, *N*-*p*-iodobenzoyl-*o*-toluidine-iodine-131
Zone C, *N*-*p*-iodobenzoyl-*m*-toluidine-iodine-131

The column was sectioned at appropriate points to isolate the three zones, and elution was carried out with 1 to 1 1-propanol–Skellysolve C. Since considerable decomposition had been experienced, on previous runs, during the evaporation of the solvent, the three zones were analyzed by the following radioactivity method. The eluates were diluted to known volumes, in volumetric flasks, and 3-ml. samples were removed for radioactivity determination in a well-type, high sensitivity, gamma ray counter.

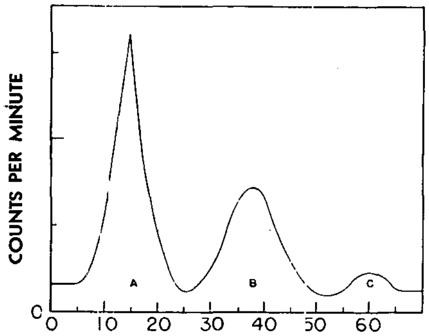

Figure 5. Smoothed recorder trace of developed chromatographic column

Zone A, acet-*p*-iodoanilide-iodine-131
Zone B, proprion-*p*-iodoanilide-iodine-131
Zone C, diacet-*p*-iodoanilide-iodine-131

The activity of these samples was compared to that of a highly purified sample of proprion-*p*-iodoanilide-iodine-131 from the same original preparation which had, therefore, the same molar degree of labeling. From the total counts per minute of the samples, the dilution factors and the equivalent weights, the weight of each component was determined. The results are given in Table I.

o- and *m*-Nitrobenzaldehyde *p*-Iodophenylhydrazone-Iodine-131. *p*-Iodophenylhydrazine-iodine-131 was prepared from *p*-iodoaniline-iodine-131 by an adaptation of the method of Bülow (*2*) for the synthesis of the corresponding 2,4-dichloro compound.

The *p*-iodophenylhydrazine was stored as the tin chloride double salt, as it appeared to be more stable to light and air than is *p*-iodophenylhydrazine. The derivatives were prepared by a published method (*3*).

A chromatographic column, 1.8 cm. in diameter and 60 cm. long, was prepared in the usual way and prewashed with one holdup volume of Skellysolve C. The mixed *p*-iodophenylhydrazones-iodine-131 were dissolved, with heating, in the smallest possible volume of 10% 1,4-dioxane in Skellysolve C and added, by means of a pipet, to the top of the column. Development was carried out with 5% 1,4-dioxane in Skellysolve C. In 5 hours the front of the ortho compound had traveled about 44 cm. As Figure 6 shows, the zones were symmetrical and the valley between them was almost at background.

Figure 6. Recorder trace of developed chromatographic column

Zone A, *o*-nitrobenzaldehyde *p*-iodophenylhydrazone-iodine-131
Zone B, *m*-nitrobenzaldehyde *p*-iodophenylhydrazone-iodine-131

The column was then allowed to run dry and air was drawn through it until no more solvent dropped from the end. The tube was sectioned to isolate the two zones and the corresponding components were eluted with diethyl ether. The latter was evaporated and the samples were dried in vacuo, recrystallized from methanol (considerable loss was experienced on attempted recrystallization from ethyl alcohol, especially of the ortho compound) and melting points taken as shown in Table I.

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Determination of Trace Metals in Oils

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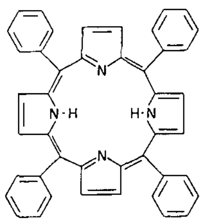
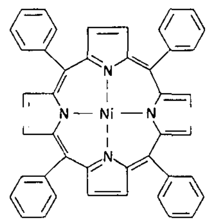
Since some metals in trace quantities have a deleterious effect on certain processes in the petroleum industry, an accurate and rapid procedure is necessary for their determination. Investigators have shown that some of these metals are present as organic complexes which are volatile and that a wet-ash procedure recovers more of the metals present in petroleum fractions than a dry-ash technique. It has never been shown conclusively that a wet-ash procedure recovers quantitatively the metals from volatile porphyrinlike complexes. This paper presents the results of the wet- and dry-ash procedures on synthetic blends of nickel, vanadium, iron, and copper derivatives of porphyrins. Information on the preparation of tetraphenylporphyrin and its metallic derivatives is presented.

THE importance of trace metals in petroleum processing has been reported by several investigators. A review of these reports has been presented by Milner, Glass, Kirchmer, and Yurick (*6*), Karchmer and Gunn (*4*), and Gamble and Jones (*3*). As the deleterious effects of metals such as nickel, vanadium, iron, and copper on catalytic cracking have been more fully realized, attempts have been made to produce cleaner feed stocks for charging to catalytic cracking units. This has been done primarily by the elimination of entrainment in distillation columns. Several years ago, it was not uncommon to encounter overhead distillates containing as high as 25 p.p.m. of metallic contamination. Fractionation efficiency has been so increased that at present it is very rare if the metallic content exceeds a few parts per million. Data have been presented by Woodle and Chandler (*13*), by Wrightson (*14*), and by Gamble and Jones (*3*) to support the postulate that metals appear in the overhead fractions by volatilization during vacuum distillation. It has been noted that porphyrin compounds and their metallic complexes are present in crudes (*1, 2, 6, 11*). It is also known that these compounds are volatile and consequently such metal-containing chelates can be expected to be present in the overhead petroleum fractions.

The procedure for ashing petroleum fractions developed by Karchmer and Gunn (*4*) was shown by these workers to be applicable to the type of stock encountered in the refinery several years ago. However, in the relatively noncontaminated overhead streams produced currently, the metallic impurities are present primarily as soluble, nonfilterable types, presumably volatile metallo-organic complexes. Hence, the conventional dry-ash procedure would not be expected to give quantitative results on these clean overhead fractions and, indeed, has been shown by Milner (*6*) and Gamble and Jones (*3*) to give results which are lower than these obtained by wet-ashing techniques. This is further proof that the metal constituents in these fractions are volatile. However, it has never been shown conclusively that the wet-ash procedure recovers all of the volatile constituents quantitatively. Hence, it was the purpose of this investigation to prepare volatile metallic tetraphenylporphyrin derivatives and to analyze synthetic blends containing these derivatives to determine if the wet-ash procedure gave quantitative results. Samples of tetraphenylporphyrin (TPP), nickel tetraphenylporphyrin (Ni TPP), vanadium oxide tetraphenylporphyrin (VO TPP), iron chloride tetraphenylporphyrin (FeCl TPP), and copper tetraphenylporphyrin (Cu TPP) were synthesized. Tetraphenylporphyrin was chosen because it is relatively easy to synthesize. The structure of tetraphenylporphyrin and its nickel complex may be represented as follows:





VOLATILITY OF METALLIC TETRAPHENYLPORPHYRINS

Evidence of the volatility of metal derivatives of tetraphenylporphyrin was demonstrated by a molecular still distillation of a sample of refined coastal residuum in which 187 p.p.m. of nickel tetraphenylporphyrin was dissolved. Analyses by visible spectroscopy of the 16 fractions obtained from this distillation are presented in Figure 1 and show definitely that this particular metallic porphyrin is volatile, but that no appreciable quantity is distilled below 1150° F. (temperature corrected to atmospheric pressure). The volatilities of nickel, vanadium, and copper tetraphenylporphyrins were proved further by test tube experiments, which showed that these metallic derivatives sublimed under reduced pressures at elevated temperatures without decomposition.

PREPARATION OF MATERIALS

Synthesis of Tetraphenylporphyrin. The method employed for the preparation of tetraphenylporphyrin was a modification of the procedure developed by Rothemund and Menotti (*8*). A mixture of 100 ml. of freshly distilled pyrole (Eastman reagent), 200 ml. of benzaldehyde (Eastman reagent), and 200 ml. of pyridine (Baker reagent) was deaerated and placed in a glass cylinder liner from which the oxygen was displaced with Seaford grade nitrogen. The glass liner and its contents were set in a 1-liter bomb (Parr autoclave equipment, Model H.C.-11) from which oxygen had been displaced with Seaford grade nitrogen. One hundred milliliters of pyridine were put in the annular space between the glass liner and the bomb. Any air in the bomb was removed by either evacuation or by pressuring the bomb to 300 pounds per square inch gage with Seaford grade nitrogen and releasing to atmospheric pressure. In case of the latter procedure, the operation was repeated several times.

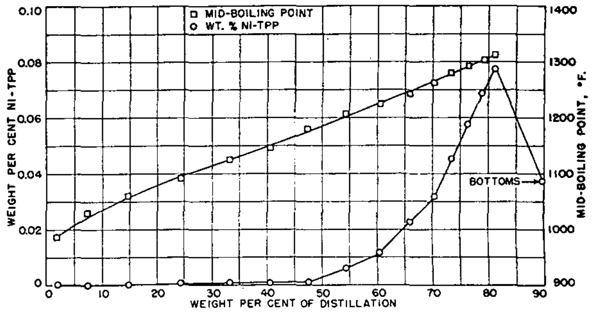


Figure 1. Molecular distillation of nickel tetraphenylporphyrin in refined coastal residum

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The bomb was heated to 360° F. for 48 hours and allowed to cool slowly in the autoclave for 24 hours. The contents in the glass liner were filtered through a glass-fritted disk filter and washed with a mixture of 90 to 10 volume % of ether and acetone until the filtrate was only a light pink color. The crystals were washed with hot methanol and dried in a vacuum oven. The average yield of crude tetraphenylporphyrin was 17.4 grams. These crude crystals were purified by extraction with 200 ml. of benzene in a Soxhlet extractor. The benzene was allowed to cool slowly to about 76° F. (room temperature), and the crystals were removed from the benzene by filtration. These crystals were re-extracted with fresh benzene and recrystallized. The dry crystals from the second extraction were essentially chemically pure tetraphenylporphyrin as shown by the following analysis.

Formula		Mol. Wt.	C	H	N
Tetraphenylporphyrin	Theoretical (calcd.)	614.71	85.97	4.92	9.1
$C_{44}H_{30}N_4$	Found (anal.)	..	85.87	5.15	9.07

Synthesis of Nickel Tetraphenylporphyrin. Nickel tetraphenylporphyrin was prepared by a modification of Method 2 of Rothemund and Menotti (*9*).

Five grams of pure tetraphenylporphyrin were dissolved in 300 ml. of pyridine (Baker reagent), and 5 grams of nickel acetate (Baker reagent) were dissolved in 200 ml. of pyridine. These solutions were mixed and deaerated by applying reduced pressure before charging to an oxygen-free glass liner for a 1-liter Parr autoclave bomb (Model H.C.-11). The glass liner and contents were placed in the bomb from which air had been displaced with Seaford grade nitrogen. One hundred milliliters of pyridine were put in the annular space between the glass liner and the bomb. The bomb was pressured to 300 pounds per square inch gage with Seaford grade nitrogen and then released to atmospheric pressure. This procedure was repeated several times to be sure that the oxygen content of the bomb was negligible. The bomb was repressured to 300 pounds per square inch gage with nitrogen and heated to 400° F. for 24 hours. The bomb and contents were allowed to cool slowly in the autoclave for 24 hours to room temperature. The contents of the glass liner were removed and the liquid volume decreased approximately 50% by distilling under reduced pressure. After cooling to room temperature (about 76° F.), the crystals were filtered from the liquid and purified by extraction with benzene in a Soxhlet extractor. The crystals obtained from the benzene extract were re-extracted with fresh benzene. About 5.2 grams of chemically pure nickel tetraphenylporphyrin were obtained from the second purification step as indicated by the following data.

Formula		Mol. Wt.	C	H	N	Ni
Ni tetraphenylporphyrin	Theoretical (calcd.)	671.38	78.71	4.20	8.35	8.74
$C_{44}H_{28}N_4Ni$	Found (anal.)		78.85	4.10	8.16	8.78

Synthesis of Iron Tetraphenylporphyrin. The procedure employed for the preparation of iron tetraphenylporphyrin was Method 1 used by Rothemund and Menotti (*9*). Five grams of pure tetraphenylporphyrin and 1 gram of sodium chloride (Baker reagent) were placed in a 1-liter three-necked flask, equipped with a glass mechanical stirrer, a glass reflux condenser, and a gas inlet tube. Four hundred milliliters of chloroform (Baker reagent) were added and the mixture was heated and stirred under a blanket of Seaford nitrogen until all of the tetraphenylporphyrin was dissolved. In a similar apparatus 2.2 grams of pure iron powder were dissolved in 400 ml. of glacial acetic acid (Baker reagent) under a blanket of Seaford nitrogen at the reflux temperature. The ferrous acetate solution was forced through a glass wool filter into the flask containing the chloroform solution of tetraphenylporphyrin and sodium chloride by means of nitrogen pressure. This mixture was refluxed for 2 hours, and the amount of liquid was reduced to slightly less than 400 ml. by distillation. The contents of the flask were allowed to cool slowly overnight.

The crystals were separated by filtration and were washed consecutively with a 50/50 mixture of glacial acetic acid and water, a small amount of glacial acetic acid, and acetone and then were dried in a vacuum oven. The dried crystals were placed in a Soxhlet extractor and extracted with chloroform. The amount of chloroform was reduced to 50 ml., and 800 ml. of hot methanol were added. This mixture was stirred for about 30 minutes and then allowed to cool slowly. The precipitate was sucked dry and re-extracted with chloroform, then was reprecipitated with methanol as before. About 3.2 grams of iron chloride tetraphenylporphyrin of the following purity were obtained.

Formula		Mol. Wt.	C	H	N	Cl	Fe
FeCl tetraphenylporphyrin	Theoretical (calcd.)	704.00	75.06	4.01	7.96	5.04	7.93
$C_{44}H_{28}N_4FeCl$	Found (anal.)	..	71.28	3.94	7.38	9.54	7.44

Synthesis of Copper Tetraphenylporphyrin. The procedure employed for the preparation of copper tetraphenylporphyrin was Method 1 used by Rothemund and Menotti (*9*). Five grams of pure tetraphenylporphyrin were placed in a 1-liter three-necked flask that was fitted with a glass mechanical stirrer, a glass condenser, and a gas inlet tube. Four hundred milliliters of chloroform (Baker reagent) were added. This mixture was heated by means of a heating mantle and stirred under a Seaford nitrogen blanket until all of the tetraphenylporphyrin was dissolved. In a similar apparatus 2.5 grams of cupric acetate (Baker reagent) were dissolved in 400 ml. of glacial acetic acid (Baker reagent). This solution was then forced through a glass wool filter into the flask containing the chloroform solution of tetraphenylporphyrin by applying nitrogen pressure. This mixture was stirred for 2 hours at the refluxing temperature and then allowed to cool slowly overnight to room temperature (about 76° F.). The crystals were separated from the liquid by filtration, washed with a 50/50 mixture of glacial acetic acid and water, and glacial acetic acid, and then sucked dry. The copper tetraphenylporphyrin was purified by benzene extraction in a Soxhlet apparatus, followed by crystallization, re-extraction with fresh benzene, recrystallization, and drying in a vacuum oven. About 5.5 grams of chemically pure copper tetraphenylporphyrin were obtained from the second purification step

Formula		Mol. Wt.	C	H	N	Cu
Cu tetraphenylporphyrin	Theoretical (calcd.)	676.26	78.12	4.18	8.29	9.41
$C_{44}H_{28}N_4Cu$	Found (anal.)	..	78.28	4.20	8.23	9.39

Synthesis of Vanadium Tetraphenylporphyrin. The method used for the preparation of vanadyl tetraphenylporphyrin is as follows:

Ten grams of c. p. vanadium pentoxide were placed in a 500-ml. three-necked flask fitted with a glass Trubore stirrer, reflux condenser, and gas inlet tube; 50 ml. of concentrated hydrochloric acid were added and the mixture was warmed and stirred gently. After 15 minutes the oxide had usually dissolved. If not, additional acid was added and heat applied until a clear green solution resulted. Two milliliters of ethyl alcohol (*7*) were added, and the solution was refluxed until a clear blue solution was obtained; this generally required 20 minutes. The heating mantle was removed and the stirred solution was neutralized to pH 5 to 7 by the addition of a saturated aqueous solution of sodium carbonate. The light cocoa-colored precipitate was filtered on a sintered-glass filter under vacuum, and washed with two 50-ml. portions of distilled water and two 25-ml. portions of absolute ethyl alcohol. The precipitate was sucked dry and immediately scraped out of the funnel into a 200-ml. round-bottomed flask containing 5 grams of tetraphenylporphyrin (twice extracted with benzene) and 30 grams of c. p. naphthalene.

Immediate transfer of the precipitate was found to be very important, because prolonged exposure of the solid to air resulted in darkening of its color and failure of the subsequent reaction. The flask was fitted with a long air condenser and was heated with a free flame. Water and naphthalene boiled out the top of the condenser until all the water was gone. More naphthalene was added at this point if necessary to maintain a convenient liquid volume. The mixture was then refluxed for 2 additional hours, or until a bright red melt was obtained. The contents of the flask were poured into water, and the lumps were broken up and placed in a Soxhlet extractor. Naphthalene was extracted with either acetone or alcohol; the extraction flask was changed and the porphyrin was extracted with 300 ml. of chloroform.

The extraction required about a week because the sintered-glass extraction thimble became clogged with very finely divided black material, probably unreacted vanadium oxides. This material was so finely divided that it passed through the sintered-glass thimble (it also passed through conventional paper thimbles) and contaminated the solid porphyrin in the flask. The black material was not volatile whereas the porphyrin was; thus, vacuum sublimation was chosen as the method of purification.

Filtration of the chloroform extract gave about 3 grams of material which were dried in vacuo at 110° C. and purified by sublimation at 450° C. and 1 to 5 microns of pressure. A nonvolatile, black residue remained behind. The dark blue-violet, crystalline sublimate was triturated to a dark red-violet powder prior to analysis. The powdered sublimate was found to be essentially chemically pure.

Formula		Mol. Wt.	C	H	N	O	V
VO tetraphenylporphyrin	Theoretical (calcd.)	679.65	77.75	4.15	8.24	2.36	7.50
$C_{44}H_{28}N_4VO$	Found (anal.)	..	77.40	4.25	8.45	2.12	7.68

Preparation of Synthetic Blends. Synthetic metallic tetraphenylporphyrin blends in gas oil were prepared for subsequent analytical studies. The gas oil used in these blends had a boiling range between 600° and 1100° F. and contained 0.32 p.p.m. of nickel, 0.04 p.p.m. of vanadium, 1.0 p.p.m. of iron, and no copper as determined by several analyses by the wet-ash procedure. Prior to the preparation of the blends the gas oil was heated to approximately 300° F. under a nitrogen blanket and filtered through a medium porosity glass-fritted disk filter. To individual-weighed samples of this filtered gas oil accurately weighed quantities of nickel tetraphenylporphyrin, vanadium tetraphenylporphyrin, iron tetraphenylporphyrin, and copper tetraphenylporphyrin of known purities were added. These mixtures were agitated mechanically in glass equipment under a Seaford nitrogen blanket at approximately 300° F. for 48 to 72 hours, followed by filtration through a medium porosity glass-fritted disk filter by means of reduced pressure. The residue on the fritted disk was dissolved by sulfuric acid digestion and analyzed to determine the amount of the particular metal that did not dissolve in the oil. This quantity of metal was subtracted from the amount originally added to obtain the actual metal content of the filter gas oil.

ASHING PROCEDURES

The procedure of Karchmer and Gunn (*4*) for the determination of ash in petroleum was used for the dry-ashing technique. Normally, 1 quart of material was burned. The wet-ashing procedure was a modification of the method reported by Milner (*6*). The modified procedure is simple, rapid, and adaptable to routine operation. It has been in routine use in this laboratory for over a year.

Modified Wet-Ash Procedure. An appropriate amount of oil was weighed into a 700-ml. Vycor beaker and 1.0 ml. of concentrated sulfuric acid was added for each gram of sample. (The size of sample for analysis depends on the expected concentration of metals, but usually 100 grams are used when analyzing for trace amounts. No more than 100 grams should be treated with sulfuric acid at one time. If more sample is required, additional 100-gram batches should be treated and the subsequent solutions combined.) The beaker containing the sample and acid was placed on a high temperature hot plate (about 800° F.) and heated as rapidly as possible. When boiling or frothing started the mixture was stirred vigorously with a Vycor stirring rod and, by use of large beaker tongs, the container was removed alternately off and on the hot plate to control foaming. How hot and when to stir definitely affects the sludging characteristics of most oil samples. In most cases a few minutes were required before a dry coke was obtained and all danger of spattering and foaming was passed.

When the sludge began to thicken, the beaker was heated on the hot plate at a high temperature and was stirred intermittently. At first, heavy fumes of sulfur oxides were emitted; however, the organic sludge soon began to crack and the ignitable hydrocarbon vapors were ignited and allowed to burn until a completely dry coke remained. The burning could be aided considerably by stirring periodically. The beaker containing dry coke was removed to a muffle furnace and reduced to an inorganic ash at 1000° F. aided by a slow flow of air or oxygen. This work, up to placing in the muffle furnace, must be done under a well constructed hood. By elimination of the sulfur oxide fumes and burning the hydrocarbon vapors, the final ashing step in the muffle furnace may be done in the open laboratory.

After the sample had been reduced to an inorganic ash it was removed very carefully from the muffle furnace and, after cooling, 20 ml. of 1 to 1 hydrochloric acid were added, and the sample was boiled to effect solution. Then it was transferred quantitatively to a 250-ml. beaker, reduced to 3 to 4 ml., filtered, and made to a convenient volume for aliquoting. Transferring from the 700-ml. beaker to the 250-ml. beaker is done because of the danger of forming dry spots in the large beaker. If a large amount of acid insoluble material is present it may be necessary to fuse the sample to effect solution. However, this procedure was not necessary in the samples used in this study.

Baker's C. P. hydrochloric acid and Du Pont C. P. sulfuric acid were used. The over-all reagent blank was approximately 0.025 p.p.m. of iron, 0.035 p.p.m. of nickel, and no vanadium. Of these amounts the sulfuric acid contained 0.020 p.p.m. of iron and 0.014 p.p.m. of nickel. Blanks on copper were high and hence copper in low concentrations was not determined.

The dry and wet ashes were then analyzed chemically for the desired metals. Nickel was determined by dimethylglyoxime (*6*), iron by thiocyanate (*10, 12*), vanadium by phosphotungstate (*6*), and copper by polarography (*5*). All reagent blanks were carefully determined and subtracted from the readings obtained on samples.

Accuracy of Wet-Ash Analyses. During the work on the synthesis of the metallic porphyrin compounds, preliminary results indicated that nickel was one of the more volatile metal derivatives of tetraphenylporphyrin. Therefore, the ability of the wet-ash procedure to determine accurately the amount of volatile metallo-organic complexes introduced into an oil was first tested on blends of nickel tetraphenylporphyrin. Blends 1 and 2 were prepared in white oil containing no detectable amount of extraneous nickel; blend 3 was prepared in a gas oil. The data in Table I present the results obtained.

Table I. Accuracy of Wet-Ash Procedure on Synthetic Blends Containing Nickel Tetraphenylporphyrin

Sample No.	1	2	3
Composition, p.p.m. Ni			
By synthesis	0.22	1.07	10.42
By wet-ash	0.23	1.05	10.15
	0.24	1.10	10.43
	0.23	1.05	10.41
	0.23	1.04	10.41
	0.22	1.03	
	0.20		
Av.	0.225	1.054	10.35
Recovery, % of synthetic value	102	99	99

It is seen from these data that the nickel was accurately and totally determined in all concentrations studied, to within the reproducibility of the test.

Comparison of Dry- and Wet-Ash Results. In order to test further the accuracy of the wet-ash procedure and to compare the results with the dry-ash technique, blends containing nickel, iron, vanadium, and copper in gas oil were prepared. The results of both types of analyses are compared in Table II.

Table II. Comparison of Wet-Ash and Dry-Ash Results at High Concentrations of Metals

Sample No.	4		5		6		7	
Metal added	Ni		V		Fe		Cu	
	Compn. by Synthesis, P.P.M.							
	15.62		12.44		15.20		16.20	
	Compn. by Analysis, P.P.M.							
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	16.1	8.7	11.5	11.0	15.5	10.4	16.7	7.7
	16.4	8.5	11.2	11.0	15.6	9.9	16.3	7.2
		8.2	11.7	11.0	15.4	10.3	16.0	6.7
			11.8	10.8	15.8	10.4	16.4	
			12.3			9.8		
						9.7		
Av.	16.25	8.47	11.70	10.95	15.55	10.09	16.35	7.20
Recovery, % of synthetic value	104	54	95	88	102	72	101	45

The data show that all the metals studied were quantitatively recovered by the wet-ash procedure but not by the dry-ash technique. In the case of vanadium, very little was lost by dry-ashing; however, the difference in the dry- and wet-ash value is statistically significant.

The comparison of wet- and dry-ash results was extended to cover low concentration of nickel, iron, and vanadium. Copper was not studied in low concentration because of reagent blank difficulties. The samples for this study were prepared by dilution of samples 3, 5, and 6 with a catalytic cycle stock containing 0.02 p.p.m. of iron, 0.03 p.p.m. of nickel, and no detectable amount of vanadium. The three blends were then mixed to make sample 8. Table III shows the results obtained on this sample.

Table III. Comparison of Wet-Ash and Dry-Ash Results at Low Concentrations

(Sample 8)

Metal added	Ni		V		Fe	
Compn. by Synthesis, P.P.M.	0.53		0.31		0.52	
Compn. by Analysis, P.P.M.	Wet	Dry	Wet	Dry	Wet	Dry
	0.52	0.41	0.29	0.31	0.51	0.46
	0.50	0.44	0.33	0.29	0.51	0.44
	0.54	0.41	0.33	0.29	0.53	0.43
	0.52	0.39	0.33	0.30	0.53	0.41
Av.	0.52	0.41	0.32	0.30	0.52	0.44
Std. dev.	0.0163	0.0206	0.0200	0.0096	0.0115	0.0208
Recovery, % of synthetic value	98	76	103	97	100	85

These data show that significant losses of nickel and iron were obtained by the dry-ash method, whereas the loss of vanadium was not statistically significant. By comparing the dry-ash results on nickel in Tables II and III, it can be seen that 76% recovery of nickel was obtained on the 0.5-p.p.m. samples, whereas only 54% recovery was obtained on the 15-p.p.m. samples. Similarly, the recoveries of iron and vanadium were greater at the low concentration level (see Tables II and III). The higher recoveries of nickel, iron, and vanadium by the dry-ash procedure at the 0.5-p.p.m. level as compared to the 15-p.p.m. level cannot be fully explained at this time. One explanation may be that 100-gram samples of the 0.5 p.p.m. blends were dry-ashed, whereas 1-quart (approximately 800 grams) samples were used in the work at the 15-p.p.m. level. The initial dry ashing results on the concentrated blends were obtained at an early stage of the experimental work by the regular routine procedure which specifies 1 quart of sample. However, as the work progressed it was necessary to reduce the amount of sample used to 100 grams, because of sample availability. In order to test this possibility, a series of six dry-ash determinations were made using 100-gram portions of sample 4 which contained 15.62 p.p.m. of nickel. The amount determined by dry ash was 11.8 p.p.m. (average of 12.2, 12.1, 10.5, 11.6, 11.9, 12.5). This represents a recovery of 76%, which checks exactly with the value of 76% obtained on sample 8 when using 100-gram portions during dry-ashing.

These data are in agreement with the results of Gamble and Jones (*3*), who have shown that the recovery of metals by dry-ashing is dependent on the amount burned at one time, the size of the dish in which the sample is burned, and the rate of burning. Inasmuch as the modified wet-ash procedure described in this work determines volatile metals quantitatively and in some cases is as fast or actually faster than the dry-ashing technique, further studies on improving the recovery by dry-ashing were of limited interest and hence not pursued.

Recovery of Metals from Natural Petroleum Stocks. Numerous experiments on petroleum fractions produced in the refinery show losses of nickel and iron by the dry-ashing techniques similar to the losses reported in Tables II and III on the synthetic blends. However, losses of vanadium on some natural petroleum fractions were higher than those experienced with the synthetic blends. These data are presented in Table IV.

Table IV. Recovery of Vanadium from Natural Petroleum Fractions

Sample No.	11		12		13	
Type of stock	Gas Oil		Deasphalted Residua			
Ashing method	Wet	Dry	Wet	Dry	Wet	Dry
Vanadium, p.p.m.	0.37	0.32	8.48	7.60	19.2	17.2
	0.39	0.34	8.48	7.50	19.6	16.2
	0.35	0.30	8.58	7.20	19.4	17.1
	0.38	0.30	8.60	7.40	19.2	16.6
Av.	0.372	0.315	8.535	7.425	19.35	16.77
Std. dev.	0.0171	0.0191	0.064	0.171	0.195	0.465
Loss by dry-ashing, % of wet ash value	15		13		13	

The dry-ash determinations reported in Table IV were done on 100-gram portions and hence should be compared to the vanadium results in Table III. By this comparison it is seen that significant losses on vanadium were realized from the natural stocks, whereas no significant loss was obtained on the synthetic sample. Likewise, the losses of nickel and iron, although similar, are not the same for all types of refinery fractions. Hence, it is not feasible to use a single dry-ash to wet-ash conversion factor for all stocks.

Effect of Ignition after Initial Sulfonation. After the initial sulfonation step in the wet-ash procedure, the unreacted organic vapors must be driven off before the sample can be put into the muffle furnace for reduction to ash. This operation usually requires several hours. In an effort to speed up the procedure, ignition of the vapors after sulfonation and prior to introduction into the muffle furnace was studied. The results are presented in Table V.

Table V. Effect of Ignition after Sulfonation

(Sample 8)

Metal added	Ni		V		Fe	
Compn. by Synthesis, P.P.M.	0.53		0.31		0.52	
Compn. by Analysis, P.P.M.	Ign.[a]	Non-ign.[b]	Ign.	Non-ign.	Ign.	Non-ign.
	0.52	0.52	0.29	0.29	0.51	0.47
	0.50	0.50	0.33	0.33	0.51	0.51
	0.54	0.52	0.33	0.26	0.53	0.53
	0.52	0.50	0.30	0.29	0.53	0.45
Av.	0.52	0.51	0.31	0.29	0.52	0.49
Std. dev.	0.0115	0.0115	0.0206	0.0282	0.0115	0.045

[a] Ignition.
[b] Nonignition.

These data show that there is no significant difference in the values obtained when either igniting or not igniting the vapors. Hence, the procedure can be greatly speeded up by igniting the oil remaining after the initial sulfonation.

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Paper Chromatographic Method for Determining Alkaloids in Tobacco

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A method of determination of the kinds of alkaloids and their approximate amounts in tobacco and related species of *Nicotiana* is described. This method is designed for the analysis of the large number of samples required in connection with a plant-breeding program. Sufficient accuracy for this purpose is obtained by visual comparison with known spots after separation by paper chromatography.

NUMEROUS methods have been devised for the determination of nicotine in tobacco and insecticides, and many of them give satisfactory results when nicotine is the predominant alkaloid present (*5*). If other related compounds are present the numerical value obtained is usually intermediate between the true nicotine content and the total alkaloid value. The most frequently used methods provide no indication of which samples contain nicotine and which may contain principally related alkaloids. It has been known for some time that certain strains of commercial tobacco contain principally nornicotine (*8*), and that the predominant alkaloid in most of the species of the genus *Nicotiana* investigated is not nicotine. In most cases nornicotine is predominant, in some nicotine, and in a few anabasine (*10*).

Plant breeders have found that certain of these wild species offer better sources of parental material from which to obtain resistance to many of the important tobacco diseases than does commercial tobacco, *Nicotiana tabacum*, and have made interspecific crosses with commercial tobacco in the development of new varieties which are being released for commercial use. If either parent produces predominantly nornicotine, the first generation of the crosses which have been studied contain predominantly the non-nicotine alkaloid, but the inheritance of the factors controlling alkaloid type in later generations requires further study. Information concerning the physiological effects on the smoker and the effects on smoking quality of these alkaloids, other than nicotine, is limited and selection of varieties similar to present commercial varieties in proportion of different alkaloids cannot be conducted without improved analytical methods.

Several methods have been devised which differentiate among pyridine alkaloids. Some have utilized reactions differentiating secondary and tertiary amines (*1, 2*) which divide the tobacco alkaloids into two classes usually referred to as nicotine and nornicotine. The determination of nicotine by these methods appears to be satisfactory, but several secondary amines are often present and nornicotine is not necessarily the one present in greatest amount. Others have used various column (*4*) or paper chromatography (*3, 7, 9, 11*) methods. The latter type of method is capable of distinguishing at least 20 to 40 compounds present in some tobacco samples, which are probably related to nicotine (*6*). There is no evidence at the present time that significant quantities of most of these constituents are present in any tobacco, but it is advantageous to use a method capable of detecting and distinguishing between various pyridine compounds should they appear in quantity in future breeding material. Observations in this laboratory indicate that the types of alkaloid formed by a tobacco strain are characteristic of the strain and that the amount present is dependent on enviromental or cultural conditions. In many instances the same plants must be used for analysis and for seed. Thus topping is impossible, resulting in a much lower total alkaloid content than would be attained in farm practice, and consequently precision is less important than simplicity, reliability, and qualitative selectivity. On the basis of 3 years of experience with various paper chromatographic methods and modifications, the following methods seem best adapted to this use.

APPARATUS

Paper Chromatographic Chamber, either ascending or descending type.

Cyanogen Bromide Tank, cylindrical jar with ground edge and plate glass cover, at least 9 inches in diameter and 19 inches high, if full-sized sheets are used.

Tracing Box, or other means of observing papers by uniform transmitted light.

Pipets, of 10-μl. delivery.

Waring Blendor, or equal (if green leaf samples are used).

REAGENTS AND SUPPLIES

Chromatographic Filter Paper, Whatman No. 1, full-sized sheets.

Acetone, according to ACS specifications.

Acetone Solution, 50%.

Paper Pretreating Solution, equal parts of 0.1*M* pH 6.5 phosphate buffer and methanol.

Developing Solution, 100 ml. of *tert*-amyl alcohol, 20 ml. o water, and 0.3 gram of ethyl *p*-aminobenzoate.

Chamber Saturating Solution, 100 ml. of water and 12.5 ml of *tert*-amyl alcohol.

Sodium Acetate Buffer, 0.2*M*, pH 5.6.

Cyanogen Bromide Crystals.

Stock Solutions of Nicotine, Nornicotine, and Anabasine, each containing 1 mg. of alkaloid per ml. in 50% acetone From these, four standards, also in 50% acetone, are made up containing the following numbers of micrograms of alkaloid pe 10 μl. (or mg. per 10 ml.).

γ/10 μl.	
Nicotine	Nornicotine
8	1
4	4
2	2
	Anabasine
1	0.2

The stock and standard solutions are kept in the refrigerator when not in use and can be kept at least 6 months if protected from evaporation or condensation.

PROCEDURE

Dry Samples. Weigh 0.25- to 0.5-gram samples of dry powdered tobacco into 50-ml. flasks. If the alkaloid content is below 1% use more sample, up to 2 grams. Add 25 ml. of 50% acetone, then let stand for 30 minutes with occasional shaking. When filtered into test tubes it is ready to apply to paper.

Fresh Leaf. Larger samples must be used, greater precautions must be taken to ensure reasonably representative samples, and the moisture content of the leaf must be considered. From 50 to 100 grams of green tissue are placed in a Waring Blendor with 100 ml. of 50% acetone. An amount of pure acetone approximately equal to the volume of water in the fresh sample is added in order to make the final solution 50% acetone. In practice this can be approximated with fresh tissue by adding 90% as many milliliters of pure acetone as grams of fresh tissue. After maceration the mixture is filtered and the resulting solution is ready for application to the paper.

For the chromatographic separation large sheets of Whatman No. 1 paper are pretreated by dipping in a solution composed of equal parts of 0.1*M* pH 6.5 phosphate buffer and methanol, draining, drying, and pressing. Ten-microliter portions of the 50% acetone solutions, obtained in either of the above ways, are placed on the sheets at intervals of about 1.5 inches (3.8 cm.) along a line 1.5 inches from one edge. Four standards, prepared as described, are included on each sheet. The samples are normally placed on the paper on the day on which they are extracted or on the following day. However, no breakdown (*7*) of refrigerated extracts in this solvent has been observed though no extracts are kept more than a week, at which time the apparent concentration of alkaloids is usually slightly higher, presumably because of evaporation of solvent.

These sheets may be developed overnight by either ascending or descending methods using a one-phase developing solution containing 100 ml. of *tert*-amyl alcohol, 20 ml. of water, and 0.3 gram of ethyl *p*-aminobenzoate. The alcohol is about nine tenths saturated with water. Water saturated with *tert*-amyl alcohol (12.5 ml. of alcohol to 100 ml. of water) is placed in the bottom of the tank. In order to obtain the best equilibrium the tank should be prepared 4 to 16 hours before use. If a Chromatocab is used in the descending method, a blank sheet is inserted in each of the outside troughs when the tank is prepared. If cylindrical glass tanks are used in the ascending method a liner is allowed to contact the water phase in the bottom.

The following morning the paper is removed from the tank, dried in air, lightly sprayed with 0.2*M* pH 5.6 acetate buffer, and placed immediately in a tank containing crystals of cyanogen bromide and located in the hood. The stabilization of pH and the presence of acetate and of moisture in the paper resulting from this spraying all appear to assist in obtaining maximum sensitivity and reproducibility of the color reaction. The papers are removed from the cyanogen bromide tank in about 5 minutes, aerated in the hood, and promptly read by transmitted light from fluorescent daylight tubes through frosted glass on a tracing box.

By this procedure nicotine produces a lemon-yellow spot, R_f about 0.6; anabasine a yellow spot, R_f 0.16 which turns pinkish on standing; and nornicotine a yellow spot at R_f 0.10. Since four levels of nicotine, three of nornicotine, and one of anabasine are included, the amount of the principal alkaloids in a sample can be estimated visually, usually within 10% and always within 25% of the actual amount. The estimates are most reliable in the range of 2 to 4 γ. If the amounts are found to be outside the range of the standards, the determinations are repeated after dilution of the sample or the addition of up to 10 successive 10-μl. portions of the sample on the same spot. This latter can be accomplished by placing the paper on a frame so that an electric strip heater, about 40 cm. long, drawing about 300 watts is about 3 cm. below and parallel to the line of spots. One 10-μl. portion of each sample is added to the corresponding spot and by the time all unknown samples have been so treated the first spot is dry and can receive another addition without increasing the spot size appreciably. No noticeable loss of alkaloid occurs if the acids dissolved from the leaf are present. If a 0.5-gram dry tobacco sample and a 10-μl. spot have been used, the number of micrograms of alkaloid estimated to be present on the paper may be divided by 2 to yield the percentage by weight of this alkaloid in the original sample. For a fresh sample the total volume of the solution is approximately the sum of the grams of water in the sample expressed as milliliters, the milliliters of 50% acetone added, and the milliliters of pure acetone added. The micrograms of alkaloid per spot or per 10 μl. are estimated directly from the paper. The percentage of alkaloid on a fresh-weight basis equals

$$\frac{\text{Total vol. of solution (in ml.)}}{\text{Vol. of extract per spot (in ml.)}} \times \frac{\text{alkaloid found per spot (in grams)}}{\text{fresh wt. of sample (in grams)}} \times 100$$

Since the standard volume of extract per spot is 0.01 ml., this becomes:

$$\frac{\text{Total vol. (in ml.)} \times \text{micrograms alkaloid per spot}}{100 \times \text{wt. of sample (in grams)}}$$

If the percentage of dry weight of the sample is determined on a similar sample of fresh tissue, this result can be converted to a dry-weight basis. Since the differences in quantity of alkaloids between different plants of tobacco are so great, the accuracy of

Table I. Comparison of Alkaloid Content of Samples of Maryland Tobacco

[Determined by method of present paper, Bowen and Barthel (*1*), and Willits and coworkers (*12*)]

		Nicotine, %		Nornicotine, %		Other Alkaloids,	Total Alkaloids, %			Nicotine/Total Alkaloids	
Variety	Grade	Paper	B & B	Paper	B & B	Paper, %	Paper	B & B	Willits	Paper, %	B & B, %
Catterton	Seconds	1.45	1.69	0.25	0.41	0.10	1.80	2.10	2.20	0.806	0.805
		1.75	2.08	0.10	0.18	0.10	1.95	2.26	2.31	0.897	0.920
		1.90	1.93	0.15	0.42	0.10	2.15	2.35	2.46	0.883	0.821
	Dull bright	2.05	2.15	0.15	0.19	0.03	2.23	2.34	2.56	0.920	0.919
		2.80	3.20	0.10	0.40	0.05	2.95	3.60	3.63	0.955	0.889
		2.75	2.96	0.12	0.14	0.01	2.88	3.10	3.38	0.955	0.955
Robinson	Seconds	0.45	0.51	1.65	1.50	0.15	2.25	2.01	2.69	0.200	0.254
		0.35	0.26	2.15	1.98	0.20	2.70	2.24	2.75	0.130	0.116
		0.25	0.14	1.90	2.31	0.15	2.30	2.45	2.82	0.109	0.057
	Dull bright	0.85	0.76	1.45	1.31	0.10	2.40	2.07	2.38	0.354	0.367
		0.90	0.69	2.60	3.24	0.15	3.65	3.93	4.35	0.247	0.176
		0.40	0.38	2.15	2.21	0.10	2.65	2.59	2.69	0.151	0.147

	Coefficients of Correlation, *r*
Present paper *vs.* Bowen and Barthel	
Nicotine	0.99**
Nornicotine	0.97**
Total alkaloids	0.87**
Nicotine/total alkaloids	0.99**

	Coefficients of Correlation, *r*
Willits method *vs.* Bowen and Barthel	
Total alkaloids	0.95**
Willits method *vs.* present paper	
Total alkaloids	0.93**

$r_{0.01}$ with 10 D.F. = 0.71

$r_{0.05}$ with 10 D.F. = 0.57

** Significant at the 1% level

the results can be enhanced more dependably by complete repetition on other samples than by replicate chromatography on the same extracts or by more precise means of measurement of a given spot.

Certain samples have been observed in which the known alkaloids, especially those of low R_f, are displaced. This appears to be due to the presence in these samples of substances which modify the movement of the alkaloid. These can be eliminated by transferring the alkaloid into ether. If the spot is still found at a different R_f from known alkaloids it is because of an unknown compound. Several of these have been found, but never in large quantities. Since the available evidence indicates that they are 3-pyridines, probably closely related to the known tobacco alkaloids, the quantity is estimated on the assumption that a given quantity of the unknown causes the same size and intensity of spot as with nornicotine. This assumption has not resulted in serious errors as the amount of these unknowns has always been small.

COMPARISON OF METHODS

An example of the importance of the ability of the paper chromatographic method to distinguish between alkaloids may be given for a sample of *N. debneyi.* Using the method here described the sample analyzed 0.05% of nicotine, 0.37% of nornicotine, and 0.75% of anabasine. By the modified method of Bowen and Barthel (*1*) it analyzed 0.02% of nicotine and 0.87% of nornicotine. That method distinguishes only between tertiary amines and those which are primary or secondary; thus it, and probably that of Cundiff and Markunas (*2*), report most of the anabasine as nornicotine. Similarly the total alkaloid value by the Willits method (*12*) was 1.13%, which would be reported as nicotine by the analyst in the absence of information to the contrary. Thus most methods in use today would report the sample as nicotine, those based on a separation of tertiary and other amides, as nornicotine, and only paper chromatographic methods specially designed to give good separation of anabasine and nornicotine would report it correctly as principally anabasine.

A comparison of other results obtained by the above-mentioned methods is given in Table I. Two commonly used varieties of Maryland Broadleaf cured tobacco were analyzed, one of which (Catterton) contains predominantly nicotine and the other (Robinson) predominantly nornicotine. Separate samples were analyzed of each of two different grades from each of three different plots of each variety grown in the same field. Results are presented for nicotine and nornicotine and for the sum of the other alkaloidlike substances by the present method. Anabasine was not found in these samples, but small amounts of two other substances which give color with cyanogen bromide were found. Results are also given for nicotine and total alkaloid by the modified Bowen and Barthel method (*1*) and, by difference, a figure for nornicotine. The total alkaloid content by the method of Willits, Swain, Connelly, and Brice (*12*) is also reported, as well as the sum of the determined alkaloids by the present method. The ratio of nicotine to total alkaloids is also given, as this is a recognized measure of the extent of conversion of nicotine into nornicotine. Correlation coefficients, *r*, were computed for the percentages of nicotine, nornicotine, and total alkaloids and the ratio of nicotine to total alkaloids between the various analytical methods. These are also presented in Table I. All correlation coefficients are highly significant based on 10 degrees of freedom and indicate that the method here described gives results which are sufficiently similar to the established methods to be acceptable. Naturally the correlation between total alkaloid results by the older methods is closer than with the new method, as these other total alkaloid values are obtained directly by analysis of the same steam distillate. The results by the chromatographic method were derived from a different extract and the values for total alkaloid were obtained indirectly by adding together the values for individual alkaloids. Nevertheless the agreement is satisfactory when the following are considered: the complexity of the material analyzed, the complete difference in principle of every step of the separation and determination, and the fact that the estimation in the proposed method is by visual comparison. At the same time the new method is able to give additional information of equalitative nature available only by means of chromatographic methods.

DISCUSSION

The advantages of the proposed method are as follows:

This method distinguishes between a larger number of different pyridine compounds than other methods adapted to use on a large number of samples. It does not distinguish as many constituents as does another chromatographic method published from this laboratory (*11*), but is much more rapid.

Approximately 300 ground samples a week can be handled regularly by one worker with adequate equipment.

Either cured or green samples may be used. Generalizations should not be made as to the alkaloid composition of a strain of tobacco from analysis of only one form, because of the frequency of alkaloid conversion at some stage of growth or curing.

The extraction solvent used, 50% acetone, permits essentially complete extraction of alkaloids and also dissolves sufficient of the organic acids so that the alkaloids are not volatilized appreciably, even on concentration during the drying of the initial spots.

Pretreatment of the paper with buffer greatly increases uniformity of the position of the spots and decreases "tailing."

The intensifier used, ethyl *p*-aminobenzoate, may be added to the chromatographic developing solution and so applied more uniformly than is possible by spraying.

As little as 0.25 γ of any of the three commonest alkaloids can be detected as a spot on the paper. This corresponds to 0.2% of the particular alkaloid in the original sample as normally run, but if a 2-gram sample of tobacco is used instead of 0.5 gram and ten 10-μl. portions are placed on the original spot, 0.01% of an alkaloid can be detected.

If greater precision of estimation of the concentration of the alkaloids in a given sample becomes desirable the remainder of the extract can be placed on the paper as a stripe, chromatographed, eluted, and determined spectophotometrically as previously described (*6*).

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Polarographic Determination of Acrylonitrile in Presence of Potassium Persulfate

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A polarographic method of analysis has been developed for the determination of acrylonitrile in the presence of potassium persulfate, hydrogen cyanide, formaldehyde, and other products resulting from the persulfate-initiated attack of oxygen on acrylonitrile. Tetramethylammonium iodide is used as the supporting electrolyte, and the oxidation of the latter is prevented by the addition of hydroquinone. Rigorous control tests show that under the conditions used, hydroquinone and the known oxidation products of acrylonitrile do not interfere with the analysis. The potassium ion was found to interfere, because it forms a continuous polarographic wave with acrylonitrile. The diffusion currents of acrylonitrile and potassium ion were shown to be algebraically additive over a wide range of concentrations; therefore, a calibration equation can be used to determine the concentration of acrylonitrile in aqueous solutions in the presence of known amounts of potassium ion.

IN A continuation of previous work (*10*) on the effect of oxygen on aqueous solutions of acrylonitrile in the presence of potassium persulfate, a suitable method of quantitative analysis for acrylonitrile was required. The literature describes several methods of analysis, but none appeared to be entirely satisfactory in the presence of persulfate and the known oxidation products of acrylonitrile.

One method, which is based on the cyanoethylation of dodecylmercaptan (dodecanethiol) (*1*), has found wide use for the quantitative determination of acrylonitrile in high purity samples and in dilute solutions. It has been modified recently for analysis of colored solutions (*6*). However, oxidizing agents interfere and the amount of water present must be limited to 25%. A method involving a modified Kjeldahl reaction (*9*) has been reported, but other nitrogen-containing compounds interfere. Oxidative titration with potassium permanganate (*3*) is obviously unsuited to the present system. A very recent method (*13*) based on the reaction between acrylonitrile and an aqueous solution of sodium or potassium glycocholate is not suitable for kinetic studies because of the time element.

Bird and Hale (*2*) used the polarographic method to determine acrylonitrile in aqueous solutions. With 0.02M tetramethylammonium iodide as supporting electrolyte, a half-wave potential of -2.05 volts against the saturated calomel electrode was obtained, and the diffusion current was shown to be proportional to the concentration of acrylonitrile. It was reported that the method did not measure other nitriles that might be present. This method of analysis was chosen as the most suitable for the present system; however, it required modification and extensive study in order to establish the reliability of the method in the presence of a variety of reducible and nonreducible compounds. The major modification involved a method of compensating for the reduction of potassium ion by use of suitable calibration studies.

APPARATUS AND MATERIALS

Apparatus. The Heyrovský polarograph, Model XII, made by E. H. Sargent and Co., was used for all acrylonitrile determinations. The dropping mercury electrode was of the Heyrovský type. A length of Corning marine barometer tubing (bore = ca. 0.05 mm.) was used for the dropping mercury electrode and was connected to the reservoir by neoprene tubing. The Sargent-Heyrovský Erlenmeyer-type electrolysis vessel was placed in a circulating water bath maintained at 25.0 ± 0.1° C.

The drop time, t, of the dropping mercury electrode immersed in a solution which was 0.02M with respect to tetramethylammonium iodide was 5.11 seconds. The mercury mass, m, was 1.0467 mg. per second in air. The value of $m^{2/3}t^{1/6}$ was 1.35 mg.$^{2/3}$ sec.$^{-1/2}$.

Materials. MERCURY. The purity of the mercury used was shown by the formation of a stable foam lasting from 10 to 15 seconds when shaken with distilled water.

ACRYLONITRILE. Eastman Kodak Co. acrylonitrile was dried over calcium chloride, distilled twice through a 50-cm. Vigreux column, stabilized with hydroquinone, and stored over calcium chloride. Samples for analyses were redistilled immediately before use. The fraction used boiled at 77.2–77.4° C. at 760 mm.; n_D^{25} 1.3886.

POTASSIUM PERSULFATE. Potassium persulfate, Baker and Adamson reagent grade, was recrystallized from water at 50° C. and dried in a vacuum desiccator over anhydrous calcium chloride for several days. Iodometric analysis indicated a purity of better than 99.5%.

TETRAMETHYLAMMONIUM IODIDE. Eastman material was recrystallized twice from 50% aqueous ethyl alcohol and dried in a vacuum desiccator over calcium chloride.

GLYCOLONITRILE. The glycolonitrile was prepared by a standard method (*4*), stored with a small amount of ethyl alcohol, and distilled at 14 to 15 mm. of pressure immediately before use; n_D^{20} 1.4102.

EXPERIMENTAL

General. The polarographic determinations were performed on aqueous solutions of acrylonitrile, using 0.02M tetramethylammonium iodide as the supporting electrolyte. The half-wave potential against the mercury pool anode was found to be -1.75 volts. As the presence of oxygen did not interfere with the well-defined acrylonitrile wave, determinations were made without the removal of oxygen. With the polarograph used in this work a shunt ratio of 10 gave the maximum sensitivity without an excessive range of oscillation of current at any voltage during the analysis. Photographic recordings were used for qualitative analyses. Diffusion current (i_d) values were taken as the difference between the mean current values (measured in millimeter-scale divisions) at applied voltages of -1.5 and -2.0 volts.

The voltages at which manual readings were taken were found to be critical. The chief reason is that certain substances cause the supporting electrolyte to start decomposing at a somewhat less negative potential than usual. None of the substances tested in this work affected the reading taken at -2.0 volts, although a slightly higher voltage led to error. When employing this method of analysis with a polarograph which shows a different half-wave potential for acrylonitrile, the proper voltages could be determined by applying the correction found for the half-wave potential. Observations from this work showed that the shifts of potential due to the use of a different instrument were consistent for the whole region covered.

EFFECT OF POTASSIUM ION. The half-wave potential for acrylonitrile was found to be -1.75 volts against the mercury pool, while that of potassium ion was -1.90 volts. Thus, the diffusion of the potassium ion begins at the point where the acrylonitrile diffusion has reached a maximum, and the result is a continuous polarographic wave when the two substances are present. Photographic recordings show a slight inflection in the current-voltage curves, essentially dividing the polarographic wave into two sections, although the division is not distinct enough to permit measurement. However, calibration studies have shown that the diffusion currents due to the potassium ion and the acrylonitrile are algebraically additive. Therefore, the concentration of the acrylonitrile can be determined if the potassium ion concentration is known.

Calibration studies were carried out with aqueous solutions containing acrylonitrile and potassium persulfate at varying concentrations. A graph of diffusion current against the potassium ion concentration at various levels of acrylonitrile concentration showed straight lines over the entire range of concentrations studied.

For use in the analysis of unknown samples, an equation was developed to relate the diffusion current to the concentrations of both acrylonitrile and potassium ion.

EFFECT OF OTHER SUBSTANCES. Since the tetramethylammonium iodide was subject to oxidation by persulfate ion under the conditions of analysis, hydroquinone was added to prevent this oxidation. A concentration of $6.00 \times 10^{-4}M$ hydroquinone was found to be sufficient for a potassium persulfate concentration as high as $4.00 \times 10^{-4}M$ in solutions for analysis. It was also shown that this concentration of hydroquinone did not affect the diffusion currents of acrylonitrile and potassium ion.

The method of analysis for acrylonitrile was required to be applicable in the presence of potassium persulfate, water, and the various products of oxidation of acrylonitrile (*10*), such as formaldehyde, hydrogen cyanide, glycolic acid, glycolonitrile (formed by reaction of formaldehyde and hydrogen cyanide), carbon dioxide, and carbon monoxide.

Some of these substances may be disregarded for various reasons. Carbon monoxide is not reducible at the dropping mercury electrode, and glycolic acid produces only hydrogen waves (*8*). Possible interference by carbon dioxide was calculated to be negligible for the mixtures analyzed in this work, in which the maximum carbon dioxide concentration was never more than 3% of the acrylonitrile. These calculations were based on the data of Van Rysselberghe, Alkire, and McGee (*14*) for the polarographic determination of carbon dioxide with a quaternary ammonium salt as the supporting electrolyte.

Detailed studies (*12*) showed that the remaining substances, with the exception of potassium ion, did not interfere with the determination of acrylonitrile. The acrylonitrile concentration was from 5×10^{-4} to $1 \times 10^{-3}M$ and the maximum concentrations of the other substances tested were as follows: persulfate ion, $4.00 \times 10^{-4}M$; formaldehyde, $2.40 \times 10^{-4}M$; cyanide ion, $1.00 \times 10^{-3}M$; glycolonitrile, $1.93 \times 10^{-4}M$.

PROCEDURE FOR CALIBRATION STUDIES. A $0.100M$ stock solution of acrylonitrile (containing 6.58 ml. of acrylonitrile per liter of solution) was diluted to give a series of reference solutions in the concentration range from 1.00×10^{-3} to $1.00 \times 10^{-2}M$. The $8.00 \times 10^{-3}M$ stock solution of potassium persulfate (containing 1.0813 grams per 500 ml. of solution) was diluted to give two reference solutions: $8.00 \times 10^{-4}M$ and $5.00 \times 10^{-4}M$. No stock solutions of either acrylonitrile or potassium persulfate were used for a period of time longer than 24 hours. The hydroquinone reference solution was $0.0150M$, and the tetramethylammonium iodide solution was $0.200M$.

By making the proper dilutions of reference solutions, a series of samples was prepared for polarographic analysis. The concentration of acrylonitrile in the samples was varied from zero to $1.00 \times 10^{-3}M$ and that of the potassium persulfate was varied from zero to 4.00 to $10^{-4}M$. The volume of sample prepared in each case was 50 ml. All samples were $6.00 \times 10^{-4}M$ with respect to hydroquinone and $0.020M$ with respect to tetramethylammonium iodide, which served as the supporting electrolyte. A 12.0-ml. sample was pipetted into the electrolytic cell for each analysis, and 10 minutes were allowed for the sample to reach equilibrium temperature after being placed in the constant temperature bath. Manual readings were taken to determine the diffusion current.

PROCEDURE FOR ANALYSIS OF SAMPLES. A sample may contain a known concentration of potassium ion along with acrylonitrile and various oxidation products of acrylonitrile. The volume of sample is chosen so that the diluted sample for analysis produces a diffusion current value in the range covered by the calibration studies. A prior dilution may be necessary in some cases. The chosen volume of sample is pipetted into a 50-ml. volumetric flask, 2 ml. of $0.0150M$ hydroquinone solution is added, and the whole is mixed thoroughly. Then 5 ml. of $0.200M$ tetramethylammonium iodide is added, and the mixture is diluted to 50 ml. An aliquot of this diluted sample is placed in the electrolysis vessel in a constant temperature bath at 25.0 ± 0.1° C. After 10 minutes the diffusion current is determined manually by reading the current at two predetermined voltages. The concentration of acrylonitrile in the sample is calculated by applying the values for diffusion current and potassium ion concentration to the proper equation derived from the calibration studies.

DISCUSSION

Graphical methods may be employed for the determination of acrylonitrile concentration in the presence of known amounts of potassium ion by applying the values for diffusion current to suitable calibration curves. However, better results are obtained by developing an equation which relates the three variables. In the present investigation the results of 93 calibration analyses were examined by statistical procedures. The method of multiple linear regression analysis was employed, since the available theory suggested that the data should conform to the underlying hypotheses of this method. In the derivation which follows, the statistical symbols conform to the usage of Goulden (*5*).

Let Y = an observed value of diffusion current (measured as millimeters wave height)
X_1 = potassium ion concentration ($M \times 10^5$) at which Y was observed
X_2 = acrylonitrile concentration ($M \times 10^4$) at which Y was observed
Y_o = true mean of all possible Y's
Y_c = calculated value of Y_o

then the true relationship will be

$$Y_o = A + B_1X_1 + B_2X_2$$

and the best estimate will be

$$Y_c = a + b_1X_1 + b_2X_2$$

From the data of the present studies the following expression was obtained by procedures detailed in Goulden (*5*):

$$Y_c = 0.301 + 0.721\ X_1 + 11.84\ X_2$$

Statistics appropriate to gage the validity and reliability of this equation are the following: the coefficient of multiple determination, $R^2_{Y.12} = 0.9971$; the partial coefficients of determination, $r^2_{Y1.2} = 0.9893$, and $r^2_{Y2.1} = 0.9969$. These values support the idea that the proposed equation does in fact represent the relationship. The variance of estimate, $s^2_{Y.12} = 0.002346$, is added evidence that departures from the linearity and independence implied by the equation are slight.

The statistical principles employed in the derivation demanded that the equation of the surface be obtained with X_1 and X_2 as independent variables. However, when determining acrylonitrile it is desirable that values of X_2 may be estimated from observed values of Y and X_1. The proper equation was obtained by algebraic transformation:

$$X_2 = 0.0845\ Y - 0.0609\ X_1 - 0.0254$$

It must be emphasized that the specific equations derived here apply only to the particular instrument and experimental conditions employed in the current investigation. The quantity of data to be compiled by other workers for the derivation of similar calibration equations should be governed by the degree of reliability desired.

This method of analysis is being used in this laboratory for the determination of acrylonitrile in the presence of a known concentration of potassium persulfate, along with the various oxidation products of acrylonitrile mentioned earlier. The precision of the method compares favorably with the usual precision of polarographic analyses. For 31 sets of duplicate observations of diffusion-current values in the range of 80 to 130 (expressed as millimeters wave height), the variance of measurement was 0.2337.

The method should be applicable to polymerization systems containing acrylonitrile and potassium persulfate initiator. It should also be suitable for solutions containing methacrylonitrile instead of acrylonitrile, since the half-wave potentials of these two compounds differ only by about 50 mv. (*11*). As sodium, rubidium, and cesium ions exhibit half-wave potentials very close to potassium ion (*7*) it should be possible to determine acrylonitrile or methacrylonitrile in the presence of any one of these ions when the concentration of the alkali metal ion is known.

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Polarographic Behavior of Monohaloacetones

Analysis of Mixtures

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Iodoacetone, bromoacetone, and chloroacetone each give one polarographic wave in the pH range 1.5 to 10. Rapid hydrolysis of the compounds prevents measurement at higher pH. The reductions are pH-independent, irreversible, and diffusion-controlled; a two-electron transfer is indicated for the reactions resulting in each wave, with the final product being acetone, which is nonreducible in the potential range investigated under the conditions used. The polarographic behavior of the three compounds is summarized and compared with respect to the factors of pH, ionic strength, buffer component nature and concentration, ketone concentration, temperature, and drop time. The factors involved in the simultaneous analysis by polarographic measurement of mixtures of the three haloacetones with satisfactory precision are discussed. Sodium acetate–acetic acid buffer (pH 4.6, ionic strength 0.5) is recommended as the preferable medium for such an electrolysis. Within the allowable limits of concentration, mixtures of all three ketones can be analyzed with an error of less than 2 relative %.

THE polarographic behavior of the three monohalogenated acetones—iodoacetone, bromoacetone, and chloroacetone—has been investigated in order to determine the relationship of half-wave potential, $E_{1/2}$, and diffusion current, i_d, to pH, ionic strength and buffer concentration effects, the probable nature of the reduction, and the extent of applicability of polarography as a method of analysis for such compounds, individually and in mixture.

The ketones have been studied polarographically in unbuffered solution by Winkel and Proske (*16*), who gave reduction potentials and claimed that the introduction of halogen made possible reduction of the carbonyl group, which in acetone itself is not reducible polarographically. Pasternak and Halban (*12*), without giving experimental conditions or data, stated that benzyl bromide and chloride give waves of pH-independent $E_{1/2}$ resulting from two-electron processes. On the basis of this pH independence, they surmised that the halogen is being reduced in the haloacetones and not the carbonyl group. The present investigation serves to substantiate the latter opinion in regard to the fundamental feature of the haloacetone reduction.

EXPERIMENTAL

Apparatus. Sargent Model XII and XXI polarographs and a Leeds and Northrup Type E Electrochemograph were used. Potential measurements with the Sargent instruments were checked with a potentiometer. Beckman Model G and H-2 pH meters were used for pH measurement; a Type E electrode was used above pH 10. Capillaries used for the dropping mercury electrodes were prepared from Corning marine barometer tubing; *m* values (open circuit in distilled water) were: at 25° C., 1.02 mg. per second at 60 cm. of mercury and 1.58 mg. per second (90 cm.); at 25° C., 1.39 mg. per second (45 cm.); at 0° C., 1.09 mg. per second (55 cm.). Jacketed H-type polarographic cells (*7*) containing reference saturated calomel electrodes were used; water at 25.0° ± 0.1° or 0.0° ± 0.1° C. was circulated through the jacket. Resistance of the cell solution system was measured with a General Radio Co. Type 650-A impedance bridge. All potentials are reported *vs.* the saturated calomel electrode and are corrected for internal resistance drops.

Reagents. Iodoacetone was prepared from Eastman Kodak practical grade chloroacetone by an exchange reaction with potassium iodide in 95% ethyl alcohol solution. Vacuum distillation of the reaction mixture after removal of the bulk of the solvent yielded a product with the following physical properties: boiling point (4 mm.) 45° C., d_4^{25} 2.0324 [literature values (*2*,

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Table I. Composition of Buffer Solutions[a]

Number	pH	Components
1	1.5	0.45M KCl + HCl
2	4.6	0.50M NaOAc + HOAc
2a	4.6	0.10M NaOAc + HOAc
3	7.0	0.27M KH_2PO_4 + NaOH
4	8.2	0.18M KH_2PO_4 + NaOH
5	8.2	0.50M NH_4Cl + NH_3
5a	8.2	0.10M NH_4Cl + NH_3
6	8.8	0.50M NH_4Cl + NH_3
7	9.5	0.50M NH_4Cl + NH_3
8	10.5	0.16M Na_2HPO_4 + NaOH
9	11.5	0.14M Na_2HPO_4 + NaOH
10	12.5	0.10M Na_2HPO_4 + NaOH
11	12.6	0.45M KCl + NaOH

[a] Nonbuffered background solutions were prepared using NH_4Cl, LiCl, and KCl as electrolyte. Their pH was between 4.6 and 5.0 when chloroacetone was present, between 3.3 and 3.6 for bromoacetone, and between 3.5 and 3.8 for iodoacetone.

13): boiling point (11 mm.) 58.4° C., d^{15} 2.17]. Bromoacetone (Jasonols Chemical Corp. and the Delta Chemical Works) was vacuum distilled: boiling point (13 mm.) 38° C., d_4^{25} 1.6132 [literature (*3*, *8*): boiling point (8 mm.) 31.5° C., d^{23} 1.634]. Chloroacetone was prepared from pure acetyl chloride by reaction with diazomethane and dry hydrogen chloride in ether solution (*15*); distillation of the reaction mixture yielded a product of boiling point (736 mm.) 118–9° C., d_4^{25} 1.1260 [literature (*1*): boiling point 118–20° C., d_4^{25} 1.123].

Stock aqueous solutions of the ketones (approximately 5mM) were relatively stable to hydrolysis over periods of 3 or 4 hours when prepared by the initial dissolution of the ketone in a minimal quantity of pure acetone before dilution with water, and when stored out of contact with light; the acetone concentration in each stock solution was not greater than 10mM. Acetone in cell solution concentrations up to 50mM was polarographically nonreducible under all conditions used in the investigation.

Buffer solutions (Table I) were prepared by initial dilution to within a few milliliters of the desired final volume of calculated amounts of analytical reagent grade chemicals, plus sufficient potassium chloride, where necessary, to give the desired ionic strength. The solution pH was then adjusted to the desired value by careful addition of buffer components, followed by dilution to the final volume. Ionic strength values were calculated in the usual manner, using the ionization constants listed in the literature.

Nitrogen used for deoxygenating was purified by bubbling it through concentrated sulfuric acid, alkaline pyrogallol, distilled water and, finally, a portion of the test solution being investigated.

Procedure for Polarographic Examination. The test solution was prepared by mixing an accurately measured volume of stock standard ketone solution with the buffered or nonbuffered background solution. The test solution in the H-cell was purged for 5 minutes with nitrogen and then electrolyzed; a nitrogen atmosphere was maintained above the solution throughout the electrolysis. Values of t, the drop life, determined for the limiting current portions of each polarogram, were used to correct i_d values for the effect of the electrocapillary curve where necessary.

POLAROGRAPHIC BEHAVIOR

Each ketone gave one well defined cathodic wave in the pH range covered, which was 4.6 to 10.5 for chloroacetone and 1.5 to 9.5 for bromo- and iodoacetone. No wave was found at higher pH because of hydrolysis of the compounds, probably to hydroxyacetone which is not reducible in the potential range investigated, although Winkel and Proske (*17*) reported it to be polarographically reduced at approximately −1.8 volts. Measurements in the vicinity of this potential could not be made because of the concurrent or prior discharge of hydrogen ion or the cation of the background electrolyte. No chloroacetone wave was found at pH 1.5 because of the confluence of the wave with a water wave (*11*) and subsequent hydrogen ion discharge.

The n value (number of electrons transferred per molecule of haloketone reacted) approximates 2 over the entire pH range for all three ketones. Values of D were estimated by comparison of the molecular volumes of the haloacetones with those of the corresponding haloacetic acids as being 1.2×10^{-5}, 1.2×10^{-5}, and 1.1×10^{-5} for chloro-, bromo-, and iodoacetone, respectively; the corresponding n values, based on i_d at various experimental conditions and calculated from the Ilkovič equation, are 1.5 ± 0.2, 1.7 ± 0.2, and 1.7 ± 0.2. (These values are, of course, open to considerable error because of the method and assumptions made in estimating D values.)

Delayed maxima were observed in the neighborhood of -0.5 volt at higher concentrations of iodoacetone—i.e., in all cases the maximum in question appeared approximately 0.2 to 0.3 volt beyond the point where the limiting current was first reached. No attempt was made to suppress these maxima, since in no case did their presence affect the wave itself (this was carefully checked) or interfere with the measurement of the limiting current; they did not appear in polarograms of mixtures. The maxima resembled a small water wave, but did not occur in the right location for a water wave and were obviously related to the concentration of iodoacetone, being most sharply defined with the higher concentrations of iodoacetone [see (*10*) for a discussion of similar maxima]. As the concentration was reduced from 0.5 to 0.3mM, the height of the maximum diminished until, at the latter concentration, it was just barely in evidence. In general, the maxima were greatest in the acid region, although present throughout the pH range. Similar maxima have been encountered with iodoacetic acid (*5*). At concentrations of bromoacetone greater than about 1mM maxima developed, which were greatest in magnitude in the acid region.

Current-Controlling Processes. Ratios of the currents at different drop times and at different temperatures were of the magnitudes expected for diffusion-controlled processes. The current ratios at two drop times—i.e., heads of mercury—in each of buffers 1 to 7 were 1.24 ± 0.04, 1.24 ± 0.02, and 1.28 ± 0.02 compared to calculated values of 1.00 for a kinetic-controlled process, 1.23 for diffusion control, and 1.52 for adsorption control; these are based on h values of 60 and 90 cm. where the back-pressure corrections were 1.8 and 1.7 cm., respectively. The percentage temperature coefficients for the current change between 0° and 25° C. in buffers 2 and 5 were 1.5 and 1.5, 2.2 and 2.0, and 2.5 and 2.2 for the chloro-, bromo-, and iodoketones, respectively; temperature coefficients were calculated by the usual compound interest formula. For all three ketones, $E_{1/2}$ values at 0° C. were slightly more negative than those at 25° C.—e.g., 0.02, 0.05, and 0.03 volt for the chloro-, bromo-, and iodoketones, respectively.

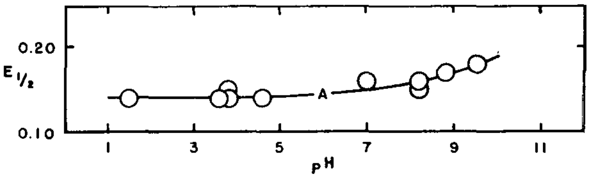


Figure 1. $E_{1/2}$-pH relationship for iodoacetone at 25° C. and ionic strength 0.5

Variation of $E_{1/2}$ with pH. The $E_{1/2}$-pH relation for each ketone is a straight line, indicating pH independence of the reduction process over the pH range investigated; $E_{1/2}$ is −0.14 volt for iodo-, −0.35 for bromo-, and −1.15 for chloroacetone. There are, however, two significant exceptions. At pH 8.8 and 9.5, $E_{1/2}$ for iodoacetone (Figure 1) becomes more negative by approximately 0.03 volt per pH unit; these values have been checked repeatedly. Such behavior may indicate interaction between the electroactive species and ammonia, as the ammonia concentration in the buffer increases rapidly from pH 8.2 to 9.5 and at the latter pH is approximately 0.8M. If the interaction product were more difficult to reduce than the noninteracted ketone, the change in $E_{1/2}$ might reflect the extent of interaction. Imine formation which has been postulated for ketones in ammonia solution would probably result in a species possessing two

Table II. Polarographic Analysis of Mixtures of Three Monohaloacetones in Acetate Buffer of pH 4.6 and Ionic Strength 0.5

Mixture No.	Chloroacetone Taken, mmole	Chloroacetone i_d μa.	Chloroacetone Found[a], mmole	Chloroacetone Error, %	Bromoacetone Taken, mmole	Bromoacetone i_d μa.	Bromoacetone Found[a], mmole	Bromoacetone Error, %	Iodoacetone Taken, mmole	Iodoacetone i_d μa.	Iodoacetone Found[a], mmole	Iodoacetone Error, %
1	0.89	3.90	0.88	1.1	0.58	2.96	0.58	0.0	0.65	3.24	0.65	0.0
2	0.92	4.03	0.91	1.0	0.59	2.99	0.59	0.0	0.62	3.07	0.62	0.0
3	0.181	0.78	0.18	0.6	0.121	0.62	0.12	0.9	0.123	0.60	0.12	0.6
A	0.237	1.03	0.23	3.3	0.59	2.99	0.59	0.0	0.62	3.09	0.62	0.0
A′[b]	0.237	1.03	0.23	3.3	0.58	3.03	0.59	1.7	0.65	3.19	0.64	1.5
B	0.92	3.95	0.90	2.0	0.121	0.61	0.12	0.9	0.62	2.93	0.59	4.8
B′	0.89	3.92	0.89	0.0	0.121	0.59	0.12	0.9	0.65	3.06	0.61	6.1
C	0.92	4.12	0.93	1.0	0.59	2.92	0.57	3.4	0.122	0.62	0.12	1.6
C′	0.89	4.01	0.91	2.2	0.58	2.85	0.56	3.4	0.131	0.63	0.13	0.8
D	0.096	0.411	0.09	6.7	0.121	0.61	0.12	0.9	0.250	1.27	0.26	4.0
D′	0.181	0.79	0.18	0.6	0.121	0.61	0.12	0.9	0.131	0.64	0.13	0.8
E	0.181	0.78	0.18	0.6	0.121	0.59	0.12	0.9	0.122	0.60	0.12	1.6
	Average			1.4				1.2				1.8

[a] Values based on diffusion current constants found in pure solutions of individual ketones at 0.5mM concentration level: 2.94 for chloro-, 3.29 for bromo-, and 3.26 for iodoacetone.

[b] Polarograms designated by primes shown in Figure 5.

reducible groups, although the imine reduction wave may be masked by the background electrolyte discharge. Unfortunately, the rapid hydrolysis of iodoacetone in this pH region prevents investigation at higher pH values to determine whether the change in $E_{1/2}$ with pH is a true pH dependency or the result of an interaction with the buffer components.

Perhaps a more probable explanation for the apparent pH dependency is that $E_{1/2}$ appears to shift, because of geometrical interference of the crest of the chloride wave with the foot of the iodoacetone wave, which immediately follows this chloride wave. An analogous case is that of the effect of the iodoacetone wave on the bromoacetone wave in mixtures of the three ketones; for mixture 1 of Table II, $E_{1/2}$ for bromoacetone was approximately 0.05 volt more negative than when measured in a solution containing bromoacetone only, whereas $E_{1/2}$ for iodoacetone in the same mixture becomes more positive by 0.02 volt.

$E_{1/2}$ values for bromoacetone determined in phosphate buffer (pH 7.0 and 8.2) were approximately 0.08 volt more positive than $E_{1/2}$ determined in other buffers of corresponding pH. This effect is associated with the presence of phosphate. As the ionic strength of the medium is increased by adding potassium chloride, this "phosphate effect" is diminished until, at an ionic strength of 2.0, $E_{1/2}$ values in the phosphate buffers agree with those determined in other buffers at the same ionic strength. Such behavior was not observed with chloroacetone, but is evident to a slight degree with iodoacetone. In the latter case, $E_{1/2}$ values in phosphate buffer at pH 7 and 8 are slightly more negative than the corresponding values in other buffers.

A possible explanation, based on interaction of phosphate with ketone to form a more easily reduced species, is not supported by the experimental data, inasmuch as the effect appears to be independent of phosphate concentration. In addition, such an interacted species would necessarily have to show behavior with increased ionic strength, which is practically identical with that of the noninteracted form, in order to produce the $E_{1/2}$ values obtained at ionic strength 2.0.

Variation of Diffusion Current with pH. The diffusion current constant ($I = i_d/Cm^{2/3}t^{1/6}$) for chloroacetone decreases by about 6% from pH 4.6 to 8.2, above which it decreases rapidly to 0 at pH 11.5 as the rate of hydrolysis increases with increasing alkalinity of the solution (Figure 2). Ammonia buffers give I values about 10% greater than do other buffers with a similar tendency to decrease as pH increases.

Bromoacetone I values decrease about 15% as pH increases up to 8.8, and then decrease rapidly to 0 at pH 10.5 because of increased hydrolysis rate. Buffer 5 (pH 8.2) produced a greater value for I than all other buffers investigated except buffer 1.

I for iodoacetone increases slightly from pH 1.5 to 4.6 and sharply (about 10% up to pH 8.2, after which it drops rapidly to 0 at pH 10.5 because of hydrolysis. Values in nonbuffered solutions are approximately 5% greater than those in buffered solutions in the same pH region.

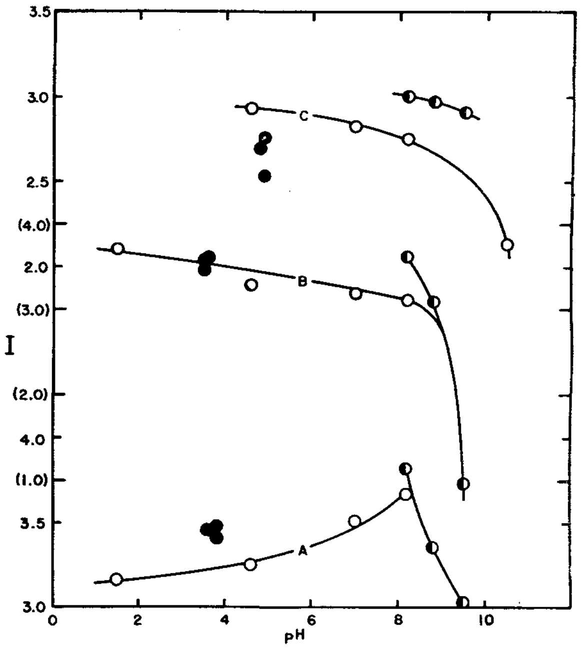


Figure 2. Effect of pH on diffusion current constant values for monohaloacetones at 25° C. and ionic strength 0.5

A. Iodoacetone
B. Bromoacetone (I scale in parentheses)
C. Chloroacetone
● Nonbuffered solutions
◐ Solutions buffered with ammonia buffers

Effect of Ketone Concentration. The variation of I with concentration is rather unexpected (Figure 3). I for iodoacetone is relatively constant for each buffer over the concentration range investigated. In the case of bromoacetone, I is constant in buffers 1 and 5, but decreases appreciably with decreasing bromoacetone concentration in the other buffers. With chloroacetone, I decreases appreciably with decreasing concentration for buffers 2 and 5; with all other buffers a slight decrease is observed from 0.50 to 0.25mM chloroacetone, followed by a relatively sharp increase when the concentration is further reduced to 0.10mM.

With all the buffers used, increasing the ketone concentration causes $E_{1/2}$ to become slightly more negative, approximately 0.02 volt. This effect is probably a result of the irreversible character of the reduction process and the accompanying unsymmetrical nature of the waves obtained. Such behavior did not occur in the analysis of mixtures of the three compounds,

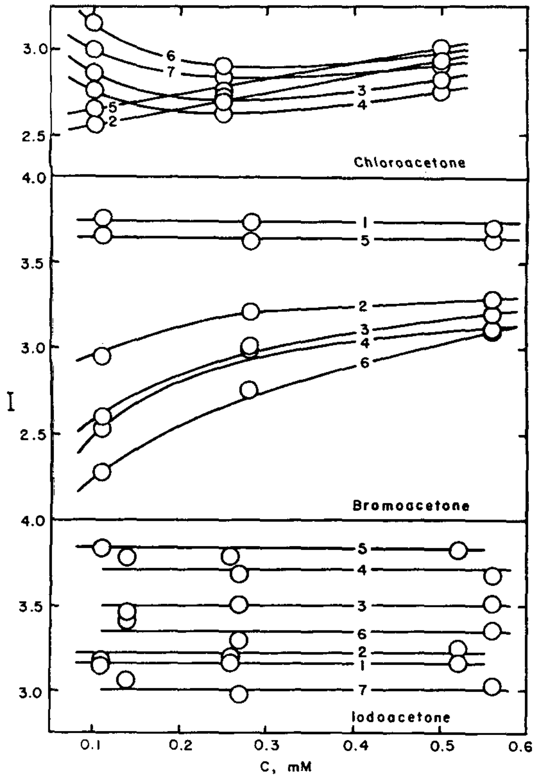


Figure 3. Relation of diffusion current constant values to concentration of monohaloacetones at 25° C. and ionic strength 0.5

Numbers refer to buffer solutions as designated in Table I.

where unsymmetrical wave shapes could be more easily accounted for—i.e., the limiting current portion of one wave becomes the introductory portion of the following wave, etc. (Table II).

Effect of Ionic Strength. Increasing the ionic strength of the solution renders chloroacetone more easily reducible throughout the pH range investigated (Figure 4), while the I values, in general, tend to increase slightly; similar behavior is observed in nonbuffered solutions where the ionic strength was varied by addition of the background electrolyte. These effects are the reverse of the behavior of bromoacetone under similar conditions and are more pronounced than in the case of iodoacetone. The effect of lower buffer component concentration is slight but definite. With buffers 2a and 5a, $E_{1/2}$ for chloroacetone is approximately 0.01 volt more negative throughout the range of ionic strengths studied than with buffers 2 and 5. I values for buffer 2a are slightly greater than those for buffer 2, whereas they are of about the same magnitude for buffers 5 and 5a. There is no significant change with increasing ionic strength in α, determined as being 0.48 ± 0.04 from the slope of the wave ($E_{1/4} - E_{3/4} = 0.056/\alpha$ at 25° C.).

Increase in ionic strength in both buffered and nonbuffered solution causes $E_{1/2}$ for bromoacetone to become more negative, whereas I generally decreases. In buffers 2a, 3, and 4, I increases slightly with increasing ionic strength. Changes in buffer component concentration have little effect with buffers 2 and 5, indicating little if any interaction between reducible species and buffer components. There is no significant change in α (0.70 ± 0.05) with increasing ionic strength except for phosphate buffers 3 and 4, where α at ionic strength 0.5 is appreciably smaller (0.56) than with any other buffer at the same ionic strength. Such behavior may account, in part, for the discrepancy in $E_{1/2}$ observed with these buffers at low ionic strength.

Increase in ionic strength of the medium has no appreciable effect on $E_{1/2}$ of iodoacetone, especially in the region of ionic strength 0.5 to 2.0. The tendency at lower ionic strength is for the reduction to become less difficult with increasing ionic strength. I increases slightly as ionic strength is increased except in nonbuffered solution, where the reverse is true. The effect of decreased buffer component concentration is noticeable; $E_{1/2}$ is about 0.02 volt more negative in buffer 2a than in buffer 2, whereas the average I values are about 20% greater over the ionic strength range investigated. Such behavior may indicate interaction between active species and buffer component. Specifically, in buffer systems with low buffer component concentrations, the postulated interaction might remove enough of the reacting buffer component (presumably the acid or base) so that the background electrolyte becomes essentially the salt component. Under such circumstances, the resulting behavior might well resemble that of a nonbuffered system, especially in view of the relatively small amount of ammonia or acetic acid needed to give the required pH for a 0.1M buffer. The fact that the $E_{1/2}$ values in the lower buffer component concentration runs were somewhat more negative than in nonbuffered runs at approximately the same pH lends some credence to this theory. Smaller buffer component concentration and increasing potassium chloride concentration enhance the possibility of behavior of the type indicated. Further support for the postulated behavior is afforded by the α values for the wave in buffer 2a (1.25 ± 0.12), which agree almost exactly with those for nonbuffered solution (1.19 ± 0.08), whereas corresponding values in buffers other than 2a are considerably smaller (0.88 ± 0.13). $E_{1/2}$ values in buffer 5a are about 0.03 volt more negative than those in buffer 5, whereas I values are of the same order of magnitude.

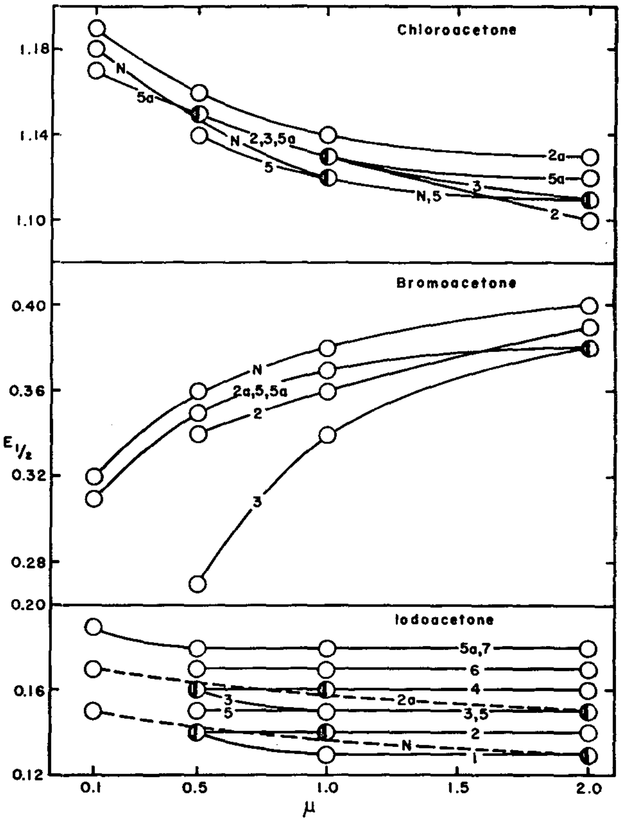
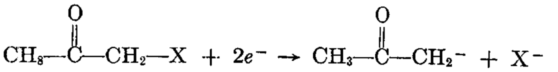


Figure 4. Effect of ionic strength and buffer component concentration of half-wave potential of monohaloacetones at 25° C.

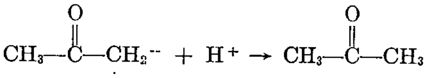
Numbers refer to buffer solutions as designated in Table I; N refers to nonbuffered ammonium chloride solution; and half-shaded points are common to two or more curves.

Reduction Mechanism. On the basis of the observed polarographic behavior, the reduction of a monohaloacetone at the dropping mercury electrode is an irreversible, pH-independent, diffusion-controlled process involving the fission of the carbon-halogen bond with acetone as the reduction product. This conclusion is substantiated by the similarity to the polarographic reduction of other halogen-containing carbon compounds (*4*), as well as by the electrolytic reduction of chloroacetone to acetone in hydrochloric acid solution with graphite or lead electrodes (*14*) and the chemical reduction of chloroacetone to acetone by zinc and hydrochloric acid (*9*). The reduction process probably involves fission of the carbon-halogen bond to form a carbanion:



$$CH_3-\overset{\overset{O}{\|}}{C}-CH_2-X + 2e^- \rightarrow CH_3-\overset{\overset{O}{\|}}{C}-CH_2^- + X^- \quad (1)$$

followed by the rapid combination of this species with hydrogen ion from the solvent:



$$CH_3-\overset{\overset{O}{\|}}{C}-CH_2^- + H^+ \rightarrow CH_3-\overset{\overset{O}{\|}}{C}-CH_3 \quad (2)$$

This mechanism accounts for the pH independence of the reduction processes, since Reaction 1 is the slow, potential-determining step with the electrons acting as a displacement reagent. (It is immaterial whether the electrons add to the carbon atom simultaneously or in rapid succession and whether Equation 2 involves H^+ or H_2O.) The two principal energy steps are the rearward approach of the electrons to the alpha carbon and the simultaneous fission of the carbon-halogen bond. The formation of the carbon-hydrogen bond (Equation 2) does not figure in the controlling electrode reaction—i.e., the reduction is pH independent. Increasing the electron density on the alpha carbon increases the energy required for the first phase of the reaction. The electron density at the alpha carbon is determined by the substituent halogen; therefore, the relative difficulty of reduction should be: chloroacetone>bromoacetone>iodoacetone; the experimental results verify this conclusion.

ANALYTICAL APPLICATIONS

Determination of Individual Haloacetone. The data cited on the $E_{1/2}$ and I behavior of the haloacetones serve to indicate the general feasibility of determining any of the monohaloacetones when present in admixture with other substances, some of which may be also polarographically reducible. Such data also indicate the critical conditions involved in developing a specific analytical procedure. In general, any of the buffers studied are satisfactory for analytical work involving the determination of iodoacetone, with buffers 2, 4, and 6 being preferred on the basis of minimum I variation with concentration changes and ionic strength effects. On a similar basis, buffers 1 and 5 are best suited for polarographic analytical work involving bromoacetone. None of the buffers investigated are ideal for analytical work with chloroacetone; on comparison of ionic strength effects and constancy of I values with ketone concentration change, buffers 3 and 4 would be most suitable.

Analysis of Mixtures of Haloacetones. As suggested by Winkel and Proske (*16*), it is possible to analyze mixtures of iodoacetone, bromoacetone, and choroacetone by the polarographic method. The major problem in the analysis of such a multicomponent mixture is the selection of a suitable background electrolyte for the electrolysis. It is desirable to choose a system in which the waves are sufficiently well defined for accurate measurement and in which the diffusion currents obtained are, if possible, directly proportional to concentrations of the electroactive species. In the case of the haloacetones, it was not possible to choose a medium in which both of these conditions would be perfectly met. The chloroacetone wave cannot be obtained in buffer 1 because of the hydrogen discharge preceding the desired wave; buffers 5, 6, and 7 are eliminated because of geometrical interference of the chloride wave of the buffers with the iodoacetone wave; and buffers 3 and 4 cannot be used since the more positive wave for bromoacetone in these media results in inadequate separation of the iodo- and bromoacetone waves. Thus, acetate buffer 2 is the only logical choice for use in simultaneous analysis of mixtures of the three compounds.

The acetate buffer used was prepared of such ionic strength as to produce a test solution of ionic strength $0.5M$ when mixed with the sample.

A number of mixtures of the three haloacetones were analyzed with the results shown in Table II and Figure 5. The concentrations of the three ketones used in mixture 1 are approximately the highest concentrations of the three ketones which do not result in maxima effects in the waves. The concentrations shown for mixtures 3 and E are approximately the minimum concentrations measurable without sacrifice in accuracy through interference of bromo- with iodo- or iodo- with bromoacetone waves.

The I values for each of the three waves in the mixtures remain relatively constant over the range of concentrations investigated; the average deviation of these values from the I value in $0.5mM$ solutions of the individual ketones is of the order of 2% or less; thus, the most important criterion for analytical application of the method is satisfied. Although a relatively large shift due to the geometry of the waves is observed for the $E_{1/2}$ of bromoacetone, no adverse effect is introduced with regard to the current measurements.

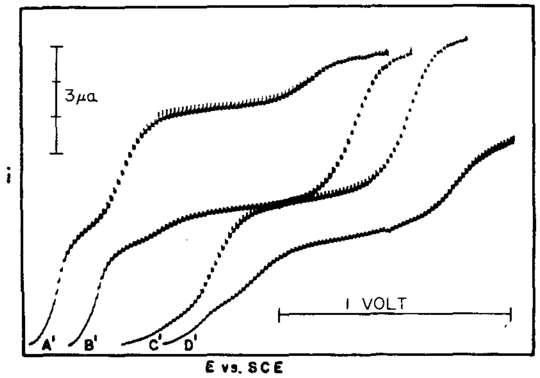


Figure 5. Polarograms obtained for various mixtures of monohaloacetones

Letters refer to mixture as designated in Table II.

Possible Interferences. Interference due to the possible presence of polarographically reducible compounds, other than the three ketones, in any solution for which haloketone analysis is desired, may be readily predicted from the $E_{1/2}$ values for such compounds. For example, iodoacetic acid and ethyl monobromoacetate would interfere whereas the bromo- and chloroacids, chloroesters, and chloroacetaldehyde would not. Acetaldehyde, the only polarographically reducible parent of any of the halogenated compounds listed, would not interfere since it does not give a polarographic wave in the acid pH region (*6*); in any case, the wave for acetaldehyde occurs at a much more negative potential (−1.7 volts) than the chloroacetone wave and the purging of the solution with nitrogen before electrolysis would probably remove the bulk of the aldehyde.

ANALYTICAL PROCEDURE

Measure out a sample containing not more than 10 mg. and not less than 2 mg. of each of the three ketones (iodo-, bromo-, and chloroacetone). Transfer the sample to a 100-ml. calibrated volumetric flask, add 70 ml. of $0.7M$ sodium acetate–acetic acid buffer (pH 4.6), and dilute to the mark with distilled water.

Rinse the cell and electrode several times with the solution to be analyzed. After purging for 5 minutes with nitrogen, electrolyze the solution over the potential range of 0 to −1.5 volts *vs.* the saturated calomel electrode, maintaining the nitrogen atmosphere above the solution. If the electrocapillary curve for the base solution is not known, note *t*, the drop time, at potentials of −0.2, −0.5, and −1.5 *vs.* S.C.E. Run a similar curve on the base solution and, if necessary, correct the sample curve for the latter curve.

Using the intercept method, determine the diffusion current for each of the three waves. Calculate the amount of each ketone present from its diffusion current constant, $C = i_d/Im^{2/3}t^{1/6}$, where C is the ketone concentration in millimoles per liter, i_d the measured diffusion current in microamperes, I the diffusion current constant for the ketone (Table II), m the mass of mercury in milligrams per second flowing from the capillary, and t the drop time in seconds measured at −0.2, −0.5, or −1.5 volts.

The weights or percentages of the three ketones present may then be calculated from the millimolar concentrations by conversion to grams and correcting for the dilutions involved.

ACKNOWLEDGMENT

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Polarographic Nitrate Determination

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A new polarographic wave has been found for the induced reduction of nitrate ion when present in a chromium(III)–glycine complex solution. The current measured from this wave is proportional to the nitrate ion concentration. Procedures have been developed for determining nitrate in the concentration range 1.0×10^{-5} to 2.5×10^{-3} mole per liter, with as good or better precision than the older polarographic methods for nitrate. The effects of gelatin, pH, and interfering ions on this reduction wave have been studied. The nitrite ion gives a similar reduction wave. As a result of the study of nitrate and nitrite reduction a rough calculation has been made of the number of electrons involved in the reduction processes.

A NUMBER of polarographic methods (*1, 4–7*) for the determination of nitrate by means of reduction induced in the presence of polyvalent cations have been described. The original observation of Tokuoka (*8*) and Tokuoka and Ruzicka (*9*) showed that the reduction potential of nitrate was shifted to more positive potentials when certain polyvalent cations were present. Lanthanum (*9*), uranyl (*5*), molybdate (*1, 4*), zirconyl (*6*), and cerium (*7, 9*) ions have been studied. Collat and Lingane (*2*) have recently made a more thorough study of the reduction products in the case of lanthanum, cerium, and uranyl induced reductions of nitrate and have reported that nitrate can be reduced directly from acid solutions.

In the course of a study of the complex ions of chromium(III) with glycine it was observed (*3*) that a chromium(III)–glycine complex, prepared by aging a 1 to 3 mixture, gave an induced nitrate reduction wave which had some unique properties that should make it especially valuable for the determination of nitrate. This paper is a description of the polarographic method which has been developed as the result of this new wave. Further work is in progress in this laboratory in an attempt to establish the nature of the processes that are responsible for the unusual shape of the wave; however, the present work has established a reproducible and highly sensitive method for the determination of nitrate or nitrite.

EXPERIMENTAL

Equipment. A Sargent, Model XXI, polarograph was used throughout this investigation. A Beckman, Model G, pH meter, calibrated with 0.05*M* potassium acid phthalate solution, was used for making all pH measurements.

Analyses were performed in an H-type cell which had a saturated calomel reference electrode in one side, the experimental solution in the other side, and a saturated potassium chloride–3% agar bridge in the cross member. This cell was suspended in a constant temperature bath which was maintained at 25.0° ± 0.1° C. for making all measurements. Dissolved oxygen was removed from the experimental solution by bubbling purified hydrogen through the cell for about 5 minutes before the recording of a polarogram.

An external potentiometer circuit was used for determination of the exact potential applied to the cell, where measurements of potential were taken.

The dropping mercury electrode used was a piece of marine barometer tubing with the characteristics, $m^{2/3}t^{1/6} = 1.65$ mg.$^{2/3}$ sec.$^{-1/2}$.

Reagents. All of the reagents used except the following were reagent grade chemicals.

Chromium perchlorate, hydrated $Cr(ClO_4)_3$, G. Frederick Smith Chemical Co.

Glycine, Eastman Kodak (white label).

Gelatin, Baker and Adamson.

A stock solution which was 0.100*M* in potassium nitrate was prepared from a dried and weighed sample of analytical reagent grade potassium nitrate. More dilute solutions were prepared from this by normal volumetric techniques. A stock solution of sodium nitrite which was approximately 0.10*M* in nitrite was prepared from reagent grade sodium nitrate (assay 98.8% $NaNO_2$). This solution was analyzed for nitrite by oxidizing with excess standard permanganate in acid solution and back-titration of the excess permanganate with standard ferrous solution. More dilute nitrite solutions were prepared from this solution by normal volumetric techniques.

Preparation of Complex. The chromium(III)-glycine complex used as a reagent in the nitrate determination was prepared by mixing 50 ml. of 0.1M perchloric acid solution, 50 ml. of 0.1M chromium(III) perchlorate solution, and 50 ml. of 0.3M glycine solution in a 500-ml. Erlenmeyer flask. The flask was placed in a boiling water bath and heated for 2 hours. During the heating period the violet hexaaquochromium solution gradually became wine-red in color. After cooling, the wine-red solution was neutralized with 0.5M sodium hydroxide solution to a pH of about 6. The resulting solution was diluted to 500 ml. This solution as normally prepared was clear, but occasionally a small amount of chromic hydroxide precipitate would form. If a precipitate formed it was ignored, since it was found to have no effect on the results obtained. A sample of complex prepared in this manner was found to be still completely effective after a period of 6 months.

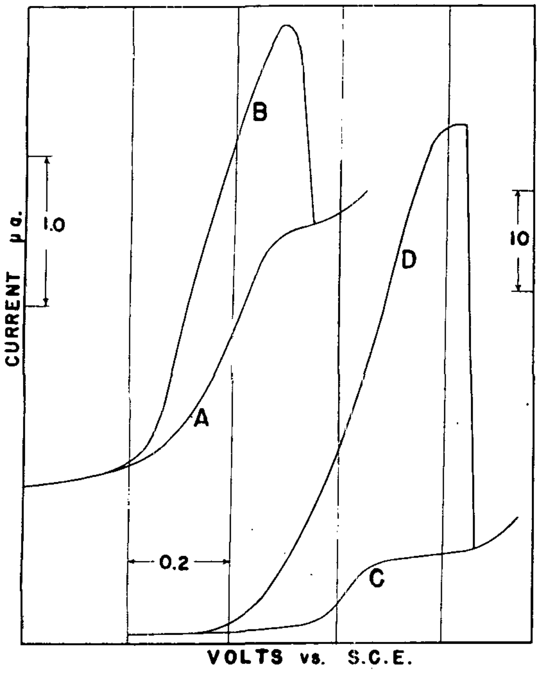

Figure 1. Polarograms with each curve starting at −1.2 volts

A. 0.5 × $10^{-3}M$ complex, no nitrate
B. Solution A with 4 × 10^{-4} moles per liter of nitrate
C. 2.5 × $10^{-3}M$ complex, no nitrate
D. Solution C with 1.6 × 10^{-3} moles per liter of nitrate

Procedure. Nitrate or nitrite was determined by adding an appropriate quantity (5 to 25 ml.) of the complex, prepared as above, 50 ml. of saturated potassium perchlorate solution, 0.4 ml. of 1% gelatin solution, and a pipetted sample of the nitrate or nitrite solution to a 100-ml. volumetric flask. The volume was adjusted to the mark, the solution was mixed, and the H-cell was rinsed thoroughly with this solution before filling. After placing the cell in the constant temperature bath and removing dissolved oxygen by bubbling hydrogen through the solution for about 5 minutes, a polarogram was recorded by scanning over the potential range −1.2 to −2.0 volts *vs.* saturated calomel electrode. The solution should have a pH between 3.4 and 7.5 for best results. The nitrate wave height was taken as the vertical distance from the peak of the maximum to the blank curve, or to the level where the current dropped. The latter was easy to determine even when no blank was run. For the reagents used in this investigation, it was found necessary to run and subtract a blank determination for low nitrate concentrations, because small amounts of nitrate were present in the reagents.

Table I. Variation of Nitrate Diffusion Current with pH

(In 1.0 × $10^{-3}M$ KNO_3, 2.5 × $10^{-3}M$ complex, 5 × $10^{-2}M$ $KClO_4$, and 0.004% gelatin)

pH	Microamperes
3.30	21.6
3.38	29.5
3.60	29.8
3.88	29.6
4.25	29.4
4.67	29.2
5.50	29.3
6.70	29.4
7.11	29.0
7.46	30.4
7.98	31.4
8.11	32.5

RESULTS AND DISCUSSION

The polarographic waves that resulted from solutions containing the chromium(III)-glycine complex described are shown in Figure 1. There are two sets of curves shown; *A* and *C* were from solutions which contained no nitrate, whereas *B* and *D* had nitrate added, 4.0 × 10^{-5} and 1.6 × 10^{-3} mole per liter, respectively. The complex concentration was 0.5 × 10^{-3} molar for *A* and *B* and 2.5 × 10^{-3} molar for *C* and *D*. The unique part of these waves is the way in which the current drops suddenly from the high value to a reduction current which is identical to the current given by a solution containing no nitrate. With the nitrate present the reduction wave obtained appears to be a maximum in the reduction wave from the chromium(III)-glycine complex alone; however, it differs from maxima in general in that its height is proportional to the nitrate ion concentration. A tentative explanation for this reduction wave may be proposed by assuming that the complex is reduced at the dropping mercury electrode to some intermediate unstable state, and that in some way an electron is transferred to the nitrate. Once the nitrate has accepted this electron, further reduction proceeds rapidly by a series of steps until the final reduction product or products are formed. At some more negative potential this process becomes competitive with the reduction of the complex to a stable state and the nitrate can no longer get the first electron to start its reduction.

Kolthoff, Harris, and Matsuyama (*5*) found that the potential at which a sudden current increase took place for nitrate reduction induced by lanthanum was a function of the direction of scanning the polarograms. In the present case, on the contrary, it was found that the potential at which the sudden current decrease occurred was essentially independent of the direction of scan. With a rate of voltage scan as low as possible, the difference in the potential at the sudden current change, for forward and backward scan, was found to be only 0.004 volt. This difference was more likely to be a lag in the recorder than a difference in the system. In all other ways the curves obtained appeared to be independent of the direction of voltage scan.

Effect of Gelatin. The gelatin was found to have a suppressing effect on both the blank and the maximum; however, without a small amount of added gelatin the maximum was difficult to measure because of irregularities. A small amount (0.004%) of gelatin was found to smooth out the wave and still cause very little supressing action. As much as 0.01% gelatin reduced the height of the complex wave (blank) from 5.4 to 4.4 μa. and reduced the height of the nitrate wave from 29.8 to 22.0 μa. For all of the determinations reported 0.004% gelatin was used.

Effect of pH. In order to study the necessity of carefully controlling the pH in making nitrate determinations, a series of samples was analyzed polarographically over a range of pH while all other variables were held constant. These runs are reported in Table I.

These results show that for any pH in the range 3.4 to 7.5, within experimental error, essentially constant results can be obtained for a given amount of nitrate, and careful buffering of the solutions for a nitrate determination is not necessary.

Nitrate Determination. The results of measuring the height of the maximum polarographic current at the point of change as a function of nitrate ion concentration are shown in Table II for two different values of the complex concentration. At complex concentrations in the range between these values similar results were obtained.

Table II. Nitrate Diffusion Current

(In 0.05M $KClO_4$ and 0.004% Gelatin Solution)

Complex, Mmoles/Liter	Nitrate, Mmoles/Liter	Diffusion Current, μa.	$k = i_d/C$, μa./Mmoles/Liter
2.5	0.100	3.04	30.4
	0.200	5.92	29.5
	0.400	11.9	29.8
	0.800	24.2	30.2
	1.40	42.7	29.5
	1.80	52.9	29.5
	2.40	67.9	28.3
	3.00	82.1	27.4
0.5	0.020	0.61	30.6
	0.040	1.25	31.4
	0.060	1.81	30.1
	0.080	2.35	29.4
	0.160	4.83	30.2
	0.260	7.72	29.7
	0.360	10.8	30.0
	0.460	13.6	29.5
	0.560	16.4	29.3
	0.660	18.7	27.4

Kolthoff, Harris, and Matsuyama (*5*) observed that it was necessary to have a certain minimum uranyl ion concentration in order to get a diffusion current proportional to the nitrate ion concentration. The results in Table II show when the nitrate to complex concentration ratio became greater than about one, the proportionality of wave height to concentration did not hold. At the other end of the scale it was found that when the nitrate to complex concentration ratio became less than 1/25 it became difficult to measure the curves with accuracy. For this reason the amount of complex used was decreased for low values of nitrate ion concentration.

Nitrite Determination. The nitrite ion gave similar polarographic waves when reduced in the presence of the chromium(III)-glycine complex. Table III lists the data obtained using this method for nitrite ion.

Table III. Nitrite Diffusion Current

(In 0.05M $KClO_4$, 2.5 × $10^{-3}M$ complex, and 0.04% gelatin)

Nitrite, Mmoles/Liter	Diffusion Current, μa.	$k = i_d/C$ μa./Mmoles/Liter
0.101	2.10	20.8
0.202	4.20	20.8
0.404	8.20	20.3
0.808	17.4	21.5
1.62	32.7	20.2

It may be assumed that the end products should be identical for the nitrate and the nitrite reductions. Since a single capillary was used under identical conditions, the ratio of the diffusion current constants should give the ratio of the number of electrons involved in the two cases. This ratio for nitrite to nitrate was 0.698. The ratio would be 0.75 if 6 and 8 electrons were involved, or 0.667 if 4 and 6 electrons were involved. This rough method of calculation indicates that in this induced nitrate reduction there is general agreement with the results of Collat and Lingane (*2*) for the uranyl and lanthanum induced reductions, where the actual end product was a mixture of substances, but in part was reduction to ammonia.

Interfering Ions. Previous work has indicated that the sulfate ion interferes with the lanthanum induced reduction of nitrate, and that 20 times or more excess of sulfate over nitrate interferes with the uranyl induced reduction of nitrate (*5*). The data given in Table IV show the list of substances and concentrations which have been checked for interference in the course of this investigation.

Chloride, sulfate, oxalate, and acetate may be present in concentrations many times that of the nitrate being determined, however, there was evidence that the highest chloride and sulfate concentrations investigated gave slightly low results because the current did not drop completely to the blank. This effect may have been caused by the high ionic strength of the solution. Phosphate and borate which reacted with the chromium-glycine complex to form more stable chromium complexes prevent this method from being effective. The values found for phosphate and borate are given in parentheses because the actual values found would depend upon the time between mixing and actually making the measurements, as the reactions between these ions and the chromium-glycine complex is a slow one. The presence of metals which are reduced at the dropping mercury electrode at potentials more positive than the potential at which nitrate reduction takes place would interfere if they were present in large amounts. Small amounts of these metals would only increase the currents by a small amount and would not seriously interfere.

Table IV. Interferences in Solutions Containing 1.00 × 10^{-3} M Nitrate

Salt Added	Mmoles/Liter	Nitrate Found, Mmoles/Liter
KCl	5.05	1.01
	29.9	1.02
	78.0	1.00
	159	0.97
K_2SO_4	19.3	1.00
	28.0	1.01
	55.2	1.00
	107	0.96
K_2HPO_4	0.20	(0.97)
	1.54	(0.79)
	6.92	None
$K_2C_2O_4$	11.0	1.02
$KC_2H_3O_2$	20.0	0.99
$Na_2B_4O_7$	0.50	(0.95)
	1.62	(0.61)

ACKNOWLEDGMENT

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Polarography of Carbonyl Compounds in Methanol

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The use of organic solvents in polarography has recently become of interest in the analysis of materials that have an inadequate water solubility, particularly organic compounds. The study confirmed the suitability of anhydrous methanol as the solvent for polarographic reductions of a variety of carbonyl compounds. Well defined waves with reproducible half-wave potentials and diffusion current constants were obtained for alpha and beta dicarbonyls and carbonyl compounds conjugated with a double bond. Wave heights proved to be greater in methanol than in water, as anticipated from the viscosity coefficients. However, in a series of methanol-water mixtures of varying composition the product, $i_d \times \eta^{1/2}$, was not constant. It was also demonstrated that cell resistances of methanolic solutions can be maintained at a sufficiently low level to prevent the formation of long drawn-out waves.

ORGANIC solvents have largely been avoided for polarographic procedures, because of conditions which do not compare favorably with the aqueous medium. The former present difficulties caused chiefly by their limited solvent ability for the electrolytes required in polarographic procedures, the high resistances of their solutions, and their relatively greater viscosities. Nevertheless, an organic solvent must frequently be employed where substances are otherwise insoluble.

Bachman and Astle (*3*) and Hala (*5*) obtained normal waves for several cations in glacial acetic acid. Gentry (*4*) reported similar results with ethylene glycol as solvent. The glycol ethers, particularly Cellosolve, were recommended by Parks and Hansen (*9*) in their description of a direct determination of tetraethyllead and naphthalene in gasoline.

A methanol-benzene mixture was employed by Lewis, Quackenbush, and De Vries (*7*) in polarographic studies of organic peroxides in rancid fats. A similar use of this mixed solvent was reported by Willets and coworkers (*13*) and Radin and De Vries (*10*). Other solvents investigated include acetone (*2*) and glycerol (*10*).

Polarographic procedures in absolute methanol have been briefly indicated for a few cations (*2*, *11*), dissolved oxygen gas (*6*, *12*), and some nitro compounds (*10*). The purpose of this investigation was to determine the behavior of methanol as solvent in polarographic reductions of organic compounds containing carbonyl groups.

EXPERIMENTAL

The organic compounds, Eastman Kodak White Label grade, were used as received with the exception of tetraethylammonium bromide, which was recrystallized from 90% ethyl alcohol until the polarogram showed no anomalous waves.

Reagent grade Baker and Adamson lithium chloride and methanol were found to be of sufficient purity. The mercury was redistilled.

Current-voltage curves were obtained with a Sargent-Heyrovský polarograph Model XII. The electrolytic cell consisted of a simple test tube, 1 inch in diameter and approximately 2 inches in length. This was fitted with a four-hole rubber stopper to provide for nitrogen inlet and outlet tubes and the dropping mercury cathode, and lead to the reference anode, a quiet pool of mercury. The temperature of the cell was maintained at 25° ± 0.2° C.

The dropping mercury electrode was cut from "marine barometer capillary tubing" made by Corning Glass Works. A 6-cm. segment, immersed in methanolic solutions, showed $m^{2/3}t^{1/6}$ values of 1.12 mg.$^{2/3}$ sec.$^{-1/2}$ in 0.3*M* lithium chloride and 1.10 mg.$^{2/3}$ sec.$^{-1/2}$ in 0.3*M* tetraethylammonium bromide. The capillary constants were determined at 25° C. with closed circuit at zero applied potential.

With the cell in operation, the tip of the capillary was at a distance of 15 ± 1 mm. from the mercury pool, which had a calculated area of 4.2 sq. cm. In this position resistance measurements of the electrolytic solutions were made with a Wheatstone bridge and oscillator. The values thus obtained for the methanolic solutions were 108 ohms for 0.3*M* lithium chloride and 72 ohms for 0.3*M* tetraethylammonium bromide.

The degassing procedure recommended by Arthur and Lyons (*2*) was followed. Oxygen was removed by vigorous bubbling of purified nitrogen through the solution in the cell prior to the addition of the mercury for the pool. A final minute of nitrogen flow was allowed before the exhaust outlet was closed and the polarogram recorded.

Viscosities were measured with an Ostwald viscometer and densities with a Westphal balance.

RESULTS AND DISCUSSION

Well defined waves were recorded for alpha- and beta-dicarbonyls and for simple carbonyl compounds conjugated with a double bond. The reproducibility of half-wave potentials and diffusion current constants was established for several concentrations in unbuffered solutions within the thousandth molar range (Tables I and II). As a basis of comparison some values from Adkins and Cox (*1*) are included in the tables. These values were obtained in a one to one mixture of water and ethyl alcohol with a mercury pool as the reference electrode. The supporting electrolytes were 0.2*M* tetramethylammonium hydroxide and 0.1*M*

Table I. Polarographic Studies in Anhydrous Methanol, 0.3*M* in Tetraethylammonium Bromide

	Concn., m*M*	$E^{1/2}$ (Volts) vs. Hg Pool		$\frac{i_d}{cm^{2/3}t^{1/6}}$
Cadmium chloride	3.00	−0.42		2.97
Acetophenone	3.25	−1.32	(−1.52)[a]	5.35
9,10-Phenanthraquinone	2.50	−0.13	(−0.4)[a]	5.97
Pyruvic acid	3.00	−1.80		0.76
n-Butyraldehyde	No wave below	−2.2	(−1.90)[a]	
Benzoin	3.00	−1.26		5.30
Benzil	3.00	(1) −0.53	(−0.7)[a]	5.09
		(2) −1.26	(−1.4)[a]	3.34
Acetylbenzoyl	3.00	−0.60		4.49
Diacetyl	3.00	−0.74		4.09
Dibenzoylmethane	3.00	(1) −1.02	(−1.60)[a]	1.46
		(2) −1.42		4.12
Benzoylacetone	3.00	(1) −1.22	(−1.80)[a]	2.49
		(2) −1.62		1.46
Acetylacetone	6.00	(1) −1.53		1.57
		(2) −1.90		0.50
Acetonylacetone	No wave below	−2.2		

[a] Adkins and Cox (*1*) 0.2*M* tetramethylammonium hydroxide in a 1 to 1 mixture of water and ethyl alcohol.

Table II. Polarographic Studies in Anhydrous Methanol, 0.3*M* in Lithium Chloride

	Concn., m*M*	$E^{1/2}$ (Volts) vs. Hg Pool		$\frac{i_d}{cm^{2/3}t^{1/6}}$
Cadmium chloride	3.00	−0.61		3.90
Acetophenone	3.25	−1.44	(−1.56)[a]	2.30
9,10-Phenanthraquinone	2.50	−0.38		4.50
Pyruvic acid	3.00	−1.51	(−1.14)[a]	1.46
n-Butyraldehyde	No wave below	−2.1		
Benzoin	3.00	−1.50		4.08
Benzil	3.00	(1) −0.71		5.21
		(2) −1.48		1.72
Diacetyl	3.00	−0.84	(1) (−0.93)[a]	3.51
			(2) (−1.68)[a]	

[a] Adkins and Cox (*1*) 0.1*M* NH_4Cl in a 1 to 1 mixture of water and ethyl alcohol.

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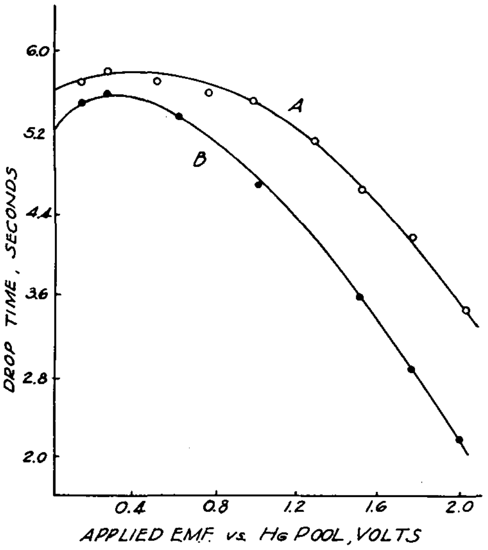

Figure 1. Electrocapillary curves in anhydrous methanol

A. 0.3M lithium chloride
B. 0.3M tetraethylammonium bromide

ammonium chloride, respectively. Maxima occurred only in the cases of benzoin and pyruvic acid. These were not suppressed by methyl red, but the wave heights were estimated successfully by utilization of the flat portions of the curves.

Only single waves were obtained for diacetyl and pyruvic acid in contrast to their behavior in buffered aqueous solutions. The attempt to elicit second waves in methanol by using a buffer mixture, lithium acetate–acetic acid, resulted in curves that rose sharply at about −1.4 volts, presumably because of the hydrogen discharge. Evidently the same limitation, as noted by Bachman and Astle (*3*) and Hala (*5*) for glacial acetic acid as solvent, applies to the use of acetic acid in methanol.

Neither *n*-butyraldehyde nor acetonylacetone showed waves below −2.2 volts, where the supporting electrolytes begin to decompose. Simple saturated carbonyl compounds exhibit extremely negative half-wave potentials in aqueous solutions, in which, however, quaternary ammonium salts permit a wide span of applied voltages. In this respect methanolic solutions of tetraethylammonium bromide (decomposition potential, −2.2 volts) showed no significant advantage over those of lithium chloride (decomposition potential, −2.1 volts). Moreover, lithium chloride displayed a more favorable electrocapillary curve (Figure 1).

The half-wave potentials of the alpha- and beta-dicarbonyls indicate the comparatively greater influence of the phenyl group as opposed to the methyl group on the ease of reduction of the carbonyl compound. Considering only first waves, when more than one occurs, progressively more negative potentials are demonstrated in the series: benzil (−0.53 volt), acetylbenzoyl (0.60 volt), and diacetyl (0.74 volt). The same effect is noted in the analogous beta dicarbonyls—namely, dibenzoylmethane (−1.02 volts), benzoylacetone (−1.22 volts), and acetylacetone (−1.52 volts). In another sense, the effect of increasing the separation of the carbonyl groups is reflected in the more negative potential of acetylacetone as compared to diacetyl and finally in the complete failure to reduce acetonylacetone.

No evaluations were made of the number of electrons involved in the various reductions. The case of benzil, however, where a second wave appears at the same half-wave potential as the single benzoin wave, indicates the reduction of benzil to benzoin as a first step.

Table III. Variation of Diffusion Currents of 0.005M Diacetyl in Methanol-Water Mixtures, 0.3M Lithium Chloride, with Viscosities

Wt. % Water[a]	i_d, μa.	η, Millipoises	$i_d \times \eta^{1/2}$
0.0	19.65	6.79	51.2
17.9	14.20	10.83	46.6
34.2	12.28	14.02	46.0
49.5	10.92	16.08	43.8
61.9	9.56	16.38	38.7
75.2	9.28	15.00	35.9
87.8	10.37	12.26	36.3
100.0	11.19	8.92	33.4

[a] Grams of water per 100 grams of methanol plus water.

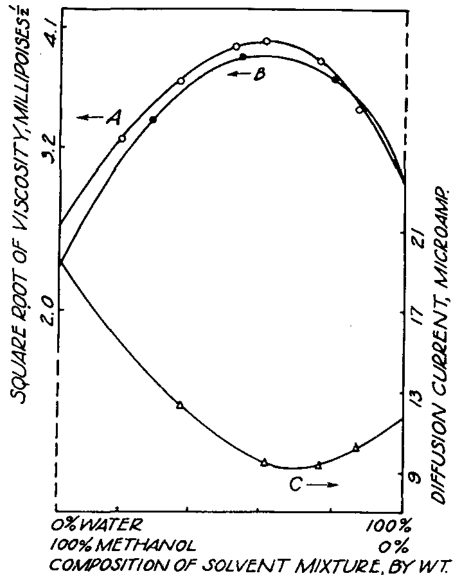

Figure 2. Relation of diffusion current of diacetyl, 0.005M in 0.3M lithium chloride to square root of viscosity of methanol-water mixtures

A. Lithium chloride solutions
B. Solvent mixture, alone
C. Diffusion current

The influence of viscosity on diffusion currents is anticipated by combining the Ilkovič and Stokes-Einstein equations. Grouping all constants into K, the relationship may be expressed as follows:

$$i_d = K\eta^{1/2}r^{1/2}$$

where r is the effective radius of the diffusing particle. With the same concentration of diacetyl in methanol-water mixtures of varying composition the diffusion current was greatest in anhydrous methanol, almost twice that in pure water. As Figure 2 indicates, the diffusion current drops as viscosity increases and reaches a minimum where the viscosity is at its maximum. However, the product, $i_d \times \eta^{1/2}$, is not constant, becoming lower with greater proportions of water (Table III). Presumably, the radius of the diffusing particle is altered because of the changes in degree or type of solvation or to complex formation as the methanol is replaced by water in the solvent mixture. This variance is in agreement with a polarographic study of the behavior of metallic cations in a series of ethyl alcohol–water mixtures (*8*).

High cell resistances during polarographic procedures are generally avoided. *IR* corrections must be applied to arrive at reproducible half-wave potentials. A more undesirable feature of a high resistance lies in the long drawn-out wave it causes. In extreme cases, the polarogram is difficult to evaluate and of doubtful analytical value. Methanolic solutions have favorable conductances as compared to other organic solvents. The semimicro cell arrangement utilized in this study, which provided for a large area of the mercury anode set at a short distance from the dropping mercury cathode and the exclusion of a liquid junction, resulted in low and negligible resistances. This was further manifested by the sharply defined waves with half-wave potentials that were reproducible without *IR* correction at different concentrations of electroactive material.

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Polarographic Determination of Small Amounts of Tungsten in Ores

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This work was undertaken as part of a research program on the recovery of tungsten from low-grade ores, which are tailings from a granitic ore that has been treated by flotation to recover molybdenite on a commercial basis. The tungsten minerals present are wolframite and hubnerite. Existing methods of tungsten analysis in ores containing 0.001 to 0.100% tungstic oxide proved to be too inaccurate and too time-consuming for this purpose. The procedure involves extracting interfering ions from the ore with concentrated nitric acid and extracting tungsten from the ore with concentrated hydrochloric acid. The tungsten is determined polarographically from a known volume of constant-boiling hydrochloric acid as the supporting electrolyte. Few ions produce polarographic waves that interfere with the tungsten wave, and those that do interfere are removed by the procedure employed here. The time necessary to complete one analysis is about 6 hours, but on a routine basis averages less than 1 hour.

THE regular gravimetric determinations of tungsten in ores containing from 0.001 to 0.100% tungstic oxide involve very long procedures that leave much to be desired in accuracy even when possible interfering ions are not present in the ore. The development of a polarographic or colorimetric procedure appeared to offer the best solution to the problem, although the possibility of a spectrographic, x-ray, or improved gravimetric method was not ruled out.

Most colorimetric procedures are based on the determination of tungsten by development of the yellowish green tungsten thiocyanate complex ion first proposed by Feigl and Krumholz (*3*). The tungsten cyanate complex ion is readily formed at a temperature of 90° to 95° C. followed by an isopropyl ether extraction of the complex ion from the cool aqueous solution. The difficulties, however, of applying this procedure to low grade tungsten ores are not easily overcome, since the absorption of light by the tungsten thiocyanate complex is dependent not only on variables commonly associated with this complex, such as temperature, acidity, organic acids, phosphoric acid, etc., but also on variables encountered in separating all of the small quantities of tungsten from the ore and removing all the possible interfering ions. However, a successful thiocyanate colorimetric determination for low grade tungsten ores has recently been perfected by the Climax Molybdenum Co.

The gravimetric procedure necessary to determine tungsten in these low grade ores employs a dilute hydrochloric acid or concentrated nitric acid extraction of the ore to remove interfering ions, then a concentrated hydrochloric acid extraction to place the tungsten in solution. After purification by precipitation of tungstic acid in the presence of cinchinone, the tungsten is weighed as lead tungstate.

Analytical possibilities using the polarograph have been applied to specific tungsten compounds and alloys with a great deal of success. The first contributions to the polarography of tungsten were made by a study of the tungstic heteropoly acids (*11–15, 17*) with some analytical applications (*15*). Pagotsky and Jofa (*9*) noticed the occurrence of free atomic hydrogen at the mercury cathode from solutions containing tungsten, and from this proposed a mechanism for the cathodic reduction of tungsten trioxide. The first study of the analytical determination of tungsten in alloys was made by Emerson (*2*) whose findings were related to molybdenum-tungsten and cobalt-molybdenum-tungsten steels. Also, von Stackelberg, Klinger, Koch, and Krath (*16*) developed a good polarographic analysis of tungsten in steel by precipitating tungstic acid, igniting it to the oxide, dissolving the oxide in a base, and then making the solution about 9*N* hydrochloric acid for a polarographic analysis.

The basic study of the polarography of tungsten has been made by Lingane and Small (*7*). Here, special attention is paid to the characteristics of the various oxidation states of tungsten. They found that in 4*N* hydrochloric acid, tungsten(VI) is reduced stepwise to the tungsten(V) and (III) oxidation states. The half-wave potential of the second wave is −0.66 volt *vs.* saturated calomel electrode (S.C.E.).

It has been observed that tungsten in the presence of peroxides gives a catalytic reduction wave (*4–6*). This wave is very large compared to the normal tungsten wave and would be ideal for analysis of very dilute solutions. The analytical chemistry of these catalytic waves, however, has not been completely worked out. A further study on the oxidation states of tungsten in hydrochloric acid has been made by Laitinen, Oldham, and Ziegler (*5*), who have proposed a mechanism of reduction at the dropping mercury electrode. Reichen (*10*) has developed a polarographic determination of tungsten in rocks by using a tungsten wave produced from the supporting electrolyte of dilute hydrochloric acid containing tartrate ion. The procedure is short and the accuracy good, although the concentration of tungsten in the ores must be high. Only iron and vanadium are removed in the preparation of the polarographic solution.

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Table I. Comparison of Polarographic and Gravimetric Determinations of Tungsten from Ores

Sample	% WO_3 Determined Gravimetrically	i_d (μa.)	i_d/C (μa./millimole)	% WO_3 Determined Polarographically[a]
1	0.004	0.253	4.40	0.0045
		0.245	4.26	0.0043
		0.245	4.26	0.0043
		0.253	4.40	0.0045
		0.249	4.33	0.0044
2	0.006, 0.009	0.514	4.75	0.0091
		0.514	4.75	0.0091
		0.510	4.71	0.0090
3	0.015, 0.012, 0.012	0.866	4.63	0.015
		0.855	4.57	0.015
		0.874	4.67	0.015
4	0.013, 0.014, 0.014	0.805	4.12	0.014
		0.805	4.12	0.014
5	0.015, 0.017	0.816	3.54	0.015
		0.831	3.60	0.015
6	0.015, 0.017	0.934	4.05	0.017
		0.923	4.00	0.016
		0.919	3.98	0.016
7	0.020, 0.023	1.22	3.94	0.022
		1.25	4.04	0.022
		1.23	3.96	0.022
		1.22	3.94	0.022
8	0.020, 0.025	1.26	3.91	0.022
		1.30	4.03	0.023
9	0.022, 0.024	1.28	4.23	0.023
		1.31	4.35	0.023
10	0.026, 0.025, 0.020, 0.020	1.50	4.54	0.027
	0.020, 0.026, 0.034, 0.019	1.74	5.25	0.031
	0.013, 0.023, 0.026, 0.023	1.66	5.01	0.029
	0.028, 0.021, 0.023, 0.020	1.66	5.01	0.029
11	0.021, 0.025	1.33	4.02	0.024
		1.33	4.02	0.024
12	0.023, 0.026	1.26	3.59	0.022
		1.28	3.62	0.023
		1.28	3.65	0.023
13	0.025, 0.026	1.37	3.75	0.024
		1.34	3.64	0.024
		1.34	3.64	0.024
		1.34	3.67	0.024
14	0.024, 0.028	1.51	4.04	0.027
		1.57	4.20	0.028
15	0.025, 0.029	1.45	3.73	0.026
		1.45	3.73	0.026
16	0.031, 0.034, 0.032, 0.028	1.81	4.44	0.032
	0.026, 0.027, 0.030, 0.028	1.82	4.45	0.032
	0.021, 0.020, 0.028, 0.031			
	0.029, 0.027, 0.033, 0.029			
17	0.028, 0.033	1.75	4.00	0.031
		1.78	4.06	0.032
18	0.056, 0.067	3.47	3.96	0.062
		3.51	3.97	0.062
19	0.139, 0.142, 0.147	6.59	3.11	0.117[b]

[a] Calculated from formula (*1*).

[b] This value is low because at this concentration the final solution has become saturated and tungstic acid has precipitated. Not used in the calculation of the average value of i_d/C.

APPARATUS AND REAGENTS

Polarographic data were obtained from a Leeds and Northrup Electro-Chemograph Type E, and H-type polarographic cell with a saturated calomel electrode. The cell was kept at a temperature of 25.0° ± 0.1° C. in a constant-temperature water bath. Dissolved oxygen was removed satisfactorily by bubbling nitrogen saturated with constant-boiling hydrochloric acid through the solution for 10 minutes before each polarographic determination. Oxygen was removed from the nitrogen stream by bubbling tank nitrogen through a vanadous sulfate solution (*8*). Characteristic polarographic properties of the capillary used were: drop time, 4.62 seconds per drop; *m* for capillary, 1.31 mg. per second; and 1.55 mg.$^{2/3}$ sec.$^{-1/2}$ for $m^{2/3}t^{1/6}$ at h = 63.3 cm. Constant-boiling hydrochloric acid was the supporting electrolyte used in all cases.

To prepare the constant-boiling hydrochloric acid 1000 ml. of hydrochloric acid (specific gravity, 1.19) were added to 850 ml. of distilled water and boiled for 0.5 hour.

The standard tungsten solutions were made from Baker's C.P. sodium tungstate and purified tungstic acid, which were standardized gravimetrically.

All other chemicals used were of reagent grade.

PROCEDURE

Ten grams of ore are extracted with 50 ml. of concentrated nitric acid by gently boiling in a tightly covered 600-ml. beaker for 1 hour. The solution is filtered with suction through a fine fritted-glass filter and the residue washed five times with hot water. The residue is removed from the fritted-glass filter and placed back in the original 600-ml. beaker, where it is extracted with 250 ml. of concentrated hydrochloric acid for 2 hours at a temperature of 80° C. The resulting solution is filtered through the same fritted-glass filter, and the residue is washed five times with 1 to 1 hydrochloric acid. The filtrate and washings are transferred to a 600-ml. beaker, and a few glass beads are added. This solution is boiled down to a volume of about 15 ml. The cover glass and sides of the beaker are washed down with about 10 ml. of constant-boiling hydrochloric acid. The volume is then made up to exactly 30 ml. in a calibrated graduated cylinder with more constant-boiling hydrochloric acid, and a polarogram is made of this solution over the range of −0.4 to −0.8 volt *vs.* S.C.E. The wave height is converted to microamperes and the per cent tungsten in the sample found from the equation

$$\% \ WO_3 = 0.167 \ i_d \ (\mu a.)/\text{sample weight (grams)} \qquad (1)$$

RESULTS AND DISCUSSION

Ten grams of ore appear to be about the correct weight of sample for the concentration range of 0.005 to 0.040% of tungstic oxide. Above 0.040% of tungstic oxide it is suggested that smaller weight samples be used, since it is at about this equivalent concentration that a precipitate of tungstic acid is formed from the final 30 ml. of constant-boiling hydrochloric acid solution. The smallest weight of sample is governed by the per cent of residue lost in the mechanical removal of the residue from the fritted-glass filter. For ores containing more than 0.04% tungstic oxide either a smaller weight sample may be taken or the filtered, concentrated hydrochloric acid solution is not evaporated all the way down to 15 ml. The final solution is then greater than the 30 ml. used here. For very low grade ores or with smaller weight samples a smaller polarographic cell may be used.

It was found necessary to use a fritted-glass filter in both filtrations, because any trace of carbohydrate material, or of its decomposition products in the final solution used in the polarographic analysis, produces a wave overlapping the tungsten wave. This wave was identified as being the result of the reduction of 5-hydroxymethylfurfural, which is a decomposition product of almost all acid-treated carbohydrate material. Not being able to use filter paper in the concentrated hydrochloric acid extraction of the ore means that more time is needed for this extraction. From a series of tests it was decided 2 hours was enough time. The per cent tungstic oxide calculated from several samples, in which only the heating time for the concentrated hydrochloric acid extraction was varied, indicated that practically all the tungsten had gone into solution in 1.5 hours.

By using a fritted-glass filter it is possible to stir the wash solution and residue thoroughly and thus increase the efficiency of the washings. However, when transferring the residue from the filter, a small amount is lost—this amount certainly being less than 0.1% of the total. There are two ways of removing the residue from the filter crucible. If the residue has had a chance to become fairly dry—e.g., standing overnight—it may be removed mechanically with a spatula and washed with a jet of water. Otherwise, it may be removed just by washing. Glass fiber filter paper may be used in the first extraction of the ore with nitric acid. This increases the ease of removal of the residue from the crucible; however, these residues cannot be stirred while washing with hot water; thus, the number of washings has to be increased.

By using a supporting electrolyte containing approximately 6*N* hydrochloric acid, few ions interfere with the tungsten wave. Over 30 of the more common cations were tested for possible interfering effects by adding small quantities of the cations in their various oxidation states to 100 ml. of constant-boiling hydrochloric acid and observing whether a polarographic wave was obtained in the region of the tungsten wave. Waves produced by nickel, vanadium, tin, lead, copper, arsenic, and molybdenum were found that were close enough to the tungsten wave to have possible interfering effects. Aluminum, silver, gold, boron, barium, bismuth, calcium, cadmium, cobalt, chromium, iron, mercury, magnesium, manganese, phosphorus, platinum, rhodium, antimony, strontium, titanium, uranium, zinc, and zirconium were not expected to interfere in this analysis and did not give waves close to the tungsten wave in the supporting electrolyte used here.

Nickel, vanadium, lead, copper, arsenic, and molybdenum are separated from tungsten by extracting the ore with concentrated nitric acid. Tin remains in the ore and is thus separated from the tungsten as it is not extracted by either concentrated nitric acid or concentrated hydrochloric acid. Small amounts of these interfering elements can be tolerated in the final solution used for polarographic analysis, although it is believed that the separation of these elements from the tungsten is quantitative. One tenth of a gram of each of the above-mentioned elements in their various oxidation states were mixed and added to a standard sodium tungstate sample. Using the procedure employed here for tungsten, a value of 4.09 was obtained for i_d/C for the tungsten wave, which indicated that these elements do not interfere.

The values of i_d/C for the tungsten ores containing 0.004 to 0.06% tungstic oxide show an excellent reproducibility for a given sample with an average value of 4.15 for the 49 determinations made in this concentration range (see Table I). The average value of i_d/C for 22 standard sodium tungstate solutions in the supporting electrolyte used here was 4.19 over a similar equivalent concentration range of tungstic oxide in the ores.

The polarographic procedure for the determination of small quantities of tungsten in ores as developed here requires about half the time of the gravimetric procedure, has a better precision as shown in Table I, and has a better accuracy especially in the low grade ores as shown by the use of sodium tungstate as a standard. The preparation of the final solution for the colorimetric analysis developed by the Climax Molybdenum Co. requires about as much time as the preparation of the solution used in the polarographic analysis. The precision and accuracy for the polarographic method are better than those for the colorimetric method in the low grade ores, but not as good in the high grade ores. They are not as good as in the gravimetric method for ores containing more than 10% tungstic oxide, because no better than the usual polarographic precision of 1 to 2% can be expected in any concentration range for a given sample.

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Determination of Molybdenum by Ion Exchange and Polarography

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A method for determining molybdenum, particularly in steels, is based on polarographic reduction of molybdenum(VI) in 0.1*M* sulfuric acid–0.5*M* citric acid. Well formed doublet waves are obtained for the reduction to molybdenum(V) and (III). Diffusion currents are directly proportional to concentration, but slightly dependent on pH and ionic strength. A standard addition technique eliminates careful control of experimental conditions and nonlinear calibration curves. All interfering cations are removed with an ion exchange resin. The final solution contains only molybdate and possibly tungstate and the supporting electrolyte. NBS steel samples have been analyzed with relative errors of less than 3%.

THE polarographic determination of molybdenum has been difficult because of the lack of a suitable supporting electrolyte in which the waves are well formed with the diffusion current proportional to concentration. Holtje and Geyer (*4*), von Stackelberg and others (*9*), Stepien (*10*), and Carritt (*1*) have discussed the molybdenum waves occurring in highly acidic solutions. Analytically useful waves are obtained in solutions containing 10*N* or more sulfuric acid, and have been used for the determination of molybdenum in steels. However, von Stackelberg's procedure (*9*) involves its prior separation as molybdenum disulfide and conversion to molybdenum trioxide by a time-consuming series of steps. Stepien's procedure (*10*) involves its separation as molybdenum trisulfide and conversion to sulfomolybdate followed by determination in 18*N* sulfuric acid. Parry and Yakubik (*7*) have described a method for steels using a tartaric acid supporting electrolyte; however, the method was tested on composited solutions and not actual steel samples.

The perchlorate wave produced in the presence of molybdenum has been utilized by Haight (*2*) for iron and steel samples. The method requires careful control of anions and the wave is not proportional to the concentration of molybdenum. A nonlinear calibration curve must be prepared for each set of conditions.

A rapid polarographic determination of molybdenum in the presence of large amounts of tungsten was described by Meites (*6*), based on the wave produced in citrate buffers at pH 7. However, the result must be obtained from a nonlinear calibration curve. The method was not applied to samples other than reagent grade sodium tungstate.

The separation of molybdate from all interfering cations by ion exchange has been described by Klement (*5*), who completed the determination gravimetrically. Volumetric methods failed, as did most common precipitating reagents, but good results were obtained with 8-quinolinol.

This paper describes a method applicable to a wide variety of samples. The molybdate is separated by a simple ion exchange technique. The determination is made from the polarographic wave obtained in a $0.1M$ sulfuric acid–$0.5M$ citric acid solution. In this medium the double wave is well formed and the diffusion currents are directly proportional to the concentration of molybdenum. The diffusion current constant is somewhat dependent on pH and ionic strength, but with the dilution technique proposed, none of the conditions are critical.

EXPERIMENTAL

A stock solution of sodium molybdate ($0.1M$) was standardized by redox titration using a Jones reductor and standard permanganate (*3*). All chemicals were reagent grade. The ion exchange column was prepared in a 50-ml. Kimble Exax buret, and contained about 20 ml. of analytical grade Dowex 50-X12, 50- to 100-mesh cation exchange resin supported on glass wool. Polarograms were recorded on a calibrated Sargent, Model XXI, polarograph using a three-compartment cell (*8*). Measurements were made at 25° ± 0.1° C. The capillary had a flow rate of 1.45 mg. per second and a drop time of 5.55 seconds at an applied potential of −0.2 volt. Solutions were deaerated with purified nitrogen and no maximum suppressors were added. All potentials were measured and reported *vs.* the saturated calomel electrode.

All pH measurements were made with a Beckman, Model G, pH meter.

PREPARATION OF SAMPLE

Samples which do not contain tungsten can be readily dissolved in dilute sulfuric acid, provided some ammonium persulfate is added after the initial reaction subsides. The persulfate oxidizes molybdenum, chromium, vanadium, manganese, and iron to their highest oxidation states. In order to reduce the chromium, vanadium, and manganese, which are anionic after the dissolution, to stable cations, a small amount of sodium bisulfite is added and the excess sulfur dioxide is boiled off. Citric acid is added for the dual purpose of keeping the molybdate in solution in the acidic medium and for its effect on the subsequent polarogram.

The solution is then made up to a known volume and poured through the ion exchange column which has been previously rinsed with $0.5M$ citric acid solution. The first 10 to 15 ml. of the filtrate is discarded, after which the filtrate is essentially uniform in composition until the breakthrough of the large amount of iron in the sample. The use of a buret for the column facilitates the measurement of exactly 10 ml. of the filtrate into the polarographic cell. It is not necessary to run the entire sample through the column; therefore, the capacity of the resin can be safely reduced or the rate of flow can be increased.

Samples containing tungsten are dissolved in dilute hydrochloric acid followed by the minimum amount of concentrated nitric acid required to oxidize the metals to their highest oxidation state. The nitric and hydrochloric acids are then removed by fuming in the presence of sulfuric acid. The remainder of the procedure is the same as above.

PROCEDURE FOR STEEL SAMPLES

Weigh the sample (0.1 to 0.5 gram if more than 1% molybdenum; 0.5 to 1.0 gram if less than 1% molybdenum) into a tall-form 200-ml. beaker.

Samples without Tungsten. Add 5 ml. of $6N$ sulfuric acid and cover beaker with a watch glass. Warm gently on a hot plate until the reaction diminishes. Add 1 gram of ammonium persulfate and boil gently for 10 minutes.

Samples with Tungsten. Add 10 ml. of $6N$ hydrochloric acid and cover with a watch glass. Warm gently on a hot plate until the reaction subsides. Add 1 to 2 ml. of concentrated nitric acid and heat to boiling. Evaporate to incipient dryness. (The use of an infrared lamp prevents bumping.) Add 10 ml. of $6N$ sulfuric acid and again evaporate to incipient dryness. Dissolve the residue in 5 to 10 ml. of $6N$ sulfuric acid.

Table I. National Bureau of Standards Steel Samples

Sample No.	Sample % Mo	Composition, %	% Mo Found	Av.	Error, %
111b	0.255	0.706 Mn, 1.81 Ni 0.028 Cu, 0.070 Cr 0.043 Al, 0.003 V	0.254, 0.254 0.278, 0.259	0.261	+2.4
159	0.414	0.807 Mn, 0.137 Ni 0.181 Cu, 1.00 Cr 0.090 Ag, 0.054V	0.424, 0.432 0.441, 0.440	0.434	+4.9
106a	0.203	0.546 Mn, 0.277 Ni 0.156 Cu, 1.15 Cr 1.08 Al, 0.002 V	0.195, 0.196 0.202, 0.197	0.198	−2.4
50b	0.401	0.325 Mn, 0.089 Ni 0.110 Cu, 4.08 Cr, 1.02 V, 18.05 W	0.430, 0.420 0.417, 0.371 0.410	0.410	+2.2
123a	0.12	0.75 Cb, 0.02 Ta 0.11 W, 0.037 V	0.113, 0.129 0.125, 0.114	0.120	0
36a	0.92	0.432 Mn, 0.243 Ni 0.114 Cu, 2.41 Cr 0.011 Sn, 0.006 V	0.89, 0.93 0.92, 0.92 0.92, 0.94	0.92	0
132a	4.50	0.27 Mn, 0.14 Ni 0.12 Cu, 4.21 Cr 6.20 W, 1.94 V	4.62, 4.55, 4.65, 4.40	4.55	+1.1
153	8.38	0.219 Mn, 0.107 Ni, 4.14 Cr, 8.45 Co 1.58 W, 2.04 V	8.85, 8.15 8.88, 8.20	8.52	+1.7

If the sample contains a high percentage of tungsten—e.g., NBS 50b in Table I—and does not dissolve with procedure of samples with tungsten, some molybdenum will be retained by the tungstic acid precipitate. To avoid this loss, add 10 grams of sodium citrate and sufficient sodium hydroxide to dissolve the tungstic acid upon gentle boiling. The sample may then be acidified with sulfuric acid without danger of reprecipitation.

Dilute to 50 ml. with water and add 0.1 gram of sodium bisulfite. Boil until the odor of sulfur dioxide can no longer be detected. Add 7 grams of citric acid and 3 grams of sodium citrate. The pH should be between 1 and 2. Transfer to a 100-ml. volumetric flask, filtering if necessary to remove silica and carbon. Make up to the mark.

Pour several 2- to 3-ml. portions of the solution into the ion exchange column, allowing each portion to drain to the top of the resin. Discard the first 10 to 15 ml. of the filtrate. Fill the column to the zero mark on the buret and allow the next 10.00 ml. to run into a dry polarographic cell. Record the polarogram from +0.2 to −0.5 volt. Add exactly 1.00 ml. of a standard sodium molybdate solution to the 10 ml. of test solution in the cell. (The concentration of the standard molybdate solution should be such that the addition approximately doubles the concentration of molybdenum in the cell. For accurate work the standard should contain $0.1M$ sulfuric acid and $0.5M$ citric acid.) Record a second polarogram and measure the diffusion currents of the first wave. The result may be obtained from the formula:

$$P = \frac{100\,M\,V_oV_sC_si_1}{S(i_2V_1 + i_2V_s - i_1V_1)}$$

where P is the per cent of molybdenum in the sample, M is the atomic weight of molybdenum, V_0 is the volume in milliliters to which the sample is made up, V_1 is the volume in milliliters of the sample solution in the polarographic cell, V_s is the volume in milliliters of standard solution added, C_s is the molar concentration of the standard solution, S is the sample weight in grams, and i_1 and i_2 are the heights of the polarographic waves before and after the addition of the standard, respectively. Using the standard procedure described above, the formula reduces to:

$$P = \frac{96000\,C_si_1}{S(1.1\,i_2 - i_1)}$$

RESULTS

These procedures have been tried on synthetic samples containing large amounts of chromium, vanadium, copper, nickel, tungsten, manganese, and cobalt with satisfactory results. The results obtained on National Bureau of Standards steel samples are shown in Table I.

POLAROGRAPHIC DATA

The polarograms of molybdenum in dilute sulfuric acid (pH 1), with and without the addition of citric acid, are shown in Figure 1. A detailed discussion of the polarographic behavior of molybdenum in this medium is beyond the scope of this paper and will be submitted elsewhere. Other hydroxy acids—e.g., tartaric acid and gluconic acid—have the same effect on the wave as does citric acid, and it would appear from the work of Strickland (*11*) that the numerous polynuclear complexes present in dilute sulfuric acid are changed into simple complexes in the presence of citric acid. The exact nature of this complex has not yet been elucidated.

The ratio of the two waves is approximately 1 to 2, indicating that reduction proceeds from the +6 state to the +5 state, and then further to the +3 state. Either wave may be used in a determination. At concentrations greater than $10^{-4}M$, the first wave can be measured more accurately and is less subject to

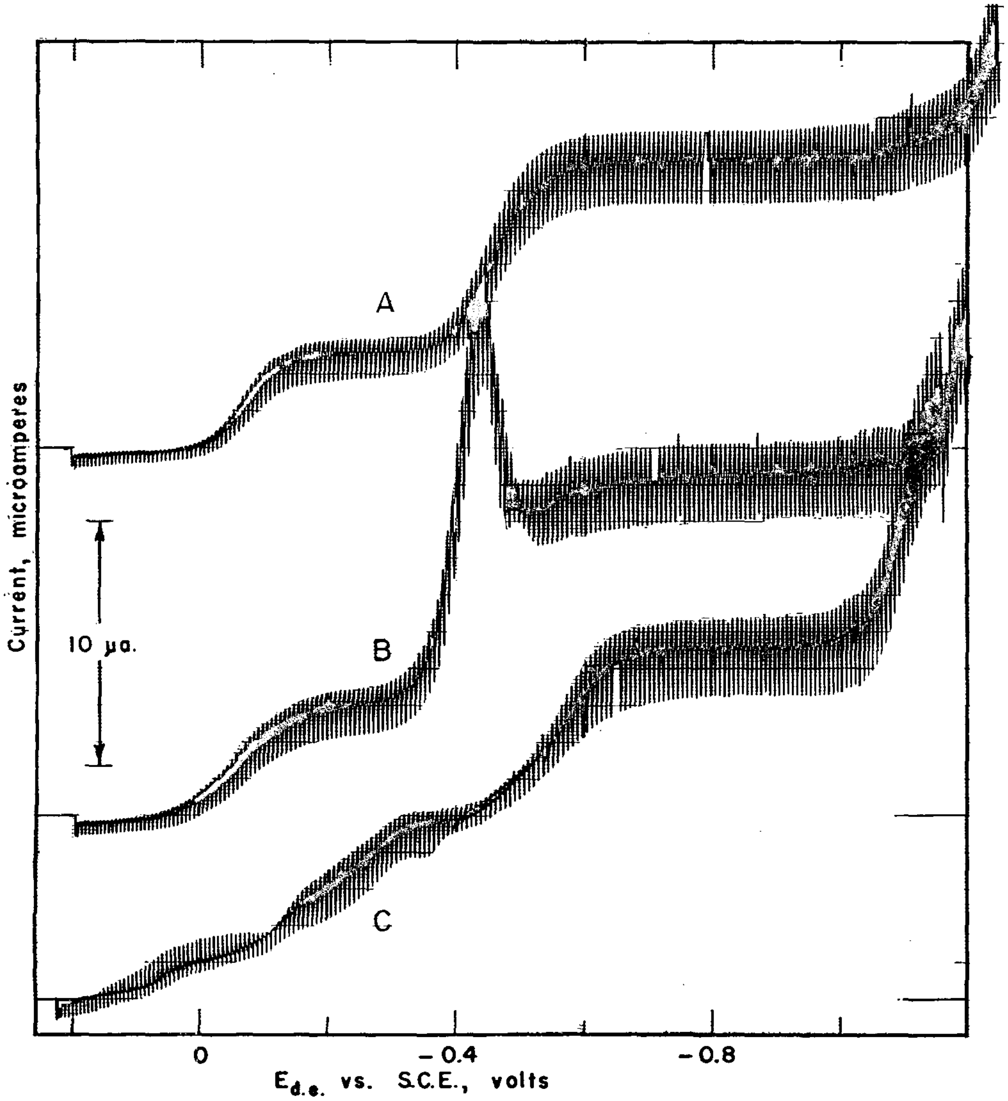

Figure 1. Polarograms of 1mM molybdenum(VI) in 0.1M sulfuric acid

A. With 0.5M citric acid
B. 0.05M citric acid
C. No citric acid
Solutions adjusted to pH 1

interference from tungsten than the second. Tungstate is not reducible in this medium when present alone. In the presence of tungstate, the second molybdenum wave does not become parallel to the residual current line but continues to increase. In solutions more dilute than $10^{-4}M$, the uncertainty of the residual current makes it preferable to use the second wave.

The half-wave potentials of the two waves in $0.1M$ sulfuric acid–$0.5M$ citric acid, pH 1, are -0.070 and -0.430 volt, respectively. The first wave is reversible in $1M$ sulfuric acid–$0.5M$ citric acid, but becomes increasingly irreversible as the pH is increased or the citric acid concentration is decreased.

The diffusion current constant, I, is 1.50 for the first wave and 3.04 for the second wave ($m^{2/3}t^{1/6} = 1.965$). These values of I increase slightly with acidity and decrease with ionic strength; however, under any given conditions, the diffusion current is exactly proportional to concentration of molybdenum in the range 10^{-4} to $5 \times 10^{-3}M$. In order to simplify the procedure and increase the accuracy, a standard addition technique is proposed in which a known amount of molybdenum is added to the sample solution and a second polarogram taken.

Both nitrate and perchlorate yield large catalytic waves which interfere with the measurement of the molybdenum wave and must be removed if present. Chloride ion must not be present in large amounts because the anodic dissolution of mercury to form calomel masks the first molybdenum wave. In the procedure given, none of these anions need be present.

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Simplified High Temperature Sampling and Use of pH for Solubility Determinations

System Uranium Trioxide–Sulfuric Acid–Water

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The solubility of uranium trioxide in aqueous sulfuric acid at elevated temperatures, 150° to 290° C., has been determined by directly sampling a solution equilibrated with solid uranium trioxide hydrate at the respective temperatures and comparing the pH of the isolated sample at 25° C. with control pH data. Isotherms of the solubility of uranium trioxide hydrate in aqueous sulfuric acid have been obtained from 1.0 to 0.00023 molal acid. In the apparatus designed for solubility determinations particular solutions are sampled with rapidity and ease. The solution sample is delivered at room temperature, but removed from the saturated solution at the equilibration temperature. This sampling method is reproducible and apparently reliable. The control pH data of uranium trioxide–sulfuric acid–water solutions at 25.00° ± 0.04° C. have been determined to a precision within ± 0.004 pH unit from 0.024 to 0.00024 molal sulfuric acid. These data have been obtained by the use of a vibrating reed electrometer and accessory equipment.

THE purpose of this investigation was to obtain experimental solubility data in the dilute region for the system, uranium trioxide–sulfuric acid–water, at elevated temperatures and, in so doing, to devise a method for sampling an equilibrated solution intermittently with rapidity and ease. In designing the pressure bomb for containing solutions the interior volume had to be kept relatively small in order to minimize the bulk size necessary to withstand pressures of approximately 1246 pounds per square inch at 300° C.

The bomb contained a length of thin-walled capillary tubing attached at one end to a pressure valve equipped with a sampling tip. The other end of the capillary passed through the bomb head and was inserted into the equilibrated solution. Part of the capillary between the bomb head and the pressure valve passed through a wet ice bath. By means of this design the solution was rapidly cooled during the sampling process, thereby eliminating a separate cooling or isolation chamber and facilitating the procurement of many samples per run. This method appears more advantageous than the "one sample per experiment" filter bomb method which relies either on a difference in vapor pressure between two internal cells or on gravitational flow to accomplish filtration at or near the equilibration temperature (*2*, *3*). In the use of a vapor pressure difference there is an uncertainty whether solvent distills to the filtrate during the filtering process. With the direct sampling technique there is neither a distillation problem nor a need for correction for loss of solvent or other components to the vapor phase at the equilibration temperature, as the liquid phase alone is sampled at the elevated temperature and can be analyzed for all components. However, with systems having a positive temperature coefficient of solubility the precipitation characteristics and solubility range of the individual system would have to be considered and evaluated.

Solid phase particles might be withdrawn through the capillary during the sampling process. In the system under study, the uranium trioxide hydrate settles rapidly (within seconds) at the high temperatures, leaving a clear liquid from which the sample is obtained. This settling action has been observed visually by means of the semimicro-phase study apparatus (*10*). Also, because the dissolving rate of uranium trioxide solid is sufficiently slow, any solid which may be withdrawn can be seen in the liquid sample. No solid was observed. If settling rates are slow for other systems, a filter may be attached to the tip of the capillary tubing within the bomb.

Solubility studies of uranium trioxide in aqueous sulfuric acid were contemplated in concentrations as low as 0.0002 molal sulfuric acid. At this dilution, standard analytical methods for uranium would not achieve the desired accuracy within ±0.5% on 5-ml. samples. The quantitative use of precise pH control curves appeared suitable, yielding pH data at 25° C. adaptable for calculation of dissociation or hydrolysis constants.

By the use of the above techniques the solubility of uranium trioxide in sulfuric acid has been determined from 1.0 to 0.00023 molal sulfuric acid between 150° and 290° C. Two empirical equations have been derived to express the data as a function of temperature, uranium trioxide molality, and sulfuric acid molality. As a requisite for the solubility determinations, the 25° C. pH data for uranium trioxide–sulfuric acid–water mixtures have been determined from 0.70 to 1.20 mole ratio, UO_3/H_2SO_4, and from 0.024 to 0.00024 molar sulfuric acid.

Table I. pH of Uranium Trioxide–Sulfuric Acid–Water Solutions at 25.00° ± 0.04° C.

Molarity H_2SO_4	pH Values[a]					
	Mole Ratios UO_3/H_2SO_4					
	0.701_7	0.803_0	0.903_8	1.004_5	1.106_0	1.203_9
0.02425	1.994	2.150	2.424	3.043	3.613	3.824
0.01210	2.259	2.421	2.692	3.267	3.732	3.923
0.004852	2.619	2.784	3.046	3.527	3.875	4.039
0.003645	2.730	2.900	3.155	3.605	3.914	4.075
0.002430	2.905	3.065	3.321	3.709	3.978	4.125
0.001212	3.174	3.348	3.593	3.907	4.094	4.215
0.0004836	3.588	3.716	3.904	4.131	4.251	4.355
0.0003635	3.672	3.823	3.967	4.202	4.305	4.396
0.0002417	3.852	4.005	4.128	4.327	4.412	4.501

[a] These values and pH values in Table II are based on a determined pH of 2.075 for 0.01000*M* HCl, 0.0900*M* in KCl (*1*).

The techniques described are suggested as relatively simple methods for determinations of phase solubility, other types of equilibria, and rate processes at elevated temperature and pressure in either aqueous or nonaqueous systems. The sparsity of solubility data alone in aqueous systems at high temperature is indicated by Booth and Bidwell's statement (*3*) that "of the great number of sparingly soluble substances known the solubilities of a mere half dozen have been measured to above 300° C. . ."

EXPERIMENTAL

The pH's at 25.00° ± 0.04° C. were measured by use of a vibrating reed electrometer coupled with a Leeds and Northrup K-2 potentiometer and Brown recorder for making a record of the final potential obtained by means of the apparatus of Kraus, Holmberg, and Borkowski (*7*). Thermostated water was circulated around both the calomel electrode and the test tube containing the sample solution, the salt bridge tip, and the glass electrode. A schematic drawing is shown in Figure 1. The fluctuation of potential on the Brown recorder was of the order of ±0.02 mv. (or ±0.0003 pH equiv.), but afforded actual readings of ±0.004 mv.

Mallinckrodt uranium trioxide hydrate, which has been washed thoroughly with hot water to remove as much nitrate impurity as possible (NO_3^- after washing <24 p.p.m.), was analyzed (78.95% uranium), weighed, and dissolved in weighed amounts of 0.02437 molal sulfuric acid (0.02428 molar) solutions to produce the desired mole ratios, UO_3/H_2SO_4. The molarities reported in Table I were obtained by correcting for the slight volume and weight changes upon dissolving uranium trioxide hydrate in sulfuric acid solutions. In making these corrections the absolute densities at 25° C. of uranyl sulfate solutions, and of water, and weights of the individual components were used. As one example, the 0.02428 molar sulfuric acid solution was multiplied by 0.9988 to obtain the corrected value reported in Table I. These initial uranium trioxide–sulfuric acid–water solutions were diluted successively by the use of calibrated volumetric pipets and flasks.

Three control hydrochloric acid–potassium chloride solutions were used for relating the measured potentials to pH: 0.009928, 0.003157, and 0.001005*M* hydrochloric acid solutions to which sufficient c.p. potassium chloride was added to obtain a total chloride concentration of 0.100*M* in each solution. The pH value used for the 0.009928*M* hydrochloric acid (0.100*M* in chloride) solution was obtained by adding 0.003 pH unit (the logarithmic difference between 0.01000 and 0.009928) to the value, 2.075 (*1*), given for a 0.0100*M* hydrochloric acid, 0.0900*M* potassium chloride solution. The pH values of 2.576 and 3.073, respectively, were obtained by the same procedure, which was justified by virtue of the constant ionic strength of all hydrochloric acid–potassium chloride solutions. The potentials of these solutions were determined before and after all series of experimental measurements, and the sample potentials and pH's were related to these values. The Nernst slope was found to lie between 59.00 and 59.50 mv. per pH unit, but precise to ±0.10 mv. per pH unit for any series of measurements. The pH's of uranium trioxide–sulfuric acid–water control solutions were measured and are believed to be reproducible within ±0.002 to ±0.004 pH unit.

Apparatus for Measuring Solubilities. A schematic diagram of the bomb and assembly for measuring solubilities at elevated temperature is shown in Figure 2. One of the two unique features is the use of pure Teflon gasketing fitted into a circular groove in the bomb head. This groove prevents the Teflon from extruding when subjected to high temperature and pressure. There is no need for auxiliary screws to tighten on a pressure plate since, when the bomb is heated to temperatures above 100° C., the Teflon expands and effects a leakproof seal. Manual

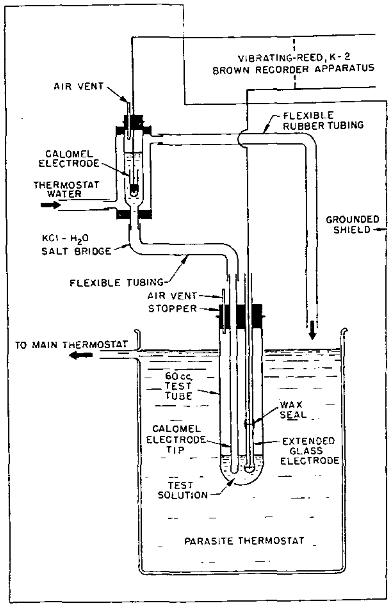

Figure 1. Schematic drawing of thermostated electrodes

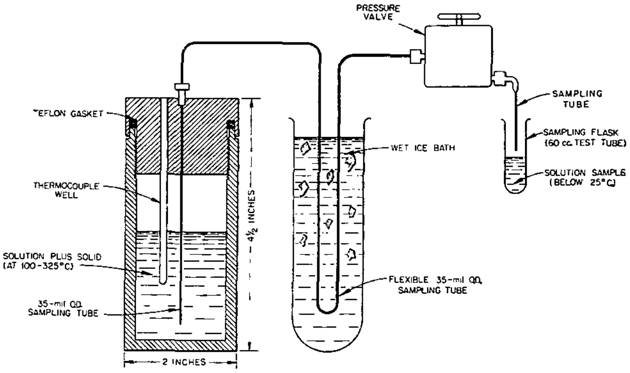

Figure 2. Schematic drawing of aqueous solution sampling assembly

tightening of the bomb prevents any leakage. Upon cooling back to room temperature the Teflon contracts, thus allowing the bomb also to be opened manually. However, a wrench is usually used for tightening and loosening the bomb head, in which event leakproof sealing is accomplished at or very near room temperature. After an experimental run the bomb assembly is cooled within several minutes by removing it from the thermostat and placing it directly in a wet ice bath.

The Teflon gasket can be used repeatedly under the above conditions to approximately 325° C., at which temperature a physical change occurs within the Teflon (*5*). Experiments with this bomb have been performed on aqueous systems at 4500 pounds per square inch without failure until 325° C. is reached. These observations indicate that it is not the water vapor pressure (the pressure of saturated steam at 325° C. is 1749 pounds per square inch) which causes the leakage at 325° C., but rather this physical change of Teflon. It is of course possible, with suitable modification of the bomb and/or elimination of Teflon gasketing, to operate at temperatures over 325° C.

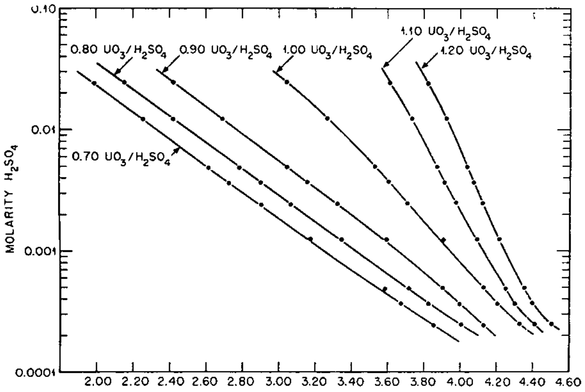

Figure 3. pH at 25.0° C. of uranium trioxide–sulfuric acid–water solutions

Sulfuric acid molarity *vs.* pH

The other feature is the use of 35-mil outer diameter, 20-mil inner diameter commercial capillary tubing for sampling solutions. By virtue of the small size of the capillary tubing the solution sample, regardless of the temperature of the system, is cooled rapidly as it passes through that portion of capillary inserted in the wet ice bath. The internal pressure of the system affords the necessary pressure for pushing the liquid phase through the capillary. A high pressure valve is opened slowly and solution comes out either dropwise or in a stream to deliver as much as 15 to 20 ml. per minute cooled below room temperature.

Procedure for Determining Solubilities. A 60.00-ml. volume of standard sulfuric acid solution and a weighed quantity of uranium trioxide hydrate were placed in the bomb, such that the mole ratio of total uranium trioxide to total sulfuric acid was approximately 3 to 1. The bomb, loaded with excess solid and solution, was inserted into an insulated, thermostated aluminum cylinder. The temperature was controlled to ±1° C. by means of a Brown Pyr-O-Vane controller. The whole assembly, including bomb, thermostat, capillary tubing, and sampling valve, was attached to a large shaker which was run at a rate of 100 cycles per minute with a displacement of 8 cm., thereby affording ample mixing of the solution and solid.

Solution and solid were equilibrated at a given temperature, a 3-ml. holdup sample was removed, and a 5-ml. sample of the equilibrated solution was delivered to a standard 60-ml. test tube. This sample solution was thermostated at 25° C. and the pH determined in the manner described. The mole ratio, UO_3/H_2SO_4, was determined from control curves of pH *vs.* ratio drawn from the curves shown in Figure 3 for the particular concentration of sulfuric acid. The pH data of Orban (*11*) were used for concentrations greater than 0.023 molal. All uranium trioxide and sulfuric acid concentrations reported in Table II have been corrected for slight dilution effects incurred by dissolving uranium trioxide in standard sulfuric acid solutions.

It was found by consecutive sampling that after the major portion of uranium trioxide had dissolved in the sulfuric acid solution the system equilibrated within 15 to 20 minutes between 150° and 290° C. when the temperature was raised. This was evidenced by agreement of samples obtained at these times with those obtained several hours later. For the most part, the system was allowed to equilibrate 1 to 2 hours before sampling and in some cases overnight. The equilibration temperature was approached from higher and lower temperatures and the solubilities thus obtained indicated relatively rapid and complete reversibility with a somewhat slower rate upon lowering the temperature.

RESULTS AND DISCUSSION

pH Data at 25° C. The pH data at 25.00° ± 0.04° C. are given in Table I and shown in Figure 3. There is a satisfactory overlap of data for stoichiometric uranyl sulfate (UO_2SO_4) obtained by previous investigators (*6*, *8*, *9*). However, these previous data lacked the precision of the measurements obtained using the present apparatus. A precision within ±0.002 to ±0.004 pH unit for the present data is approximately five times

Table II. Solubility of Uranium Trioxide in Sulfuric Acid–Water Solutions

Temp., ° C.	Molar Concentrations[a]		UO_3/H_2SO_4 Mole Ratio	pH at 25.0° C.	Molal Concentrations	
	H_2SO_4	UO_3			H_2SO_4	UO_3
150						
	1.000	1.318	1.318	3.111		
	0.2883[b]	0.3644[b]	1.264[b]		0.294[b]	0.372[b]
	0.1000	0.1205	1.205	3.628	0.1008	0.1215
	0.05043[b]	0.05951[b]	1.180[b]		0.05061[b]	0.05972[b]
	0.02293	0.02683	1.170	3.785	0.02303	0.02696
	0.01147	0.01317	1.148	3.844	0.01151	0.01321
	0.001148	0.001230	1.071	4.054	0.001152	0.001234
175						
	1.000	1.300	1.300	3.081		
	0.2861[b]	0.3551[b]	1.241[b]		0.292[b]	0.323[b]
	0.1000	0.1174	1.174	3.566	0.1008	0.1183
	0.05043[b]	0.05821[b]	1.154[b]		0.05061[b]	0.05841[b]
	0.02293	0.02575	1.123	3.677	0.02303	0.02586
	0.01147	0.01254	1.093	3.700	0.01151	0.01258
	0.004590	0.004786	1.043	3.701	0.004607	0.004803
	0.0002289	0.0002080	0.909	4.154	0.0002297	0.0002087
200						
	1.000	1.285	1.285	3.053		
	0.2912[b]	0.3539[b]	1.215[b]		0.297[b]	0.361[b]
	0.1000	0.1151	1.151	3.504	0.1008	0.1160
	0.05043[b]	0.05686[b]	1.128[b]		0.05061[b]	0.05706[b]
	0.02293	0.02495	1.088	3.544	0.02303	0.02505
	0.01147	0.01193	1.040	3.484	0.01151	0.01197
	0.004590	0.004459	0.971	3.368	0.004607	0.004475
	0.001148	0.001015	0.884	3.560	0.001152	0.001019
	0.0002289	0.0001704	0.744	3.954	0.0002297	0.0001710
225						
	1.000	1.268	1.268	3.019		
	0.2951[b]	0.3471[b]	1.176[b]		0.301[b]	0.355[b]
	0.1000	0.1132	1.132	3.443	0.1008	0.1141
	0.02293	0.02389	1.042	3.302	0.02303	0.02399
	0.01147	0.01137	0.991	3.191	0.01151	0.01141
	0.004590	0.004188	0.912	3.103	0.004607	0.004203
	0.001148	0.000902	0.785	3.331	0.001152	0.000905
	0.0002289	0.0001497	0.654	3.794	0.0002297	0.0001502
250	1.000	1.243	1.243	2.967		
	0.2983[b]	0.3466[b]	1.162[b]		0.305[b]	0.3541[b]
	0.1000	0.1114	1.114	3.372	0.1008	0.1123
	0.02293	0.02315	1.010	3.095	0.02303	0.02325
	0.01147	0.01079	0.941	2.868	0.01151	0.01083
	0.004590	0.003898	0.849	2.908	0.004607	0.003913
	0.001148	0.000831	0.724	3.224	0.001152	0.000834
270						
	0.1000	0.1105	1.105	3.330	0.1008	0.1114
	0.02293	0.02253	0.983	2.895	0.02303	0.02263
	0.01147	0.01036	0.903	2.709	0.01151	0.01039
	0.004590	0.003705	0.807	2.813	0.004607	0.003719
	0.001148	0.000760	0.661	3.137	0.001152	0.000762
290						
	0.1000	0.1098	1.098	3.293	0.1008	0.1107
	0.02293	0.02210	0.964	2.762	0.02303	0.02220
	0.01147	0.01007	0.878	2.622	0.01151	0.01011
	0.004509	0.003594	0.783	2.765	0.004607	0.003608
	0.001148	0.000716	0.624	3.088	0.001152	0.000719

[a] Molarities are on a 25° C. basis.
[b] Gravimetric and titrimetric analytical values.

more precise than can be obtained using standard laboratory pH instruments.

Solubility Data. The solubilities are given in Table II and, in Figure 4, the log molality sulfuric acid is plotted against the mole ratio, UO_3/H_2SO_4. The two-liquid-phase region indicated in Figure 4 was found experimentally and is characteristic of this system (*12*).

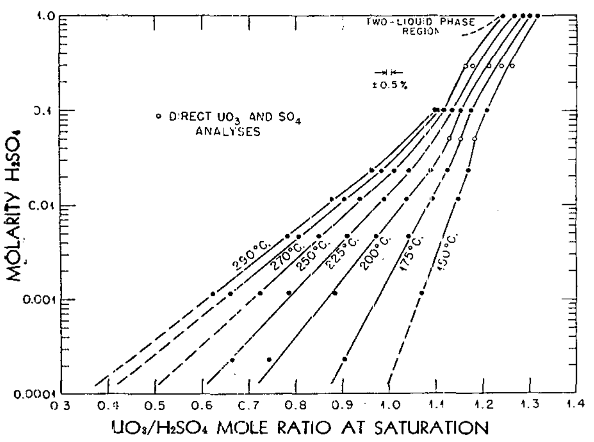

Figure 4. Solubility of uranium trioxide in sulfuric acid–water solutions from 150° to 290° C.

Sulfuric acid molality *vs.* mole ratio

In making a run a consecutive series of liquid samples at various temperatures was withdrawn for analysis. At high temperature the water in the vapor phase which originates from the liquid is not negligible and each depletion of liquid volume by sampling necessitates further removal of water to the vapor and, consequently, a higher concentration of unsaturated components (other than water) in the liquid phase. After careful consideration of all factors, it is apparent that a marked change in sulfuric acid concentration in the liquid phase will produce only a small change in solubility mole ratio, UO_3/H_2SO_4. This change, moreover, will be nearly self-correcting if the pH control curve for the concentration of sulfuric acid neglecting loss of water to the vapor is used and if the solubility ratio data are plotted against this same sulfuric acid concentration (Figure 4). The data in Figure 4 are believed to represent the true solubility relationships along the solubility curves to the stated precision and to the same degree of accuracy.

Usually, phase equilibria data of this type would be shown on a three-component triangular diagram—for example, as the system, uranium trioxide–sulfuric acid–water. However, the data are presented in Figure 4 to show the striking linearity in dilute concentrations of the various isotherms and to emphasize the precision and apparent accuracy of the measurements when plotted in this manner. These data are reproducible to ±0.5%, except at 0.00023 molal sulfuric acid, at which concentration there is a somewhat wider divergence. Gravimetric data for uranium by ignition at 900° C. to U_3O_8 and titrimetric analyses for sulfate (*4*), both determined after direct sampling, are included in Table II and Figure 4, and substantiate the pH analytical data at least at the higher concentrations. The data in the dilute region show considerably better precision than could have been obtained by standard analytical methods for uranium on 5-ml. solution volumes. On the basis of plots of log $[H_2SO_4]$ *vs.* $[UO_3]/[H_2SO_4]$ there appears to be a direct correlation between the hydrogen ion activity at a particular temperature and the mole ratio, UO_3/H_2SO_4.

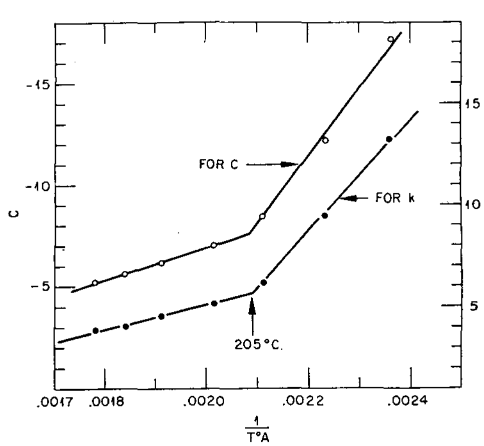

Figure 5. Variation of *C* and *k* with $1/T°$ A.

The straight-line portions of given isotherms in Figure 4 can be represented by the equation,

$$\text{Log } [H_2SO_4] = k[UO_3]/[H_2SO_4] + C$$

If the slopes, k, are plotted against $1/T°$, where T = ° A., a break in the resultant curve occurs at 205° C. as shown in Figure 5. A similar break appears at 205° C. on plots of C *vs.* $1/T°$ A. (Figure 5). Approximate linear relationships are observed on both sides of 205° C. for both k and C plots regardless of whether $1/T°$ A. or $1/t°$ C. is used for the temperature function. J. O. Blomeke of this laboratory has observed a transition of uranium trioxide hydrate heated in water occurring in the neighborhood of 200° C. and, by means of x-ray diffraction patterns, has identified the hydrates as $\alpha UO_3.H_2O$ (at the lower temperature) and $\beta UO_3.H_2O$, both observed by Zachariasen (*13*). If these two solids are really the stable hydrates in the presence of the saturated uranium trioxide–sulfuric acid–water solution, then the breaks at 205° C. presumably represent the transition from $\alpha UO_3.H_2O$ to $\beta UO_3.H_2O$.

Equations for k and C as a $f(t)$ are determined, from which final empirical equations have been derived to express the solubility data. These equations are believed accurate to ±1% in mole ratio in the respective temperature and concentration ranges with the exception of 290° C., at which temperature the particular equation is in error by −0.01 to −0.03 mole ratio unit. Both equations are given as follows and are applicable from approximately 0.05 to 0.0001 molal sulfuric acid:

(For use between 150° and 205° C.)

$$\log [H_2SO_4] = \left(\frac{4150}{t°\text{ C.}} - 14.42\right)\frac{[UO_3]}{[H_2SO_4]} - \frac{5230}{t°\text{ C.}} + 17.74 \quad (1)$$

(For use between 205° and 290° C.)

$$\log [H_2SO_4] = \left(\frac{1400}{t°\text{ C.}} - 1.096\right)\frac{[UO_3]}{[H_2SO_4]} - \frac{1880}{t°\text{ C.}} + 1.324 \quad (2)$$

where $[UO_3]$ and $[H_2SO_4]$ are expressed as molalities. The equations also are applicable to concentrations of sulfate as high as 1 molal but approach a maximum deviation of +8% at 290° C. at the highest concentration.

The pH's at 25° C. of solution mixtures saturated with uranium trioxide at the elevated temperatures are given in Table II and are shown in Figure 6 as a series of isotherms. The points shown in Figure 6 were obtained by use of the drawn isotherms of Figure 4 and the particular pH-mole ratio curves. These isotherms indicate a reasonable minimum acidity at 25° C. which must be maintained at a particular concentration of sulfate in order to prevent hydrolytic precipitation of uranium trioxide. This rela-

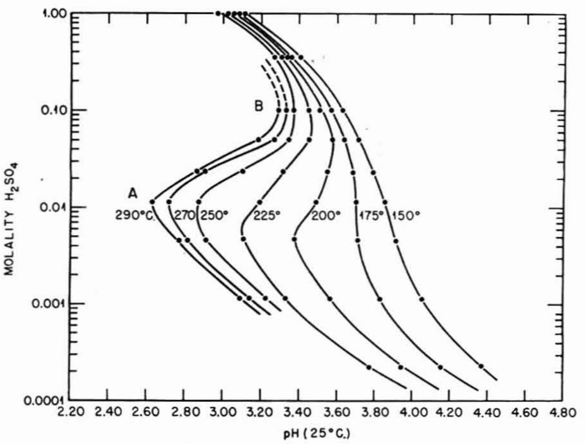

Figure 6. pH at 25° C. of sulfuric acid–water solutions saturated at various temperatures with uranium trioxide

tionship should approach ideality as the sulfate concentration approaches infinite dilution. The deviations expected at finite concentrations would be attributed to the changes in activity coefficients of the solution components and to the extent of complexing at the elevated temperature.

The "reverse S"-shaped curves of pH data at 25° C. for the saturated solutions at elevated temperature (Figure 6) might well not exist if pH data were measured at the elevated temperature. If, however, this type of curve does exist at the high temperature, the isotherms shown in Figure 6 could represent the general character of curves of hydrogen ion activity measured at elevated temperature *vs.* log molality of sulfuric acid, but would not represent the actual hydrogen ion activity at high temperature. The assumption is made that the hydrogen ion activity of various solution concentrations changes proportionally to each other as a function of temperature. In this event the reversal in curvature as shown in region *A* of Figure 6 is characteristic of any acid-base system in which the ratio, base/acid, increases as a particular function of increase of acid or base concentration. A minimum in pH as a function of concentration must necessarily appear in the system in which complexing does not occur or is a minor influence on acidity relative to change of ratio. The second reversal in curvature shown in region *B* is indicative of complexing in the particular system and, in this case, might imply and substantiate polymerization of uranyl ion by equilibria of the following type:

$$UO_2^{++} + UO_2(OH)_2 \rightleftharpoons U_2O_5^{++} + H_2O$$

This type of equilibria, known to exist at low temperature, would decrease the concentration of the base component and, effectively, increase the acidity of this system.

The solubility relations for the system under investigation, uranium trioxide–sulfuric acid–water, fall within the most sensitive range of pH variation with mole ratio. For the application of this method to other systems the sensitivity of pH in the particular solubility range must be considered. There are, however, possible modifications of the analytical method. For example, an acid-base system, in which solubilities do not fall in the pH-sensitive range, might be investigated using pH control curves by adding a known quantity of standard acid or base to the liquid phase sample to make the sample pH fall within the sensitive range for analysis and then correcting for the addition.

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Direct Determination of Moisture in Sodium Hydroxide

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A simple, direct method for the determination of the moisture content of anhydrous caustic soda has been developed. The method involves heating the caustic to about 350° C. in a closed nickel container. The moisture is purged from the caustic into tared anhydrous magnesium perchlorate filled U-tubes by means of a stream of dry nitrogen. The elapsed time for analysis is about 45 minutes. Individual determinations fall within ±0.012% of the true moisture content 95% of the time.

THE established technique for the determination of the moisture content of anhydrous caustic soda (sodium hydroxide) has been the indirect method. This consists of subtracting the sum of all components analyzed in the caustic sample from 100% and calling the difference moisture. It is evident that this method gives, at best, only an approximation of the true content.

The possibility of using an oven drying method is ruled out in this case, because of the carbon dioxide present in the air. Several distillation-extraction methods using an immiscible solvent have been tried. Various stills and traps (*1–3*, *5*,) have been used, but the difficulty seems to lie in the fact that significant amounts of water remain in the condenser. Collecting a large volume—i.e., several milliliters—is one way of minimizing this error. However, if the water content of the sample is low, this requires extremely large samples.

Suter (*4*) devised a distillation-titrimetric method whereby he used xylene to distill the water from the sample and then titrated the xylene-water mixture with Karl Fisher reagent. The author claimed an accuracy of about 5% of the water present. However, the procedure takes over 4 hours per sample.

The apparatus and procedure described have been used in this laboratory for over a year, having been used for the analysis of caustic soda samples which have ranged from 0.02% to over 1% water.

APPARATUS

Complete assembly of the apparatus is shown in Figures 1 and 2. Detail of the head assembly is given in Figure 3.

PROCEDURE

Thoroughly dry the nickel container and assemble the apparatus as shown.

Adjust a flow of nitrogen to 2000 cc. per minute. After several minutes of purging, run a blank on the system. Disconnect the U-tubes and weigh them on an analytical balance, then place them back in position and allow the nitrogen to flow at the same rate for 45 minutes. Again disconnect the tubes and weigh them. If the tubes have gained weight, a larger dryer for the nitrogen gas is needed.

If there is no blank on the nitrogen, check the system for leaks by introducing a weighed sample of water into the container. Next place the tared U-tubes in position and adjust the nitrogen flow to 2000 cc. per minute. Then heat the container to 350° C. and maintain this temperature for 30 minutes, after which reweigh the U-tubes. If the recovery of water is not quantitative, there is probably a leak in the system which must be located.

When the system has been found to be leak-free, it is ready for use. After the system has been purged and an initial weight obtained on the U-tubes, place them in position and adjust the nitrogen flow to 2000 cc. per minute.

Open the container and quickly pour in the weighed sample (50 to 100 grams) of caustic. Replace the cover on the container immediately and tighten.

Begin heating the container and raise the temperature to 350° C. Maintain this temperature for 45 minutes. At the end of this time, disconnect the U-tubes and reweigh. The gain in weight of the U-tubes is equal to the water content of the caustic.

Table I. Precision of Determination of Water in Caustic Soda with High Moisture Content

(500° C. for 60 minutes)

Run No.	% H_2O	Deviation from Mean
1	0.97	0.02
2	0.95	0.00
3	0.92	0.03
4	0.96	0.01
Mean	0.95	
Av. dev. from mean (1.6% of water present)		0.015

Table II. Precision of Determination of Water in Caustic Soda with Low Moisture Content

Run No.	Time, Minutes	Temperature, ° C.	% H_2O	Deviation from Mean
1	60	500	0.067	0.002
2	60	500	0.070	0.001
3	60	500	0.068	0.001
4	60	500	0.068	0.001
5	60	500	0.066	0.003
6	60	500	0.071	0.002
7	30	350	0.077	0.008
8	30	350	0.064	0.005
9	30	350	0.073	0.004
10	30	350	0.062	0.007
	Mean		0.069	
	Av. dev. from mean (4.3% of water present)			0.003

While the caustic is still molten disconnect the container and pour the caustic into a dry metal container. Later cool the container in water and wash out the remaining caustic. After drying, the container is again ready for use.

As spattering may occur, extreme caution is necessary when handling the hot molten caustic; thus, wear a face shield and gloves when cleaning out the container.

EXPERIMENTAL

It was believed that the temperature necessary to drive off the moisture from the caustic in a reasonable time would probably be above its fusion temperature of approximately 318° C.; however, too high a temperature may cause decomposition of the caustic resulting in a release of water.

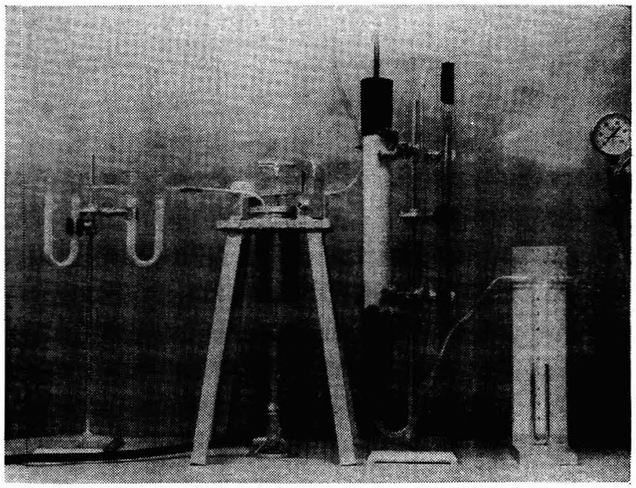

Figure 1. Complete assembly

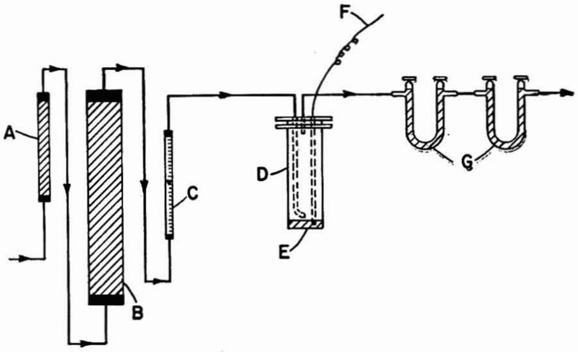

Figure 2. Schematic diagram of apparatus

A. Ascarite column, 1 by 10 inch tubing
B. Anhydrone column, 2 by 20 inch tubing
C. Flowmeter
D. Nickel container, $2^5/_8$ by 6 inches with $^1/_8$-inch wall thickness
E. Caustic sample
F. Thermocouple leads
G. 150-mm. Anhydrone-filled U-tubes

A sample of high moisture content caustic (ca. 1%) was run at 500° C. for 60 minutes. These results are shown in Table I. More runs were made at lower temperatures and shorter run times. At 350° C., all the moisture was recovered in 30 minutes.

When making the runs shown in Table II, two different conditions were used. The first six runs were made at 500° C. for 60 minutes, and the last four runs were made at 350° C. for 30 minutes. As there was no significant variation in the results, it was decided to use 350° C. and 45 minutes in the method.

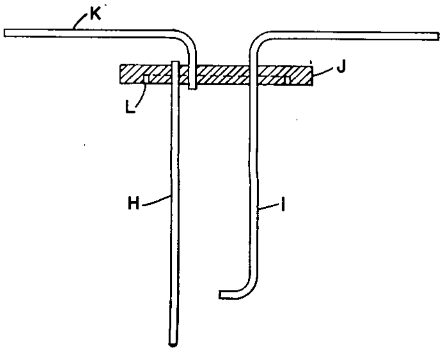

Figure 3. Detail of head assembly

H. Thermocuple well
I. Inlet tube for gas stream
J. Cover plate, $^3/_8$-inch nickel plate
K. Exit tube for gas stream
L. Recessed circular well cut to snugly fit lip of nickel container. The well is lined with an asbestos gasket

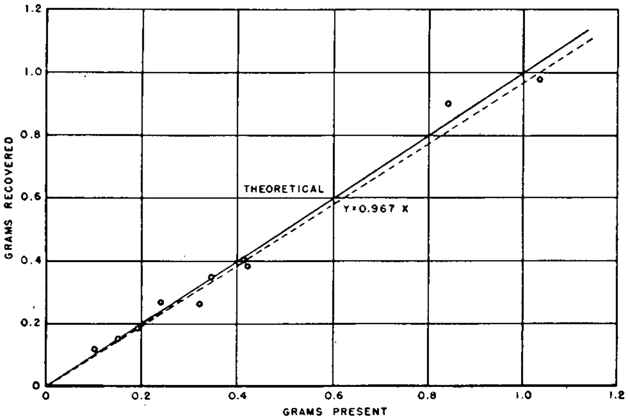

Figure 4. Recovery of water

Any carbon dioxide in the nitrogen purge gas reacting with the caustic would release additional water. To eliminate this possibility an Ascarite column was placed ahead of the magnesium perchlorate drying train.

PRECISION

Several determinations were made on one sample of caustic soda which was kept in a tightly stoppered bottle. The results are shown in Table I.

Another sample of caustic which had a low moisture content was used to evaluate the method. A series of determinations were made on this sample. The results are shown in Table II.

ACCURACY

No samples of caustic soda were available that were known to be completely anhydrous. Therefore, it was necessary to use the sample of caustic which had been analyzed by this method (Table II) in order to ascertain the accuracy of the method. Known quantities of water were added to varying weights of sample with the average of the 10 determinations shown on Table II used as the original water content. The sample was weighed and placed in the nickel container to which the varying weights of water were immediately added from a weight pipet. The addition was made by distributing the water over a large area to minimize local overheating and to prevent any significant loss of water. The container was then immediately closed and the determination completed in the usual manner. The results of these runs are shown in Table III.

Table III. Recovery of Water Added to Caustic Soda

(350° C. for 45 minutes)

I	II	III	IV	V	VI	VII	VIII
		H_2O, Grams				H_2O, %	
Run No.	Sample, Grams	In sample	Added	Total present	Recovered	Calcd.	Found
1	54.9	0.0377	0.1558	0.1935	0.1866	0.35	0.34
2	54.7	0.0376	0.3770	0.4146	0.4025	0.75	0.74
3	112.4	0.0772	0.0743	0.1515	0.1530	0.14	0.14
4	79.1	0.0543	0.9852	1.0395	0.9753	1.30	1:23
5	36.8	0.0253	0.0665	0.0918	0.1190	0.25	0.32
6	126.0	0.0866	0.2379	0.3245	0.2601	0.26	0.21
7	62.8	0.0432	0.3802	0.4234	0.3895	0.67	0.61
8	86.9	0.0596	0.1812	0.2408	0.2690	0.28	0.31
9	143.0	0.0982	0.7454	0.8436	0.8665	0.59	0.60
10	52.6	0.0362	0.3127	0.3489	0.3511	0.66	0.66

Table IV. Comparison of Duplicate Analyses

(350° C. for 45 minutes)

Sample No.	% H_2O	Difference between Duplicates
1	0.082 0.078	0.004
2	0.036 0.050	0.014
3	0.047 0.049	0.002
4	0.060 0.072	0.012
5	0.039 0.041	0.002
6	0.306 0.314	0.008
7	0.037 0.038	0.001
8	0.075 0.078	0.003
9	0.129 0.123	0.006
10	0.145 0.137	0.008
11	0.267 0.278	0.011

The data from columns V and VI of Table III are shown on Figure 4. Since circumstances required that the water content of the original caustic sample be determined by the analytical method being evaluated, it is necessary to assume that there is no constant error or bias—i.e., line passes through the origin. The straight line, restricted to pass through the origin, that best fits the data is shown on Figure 4. The slope of this line does not differ significantly from the theoretical. This gives rise to the conclusion that there is no inherent error in the method of analysis. Thus, there is no correction to be applied to the results. If this is true, the deviations represent only experimental error.

Examination of data obtained from duplicate determinations, for regular caustic samples tested under routine laboratory conditions, indicates that individual determinations fall within ±0.012% of the true moisture content 95% of the time (Table IV). This estimate of error is less than would be predicted from the data in Table III for synthetic samples. There is more chance for error when performing the additional manipulations involved in the preparation of synthetic samples, and this no doubt explains the larger error associated with the analysis of such samples.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance of Ralph E. Swackhamer in statistically evaluating the data given.

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Rapid Estimation of Dialdehyde Content of Periodate Oxystarch through Quantitative Alkali Consumption

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A rapid and reliable method was required for determination of the extent of oxidation of numerous samples of periodate oxidized starches prepared in the course of a research program on these materials. Such a method, described here, is based on the discovery of reaction conditions under which a mole of alkali is consumed per mole of dialdehyde unit in oxystarch. There was good agreement between results obtained by this method and by other independent procedures over a wide range of dialdehyde contents. The simplicity of this method permits a single determination to be made in 10 minutes.

JACKSON and Hudson (*6*) have established that oxidation of starch by periodate results in cleavage of the anhydroglucose units between carbon atoms 2 and 3, and have assigned the structure shown in Figure 1 to the repeating unit of the modified starch. In connection with a research program on periodate oxidized starches now under way at this laboratory, a rapid and reliable analytical method was required for determining, on a routine basis, the extent of conversion of anhydroglucose residues in starch to dialdehyde units. Although a number of procedures for carbonyl determination using hydroxylamine (*3*), sodium chlorite (*1*), cyanide (*1*), bisulfite (*1*), hypoiodite (*1*), sodium borohydride (*7*), or hydrolysis techniques (*4*) were considered, and some were tested experimentally, none had the rapidity and simplicity of the method described here, which allows analysis of an oxystarch sample in less than 10 minutes.

EXPERIMENTAL

From 0.1500 to 0.2000 gram of oxystarch of known moisture content is weighed into a 125-ml. Erlenmeyer flask. To the flask is added, via pipet, 10 ml. of standardized, carbonate-free 0.25N sodium hydroxide, and the flask is gently swirled and immediately placed on a steam bath having a circular opening 5.5 cm. in diameter for exactly 1 minute. There should be rapid flow of steam during the heating period. The flask is then cooled immediately under running tap water with rapid swirling for 1 minute, and 15 ml. of standardized 0.25N sulfuric acid is added by pipet. To the contents of the flask are added 50 ml. of water and 1 ml. of neutral 0.2% phenolphthalein. Titration of the acid solution is carried out using 0.25N sodium hydroxide delivered from a 10-ml. microburet, graduated to 0.02 ml. Alternatively, the titration may be carried out potentiometrically if desired. The percentage of dialdehyde units in the oxystarch is given by the equation:

$$\frac{\text{Total meq. base} - \text{total meq. acid}}{\dfrac{\text{dry sample wt., mg.}}{161}} \times 100 = \%\ \text{dicarbonyl units}$$

where 161 is the average molecular weight of the repeating unit in starch, 50% of which has been converted to dialdehyde units.

RESULTS

In the course of investigating the reaction of alkali with periodate oxidized starch for analytical purposes two reaction temperatures were employed, each having been tested for varying time periods and on products oxidized to different dialdehyde contents. The results are summarized in Figure 2. For the reactions which are indicated as having been carried out at 70° C., the flasks were immersed to a depth of 1 cm. in a water bath held at that temperature. The temperatures attained by the liquid reaction mixtures under conditions used were as follows.

Heating Time, Seconds	Temperature (° C.) of Reaction Mixture When Heated	
	On steam bath	In 70° C. bath
15	47	45
30	60	49
45	67	55
60	72	58
120	79	63

It can be seen in Figure 2 that the reaction of periodate oxidized starch with alkali does not cease upon consumption of 1 mole of alkali per dialdehyde unit. Yet there is apparently a definite primary reaction which occurs involving this stoichiometric ratio and which is manifest in an inflection point of the curves (steam bath) or in the markedly lowered rate of reaction (70° C.) beyond that point. The rate of secondary reaction is considerably more pronounced at the higher temperature. Heating times might be selected to form the basis of an analytical method to be carried out at 70° C. At this temperature it is obvious that higher precision might be expected, since slight deviation from the time selected would have less influence on the alkali consumption. However, for convenience, and because the method was required in these studies primarily as a guide, most

Table I. Per Cent Oxidation of Periodate Oxystarch Determined by Three Independent Methods

Per Cent Oxidation[a]		
Periodate consumed in preparation[b]	Hydroxylamine[c]	Alkali consumption
1.0	2.2	1.4
5.0	6.0	6.0
10	11	11
20	21	22
40	41	41
48	50	50
59	60	63
73	71	78
76	77	78
78	73	76
89	87	86
96	94	96
97	93	96
98	90	93
[d]	94	94
[e]	89	95

[a] Number of dialdehyde units in 100 repeating units.
[b] Corrected for additional periodate consumed at nonreducing end groups, as estimated by formic acid production during the oxidation.
[c] Determined by slight modification of the method employed by Gladding and Purves (*3*).
[d] Periodate oxidized cellulose.
[e] Periodate oxidized dextran.

Table II. Comparison of Dialdehyde Contents of Selected Oxystarch Samples Measured by Alkali Consumption (AC)

(Under two reaction conditions with values obtained by other methods)

Sample (Figure 2)	AC, Steam Bath, 1 Min.	AC, 70° C., 2 Min.	Sodium Borohydride (*7*)	Hydroxylamine	Periodate Consumed in Preparation
A	99	97	99	85	..
B	63	60	56	60	59
C	11	11	12	12	10

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of these analyses were carried out using the steam bath temperature as described in the experimental section.

That the consumption of alkali in the initial reaction phase is actually a measure of dialdehyde content is confirmed by the good agreement in most cases between analytical values obtained by the alkali consumption method and those from three independent procedures for assaying the dicarbonyl content of oxypolysaccharides. Comparative data are shown in Tables I and II.

Table III. Precision of Analyses by Alkali Consumption Method

Approximate Dialdehyde Content, %	No. of Samples in Set	Av. Dev.	Std. Dev.	Reaction Conditions	Operator
99	9	1.53	2.14	Steam bath, 1 min.	1
99	10	1.74	2.12	Steam bath, 1 min.	1
99	10	0.88	1.25	Steam bath, 1 min.	2
99	9	1.58	1.99	Steam bath, 1 min. potentiometric to pH 7.1	1
60	10	0.71	0.92	70° C., 80 sec.	1
42	9	0.97	1.34	Steam bath, 1 min.	1

Values obtained by the alkali consumption procedure might be expected to be somewhat high, since the secondary reaction with alkali is undoubtedly proceeding during the initial heating period. This is shown by the trend in Table II for values obtained at steam bath temperature to be higher than those when reaction was at 70° C. However, values were not consistently higher than those obtained from the independent methods used.

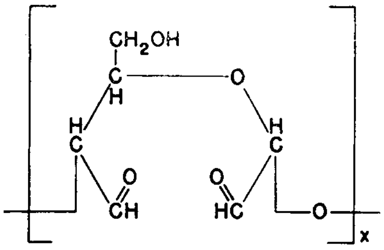

Figure 1. Repeating unit of periodate oxystarch

The precision to be expected of the alkali consumption method is exemplified by the data of Table III, which include analyses run on different samples, different days, by different operators, by use of indicator or potentiometer, and at 70° C. versus steam bath temperature. The average standard deviation of the analyses for essentially completely oxidized samples was 1.88.

DISCUSSION

Literature reports (*2*, *5*) have indicated the occurrence of an internal Cannizzaro reaction when certain polyaldehydes produced by the periodate oxidation of carbohydrates were treated with alkali. In one case (*2*) stoichiometric consumption of 1 mole of alkali per mole of the simple dialdehyde, L′-methoxy-L-methyldiglycolaldehyde (prepared by periodate oxidation of α-methyl-L-rhamnopyranoside), was demonstrated. Acid hydrolysis of the product from the alkali treatment, and identification of the fragments, confirmed the occurrence of a Cannizzaro reaction. However, treatment of starch in a similar manner resulted in consumption of only 0.7 mole of alkali per dialdehyde repeating unit. In general, periodate oxidized starch, cellulose, and structurally related polysaccharides are characterized by instability in even mildy alkaline solution, manifested by degradation, production of acidic groups, and formation of colored products. Standardized conditions have been developed under which 1 mole of alkali is consumed for each dialdehyde group in oxystarches having widely varying degrees of oxidation. Indications are that the conditions are valid also for periodate oxidized dextran and cellulose. Although the mechanism of action and reaction products resulting from this alkaline reaction are unknown at this time, it is probable that the predominating reaction is a dismutation of the Cannizzaro type.

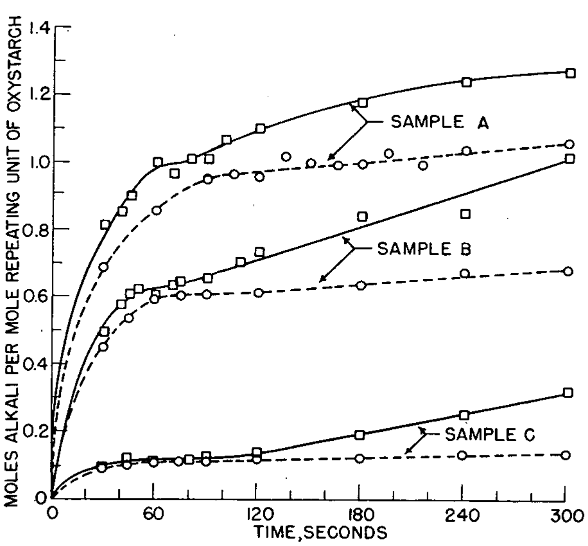

Figure 2. Effect of reaction time on consumption of alkali at different temperatures and dialdehyde contents

— Analyses carried out on steam bath
--- Analyses carried out in 70° C. water bath

The alkali-consumption procedure is currently in routine use at this laboratory for assaying periodate oxidized starches in research directed toward improved procedures for preparing these materials. Future work on the underlying chemical changes involved in this alkali reaction is contemplated.

ACKNOWLEDGMENT

The authors are indebted to R. L. Mellies and J. C. Rankin for the determinations of dialdehyde content of oxystarches by means other than the alkali consumption method, and to P. R. Watson for his assistance in evaluating the precision of the present method.

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Colorimetric Determination of Palladium with Alpha-Furildioxime

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A spectrophotometric method for the determination of microgram quantities of palladium with α-furildioxime was developed. Palladium forms with the reagent in a 0.1 to 1.4*N* hydrochloric acid medium, a yellow-colored complex which obeys Beer's law at 420 mμ. The colored complex is readily extractable into chloroform, in which medium it adheres to Beer's law at the 380 mμ wave length. In the aqueous phase, in the presence of 10% ethyl alcohol, up to 5 γ of palladium per ml. of final volume, the colored complex is stable for 20 minutes, after which time the absorbance decreases slowly. In the organic phase the colored complex is stable for at least 24 hours. This reagent is highly specific for palladium. Platinum group metals and gold do not interfere. In the presence of highly colored ions, extraction with chloroform eliminates all interference, except interference of cyanide ions, which prevent the formation of the palladium α-furildioxime complex. The method is very sensitive, as concentrations as low as 0.1 p.p.m. of palladium can be determined in 5-cm. cells. The precision at the optimum spectrophotometric range is within 1%.

Of THE several methods generally recommended for the determination of microgram quantities of palladium, as indicated in a recent article by Beamish and McBryde (*1*), the most sensitive reagent is *p*-nitrosodiphenylamine (*5*). The method in which this reagent is used requires exact control of pH and, furthermore, its utility is decreased somewhat by the interference of high salt concentration, oxidizing agents, and gold. Recently, Yoe and Kirkland (*4*) described a method in which palladium is determined with *p*-nitrosodiphenylamine after the separation of the palladium from most of the platinum group metals by extraction of the diethyldithiocarbamate salts of these metals.

Reed and Banks used α-furildioxime (*3*) for the gravimetric estimation of palladium and nickel, and also demonstrated that this reagent can be utilized as a very sensitive, qualitative test for these elements. It was the purpose of this investigation to determine the applicability of the α-furildioxime reaction to the quantitative estimation of palladium in an aqueous solution and in an organic solvent extract.

APPARATUS AND REAGENTS

Spectrophotometer. Absorbance measurements were made with a Beckman spectrophotometer, Model DU, in 1-cm. cells. The absorption spectrum curves were made with the Warren Spectracord attachment to the Beckman spectrophotometer.

Standard Palladium Solution. A standardized stock solution was prepared by dissolving palladium chloride in concentrated hydrochloric acid. The palladium content of this stock solution (2.97 mg. of palladium per ml.) was established gravimetrically by the dimethylglyoxime method (*3*). The standard solutions, which were used in this work, were made by appropriate dilution of aliquots of this stock solution.

Reagent Solution. A 1% solution of α-furildioxime was prepared by dissolving 1 gram of the purified reagent in 30 ml. of absolute ethyl alcohol, followed by dilution with water to a volume of 100 ml. The purification procedure as outlined by Reed and Banks (*3*) for the α-furildioxime reagent was followed in its entirety.

Solutions of Diverse Ions. Reagent grade salts were employed in the preparation of solutions of inorganic ions. All interfering cations were added as chlorides except thorium nitrate, lead nitrate, nickelous sulfate, manganous sulfate, and zirconium perchlorate. Sodium salts were used for the anion studies.

Other Reagents. All other reagents were analytical grade and were used without further purification.

COLOR REACTION

The reaction of palladium with α-furildioxime in an acid medium was found to give a yellow color which develops very rapidly. This same yellow color was found to be extractible into chloroform.

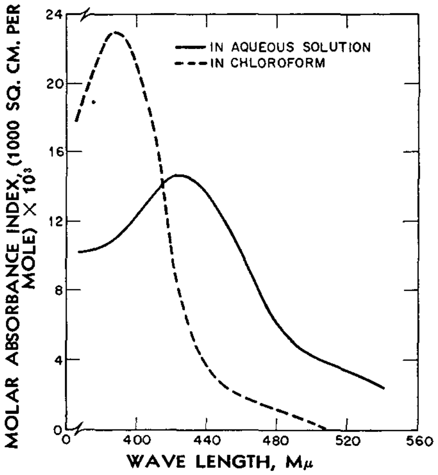

Figure 1. Molar absorbance index of palladium α-furildioxime

Palladium α-Furildioxime in Aqueous Solution. The yellow complex of palladium α-furildioxime was formed by transferring 59.0 γ of palladium (as palladium chloride) to a 25-ml. volumetric flask, and adding successively 1 ml. of concentrated hydrochloric acid, 2.5 ml. of 100% ethyl alcohol, 10 ml. of water, and 1 ml. of reagent solution of α-furildioxime, followed by dilution with water to the calibrated volume. As shown in Figure 1, the complex exhibits an absorption peak at 420 mμ, when measured against a reagent blank solution. The purified reagent does not absorb at this wave length, whereas the unpurified reagent absorbs only slightly.

Palladium α-Furildioxime in Organic Solution. The yellow complex of palladium α-furildioxime was formed by transferring 74.0γ of palladium (as palladium chloride) to a 30-ml. separatory funnel, and adding successively 1 ml. of concentrated hydrochloric acid and the requisite amount of α-furildioxime. A 10-ml. portion of chloroform is sufficient to remove the palladium complex from the aqueous solution after a 20-second shaking period. The organic layer is removed and collected in a 25-ml. volumetric flask. Approximately 0.5 gram of anhydrous sodium sulfate is added and the extract is diluted to volume with chloroform. The complex, as shown in Figure 1, exhibits an absorption peak at 380 mμ, when measured against chloroform, the reagent blank solution.

Stability of Color. To test the stability of the color in the aqueous medium, the colors of solutions containing 15, 47, 83, and

120 γ of palladium were developed by the above procedure. Complete color development was reached before measurement of the color intensity could be made, although the absorbance measurements were taken immediately, as well as over a period of several hours. The solutions containing low concentrations of palladium (15 γ) were stable for 2 hours, but in solutions containing the higher concentrations of palladium a gradual decrease in absorbance was observed after 20 minutes. The color in the chloroform extract was stable for the 24-hour period tested.

Effect of Temperature. The solutions were maintained at room temperature through the experiment, but it was observed that when a test solution was heated to 80° C., the palladium α-furildioxime precipitated immediately.

Effects of Hydrochloric Acid. The effect of hydrochloric acid on the color reaction was studied to determine the optimum concentration of this acid. In aqueous solutions it was noted that when the concentration of hydrochloric acid was made greater than 1.4N, a marked decrease in absorbance occurred. It was also observed that if the color reagent is added to a strong hydrochloric acid solution, cloudiness occurs. This cloudiness was eliminated by diluting the sample to 20 ml. prior to the addition of the chromogenic reagent. The development of cloudiness in the samples prior to extraction with chloroform did not impair the efficiency of the extraction. If a precipitate of palladium α-furildioxime is formed in the aqueous phase, it is of no significance in the method, since it is readily soluble in the organic phase.

Ethyl Alcohol Concentration. It was observed that the colored complex of palladium α-furildioxime precipitates when concentrations of palladium in excess of 30 γ were tested. To increase the working range, various concentrations of ethyl alcohol were tried. It was observed that in a 10 (v./v.) % solution of ethyl alcohol, concentrations as high as 120 γ of palladium in a final volume of 25 ml. could be analyzed without the occurrence of precipitation, but higher concentrations of ethyl alcohol only delayed the color formation and did not increase the stability of the color. The presence of ethyl alcohol in the aqueous solution, however, in the extraction procedure is detrimental. Its presence in excess of 1% by volume reduced the efficiency of the extraction.

Table I. Absorbance Index of Palladium α-Furildioxime

Pd, γ	Absorbance, A	Absorbance Index, A_s
11.8	0.067	142
23.6	0.137	145
35.4	0.199	140
47.2	0.267	141
59.0	0.327	138
74.0	0.408	138
	Av., $\bar{x}$	141
	Std. dev., S	3.0
	Coefficient of variation, V	2%

Table II. Extraction of Palladium α-Furildioxime in Chloroform[a]

Pd, γ	Absorbance, A	Absorbance, Index, A_s
14.8	0.133	226
29.6	0.265	223
44.4	0.394	222
59.2	0.525	222
74.0	0.650	220
	Av., $\bar{x}$	223
	Std. dev., S	2.3
	Coefficient of variation, V	1%

[a] For method of calculation see Table I.

Reagent Concentration. A molar excess of at least 20 to 1 of reagent over palladium in the aqueous solution is required in order to obtain the maximum absorbance within 3 to 5 minutes. In the presence of a smaller excess of the reagent, the colored complex was slow to develop. Large excess of the reagent, as much as 72M excess, did not affect either the rate or the color intensity of the compound. Similarly, it was found that for extraction of the colored compound with chloroform, a large excess of reagent was required. When solutions were heated on a water bath at 80° C., however, a 2 to 1M ratio of α-furildioxime to palladium was sufficient to yield maximum absorbance in the organic phase.

EXPERIMENTAL

Calibration Curve for Aqueous Solutions. Aliquots of a standardized, concentrated solution of palladium were transferred into 25-ml. volumetric flasks, to which 1 ml. of hydrochloric acid, 2.5 ml. of ethyl alcohol, and sufficient water to give a 20-ml. volume were added. Then 1 ml. of α-furildioxime solution was added, and the volumetric flask was filled to its calibrated volume with water. The absorbance was measured in a 1-cm. cuvette within 10 minutes at a wave length of 420 mμ against a reagent blank solution. The calibration curve is linear and follows Beer's law. In a range of concentrations from 12 to 74 γ of palladium in a volume of 25 ml., as shown in Table I, the coefficient of variation for the absorbance index is 2%.

Table III. Effect of Certain Cations on Determination of Palladium in Aqueous Solution by α-Furildioxime Method

Duplicate determinations.
All solutions contained 59.0 γ of palladium.
Not in order determined—arranged in order of differences.

Element	Amount Present, Mg.	Pd, Found, γ	Difference[a] γ	Difference[a] %
Ir^{+4}	0.06	59.0	0.0	0.0
Pt^{+4}	0.7	59.0		
Fe^{+++}	2.0	59.0		
Cd^{++}	2.1	59.0		
Os^{+4}	3.3	59.0		
Cu^{++}	3.7	59.0		
Na^{+}	3.8	59.0		
U^{+6}	8.4	59.0		
Th^{+4}	25.0	59.0		
Rh^{+++}	0.8	58.1	−0.9	−1.6
Zr^{+4}	1.9	58.1	−0.9	−1.6
Pb^{++}	2.3	58.1	−0.9	−1.6
Mo^{+6}	4.0	59.4	+0.3	0.6
Cr^{+++}	4.3	59.4	+0.3	0.6
Mn^{++}	1.0	59.9	+0.9	1.5
Ni^{++}	1.1	59.9	+0.9	1.5
Au^{+++}	2.5	61.6	+1.6	2.7
Co^{++}	2.5	60.8	+1.8	3.1
Av., $\bar{x}$		59.2		
Std. dev., S		0.3		
Coefficient of variation, V		0.6%		

[a] Differences are not statistically significant at the concentration level shown above.

Purified α-furildioxime was used as the chromogenic reagent for the aqueous solution. It was noted that when the purified reagent is used the sensitivity is increased by 5% and does not introduce the necessity for a correction for the absorbance of the reagent blank.

$$A_s = \frac{A}{Cb}$$

where A_s = absorbance index
A = absorbance
b = length of absorbing layer of material, cm.
C = concentration in grams/1000 grams of solution

Calibration Curve for Organic Solution. Aliquots of the standardized concentrated solution of palladium were transferred into a 30-ml. separatory funnel to which 1 ml. of hydrochloric acid, 1 ml. of α-furildioxime, and 10 ml. of chloroform were added. A chloroform-insoluble stopcock grease was used, prepared from starch and glycerol (*2*). The solutions were shaken for 20 seconds, following which the chloroform phase was removed and collected in a 25-ml. volumetric flask. With 10 ml. of chloroform droplets of chloroform were removed from the aqueous layer and combined with the first extract. The organic phase was freed of traces of water by the addition of 0.5 gram of anhydrous sodium sulfate. The flask was filled to its calibrated volume with chloroform, following which the absorbance was measured in a 1-cm. curvette at a wave length of 380 mμ against a chloroform blank solution. The calibration curve, although not shown, is linear and follows Beer's law. The data from which this curve can be constructed are shown in Table II. The coefficient of variation for the absorbance index in the concentration range from 15 to 74 γ of palladium is 1%.

EFFECT OF DIVERSE IONS

To the extent that the effects of other ions were examined (Tables III, IV, and V), no serious interference was observed.

Table IV. Effect of Certain Anions on Determination of Palladium in Aqueous Solutions by α-Furildioxime Method

(All solutions contained 59.0 γ of palladium)

Anion	Amount Present, Mg.	Pd, Found, γ	Difference[a] γ	Difference[a] %
F^-	7.15	58.1	0.0	0.0
SO_4^{--}	6.90	58.8	0.2	0.3
NO_3^-	7.29	58.6	0.4	0.7
ClO_4^-	7.15	58.1	0.9	1.5
CN^-	4.0	0	59.0	100
Av., $\bar{x}$			58.6	Excluding CN^-
Std. dev., *S*			0.4	Excluding CN^-
Coefficient of variation, *V*			0.7%	Excluding CN^-

[a] Except in the case of CN^-, the differences are not statistically significant at the concentration tested.

The platinum group metals, including gold, do not form a complex with the reagent. The chlorides of ruthenium, rhodium, iridium, platinum, and gold do, however, form deeply colored solutions, and in their presence a sample blank must be used as a reference solution. The interference due to the presence of highly colored ions is best eliminated by extraction with chloroform, as shown by the data presented in Table V. As shown in Table IV, large quantities of anions can be tolerated with the exception of the cyanide ion.

DISCUSSION

In a dilute hydrochloric acid medium, microgram quantities of palladium form a yellow-colored complex with α-furildioxime which obeys Beer's law when absorbance measurements are made at 420 mμ. In aqueous solutions containing 10% by volume of ethyl alcohol, with 1 to 5 γ of palladium per ml., a colored complex is formed which is stable for at least 20 minutes. After that period a gradual decrease in absorbance is noted. A large excess of the reagent is required for the rapid formation of color. In the case of purified reagents, this large excess can be tolerated, because it does not absorb in the wave-length region around 420 mμ. The reagent, under the conditions of this method, is specific for palladium, except that colored ions absorbing at this wave length present some minor difficulties. The absorbance of the interfering substances can be compensated for by incorporating them in reference solutions. To achieve initial instrument balance, however, either the slit must be widened, the intensity of the light source increased, or the sensitivity of the photosensitive detector increased. This imposes an upper limit on the quantities of colored ions which can be handled successfully.

The palladium complex can be extracted readily from the aqueous phase with chloroform. The absorbance of this compound obeys Beer's law when measured at a wave length of 380 mμ. The quantity of reagent may be reduced to a 1 to 2 ratio of palladium to α-furildioxime, but then the solution must be heated to allow the complex to form and precipitate prior to the extraction. This would indicate that the compound extracted is $Pd(C_{10}H_7O_4N_2)_2$ as reported by Read and Banks (*3*) in the gravimetric method for estimating palladium. The advantage of the extraction procedure over the aqueous color system is that all interferences, due to the absorbance of colored ions which may be present in the solution, is thus eliminated.

Table V. Effect of Certain Highly Colored Cations on Determination of Palladium in Chloroform Extracts by α-Furildioxime Method

(All solutions contained 74.0 γ of palladium)

Element[a]	Amount Present, Mg.	Pd, Found, γ	Difference γ	Difference %
Pt	7.31	74.0	0.0	0.0
Rh	3.94	75.0	+1.0	1.3
Ir	2.85	72.7	−1.3	1.7
Au	13.30	75.3	+1.3	1.7
Ru	5.45	71.8	−2.2	2.8
Av., $\bar{x}$		73.8		
Std. dev., *S*		1.4		
Coefficient of variation, *V*		2%		

[a] No attempt was made to check any ion that was not highly colored.

The precision of the method over the range of palladium concentrations, yielding for spectrophotometric measurement, a minimum relative error is 1 to 2%.

CONCLUSION

α-Furildioxime can be utilized as the reagent in a highly selective method for the spectrophotometric estimation of palladium. Cyanide interferes seriously and must be removed prior to the addition of the α-furildioxime. At relatively low concentrations of substances which yield colored ions in solution, no significant interference occurs. On the other hand, in the chloroform extraction procedure none of the commonly encountered ions interfere even when present in relatively high concentrations. . The palladium complex is stable for at least 24 hours in the chloroform extract, whereas in aqueous solutions a gradual decrease in stability occurs after 20 minutes. The precision of the method under the conditions reported herein is within 1%.

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Improvements in Isotopic Carbon Assay and Chemical Analysis of Organic Compounds by Dry Combustion

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Modifications in the apparatus and combustion procedures used in this laboratory afford certain advantages over the original design. Improvements are described with regard to the combustion tube filling and arrangement, the removal of nitrogen oxides, the trap and manometer system, and the carbon-14 assay method, using proportional gas counters.

IMPORTANT modifications have been made in the apparatus (*1*) by which micro samples of organic compounds are burned to carbon dioxide and water, the latter being measured in a low pressure gas manometer and then employed directly for isotopic assay. In the search for improvements in what has proved in 4 years to be a basically sound apparatus, several changes have afforded advantages in rapidity of operation as well as accuracy and reproducibility of results. The alterations are chiefly in the combustion tube and filling, the removal of nitrogen oxides, the trap and manometer system, and the isotopic assay for carbon-14.

COMBUSTION TUBE AND FILLING

The combustion tube, its filling, and operation have been changed as described by Kirsten (*8, 9*) (Figure 1). This system operates at 930° to 970° C. with a minimum of tube filling and on the principle of thermally cracking the organic compound first and then oxidizing the reactive fragments in the flowing oxygen. As a result, combustions are complete in much shorter times, preconditioning time for new tubes is greatly reduced, and the likelihood of radioactive contamination from sample to sample is essentially eliminated. The sample is placed inside a platinum thimble and run directly into the main combustion furnace. The oxygen flow is about 30 cc. per minute, and the total combustion time is only 30 to 40 minutes. This filling, which consists of a nickel fin and sleeve, has been satisfactory with all compounds tried so far and is less tedious to use than the standard copper oxide filling. An automatic advancing mechanism is used to move an electromagnet which pushes the sample into the furnace; it is capable of varying speeds, but normally about 10 minutes is required to move the sample into the furnace.

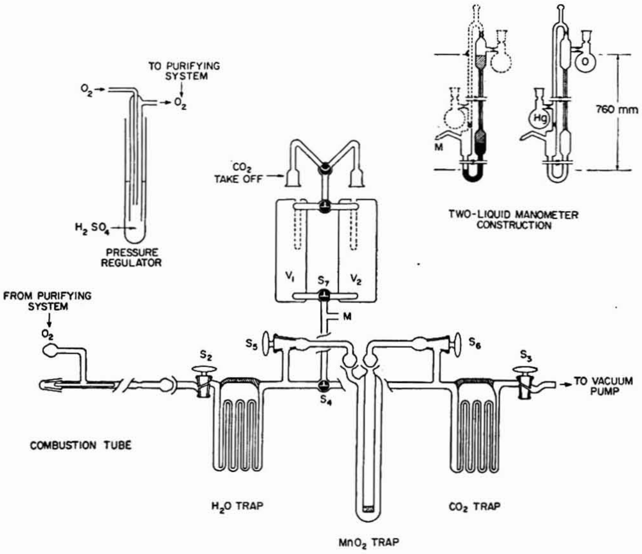

Figure 1. Combustion line and gas measurement system, manometer construction, and pressure regulator

The parts used inside the combustion tube are conveniently prepared by hand from pure 10-mil nickel foil. Just prior to insertion in the quartz tube, these nickel components are heated to about 130° C. for 30 minutes in concentrated sulfuric acid and washed, as recommended by Kirsten, to decrease the time required for obtaining a low blank. The silver, used in the form of balls of fine wire in the tube, must also be pretreated to reduce the blank. The procedure of Niederl and Niederl (*11*) is satisfactory and consists of heating the silver components in porcelain boats in a quartz tube to 400° C. for 1 hour with hydrogen and then 1 hour with oxygen. When the parts are treated in this manner, a blank of less than 1 mm. in the carbon dioxide trap is found in 40 minutes, following a preconditioning time of 1 to 2 days. A small blank persists throughout the life of the tube, but this is also observed when a Pregl filling is used, and its magnitude is such that it is ignored in actual practice. This blank is not due to water, since if the oxygen purification train is functioning properly no detectable water blank is observed. A list of specimen analyses with this filling is shown in Table I.

Table I. Specimen Analyses

Compound	Sample, Mg.	C, % Theoret.	C, % Found	H, % Theoret.	H, % Found
Benzoic acid	5.255	68.84	68.87	4.95	4.83
	8.237		69.13		4.89
	5.901		69.12		4.91
	10.680		68.86		4.93
	6.067		68.44		4.99
Isocaproanilide	4.724	75.35	75.23	8.95	9.02
	6.894		75.16		8.96
o-Chlorobenzoic acid	6.028	53.64	53.40	3.22	3.22
	7.702		53.84		3.21
Anthracene	6.340	94.34	93.50	5.66	5.69
Acetanilide	9.026	71.08	70.95	6.71	6.76
	7.519		70.84		6.72
	5.205		71.91		6.76
	6.769		71.08		6.66
Glutaric acid	4.450	45.45	45.57	6.10	6.04
	4.359		45.62		6.07
Acridine	4.442	87.12	87.28	5.06	5.13
	5.950		87.91		5.23
Benzanilide	4.654	79.16	78.60	5.62	5.69
	7.103		78.90		5.72

If the temperature observed on the thermometers in the wells of the volumes (V_1 and V_2, Figure 1) is not reasonably constant (within about 2° C.), the calibration and analysis measurements should all be corrected to a standard reference temperature by a gas law calculation.

Certain compounds which contain other elements in addition to carbon, hydrogen, oxygen, and nitrogen often prove intractable or cause undue attrition on the standard combustion tube. Iodoform might be cited, as well as alkali salts. The latter leave a residue which attacks the quartz tube, affects water values, and may form active carbonate which can undergo exchange and thus introduce cross contamination. Such compounds may be handled by wet combustion, but the following procedure has been found satisfactory:

The sample to be burned is weighed by difference into a quartz capsule, which can be introduced into a 25 × 200 mm. quartz tube with a male 19/38 standard taper joint. The compound is covered and mixed with 200 to 500 mg. of cupric oxide wire. The main tube is fixed with Apiezon wax to a 4-mm. pressure stopcock with two joints, one for the tube and the other for attachment to the loading line (Figure 2). The tube is pumped out, if the compound is not volatile, and 0.5 atmosphere of oxygen is introduced. With volatile compounds, it must be flushed out with oxygen and closed at 1 atmosphere. The quartz tube is then heated to 700° C. for 1 hour and attached while hot to the loading line, and the evolved carbon dioxide is trapped, measured, and put into counting tubes. The combustion apparatus can then be disassembled, the cupric oxide discarded, and the tube decontaminated by heating in a concentrated nitric acid–concentrated sulfuric acid bath, washed, steamed, and dried for reuse.

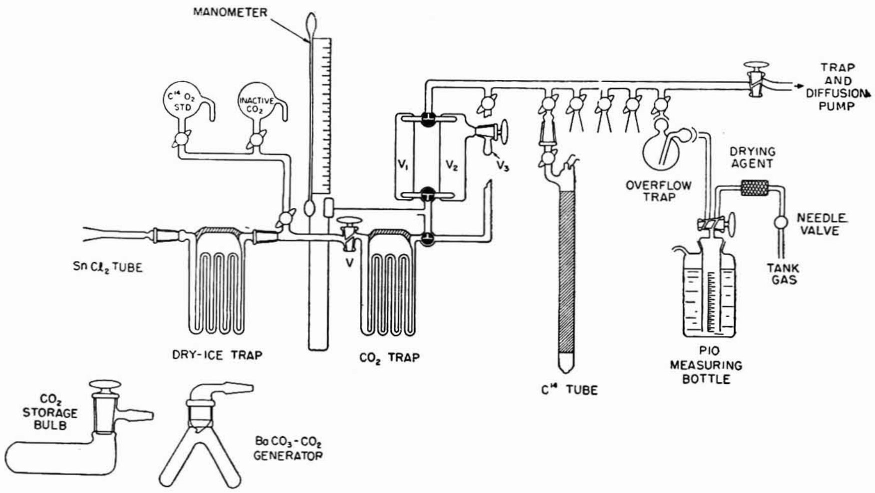

Figure 2. Sample loading and dilution line

REMOVAL OF NITROGEN OXIDES

The problem of removal of nitrogen oxides during combustions of organic compounds has not been satisfactorily solved in the past. The problem is magnified when the products of the combustion are desired for isotopic assay, because the usual methods, such as heated lead dioxide, cannot be employed. Such reagents involve an equilibrium with carbon dioxide, as well as water (*12*), and would therefore introduce cross contamination between samples if used for this purpose. Furthermore, when it is desired to obtain the carbon dioxide in the gaseous form, by freezing it out with liquid nitrogen, the use of any reagent for the removal of nitrogen oxide involving the formation of any volatile product which freezes at liquid nitrogen temperature is precluded. With these restrictions, the method for the removal of nitrogen oxide which has been in general use on trains used for isotopic assay has been reduction over hot copper (*1, 6*). This method is time-consuming, because of the necessity of freezing out all volatile products and pumping off the oxygen present before passing the combustion products over the hot copper. It is also extremely sensitive to temperature. In many cases, unsatisfactory carbon results have occurred when the temperature of the copper varied as little as 5° C. from the optimum temperature (about 455° C).

Belcher and Ingram (*2*) noted the feasibility of using manganese dioxide for the absorption of nitrogen oxide, and Kirsten (*8*) recommends the use of this compound. Results indicate that it is completely satisfactory under the conditions prevailing in the present system. It not only appears to give complete removal of nitrogen oxide, but it can be used at room temperature, and continuously during the combustion period, thus eliminating the necessity of the extra step of freezing out and pumping off of oxygen before the removal of the nitrogen oxide.

The manganese dioxide trap is placed between the water trap and carbon dioxide trap in such a way that it can be isolated from the system by means of stopcocks S_{-5} and S_{-6} (Figure 1) if nitrogen is absent. When nitrogen is present, the sample is placed in the fore part of the combustion tube, and the oxygen flow is adjusted as usual by means of stopcock S_{-2}, using the manometer as a flow meter (see following section). S_{-5} and S_{-6} (2-mm. vacuum stopcocks) are closed during this operation. After the flow is correct, the dry ice and liquid nitrogen baths are put in place, S_{-5} and S_{-6} are opened, and S_{-4} is turned so that it faces the carbon dioxide trap. This still gives a pressure reading on the manometer, to indicate the oxygen flow, but forces the combustion gases to pass through the manganese dioxide trap. At the end of the combustion, S_{-5} and S_{-6} are closed first, then S_{-2} is closed, and S_{-4} is turned so that the water trap will also be pumped out. The amount of carbon dioxide and water is then measured normally, and the carbon dioxide either placed in counting tubes directly or in bulbs for other treatment.

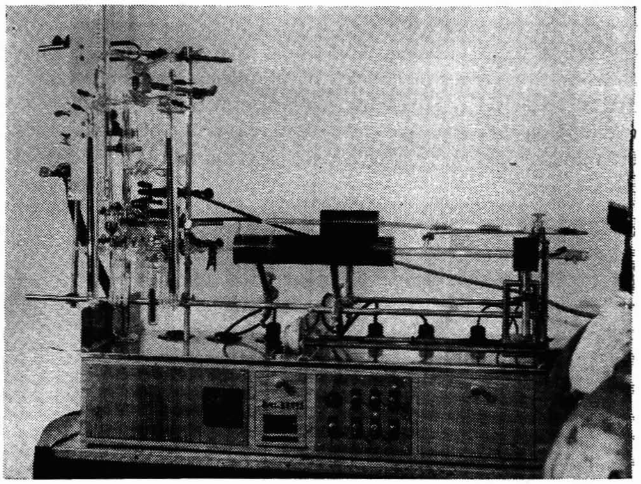

Figure 3. Combustion line as designed by Kirsten and modified by present gas measurement and collection system

The manganese dioxide trap is not pumped out during an analysis, because a slight additional blank is contributed to the carbon dioxide by such a procedure, and it has been found unnecessary even from the standpoint of possible holdup of carbon dioxide in this trap. To test this, a sample of benzoic acid containing 12 millimicrocuries of carbon-14 per milligram of carbon (an amount of carbon dioxide producing 70,000 c.p.m.) was burned and its carbon dioxide passed through the trap. Without pumping on the manganese dioxide trap, a sample of inactive acetanilide was burned and the products were passed through the trap. The carbon dioxide so obtained was placed directly in a counting tube and gave a count of 81.6 ± 1.1 c.p.m., as against a background of 82.5 ± 0.9 c.p.m. with the same tube filled only with P-10 gas, with standard deviations as shown. This clearly eliminates any need to pump down the manganese dioxide trap between combustions.

The manganese dioxide trap (Figure 1) is 15 cm. long over-all, 22 mm. in outside diameter, with an inner tube about 12 mm. in outside diameter, having a coarse glass frit sealed into its bottom end. About 2.5 grams of 10- to 20-mesh manganese dioxide is placed in the inner tube, covered by a plug of glass wool, which is topped with a 5- to 6-cm. layer of Anhydrone followed by another plug of glass wool. The quality of the manganese dioxide is critical in this application. Ordinary manganese dioxide suspended on asbestos gives erratic results. On the advice of the Shell Development Co. (*13*) specially prepared manganese dioxide (Laboratory Equipment Co., St. Joseph, Mich., No. 501-C) was tried. Experience of the Shell Development Laboratories indicates that this manganese dioxide is sufficiently pretreated and regenerated by heating to 120° C. overnight (*2*, *13*) and that under normal combustion conditions the capacity of this material is 30 to 90 mg. of nitrogen dioxide per gram of manganese dioxide. This manganese dioxide works equally well under present conditions, where the trap pressure is about 1 cm. It is replaced when 30 mg. of nitrogen dioxide per gram of manganese dioxide has been trapped, although it has not been exhausted by that time.

This calculation is made on the basis of nitrogen as nitrogen dioxide, although this may not be the oxide present. If the nitrogen oxide were held in the water trap, incorrect hydrogen values would be expected. However, no effect has been noted on hydrogen analyses of any nitrogen-containing samples. On the other hand, it has been stated (*4*) that 1 part of nitrogen dioxide in 10,000 parts of carbon dioxide will lower the counting rate several per cent in proportional counting tubes of design similar to the present tubes, although no filling gas was employed in that case. As no depression in the expected counting rate has been noted with nitrogenous samples under the present conditions, it is apparent that no nitrogen dioxide is present in the carbon dioxide obtained with the present apparatus. Furthermore, if nitrogen oxides were present in the carbon dioxide, an incorrect determination of the amount of carbon dioxide placed in the counting tubes would ensue, with a consequent incorrect specific activity value being obtained even if the nitrogen oxide had no untoward effect on the counting of the sample. With the use of manganese dioxide, the expected specific activity has been obtained in all cases where it was possible to calculate this value, within the experimental error.

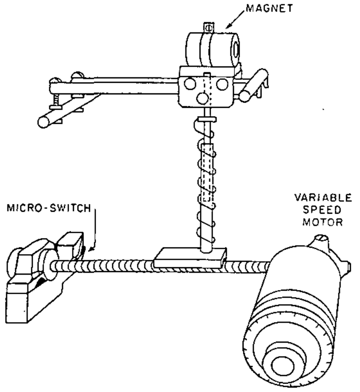

Figure 4. Automatic sample advancing mechanism

Belcher and Ingram (*2*, *7*) have stated that nitrogen dioxide, and not nitric oxide, is absorbed by manganese dioxide at room temperature. However, nitrogen dioxide, with a melting point of −9.3° C., would not be expected to pass through the dry ice-cooled water trap used in this apparatus. Therefore, the question of the identity of the nitrogen oxide present at the various stages of this absorption system will be investigated further, in an effort to determine the actual processes involved in this procedure.

TRAP AND MANOMETER SYSTEM

Various modifications of the original system have been made in the interest of efficiency and convenience. For example, the traps for collecting the carbon dioxide and water have been changed to multiple U-traps of the type described by Buchanan and Nakao (*5*) (Figure 1).

A convenient method of calibrating the two-liquid, low pressure manometer for use as a flow meter is to measure the inner diameter of the open-ended tube in the pressure regulator (Figure 1) before the oxygen prepurifying furnace, then to calculate the volume of a height of 2 or 3 cm. in the tube. When this point is marked on the outer tube, which contains concentrated sulfuric acid, it is possible to measure the length of time taken for the acid to rise and fill the calculated volume by shutting off the oxygen flow at the tank when the meniscus is at the bottom of the inner tube. In this way, the oxygen flow in cubic centimeters per minute can be determined for any given reading on the manometer.

In the filling of the low pressure, two-liquid manometers (Figure 1), several changes in procedure are advantageous. In order to prevent any of the oil from getting into the lower part of the manometer, all of the mercury is now distilled in first. If each of the lower bulbs is filled somewhat more than half full, a sufficient amount of mercury must be present. The constriction below the flask from which the mercury is distilled is then sealed off, to prevent any oil from being carried over to that side of the manometer. Only then is the oil degassed, by alternate freezing and thawing. It is almost impossible to keep the oil from bumping into the manometer occasionally during this procedure, making the above treatment of the mercury desirable.

After the oil (commonly dibutoxytetraethylene glycol) (*1*) has been completely degassed, it is distilled into the manometer until approximately the correct amount is present. Before the top of the manometer is sealed off above the upper bulb, an advantage in the ultimate vacuum obtained above the oil may be accomplished by slowly allowing air to enter above the mercury, thus driving the oil into the upper reservoir. The top constriction, above the upper reservoir, is then sealed off, after the bulb containing the unused oil has been sealed off and removed. The mercury filling bulb is also removed and the manometer removed from the vacuum line. The glass tube arch connecting to the vacuum line may be left in place for additional support for the manometer, if desired.

It is not necessary to be precise about the amount of oil distilled into the manometer, as the zero level of the manometer can be easily adjusted by addition or removal of mercury at any time. If desired, a ball joint can be added to the top of the bulb above the mercury to facilitate this adjustment, with the joint normally being closed by a stopper sealed in place with Apiezon wax.

It is best to make the capillary portion of the manometer somewhat longer than 50 cm., if a 50-cm scale is to be used with the apparatus, to allow free movement of the sliding vernier at both ends of the scale.

The arrangement of the calibrated bulbs is a matter of personal preference. At present, the manometer connects into the system between S_{-4} and S_{-7}, as shown in Figure 1. It is convenient to make one of the two bulbs of such a size that it can be used alone under ordinary conditions (V_2, Figure 1). A bulb with a volume of about 400 cc. will produce a rise per milligram of carbon of about 4.5 to 5 cm. on the manometer, and is a convenient size. The second bulb (V_1), about 200 cc. in volume, is then used in case the pressure of one of the gases becomes too high at some time. With water, furthermore, erratic performance occurs if more than about 0.6 mg. of hydrogen (as water) is present in the 400-cc. volume only, because of condensation on the walls. When a relatively large amount of water is present, it can be allowed to expand into both volumes. It is best to calibrate the system for both volume combinations for both carbon dioxide and water, when this arrangement is used.

If a stopcock and hose attachment is provided on the portion of the system to which the counting tubes are attached (above the volumes), this portion of the system can be pumped out by a separate vacuum pump even during a combustion. This can result in a considerable saving of time, as the pumping out of air in this space through the volumes and carbon dioxide trap is rather slow.

Figure 3 is a picture of the apparatus designed by Kirsten (*9*), as modified by the substitution of the authors' measurement and gas collection system.

A combustion line incorporating similar features, made by laboratory personnel, is also in use in this laboratory. The main problem involved is in providing a combustion furnace which operates well at 950° C., since the micro combustion furnaces generally available in this country do not operate satisfactorily, if at all, at such temperatures. At present, the simplest solution seems to be the winding of a furnace on a ceramic base with Nichrome or Kanthal resistance wire, with the temperature being controlled by means of a Variac and measured with a thermocouple.

In this apparatus, the automatic sample-moving action is provided by means of a governor-controlled, variable-speed motor which is attached directly to a screw drive (Figure 4). A threaded shoe fits over the top half of the screw and is attached, through a tube fitting over a shaft, to a platform which moves on a track. The magnet (Ace Glass, Inc., Vineland, N. J., catalog No. 6556) is mounted on the platform. The shoe and the tube attached to it can be raised over the shaft attached to the platform, so that the magnet can be moved back to the front end of the combustion tube after each analysis. The shoe is held down on the screw by a spring which is attached to a collar on the shaft below the platform, and to the shoe itself. A microswitch is provided which turns off the motor when activated by the shoe as it approaches the combustion furnace.

ISOTOPIC ASSAY

Ordinarily the carbon dioxide from a sample can be loaded directly into the counting tube(s) (*3, 15*) on the combustion line, with no loss in counting efficiency. If erratic counting behavior has been encountered with any sample, the carbon dioxide is collected in a glass bulb and then passed through a tube containing stannous chloride (*10*), then through a dry ice multiple U-trap, and finally collected in a liquid nitrogen trap. This is done on the same vacuum system used to fill the tubes with the filling gas, and this loading line is provided with a two-liquid manometer and a volume system similar to that used on the combustion lines, to measure the amount of carbon dioxide put into the counting tubes (Figure 2). A mercury diffusion pump is used on this line, to facilitate the pumping out of counting tubes after use. Unless a very active sample has been in a tube, about a half hour of pumping under these conditions is sufficient to remove all traces of the activity. The same can be said about the line itself.

It is also possible to decompose barium carbonate samples on this vacuum line. Concentrated sulfuric acid appears to be a satisfactory agent for the liberation of carbon dioxide. The carbon dioxide evolved is collected in the usual manner in a liquid nitrogen–cooled trap and distilled into counting tubes.

If difficulty is encountered in the counting of any counting tube, or if the carbon dioxide therein is desired for any reason, the counting tube itself can be placed on the sample-loading line and the gases pumped out through the liquid nitrogen trap. The carbon dioxide is retained by the trap and the filling gas is pumped off, after which any desired disposition may be made of the carbon dioxide obtained.

The use of P-10 gas (90% argon, 10% methane) instead of methane as a filling gas offers the advantage that the proportional plateau occurs at about 2100 volts rather than at 3800 to 4000 volts. This allows the use of a 3000-volt power supply, and decreases the possibility of arcing or other high voltage effects. The counting efficiency starts to fall off with a tube filling of more than 8% carbon dioxide, but such an amount is adequate for most samples (about 4 mg. of carbon in a 100 ml. of counting tube). Another property of this filling gas is that the plateau is much sharper than that obtained with methane, although somewhat shorter. This makes it much easier to recognize the plateau, as the effect on the counting rate of being only slightly above or below the plateau is relatively great. It is advisable to take a plateau with each filling of each counting tube, for accurate work. This can be done rapidly by taking three or four 1-minute counts, with an external source if necessary.

The external check source consists of a small amount of a cesium-137 salt evaporated onto a copper strip, the activity covered with polymer film, and the strip soldered to a hose clamp which in turn can be slipped over a counting tube and fastened. The strip is bent into a U-shape, so that the upper edge of the arm again rests against the side of the counting tube when the source is in use. This holds the activity at a reproducible distance from each tube. The source is held about 1.5 inches from the outside of the tube and approximately opposite the middle of the cathode volume, and its strength adjusted to give 5000 c.p.m. or more. If the counting is done in a room in which there are scintillation counters, it is preferable to use a strontium-90 source (pure beta emitter), but in this case each tube should be calibrated against this external source.

A paper has appeared recently (*14*) giving a complete discussion of the use of P-10 gas in glass proportional counting tubes, as well as a description of the amplifier circuit used here for proportional counting.

Provision is also made on the loading line (Figure 2) for the dilution of samples which are too active to be counted in their original concentration. This condition is a function of the scaling circuit on the counter but with a decade scaler of the type commonly used, the maximum safe rate is about 200,000 c.p.m., at which point a coincidence correction of 1.6% applies with a scaler of 5 microseconds resolving time. When the activity is such that more than the maximum counting rate would be observed, the sample is put into the loading line in the usual way, except that the absolute amount of carbon dioxide present from the sample is noted. A small separate volume (V_3) is provided on the system in such a way that it can be closed off from the rest of the manifold, and this is now done. The small volume and the larger ones to which it is attached have been previously calibrated by weighing with and without water (*1*), so that the fraction of the small volume to the rest of the system can be determined. The fraction of the active sample which is trapped in the small volume can thus be calculated.

The remainder of the active sample is removed, either by freezing it into a bulb or by pumping it off (the system should be pumped on for at least 15 minutes in any case, to remove all of the active sample). Inactive tank carbon dioxide, which is passed through a dry ice–cooled, multiple U-trap before storage, is next introduced from a storage bulb attached to the system, and again the absolute amount present is noted. When the active and inactive carbon dioxide is mixed, the dilution factor for the active carbon dioxide is the ratio of the amount of active carbon dioxide to total carbon dioxide times the ratio of the small volume to the volume of the entire system used. In order to ensure thorough mixing of the active and inactive carbon dioxide, it is necessary to freeze and thaw the gas at least five times before loading it into counting tubes. The results obtained by this method agree closely with those obtained by chemical dilution methods, and the procedure is much less time-consuming and less liable to accidents.

In addition to the mixing for the dilution step above, proper mixing of the carbon dioxide and the filling gas, either methane or P-10, is essential. As a general procedure, the filled tubes are removed from the vacuum line, the bottom end is cooled in liquid nitrogen for 1 minute, and the tube is turned upside down and held in a rack for several hours, or preferably overnight. After such a procedure there is no further variation in counting rate with time.

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Inherent Errors and Lower Limit of Activity Detection in Gas-Phase Proportional Counting of Carbon-14

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The precision of the method of carbon-14 assay used in this laboratory—by measurement with gas proportional counters—is discussed from the standpoint of the identity and magnitude of the various errors involved. The lower limit of activity detection in these gas proportional counting tubes has been investigated.

IN VIEW of the interest which has been manifested in the carbon-14 assay method used in this laboratory (*1, 4*), a discussion of the errors inherent in the system seems appropriate. The systematic errors affect all analyses equally and are therefore important only from the standpoint of the correctness of the absolute activity values reported. Others do not affect different analyses equally and therefore contribute to the deviation in results between samples. In this article, the errors are discussed approximately in the order in which they are encountered in practice, followed by a discussion of the lower limit of activity detection using the present method of analysis.

The equation used to calculate the specific activity of a given sample is:

$$\text{M}\mu\text{c./mg. C} = \frac{\text{net counts per minute}}{2220 \times Ve \times E \times \text{mg. C}}$$

The constant 2220 is the number of disintegrations per minute in 1 mμc. of activity. *Ve* is the fraction of the total counting tube volume, V_t, which is contained within the silvered cathode volume, V_s and is therefore defined by $Ve = V_s/V_t$ (*2*). *E* is the apparent counting efficiency of the tubes within the volume fraction *Ve*, and is now determined by comparison with a standard supplied by the National Bureau of Standards (*3, 7, 8*). The milligrams of carbon present in the counting tube is determined by pressure-volume-temperature measurement by means of a calibrated manometer (*1, 4*). Each of these factors, except the constant 2220, is subject to one or more errors of various types.

In no case does the original weighing of the combustion sample have any bearing on the isotopic assay, so long as the analytical result indicates reasonably complete combustion of the sample. This holds because the sample is not quantitatively transferred to counting tubes, but rather the amount which is placed in the counting tubes is measured by the pressure change on a calibrated manometer (*1, 4*).

The manometer on the sample loading line (*4*) is calibrated by observing the rise obtained from carbon dioxide evolved from weighed amounts of barium carbonate, by decomposition with sulfuric acid. The purity of this barium carbonate, in terms of carbon dioxide content, must therefore be accurately known. It is also necessary to ensure quantitative evolution and trapping of the carbon dioxide evolved. As this is done at a pressure of about 10^{-3} mm. of mercury, great care must be taken to prevent spraying of the barium carbonate powder when it is first attacked by the sulfuric acid. At the end of the reaction, the acid must be heated until solution of the carbonate occurs, in order to ensure its complete decomposition.

The result of such measurements is expressed as centimeters of rise per milligram of carbon present, and the root mean square deviation from the mean of 12 determinations made on the sample loading line is ± 0.73%. This error in the standard rise affects all samples equally and so need not be taken into consideration when comparing activities of different samples among themselves.

A similar error occurs in the calibration of the manometer on the combustion line itself, where the standard rise per milligram of carbon is determined by averaging the rise obtained from a number of standard carbon-hydrogen sample runs and/or from carbon dioxide evolved from barium carbonate. The reproducibility of these determinations is of a similar order to that obtained with barium carbonate on the loading line, so for counting tubes loaded directly from the combustion line and not on the loading line a similar error must still be considered.

Correction should be made on both calibration and analysis measurements to a standard reference temperature, in order to minimize the effect of temperature on the observed gas pressure. If this is done, the effect of this error is small (probably less than 0.2%).

On the two-liquid manometers previously described (*1, 4*), the reproducibility of a given reading of the manometer is about ± 0.01 cm., with two readings being involved in each tube filling and each calibration determination. The percentage effect of this error decreases as the amount of carbon dioxide being measured increases, but with a filling of 1 mg. of carbon on a system where the standard rise is about 5 cm. per milligram of carbon, it amounts to ± 0.2%.

Another error stems from the calibration of the volume fraction, *Ve*, within the cathode volume of each counting tube. It is measured by filling the tube to the various levels necessary with redistilled toluene from a buret, then calculating the fraction from the relative volumes so determined. As the use of buret readings taken to 0.05 ml. gives results nearly identical to those obtained by weighing the tube at each stage, the more rapid buret readings are considered sufficient. However, while the root mean square deviation in results on a given tube, as determined by any one person, averages about ± 0.25%, the deviation when the tube is calibrated by several different persons is ± 0.4%. Results obtained by four different persons on one tube are as follows: 0.838 ± 0.002; 0.845 ± 0.001; 0.844 ± 0.004; 0.847. The over-all value is 0.843 ± 0.0035 (root mean square deviation calculated separately, on the basis of 12 values). Since a tube is ordinarily calibrated once or twice by only one person, the assignment of uncertainty from this source is ± 0.5%. This error, and that in the manometer readings, are the chief operational errors which do not affect each sample equally.

An uncertainty exists in the calibration of the counting efficiency within the "active volume" of the counting tubes. Its determination is subject to all of the above operational errors, and to the effect of the counting statistics involved, as the calibration must be made on the basis of counting carbon dioxide evolved from a standard sample. This determination has been made a number of times, and the root mean square deviation of the results of these determinations is therefore a good measure of the actual effect of the operational and statistical errors on this system. The results of 30 different determinations of three different treatments of the standard sample show a root mean square deviation of ± 1.93% from the average value obtained.

In any counting procedure, the statistics of the counting must be considered. The standard deviation of any count or series of counts may be easily calculated by standard methods (*5*). In the present work, with tubes of about 100-cc. volume, the background is approximately 70 to 80 counts per minute. When the sample counting rate is below 3000 counts per minute, the standard deviation due to the background must also be taken into consideration. Above that counting rate, the uncertainty in the background becomes insignificant, statistically, and may be ignored.

It should also be recognized that, at very low sample counting rates, deviations due to such untoward effects as electronic aberrations and external sources of various types become increasingly important. This does occur occasionally, and must be watched for at all times. The use of calibrated external sources to determine that the counter is behaving normally is of value in this connection (*4*).

The precision with which a sample can be determined varies with the net counting rate of the sample. Table I lists the approximate precision at various counting rates, for a typical sample counted in two counting tubes to a point where 10,000 total counts are registered, or at least 3 minutes has passed (to reduce possible errors in the counting time), on each tube. This precision includes operational errors and statistical counting errors, but *does not* include any errors which affect the samples equally. With activities of about 300 counts per minute or more, it is possible to approach a precision of ± 2% by burning and counting a sample a number of times, as statistical errors are thereby minimized and the operational errors become the chief contributors. At very low net counting rates, the operational errors become relatively insignificant, with counting statistical deviations and nonstatistical events playing the dominant role in the precision obtained.

At low net counting rates, another factor operates to lower the precision which is attainable. At a gross counting rate of about 100 counts per minute, each count by which the background (70 counts per minute) is incorrect contributes an error to the net sample counts of about 3%; and, since the background cannot be counted simultaneously with the sample, on the same counter and in the same counting tube, some uncertainty in its actual value is inevitable. With a given counting tube on a given counter, the background may vary 1 to 2 counts per minute from hour to hour, about 3 to 4 counts per minute in a day. Occasionally a variation of as much as 10 counts per minute has been observed with 1-hour counts taken on different days.

The actual counting efficiency, *E*, is determined by the magnitude of the end effects of the counting tubes, but in practice is based on a comparison with the National Bureau of Standards sodium carbonate carbon-14 standard. The absolute value of this standard is at present open to question, however (*3, 7, 8*), and until the discrepancy concerning it is resolved, an unknown and possibly large error in the absolute specific activity values obtained by this method must be considered. At present, the value given by the National Bureau of Standards for this standard—namely, 1280 disintegrations per second per milliliter—is used to calculate the counting efficiency of 0.975. This error does not affect relative values between samples assayed on the same system and may be ignored from that standpoint.

Table I. Precision of Activity Determination at Various Operational Error Levels

Net Counting Rate, C.P.M.	Precision of Specific Activity[a,b,c] for Several Operational Error Levels, ±%[a,b,c]		
	2%	3%	4%
50	5.1	5.5	6.1
100	3.3	4.0	4.8
300	2.5	3.4	4.3
500	2.4	3.3	4.2
1,000	2.2	3.2	4.1
3,000	2.2	3.1	4.1
10,000	2.1	3.1	4.0

[a] Standard deviation of the background taken to be ±2 c.p.m. in each case.

[b] For one sample in two counting tubes, counted for 3 minutes or 10,000 counts (gross).

[c] Includes operational and statistical errors, but no correction for absolute counting efficiency.

LOWER LIMIT OF ACTIVITY DETECTION

It was considered desirable to determine the reasonable lower limit of detectable activity with this counting system, and to determine the precision with which such measurements could be made. To this end, benzoic acid, the activity of which had been determined 13 times and was known to within ± 2%, was diluted with inactive benzoic acid to obtain material which contained 10.1 ± 0.2 and 2.02 ± 0.04 counts per minute per milligram of carbon. About 1 mg. of carbon, as carbon dioxide, from each sample, was placed in each of two counting tubes and counted under optimum conditions. For this purpose, counting tubes were selected which showed long counting plateaus and stable background characteristics. The results are shown in Table II.

These results show that, under these conditions, even 2 counts per minute can be detected with a standard deviation of about 20%. Variation in the actual division of counting time between sample and background is allowable, but it is desirable to count the background as long as the sample itself in such cases (*6*). In this case, the background was counted both before and after the sample was counted, with the time period of each back-

Table II. Lower Limit of Activity Detection

		Background		Sample			
Sample No.	Nature of Sample[b]	Background counts, c.p.m.[a]	Time counted, min.	Sample counts, c.p.m.[a]	Time counted, min.	Net sample counts, c.p.m.[a]	Av. net sample counts/mg. C, c.p.m.[a,c,d]
1	Inactive	72.6 ± 0.30	791	73.0 ± 0.64	176	0.4 ± 0.71	0.6 ± 0.51
		73.9 ± 0.31	785	74.7 ± 0.66	172	0.8 ± 0.72	
2	Inactive	70.8 ± 0.38	493	71.0 ± 0.65	165	0.2 ± 0.75	0.6 ± 0.54
		73.2 ± 0.39	479	74.2 ± 0.68	158	1.0 ± 0.78	
3	Inactive	77.0 ± 0.44	398	76.3 ± 0.58	224	−0.7 ± 0.73	0.2 ± 0.55
		75.5 ± 0.46	360	76.6 ± 0.67	169	1.1 ± 0.81	
4	10.3 ± 0.2	71.5 ± 0.44	370	82.1 ± 0.67	181	10.6 ± 0.80	10.0 ± 0.56
	10.2 ± 0.2	73.6 ± 0.42	420	83.2 ± 0.68	182	9.6 ± 0.80	
5	2.08 ± 0.04	72.0 ± 0.44	364	73.5 ± 0.64	177	1.5 ± 0.78	2.7 ± 0.53
	2.14 ± 0.04	74.1 ± 0.45	365	78.2 ± 0.66	178	4.1 ± 0.80	

[a] Deviations shown are calculated standard deviations.

[b] Where expected counts are listed, they are based on original activity and standard deviation, then calculating activity of sample from dilution by weight with inactive material. Corrected for actual amount of carbon dioxide present.

[c] With inactive samples, no correction is made for milligrams of carbon present.

[d] Expected counts per minute per milligram of carbon are: sample 4, 10.1 ± 0.2; sample 5, 2.02 ± 0.04.

ground counting period and the sample counting period being approximately equal. This means that for each sample the background was counted approximately twice as long as the sample, and each sample was counted to well over 10,000 counts. For the calculations, the total background time was taken for each tube, rather than considering the two background periods separately.

METHOD OF CALCULATING σ

The standard deviations shown in the tables were calculated by the usual methods (*5*). In Table I, the deviations are a combination of the standard deviation of the gross counts involved, the standard deviation of a normal background count (taken to be ± 2 counts per minute in all cases), and the indicated contributions from the operational errors. The percentage deviations listed were determined from the square root of the sum of the squares of the numerical values for each of these deviations. It has been shown that under the present conditions the operational errors are about 2%, and the column under that heading is taken to apply.

In Table II, the deviations shown are the calculated standard deviations, in counts per minute, based on total counts in each case. The deviation for the net sample counts for each tube is the square root of the sum of the squares of the appropriate background and sample deviations, and the average net sample deviations are half the square root of the sum of the squares of the deviations of the two values used to obtain the average value. With the inactive samples, no correction is made in the average value for the amount of carbon dioxide in the counting tube, as this contributes no statistical variations to the background count so obtained.

Present experience indicates that with very low counting rates, such as those under discussion, it is best to do all of the counting, during the working day. When these counting tubes were left counting overnight, either on background or on sample counts, deviations were often observed which were far outside of the expected statistical deviations. As a result, all of the data presented in this paper were taken during the working day, when external conditions appear to have remained more or less constant. The counting rates tend to be somewhat lower during the night than during the day, although this is not found to be universally true.

It may be significant that the deviation from the expected counts per minute is generally positive, including those cases where inactive carbon dioxide was employed. The filling gas was introduced into the counting tubes from the same vacuum line in all cases, for both samples and backgrounds, so it is unlikely that this effect is due to contamination from the line. Sample 3 was obtained by burning an inactive sample on a combustion line which had never been used with an active sample. It may be that the presence of carbon dioxide in these counting tubes causes a slight rise in the counting rate observed.

In view of these results, it appears that as little as 2 counts per minute can be detected in a counting tube of this type with the background conditions prevailing here. Those samples which show counts indistinguishable from background, in terms of the standard deviation, are considered inactive. However, even 1 count per minute might be detected by this method, with somewhat longer counting periods. Certainly it is possible, with the counting conditions described, to state definitely that a sample has less than 2 counts per minute, if such is the case, and for practical purposes 2 counts per minute is considered the lower limit of activity detection by the method described.

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Laboratory Methods for Evaluation of Evaporation Losses of Petroleum Products

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Two laboratory methods have been developed for the accurate determination of evaporation losses. The first procedure is a modified Chenicek-Whitman vapor pressure method, in which measured amounts of dry air are bubbled through a known amount of a hydrocarbon mixture at a fixed temperature and at a rate to ensure saturation of the air with hydrocarbon vapors. The vapor pressure of the hydrocarbon mixture is calculated from the volume of the vapor evolved, the total amount of air and vapor, and the pressure of the gas-measuring device. In the second procedure a density-evaporation curve is established to determine the effect of the evaporation loss on the density of the sample under investigation. Both procedures are accurate, convenient, and applicable to almost any type of evaporation problem. The vapor pressure method can accurately determine evaporation losses as low as 0.04%; density method can detect losses as low as 0.01%.

ECONOMIC and weather conditions may require extended storage of crudes and finished petroleum products. The relatively high volatility and values of these materials justify the attention paid to evaporation-control measures.

The magnitude of the evaporation losses in handling and storage of petroleum and its products has to be determined accurately in order to provide economic justification for evaporation-loss prevention programs in balancing the savings against the cost of equipment and preventive measures. Methods which can be applied to determine these evaporation losses may be based on direct measurement, such as by gaging, where the volume is measured prior to and after the loss has been incurred, or on some change in physical property such as vapor pressure or density.

The petroleum industry has long felt the gaging method to be unsatisfactory as a practical procedure in spite of its suitability as to theory. The most significant factor contributing to its

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inadequacy is probably the correction to be applied for temperature which has a wide variation within the tank. Another objection to the use of the gaging method for evaporation studies is that it does not differentiate between mechanical and evaporation losses.

The vapor pressure of a liquid consisting of a mixture of hydrocarbons is very sensitive to evaporation losses. The light compounds which contribute most to the vapor pressure are the ones which are most readily evaporated.

The apparatus and technique as developed by Standard Oil Co. of Indiana (*3, 4*) make it possible to interpret vapor pressure data in terms of evaporation loss. A vapor pressure–evaporation loss curve can be constructed for any single sample by measuring the lowering of vapor pressure with different percentages of sample evaporated.

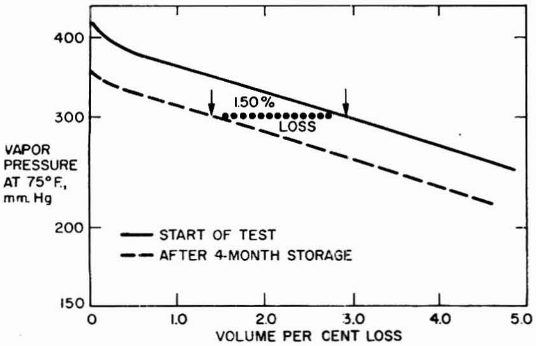

Figure 1. Standing-storage evaporation loss of crude oil No. 1

Weathering losses occurring during storage or transport can be determined by means of a vapor pressure–evaporation curve of the hydrocarbon mixture prior to and after storage or transfer. Any loss of the lighter components would result in a decrease of vapor pressure and would be reflected in a lower vapor pressure–evaporation curve on the depleted hydrocarbon mixture. If the curves of the original and depleted samples are plotted on the same scale, as in Figure 1, the horizontal distance between the curves at any given vapor pressure represents the per cent of the volume of the hydrocarbon mixture which has been lost by evaporation.

It is difficult to obtain accurate results with the vapor pressure method on samples which show small differences in vapor pressure for successive increments of sample evaporated, such as materials with low vapor pressure. On samples of this type, it is possible to estimate the losses more accurately by the density method, since densities can be determined with greater accuracy than the vapor pressure.

Densities of liquids consisting of hydrocarbon mixtures are also sensitive to evaporation losses. However, it is necessary to establish a density-evaporation curve for each sample under investigation in order to determine the effect of the evaporation loss on density.

The modification of the vapor pressure method involves the substitution of a sintered disk for the capillary in the air-bubbling or sample buret to ensure a better distribution of the air throughout the sample, the use of a metal needle valve for closer control of the air rate, and the use of mercury lifts to simplify the air measuring system and facilitate maintenance of atmospheric pressure in the operation of the air and vapor buret.

Both of these procedures are sensitive to evaporation effects, and it is necessary to obtain representative samples of the stock in the field in order to take full advantage of the accuracy of the methods. The samples should be taken as described by the American Petroleum Institute (*1*).

The samples, when received in the laboratory, should be kept in a refrigerator wherever possible. All precautions should be used to reduce losses to a minimum during storage and transfer to the apparatus.

VAPOR PRESSURE METHOD

Apparatus. The general appearance of the apparatus used for measuring the vapor pressure by evaporation loss is shown in Figure 2. The design of the apparatus is given in Figure 3. The essential parts of the apparatus are the air-measuring buret, the air- and vapor-measuring buret, the air-bubbling or sample buret, a drying tube, and a constant temperature circulating system.

The air- and the air- and vapor-measuring burets are conventional gas burets of 100-ml. and 200-ml. capacity calibrated to read to 0.1 and 0.2 ml., respectively. Mercury is used as the confining liquid in the burets. Mercury lifts consisting of stainless steel tanks and valves are used for controlling the flow of mercury. For carrying out this operation, the laboratory-compressed air and vacuum were found to be adequate. A three-way cock directs either pressure or vacuum to each tank; the arrangement is such that flow of mercury in the buret is indicated by the "down" or "up" position of the valve handle. The flow of mercury in and out of the burets is controlled by two stainless-steel blunt needle valves.

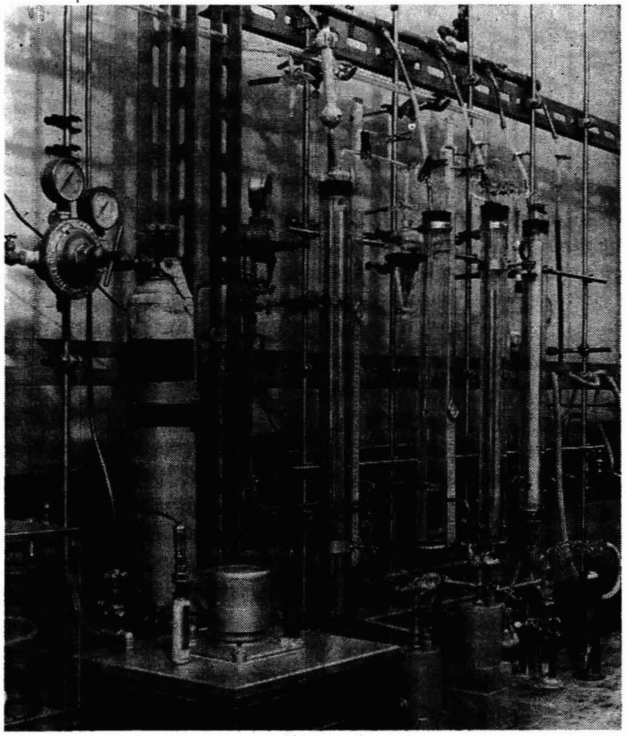

Figure 2. Vapor pressure apparatus

Leveling tubes are used to facilitate adjustment of the pressure in the buret to that of the atmosphere. The leveling tubes and burets are of the same diameter and adjacent to each other. This permits quick and accurate comparison of the relative levels of the mercury, and facilitates maintenance of atmospheric pressure in the operation of the air and vapor buret.

The air-bubbling or sample buret of approximately 100-ml. capacity consists of an ungraduated section attached to a 5-ml. pipet with 0.05-ml. graduations, making it possible to estimate to 0.01 ml. A sintered disk of medium porosity through which air is admitted to the bottom of the column of sample is located at the bottom of the buret. The rate of bubbling air through the sample is controlled by the metal needle valve, which is cemented to the capillary tubing with high vacuum sealing wax. The capillary tube is attached to the sintered disk and is enclosed in the same water jacket with the buret. The stopcock located at the bottom of the buret is used for admitting the previously chilled sample, forced in by water displacement. The buret is calibrated with water with the sintered-disk cup full of air.

The manometer, *M*-1, as shown in Figure 3, is used to indicate the pressure of the air-measuring system, while manometer *M*-2 indicates the pressure differential between the sample buret, *C*, and the air and vapor buret, *D*.

Laboratory-compressed air used for bubbling through the sample is successively passed through a reducing valve, pressure regulator, sulfuric acid scrubber, and finally dried by Drierite in the drying tube, *A*, before being drawn into the air-measuring buret.

The air-drying tube, *A*, and all of the burets are enclosed in water jackets. The temperature of the water flowing through the jackets is maintained within a maximum variation of 0.1° F. by recirculation through a constant temperature water bath. The temperature at which the determinations are ordinarily made is 75° F. Other temperatures may be used. Temperatures between 40° and 110° F. can be readily maintained by the constant temperature circulating bath.

Capillary tubing is used throughout the entire unjacketed portion of the apparatus. The ball joints between the air-measuring buret and the sample buret are sealed with high vacuum sealing wax. Stopcock grease of high vacuum type is used on all stopcocks and connections, with the exception of those which come in contact with the liquid hydrocarbon sample or vapor, in which case hydrocarbon-insoluble grease is used.

All outlets to the atmosphere through which moist air may be drawn into the system are protected by Drierite-drying tubes.

Procedure for Establishing Vapor Pressure-Evaporation Curve. The apparatus is assembled as shown in Figure 3. The air-measuring system and the air- and vapor-measuring system are tested for leaks by drawing air into the respective burets, placing the system first under pressure and then under vacuum by means of proper manipulation of the mercury lifts, and noting any change in volume after the system has been under vacuum or pressure.

The sample is introduced into the air-bubbling buret, *C*, by water displacement. The charging of the sample is done carefully in order to expel all of the air from the connections to prevent bubbling any air through the sample. To ensure getting a uniform sample, approximately 150 ml. of the sample is overflowed into the separatory funnel. A small amount of sample is then withdrawn from the buret through the buret stopcock. The sample is allowed to come to the set temperature of the circulating water which requires about 10 or 15 minutes. The excess sample is drained from the buret until the liquid level is slightly above the zero mark. Any sample that may have drained into the sintered-disk cup can be forced out with air by putting the air buret under pressure and gradually opening the metal needle valve, being careful not to bubble any air through the sample. After allowing a sufficient amount of time for the complete drainage of the sides of the buret, the sample level is adjusted to the zero graduation.

A measured amount of dry air in buret *B* at a pressure of 10 to 12 cm. of mercury is bubbled through the known amount of sample contained in buret *C* at a rate of 8 to 12 ml. per minute. This rate assures essentially complete saturation of the air with hydrocarbon vapor. The resulting volume of air and vapor is collected and measured in buret *D*. The air and hydrocarbon vapors being collected in buret *D* are always kept at atmospheric pressure by maintaining the mercury levels in the buret and leveling tube equal. The operation of equalizing the mercury levels in the air and vapor buret *D* should be done carefully so as not to put the system under too large a vacuum or pressure to prevent forcing some of the sample into the sintered-disk cup.

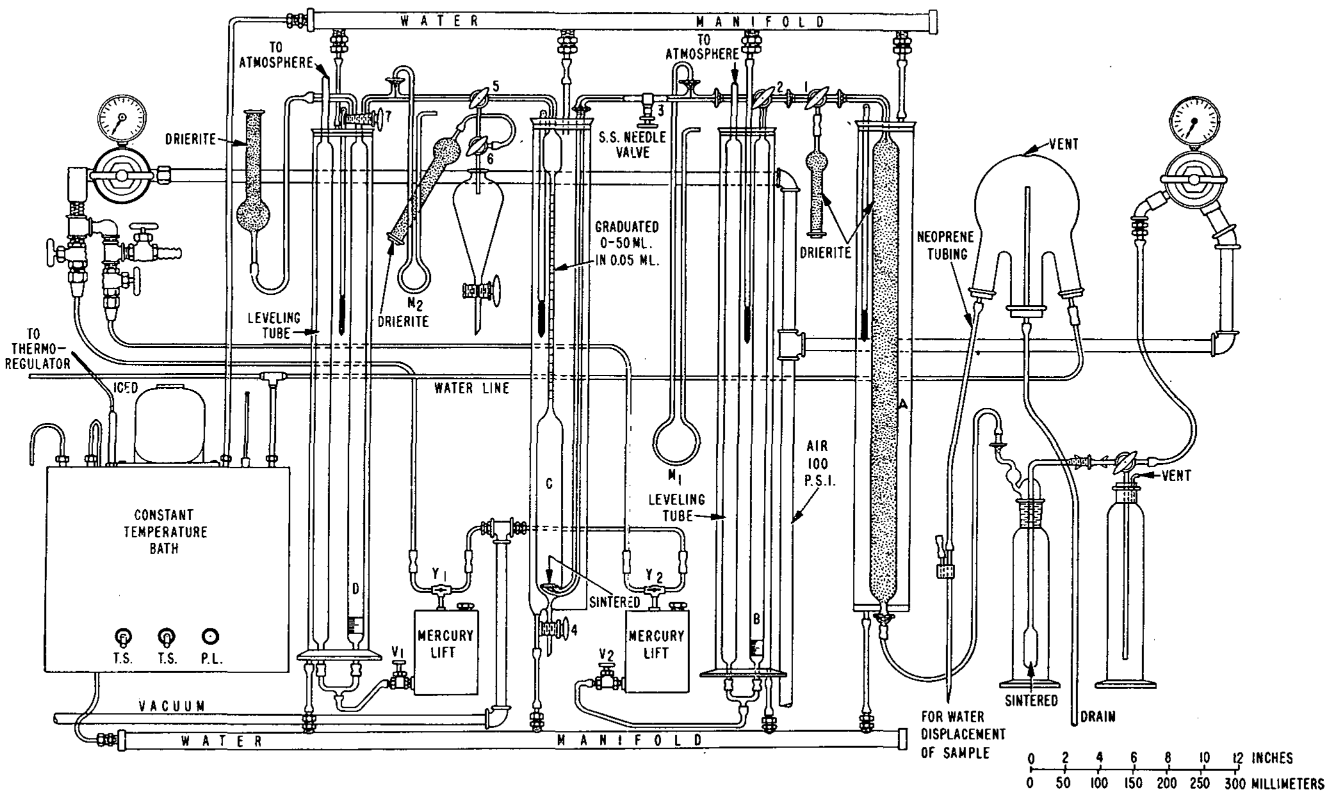

Figure 3. Modified Chenicek-Whitman vapor pressure apparatus

1, 2, 5, 6.	Straight-bore, three-way stopcocks. Type devised for use with Fisher unitized gas analyzers (Fisher Scientific Co., Pittsburgh, Pa.)
3.	Metal needle valve, Hershberg-Southworth type (Catalog No. 7537, A. H. Thomas Co., Philadelphia, Pa.)
4.	Precision grade, 4-mm. stopcock, borosilicate glass (Catalog No. 5000, Eck and Krebs, New York, N. Y.)
M-1, *M*-2.	Mercury manometers
Mercury lifts.	Stainless steel tanks, made from 4-inch stainless steel pipe, 4 × 5 inches
Y-1, Y-2.	Imperial three-way shut-off cocks, 1/4 inch (Imperial Brass Mfg. Co., Chicago, Ill.)
V-1, V-2	Vogt S.S. blunt needle valves, 1/4 inch (Henry Vogt Machine Co., Louisville, Ky.)
Constant temperature bath.	Labline constant temperature circulating system (Catalog No. 3052, Labline, Inc., Chicago, Ill.)
A.	Air-drying tube
B.	Air buret, 100 ml. calibrated gas buret laboratory type (Burrell Technical Supply Co., Pittsburgh, Pa.)
C.	Air-bubbling buret, approximately 100 ml. ungraduated section attached to 5-ml. pipet with 0.05-ml. graduations
D.	Air and vapor buret, 200 ml. calibrated gas buret (made specially by Scientific Glass Co., Bloomfield, N. J.)

The amount of sample evaporated is equal to the decrease in liquid volume in buret *C*.

The air-passing procedure is repeated until about 3 to 5 ml. of the hydrocarbon sample has been evaporated. On weathered or depleted samples of the original material, successive determinations until about 3 ml. of the sample has been evaporated usually suffice, since there is no point in carrying out the last sample to lower vapor pressures than that of the preceding one.

Several barometric readings should be taken during the course of the determinations to take into account changes in the barometric pressure.

The vapor pressure for each air-passing operation is calculated by dividing the product of the corrected barometric pressure and the total vapor volume by the total air and vapor volume. The vapor volume is the difference between the total air and vapor volume and the total air volume.

The vapor pressures of the various increments are plotted on semilog paper against the average per cent of sample evaporated, obtained by averaging the total per cent of sample evaporated at the beginning and end of each increment.

Table I. Comparison of Observed and Known Vapor Pressures of Pure Organic Liquids

(Vapor pressure in mm. of Hg at 25° C.)

Compound	Found	Literature Value
Ethyl ether	533, 533, 532	537.0
Chloroform	199, 199, 201	199.1
Benzene	93, 95, 94	94.0
Carbon tetrachloride	111, 113, 112	114.5

The vapor pressure–evaporation curves on the depleted samples of the original material are plotted in Figure 1. Loss of the lighter components in the depleted samples results in a decrease of vapor pressure and gives lowered vapor pressure–evaporation curves. The horizontal distance between these curves is a direct measure of the evaporation loss.

RESULTS AND CONCLUSIONS

The accuracy of the vapor pressure method is dependent upon the care in handling the sample, the accuracy of the measurements involved, and the degree of saturation of the air with hydrocarbon vapor. That practical saturation of the air with vapor is reached by this procedure is demonstrated in Table I by the close agreement between observed and known vapor pressures of various organic liquids.

It is shown in Table I that practical saturation of the air with vapor of highly volatile material such as ethyl ether can be obtained even at a rate three times as great as the 8 to 12 ml. per minute specified in the method.

The procedure is capable of good reproducibility. The vapor pressures of each increment of sample evaporated on successive samples of the same material when plotted on semilog

Table II. Vapor Pressure-Evaporation Loss Data of Crude Oil No. 1

(Crude sample No. 1. Middle of tank 167. Vol. charged = 99.2 ml. Jacket temp. = 75.0° F. Vol. capillary tube = 5.00 ml. Evapn. corr. = 1.00806)

		Air Volume			Sample Volume					Air and Vapor Volume				
Time	Bar.[a] Rdg.[b], Mm. of Hg	Initial rdg., ml.	Final rdg., ml.	Diff.	Initial rdg., ml.	Final rdg., ml.	Diff.	Av. % evap.	Corr. av. % evap.	Initial rdg., ml.	Final rdg., ml.	Total diff.	Net Vapor Volume	Vapor Pressure, Mm. of Hg
At Start of Storage Test														
10:00	772.0 at 77° F.	96.9	93.4	3.5	0.00	0.01	0.01	0.005	0.005	0.0	6.2	6.21	2.71	335.5
10:08	768.9 corr.	93.4	90.4	3.0	0.01	0.02	0.01	0.015	0.02	6.2	12.8	6.61	3.61	419.9
10:18		90.4	45.05	45.35	0.02	0.22	0.20	0.12	0.12	12.8	108.3	95.7	50.35	404.5
10:28		98.0	45.2	52.8	0.22	0.45	0.23	0.335	0.34	0.0	105.6	105.83	53.03	385.3
10:40		97.2	40.2	57.0	0.45	0.68	0.23	0.565	0.57	0.0	112.0	112.23	55.23	378.4
10:55	771.1 at 78° F.	96.7	40.3	56.4	0.68	0.91	0.23	0.795	0.80	0.0	108.4	108.63	52.23	369.2
11:10	767.9 corr.	95.3	37.7	57.6	0.91	1.14	0.23	1.025	1.04	0.0	108.5	108.73	51.13	361.1
12:15		98.6	34.3	64.3	1.13	1.37	0.24	1.25	1.26	0.0	118.7	118.94	54.64	353.1
12:30		98.0	36.3	61.7	1.37	1.60	0.23	1.485	1.50	0.0	111.8	112.03	50.33	344.9
12:42	769.4 at 78° F.	96.35	32.95	63.4	1.60	1.80	0.21	1.705	1.72	0.0	113.1	113.31	49.91	337.5
12:55	766.2 corr.	97.1	31.4	65.7	1.81	2.03	0.22	1.92	1.93	0.0	115.4	115.62	49.93	330.9
1:14		98.2	26.4	71.8	2.02	2.27	0.25	2.145	2.16	0.0	124.0	124.25	52.45	323.4
1:25		98.5	28.8	69.7	2.27	2.48	0.21	2.375	2.39	0.0	118.6	118.81	49.11	316.7
1:39		98.5	24.8	73.7	2.48	2.71	0.23	2.595	2.62	0.0	123.6	123.83	50.13	310.2
1:50		98.5	24.2	74.3	2.71	2.92	0.21	2.815	2.84	0.0	122.6	122.81	48.51	302.6
2:03		98.0	15.3	82.7	2.92	3.16	0.24	3.04	3.06	0.0	134.8	135.04	52.34	297.0
2:15		98.5	17.0	81.5	3.16	3.38	0.22	3.27	3.30	0.0	131.1	131.32	49.82	290.7
2:27	768.4 at 78.5° F.	98.5	15.9	82.6	3.38	3.60	0.22	3.49	3.52	0.0	131.3	131.52	48.92	284.6
2:39	765.2 corr.	98.5	14.6	83.9	3.60	3.83	0.23	3.715	3.74	0.0	131.7	131.93	48.03	278.6
2:48		98.5	13.85	84.65	3.83	4.03	0.20	3.93	3.96	0.0	131.4	131.60	46.95	273.0
2:58		98.5	13.6	84.9	4.03	4.22	0.19	4.125	4.16	0.0	130.4	130.59	45.69	267.7
3:10		98.5	15.05	83.45	4.22	4.42	0.20	4.320	4.35	0.0	126.6	126.80	43.35	261.6
3:21		98.5	13.05	85.45	4.42	4.63	0.21	4.525	4.56	0.0	127.8	128.01	42.56	254.4
3:32		98.5	40.0	58.5	4.63	4.75	0.12	4.69	4.73	0.0	87.2	87.32	28.82	252.6
3:40		98.5	9.8	88.7	4.75	4.92	0.17	4.835	4.87	0.0	131.4	131.57	42.87	249.3
After 4 Months' Storage														
9:33	772.3 at 79° F.	97.0	89.65	7.35	0.00	0.02	0.02	0.01	0.01	0.0	12.9	12.92	5.57	331.5
9:38	769.0 corr.	89.65	82.7	6.95	0.02	0.04	0.02	0.03	0.03	12.9	25.8	12.92	5.97	355.3
9:44		82.7	47.0	35.7	0.04	0.15	0.11	0.095	0.10	25.0	90.9	65.21	29.51	348.0
9:56		98.0	41.3	56.7	0.15	0.35	0.20	0.25	0.25	0.0	100.8	101.0	44.30	337.3
10:10		98.0	34.55	63.45	0.35	0.55	0.20	0.45	0.45	0.0	111.0	111.2	47.75	330.3
10:24		98.0	19.8	78.2	0.55	0.80	0.25	0.675	0.68	0.0	134.2	134.45	56.25	321.7
10:35		99.0	18.75	81.25	0.80	1.05	0.25	1.025	1.03	0.0	136.8	137.05	55.80	313.1
10:49		100.0	12.75	87.25	1.05	1.32	0.27	1.185	1.19	0.0	144.6	144.87	57.62	305.9
11:00		100.0	12.10	87.9	1.32	1.57	0.25	1.445	1.46	0.0	143.8	144.05	56.15	299.8
11:14		100.0	14.3	85.7	1.57	1.80	0.23	1.685	1.70	0.0	138.1	138.33	52.63	292.6
11:30		100.0	16.8	83.2	1.80	2.02	0.22	1.91	1.93	0.0	132.0	132.22	49.02	285.1
11:45		100.0	16.5	83.5	2.02	2.23	0.21	2.125	2.14	0.0	130.8	131.01	47.51	278.9
11:56		100.0	20.95	79.05	2.23	2.43	0.20	2.33	2.35	0.0	122.6	122.8	43.75	274.0
12:10		100.0	19.0	81.0	2.43	2.63	0.20	2.53	2.55	0.0	124.1	124.3	43.30	267.9
12:24		100.0	33.2	66.8	2.63	2.79	0.16	2.71	2.73	0.0	101.6	101.76	34.96	264.2
12:40		100.0	43.4	56.6	2.79	2.92	0.13	2.855	2.88	0.0	85.4	85.53	28.93	260.1
1:32	772.6 at 81° F.	100.0	14.75	85.25	2.90	3.11	0.21	3.005	3.03	0.0	127.6	127.81	42.56	256.1
1:45	769.2 corr.	100.0	14.9	85.1	3.11	3.31	0.20	3.21	3.24	0.0	126.4	126.6	41.50	252.1
1:56		100.0	16.3	83.7	3.31	3.48	0.17	3.395	3.42	0.0	123.4	123.57	39.87	248.2
2:08		100.0	14.4	85.6	3.48	3.67	0.19	3.575	3.60	0.0	125.2	125.39	39.79	244.1
2:20		100.0	15.15	84.85	3.67	3.84	0.17	3.755	3.79	0.0	123.0	123.17	38.32	239.3
2:34		100.0	14.25	85.75	3.84	4.00	0.16	3.92	3.95	0.0	123.1	123.26	37.51	234.1
2:46		100.0	12.2	87.8	4.00	4.17	0.17	4.085	4.12	0.0	124.9	125.07	37.27	229.2
2:59		100.0	13.3	86.7	4.17	4.34	0.17	4.255	4.29	0.0	122.5	122.67	35.97	225.5

[a] Barometer.
[b] Reading.

graph paper all fall on the same straight line, no point being more than 2 or 3 mm. of pressure from the curve. The initial vapor pressure of the sample can be estimated by extrapolation of the curve to 0% loss.

An error analysis of the results in terms of the accuracy of the various measurements justifies estimation of evaporation losses as low as 0.04%. The over-all accuracy of estimating evaporation loss varies with the slope of the curves, which are dependent upon the volatility of the hydrocarbon mixture and the temperature at which the vapor pressures are determined.

Table II shows the type of data required for establishing a vapor pressure–evaporation curve. These data were obtained on a crude oil at the start of a standing storage test and after approximately 4 months of standing storage. The data shown in Table II are plotted on semilogarithmic paper in Figure 1. The horizontal distance between the two curves represents the evaporation loss (1.50 volume %) of the crude oil at the end of the 4-month test period.

In order to demonstrate the procedure for establishing a vapor pressure–evaporation curve, all the recorded data are shown in Table II.

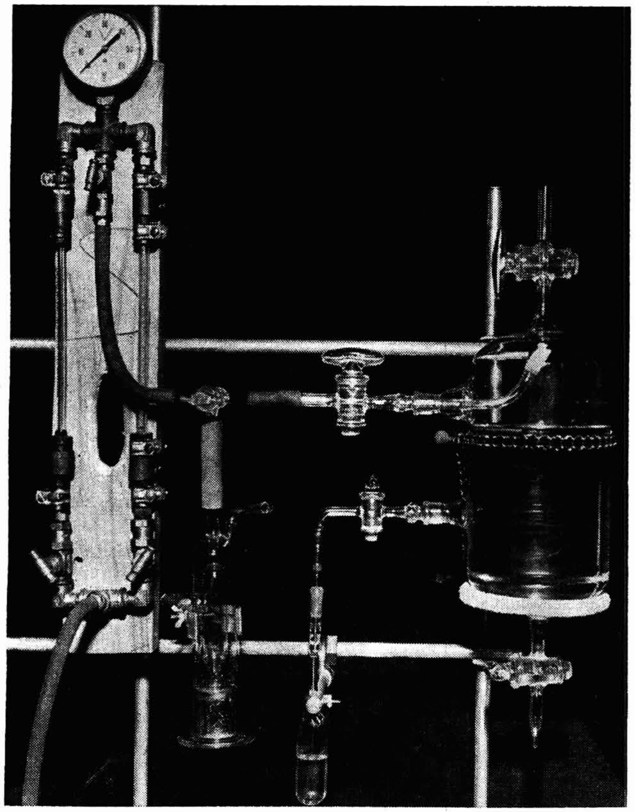

Figure 4. Apparatus for evaporation losses by density method

The vapor pressures of the sample for each increment evaporated were calculated by the following equation:

$$\text{Vapor pressure, mm. of Hg} = \text{corrected barometric pressure} \times \frac{\text{total vapor vol.}}{\text{total air and vapor vol.}}$$

Using the above relationship and the data recorded in the first row of Table II, the vapor pressure of the sample for the first increment evaporated is:

$$\text{Vapor pressure, mm. of Hg} = 768.9 \times \frac{2.71\ (\text{column } 14)}{6.21\ (\text{column } 13)} = 335.5$$

The vapor pressure–evaporation curve is obtained by plotting the vapor pressures (column 15) against the corrected average per cent evaporated (column 10) on semilog graph paper. The average per cent evaporated figure is used, because the vapor pressure recorded is the average pressure of each increment of sample evaporated. The first increment is not used in plotting the curve, since the sample may be supersaturated or undersaturated with air at the temperature of the vapor pressure determination.

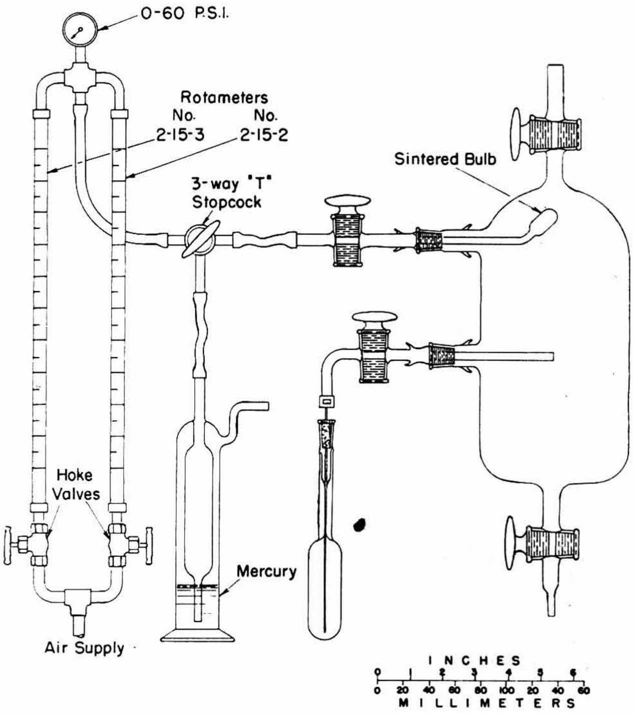

Figure 5. Diagram of apparatus for evaporation losses by density method

The vapor pressure–evaporation curves on depleted samples of an original material are plotted on the same graph paper. Loss of the lighter components in the depleted samples results in a decrease of vapor pressure and gives lowered vapor pressure–evaporation curves.

DENSITY METHOD

Apparatus. The general appearance of the apparatus used for determining the relationship between density and evaporation loss is shown in Figure 4. The sketch of the apparatus is shown in Figure 5. The apparatus consists of a supply of compressed air, a rotameter, pressure regulator, a sample container, and a 25-ml. Bingham-type pycnometer for the accurate determination of fifth-place densities.

In the initial setup of the apparatus two rotameters, as shown in Figure 5, were used. With these rotameters it was possible to control the rate of flow of the air through the gas dispersion fritted cylinders from 0 to 1500 ml. per minute. However, it was found that keeping the rate constant at 500 to 600 ml. per minute was sufficient to give approximately a 1% loss for a 1-hour period for the type of motor gasolines and crudes which were being studied.

The sample container of approximately 2-liter capacity is constructed to simulate to some extent conditions of evaporation existing in field tests. The rate of vapor removal from the vapor phase without disturbing the liquid surface is controlled by the flow of air through a coarse gas dispersion fritted cylinder. A sampling device for obtaining samples for density measurement is located halfway between the air inlet tube and the botton stopcock of the sample container.

Hydrocarbon-insoluble grease is used on all the stopcocks and connections.

Procedure for Obtaining Density–Evaporation Loss Curve. The apparatus is assembled as shown in Figure 5. The precooled sample is charged by water displacement or air pressure into the tared sample container. About 100 to 200 ml. of the sample is allowed to overflow through the sampling device. With the stopcock of the sampling device closed, the sample (about 1500 ml.) is charged to a point just below the air inlet tube connection.

Table III. Data Sheet for Obtaining Density-Evaporation Curve for Typical Premium Gasoline

	Evaporation		
	First	Second	Third
Initial wt. of sample container and contents, g.	1465.136	1440.638	1389.213
Wt. of container, g.	686.671	686.671	686.671
Initial wt. of sample, g.	778.465	753.967	702.542
Initial d. of sample, g./ml. at 25° C.	0.72473	0.72531	0.72684
Initial vol. of sample, ml.	1074.145	1039.510	966.570
Final wt. of sample container and contents, g.	1462.043	1432.285	1376.434
Final wt. of sample, g.	755.372	745.614	689.763
Final d. of sample, g./ml. at 25° C.	0.72531	0.72684	0.72928
Final vol. of sample, ml.	1069.022	1025.830	945.814
Evaporation loss, vol. %	0.477	1.316	2.147
Evaporation loss, vol. %, based on original vol.	0.477	1.360	2.386
Total evaporation loss, vol. %, based on original vol.	0.477	1.837	4.223

After the container and contents have come to room temperature, the initial density of the hydrocarbon is determined on a sample drawn into the bottom of the Bingham-type pycnometer through a hypodermic needle connected to the end of the sampling device. In order to obtain a steady flow of sample through the hypodermic tubing, it is necessary to place the sample container under a pressure of approximately 2 inches of mercury. The sample is allowed to flow into the overflow reservoir of the pycnometer until about 25 ml. of sample has been withdrawn through a steel drawoff needle. The fifth-place density measurements are made according to the ASTM procedure (*2*).

Table IV. Relationship between Density and Evaporation Loss on Typical Premium Gasoline

Container 1		Container 2	
Vol. % loss	Density at 77° F. g./ml.	Vol. % loss	Density at 77° F., g./ml.
0.000	0.72473	0.000	0.72474
0.477	0.72531	0.989	0.72588
1.837	0.72684	2.828	0.72785
4.223	0.72928	5.322	0.73037

An alternative procedure that can be employed for obtaining samples from the sample container for density measurements is to use a 30-ml. hypodermic syringe equipped with a needle made from stainless steel tubing (No. 18 or No. 20 Stubb's gage) and sufficiently long to extend to the same point as the sampling device. A 10-inch needle was used for the apparatus shown in Figure 5.

The air inlet tube is replaced with a stopper, and the sample container and contents are weighed after the sample for the initial density has been taken. This is taken as the original weight. All of the weighings are made, to the nearest milligram, on a balance with a capacity of 2 kg. in each pan and a sensitivity of 0.2 mg. The stopper is replaced by the air inlet tube, and the rate of vapor removal from the vapor phase is adjusted by controlling the air rate to give approximately a 1% loss for a 1-hour period. At the end of this period, the air inlet tube is replaced with the stopper. The amount of evaporation loss is obtained by reweighing the container and contents. A sample is withdrawn through the sampling device or hypodermic syringe into the pycnometer for determination of the density of the depleted sample as described.

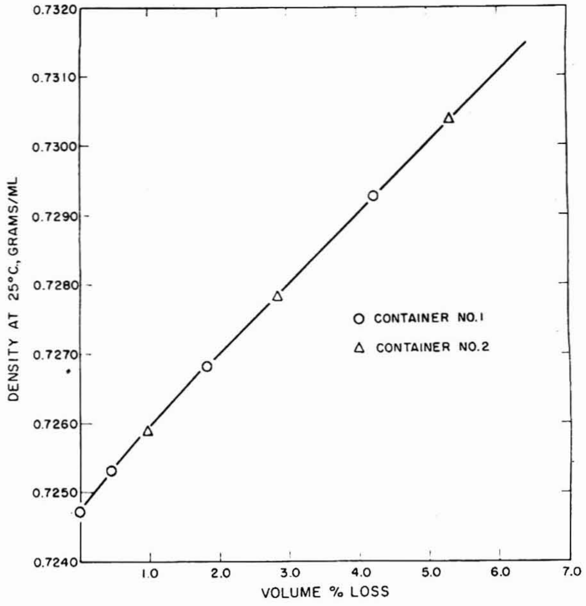

Figure 6. Density-evaporation curve for typical premium gasoline

Plot of data given in Table V

The container and contents are again reweighed and this weight is used as a basis for calculating the next loss. The procedure for obtaining the evaporation losses and densities of the depleted sample is repeated several times until 4 to 6% of the sample has been evaporated. The individual losses are calculated back to the original volume to obtain the total loss on the sample.

Table V. Evaporation Loss of Crude Oil on Standing Storage

	Volume % Loss	
Sample No.	By density method	By vapor pressure method
1	0.75	0.77
2	1.88	1.87
3	2.50	2.46
4	3.51	3.44

The density-evaporation curve is obtained by plotting the densities against the volume per cent evaporated. To obtain evaporation losses on depleted samples of the original material, the density is determined on the depleted samples and the evaporation loss read directly from the density-evaporation curve.

RESULTS AND CONCLUSIONS

Table III contains the type of data recorded for obtaining a density-evaporation curve.

The density-evaporation curve is obtained by plotting the initial density of the sample at zero evaporation loss and the final densities shown in the three columns in Table III against the total evaporation loss shown at the bottom of each column.

Two identical sample containers, as in Figure 5, were used to establish the reproducibility of the density method. The rate of vapor removal was varied in each container by controlling the flow of air through the coarse gas dispersion fritted cylinder. The evaporation loss and density data for both containers are given in Table IV and plotted on Figure 6. The procedure is capable of good reproducibility, since all the plotted points fall in the same curve.

The ASTM procedure (*2*) for the determination of fifth-place densities by means of the Bingham-type pycnometer has been well established. The repeatability of the density measurement with a 25-ml. pycnometer is within ±0.00001, the accuracy ±0.00002. In terms of the accuracy of the density measurement, it should be possible to estimate evaporation losses as low as 0.01%. In actual practice on gasoline samples of high volatility, the evaporation losses can only be estimated as low as 0.03%.

Comparative data by the two methods were obtained on periodic samples of crude oil on which an extended storage test was being investigated. The data, shown in Table V, indicate good agreement between the two methods.

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Type Carbon Atom Analysis of Heavy Hydrocarbon Products

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A method is described for determining the percentages of aromatic, naphthenic, and alkyl carbon atoms in hydrocarbon products having molecular weights above 150. This method may be used on highly olefinic materials, and does not require the assumptions that the rings present are six-membered and *kata*-condensed. Selective hydrogenation is used to differentiate between olefinic and aromatic double bonds. The ring content is computed from the molecular formula after complete hydrogenation. The ring size is determined by using the density–molecular weight correlations of Kurtz and Lipkin. In some cases the extent of ring condensation is estimated from an equation based on measurable quantities; in other cases it is assumed that 50% of the condensable ring carbon atoms are condensed. The errors which may result from the use of this method are shown to be generally less than the errors caused by assuming six-membered, *kata*-condensed rings.

HEAVY hydrocarbon products are used extensively in commercial formulations. Of these heavy materials, some are the primary products of controlled manufacturing processes, and are tailor-made to definite specifications. Many of them, however, are the incidental by-products of pyrolytic or catalytic conversions, treating processes, and miscellaneous reactions employed in industry. Owing to a wide range of properties they are useful in diverse materials, such as plastic compositions, protective coatings, rubber compounds, asphalt tiles, core oils, and reclaiming oils.

The use of these hydrocarbon products in formulating other materials is largely an empirical art. Conversion of this art to a science requires the ability to correlate service behavior with molecular structure. In making such correlations it is desirable to express the complex structures present in these products in terms of simple features, such as the amounts and types of rings, the numbers of olefinic and aromatic double bonds, and the percentages of type carbon atoms.

A type carbon atom analysis determines the percentages of aromatic, naphthenic, and alkyl (paraffinic) carbon atoms in a hydrocarbon product. It is not generally applied to lower-boiling mixtures, whose compositions may be expressed adequately in terms of whole molecular types. However, materials boiling above approximately 200° C. or having molecular weights over 150, are apt to contain molecules which are difficult to classify as pure types, owing to their multiplicity of functions. The compositions of these materials, referred to in this paper as "heavy" hydrocarbon products, are more accurately defined in terms of the percentages of type carbon atoms.

Olefinic carbon atoms may be either naphthenic or alkyl. As the scheme described in this paper does not differentiate between these species, the type carbon atom percentages are supplemented by the number of olefinic double bonds per molecule.

The need for a method of characterizing complex hydrocarbon products in this laboratory led to a thorough survey of the literature dealing with this subject. Only four of the published methods proved to be broad enough in scope to be classed as general methods for type carbon atom analysis, but none of these was entirely adequate for the products encountered.

Three of the literature procedures—viz., the methods of Waterman (*32*, *33*) and of Deanesly and Carleton (*5*), and the *n-d-M* method (*27*)—are based primarily on correlations of physical and chemical properties. The fourth scheme, referred to by van Nes and van Westen (*22*) as the direct method, uses computations derived directly from ultimate analysis and molecular weight.

With the exception of the Deanesly-Carleton procedure, the other three methods require the absence of olefinic double bonds; moreover, they assume that the rings present are exclusively six-membered and condensed as in naphthalene, anthracene, and naphthacene ("*kata*"-condensation).

The Deanesly-Carleton method is applicable to cracked products. In addition, it provides a correlation which presumably makes it unnecessary to assume that all the rings present are six-membered and *kata*-condensed.

Because of its much broader scope, the Deanesly-Carleton method was studied extensively in this laboratory. Unfortunately, it was found unsuitable, except in limited cases, for the types of products referred to. The reasons for its failure may be summarized as follows:

The olefinicity is computed by Deanesly and Carleton from the bromine number. This procedure may lead to erroneous values for the content of olefinic double bonds (*30*), and this, in turn, affects the properties of the computed olefin-free portion of the molecule. In particular, it was observed in this laboratory that halogen addition values on the conjunct polymers and their partially hydrogenated products furnish olefinic double bond contents as high as 300% of the true value found by hydrogenation.

The correction made to the observed specific dispersion in order to place the computations on the olefin-free basis may lead to erroneous values of aromaticity, as measured by hydrogenation. This difficulty cannot be attributed solely to the wrong value of olefinicity; it was observed in this laboratory even when "corrected" bromine numbers, based on the true olefinicity found by selective hydrogenation, were used.

The increment of molecular refraction per double bond (termed k), which is used to arrive at the type of ring system involved, is based on a limited number of ring types. Thus, while several classes of monocyclic compounds are used in deriving the correlation, the only polycyclics used are indene, biphenyl, alkyl naphthalenes, and alkyl anthracenes. Condensed and uncondensed five-membered ring systems are absent.

The correlation is limited to three rings per molecule. Many useful hydrocarbon products extend beyond this value.

For oils containing no aromaticity, the terms involving k may reduce to zero, in which case k is indeterminate and the ring type cannot be ascertained.

Many hydrocarbon products are too dark in color to allow an accurate determination of refraction and dispersion.

Table I. Explanation of Symbols Used in This Paper

Symbol	Explanation
H_0	Weight % hydrogen in original product
H_1	Weight % hydrogen in selectively hydrogenated product
H_2	Weight % hydrogen in totally hydrogenated product
M_0	Molecular weight of original product
M_1	Molecular weight of selectively hydrogenated product
M_2	Molecular weight of totally hydrogenated product
Q	Average number of double bonds per molecule
Q_O	Average number of olefinic double bonds per molecule
Q_A	Average number of aromatic double bonds per molecule
n	Average number of carbon atoms per molecule
R	Average number of rings per molecule
r^a	Average ring size, expressed as number of carbon atoms
F^a	Degree of condensation or fusion of rings, expressed as per cent of condensable ring carbon atoms which are actually condensed
$F_{\text{min}}{}^a$	Minimum possible degree of condensation of condensable ring carbon atoms for the system under consideration
$F_{\text{est}}{}^a$	Estimated degree of condensation of condensable ring carbon atoms
r_C^a	Average number of carbon atoms per ring for maximum possible condensation
$(r_C)_F^a$	Average number of carbon atoms per ring for the F degree of condensation
C_R	Average number of ring carbon atoms per molecule
C_A	Average number of aromatic carbon atoms per molecule
C_N	Average number of naphthenic carbon atoms per molecule
C_P	Average number of alkyl (paraffinic) carbon atoms per molecule

[a] Symbols new in this work.

The existing methods of type carbon atom analysis were developed primarily for the examination of the heavy fractions obtained from petroleum. For such materials it has long been considered reasonable to assume that the rings present are six-membered and *kata*-condensed (*23*). However, these assumptions are unsafe when applied generally to heavy hydrocarbon products. Although some of these products are petroleum derivatives, they may have undergone isomeric or skeletal changes by virtue of the treatment to which they have been subjected. Consequently, they cannot be assumed to have the same basic structures as those present in fractions isolated from petroleum by simple physical processes. In fact, it has been proved that the rings present in some of these materials are predominantly five-membered, and are condensed according to a system which is not *kata;* moreover, some of these products are known to contain uncondensed ring systems, and many are highly olefinic. For these reasons, before adequate characterizations of the heavy hydrocarbon products could be made, it was necessary to develop a new method for type carbon atom analysis which would be more general than the methods currently in the literature.

The method developed in this laboratory eliminates the objections of the previous methods by the following means:

To avoid assuming only condensed rings, some new concepts are presented regarding ring condensation and the means for expressing it quantitatively. Equations are provided for calculating or estimating the extent of condensation in some ring systems. For other cases, the compromise assumption of 50% condensation is made.

To avoid assuming that the only type of condensation involved is *kata*, the new scheme employs a compromise between several possible types of ring fusion.

To avoid assuming that the rings present are six-membered, the actual ring size is determined by the method of Kurtz and Lipkin.

To make the method applicable to olefinic products, selective hydrogenation is an integral part of the procedure.

The method of analysis described in this paper has been used on hydrocarbon products with molecular weights as high as 1000, and containing as many as 10 rings per molecule. Theoretically, there is no upper limit to its application, but in practice the uncertainty increases above molecular weights of 700, since the ring size correlations have not been proved entirely valid above this level.

The method is especially useful for highly olefinic products, particularly the conjunct polymers. It is of no great advantage for the analysis of petroleum fractions, or other products in which it may be assumed that the rings present are exclusively six-membered and *kata*-condensed. For materials of this type, a rapid procedure, such as the *n-d-M* method, is preferred. The method described herein is time-consuming and of little use for routine; it should be considered primarily as a research tool.

DESCRIPTION OF METHOD AND DISCUSSION

Notation. For convenience in following the development of this method, the symbols used are listed in Table I. In general, the notation was selected to conform with that usually used in the conventional methods of type carbon atom analysis. New symbols which were adopted in this work are indicated by a footnote in the table.

Preliminary Examination. An inspection of the hydrocarbon product prior to analysis will often reveal useful information. As considerable time may be gained if the hydrogenation need not be selective, it is of particular value to know whether both olefinic and aromatic unsaturation are present.

Olefinic unsaturation is revealed by a positive halogen addition value. This is suitable as a qualitative guide, but should not be relied upon quantitatively. Aromaticity is detected by the presence of strong absorption in the ultraviolet region around 250 mμ and higher. Care must be taken not to confuse olefinic conjugation with aromaticity, since some conjugated cyclic hydrocarbons may absorb around 225 to 250 mμ and even higher. Conjugated hydrocarbons should show a positive maleic anhydride value. If any doubt exists, the sample should be hydrogenated selectively.

With this preliminary information as a general guide, the following criteria may be used for specific cases to decide whether or not the hydrogenation need be selective:

If the unknown sample has a bromine or iodine number greater than 1 and shows no ultraviolet absorption above 240 mμ, a rapid, nonselective hydrogenation should be carried out. The double bond content should be computed entirely as olefinic double bonds.

If the halogen addition value is less than 1 and the sample shows absorption above 240 mμ, a nonselective hydrogenation should be employed. The double bond content should be computed entirely as aromatic double bonds.

If the sample has a positive bromine or iodine number and also absorbs in the aromatic region or shows other indications of aromaticity, the hydrogenation should be made selective. After the olefinic double bonds have been completely saturated, the selectively hydrogenated product should then be totally hydrogenated to saturate the aromatic double bonds.

Occasionally a highly aromatic material may exhibit a positive but relatively low value of bromine or iodine number, even though olefinic double bonds are absent. Consequently, all materials having high aromaticity and relatively low values of bromine or iodine number should be considered of doubtful olefinic content, and should be hydrogenated selectively. However, in these cases the fact that the bromine or iodine number is not decreased under selective hydrogenation conditions will rule out the presence of olefinicity.

On the other hand, if a highly olefinic material is believed to contain a relatively low order of aromaticity, owing to its specific dispersion or to a poorly defined absorption band in the ultraviolet, the hydrogenation should be made selective. Any residual aromaticity will be revealed after no further hydrogenation occurs under selective conditions (see Example 2 under Illustrations of the Method).

Selective and Total Hydrogenation. Aromatic hydrocarbons, with but few exceptions, require temperatures above 180° C. for complete hydrogenation over nickel catalysts (*1*). Olefins, on the other hand, are completely and rapidly hydrogenated at much lower temperatures. In the hydrogenation of products containing both types of double bonds, it is desirable to carry out the first stage of the hydrogenation at as low a temperature as practical, in order to assure selectivity. In this work, a temperature of 130° ± 5° C. was adopted as the temperature for selective hydrogenation of olefinic double bonds.

Pressure and catalyst concentration usually have no critical effect on selectivity. The hydrogenations described in this paper employed pressures of 2000 to 3000 pounds per square inch of hydrogen and concentrations of 20 to 30% by weight of nickel catalyst.

The refractive index, combined with bromine or iodine number, is used as the criterion for completion of the selective process. After the bromine or iodine number reaches zero or levels off to a low value, filtered samples of the product are periodically tested for refractive index. The sample is considered selectively hydrogenated when the refractive index is lowered no more than 0.0002 after a further hydrogenation for at least 6 hours at the same temperature and using a fresh batch of catalyst. This criterion applies also to total hydrogenation of olefinic, nonaromatic products. An alternative criterion is presented in Example 7 under Illustrations of the Method.

Total hydrogenation of the selectively hydrogenated product is carried out at temperatures considerably above the selective temperature. Higher temperatures decrease the time required for complete saturation of aromatic double bonds. However, it is desirable not to exceed a temperature of about 275° C. in order to obviate the possibility of changes in the skeleton structure of the product.

Probably the best criterion for complete hydrogenation of aromatic nuclei is the lowering of ultraviolet absorption to zero (*19*, *20*). The hydrocarbon product is hydrogenated until its absorptivity in the aromatic region is nil or extremely low. Thus, many aromatic products which were hydrogenated in this laboratory to (F-C) dispersions below 98 were found to have absorptivities of 4 to 5 at 250 to 260 mμ or near 220 mμ. However, it was always possible to reduce the absorptivity throughout the 220- to 350-mμ range to about 0.01 or less by further, prolonged hydrogenation. For stubborn cases, or even as a general practice, it is advisable to use large ratios of catalyst to hydrocarbon, as recommended by Lipkin, Martin, and Worthing (*19*).

In rare cases, selective hydrogenation over nickel may be impossible. If a pronounced decrease in the absorptivity of the aromatic band is observed at the selective temperature, attack on susceptible aromatics is indicated. In such instances, copper-chromium oxide (*1*) or other selective catalysts (*4*, *12*, *29*, *31*, *34*) are advisable in place of nickel.

Relatively few hydrocarbons undergo hydrogenolysis below 125° C. (*2*). If appreciable hydrogenolysis occurs, it is detectable by a decrease in molecular weight. Van Nes and van Westen (*25*) have shown how to correct for slight cracking during hydrogenation. However, if the extent of hydrogenolysis is severe, it is advisable to hydrogenate under milder conditions, using a noble metal catalyst.

Average Number of Double Bonds per Molecule. Lipkin, Sankin, and Martin (*20*) have presented an equation for computing the total number of double bonds per molecule from the increase in hydrogen content after complete hydrogenation. Modification of their equation leads to Equations 1 and 2 for computing the number of olefinic (Q_O) and aromatic (Q_A) double bonds per molecule from selective hydrogenation data. Equation 3 is used to convert the number of aromatic double bonds to the number of aromatic carbon atoms (C_A) per molecule.

$$Q_O = \frac{M_0\,(\mathrm{H}_1 - \mathrm{X}_0)}{2.016\,(100 - H_1)} \tag{1}$$

$$Q_A = \frac{M_1\,(\mathrm{H}_2 - \mathrm{H}_1)}{2.016\,(100 - \mathrm{H}_2)} \tag{2}$$

$$\mathrm{C}_A = 2Q_A \tag{3}$$

Average Number of Rings per Molecule. The percentages of carbon and hydrogen in the completely hydrogenated product are corrected for minor amounts of nonhydrocarbon constituents or for slight experimental errors in the analysis (*5*) as shown in Table VII, footnote [g]. The corrected carbon and hydrogen contents are used with the molecular weight to derive the molecular formula of the hydrogenated product. This is converted to the conventional type formula, C_nH_{2n-x}, from which the average number of rings per molecule, R, is obtained by Equation 4.

$$R = 1 + \frac{x}{2} \tag{4}$$

Although R is obtained in some schemes by means of physical property correlations, the determination according to Equation 4 is more fundamental and, in principle, more accurate. It apparently applies to any type of ring system except the complex cyclic hydrocarbons whose rings cannot be represented graphically in the same plane, examples of which are generally rare (*7*, *16*).

Average Ring Size. Prior to 1939 no method was described in the literature for determining the average size of the rings in a polycyclic naphthene system. Since that date, the method of Kurtz and Lipkin (*13*, *14*, *18*) has been available for computing ring size from molecular weight, density at 20° C., and average number of rings per molecule.

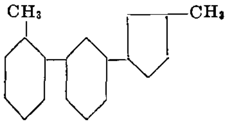
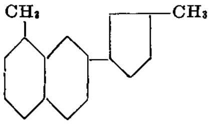
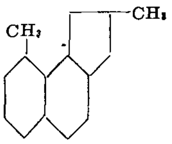
The Kurtz-Lipkin graphs of molecular weight versus density apply to five- and six-membered ring systems containing up to five rings per molecule and having molecular weights up to 700. The general molecular volume equation from which Kurtz and Lipkin derived these graphs is valid for ring sizes from 3 to 8. Both the graphs and the equation assume the absence of carbon atoms common to more than two rings ("buried" carbon atoms) and spirane systems.

The molecular weight–density graphs were extrapolated in this laboratory to eight rings per molecule and to molecular weight 1000. The ring sizes of products having molecular weights above 1000 or ring contents greater than eight were computed from the use of the general molecular volume equation (see Example 6 under Illustrations of the Method). It is understood that all such cases involve greater uncertainty than those falling within the scope of the Kurtz-Lipkin graphs.

The ring size of a solid hydrogenated product may be determined from the density of the molten material at any temperature below 200° C. The computations make use of Kurtz and Sankin's recent extension of the molecular volume equation to higher temperatures (*15*). An illustration of this is presented in Example 6.

Kurtz and Lipkin referred to the numerical value obtained from their graphs as average number of carbon atoms per ring. In this paper, the same number is referred to as average ring size, while the term used by Kurtz and Lipkin is employed in another sense.

Table II. Illustration of Terminology

			
R	3.00	3.00	3.00
C_R	17.00	15.00	13.00
n	19.00	17.00	15.00
Average ring size, r	5.67	5.67	5.67
Average number of carbon atoms per ring, $(r_C)_F$	5.67	5.00	4.33
r_C[a]	4.33	4.33	4.33
$r_C R$	12.99	12.99	12.99
rR	17.01	17.01	17.01
$(r_C)_F R$	17.01	15.00	12.99
F	0	50	100

[a] Computed from graphs 5A and 6A of Figure 1.

Table II illustrates the difference between these two concepts for three tricyclic molecules made up of the same rings but differing in the extent of ring condensation. The average ring size, r, of each molecule is identical. It is found simply by taking the arithmetic average of the individual ring sizes and is thus equal to (6 + 6 + 5) ÷ 3, or 5.67. However, the average number of carbon atoms per ring is found by dividing the number of ring carbon atoms per molecule, C_R, by the number of rings per molecule, R. The average number of carbon atoms per ring thus varies from 5.67 for the uncondensed case to 4.33 for the condensed system.

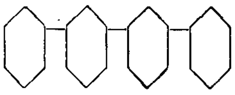
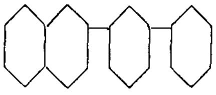
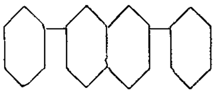
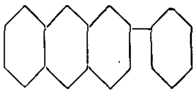
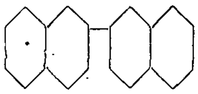
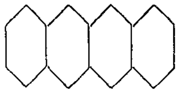
Average Number of Carbon Atoms per Ring. The means for determining the average number of carbon atoms per ring on the completely hydrogenated product is the key to this analytical scheme. As this quantity is dependent on the extent of ring condensation, as illustrated in Table II, its value is first determined for the hypothetical fully condensed system analogous to the one under consideration, and adjustment is then made for the actual or estimated extent of condensation. The symbol r_C is used to designate the average number of carbon atoms per ring for the fully condensed ring system.

Figure 1 presents a family of graphs which is used for determining the value of r_C for fully condensed ring systems made up of four-, five-, and six-membered rings containing no buried carbon atoms or spiranes. If the type of ring condensation involved is known, or if a certain type is considered most probable, the individual graph of Figure 1 corresponding to that type may be used (see Example 5). However, for completely unknown products it is necessary to employ curves which are compromises between the several possible types of condensation. These are labeled "average C_5" and "average C_6" in Figure 1. They are strictly arithmetical averages, inasmuch as statistical data showing the probability of encountering the different types of ring condensation are not available.

Degree of Ring Condensation. There is no general method in the literature for detecting and determining the degree of ring condensation in a polycyclic hydrocarbon system. The scheme offered in this paper is applicable to a limited number of cases, and for all other cases a compromise assumption of 50% condensation is made.

The symbol F is used to express the degree of ring condensation or fusion. In an effort towards simplification, F could be expressed in terms of the percentage of rings present which are condensed. However, such a definition is apt to lead to confusion. An illustration of this is seen in Table III for a series of tetracyclic molecules composed of six-membered rings in various stages of *kata*-condensation. By defining F in terms of the percentage of rings which are condensed, Examples 2 and 3 would be 50% condensed and 4 would be 75% condensed. However, Example 5, with each of its four rings fused to another ring, would of necessity be considered 100% condensed, despite the fact that further condensation, as in Example 6, is possible.

Table III. Illustration of Degree of Ring Condensation

Example Number	Ring System	Condensable Ring Carbon Atoms	Condensed Ring Carbon Atoms	F, %
1		6	0	0
2		6	2	$33^{1}/_{3}$
3		6	2	$33^{1}/_{3}$
4		6	4	$66^{2}/_{3}$
5		6	4	$66^{2}/_{3}$
6		6	6	100

For this reason, F is defined in terms of ring carbon atoms rather than rings. Every polycyclic hydrocarbon system is considered to have a maximum number of condensable ring carbon atoms. This number is equivalent to the number of ring carbon atoms which would be condensed or shared between rings if the system were as fully condensed as possible without involving buried carbon atoms. The degree of ring condensation, F, is then defined as the percentage of condensable ring carbon atoms which are actually condensed. The application of this definition may be seen with reference to the examples of Table III.

Criteria for Diagnosing Ring Condensation. If the average number of carbon atoms per ring is multiplied by the average number of rings per molecule, the average number of ring carbon atoms per molecule is obtained. If this generalization is applied to the two extreme structural configurations possible in a ring system—namely, to completely condensed and completely uncondensed rings—algebraic expressions are obtained which denote the minimum and maximum number of ring carbon atoms the system may possess.

Consider a general hydrocarbon system containing R rings per molecule with average ring size r. Let r_C represent the average number of carbon atoms per ring for the completely condensed case. Then $r_C R$ defines the minimum number of ring carbon atoms possible in the system. As the ring size is identical with the average number of carbon atoms per ring for an uncondensed system, rR represents the maximum number of ring carbon atoms the system may possess. The actual number of ring carbon atoms in the system has a value limited by the two extremes $r_C R$ and rR. Table II may be referred to for illustration of these concepts.

Now introduce another parameter, n, the total number of carbon atoms in the molecule. It is obvious that n can never be less than the product $r_C R$, as this would be equivalent to saying that there are fewer carbon atoms in the molecule than the minimum possible number of ring carbon atoms. Hence, the following generalization may be presented for any hydrocarbon system containing rings:

$$r_C R \leq n \tag{5}$$

Equation 5 provides two general possibilities. On the one hand, a product may occasionally be encountered in which $r_C R = n$, within the experimental limits imposed by Figure 1. In such a case it is clear that one is dealing with a completely condensed ring system. Inasmuch as the total number of carbon atoms is equal to the minimum possible number of ring carbon atoms, the system is also free of alkyl carbon atoms. Hence, for such a case, $F = 100\%$ and $C_P = 0$.

On the other hand, most products dealt with probably have systems in which $r_C R < n$. Such systems have either (1) completely condensed rings with alkyl carbon atoms, (2) partly condensed rings, or (3) completely uncondensed rings. The value of F may vary from 0 to 100% for such cases, and additional information is required in order to estimate F.

Fortunately, this additional information may sometimes be obtained by examining the relationships between n and the product rR. Three general cases are possible, depending on whether n is greater than, equal to, or less than rR.

Suppose that $n = rR$ in a saturated hydrocarbon system. From the preceding discussion, it is apparent that such a system could consist of completely uncondensed rings without alkyl carbon atoms, or of rings which are at least partly condensed and which contain alkyl carbon atoms. The key to differentiating these two possibilities lies in detecting the presence or absence of alkyl carbon atoms. This may be accomplished by examining the infrared absorption spectrum of the material.

Most alkyl groups give rise to a band in the vicinity of 7.25 microns, which is attributed to the methyl group. This band occurs even when the CH_3 is present as a methyl branch, or as the terminal methyl of a long alkyl group. The band may be rather weak if the only methyl group present is the terminal methyl of a long n-alkyl group. However, chains of alkyl carbon atoms having at least four CH_2 groups exhibit a characteristic band of their own at or near 13.8 microns. Consequently, if a system in which $n = rR$ shows no absorption bands at both the 7.25- and 13.8-micron positions, it is reasonably safe to conclude that alkyl carbon atoms are absent, and, therefore, the rings are completely uncondensed.

However, if a saturated system in which $n = rR$ shows absorption at 7.25 and/or 13.8 microns, alkyl carbon atoms are probably present. Such a system must be at least partly condensed, but the exact value of F is indeterminate.

If $n > rR$, in most cases the value of F cannot be determined. An exception is the specialized case in which the alkyl carbon atom or atoms link together more than one ring, such as in dicyclohexylmethane or 1,2-dicyclopentylethane. These systems have values of n which are greater than rR. They contain no methyl groups, and provided the chain linking the rings has no more than three carbon atoms, do not have an absorption band at 13.8 microns. Therefore, if a system in which $n > rR$ shows no absorption at both the 7.25- and 13.8-micron positions, the rings must be uncondensed. If absorption is present at 7.25 microns, F cannot be determined, because both condensed and uncondensed systems in which $n > rR$ may contain alkyl side chains. Likewise, if there is an absorption band at 13.8 microns but none at 7.25 microns, F cannot be determined. In this case, the system consists of a chain containing at least four methylene groups linking together two or more rings or groups of rings. If these terminal rings were always single rings, such as in dicycloalkylalkanes, F would always be 0. However, the terminal rings may themselves be condensed, as in 1,5-didecalylpentane, and the element of uncertainty is again present.

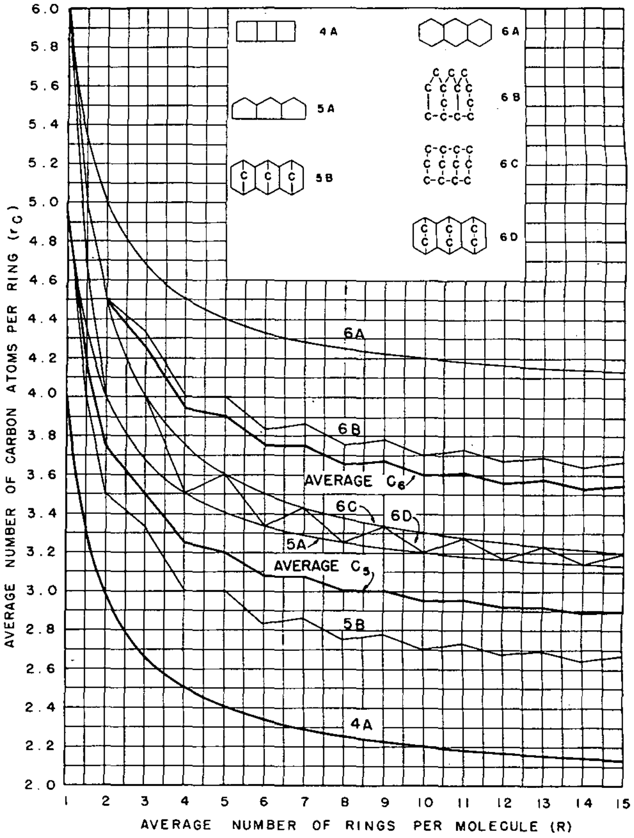


Figure 1. Relation between the average number of carbon atoms per ring and the average number of rings per molecule for condensed ring systems

Finally, since rR represents the number of ring carbon atoms for the completely uncondensed system, it follows that if $n < rR$ it is mathematically impossible for all the ring carbon atoms in the system to be uncondensed. For such cases one may diagnose at least partial ring condensation and the minimum possible extent of condensation may be determined by interpolating between the extremes for $F = 0$ and $F = 100$.

Derivation of Equation Defining *F*. For a ring system in which $n < rR$, and for which, therefore, F has a value greater than 0, let the average number of carbon atoms per ring be denoted by $(r_C)_F$. Then the average number of ring carbon atoms for this case is equal to $(r_C)_F R$. The number of condensable ring carbon atoms may be found as the difference between the maximum and minimum number of ring carbon atoms for the system—that is, as $(rR - r_C R)$. This may be understood by a study of Tables II and III. Likewise, the actual number of condensed ring carbon atoms is equal to $[rR - (r_C)_F R]$. Hence, by equating according to the definition previously given,

$$F = \frac{100\ [rR - (r_C)_F R]}{rR - r_C R} \tag{6}$$

Equation 6 would enable F to be computed if $(r_C)_F$ could be determined. As this is not possible, Equation 6 is modified by computing the value of F for the special case where $n = (r_C)_F R$. This defines the minimum possible extent of condensation in the system, which is designated as $F_{\min}$.

$$F_{\min} = \frac{100\,(rR - n)}{rR - r_C R} \tag{7}$$

Finally, as the maximum possible degree of condensation in any system is always 100%, one may estimate the degree of condensation, referred to as F_{est}, as a compromise between $F_{\min}$ and 100%. Thus,

$$F_{\text{est}} = \frac{F_{\min} + 100}{2} \tag{8}$$

Table IV summarizes the method of arriving at a numerical value for F. It can be seen that cases 3B and 4B have no diagnostic value for ring condensation. Unfortunately, it is probably true that the majority of hydrocarbon products analyzed are apt to fall in one of these classes. However, even for such cases, instead of assuming completely condensed rings, F_{est} is arbitrarily taken as 50%.

Example 6 under Illustrations of the Method is that of a hydrocarbon resin examined in this laboratory for which n was found to be $<rR$ and for which $F_{\min}$ could, therefore, be computed.

Average Number of Ring Carbon Atoms per Molecule. By rearranging Equation 6 and solving for $(r_C)_F$, Equation 9 is obtained. The value of $(r_C)_F$, the average number of carbon atoms per ring for the system having the F degree of condensation, is computed by substituting the proper values for r, r_C, and F or F_{est}.

$$(r_C)_F = r - \frac{F\,(r - r_C)}{100} \tag{9}$$

Equation 10 then provides the means for computing C_R, the average number of ring carbon atoms per molecule.

$$C_R = R\,(r_C)_F \tag{10}$$

Completion of Analysis. The remainder of the analytical scheme is practically routine. Since C_A has already been determined by hydrogenation, its value is subtracted from C_R, as in Equation 11, in order to obtain C_N, the average number

$$C_N = C_R - C_A \tag{11}$$

$$C_P = n - C_R \tag{12}$$

of naphthenic carbon atoms per molecule. Equation 12 leads to the value of C_P, the average number of alkyl carbon atoms per molecule. Finally, inasmuch as

$$C_A + C_N + C_P = n \tag{13}$$

the percentages of type carbon atoms are computed from Equations 14, 15, and 16, as in the conventional methods of type carbon atom analysis.

$$\%\ C_A = \frac{100\ C_A}{n} \tag{14}$$

$$\%\ C_N = \frac{100\ C_N}{n} \tag{15}$$

$$\%\ C_P = \frac{100\ C_P}{n} \tag{16}$$

Table IV. Estimating Degree of Ring Condensation

Case	Observation	Absorption at 7.25 and/or 13.8 μ	Diagnosis
1	$r_C R = n$	...	$F = 100\%$; $C_P = 0$
2	$r_C R < n < rR$	...	$F > 0$ Determine $F_{\min}$ by Equation 7 and F_{est} by Equation 8
3A	$r_C R < n = rR$	Absent	$F = 0$; $C_P = 0$
3B	$r_C R < n = rR$	Present	$F > 0$; $C_P > 0$ $F_{\text{est}} = 50\%$
4A	$r_C R < n > rR$	Absent	$F = 0$ Polycycloalkylalkane type, $C_P < 4$
4B	$r_C R < n > rR$	Present	$F_{\text{est}} = 50\ \%$

Table V. Errors Introduced into Determination of Naphthenic Carbon Atoms by Use of Erroneous Value of Average Ring Size

Type of Ring System	C_R, Computed[a] For True Value of r		For $r + 0.5$		For $r - 0.5$		Relative Error, %, in Determining C_R for Erroneous Case[b]	
	2 R	10 R	2 R	10 R	2 R	10 R	2 R	10 R
C_4 rings, uncondensed	8.00	40.00	9.00	45.00	...	...	12.5	12.5
C_4 rings, condensed	6.00	22.00	6.76	25.80	...	...	12.7	17.3
C_5 rings, uncondensed	10.00	50.00	11.00	55.00	...	...	10.0	10.0
C_5 rings, condensed	7.50	29.50	8 26	32.80	...	...	10.1	11.2
C_6 rings, uncondensed	12.00	60.00	...	...	11.00	55.00	8.3	8.3
C_6 rings, condensed	9.00	36.00	...	...	8.26	32.80	8.2	8.9

[a] Using values of average number of carbon atoms per ring from Figure 1.
[b] Relative errors ±.

Estimate of Errors. Two general kinds of errors may affect the determination of type carbon atoms—experimental errors in the physical measurements and errors arising from the assumptions and correlations used.

Possible errors which may result in determining molecular weights and carbon-hydrogen contents have been discussed thoroughly by van Nes and van Westen (*26*) with regard to the direct method. They have also considered the effects of the presence of oxygen, sulfur, and nitrogen upon the accuracy of the results (*24, 26*). These considerations also apply to this method.

The principal errors which may be introduced by using the assumptions of this method are associated with the determination of ring size, the use of average C_5 and C_6 curves in determining r_C, and the assumption of 50% condensation for the indeterminate cases.

EFFECT OF ERROR IN DETERMINATION OF RING SIZE. The determination of average ring size is subject to an error which is usually about ±0.2, but, in exceptional cases, may be as great as ±0.5. In order to estimate the effect of such an error on the determination of ring carbon atoms, test cases were set up of hypothetical polycyclic naphthene systems containing no alkyl carbon atoms. These are shown in Table V, wherein examples of systems comprising 2 and 10 rings per molecule are considered. The number of ring carbon atoms was computed for each of the 12 examples on the basis of its true ring size and on the basis of a ring size either 0.5 higher or 0.5 lower than the true value.

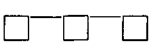
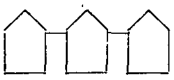
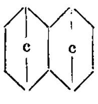
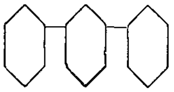
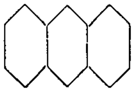
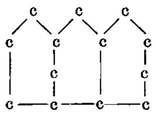
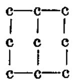
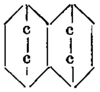
The final two columns of Table V show the percentage errors in the computed values for naphthenic carbon atoms, assuming a 0.5 error in ring size. With but one exception, these errors are between 8.2 and 12.7%.

EFFECT OF PARAFFINS ON ACCURACY OF RING SIZE DETERMINATION. By considering a number of hypothetical cases, it was demonstrated that the presence of as much as 75 mole % of paraffins in a mixture of cyclic and acyclic hydrocarbons affects the computed value of average ring size by no more than approximately ±0.15.

ERRORS INTRODUCED BY ASSUMING AN AVERAGE TYPE OF CONDENSATION. A study was made to determine the relative errors caused by using the "average C_5" and "average C_6" curves of Figure 1. The value of C_R was computed for dicyclic and decacyclic molecules for each of the six types of five- and six-membered ring systems of Figure 1, using both the "average" curves and the actual curves for each type. The relative errors caused by using the average curves varied from −14.3 to +12.5% for the twelve examples.

ERRORS INTRODUCED BY COMBINED

Table VI. Errors in Determination of Per Cent Naphthenic Carbon Atoms by Assumption of 50% Condensation and Use of Average Curves of Figure 1

Type of Ring System under Consideration		No. of Rings per Molecule	% Error in Determination of Naphthenic Carbon Atoms	
			By combined assumptions of this method	By assumption of *kata*-condensed C_6 rings
C_4 rings, uncondensed		2 10	−12.5 −22.5	+25.0 +5.0
C_4 rings, condensed		2 10	+16.7 +40.9	+66.7 +90.9
C_5 rings, uncondensed		2 10	−12.5 −20.4	0.0 −16.0
C_5 rings condensed		2 10	+9.4 +24.4	+25.0 +31.2
C_5 rings, condensed		2 10	+25.0 +47.4	+42.9 +55.6
C_6 rings, uncondensed		2 10	−12.5 −20.0	−16.7 −30.0
C_6 rings, condensed		2 10	+5.0 +14.3	0.0 0.0
C_6 rings, condensed		2 10	+16.7 +29.7	+11.1 +13.5
C_6 rings, condensed		2 10	+16.7 +45.5	+11.1 +27.3
C_6 rings, condensed		2 10	+31.3 +50.0	+25.0 +31.3

ASSUMPTIONS OF THIS METHOD. As it is expected that most unknown hydrocarbon products will require the adoption of $F_{est} = 50\%$ and the use of the "average C_5" or "average C_6" curves of Figure 1, it is helpful to know what errors are involved in the determination of naphthenic carbon atoms by the combined use of these assumptions. Table VI presents these data.

The errors were calculated by first computing r_C for each case, and then $(r_C)_F$ at $F = 50\%$. The values for C_R were then obtained, both by this method and by assuming six-membered *kata*-condensed rings. The computed values of C_R were compared with the correct values of C_R for each type of system. As seen in Table VI, the assumptions of this method may lead to possible relative errors varying from 22.5% too low to 50.0% too high. However, by using the alternative assumptions of C_6-*kata*-condensation, the possible errors vary from 30.0% too low to 90.9% too high

TOPICS FOR FURTHER INVESTIGATION

The method of analysis described in this paper is an attempt to provide a type carbon atom analysis scheme which is general for all heavy hydrocarbons. As such, the method falls short in some respects. Improvement of the method is possible along the following lines.

1. Average ring size. Graphs for four-, seven-, and eight-membered rings should be made available. Extension of the correlations should be made to at least 15 rings per molecule and molecular weight 2000. A means should be provided for determining ring size directly

Table VII. Properties of Hydrocarbon Materials Characterized

Example number	1	2	3	4	5	6	7
Material	Gray tower polymer	HF conjunct polymer	Alkylation sludge polymer	Aromatic recycle oil	Alkylated naphthalene	Hydrocarbon resin I	Hydrocarbon resin II
Boiling range, ° C.	92–278 at 0.6 mm. Hg	166 at 760 mm., 284 at 6 mm. Hg	...	103–189 at 0.5 mm. Hg	...	...	...
°API, 60° F.	14.2	21.2	22.1	2.9	...	...	...
Density, 25° C.	0.9705	0.9195	0.9064	1.0502[a]	0.9441[a]	...	...
Refractive index, 25° C.	1.5435	1.5179	1.5010	1.6405[a]	1.5571[a]	...	...
Specific dispersion (*F–C*), 25° C.	161.5	130.2	106.4	314.2[a]	218.2[a]	...	...
Bromine number	69.0	188	179	Negligible	Negligible	...	8.2
Iodine number	196	437	314	Negligible	...	...	18
Maleic anhydride value[b]	30	354	253	...	...	...	...
Color, ASTM	5½	>8	>8	4½	...	...	...
Viscosity, Stokes, 25° C.[c]	4.5	0.14	0.65	...	...	...	...
Molecular weight	325	265	362	223	258	512	694
Ultraviolet absorption maximum, mμ	265	248	245	256[d]	...	260[e]	266
Carbon, %	89.16	88.12	81.58	92.03	89.44	90.10	89.91
Hydrogen, %	10.72	11.29	11.45	7.82	10.46	8.78	9.97
Total, % C + % H	99.88	99.41	93.03[f]	99.85	99.90	98.88	99.88
Corrected % C[g]	89.27	88.64	87.69	92.17	89.53	91.12	90.02
Corrected % H[g]	10.73	11.36	12.31	7.83	10.47	8.88	9.98
Molecular formula	$C_{24.16}H_{34.60}$	$C_{19.56}H_{29.87}$	$C_{26.43}H_{44.21}$	$C_{17.11}H_{17.33}$	$C_{19.23}H_{26.80}$	$C_{38.85}H_{45.11}$	$C_{52.02}H_{68.71}$
Type formula	$C_nH_{2n-13.72}$	$C_nH_{2n-9.25}$	$C_nH_{2n-8.65}$	$C_nH_{2n-16.89}$	$C_nH_{2n-11.66}$	$C_nH_{2n-32.59}$	$C_nH_{2n-35.33}$

[a] At 20° C.

[b] MAV = mg. maleic anhydride reacting with 1 g. material under reflux in toluene solution.

[c] Computed from Gardner "bubble tube" viscosities.

[d] Considerable absorption present throughout 215- to 300-mμ range; absorptivity at 229 mμ = 170.

[e] Absorptivity at 260 mμ = 8.

[f] Oil contained 2.35% ash and 1.00% sulfur, balance probably oxygen.

[g] $\text{Corrected \% C} = \dfrac{\%\text{C}}{\%\text{C} + \%\text{H}}$

$\text{Corrected \% H} = \dfrac{\%\text{H}}{\%\text{C} + \%\text{H}}$

on solid products at ambient temperatures.

2. The determination of r and r_C should be made applicable to all possible types of hydrocarbons. This would include systems with buried carbon atoms, spiranes, highly fused systems having carbon atoms common to four rings, adamantane-type hydrocarbons, and stable free radicals.
3. An unequivocal means for detecting condensation and identifying the type of condensation should be sought.
4. A method should be devised for quantitatively measuring the extent of ring condensation in all cases and not merely the limited ones described in this scheme.
5. A method should be sought for differentiating cyclic and acyclic olefinic carbon atoms. This would make it possible to analyze a complex hydrocarbon product in terms of five types of carbon atoms: aromatic, saturated naphthenic, saturated alkyl, unsaturated naphthenic, and unsaturated alkyl. Inclusion of item 6 would add a sixth possible carbon atom type.
6. Provision should be made to include alkyne linkages in the scheme. Selective hydrogenation of triple bonds to double bonds might be used as the basis for this, provided a suitable means for detecting the alkyne group can be found.

Table VIII. Type Carbon Atom Analyses of Hydrocarbon Products Characterized

Example number Material	1 Gray tower polymer	2 HF conjunct polymer	4 Aromatic recycle oil	5 Alkylated naphthalene	6 Hydrocarbon resin I	7 Hydrocarbon resin II
Hydrogenation conditions						
Selective						
Temp., ° C.	130± 10	115 ± 5	...	...	...	75–100
Pressure, lb. sq. in.	2000–3000	1500–2100	...	...	...	1200–1900
Total						
Temp., ° C.	250–275	...	150–275	265–282	170–270	...
Pressure, lb./sq. in.	2000–3400	...	2000–3000	2200–2900	1500–3500	...
Catalyst						
Type	Ni/K[a]	R[a]	Ni/K[a]	Ni/K[a]	Ni/K[a]	R[a]
Weight %	30	5-5.5	30	30	30	30
Original product						
M_0	325	265	223	258	512	694
H_0 (corr.)	10.73	11.36	7.83	10.47	8.78	9.98
Selectively hydrogenated product						
H_1 (corr.)	12.19	13.33	...	...	...	10.20
Totally hydrogenated product						
M_2	331	273	243	265	524	...
d_4^{20}	0.9222	0.9029	0.9120	0.8753	0.9858[b]	...
Wt. % C (corr.)	87.12	86.67	87.07	86.36	88.59	...
H_2 (corr.)	12.88	13.33	12.93	13.64	11.41	...
Molecular formula	$C_{24.01}H_{42.29}$	$C_{19.70}H_{36.10}$	$C_{17.62}H_{31.17}$	$C_{19.05}H_{35.86}$	$C_{38.65}H_{59.31}$	...
Type formula	$C_nH_{2n-5.73}$	$C_nH_{2n-3.30}$	$C_nH_{2n-4.07}$	$C_nH_{2n-2.24}$	$C_nH_{2n-17.99}$	...
Computations						
Q	...	...	...	...	7.24	...
Q_O	2.68	2.98	0.00	0.00	...	0.84
Q_A	1.29	0.00	6.58	4.68	...	...
R	3.87	2.65	3.04	2.12	10.00	...
r	5.12	5.80	5.34	5.72	5.52	...
r_C	3.37	4.19	3.76	4.63	3.29	...
n	24.01	19.70	17.62	19.05	38.65	...
r_CR	13.04	11.12	20.09	9.82	32.90	...
rR	19.8	15.37	16.24	12.13	55.20	...
F_{min}, %	Ind.[c]	Ind.[c]	Ind.[c]	100	74.2	...
F_{est}, %	50	50	50	100	87.1	...
$(r_C)_F$	4.25	4.99	4.55	4.63	3.58	...
C_R	16.45	13.22	13.84	9.82	35.80	...
C_A	2.58	0.00	13.16	9.36	[d]	...
C_N	13.87	13.22	0.68	0.46	35.80	...
C_P	7.56	6.48	3.78	9.23	2.85	...
% C_A	10.7	0.0	74.6	49.1	[d]	...
% C_N	57.8	67.1	3.9	2.4	92.6	...
% C_P	31.5	32.9	21.5	48.5	7.4	...

[a] Ni/K = nickel on kieselguhr; R = Raney nickel.
[b] Density of the molten material at 142° C.
[c] Indeterminate.
[d] Aromatic carbon atoms included with naphthenic.

Table IX. True Unsaturation of Hydrocarbon Resin II

Sample	Original	1	2	3	4	5	6
Hydrogenation temp., ° C.	...	75 ± 5	90 ± 5	90 ± 5	100 ± 5	120 ± 5	150 ± 5
Hydrogen in sample, wt. %	9.98	10.01	9.96	10.18	10.20	10.63	10.83

ILLUSTRATIONS OF THE METHOD

Experimental Techniques. Densities were determined by means of a capillary pycnometer. For highly viscous liquids and solids, a wide-mouthed pycnometer was used (*3*). The accuracy in either case was within ±0.0005.

Molecular weights were determined in benzene solution by the Menzies-Wright method (*21*), modified by Hanson and Bowman (*8*, *9*), with an accuracy within ±1%.

Carbon and hydrogen determinations were made by the micromethod, with an estimated accuracy within 0.2%.

Bromine numbers were determined by the Francis method (*6*) and iodine numbers by the mercuric acetate-catalyzed Wijs method (*11*), using a 3-minute reaction time and 200% excess iodine.

Ultraviolet absorption spectra were determined in 2,2,4-trimethylpentane or methylcyclohexane solution in a Beckman Model DU spectrophotometer. A Perkin-Elmer Model 12B infrared spectrometer was used to record the infrared spectrograms.

All hydrogenations except that of Example 2 were performed in an American Instrument Co. superpressure rocking autoclave of 1410-ml. capacity and constructed of AISI Type 410 stainless steel. The hydrogenation described in Example 2 was carried out in a 2-liter Monel autoclave equipped with a propeller agitator rotating at 1750 r.p.m. and a water-cooled Teflon-packed stuffing box.

The catalysts used in this laboratory were nickel on kieselguhr, Raney nickel, and copper-chromium oxide. A reduced and stabilized nickel on kieselguhr (Harshaw Ni-0104) is the preferred catalyst for most hydrogenations, selective or otherwise. Although less active than Raney nickel, it requires no preliminary activation, is not pyrophoric, and is usually sufficiently active for all practical purposes. It is generally ground to a fine powder before use and employed in an amount equal to 30% by weight of the material charged.

Examples. A number of examples are presented from among the diverse products characterized in this laboratory. The properties of these products are summarized in Table VII and the hydrogenation conditions in Table VIII.

EXAMPLE 1. GRAY TOWER POLYMER. This complex oil was a vacuum-distilled "polymer" obtained in the Gray process by the clay treatment of a cracked gasoline (*28*). Its properties (Table VII) indicated both appreciable olefinicity and aromaticity, and, therefore, selective hydrogenation was employed. After selectivity was complete, a temperature rise of about 100° C. was required to effect further hydrogenation. The characterization of the oil is shown in Table VIII. The oil was found to be highly cyclic, with an average ring size somewhat larger than that of cyclopentane rings. Its content of olefinic double bonds was approximately twice as great as its aromatic double bond content.

EXAMPLE 2. HYDROFLUORIC ACID CONJUNCT POLYMER. This oil was derived from the acid layer in a catalytic process involving the contact of a nonaromatic petroleum fraction with liquid, anhydrous hydrofluoric acid at above 170° C. (*10*, *17*). The position of its ultraviolet absorption band and the high maleic anhydride value suggested conjugation rather than aromaticity. However, to eliminate any doubt, selective hydrogenation was carried out to the point of negligible bromine number, constant refractive index, and substantially no ultraviolet absorption between 220 and 310 mμ. Inasmuch as this was possible without raising the temperature above 120°, this fact was taken as evidence of the absence of aromaticity. The characterization of this interesting product is seen in Table VIII.

EXAMPLE 3. ALKYLATION SLUDGE POLYMER. This oil was obtained from the spent sulfuric acid of an alkylation unit. The sludge which separated by diluting the acid with water was extracted from the aqueous mixture by means of a light hydrocarbon, and neutralized with caustic. It contained a relatively high proportion of sulfur, oxygen, and ash, as noted in Table VII.

The use of corrected carbon and hydrogen values was believed to compensate for the presence of the nonhydrocarbon constituents. The composition of the oil was found to be quite similar to that of the hydrofluoric acid conjunct polymer, which it resembled closely in its properties.

The alkylation sludge polymer was nonaromatic. It contained 3.17 olefinic double bonds per molecule and 2.01 rings per molecule, the ring size being 5.5. On the basis of $F = 50\%$, the total oil had 36.8% naphthenic carbon atoms and 63.2% alkyl carbon atoms. Its double bonds were 59% conjugated (as found by maleic anhydride value).

EXAMPLE 4. AROMATIC RECYCLE OIL. This commercial product had properties which suggested a high degree of aromaticity and it was believed to be an aromatic recycle stock from catalytic cracking operations. Owing to negligible olefinicity, a nonselective hydrogenation was employed. Removal of the last traces of ultraviolet absorption in the neighborhood of 229 mμ was difficult and required hydrogenation at 250° to 275.°

The characterization is summarized in Table VIII. The low value of r for a highly aromatic product suggested the presence of components resembling acenaphthene ($r = 5.7$) or its homologs, as an error of a few tenths in r is possible.

EXAMPLE 5. ALKYLATED NAPHTHALENE. This product was known to be essentially an alkylated naphthalene, but was believed to contain a small proportion of naphthenic carbon atoms. It was characterized in order to check the validity of the ring size determination, as well as to obtain an indication of the degree to which the material was alkylated. The results are shown in Table VIII.

The found value of r was 0.28 in error, assuming only aromatic rings. F was taken as 100%, and the value of r_C derived from Figure 1 was the interpolated value for $r = 5.72$ and $R = 2.12$, using the "average C_5" curve and the curve for *kata*-condensed C_6 rings. The value obtained for % C_P indicated that the product was highly alkylated, whereas the value found for % C_N showed a small degree of naphthenicity.

EXAMPLE 6. HYDROCARBON RESIN I. This commercial product had a softening point in the neighborhood of 100° C. Although it was believed to have a low degree of aromaticity, the product was essentially olefinic. As a nonselective hydrogenation was carried out, only the total number of double bonds is reported. The hydrogenation was effected in benzene solution because of insolubility of the resin in saturated solvents. The resulting solution, which showed negligible ultraviolet absorption, was distilled under reduced pressure to remove cyclohexane. Last traces of solvent were removed by powdering the hydrogenated resin and keeping at 60° C. and 1-mm. pressure for a number of hours. The hydrogenated resin formed a snow-white powder which required a temperature above 130° C. for complete liquefaction. Its density was measured at 142° C.

Since the product had 10.00 rings per molecule, the Kurtz-Lipkin graphs could not be used for computing the ring size. The computation was made by constructing special graphs, using the general equation for the molecular volume of saturated hydrocarbons. An equation valid for a temperature of 142° C. was first set up, using the data presented by Kurtz and Sankin (*15*). A series of hypothetical decacyclic molecules with varying ring sizes, and condensed according to the schemes of Figure 1 was studied. For each case the molecular volume was computed from the general equation, and the molecular weight calculated from the formula of the compound. In this manner, graphs of density at 142° C. *vs.* molecular weight for decacyclic molecules with ring sizes between 5.0 and 5.6 were prepared. From these graphs, by interpolating the density of 0.9858 and molecular weight 524, the ring size of the hydrogenated resin was found to be 5.52.

The completed characterization is shown in Table VIII. In view of the fact that $r_C R < n < rR$ for this sample, it was possible to estimate F by means of Equations 7 and 8, and hence $(r_C)_F$ by Equation 9. Thus the extent of condensation of condensable ring carbon atoms was placed at approximately 87%. The value of C_R, found by Equation 10 was taken to be the same as C_N, as the aromaticity was not computed. On this basis, the resin had 92.6% naphthenic carbon atoms, and 7.4% alkyl carbon atoms.

EXAMPLE 7. HYDROCARBON RESIN II. This product was a resin of low iodine number, and the only information desired was the true olefinicity; hence, only a selective hydrogenation was carried out, in methylcyclohexane solution.

The temperature was varied stepwise. At each temperature, hydrogenation was continued until no further pressure drop occurred. The point of selectivity was difficult to determine by refractive index, owing to the highly aromatic nature of the resin. It was established by recovering each sample, dissolving to a fixed concentration in a mixture of acetone and benzene, and adding a fixed amount of 2% aqueous potassium permanganate. The first sample which failed to decolorize the solution was considered to be selectively hydrogenated. This was sample 4 in Table IX. It can be seen that the hydrogen contents also leveled off at this point. (Sample 2 appeared to represent an error in analysis.)

Calculation of Q_O indicated 0.84 olefinic double bond per molecule, whereas the bromine and iodine numbers indicated 0.36 and 0.49, respectively.

ACKNOWLEDGMENT

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Determination of Molecular Weight of the *m*-Polyphenyls by Measuring Their Absorbance

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At the wave length of maximum absorbance, the absorbance of the *m*-polyphenyls decreases with increasing molecular weight, and the values are directly related, graphically. In this manner, the absorbance of the unknown is obtained and the molecular weight is read from the standard curve.

INCREASED interest in the use of organic materials as coolants in high-temperature systems has resulted in the need for more chemical information about these heat transfer agents. Engineers designing heat transfer systems would be immensely benefited if the inevitable products of pyrolysis were identified and if the physical properties (viscosity, in particular) were estimated.

Some information on the *m*-polyphenyls has appeared in the literature. The properties of the *m*-polyphenyls of three to five phenyl groups are given in papers by Woods and others (*7*), Bowden (*1*), and Gillam and Hey (*2*). Gillam and Hey also list extinction coefficients for *m*-polyphenyls with 9 to 16 phenyl groups. The melting point of a *m*-sexiphenyl has recently been reported (*3*).

The polyphenyls are characterized by their absorbance curves. As the *p*-polyphenyl molecules increase in molecular weight, the wave length of peak absorbance increases. On the other hand, the wave lengths of peak absorbance of the *m*-polyphenyls (*2*) remain within the 250- to 260-mμ range. A study was then undertaken to correlate the molecular weights of the *m*-polyphenyls with their respective absorbances, and thus show that an unknown *m*-polyphenyl may be identified by its absorbance or extinction coefficient.

REAGENTS

Cyclohexane, spectrophotometrically pure.
Chloroform, analytical reagent.
Acetone, analytical reagent.
Ethyl ether, analytical reagent.
Ethyl alcohol, analytical reagent.
m-Terphenyl, Matheson, Coleman and Bell Co.
m-Quaterphenyl.
m-Quinquephenyl.
m-Sexiphenyl.
m-Nonaphenyl.

APPARATUS

Beckman Model DU Quartz spectrophotometer and 10-mm. cuvettes.
Distilling apparatus (*6*).
Melting point and molecular weight apparatus (*5*).

EXPERIMENTAL PROCEDURE

The individual pyrolytic products of *m*-terphenyl were obtained by vacuum distillation of the sample at 5-micron pressure (*6*). Some of the fractions were further purified by solvent extraction and by crystallization, using acetone, ether, cyclohexane, alcohol, and chloroform.

Samples of the purified fractions weighing from 1 to 5 mg. were dissolved in chloroform, under reflux. The solutions were diluted to 25 ml. with chloroform. Aliquots equivalent to exactly 0.4

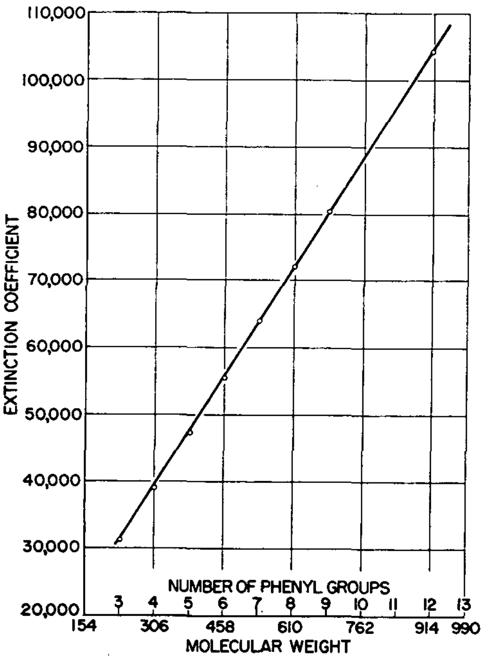

Figure 1. Extinction coefficients of *m*-polyphenyls

mg. were pipetted from the first solution and diluted to 25 ml. with chloroform. The maximum absorbances of the solutions on the 250- to 260-mμ range (with chloroform as reference) were obtained. The molecular weight of the unknown *m*-polyphenyl was then read from a molecular weight–absorbance curve.

Standard Curve. Solutions of the known *m*-polyphenyls were prepared in the exact concentration of 0.4 mg. per 25 ml. of chloroform. Absorbance was plotted, as ordinate, against the logarithm of molecular weight, as abscissa (Figure 3).

Table I. Properties of *m*-Polyphenyls

Compound	M.W.	M.P., ° C.	λ, Max., mμ	Slit Width, mμ	Absorbance	Extinction Coefficient
Terphenyl	230	85.5–86	251	0.90	2.17	31,050
Quaterphenyl	306	86	252	0.84	2.04	39,000
Quinquephenyl	382	97–98	253	0.80	1.99	47,500
Sexiphenyl	458	147.2–147.8	253	0.80	1.95	56,000
Noviphenyl	686	195–200	255	0.74	1.87	80,300
Duodeciphenyl	914	Approx. 250	256	0.72	1.81	104,000

RESULTS

The identities of the compounds (Table I) were established by careful determinations of molecular weights by the Rast (*6*) method, by melting points, by ultraviolet absorption curves, and by comparison with data already listed in the literature. The melting point determinations for the 9–*m*-phenyl and for the 12–*m*-phenyl group compounds had not been reported previously, but molecular weight and absorbance data indicate that these compounds exist. Melting points for the 9- and the 12-phenyl group compounds fall in line.

The extinction coefficients of the compounds listed in Table I were determined by using 0.4 mg. of polymer dissolved in 25 ml. of chloroform. These values were plotted against molecular weight and a straight-line plot was obtained (Figure 1).

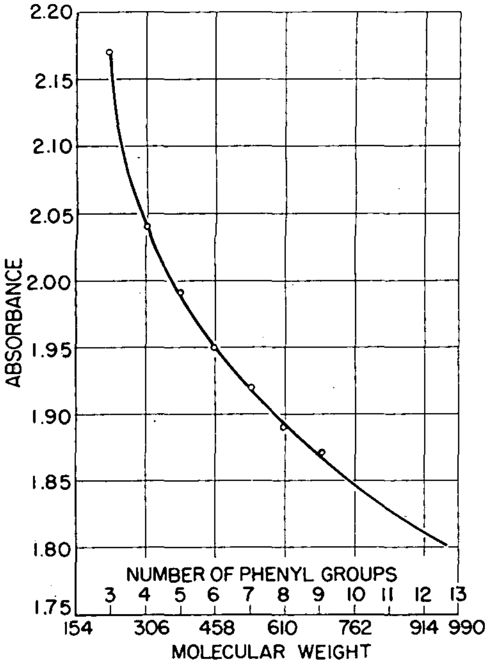

Figure 2. Absorbance of *m*-polyphenyls

The extinction coefficients of these polyphenyls change with concentration. As the concentration decreases, the extinction coefficient increases. Gillam and Hey (*2*) obtained higher results by using a spectrograph and photographic technique. Their extinction coefficients can be duplicated by using very dilute solutions (0.0008 mg. per 25 ml. of chloroform). Woods and coworkers (*7*) obtained higher results (Table I), which can be duplicated by using a concentration of about 0.002 mg. per 25 ml. of chloroform. The reason for using 0.4 mg. per 25 ml. is the technique convenience as shown in the procedure.

The curve in Figure 2 is a plot of absorbance against molecular weight and this curve (or the log plot in Figure 3) becomes the working curve for routine analysis of pyrolyzed *m*-terphenyl samples. The decrease in the slope of the curve (Figure 2) as the molecular weight increases is attributed to the decrease in the number of molecules per unit volume of solution.

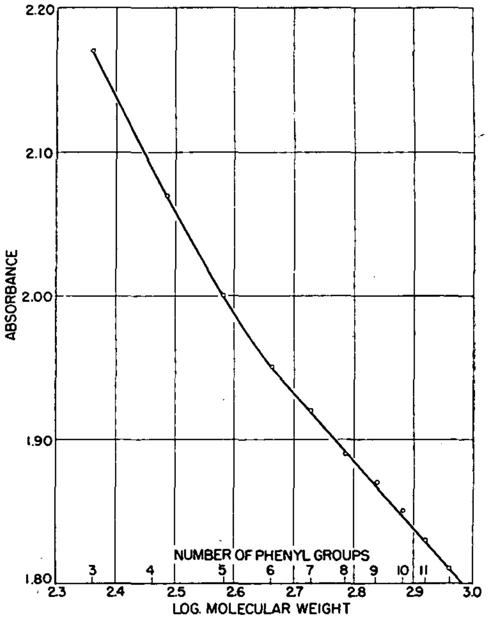

Figure 3. Absorbance of *m*-polyphenyls

It is essential that the pyrolysis products be separated carefully and purified. Then the absorbance at the maximum (250 to 260 mμ) can be read and the molecular weight may be determined directly from the standard curve. The identity of all of the *m*-polyphenyls shown in Table I, which have been separated from numerous pyrolyzed *m*-terphenyl samples, has been established. By using more dilute solutions, the possibility of extending the curves in Figures 1, 2, and 3 to include *m*-polyphenyls containing more than 12-phenyl groups becomes evident.

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Isotope Analysis Using Dimethylmercury

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A simple technique is described for the complete conversion of milligram quantities of elemental mercury to dimethylmercury and for mass spectrometric analysis of isotope abundance ratios. Mercuric chloride, prepared by direct combination of the elements, is reacted with a slight excess of dimethylzinc to form the mercury alkyl. Experiments using mercury-198 establish the absence of measurable fractionation in the synthesis, or of exchange, with mercury vapor during analysis. Measurements of isotope abundance ratios using dimethylmercury are compared with results obtained by other laboratories measuring mercury vapor.

VOLATILE organometallic compounds for isotope abundance measurements were first used by Aston (*1*) in his early studies of the isotopic constitution of the elements. Although measurements were usually made on the atom ions, the formation of hydride ions by rearrangement complicated the mass spectrum and the calculation of abundance ratios. Consequently, the use of the elements or appropriate inorganic compounds have been preferred especially for establishing upper limits to the existence of rare isotopes.

In 1951, however, on the basis of accumulated experience in mass spectra and the development of semimicrotechniques for the preparation of tetramethyllead, this compound was suggested (*2*, *3*) for use in routine isotopic analysis of milligram quantities of lead. Since that time, a number of laboratories have attained considerable experience with this method and at least one study (*4*) has shown that analytical results obtained by using the volatile lead alkyl are comparable with those obtained by evaporating lead chloride or lead iodide.

Although no measurable variation in the natural abundance of mercury isotopes has been reported, the element is now available in a wide range of isotopic compositions for various research purposes, including nearly pure mercury-198 and -202 for use in spectroscopic lamps as standards of length. Necessary isotope abundance measurements associated with these researches are commonly made by using the vapor of the element. However, accurate measurements on samples differing appreciably from normal composition require rigorous exclusion or removal of mercury background, usually accomplished by prolonged bakeout and frequent cleaning of the ion source. Furthermore, laboratories also engaged in the precise measurement of lead-204 abundance using lead atom ions prefer to exclude mercury vapor from their mass spectrometers at all times. Thus, the use of a volatile organometallic compound appears to be particularly attractive for routine abundance measurements of mercury isotopes.

It is the purpose of this paper, therefore, to describe a simple method for the preparation of milligram quantities of dimethylmercury and a mass spectrometric technique using this compound for isotope assay. Further, the method is evaluated by experiments using a slightly contaminated sample of mercury-198 and by means of comparative measurements by several laboratories on identical samples of a stock of ordinary mercury prepared as a density standard at the National Physical Laboratory, Teddington.

EXPERIMENTAL DETAILS

Preparation of Dimethylmercury. A schematic diagram of the apparatus used in the preparation of the mercury alkyl is shown in Figure 1. The manifold and connections are constructed of borosilicate glass tubing, 5 mm. in inside diameter. All stopcocks and joints are lubricated with a low vapor pressure fluorocarbon grease. Several 15-cm. lengths of 3-mm.-outside diameter borosilicate glass tubing, *A*, are flame-sealed at one end (the addition of a 2-ml. bulb at this end facilitates vacuum handling of the volatile reactants) and sealed to inner 14/20 standard tapers, *B*, at the opposite end. One or 2 mg. of the mercury to be analyzed is placed in each dry, clean tube by means of a calibrated micropipet. The sample tubes are then connected to the manifold as shown in Figure 1. The manifold and tubes are evacuated to a few microns pressure by means of a mechanical pump through a trap refrigerated with solid carbon dioxide. Stopcock *C* is then closed, and chlorine gas is admitted to the system at a pressure slightly less than 1 atm. All *D* stopcocks are then closed and the mercury droplets warmed gently in the tubes with a microburner until a rapid, vigorous reaction occurs. The resultant mercuric chloride sublimes readily to the cooler walls of each tube, permitting complete reaction of the mercury. Then the excess chlorine is pumped away and dimethylzinc (commercially available in high purity) is metered in slight excess of the calculated stoichiometric quantity by means of the gas pipet, *E*, and condensed into the appropriate sample tube held at liquid nitrogen temperature. The *D* stopcocks are manipulated properly throughout the procedure to prevent cross contamination of mercury samples. Finally, the tubes are flame-sealed, cut from the standard tapers, and stored at room temperature until analyzed by the mass spectrometer.

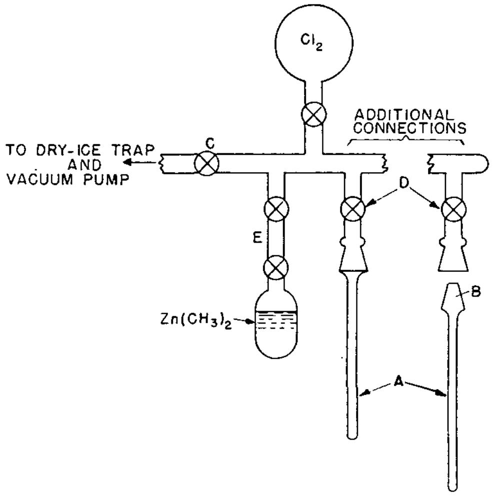

Figure 1. Schematic diagram of apparatus used in preparation of dimethylmercury

Pressure-volume-temperature measurements and mass spectrometric analyses made immediately after the tubes warm to room temperature indicate a rapid, essentially quantitative conversion to the mercury alkyl. In several preparations, small amounts of water vapor were condensed into the tubes after formation of the dimethylmercury in order to destroy any excess dimethylzinc. No significant differences in results were observed. There was also no evidence for fractionation effects in preparations using slightly less than stoichiometric quantities of dimethylzinc.

Several samples of dimethylmercury synthesized from approximately 95 atom % mercury-198 were sealed in vacuo together with droplets of ordinary mercury. No measurable decrease in mercury-198 abundance was observed in the dimethylmercury after standing several hours at room temperature.

Known mixtures of mercury-198 and ordinary mercury were also converted to dimethylmercury. The mercury alkyl exhibited the expected isotope abundance ratios within the estimated experimental errors.

Table I. Comparison of Isotope Analyses Using Dimethylmercury and Mercury Vapor

Observer	This Research	Fleming (*5*)	Von Ubisch and Sälg (*8*)	Palmer (*7*)
NPL sample number	9	8	13, 14, 15	6
Form	$Hg(CH_3)_2$	Hg vapor	Hg vapor	Hg vapor
Isotope	Abundance, Atom %			
196	0.156 ± 0.01	0.151 ± 0.002[a]	0.154 ± 0.005[b]	0.18 ± 0.02
198	10.12 ± 0.10	10.00 ± 0.02	9.98[c]	10.00 ± 0.11
199	16.99 ± 0.09	16.93 ± 0.02	16.95	16.91 ± 0.08
200	23.07 ± 0.12	23.15 ± 0.02	23.19	23.11 ± 0.06
201	13.27 ± 0.07	13.16 ± 0.04	13.21	13.18 ± 0.05
202	29.64 ± 0.15	29.83 ± 0.02	29.74	29.83 ± 0.16
204	6.79 ± 0.05	6.77 ± 0.03	6.78	6.78 ± 0.03

[a] Mean of three runs and standard deviation.
[b] Single determination.
[c] Weighted mean of three samples. Individual analyses differed by less than 0.2%, relatively.

Mass Spectrometric Measurements. The published (*3*) mass spectrum of dimethylmercury shows the Hg^+, $Hg(CH_3)^+$, and $Hg(CH_3)_2^+$ ions to be of comparable abundance. Of these, however, only the molecule ion is free of interference from mercury background and from the rearrangement hydride ions that complicate the atom ion and monomethyl ion spectra. Furthermore, it is convenient and advantageous to measure the abundances of the isotopic molecule ions by using ionizing electron energies sufficient to produce the molecule ions without forming ions by the dissociation of hydrogen atoms from the molecule. The nearly monoisotopic dimethylmercury-198, synthesized in the previous section, provides a sensitive means of determining the optimum electron energy for the measurement of $Hg(CH_3)_2^+$ ions in the absence of $Hg(CH_3)(CH_2)^+$ and other ions resulting from further loss of hydrogen atoms.

Abundance measurements are made using a 180° mass spectrometer designed for routine analysis of gases and volatile liquids. The instrument has a 5-inch radius of curvature and an estimated resolving power of 1/350. Sample vapor enters the ion source from a 4-liter reservoir through a molecular leak, and data are obtained by electrostatic scanning. The molecule ions of the dimethylmercury samples were measured several times using, alternately, 70- and 10-volt (uncorrected) electrons. The relative abundances of the mercury isotopes are calculated directly from the peak heights observed at 10 volts after correction for ions containing carbon-13 and deuterium atoms in normal abundance. However, a more precise measure of the mercury-196 abundance is obtained in the following manner: The $Hg^{196}(CH_3)_2^+/Hg^{198}(CH_3)_2^+$ ratio is measured using 70-volt electrons and a correction made for dissociated ions as determined from the pure $Hg^{198}(CH_3)_2$ spectrum at 70 volts. This ratio is then used to calculate the 10-volt $Hg^{196}(CH_3)_2^+$ peak height from the observed 10-volt $Hg^{198}(CH_3)_2^+$ peak. The calculated $Hg^{196}(CH_3)_2^+$ peak height at 10 volts usually agreed with the observed value well within the record reading error.

RESULTS AND DISCUSSION

Mean values obtained for the isotopic analysis of five samples of dimethylmercury synthesized from National Physical Laboratory mercury sample No. 9 are given in column 2 of Table I. The indicated uncertainties, which are two to five times the mean deviations for each analysis, are estimated only from possible errors in the recording system. Columns 3, 4, and 5 give results for samples from the same source analyzed, respectively, by W. H. Fleming, Hamilton College, Hamilton, Ontario; by Hans von Ubisch and S. E. Sälg, Aktiebolaget Atomenergi, Stockholm; and by G. H. Palmer, United Kingdom Atomic Energy Authority, Harwell. Fleming (*5*) used a 180° mass spectrometer of conventional design with magnetic scanning to measure mercury atom ions in mercury vapor. Von Ubisch and Sälg (*8*) and Palmer (*7*) used conventional 90° instruments with magnetic scanning to measure mercury atom ions in mercury vapor. Fleming and von Ubisch and Sälg introduced mercury vapor directly into the ion source, whereas Palmer introduced vapor through a molecular leak.

Although the abundance ratios are measured under different experimental conditions, the results obtained using dimethylmercury and routine techniques generally agree well within the stated limits with careful measurements obtained for mercury from the same source using the vapor. The mean of the collected values shown in Table I is not in complete agreement with Nier's 1950 measurements (*6*) on laboratory-supply mercury. However, the differences may result from alteration in the abundance ratio of the carefully purified sample used in this research.

The agreement shown in Table I is particularly gratifying in view of the simple synthesis for milligram quantities of dimethylmercury and the apparent suitability of the physical and chemical properties of this compound to mass spectrometric analysis. For example, the use of the mercury alkyl in a properly designed inlet system, such as that used in commercially available gas analysis mass spectrometers, avoids the uncertain degree of isotopic fractionation occurring in the evaporation of a liquid or a solid. Also, when used in sample introduction systems free of lubricated stopcocks, only elementary precautions are required in evacuating a previous sample of dimethylmercury in order to prevent memory effects in subsequent samples. Furthermore, dimethylmercury analyses are unaffected by a background of mercury vapor or absorbed mercury in the inlet system or analyzer tube. This is particularly useful in analyzing isotopic compositions differing appreciably from ordinary mercury. Finally, there is no interference between ions commonly used in the analysis of tetramethyllead—i.e., $Pb(CH_3)_3^+$ ions (m/e = 248 to 254) and the molecule ions of dimethylmercury (m/e = 226 to 234) thus permitting routine isotope analysis of both these elements using the same mass spectrometer.

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Radiochemically Pure Cerium

New Specification of Chemical Purity

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Radiochemically pure cerium was prepared as a carrier for tri- and quadrivalent ions in extremely low level radiochemical analyses, such as urinalysis. One requirement for such a reagent is that the alpha activity should not exceed one count per hour per milligram of cerium. Analyses of a series of commercial salts showed that none of the commercial compounds was suitable for such low level radiochemical work. The alpha activity in the commercial salts was due to thorium impurity, which varied from a few hundredths of a per cent to several per cent depending on the salt and its source. Radiochemically pure cerium was prepared by three methods: fractional precipitation of the iodate from homogeneous solution, fractional crystallization of the double magnesium nitrate, and fractional crystallization of ceric ammonium nitrate.

WITH the growth of radiochemistry there is an increasing need for a new specification of chemical purity, that of radiochemical purity. Many reagent grade chemicals may meet all the present specifications and yet be totally unsuitable for low level radiochemical work, because of the presence of small quantities of naturally occurring radioactive elements.

An important need for radiochemically pure compounds arose in the determination of the amounts of radioactive materials ingested by exposed personnel. For example, in the development of urinalysis procedures cerium salts were needed as carriers for tri- and quadrivalent ions (*1*). One requirement was that the alpha radioactivity should not exceed one count per hour per milligram of cerium. Although a series of commercial compounds was analyzed, none was satisfactory for such low level radiochemical work.

PROCEDURE

The following procedure was used to determine the alpha activity in the cerium compounds. A dilute nitric acid solution of each of the compounds was prepared so that the concentration of cerium was about 100 mg. of cerium per milliliter of solution. In some cases, such as hydrated oxides, oxalates, etc., it was necessary to convert the compounds to the nitrates. Twenty-five microliters of the solution, containing approximately 2.5 mg. of cerium, was mounted in 100 to 150 small droplets on a 2-inch stainless steel disk. The droplets were evaporated by placing the disk under an infrared lamp. The disk was ignited gently to convert the dried cerium compound to the oxide. Strong ignition was avoided, as high temperatures caused the oxide to shrink and lose its adherence to the stainless steel disk. The alpha activity on the disk was counted for 16 hours in a Nuclear Measurements Corp., Model PC-1 proportional counter. The cerium solution was standardized gravimetrically by precipitation of cerium oxalate followed by ignition to the oxide. The alpha activity in counts per hour per milligram of cerium was calculated.

Except for the 25-μl. micropipet, all new glassware was used for each sample. A series of blank determinations on the micropipet, forceps, disks, etc., gave an alpha activity of 1.2 ± 0.6 counts per hour. Since 2.5 mg. of cerium was mounted on each disk, the limit of detectability was approximately 0.5 count per hour per milligram of cerium. All commercial cerium compounds showed a definite count above the blank value.

ANALYSIS OF COMMERCIAL CERIUM SALTS

Fifteen commercial cerium salts were analyzed. The salts were purchased from the major cerium producers and were the highest quality obtainable. They are listed in Table I and are arranged in order of increasing alpha radioactivity. A wide range of radiochemical purities is represented by alpha activities of from 6 to 1245 counts per hour per milligram of cerium. In every case, except samples 6 and 7, the compounds came directly from the manufacturer, and the bottles had not been opened previously. A Kahlbaum sample, sample 12, was a museum specimen which had not been opened since it was originally packaged by the German concern in 1912.

In general, the cerous salts contained less radioactivity than the ceric salts. This may indicate that the manufacturers are concentrating thorium, the most probable radioactive impurity in cerium salts, in the process of producing the ceric compounds. The amount of radioactivity in the same cerium compound may vary considerably—for example, the alpha activity in the three ceric ammonium nitrate samples, samples 7, 11, and 13, varied from 89 to 202 to 693 counts per hour per milligram of cerium. Salts from the same source may vary considerably in radiochemical purity. The cerous oxide from company A, sample 1, had a count of only 6, whereas their ceric oxide, sample 14, had a count of 853, or contained over 100 times as much radioactivity.

Table I. Radiochemical Purity of Cerium Compounds

Sample	Compound	Source	Radioactivity[a]
1	$Ce_2O_3 \cdot xH_2O$	A	6
2	$Ce(NO_3)_3 \cdot 6H_2O$	B	16
3	$Ce_2(SO_4)_3 \cdot 6H_2O$	A	16
4	$Ce(NO_3)_3 \cdot 6H_2O$ (Crop No. 1)	B	16
5	$Ce(NO_3)_3 \cdot 6H_2O$ (Crop No. 2)	B	26
6	$(NH_4)_2Ce(SO_4)_3 \cdot 2H_2O$	B	30
7	$(NH_4)_2Ce(NO_3)_6$	B	89
8	$CeCl_3 \cdot 7H_2O$	C	129
9	$Ce_2(C_2O_4)_3 \cdot xH_2O$	A	143
10	$Ce(IO_3)_4$	B	186
11	$(NH_4)_2Ce(NO_3)_6$	B	202
12	$Ce(NO_3)_3 \cdot xH_2O$ (dated 1912)	D	364
13	$(NH_4)_2Ce(NO_3)_6$	A	693
14	$CeO_2 \cdot xH_2O$	A	853
15	$Ce(SO_4)_2$	C	1245

[a] Alpha counts per hour per milligram of cerium.

The radioactive impurity can be separated by fractional crystallization. This is shown by samples 4 and 5 (specially prepared by Company B) which are the first and second crop of crystals, respectively, from a series of cerous nitrate crystallizations.

If all the alpha radioactivity in the cerium compounds were due to thorium impurity, the percentage of thorium would vary from a few hundredths of 1% for sample 1 to several per cent for sample 15. The calculated alpha counts per hour per milligram of cerium for varying amounts of thorium impurity are shown in Table II.

Thorium was detected in three of the cerium compounds, samples 2, 10, and 11, by isolation and identification of its ra-

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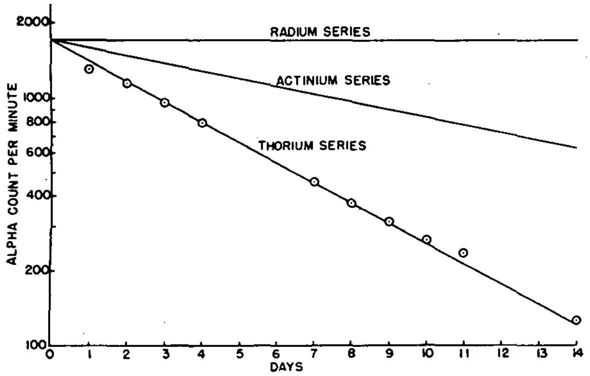

Figure 1. Decay of radium isotopes separated from ceric ammonium nitrate

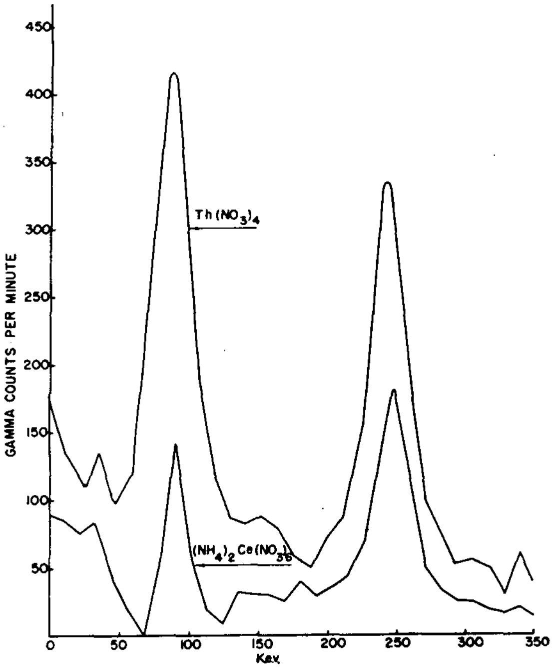

Figure 2. Gamma spectra

dium-224 daughter. Ten milligrams of barium carrier was added to a solution containing 1 gram of the cerium salt, and the barium was precipitated as the sulfate. The barium sulfate was centrifuged, washed, and mounted on a stainless steel disk. The disk was counted daily for alpha activity. The thorium content was calculated from the amounts of the radium daughter detected. The results of these calculations are shown in Table III.

The data for Sample 11 are plotted in Figure 1. The straight lines represent the decay at equilibrium of the alpha-emitting radium isotopes in each of the three natural decay series, the radium, actinium, and thorium series, respectively. It is obvious that the radium isotopes separated from the ceric ammonium nitrate decays at a rate equivalent to that in the thorium series—i.e., radium-224.

The presence of thorium in the ceric ammonium nitrate, Sample 11, was confirmed by x-ray fluorescence spectroscopy (*4*). (Arc spectrographic analysis is less sensitive than the x-ray method, because the thorium spectrum is masked by that of cerium.) The thorium was also detected by gamma pulse height analysis (Figure 2). The upper spectrum is that of thorium nitrate, whereas the lower spectrum is due to the radioactive impurity in ceric ammonium nitrate. The spectra are nearly identical.

Therefore, the major radioactive impurity in commercial cerium salts is thorium as proved by the separation and identification of radium-224, by x-ray fluorescence spectroscopy, and by gamma pulse height analysis.

PREPARATION OF RADIOCHEMICALLY PURE CERIUM

Because none of the commercial cerium salts was suitable for use in urinalysis, radiochemically pure cerium was prepared in the laboratory by three methods: fractional precipitation of the iodate from homogeneous solution (*5*); fractional crystallization of the double magnesium nitrate (*2*); and fractional crystallization of ceric ammonium nitrate (*3*).

Fractional precipitation of ceric iodate from homogeneous solution was accomplished by a method similar to that reported by Willard and Yu (*5*). Iodic acid and potassium chlorate were added to a solution of cerous nitrate in approximately 5*N* nitric acid. The solution was boiled; and as the cerium was slowly oxidized, it precipitated as ceric iodate. Several successive iodate fractions were precipitated. The cerium remaining in solution after the fifth iodate precipitation was recovered as the hydroxide. After filtration, the iodate fractions were converted to nitrates by evaporating with hydrochloric acid and then with nitric acid. The hydroxide residue was filtered, washed, and dissolved in nitric acid. After 3 to 4 weeks equilibrium was reestablished and samples of the nitric acid solutions were mounted as described. The alpha activity on each disk was determined. The results of these analyses are shown in Table IV.

Table II. Radioactivity in Cerium Compounds Due to Thorium Impurity

Thorium, %	α Activity[a], Counts/Hr./Mg. Ce
1.0	456
0.1	46
0.01	4.6
0.001	0.5

[a] Calculated; assuming radioactive equilibrium.

Table III. Analyses of Commercial Cerium Compounds

Sample	Compound	Thorium, %
2	$Ce(NO_3)_3 \cdot 6H_2O$	0.03
10	$Ce(IO_3)_4$	0.4
11	$(NH_4)_2Ce(NO_3)_6$	0.4

Table IV. Analyses of Iodate Fractions

Fraction	Total Cerium Precipitated, %	α Activity, Counts/Hr./Mg. Ce
Original	. .	16
1	9	95
2	19	0
3	39	0
4	22	0
5	3	0
Residue	3	24

One gram of cerium as the nitrate was used as the original material. The reagent grade cerous nitrate hexahydrate had an alpha activity of 16 counts per hour per milligram of cerium. The increase in activity in the first iodate fraction to 95 counts per hour per milligram of cerium is undoubtedly due to thorium which should concentrate in this fraction. The final residue should contain any actinium and radium isotopes along with their daughter activities, and for this reason showed a count of 24. The center fractions composing nearly 90% of the original cerium sample were radiochemically pure.

Analyses were made of samples from two fractionation series, NCe and CE, in which cerium had been recrystallized from concentrated nitric acid as the double magnesium nitrate [$3Mg(NO_3)_2.2Ce(NO_3)_3.24H_2O$]. It was estimated that 100 to 150 recrystallizations had been made in each series. Samples from each fraction in the two series were analyzed for alpha activity by the method described. If the radioactive impurity were thorium, it would be expected to concentrate in the more soluble end—i.e., larger numbered fractions—of a double magnesium nitrate series. The results of analyses listed in Table V show that the radioactive impurity did concentrate in the more soluble end of the series.

The cerium in about one half of each series was radiochemically pure. The alpha-emitting impurity increased by an order of magnitude in each of the last four fractions of each series.

Nine bottles of cerium samples taken from various steps in a cerium purification process, similar to a commercial process, were supplied by G. Frederick Smith. Bottle 1 contained a sample of technical grade ceric oxide. Several pounds of this material was leached with hot nitric acid, and a sample of the nitric acid insoluble residue, containing silica, some phosphate, etc., was packaged in bottle 2. To the nitric acid soluble portion was added excess ammonium nitrate to precipitate ceric ammonium nitrate, a sample of which was contained in bottle 3. A series of fractional crystallizations of the ceric ammonium nitrate yielded a second, third, fourth, and fifth crop of crystals, samples of which were in bottles 4, 5, 6, and 8, respectively. The filtrates from the first, second, and third crop of crystals were apparently discarded as no samples of these materials were available for analysis. However, samples of the filtrates from the fourth and fifth crops of crystals were supplied, bottles 7 and 9, respectively.

The radiochemical analyses of the nine samples are shown in Figure 3. The alpha activity in the cerium samples shows the distribution of thorium in the purification process. The fifth crop of crystals, bottle 8, is very pure, since it contains a maximum of 0.001% thorium. Therefore, radiochemically pure cerium can be prepared by the method of Smith, Sullivan, and Frank (*3*), provided a sufficient number of crystallizations are made. The yield in this experiment was rather low as only 3 ounces out of more than 4.75 pounds was recovered in a radiochemically pure form.

Radiochemical analyses indicate that the original cerium, bottle 1, contained about 0.75% thorium and that the first crop of crystals, bottle 3, contained only about 0.25%. The thorium appears to concentrate in the filtrates as is shown by the analyses of the samples in bottles 7 and 9. In these cases the filtrates contain 10 times more alpha activity than the crystals, bottles 6 and 8, respectively. The nitric acid leach of the technical grade ceric oxide seems to have had little effect on the separation of thorium as the alpha activity of the cerium in bottle 2 is only slightly greater than that of the original, bottle 1.

CONCLUSIONS

Radiochemically pure cerium with an alpha activity of less than one count per hour per milligram of cerium can be prepared by any of the three described methods. Fractional iodate precipitation seems suitable only as a laboratory method for rapidly preparing small quantities of radiochemically pure cerium. Even though a high yield may be obtained, the high cost of chemicals prohibits the large scale use of the method. Either fractional crystallization of the double magnesium nitrate or ceric ammonium nitrate is satisfactory for producing large quantities of radiochemically pure cerium on a routine scale. The number of crystallizations required to obtain a high radiochemical purity depends on the purity of the technical grade ceric oxide used as the source material.

Table V. Analyses of Double Magnesium Nitrate Fractions

Fraction NCe	α Activity, Counts/Hr./Mg. Ce	Fraction, CE	α Activity, Counts/Hr./Mg. Ce
25	0	25	0
26	0	26	0
27	0	27	0
28	0	28	0
29	0	29	1
30	3	30	20
31	25	31	374
32	229	32	13,474
33	2,621		

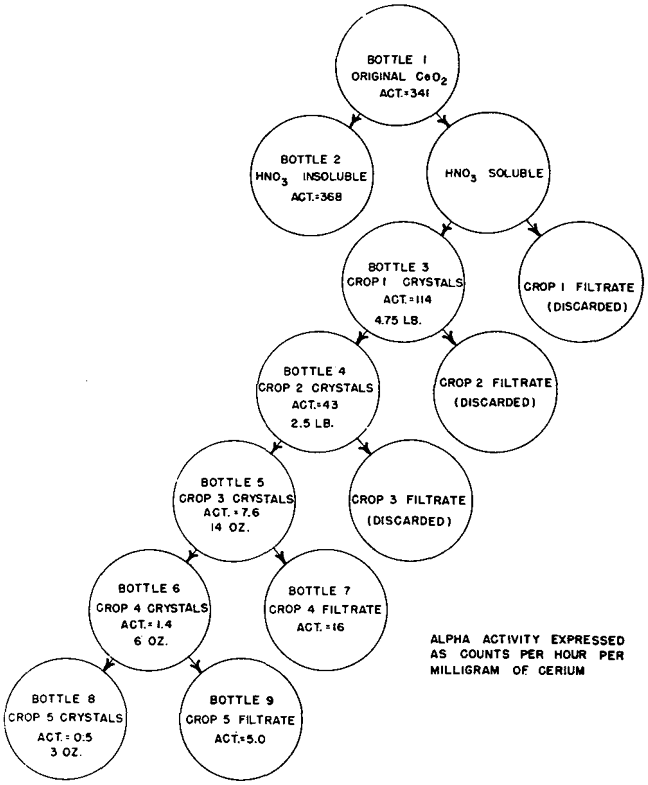

Figure 3. Ceric ammonium nitrate fractions

The detection of radioactivity in cerium salts is only one example of radioactivity in reagent grade chemicals. The authors have detected radioactivity in several common chemicals and for this reason are suggesting a new chemical purity specification, radiochemical purity.

ACKNOWLEDGMENT

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Determination of Pore Volume of Solid Catalysts

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A convenient method has been devised for the measurement of the micropore volume of porous solids at room temperature. It consists of weighing the carbon tetrachloride adsorbed by catalyst samples after equilibration with a solution in which the vapor pressure of carbon tetrachloride had been lowered 5% through cetane addition. The above type of pore volume measurement was found to be just as reliable and much more convenient than that consisting of the measurement of adsorbed nitrogen at −196° C.

IN RECENT years increasing attention has been focused on the porosity of solid catalysts. This interest in catalyst porosity created a need for a convenient, yet accurate method for making the large number of pore volume measurements used to follow variations in catalyst preparation and changes in catalyst properties during use, and to check specifications in catalyst manufacture. The development of such a method is the subject of the present study.

Table I. Pore Diameter Thresholds for Carbon Tetrachloride at 25° C.

Relative Pressure	Pore Diameter Threshold, *A*
0.995	8000
0.99	4000
0.98	2000
0.95	800
0.90	390
0.80	180

Although the pore volume of porous solids is commonly determined by measuring condensed nitrogen in a gas adsorption apparatus (*5*, *7*), this method was considered much too time-consuming to satisfy the above-mentioned needs. The most convenient way of determining pore volumes would seem to consist of directly weighing a suitable vapor condensed at its saturation pressure (*1*, *8*). It has now been found, however, that such measurements often give inordinately high pore volume values, because of the existence of interparticle condensation. This difficulty can be overcome by dissolving enough nonvolatile solute (cetane) in the saturating liquid (carbon tetrachloride) to lower its vapor pressure to the desired extent.

DATA AND DISCUSSION

Carbon tetrachloride very nearly exhibits Raoult's law behavior in the range of carbon tetrachloride—cetane composition under interest. The deviation from ideality, estimated by means of the Hildebrand-Flory-Huggins equation (*4*) is only 0.3% in a solution containing 95 mole % carbon tetrachloride. The relative pressure (partial pressure divided by vapor pressure of pure liquid) of carbon tetrachloride is therefore constant over such a solution between 20° and 30° C.

The pore volume of porous solids is conventionally pictured as consisting of a network of cylindrical micropores. Though this is undoubtedly an oversimplification, the effective pore diameter threshold below which all pores are filled can be calculated in terms of the carbon tetrachloride relative pressure by means of the Kelvin equation, which is

$$d = \frac{-4\gamma V \cos\theta}{RT \ln p/p_0}$$

where d is the pore diameter threshold, γ is the surface tension [26.1 dynes per square centimeter at 25° C. for carbon tetrachloride (*3*)], V is the molar volume (97.1 cc. per mole at 25° C.), θ is the contact angle (assumed to be 0° C.), R is the gas constant, T is the absolute temperature, and p/p_0 is the relative pressure. The resulting relation between pore diameter threshold and relative pressure is illustrated in Table I.

The pore diameter threshold at a given relative pressure changes with temperature as the change in molar volume of carbon tetrachloride is more than offset by the corresponding change in surface tension. The effect is practically negligible, however; a 1° change in temperature changes the pore diameter threshold only 0.7% at a relative pressure of 0.95.

The adsorption of carbon tetrachloride at room temperature on four different catalyst samples is plotted as a function of relative pressure in Figure 1. Adsorption was measured using the procedure described. It can be seen that carbon tetrachloride adsorption rises sharply above a p/p_0 of 0.97 for three of the four samples studied. After carbon tetrachloride had been adsorbed on the same three samples at a p/p_0 of 1, the resulting samples had a tendency to cake and did not flow freely. The above behavior strongly suggested that carbon tetrachloride vapor was condensing in the small interstices formed by catalyst particles in contact with one another. Van Nordstrand, Kreger, and Ries (*9*) have arrived at a similar conclusion on the basis of measurements of nitrogen adsorption near saturation pressure. It is

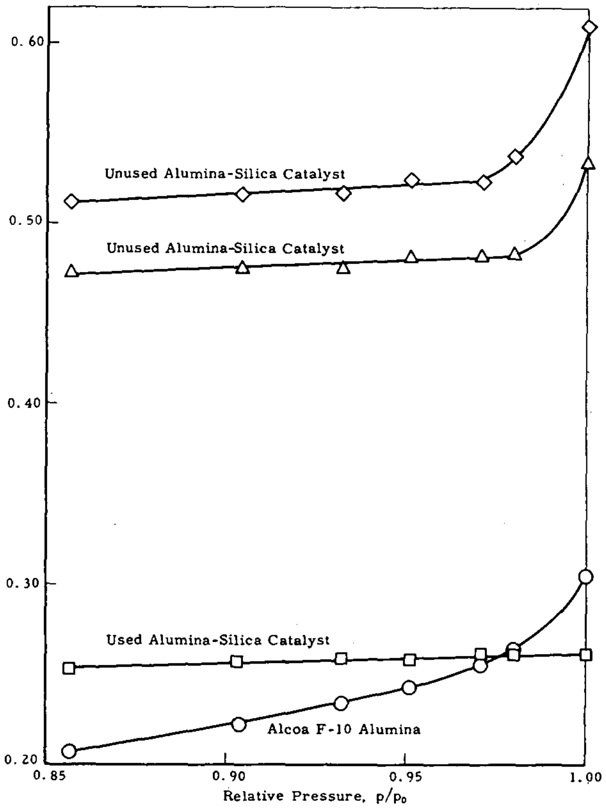

Figure 1. Carbon tetrachloride adsorption at room temperature

thus clear that the weight of vapor adsorbed at a p/p_0 of 1 is not an accurate measure of micropore volume.

For the reasons given it was decided to make pore volume measurements at a relative pressure below 0.97. Since too low a relative pressure is also undesirable, a carbon tetrachloride relative pressure of 0.95 was used in the procedure finally decided upon. As can be seen in Table I, the pore volume measured at this relative pressure includes all pores having diameters smaller than 800 A. It is emphasized that the latter definition of pore volume is arbitrary. In studying other types of porous systems it may be desirable to choose an entirely different pore diameter threshold than the one chosen in the present investigation.

The relatively high slope of the curve for alumina in the 0.85 to 0.95 relative pressure region (Figure 1) indicates that this sample has an appreciable number of 250 to 800 A. pores. This result suggests that the procedure described, if used at the appropriate relative pressures, can also be utilized to determine pore size distributions.

Materials and Procedure. Carbon tetrachloride (Baker and Adamson) was chosen as the saturating liquid, because it is nonpolar, commercially available in pure form, and essentially a spherical molecule and should therefore exhibit no unusual packing behavior. Cetane (*n*-hexadecane) was chosen as the solute because of its low vapor pressure (0.002 mm. at 25° C.) and also because it is nonpolar. The cetane adsorbed in the course of a pore volume measurement was negligible. Before use the cetane was purified by fractional recrystallization.

The basis of the procedure consisted of weighing the carbon tetrachloride adsorbed after equilibrating dried catalyst samples with a solution in which the vapor pressure of carbon tetrachloride had been lowered to 95% of its saturation pressure.

Samples (1 to 2 grams) contained in small weighing bottles (15 × 20 mm.) were first dried in a vacuum oven at 103° C. for 3 hours at a pressure of 5 inches of mercury. (Drying in an ordinary oven at 130° C. may also have been adequate, but was not tried.) Samples were then weighed and transferred to a 16-cm. vacuum desiccator (Corning No. 3118) containing 210 ml. of carbon tetrachloride–cetane mixture (see below). The desiccator, which was attached to a graduated cold trap, manometer, and vacuum pump, was then closed and evacuated until the carbon tetrachloride boiled. After testing for leaks, about 10 ml. of carbon tetrachloride was vaporized and collected in the cold trap in order to flush out all air. The desiccator contents were then allowed to equilibrate from 4 to 16 hours at room temperature.

The average temperature of the samples was noted. Air was then slowly admitted to the desiccator. The desiccator cover was removed, the weighing bottles were stoppered as rapidly as possible, and the resulting samples weighed. Pore volumes were then calculated from the gain in weight of the sample and the density of carbon tetrachloride at the desiccator temperature. The gain in sample weight was first corrected for the weight of carbon tetrachloride vapor caught upon stoppering the weighing bottle. This correction was measured by making a parallel determination using an empty weighing bottle.

A relative pressure of 0.95 was obtained by using a carbon tetrachloride–cetane solution containing 13.1 volume % (4.7 mole %) of cetane at 20° C. Before each determination an extra 10 ml. of carbon tetrachloride was added to 200 ml. of the above solution to compensate for the vapor condensed in the cold trap during the evacuation step. The relative pressure was only slightly influenced by errors of a few milliliters in this adjustment. The solution could be re-used, provided its composition was checked and readjusted using refractive index measurements as a guide.

Reproducibility of Method. Pore volume values were unaffected by any of the following variations in the procedure:

Varying the time from 1 to 20 seconds between opening the desiccator after equilibration and closing the weighing bottles.

Using sample weights that ranged from 1 to 4 grams.

Varying the equilibration period from 1 to 24 hours.

Judging from the data obtained upon checking and rechecking pore volume values of seven different samples, the accuracy of the method was estimated to be 0.01 cc. per gram. The precision was even better. If samples were measured side by side in the same desiccator, differences between duplicate samples were less than 0.003 cc. per gram in 90% of the cases.

Sources of Error. As samples were simply equilibrated in a desiccator in a room whose temperature commonly ranged from 20° to 25° C., the most likely source of error would be that resulting from temperature nonuniformity within the desiccator. It would therefore be desirable to store the desiccator in an air thermostat or a constant temperature room if higher accuracy is needed. Yet, as is evident from the smoothness of the data shown in Figure 1, pore volume measurements using the procedure are sufficiently accurate to follow even small variations in catalyst structure.

Precautions should be taken to keep catalyst samples dry, because adsorbed water, of course, reduces the pore volume. If samples are inadvertently exposed to atmospheric moisture, they should be redried directly before the equilibration step.

Heating high surface silica and alumina-silica samples to 550° C. lowers their micropore volumes as much as 10%. The desirability of standardizing the catalyst pretreatment is therefore obvious.

Comparison with Nitrogen Adsorption Method. Pore volume values obtained using the procedure are compared with those obtained from nitrogen adsorption measurements in Table II. In order to make the comparison valid, nitrogen pore volume values were determined at a p/p_0 of 0.977, where pores having diameters less than 800 A. were filled. The nitrogen pore volumes, Brunauer-Emmett-Teller (BET) surface areas (S), and average pore diameters ($\bar{d}$) were derived in the standard way (*2*, *6*) from nitrogen adsorption-desorption isotherms which were measured for each of the samples listed. All samples were originally Aerocat alumina-silica catalysts (microspheroidal type) manufactured by the American Cyanamid Co.

Table II. Comparison Between Nitrogen and Carbon Tetrachloride Methods

Catalyst History	S, Sq. Meters/G.	$\bar{d}$, A.	Pore Volume, Cc./G.	
			Nitrogen at 0.977 p/p_0	CCl_4 at 0.950 p/p_0
Unused	635	48	0.76	0.68
	541	50	0.67	0.60
	510	64	0.81	0.76
Steamed	507	71	0.90	0.88
	242	97	0.59	0.59
	135	113	0.38	0.37
	208	115	0.60	0.58
Used Steamed	94	126	0.29	0.28
	107	138	0.37	0.37
	125	157	0.49	0.47

Although the average difference between the nitrogen and carbon tetrachloride pore volume values listed in Table II is small, there seems to be no doubt that the nitrogen method gives larger pore volumes than does the carbon tetrachloride method when compared on the proper basis. Because the largest differences are observed for the catalysts having the smallest average pore diameters, it seems likely that some of the small pores in such catalysts are accessible to nitrogen but not to the much larger carbon tetrachloride molecules. These differences may or may not be desirable depending on the purpose for which the data are being obtained. If the entire void volume in a porous solid is needed, nitrogen measurement would give the better approximation. If the pore volume desired is the volume accessible to large hydrocarbon molecules, carbon tetrachloride measurement would give the more realistic value.

For most practical purposes, however, the major considerations involved in a choice between the above two methods are: Nitrogen pore volume values determined at a p/p_0 of 1 are unreliable because of the interparticle condensation phenomenon; nitrogen pore volume values determined at a p/p_0 of 0.977 are reliable but require considerable time and effort for their measurement; carbon tetrachloride pore volume values determined at a p/p_0 of 0.950 are both reliable and easy to measure.

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Determination of Zinc in Presence of Iron and Nickel

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The analysis of ferrites requires the determination of zinc, iron, and nickel to a high degree of accuracy, and although standard methods meet this requirement for iron and nickel, the accurate determination of zinc requires separation by methods that are usually tedious and erratic. Ion exchange offers a rapid and convenient method of separation, and subsequent precipitation of the zinc with quinaldic acid yields accurate results.

MOST analytical chemists are aware of the value of ion exchange as a tool for the separation of elements, but hesitate to apply the method to specific problems. By nature, zinc is one of the elements difficult to determine in the presence of iron and nickel, and recommended methods for its separation (*1*) are tedious and often erratic. However, separation by ion exchange (*2*) on Amberlite IRA-400 or Dowex-1 (a quaternary amine, polystyrene-divinylbenzene) is comparatively simple and can be made almost automatic by commercial ion exchange columns. Nickel is not retained even in 12*N* hydrochloric acid, iron is eluted by 0.5*N* hydrochloric acid, and zinc by 0.005*N* hydrochloric acid. Once separated, the zinc can be accurately determined by precipitation with quinaldic acid (*3*). The separation of zinc from other elements, as well as its determination in the gamma range, has been discussed (*2–5*).

EXPERIMENTAL PROCEDURE

Preparation of Ion Exchange. The ion exchange column can be a simple glass tube with the resin supported by glass wool resting on a one-hole rubber stopper, through which a glass tube is inserted to provide the outlet. A rubber tube and a screw clamp are used as the valve. More convenient models, which have the advantages of a reservoir and a design that does not let the column run dry, are available commercially (Microchemical Specialties Co., Berkeley 3, Calif.).

The height of resin should be about 30 cm. and its diameter 1 to 2 cm., as the total volume of resin determines the volume of solution needed to elute the ions. Amberlite IRA-400 or Dowex-1, 60 mesh, is suspended in distilled water. After the slurry is allowed to settle for a few minutes the cloudy, supernatant liquor is poured off, removing very fine particles and allowing a reasonable flow through the resin. To free the slurry of organic contaminants (a visible cloudy layer) it is poured into the column and washed with methanol. Then the resin is rinsed with distilled water to remove the methanol. The column can be stored with water for indefinite periods of time; however, it cannot remain for extended periods—i.e., overnight or weekends—in concentrated hydrochloric acid. Just before using, the column is equilibrated with 12*N* hydrochloric acid.

Separation of Elements. A solution of the chlorides of iron (as ferric), nickel, and zinc in concentrated hydrochloric acid is evaporated to 1 to 2 ml., is allowed to cool, and is transferred to the ion exchange column (previously equilibrated with 12*N* hydrochloric acid) with additional 12*N* hydrochloric acid. The column is eluted with 25 to 50 ml. of the same acid or until all the nickel has been washed through. The resin is then eluted with about 100 ml. of 0.5*N* hydrochloric acid to separate the iron fraction. The zinc fraction is eluted with about 150 to 200 ml. of 0.005*N* hydrochloric acid. The exact amounts should be checked for each column. Although the flow rates are not critical, they should be 0.5 to 1.0 ml. per minute.

Table I. Determination of Zinc after Separation by Ion Exchange

Zn Added, Mg.	Fe^{+++} Added, Mg.	Ni^{++} Added, Mg.	Zn Found, Mg.
48.8	201.3	53.1	48.5
			48.7
			48.9
			48.7
			48.4
			48.7
			48.9
			48.7
			48.6
			49.1
	Av. = 48.7 ± 0.20 (std. dev.)		
29.2	201.3	31.86	29.2
			28.6
			29.3
			28.9
	Av. = 29.0 ± 0.3 (std. dev.)		

Determination. Iron and nickel can be determined on separate samples. A trace of iron seems to persist in the zinc fraction and can be complexed with tartrate. To the solution of about 200 ml., 3 to 4 grams of ammonium chloride and 7 to 8 grams of potassium sodium tartrate are added. The amount of zinc should not exceed 0.1 gram. The solution is then neutralized to pH 7 with ammonium hydroxide and heated to boiling, and 15 ml. of 3% quinaldic acid (prepared by solution in hot water) is added with vigorous stirring and heating until precipitation is complete. The solution is cooled, filtered through a porous porcelain (Selas) crucible, and washed with cold water. The gravimetric factor, $F = 0.1529$, is weighed as zinc quinaldate, $Zn(C_{10}H_6O_2N)_2.H_2O$. The results obtained are shown in Table I.

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Colorimetric Determination of Molybdenum in Tungsten-Bearing Steels

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This study was undertaken because of the need for a rapid and simple method for the determination of molybdenum in excess of 0.24% in a wide variety of tungsten-bearing steels. Provisions have been made for the retention of tungsten in solution, the elimination of interfering light absorption caused by the yellow thiocyanate complex of reduced tungsten, and the use of a minimized correction factor for chromium to eliminate the need for the volatilization of chromium. Butyl Cellosolve is used to stabilize the orange-colored thiocyanate complex of quinquevalent molybdenum. Results are shown for tungsten-bearing standards and mixtures of standards which contain maxima of approximately 9% molybdenum, 18% tungsten, 19% chromium, and 5% cobalt. Included are results for a number of compositions which simulate various trade steels. The standard deviation of a single determination is 0.0149. The analysis time is approximately 1 hour per single sample.

EACH of the methods recently used by the Allis-Chalmers Research Laboratories for the routine determination of molybdenum in tungsten-bearing steels has certain disadvantages. The butyl acetate method, a widely used colorimetric method, depends upon the development of an orange-colored thiocyanate complex of quinquevalent molybdenum and the stabilization of this complex by extraction into a water-immiscible layer of butyl acetate (*9*). As the concentration of tungsten approaches 1%, tungsten interferes in two ways: Molybdenum is occluded in any tungstic oxide that may separate in the presence of perchloric acid (*9*), and tungsten remaining in solution causes interfering light absorption with the 410 mμ filter used in the filter photometer (Photelometer). Even if tungsten could be held in solution and its interfering light absorption overcome by the use of a filter of a different wave length, the method is still subject to undesirable anomalous color effects as reported by Hurd and Allen (*4*). In addition, the butyl acetate must be saturated with reagents (*5, 7*) and filtered immediately before use. This is a time-consuming operation. The extraction is also time-consuming, and it is difficult to prevent the formation on the absorption cells of a deposit which interferes with light transmittance.

The method most recently used by this laboratory for the routine determination of molybdenum in tungsten-bearing steels requires a lengthy hydrogen sulfide separation of the molybdenum prior to color development with hydrogen peroxide in concentrated sulfuric acid. The method does not permit the accurate determination of the small amounts of molybdenum present in some types of tungsten-bearing steel.

Other methods used are the polarographic, titrimetric, and gravimetric. Although these are good alternative methods, they are too lengthy for routine analysis.

The Cellosolve method in current use for nontungsten-bearing steels, an adaptation of the work of Kapron and Hehman (*6*), is rapid and convenient, largely because the butyl acetate extraction is eliminated by the use of water-miscible butyl Cellosolve for the stabilization of the orange-colored molybdenum—thiocyanate complex.

In spite of the desirable aspects of the Cellosolve method, direct application cannot be made to many tungsten-bearing steels. The tungsten interferes as in the butyl acetate method—by occlusion of molybdenum, and by interfering light absorption. Although the Cellosolve method of Kapron and Hehman (*6*) provides for the presence of tungsten, the method is not entirely satisfactory, because it requires considerable manipulation, and the reported upper limit for acceptable accuracy is only 0.24% molybdenum.

Inasmuch as a rapid and satisfactory method for the determination of molybdenum in excess of 0.24% in tungsten-bearing steels has not been found in the literature, it was proposed that butyl Cellosolve be incorporated into a colorimetric method based upon the thiocyanate complex of molybdenum. Modifications of current techniques included provision for the retention of tungsten in solution, the elimination of interfering light absorption caused by the yellow thiocyanate complex of tungsten, and the substitution of a correction factor for chromium to avoid the previously necessary volatilization of chromium.

APPARATUS

Transmittance measurements were made with a Cenco-Sheard-Sanford Photelometer, Type A, 1-cm. cell. This filter photometer was used because of its simplicity of operation. All measurements were made in optically matched Cenco fused absorption cells, each of which had a capacity of 8 ml. and a light path of 1 cm. A green glass light filter with a central transmittance maxima of 525 mμ was used. It was not necessary to remove the fixed diaphragm of the Photelometer.

REAGENTS AND SOLUTIONS

Unless otherwise stated, all reagents are C.P. grade or meet ACS specifications. Distilled water is used exclusively.

DISSOLVING MIX. Add 100 ml. of nitric acid and 100 ml. of hydrochloric acid to 300 ml. of water.

SULFURIC-PHOSPHORIC MIX (1 to 1). Mix equal volumes of sulfuric and phosphoric acids.

IRON SOLUTION. Dissolve 25 grams of ferrous sulfate ($FeSO_4.7H_2O$) in 200 ml. of water containing 5 ml. of sulfuric acid. Oxidize by adding 8 ml. of 30% hydrogen peroxide. Heat the solution to expel free oxygen. Cool, and dilute to 500 ml. (1 ml. of this solution is equivalent to 0.01 gram of iron).

SULFURIC ACID (1 to 1). Mix equal volumes of sulfuric acid and water.

BUTYL CELLOSOLVE. Technical grade of ethylene glycol mono-*n*-butyl ether.

POTASSIUM THIOCYANATE. Dissolve 25 grams of potassium thiocyanate (KSCN) in 300 ml. of water. Dilute to 500 ml. Filter through a Whatman No. 31 filter paper and add a few drops of chloroform to the final solution.

STANNOUS CHLORIDE. Dissolve 175 grams of stannous chloride ($SnCl_2.2H_2O$) in 125 ml. of concentrated hydrochloric acid at 40° to 50° C. Cool, and add 125 ml. of water. Dilute to 500 ml. with 1 to 1 hydrochloric acid. Add 1 to 3 grams of metallic tin to the solution.

EXPERIMENTAL

Removal or Solution of Tungsten. Four methods of eliminating tungsten residue interference were investigated by modification of the Cellosolve procedure used for nontungsten-bearing steels.

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It is generally accepted that tungstic oxide residues occlude molybdenum (*3*, *7*), but in the absence of data showing the loss of molybdenum for various tungsten to molybdenum ratios, the insoluble residue remaining after the sample had been taken into solution was removed by filtration after the color development. A loss of molybdenum was apparent from low results obtained when approximately 1% of each of tungsten and molybdenum was present in steel samples.

The use of citric acid and sodium hydroxide for keeping tungsten in solution has been reported (*1*) in connection with extraction techniques. The combination of citric acid and sodium hydroxide proved to be satisfactory when used in the Cellosolve method, and the color was successfully developed. However, this technique was not considered to be adequately rapid.

The incorporation of phosphoric acid into the dissolving mix prevented the formation of insoluble residues in the early stages of sample processing. However, a heavy precipitate, observed also by Kapron and Hehman (*6*), formed soon after the color was developed and adversely affected the transmittance readings.

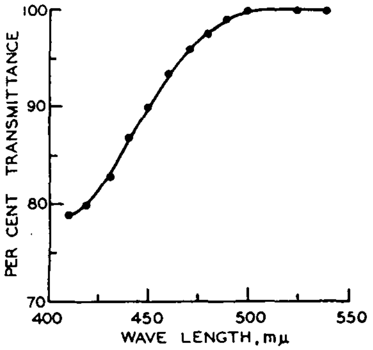

Figure 1. Spectral curve for tungsten

A mixture of nitric, hydrochloric, phosphoric, and sulfuric acids was then tried in the absence of perchloric acid, and not only provided a clear initial solution, but also prevented subsequent turbidity, and established conditions of acidity which permitted the formation of a green chromium compound which was used as the basis for a correction for chromium.

Light Absorption Caused by Tungsten. Erroneous results in the colorimetric determination of molybdenum may result from the interfering light absorption caused by the yellow thiocyanate complex of reduced tungsten when stannous chloride is added. This absorption is sufficiently pronounced under certain conditions to serve as a basis for the colorimetric determination of tungsten in steel (*8*). The extent to which the yellow complex provides interfering absorption depends upon both acid concentration and the choice of light filters or wave-length setting.

According to Hillebrand, Lundell, Bright, and Hoffman (*2*), the absorbance maximum of the molybdenum-thiocyanate complex occurs at approximately 470 mμ (frequently used for molybdenum), but in order to avoid tungsten interference, transmittance readings should be taken at 540 mμ. On the other hand, Kapron and Hehman (*6*) take transmittance readings in the presence of tungsten at 470 mμ (Corning filter No. 430). In order to resolve this discrepancy, the proposed procedure was applied to a molybdenum-free steel standard to which the equivalent of 6% tungsten was added. With a tungsten-free blank in the reference cell, transmittance readings were taken on a Coleman Model 11 spectrophotometer at intervals from 410 to 540 mμ. Two major observations were made. First, at 470 mμ a gradual intensification of the tungsten complex was observed. Second, the spectral curve (Figure 1), prepared from transmittance readings taken at various wave-length intervals, indicated that to avoid tungsten interference, readings would have to be taken at a wave-length setting of no less than 500 mμ. Inasmuch as Kapron and Hehman made no reference to absorption effects at wave lengths less than 500 mμ, it is possible that the 470 mμ filter used in their work had transmittance characteristics different from those provided by the Coleman spectrophotometer at 470 mμ.

Selection of Light Filter. Of the light filters available for use with the Photelometer, the blue filter (410 mμ) is more sensitive to the molybdenum complex than is the green filter (525 mμ). However, the spectral curve for tungsten (Figure 1) shows that tungsten causes interfering light absorption at a wave-length setting of 410 mμ, but not at 525 mμ. The assumption that tungsten interference would be prevented by the use of the green filter was verified by actual test. Consequently, even though the green filter is less sensitive to the molybdenum-thiocyanate complex than is the blue filter, it must be used to avoid tungsten interference.

Stability of Molybdenum-Thiocyanate Complex. The color of the molybdenum-thiocyanate complex has been reported (*6*) to be stable for at least 24 hours. However, since there was no necessity of maintaining stability over this period of time, stability was verified over only a 1-hour period, which provides ample time for routine manipulations.

Effect of Diverse Ions. Each of several diverse metal ions likely to be encountered—chromium, cobalt, manganese, nickel, vanadium—were added, as solutions, to molybdenum-free steel standards in an amount well in excess of that ordinarily present in the steels under consideration. The proposed procedure was applied in each case. Table I shows the extent of light absorption for each of the ions.

Table I. Effect of Diverse Ions

Diverse Ions	Added, %	Absorption of Transmitted Light, %
Chromium	20	6
Cobalt	20	3
Manganese	10	None
Nickel	20	None
Vanadium	3	None

Of the two interfering ions, cobalt is the least significant, because it absorbs a relatively small amount of transmitted light. Cobalt, 20%, in the sample absorbs approximately 3% of the transmitted light. In the presence of 10% cobalt, a 1% molybdenum value tends to be high by approximately 0.01%. When the 5% cobalt level is reached, as in the alloy Uniloy 1420, the interference from cobalt becomes negligible. Because steels containing cobalt in excess of 6% are encountered infrequently, no attempt has been made to establish a correction procedure for cobalt.

Chromium interferes sufficiently so that provision must be made to correct for its presence. Kapron and Hehman (*6*) stress the necessity of volatilization of chromium as chromyl chloride from stainless steels. However, it was found during this investigation that volatilization could be omitted and the interference from chromium could be compensated by the use of a correction curve. Because the slope of the correction curve is small, slight inaccuracies in the chromium result have little effect on the accuracy of the molybdenum determination. Inasmuch as an analysis is usually made for chromium in those tungsten-bearing samples in which molybdenum is to be determined, the correction factor can be applied readily. The simplified technique for solution and fuming of the sample eliminates sufficient steps in the procedure to more than compensate for the extra calculation involved in the application of the correction factor.

Correction for Chromium. Although perchloric acid is not used in the procedure for molybdenum, it was used in portions of the experimental work. It was used with nitric, hydrochloric, phosphoric, and sulfuric acids in the solution and oxidation steps originally applied to chromium-bearing samples. As the samples were being processed, it was observed that under certain conditions of perchloric acidity, orange, intermediate, and green colors were formed in the small volumes remaining after the required fuming of each sample.

To determine the light-absorbing effect of these variously oxidized solutions, three sets of samples containing from 0 to 20% chromium and 0.001% molybdenum were processed as follows: The first set was fumed for only 3 or 4 minutes after perchloric acid oxidation had developed the orange color of chromium; the second set was fumed to an estimated 5-ml. volume, and the intermediate colors which formed just prior to the expulsion of the last of the perchloric acid, tended toward the green rather than to the orange; the third set was fumed until the green color of reduced chromium appeared upon the expulsion of the perchloric acid. (The green color which appeared upon the complete expulsion of perchloric acid is presumed to have been caused by the sulfuric-phosphoric acid reduction of hexavalent chromium to chromic metaphosphate.) Each of the three sets was then carried through the remainder of the proposed procedure and the log per cent transmittance readings obtained against water were plotted against the corresponding chromium values (Figure 2).

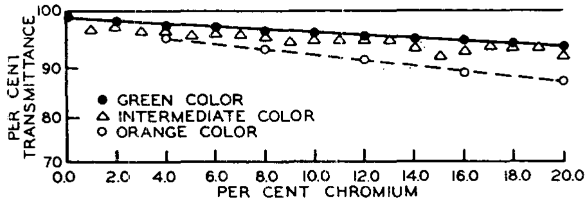

Figure 2. Stages of chromium oxidation and reduction

Straight-line curves were obtained for the orange and the green solutions. However, the slopes were markedly different; the green solutions caused less light absorption than did the orange solutions. The intermediate colors of the partially reduced chromium produced readings which fell between the straight-line curves, but which were generally closer to the curve for the green solutions. Inasmuch as absorption interference caused by the green color of reduced chromium is less than that caused by the orange color of oxidized chromium, the procedure which produced the green-colored solution was selected for use.

Because the formation of the green chromium compound was found to be independent of perchloric oxidation of chromium, and because a mixture of nitric, hydrochloric, phosphoric, and sulfuric acids obviated the necessity of using perchloric acid in sample dissolution, the procedure was modified to omit the use of perchloric acid.

The chromium correction curve adopted for use in the procedure was prepared from mixtures of National Bureau of Standards potassium dichromate 136 and National Bureau of Standards steel 16c analyzed by the proposed procedure. In order to make the curve equivalent to the net interference effect of chromium, transmittance readings were taken against a blank sample, instead of water. (The blank was prepared by application of the proposed procedure to National Bureau of Standards 16c in the absence of chromium.) The curve plotted from the data is similar to the curve for the green solution in Figure 2, with the exception that it originates at 100% transmittance for 0.0% chromium.

The chromium correction curve was tested by applying the proposed procedure to National Bureau of Standards sample 16c to which various combinations of a standard molybdenum solution and National Bureau of Standards potassium dichromate 136 were added. The observed transmittance readings were corrected as shown under Analytical Procedure and were read on a tentative calibration curve. Encouraging results shown in Table II prompted the testing of the method on molybdenum and tungsten-bearing standard steels.

Table II. Application of Chromium Correction to Synthetic Samples

Taken, %		Decrease in Transmittance Caused by Chromium, %	Molybdenum, %	
Chromium	Tungsten[a]		Taken	Found
0.0	0.5	0.0	1.00	1.00
0.0	8.0	0.0	1.00	1.00
5.0	1.0	1.7	1.00	1.00
10.0	0.0	3.1	1.00	1.00
10.0	1.0	3.1	1.00	1.02
15.0	0.0	4.6	1.00	1.00
15.0	1.0	4.6	0.50	0.51
15.0	1.0	4.6	1.00	1.00
15.0	1.0	4.6	1.50	1.50
15.0	2.0	4.6	1.00	1.00
15.0	4.0	4.6	1.00	1.01
15.0	6.0	4.6	1.00	1.01
15.0	8.0	4.6	1.00	1.00
20.0	0.0	5.9	0.25	0.24
20.0	0.0	5.9	0.50	0.50
20.0	0.0	5.9	1.00	1.01
20.0	0.0	5.9	1.25	1.25

[a] Added as standard solution.

Calibration Curve for Molybdenum. The tentative calibration curve referred to above was prepared by applying the proposed procedure to National Bureau of Standards steel 16c (0.001% molybdenum and no tungsten) to which were added varying amounts of a standard molybdenum solution. Excellent recoveries of molybdenum were obtained for various combinations of tungsten, molybdenum, and chromium added to National Bureau of Standards 16c, provided that the known amount of molybdenum was added as a standard molybdenum solution. However, when National Bureau of Standards molybdenum-bearing standard steels were tested, good results were obtained below 0.90%, but slightly high results were consistently obtained in the 0.90 to 1.40% range, the upper portion of the curve.

To overcome this difficulty, a new calibration curve was prepared by applying the procedure to standard samples and mixtures of standards containing increments of molybdenum in combination with various percentages of other alloying elements, particularly those of chromium and tungsten. Because chromium appeared to be the only element which contributed interfering absorption in the standards used, and because chromium was present in the samples used for calibration, the observed transmittance readings were corrected for chromium by the following method. The per cent transmittance equivalent to the per cent of chromium in each sample was obtained from the chromium correction curve and was substituted into the following equation:

$$\frac{\text{Observed \% transmittance} \times 100}{\text{\% transmittance equivalent to chromium}} = \text{corrected \% transmittance for molybdenum}$$

Each log per cent transmittance similarly obtained was then plotted against the corresponding per cent of molybdenum.

ANALYTICAL PROCEDURE

This method is based upon a basic sample weight of 0.10 gram. When any necessary deviations are made as indicated in the following table, the per cent molybdenum corresponding to the transmittance readings must be multiplied by a factor to make proper allowance for sample size.

Molybdenum Expected, %	Sample Size, Gram	Factor
0.00 to 1.40	0.10	1
1.40 to 2.80	0.05 plus 0.05 of molybdenum-free steel	2
2.80 to 14.00	0.10 (take a 0.1 aliquot as described in procedure)	10

(The maximum molybdenum value tested was 8.68%, because it is unlikely that higher values will be encountered. The 14.00% value is used in the table in order to maintain proportionality.)

Weigh the sample into a 150-ml. beaker. Add 5 ml. of dissolving mix. Heat to start dissolving action. If necessary, add a few milliliters of additional hydrochloric acid. (It is not necessary to add hydrofluoric acid to keep silicon in solution; the subsequently added phosphoric acid fulfills this function.) When dissolving action ceases, add 5 ml. of sulfuric-phosphoric mix (1 to 1), and heat on an asbestos pad until the sample boils down to a quiescent or sirupy condition which is accompanied by the appearance of light white fumes of sulfur trioxide, and, if a minimum of 1% chromium is present, is further characterized by the green color of trivalent chromium. Continue fuming for an additional 3 minutes to ensure complete conversion of chromium to the green compound. Cool.

If molybdenum is expected to be less than 2.80%, wash the sample into a 100-ml. volumetric flask with about 50 ml. of water and proceed with color development as outlined below. If molybdenum is expected to exceed 2.80%, wash the sample into a 100-ml. volumetric flask, dilute to the mark with water, and mix thoroughly. Pipet a 10-ml. aliquot into another 100-ml. volumetric flask. Add 9 ml. of iron solution and 4 ml. of sulfuric-phosphoric mix (1 to 1) to approach proper conditions for color development. Dilute to approximately 50 ml.

In either of the above two cases, add 15 ml. of sulfuric acid (1 to 1) and cool to room temperature. Develop the color by adding to the 100-ml. volumetric flask, in the order shown and with mixing after each addition, 15 ml. of butyl Cellosolve, 5 ml. of potassium thiocyanate solution, 5 ml. of stannous chloride solution, and water to dilute to exactly 100 ml. Both the Cellosolve and the thiocyanate may be added consecutively to an entire group of samples. However, stannous chloride must be added to only one sample at a time, and the sample must be immediately diluted to the mark with water and mixed thoroughly. Allow 15 minutes for full color development.

Transfer a portion of the colored solution to a 1-cm. absorption cell, and with the green filter in place in the Photelometer, read the per cent transmittance. The observed per cent transmittance must be corrected for the amount of chromium present in the sample. To do this, determine from the chromium correction curve the per cent transmittance equivalent to the per cent of chromium in the sample. (Because the correction curve is based upon a 0.10-gram sample, the use of a smaller sample necessitates the determination of the correction on the basis of the proportionately decreased amount of chromium.) Substitute values into the following equation:

$$\frac{\text{Observed \% transmittance} \times 100}{\text{\% transmittance equivalent to chromium}} = \text{corrected \% transmittance for molybdenum}$$

Using the calibration curve, convert the corrected per cent transmittance to per cent of molybdenum, making proper allowance for sample size.

DISCUSSION

Application of the Procedure. The proposed procedure has been applied to both tungsten-bearing and nontungsten-bearing standard samples and mixtures of standards issued by the National Bureau of Standards and by the Bureau of Analyzed Samples, Ltd. (British). Where necessary, standard solutions of tungsten and/or chromium were added to the standards in order to simulate several types of tungsten-bearing steels not represented by standard samples (Table III), and, also, to test the versatility of the method in its application to samples with compositions different from those listed in Table III. Typical results are shown in Tables IV and V. Each result represents a single determination.

Table III. Significant Components of Various Tungsten-Bearing Steels

Type of Steel	Composition, % Tungsten	Chromium	Molybdenum	Cobalt
Neor (die steel)	1.00 max.	11.00–13.00	1.00 max.	0.60 max
AISI Type 420 modified stainless steel	0.90– 1.20	12.00–14.00	0.90–1.20	...
Die steel, low carbon, Cr-Si-W-Mo	1.20– 1.70	4.75– 5.25	1.30–1.80	...
Buster (shock-resistant tool steel, 2$^1/_2$% W)	2.00– 3.00	1.10– 1.75	0.60 max.	...
Uniloy 1420	2.50– 3.50	11.50–13.00	0.15 max.	4.50–5.50
High speed tool steel, W-Mo-V	5.75– 6.50	3.75– 4.25	5.00–6.25	...
High speed tool steel, W-Mo	6.00– 6.75	3.90– 4.40	4.75–5.25	...
Vinco (high speed tool steel, 18-4-1)	17.50–18.50	3.75– 4.25	1.00 max.	...
Rex AAA (high speed tool steel, 5% Co)	17.50–18.50	3.75– 4.25	1.00 max.	4.75–5.25

Table IV. Analysis of Simulated Tungsten-Bearing Steels

Type of Steel	Taken, % Tungsten	Taken, % Chromium	Molybdenum, % Taken	Molybdenum, % Found
Neor (NBS 16c + NBS 36 + 0.5% Co[a])	1.00	12.15	0.50	0.51
Modified Type 420 stainless steel				
NBS 16c + British 254	1.00	13.48	1.16	1.17
NBS 36a	1.00	12.41	0.92	0.90
NBS 36	1.00	12.31	1.01	1.00
A-C EAWX	1.04	13.40	0.94[b]	0.94
A-C EAWX	1.04	13.40	0.94[b]	0.94
Die steel, low C-Cr-Si-W-Mo (NBS 16c + 153)	1.31	4.83	1.68	1.68[c]
Buster				
NBS 72d	3.00	1.03	0.21	0.21
NBS 30d	2.50	1.15	0.53	0.53
NBS 159	2.00	1.00	0.41	0.42
Uniloy 1420				
NBS 155 + 5.0% Co[a]	2.52	12.49	0.04	0.04
NBS 73a + 5.0% Co[a]	3.09	14.09	0.07	0.06
NBS 156 + 5.0% Co[a]	3.00	12.43	0.14	0.14
High speed tool steel, W-Mo-V (NBS 16c + 134)	6.27	4.11	6.08	6.17[c]
High speed tool steel, W-Mo (NBS 16c + 132)	6.40	4.38	4.95	4.92[c]
Vinco (NBS 50b)	18.05	4.08	0.40	0.40
Rex AAA (NBS 50b + 5.0% Co[a])	18.05	4.08	0.40	0.41

[a] Added as standard solution.
[b] Av. of gravimetric and titrimetric results.
[c] Multiplication factor of 10 applied.

Table V. Analysis of Standard Samples and Mixtures Based on Standards

Sample Identity	Taken, % Tungsten	Taken, % Chromium	Molybdenum, % Taken	Molybdenum, % Found
NBS 12f	0.00	0.07	0.01	0.01
NBS 101c	0.00	18.21	0.10	0.10
NBS 123a	0.11	~18.00	0.12	0.10
NBS 123b	0.18	~18.00	0.17	0.17
NBS 139	0.00	0.55	0.18	0.18
British Std. 257	3.00	1.72	0.32	0.32
NBS 50b + 16c	16.25	3.68	0.36	0.37
NBS 159	0.00	1.00	0.41	0.42
British Std. 258	3.00	3.07	0.42	0.43
NBS 135	0.00	5.15	0.58	0.58
NBS 16c + A-C 19-9 DL	0.86	11.48	0.74	0.74
NBS 16c + 36a	0.00	1.94	0.74	0.73
NBS 16c + British 254	1.00	0.32	0.77	0.79
	0.00	0.32	0.77	0.79
NBS 134 + 155	0.65	0.81	0.90	0.91
British Std. 253 (0.49% Cu)	3.00	0.35	0.94	0.95
	0.00	0.35	0.94	0.96
NBS 36	1.00	2.31	1.01	1.00
NBS 132 + 16c	0.94	0.62	1.06	1.06
NBS 36a + A-C 19-9 DL	1.14	15.78	1.18	1.19
NBS 16c + 160	0.00	7.68	1.18	1.17
A-C 19-9 DL (Cb 0.40, Ti 0.31)	1.43	19.13	1.24	1.22
British Std. 254	3.00	0.54	1.29	1.29
	0.00	0.54	1.29	1.29
NBS 36a + 160	0.00	5.75	1.33	1.30
NBS 50b + 160 + 16c	7.22	9.28	1.34	1.36
NBS 160	0.00	19.12	2.95	2.90[a]
	0.00	19.12	2.95	2.92[a]
NBS 132	6.29	4.11	7.07	6.98[a]
	6.29	4.11	7.07	6.98[a]
NBS 134	1.82	3.73	8.68	8.59[a]
	1.82	3.73	8.68	8.59[a]

[a] Multiplication factor of 10 applied.

Results. The results obtained over the entire range of molybdenum tested are in good agreement with the values sought, not only for the simulated tungsten-bearing steels, but also for the other combinations. The presence or absence of tungsten apparently does not influence the results. Cobalt up to 5% is also without effect. Corrections made for chromium appear to be satisfactory. The largest correction for chromium amounted to 6% of the observed per cent transmittance.

One of the samples included in Table V, A-C 19-9 DL, is not a certified standard sample, but it bears a molybdenum value which is the average of results obtained by the Research Laboratories and cooperating laboratories. The proposed method gave good results against the accepted value whenever this sample was used.

Statistical Analysis. A statistical analysis was made of 55 results for 46 different samples, not including those which required a multiplication factor of 10. The analysis of these results, made as outlined by Youden (*10*), showed the method to be free of both constant and relative errors.

The standard deviation of a single determination of molybdenum is 0.0149. Therefore, 95% of the values determined in the range of 0.01 to 1.34% will be within ±0.03% from the true value.

Aliquot Samples. Samples 132, 134, and 160 in Table V required a multiplication factor of 10, because a one-tenth aliquot of the original sample was taken. Slightly low results were obtained. However, they were comparable to the lowest values reported on the certificates for each of the samples. The results obtained for National Bureau of Standards standards 132, 134, and 160 were 6.98, 8.59, and 2.90%, respectively. The corresponding lowest values reported on the respective certificates were 7.01, 8.58, and 2.91%.

Elapsed Time. This method produces results in about 1 hour as compared with 2 hours for the peroxide-sulfuric acid method and 3 hours for the titrimetric method. Neither of these slower methods can be expected to give results which are more accurate than those obtained by the proposed method.

Limits of Application. Maximum values encountered in the testing of the method were 8.68% molybdenum, 18.05% tungsten, 19.13% chromium, and 5.00% cobalt. Although it is unlikely that higher values will be encountered in routine work, it is possible that the method can be applied satisfactorily in such cases. It may be necessary, however, to extend correction curves and/or to make other adjustments of the method.

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Use of Vanadium Pentoxide in Combustion Method for Sulfur in Refractory Materials

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A method is proposed for the determination of sulfur in inorganic materials that are highly resistant to decomposition by pyrolysis. The method is based on the reaction of sulfur with vanadium pentoxide, possibly by replacement of the sulfur through the formation of metal pyrovanadates. The oxide is mixed intimately with the powdered sample and heated in a quartz tube at 900° to 950° C. The liberated gases are absorbed in a solution of hydrogen peroxide and the resulting sulfuric acid is determined alkalimetrically. Such materials as inorganic sulfates, silica-alumina catalysts, and suspensions of barium sulfate in oil have been successfully analyzed by this method. Inorganic sulfides require the addition of chromium to the ignition mixture, to obtain complete sulfur recovery. An analysis may be completed in 35 to 45 minutes, thus offering an appreciable time-saving factor as compared to any of the conventional wet chemical methods.

COMBUSTION methods for determining sulfur have generally proved inadequate when applied to refractory materials. Well established procedures such as the Carius (*3*), the Parr oxygen bomb (*1*), heating in a horizontal (*4, 11*) or vertical tube (*5*) in the presence of oxygen or air have failed to provide a satisfactory means for determining sulfur in such substances as cracking or reforming catalysts, certain inorganic sulfates, and inorganic sulfides.

There are, however, two methods which do not use combustion tube technique. One involves fusion of the refractory sample with sodium peroxide in a metal bomb (*2*). The other requires treatment of the material with hydrofluoric and perchloric acids (*9*), reduction of sulfates to sulfides, and a final iodometric titration. Both methods are lengthy and the latter procedure is of uncertain value in estimating sulfur in forms other than sulfate.

To circumvent the disadvantages of the afore-mentioned methods, attention was directed to some rather meager information revealed by Kirsten (*6–8*), who volatilized residual sulfur from the ash of certain biological materials by heating in a combustion tube with vanadium pentoxide. It was also used by Zinneke (*12*) in his micro combustion method for sulfur in organic compounds containing phosphorus and fluorine. On the basis of this information, a rapid and simplified analytical method was developed for the total sulfur assay of inorganic and certain organic materials by combustion with vanadium pentoxide.

APPARATUS

Furnace Assembly (Figure 1). The apparatus employed in this investigation consists of a single quartz combustion tube and furnace unit similar to that described by Peters, Rounds, and Agazzi (*10*), except that a single rather than a dual unit was used.

REAGENTS

BARIUM SULFATE, reagent grade.

CALCIUM SULFATE, ($CaSO_4.2H_2O$), reagent grade.

HYDROGEN PEROXIDE, 6% solution. Dilute 200 ml. of reagent grade 30% hydrogen peroxide to 1 liter with water.

METHYL PURPLE INDICATOR, prepared solution available from E. Machlett & Co., New York 10, N. Y.

SILICA, powdered reagent grade.

SODIUM HYDROXIDE SOLUTIONS, carbonate-free, 0.10 and 0.01*N*, prepared from reagent grade sodium hydroxide.

SODIUM SULFATE, anhydrous, reagent grade.

VANADIUM PENTOXIDE, reagent grade, available from Eimer & Amend, New York, N. Y.

PROCEDURE

With the combustion tube in position and the furnace adjusted to 900° to 950° C., 30- and 10-ml. volumes of 6% hydrogen peroxide solution are added to the primary and secondary absorbers, respectively. Air flow through the combustion assembly is regulated to a rate of 2 liters per minute by applying vacuum at the absorber exit.

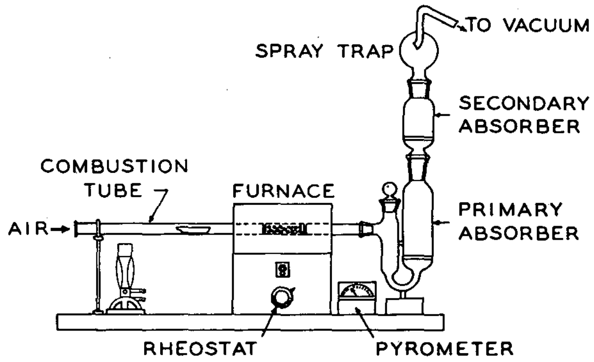

Figure 1. Combustion apparatus

Preparatory to analysis of the sample, a thin layer of silica is distributed evenly on the bottom of the combustion boat, followed by a similar layer of vanadium pentoxide (approximately 1 gram). A 0.3- to 0.6-gram portion (depending on sulfur content) of the finely-ground sample is distributed on a thin uniform layer over a prepared bed of the weighed combustion boat which is subsequently reweighed to obtain sample weight. Another layer of vanadium pentoxide, followed by a final covering of silica similar in amount to the first two layers, is added preparatory to inserting the boat in the tube. The boat is placed on a concave metal shield made of thin gage nickel and approximately as long as the boat, to protect the quartz tube against splattering. With the aid of a metal rod, the shield and boat are pushed into the tube to a point within 50 mm. of the heated zone of the furnace. A wire gauze heat-reflector is placed on the outside of the combustion tube above the sample. Heat from a Fisher blast burner is applied approximately 30 mm. in front of the boat. The burner is adjusted to provide a temperature of 900° to 950° C., similar to that obtained in the furnace. After 3 minutes, the flame is advanced slowly along the tube until the furnace is reached, allowing 25 to 30 minutes for the completion of this operation. Following this, the short exit end of the tube is also heated with the gas flame for 1 to 2 minutes. The open end of the combustion train is stoppered and the vacuum line at the absorber outlet is disconnected. The partial vacuum remaining in the system serves to draw the contents of the secondary absorber into the primary absorber. After collecting the water washings of the secondary in the primary absorber and addition of 3 drops of methyl purple indicator, the sulfuric acid is titrated directly in the absorber using 0.01 or 0.1*N* sodium hydroxide, depending on the expected sulfur content. Acidity due to compounds other than sulfuric acid must be determined in order to apply proper correction.

DISCUSSION OF METHOD

Vanadium pentoxide reacted so vigorously at elevated temperatures that it fused with the glazed surfaces of the porcelain boats rendering them unfit for more than a single determination. This difficulty was overcome, however, by lining the boat with a protective layer of silica before introducing the sample and vanadium pentoxide. Also an additional layer of silica was placed on the combustion mixture to aid in retarding the vigorous reaction when fusion of vanadium pentoxide with the sample occurred.

Extending the heating period to 55 minutes was found necessary in the case of those samples containing approximately 15% or more of sulfur.

An air purification assembly is commonly used with the apparatus prescribed for this method and is highly recommended if the presence of atmospheric sulfur is at all likely. However, the possibility of error from this source in the procedure as described was considered to be negligible. Replicate determinations of as little as 0.01% sulfur (Table II) appear to justify this conclusion.

RELIABILITY OF METHOD

Synthetic samples containing known amounts of calcium sulfate, sodium sulfate, and inorganic sulfides were mixed with finely ground silica or chromia-alumina compounds, and were subjected to the above analytical scheme. Sulfur recoveries obtained on these materials are presented in Table I. Molybdenum sulfide required the presence of chromia in the combustion mixture, in order to obtain acceptable results. This was accomplished by including a powdered chromia-alumina matrix, containing 20 to 30% chromium, in the combustion mixture (Table I), in the proportion of about five parts of chromium to one of sulfur.

Table I. Analyses of Inorganic Sulfates and Sulfide[a] in Synthetic Catalysts

Inorganic Compound Used in Mixture	Sulfur, Wt. % Added	Sulfur, Wt. % Found
Barium sulfate	0.50	0.51, 0.48, 0.49
Calcium sulfate	0.36	0.36, 0.36
Sodium sulfate	0.22	0.22, 0.22
Molybdenum sulfide	1.20	1.18, 1.23, 1.16, 1.20
	2.40	2.36, 2.42
	5.27	5.11, 5.10

[a] Sulfide values obtained by adding to combustion mixture a chromia-alumina catalyst containing 27% chromium.

Table II. Solid Catalysts and Related Substances

Material Tested	Total Sulfur, Wt. % Theory or determined	Total Sulfur, Wt. % V_2O_5 method
TCR bead catalyst[a]		
A	0.01, 0.00, 0.01	0.01, 0.01, 0.01
B	0.42	0.41, 0.41, 0.43
TCR bead catalyst plus molybdenum sulfide		
C	1.20	1.11, 1.16, 1.20
D	2.39	2.35, 2.42
Na_2SO_4-catalyst mixture	0.66	0.66, 0.66
Petroleum coke deposit	7.44, 7.50	7.39, 7.47, 7.35, 7.43

[a] 30% chromia, 70% alumina.

The agreement between the amount of sulfur added and that found by the proposed method is acceptable. These results attest to the effectiveness of the vanadium pentoxide combustion technique in quantitatively liberating sulfur from materials of high thermal stability.

Application of the proposed method to the determination of total sulfur in solid catalysts and related substances was next investigated. Several experimental catalysts were obtained and the sulfur content was determined by the vanadium pentoxide method. These results, compared to either calculated values or to values obtained by the iodometric procedure (*7*) are reported in Table II.

In order to extend the application of the method to the sulfur assay of organic systems of diverse compositions, such as may be encountered in petroleum research laboratories, a series of blended samples was analyzed. The first two samples represented lubricating oils containing a barium sulfonate additive in moderately high concentrations. The third sample contained a suspension of barium sulfate in mineral oil. Other tests were run on blends consisting of barium sulfonate mixed with an oil of high disulfide content. These blends ranged in concentration from approximately 4 to 20% sulfur.

Analytical data on these materials are given in Table III. On the basis of the above data, the standard deviation for per cent of sulfur is as follows:

% S	Std. Dev.
0 to 1	0.006
1 to 5	0.06
Over 5	0.10

INTERFERENCES

Other acid-forming constituents interfere, in which case a gravimetric determination of the evolved sulfur is required.

CONCLUSION

Total sulfur may be readily determined in materials of high thermal stability by combustion with vanadium pentoxide at 900° to 950° C. Elements such as calcium, barium, sodium, and aluminum do not interfere, although acid-forming elements do cause interference. The method is applicable to organic as well as inorganic substances. In materials containing molybdenum sulfide, the presence of chromium in the combustion mixture is necessary. Application of the method to the sulfur assay of paints, ceramics, shale, and minerals is suggested.

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Table III. Application to Organic Systems Containing Barium

Sample	Sulfur Wt. % of Added	Sulfur Wt. % of Found	Wt. % of Barium Present
Barium sulfonate in lubricating oil			
1	1.51	1.49, 1.51	4.84
2	1.82	1.80, 1.85	7.48
Barium sulfate suspended in mineral oil	0.14	0.13, 0.14	0.59
Barium sulfonate and disulfide oil			
1	4.54	4.45, 4.66, 4.65	5.4
2	9.15	9.27, 9.06, 9.13	4.5
3	15.3	15.13, 15.39	3.3
4	20.8	20.72, 20.79	2.3

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Determination of Lead in Lead Sulfide Ores and Concentrates

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The present work was begun to develop a more accurate and rapid method for the determination of lead in lead sulfide ores and concentrates. A method employing the perchloric acid dissolution of the sample, electrodeposition of lead as lead dioxide, and complexometric titration of the lead with HexaVer is proposed. The entire procedure for the determination of lead can be completed in less than one hour with a precision of about one part in a thousand. A longer procedure is given for the elimination of arsenic, antimony, and tin if these elements are present in sufficient amounts to interfere in the deposition of lead. The method avoids the time-consuming and inaccurate acetate extraction from separation of lead from barium. Not only is the complexometric titration faster than drying and weighing the lead dioxide deposit, but it also avoids the use of an empirical factor for the deposit.

THE separation of lead from barium in the presence of sulfate ions based upon the acetate extraction of the lead has been shown to be incomplete (*1*, *5*). The separation of lead as lead dioxide by electrodeposition with subsequent weighing is objectionable, because of the empirical conversion factor that varies with the conditions. This factor varies from 0.845 (*3*) up to the theoretical factor of 0.8662 (*7*).

Loomis (*4*) showed that lead and copper could be simultaneously deposited on the anode and cathode, and that the lead could be determined volumetrically using HexaVer (disodium dihydrogen 1,2-diaminocyclohexane *N*,*N*,*N*′,*N*′-tetraacetate), by adding an excess of the HexaVer and back-titrating with standard magnesium chloride solution. Flaschka (*2*) titrated lead directly using Versenate [disodium dihydrogen (ethylenedinitrilo) tetraacetate] in the presence of tartrate to keep the lead in solution at a pH of 10.

The authors found that the lead ore samples used for student analysis (prepared and sold by Hach Chemical Co., Ames, Iowa) could be dissolved rapidly and completely in about 5 minutes using boiling 72% perchloric acid, and that the lead could be quantitatively separated from the barium, iron, arsenic, bismuth, and silica present in the ores even when appreciable amounts of sulfate ion were present. The lead dioxide was washed and dissolved using nitric acid and hydroxylammonium chloride, and the lead determined by direct titration using HexaVer.

MATERIALS AND APPARATUS

Solutions. Standard HexaVer. Analytical reagent grade HexaVer (obtained from Hach Chemical Co., Ames, Iowa) was used in preparing the standard solution, by dissolving about 7.8 grams per liter of distilled water and then standardizing against the primary standard lead nitrate, standard calcium chloride solution, and the gravimetrically standardized lead perchlorate solution.

Lead Perchlorate. Twenty-one grams of test lead were dissolved by boiling in 72% perchloric acid for a few minutes. The solution was cooled and diluted with about 30 ml. of water, boiled to remove chlorine, and diluted to about 5 liters. This solution was standardized by precipitating the lead as the sulfate and weighing as such. The solution was found to be 0.01981M.

Sodium Tartrate. In 1 liter of water 46 grams of sodium tartrate monohydrate were dissolved to give approximately a 0.2M solution.

Sodium Hydroxide. To give a 5M solution 200 grams of sodium hydroxide were dissolved in water and diluted to about 1 liter.

Standard Calcium Chloride. An accurately weighed amount (0.4 to 0.5 gram) of primary standard calcium carbonate (Mallinckrodt low alkali grade, dried at 110° C. for 2 hours) was dissolved in 50 ml. of water containing 5 ml. of 5M hydrochloric acid. The solution was boiled to expel carbon dioxide, cooled, and diluted to exactly 250 ml.

Indicator. An Eriochrome Black T indicator preparation, ManVer, commercially available from the Hach Chemical Co., Ames, Iowa, was used.

Buffer (pH 10). The pH 10 buffer solution was prepared by adding 67.5 grams of ammonium chloride to 570 ml. of concentrated ammonium hydroxide and diluting to 1 liter.

Primary Standard Lead Nitrate. Test lead was dissolved by heating in dilute nitric acid, and the dissolved lead precipitated as lead nitrate by adding concentrated nitric acid. The salt was thrice recrystallized from concentrated nitric acid. The salt was then dried *in vacuo* (25 mm. of mercury) at 100° C. for 24 hours. Twice during the drying the salt was powdered. A gravimetric analysis of the salt by the sulfate method showed it to be 99.9% lead nitrate. An emission spectrum showed the absence of foreign metals.

Electrodes. The anodes used were platinum gauze (45-mesh wire, diameter 0.0077 inch) formed into cylinders $1^5/_8$ inches in diameter and 2 inches long. The cathodes were similar, but were only 0.5 inch in diameter and mounted to provide stirring during all depositions. The anodes were roughened by sandblasting.

EXPERIMENTAL

Dissolution of Ore. Concentrated, as well as dilute (1 to 1), nitric and hydrochloric acids were tried as solvents for the ore samples, but concentrated (72%) perchloric acid was found to be far superior to the nitric and hydrochloric acids as the solvent. The results of the dissolution studies are given in Table I. In each case about 0.2 gram of the ore was placed in a 300-ml. tall-form beaker, 20 ml. of the solvent were added, and the beaker was placed on an electric hot plate adjusted to give a surface temperature of about 260° C.

The silica residue formed in the perchloric acid solution method was filtered off, treated with hydrofluoric and sulfuric acids, and tested for lead using chromate. No lead was found.

To dissolve the ore samples promptly and completely 10 ml. of the 72% perchloric acid can be used. When large amounts of silica are present, bumping can be decreased by using 20 ml. of the acid, or by adding glass beads.

Current use of organic flotation reagents may leave a residue of these reagents on the lead sulfide. The question of the action of 72% perchloric acid on these organic residues immediately arises; thus, 10 different lead sulfide "flotation concentrates" were tested with hot 72% perchloric acid. One-gram samples (considerably in excess of the recommended sample size) went into solution easily and smoothly with no sign of any difficulty.

Table I. Dissolution Studies of Lead Sulfide Ores

Solvent	Dissolution Time, Minutes	Remarks
72% $HClO_4$	6	Completely dissolved except for silica residue
Concd. HNO_3	15	60 minutes additional required to dissolve sulfur bead formed
1 to 1 HNO_3	45	120 minutes additional heating failed to dissolve sulfur bead formed
Concd. HCl	Not soluble	After 120 minutes ore was not in solution
1 to 1 HCl	Not soluble	After 120 minutes ore was not in solution

Table II. Comparison of Standardization Methods for HexaVer

Method	No. of Detn.	Av. Molarity	Av. Dev.	Std. Dev.
Primary std., $CaCO_3$	8	0.01987	0.00001	0.000013
Primary std., $Pb(NO_3)_2$	9	0.01988	0.000014	0.000016
0.01981M $Pb(ClO_4)_2.3H_2O$	5	0.01989	0.000004	0.000007

Standardization of HexaVer Solution. The HexaVer solution was standardized against the standard calcium chloride solution by pipetting exactly 25 ml. of the calcium chloride solution into a clean flask and adding 1 ml. of the buffer solution and about 0.25 gram of sodium cyanide to eliminate interference from traces of copper present in the distilled water. To this were added about 25 mg. of the magnesium salt of HexaVer and about 5 drops of the indicator.

The solution was then titrated with HexaVer until the pink color turned to a pure blue. The results of eight consecutive titrations are given in summary form in Table II.

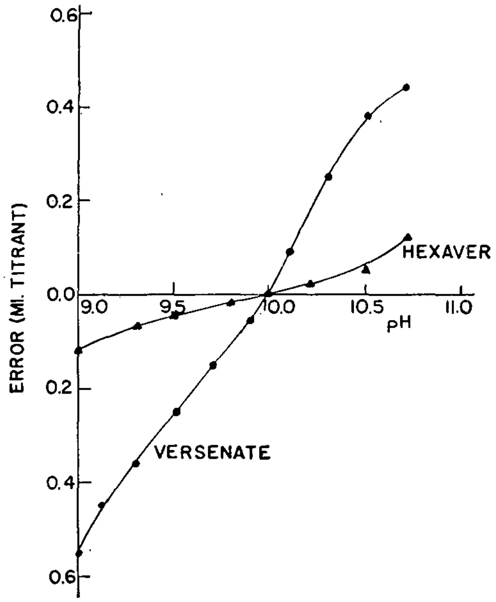

Figure 1. Effect of variation of pH on titration of lead with HexaVer and Versenate

Direct Titration of Lead with HexaVer. About 0.2 gram of the primary standard lead nitrate was accurately weighed and dissolved in about 50 ml. of water. To this were added 10 ml. of the 0.2M tartrate solution, and 5M sodium hydroxide was added dropwise until the precipitate of lead tartrate first formed just redissolved. About 1 ml. of the buffer was added, followed by about 0.25 gram of sodium cyanide, and 5 drops of the indicator. This lead solution was then titrated with the HexaVer solution until the violet color changed to a clear blue-green. The results of nine consecutive titrations are given in summary form in Table II. Also given in Table II are the results of five titrations which were similarly titrated using 50-ml. aliquots of the lead perchlorate solution.

Comparison of HexaVer and Versenate at Various pH Values. Aliquots of 25 ml. of the lead perchlorate solution were titrated with the standard HexaVer solution and with a standard Versenate solution at various pH values. The difference in the amounts of solution required to obtain the end point at various pH values (using pH 10 as the reference) is shown in Figure 1. A sharp end point was obtained only in the pH region from 9.0 to 10.7. From this study it is apparent that pH control is less critical using HexaVer. It also was observed that sharper end points are obtained with HexaVer in the direct titration of lead.

Electrodeposition Studies. Effect of Acids on Depositions. It was established that perchloric acid was the ideal solvent for the ore samples; hence, in the lead deposition studies 25-ml. aliquots of the lead perchlorate solution were used in each of the studies. Varying amounts of concentrated nitric and 72% perchloric acids were added, and the solution was diluted to about 125 ml. in order to nearly cover the anode in the 300-ml. tall-form beakers. It was anticipated that appreciable amounts of sulfate would be present in the solutions resulting from the dissolution of ore samples; thus 0.25 ml. of concentrated sulfuric acid was added to each run. In each of the studies (Table III) a current of 2.5 amperes was used for 1 hour. In each case the deposited lead dioxide was dissolved and titrated with HexaVer using the procedure for ore analysis.

It is apparent from the data presented in Table III that complete deposition does not result under the test conditions unless the solution is contained in excess of 25 ml. of concentrated nitric acid. The presence of varying amounts of perchloric acid appears to have little effect on the deposition.

Current versus Time. The deposition of the lead was studied using 3, 4, 5, 7.5, and 10 amperes of current to determine the corresponding required deposition time. In each case 35 ml. of nitric acid and 20 ml. of 72% perchloric acid were added to the 25-ml. aliquot of the lead before dilution to about 125 ml. The lead dioxide was dissolved and titrated by the procedure for ore analysis. The data (Table IV) show that about 0.1 gram of lead can be quantitatively deposited very rapidly at current rates up to 10 amperes.

In other studies as much as 0.5 gram of lead was deposited on the anode.

Interfering Element Studies. A qualitative analysis of the ore samples showed that considerable amounts of iron and barium were present, but only traces of arsenic and bismuth were found.

To determine whether these ions would interfere in the lead deposition and its subsequent titration, a series of studies was made using the primary standard lead nitrate as the lead source. To accurately weighed samples of the lead nitrate varying amounts of the foreign ions and 0.25 ml. of concentrated sulfuric acid were added. The mixture was dissolved by boiling with 20 ml. of 72% perchloric acid. The results are given in Table V. The barium ion interference resulted only when a barium sulfate precipitate was formed. The 0.25 ml. of concentrated sulfuric acid added in these studies far exceeds the amount of sulfate likely to be present or formed when ores are dissolved.

Further interference studies were made using a lead ore sample as the source of lead. To the accurately weighed sample, varying amounts of arsenic, antimony, bismuth, silver, manganese, tin, and barium were added and the mixture was analyzed, using the procedure for determination of lead in lead sulfide ore. The results are given in Table VI. The error introduced by interferences is significant if it is more than 0.2 mg. Greater amounts of interfering ions than listed in the table cause a proportionately larger amount of error in the lead deposited.

Silica interference is discussed in the section, Dissolution of Ore.

PROCEDURE FOR DETERMINATION OF LEAD IN LEAD SULFIDE ORES

The sample of the ore, containing between 0.1 and 0.15 gram of lead, is weighed out and transferred to a dry 300-ml. tall-form beaker. Approximately 15 ml. of 72% perchloric acid are added and the beaker is swirled to disperse the sample. Glass beads are added to help prevent bumping. The beaker is covered with a watch glass and placed on a Nichrome gauze. Heat is applied with a Tirrill burner adjusted so that it boils 72% perchloric acid very gently. By the time the perchloric acid begins to boil, the sample is in solution, but the solution is allowed to boil very gently for about 1 minute to expel any hydrochloric acid formed during the solution process. The beaker is then cooled for about 2 minutes, and the walls of the beaker are washed down with distilled water. The solution is diluted with water to about 50 ml. (about 1 inch in the beaker) and boiled for about 2 minutes to expel chlorine. (This is very essential.) Next, 30 to 35 ml. of concentrated nitric acid are added and the solution is placed on the deposition apparatus. Distilled water is added until the electrodes are nearly covered (about 125 to 150 ml. total volume).

The current used for deposition can vary up to at least 10 amperes. The length of time required to obtain complete deposition varies inversely with the current used (see Table IV). It is also important that the solution be stirred during the deposition. (In the analyses that follow, the current was 3 amperes and the time was 1 hour. Stirring was accomplished with a rotating platinum cathode.)

Table III. Variation of Acid Concentrations for Deposition of Lead

No.	HNO_3, Ml.	$HClO_4$, Ml.	Lead, Gram		Error, Mg.
			Taken	Found	
1	10	..	0.1026	0.1001	2.5
2	15	..	0.1026	0.1008	1.8
3	20	..	0.1026	0.1011	1.5
4	25	..	0.1026	0.1020	0.6
5	30	..	0.1026	0.1026	0.0
6	35	..	0.1026	0.1026	0.0
7	20	5	0.1026	0.1022	0.4
8	25	5	0.1026	0.1024	0.2
9	30	5	0.1026	0.1026	0.0
10	20	10	0.1026	0.1023	0.3
11	25	10	0.1026	0.1024	0.2
12	30	10	0.1026	0.1026	0.0
13	20	15	0.1026	0.1023	0.3
14	25	15	0.1026	0.1024	0.2
15	30	15	0.1026	0.1026	0.0
16	20	20	0.1026	0.1024	0.2
17	25	20	0.1026	0.1026	0.0
18	30	20	0.1026	0.1026	0.0

Table IV. Effect of Current on Time of Lead Deposition

No.	Time, Min.	Current, Amp.	Lead, Gram		Error, Mg.
			Taken	Found	
1	15	3	0.1026	0.1020	0.6
2	30	3	0.1026	0.1024	0.2
3	45	3	0.1026	0.1026	0.0
4	15	4	0.1026	0.1024	0.2
5	30	4	0.1026	0.1026	0.0
6	45	4	0.1026	0.1026	0.0
7	10	5	0.1026	0.1024	0.2
8	15	5	0.1026	0.1026	0.0
9	30	5	0.1026	0.1026	0.0
10	5	7.5	0.1026	0.1022	0.4
11	10	7.5	0.1026	0.1026	0.0
12	15	7.5	0.1026	0.1026	0.0
13	5	10	0.1026	0.1025	0.1
14	10	10	0.1026	0.1026	0.0

Table V. Interference Studies on Lead Deposition Using Primary Standard Lead Nitrate

No.	Interference		Lead, Gram		Error, Mg.
	Taken	Amount, mg.	Taken	Found	
1	Iron	20.0	0.1004	0.1004	0.0
2	Arsenic	0.5	0.1000	0.1000	0.0
3		1.0	0.1003	0.0999	0.4
4	Bismuth	2.0	0.1004	0.1004	0.0
5		5.0	0.1007	0.1004	0.3
6		7.0	0.1005	0.0995	1.0
7	Barium	10.0	0.1003	0.1003	0.0
8		20.0	0.1006	0.1003	0.3

Table VI. Interference Studies on Lead Deposition Using Lead Ore No. 1

No.	Interference		Lead, Gram		Error, Mg.
	Taken	Amount, mg.	Taken	Found	
1	Arsenic	0.5	0.1001	0.1001	0.0
2		1.0	0.1002	0.0998	0.4
3	Antimony	1.0	0.1000	0.1000	0.0
4		2.0	0.1004	0.0999	0.5
5	Bismuth	1.0	0.1003	0.1003	0.0
6		2.0	0.1005	0.1005	0.0
7		5.0	0.1003	0.1000	0.3
8		7.0	0.1002	0.0991	1.1
9	Silver	3.0	0.1003	0.1003	0.0
10		4.0	0.1002	0.1000	0.2
11		5.0	0.1002	0.0996	0.6
12	Manganese	3.0	0.1001	0.1001	0.0
13		5.0	0.1004	0.1005	0.1
14		7.0	0.1003	0.0999	0.4
15		10.0	0.1000	0.0995	0.5
16	Tin	3.0	0.1000	0.1001	0.1
17		5.0	0.1003	0.1005	0.2
18		7.0	0.1003	0.0999	0.4
19	Barium	2.0	0.1001	0.1001	0.0
20		5.0	0.1000	0.1000	0.0
21		10.0	0.1002	0.1002	0.0
22		50.0	0.1002	0.0952	5.0
23		100.0	0.1000	0.0870	13.0

Table VII. Consecutive Analyses of Lead Sulfide Ores

Lead Sulfide Ore	Lead, %	Dev. from Av.	Std. Dev.
No. 1			
1	68.95	+0.10	
2	68.78	−0.07	
3	68.83	−0.02	
4	68.77	−0.08	
5	68.89	+0.04	
6	68.90	+0.05	
7	68.81	−0.04	
8	68.85	±0.00	
Av.	68.85	±0.05	0.062
No. 2			
1	61.80	−0.11	
3	61.79	+0.06	
3	61.91	±0.00	
4	61.84	−0.07	
5	61.89	−0.02	
6	61.93	+0.02	
7	61.96	+0.05	
8	61.95	+0.04	
Av.	61.91	±0.05	0.06
No. 3			
1	44.43	+0.07	
2	44.33	−0.03	
3	44.39	+0.03	
4	44.44	+0.08	
5	44.35	−0.01	
6	44.28	−0.08	
7	44.28	−0.08	
8	44.34	−0.02	
Av.	44.36	±0.05	0.062
No. 3 with 50 mg. of arsenic added (using modified procedure)			
1	44.23	−0.08	
2	44.29	−0.02	
3	44.32	+0.01	
4	44.40	+0.09	
5	44.35	+0.04	
6	44.25	−0.06	
Av.	44.31	±0.05	0.063

After deposition is completed, the electrodes are washed with water, and the anode is placed in a 150-ml. beaker containing 25 ml. of water, 0.5 gram of hydroxylammonium chloride, and about 0.25 ml. of concentrated nitric acid. The beaker is tilted sufficiently to allow solution of the lead dioxide. The electrode is washed with water and removed. Ten milliliters of 0.2M tartrate solution are added, and 5M sodium hydroxide is added dropwise until the precipitate of lead tartrate just dissolves. (As the sodium hydroxide is added and the nitric acid neutralized, lead tartrate precipitates. With continued addition of the sodium hydroxide, the lead tartrate redissolves.) Approximately 1 ml. of the pH 10 buffer, about 0.25 gram of sodium cyanide, and 6 drops of ManVer indicator are added. The solution is titrated with 0.02M HexaVer until the indicator changes from violet to blue-green.

Modified Procedure. Arsenic, antimony, and tin that may interfere in the determination of lead (see Table VI) can be removed quantitatively as the volatile bromides (*8*). This volatilization was used advantageously by Norwitz and Norwitz (*6*) in the deposition of lead. The following procedure may be used to remove these three interferences.

The sample is put into solution as above with 15 ml. of 72% perchloric acid. Following the gentle boiling of the concentrated acid solution, the solution is allowed to cool, 20 ml. of 48% hydrobromic acid are added, and the solution is evaporated on a hot plate to the dense white fumes of perchloric acid. Another 20 ml. of 48% hydrobromic acid are added to the cooled solution, and the solution again evaporated to fumes of perchloric acid. The solution is boiled gently for 2 minutes to expel the last traces of bromide, after which it is cooled for 2 minutes, diluted to about 50 ml. of water, and boiled for about 2 minutes to expel chlorine as in the above detailed procedure. From this point the procedures are identical.

This modified procedure adds about an hour to the time required for the determination of lead.

RESULTS OF LEAD ORE ANALYSIS

Using the above procedure, three different lead ore samples were analyzed (Table VII). Also included in Table VII are the results of the determination of sample 3 with 50 mg. of arsenic added using the modified procedure. Though the average is lower than sample 3 without the arsenic, the results are within the error of the method.

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Determination of Aldehydes Using Unsymmetrical Dimethylhydrazine

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A method is presented for determining aldehydes by reaction with unsymmetrical dimethylhydrazine. An excess of hydrazine reagent is added to a sample, and, after the reaction is complete, the excess is titrated with standard acid. Ketones cannot be determined by this method. Aromatic aldehydes can be determined in the presence of ketones, but aliphatic aldehydes cannot. The hydrazine reagent is alkaline and is stable toward decomposition and oxidation, making possible a precision and recovery within ±1%. Because the method is nonaqueous in nature, its range is extended beyond just water-soluble samples. Acetals and carboxylic acids do not interfere.

THE use of hydrazines for the determination of aldehydes has been restricted by one or more factors, the most common of which are: ease of oxidation of the hydrazines, use of aqueous reagents limiting analysis to water-soluble samples, and use of hydrazonium salts which are acidic and will react with acetals as well as aldehydes. Employing dimethylhydrazine makes it possible to overcome these difficulties.

Kleber (*16*) tried the determination of some carbonyl compounds by addition of excess of phenylhydrazine and acidimetric determination of the excess. Phenylhydrazine is a poor reagent, owing to its ease of oxidation by atmospheric oxygen. Blanks can be run to account for the phenylhydrazine lost through oxidation during the reaction, but these blanks are too large for a satisfactory quantitative method.

Ardagh and Williams (*1*) used phenylhydrazine for determining carbonyl compounds. Excess phenylhydrazine was added to a sample and the excess was determined iodometrically. Because of the instability of the reagents toward atmospheric and dissolved oxygen and also partial reaction of the hydrazones, the method is unwieldy. It requires oxygen-free conditions and an extraction to remove the hydrazone before the excess hydrazine can be determined. The work of Ardagh and Williams was an extension of the work of Von Meyer (*32*) and has been converted to a microprocedure (*18*).

Several procedures for carbonyl compounds use excess phenylhydrazine and decompose the excess hydrazine with Fehling's solution to liberate nitrogen, which is collected and measured (*6, 11, 19, 20, 29, 30*). These procedures are used only for quantitative estimations and cannot be applied for precise and accurate measurement of most carbonyl compounds.

Hydrazine, as such, cannot conveniently be used, but hydrazine sulfate has been used to determine certain aldehydes (*12, 13, 23*). The acid liberated in the reaction with the carbonyl compound is measured. The reaction times are generally long and cannot be applied to acetal-containing samples, owing to the acidity of the reagent.

2,4-Dinitrophenylhydrazine has been used for volumetric methods. Clift and Cook (*4*) dissolved the hydrazone in excess standard caustic and back-titrated the excess. Espil and associates (*7, 8*) used titanous chloride to reduce the nitrohydrazones. The difficulties in these methods lie in the quantitative separation of the hydrazone and drawbacks in the use of titanous chloride. Schöeniger and Lieb (*27, 28*) determined the excess 2,4-dinitrophenylhydrazine with titanous chloride.

Table I. Results Obtained in Analysis of Various Aldehydes

	% by Dimethylhydrazine	Reaction Time, Hours	% by $NH_2OH.HCl$[a]
Formaldehyde (aqueous solution)	36.6 ± 0.1 (4)[b]	0.25	36.6
Acetaldehyde	97.3 ± 0.8 (3)[b]	0.25	97.3
Propionaldehyde	96.6 ± 0 5 (3)[b]	0.25	96.5
Butyraldehyde	94.5 ± 0.5 (3)[b]	0.25	95.1
Cinnamaldehyde	96.6 ± 0.2 (3)[b]	0.50	97.1
Furfural	97.6 ± 0.2 (3)[b]	2.0	98.2
Crotonaldehyde	93.0 ± 0.2 (4)[b]	0.5	93.2[c]
Salicylaldehyde[d]	99.6 ± 0.3 (10)[e]	0.5	99.6 ± 0.4 (10)
Anisaldehyde	95.7 ± 0.4 (3)[e]	2.0	95.9
Benzaldehyde	92.7 ± 0.3 (3)[e]	2.0	94.7
m-Nitrobenzaldehyde	98.7 ± 0.1 (3)[e]	2.0	98.7
2,6-Dichlorobenzaldehyde	98.3 ± 0.5 (3)[f]	2.0	98.8
2,4-Dimethoxybenzaldehyde	99.1 ± 0.3 (3)[f]	2.0	97.7

[a] $NH_2OH.HCl$ used was $0.5N$ in 1:1 methanol-water. Samples were refluxed 1 hour and then titrated potentiometrically with $0.5N$ NaOH.

[b] $0.2M$ DMH in ethylene glycol.

[c] Determined by bisulfite.

[d] Since salicylaldehyde is stable, it was possible to use it as a pure standard. Sample used was made from the bisulfite addition compound (Eastman Kodak), distilled once in laboratory before use.

[e] $1M$ DMH in ethylene glycol.

[f] $1M$ DMH in MeOH.

Parentheses indicate number of determinations used. Precision indicated is as average deviation arrived at from number of determinations made.

p-Nitrophenylhydrazine has been used (*24*) by isolating the derivative, reducing with stannous ion, and determining the excess stannous iodometrically. The procedure is long and suffers from the difficulty of complete isolation of the derivative.

2,4-Dinitrophenylhydrazine has been tried from a gravimetric standpoint—namely, removal of the hydrazone derivative and weighing it (*5, 9, 10, 14, 15, 25, 31*). The gravimetric methods give slightly low recovery, and are limited generally to aqueous or partially aqueous media because of the lower solubility of the hydrazones in water.

2,4-Dinitrophenylhydrazine has been used also for colorimetric determination of carbonyl compounds (*2, 3, 17, 21, 22, 26*).

In the course of the work being reported, phenylhydrazine and *p*-nitrophenylhydrazine were tried, but it was difficult to obtain a reagent stable toward oxidation. Reagent solutions using 2,4-dinitrophenylhydrazine were employed. The excess of hydrazine was difficult to determine in this case, however, as the compound is not basic enough to be titrated in solvents suitable for the reaction.

Unsymmetrical dimethylhydrazine gave a reagent which was stable for several weeks when stored in a dark bottle. The reaction with aldehydes is rapid and complete. This hydrazine also reacts with ketones, as is indicated by the evolution of heat and color development. Evidently, however, the basic strength of the hydrazones of the ketones is not too much different from the basic strength of the hydrazine itself. Therefore, on back-titration of the hydrazine, the excess hydrazine cannot be differentiated from the hydrazone formed, and a value for base is obtained which coincides with the amount of hydrazine initially added to the sample. This behavior makes possible the determination of aromatic (but not aliphatic) aldehydes in the presence of ketones (Table II).

Aromatic aldehydes yield hydrazones which exhibit no detectable basicity. Therefore on titration of the excess hydrazine, a sharp break in the titration curve is obtained when all the excess hydrazine is neutralized (Figure 1). However, the aliphatic aldehyde hydrazones are noticeably basic; on back-titration of the excess hydrazine, a more gradual break in the titration curve is obtained (Figure 1), and sometimes a second break if the titration is carried further. When ketones are present, the hydrazone of the ketone buffers the solution very noticeably. The hydrazone of the aromatic aldehyde is so weakly basic that, though the break in the titration curve is subdued by the buffering action of the hydrazone of the ketone, the break is still visible. However, in the case of the aliphatic aldehyde, the hydrazone of the ketone obliterates the already weak break.

Table II. Effect of Acids, Acetals, and Ketones on Aldehyde Determination

Mixture Analyzed	Aldehyde Added, Grams	Acid Added, Gram	Acetal Added, Gram	Ketone Added, Grams	Aldehyde Found	
					Grams	%
Formaldehyde[a]	0.1660	...	...	...	0.0609	36.7
Formaldehyde[a]-formic acid	0.1684	0.0211	...	...	0.0611	36.3
	0.1758	0.0624	...	...	0.0643	36.6
Formaldeyde[a]-methylal	0.1830	...	0.0549	...	0.0670	36.6
	0.1631	...	0.1512	...	0.0595	36.5
Acetaldehyde	0.1062	...	...	...	0.1031	97.1
Acetaldehyde–acetic acid	0.1062	0.1283	...	...	0.1036	97.6
Acetaldehyde–dimethyl acetal	0.1062	...	0.1213	...	0.1036	97.6
	0.1062	...	0.0342	...	0.1040	98.0
Salicylaldehyde	1.3053	...	...	...	1.2962	99.3
Salicylaldehyde–salicylic acid	1.1999	0.1380	...	...	1.2008	100.1
Salicylaldehyde–cyclohexanone	1.2249	...	...	0.9580	1.2233	99.9
Salicylaldehyde–benzophenone	1.2046	...	...	1.5077	1.2008	99.7

[a]Baker's formaldehyde solution, 37%.

Carboxylic acids, in general, do not interfere, as they are relatively weak acids compared with the mineral acid used in the titration (Table II). If a strong acid is present in a sample, a separate determination of this acid can be made, and a correction applied; or the sample can first be neutralized using the hydrazine reagent before the hydrazine for the aldehyde reaction is added. There is no evidence of

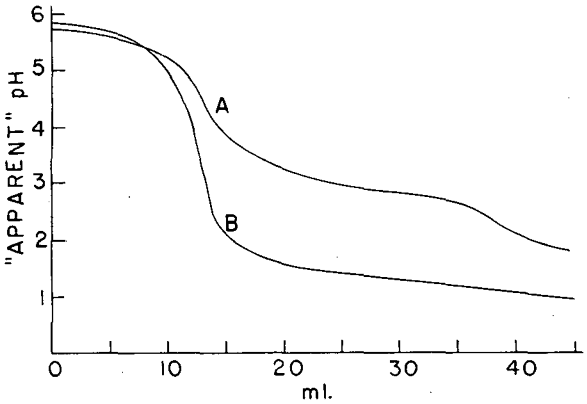

Figure 1. Titration curves

A. **Formaldehyde**
B. **Salicylaldehyde**

hydrazide formation with carboxylic acids even after 4 hours of reaction time, which is far beyond the time intervals used in this study.

Acetals and ketals do not interfere in this procedure, as the alkalinity of the reagent prevents hydrolysis to the carbonyl compounds (Table II). This is one advantage of this approach over hydroxylamine hydrochloride methods; another is ability to determine some aldehydes in the presence of ketones. The shape of the titration curves of all the aromatic aldehydes resembles those of salicylaldehyde and not those of the aliphatic aldehydes. The sharpness of the breaks makes possible the analysis with ketone present, as it can stand the buffering effect.

The reaction between the dimethylhydrazine and aldehydes was found to proceed best in polar solvents. Water can be used, but a nonaqueous solvent was desired because many organic samples may not be water-soluble and the titration breaks in the case of the aliphatic aldehydes were poor in water. Alcohols had the drawback of undergoing acetal formation with the aldehydes, giving low results. Ethylene glycol was found to operate satisfactorily both as solvent and as reaction medium and did not undergo any noticeable acetal formation. Alcohol was used in the determination of a few aldehydes (Table I) as these aldehydes were not very soluble in the glycol and had carbonyl groups which were hindered enough to undergo acetal formation slowly enough to prevent interference. Tetrahydrofuran and chlorobenzene were tried as solvents, but the reaction proceeded much too slowly. *tert*-Butyl alcohol was tried, as this is still a polar solvent but does not undergo acetal formation very readily; the reaction was somewhat slow, and poor precision was obtained. Pyridine was also tried; the reaction proceeded rapidly, as indicated by the heat evolved and color formation, but the hydrazone and the excess hydrazine titrated together, making analysis impossible.

REAGENTS

Unsymmetrical dimethylhydrazine (Westvaco Chemical Division, Food Machinery and Chemical Corp., N. Y.), $0.2M$ solution in ethylene glycol for aliphatic aldehydes, $1M$ solution in ethylene glycol for aromatic aldehydes, and $1M$ solution in methanol for disubstituted benzaldehydes. Standard 0.1 and $0.5N$ solutions of hydrochloric acid in methanol.

PROCEDURE

Twenty-five milliliters of reagent are pipetted into a glass-stoppered flask, and the weighed sample (about 0.002 mole for aliphatic and 0.01 mole for aromatic aldehydes) is added to the reagent. The reaction is allowed to proceed at room temperature for 15 minutes or longer, depending on the aldehyde being determined (Table I). When the reaction is complete, the solution is washed into a 250-ml. beaker with approximately 50 ml. of methanol, and the excess dimethylhydrazine is titrated potentiometrically with standard hydrochloric acid in methanol. Hydrochloric acid, $0.1N$, is used to titrate $0.2M$ hydrazine reagent, and $0.5N$ hydrochloric acid is used to titrate the $1M$ reagent. A blank on 25 ml. of reagent is determined in the same manner. The glass-calomel electrode system is used in all cases.

Per cent aldehyde is calculated using the following equation.

$$\% \text{ aldehyde} = \frac{(\text{ml. for blank}) - (\text{ml. for sample}) \times N \text{ of HCl} \times \text{M.W.} \times 100}{\text{weight of sample} \times 1000}$$

One molar reagent is necessary for determining aromatic aldehydes which react slowly with more dilute reagent, but it should not be used for aliphatic aldehydes, as a rise in temperature resulting from rapid reaction may cause loss of reagent. To keep the reaction time at a minimum, 100% excess of reagent should be used in all determinations.

The solubility of disubstituted benzaldehydes in ethylene glycol is not great enough to allow their determination in this solvent, but, as they do not form acetals under the conditions used, it is possible to determine them in methanol without loss of aldehyde. Most other aldehydes give low results in methanol because of acetal formation.

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New Color Reaction for Vitamins D

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Vitamins D_2 and D_3 were found to react with an iodine–ethylene dichloride reagent to produce a strong yellow color which showed maximal absorption at 450 mμ. The intensity of the color was enhanced by mercuric *p*-chlorobenzoate and certain other compounds. The reagent showed high specificity for the vitamins D, no other compound having been found to give rise to a similar color. Various sterols produced no color; vitamin A produced a weak violet color which showed some absorption at 450 mμ. While the reagent showed greater sensitivity as well as greater specificity than the glycerol dichlorohydrin reagent, it was less sensitive than the antimony trichloride reagent. It is easy to prepare and its use is not complicated by the formation of films or corrosive volatiles.

TWO interrelated problems have thus far impeded the development of a satisfactory chemical or physicochemical method of assay for the vitamins D. First, the vitamins have proved to be extremely difficult to separate quantitatively from other closely related compounds. Secondly, known reactions have lacked specificity or sensitivity, or both, for use with the vitamin D concentrates from most natural oils and synthetic irradiation mixtures.

Of the several methods of assay which have been proposed, none has come into common use. Direct spectrophotometry in the ultraviolet (*4*) and infrared (*8*) regions lacks specificity; measurement of colors derived from the vitamins has found wider acceptance. Two of these color reactions, one with antimony trichloride developed originally by Brockmann and Chen (*1*), and a second with glycerol dichlorohydrin advanced by Sobel, Mayer, and Kramer (*10*), have continuously stimulated interest. Improvements on the original antimony trichloride procedure which have been proposed by Raoul and Meunier (*9*), Nield, Russel, and Zimmerli (*7*), and others have produced a method which has good sensitivity but lacks the desired specificity. A modification of the Sobel procedure which increased the sensitivity of this reagent by a factor of 20 was introduced by Campbell (*2*); however, sensitivity is still one of its limitations.

In the present work a new color reaction has been discovered which is highly specific for vitamins D and which, it is hoped, may give rise to a procedure with fewer shortcomings than those which are currently available. This paper describes responses of pure vitamins and related sterols to the reagent.

REAGENTS AND APPARATUS

Vitamin D_2 (crystalline, 40,000,000 U. S. P. units per gram), Sterwin Chemicals, Inc.

Vitamin D_3 (crystalline, 40,000,000 U. S. P. units per gram), Sterwin Chemicals, Inc.

Ergosterol, Eastman Kodak Co. Recrystallized twice from ethyl acetate and once from ethylene dichloride before use; melting point 165–166° C.

7-Dehydrocholesterol, E. I. du Pont de Nemours & Co., Inc.

Cholesterol, Eastman Kodak Co. Recrystallized from ethyl acetate before use; melting point, 147–148° C.

Stigmasterol (melting point, 170–171° C.), The Glidden Co.

Sitosterols (purified mixture, melting point, 137° to 140° C.), The Glidden Co.

Lumisterol. Prepared by saponification of the 3,5-dinitrobenzoate, which was obtained from Smith-New York, Inc.

Vitamin A (crystalline alcohol), Distillation Products, Inc.

Iodine (C.P. resublimed), J. T. Baker Chemical Co.

Ethylene dichloride. A 750-ml. quantity of a technical grade of ethylene dichloride was shaken in a separatory funnel with two 50-ml. portions of concentrated sulfuric acid. After removal of the hypophase the ethylene dichloride was washed with distilled water and with 2% sodium bicarbonate solution, dried over anhydrous sodium sulfate, and allowed to percolate through a 20 × 200 mm. column of silica gel (28- to 200-mesh, Davison Chemical Corp.).

Mercuric *p*-chlorobenzoate. A concentrated aqueous solution of sodium *p*-chlorobenzoate was added to a saturated aqueous solution of mercuric chloride. The white precipitate was filtered and washed with water.

Iodine–ethylene dichloride reagent (subsequently referred to as "the reagent"). To a flask of ethylene dichloride were added 80 p.p.m. of mercuric *p*-chlorobenzoate and sufficient iodine to produce an absorbance at 500 mμ of 0.182 ± 0.002 (absorbance at 450 mμ of 0.076 ± 0.002). This solution was approximately 0.20m*M* with iodine.

A Beckman Model DU spectrophotometer with matched 1-cm. cells was used for all absorbance measurements. An incandescent lamp served as the source of illumination, and the slit width was varied to accord with the region of the spectrum being examined.

EXPERIMENTAL

Development and Measurement of Colored Vitamin D Complex. When a 0.10m*M* solution of vitamin D_2 or D_3 in ethylene dichloride was added to an equal volume of the reagent, the pink color of the reagent was superseded by a yellow color which developed in the solution. This colored vitamin D complex showed a sharp absorption maximum at 450 mμ (Figure 1), reached its maximal intensity in about 7 minutes, then faded slowly. Different samples of crystalline vitamins D_2 and D_3 showed essentially identical behavior.

To obtain the data for the absorption curves (Figure 1), a series of test tubes, each containing 3.0 ml. of reagent, was prepared, and to each were added 3.0 ml. of a 0.10 m*M* solution of the vitamin, but at successive intervals. After several of the tubes had been used to establish the 450 mμ reading at the time of maximal color development, each of the remaining tubes was used to obtain a point at a different wave length, the reading being taken in each case as quickly as possible after the maximum reading at 450 mμ had been reached. No attempt was made to correct the absorption curves for light absorbed by free iodine in the

Table I. Reactivity of Reagent with Vitamins and Sterols

(Readings taken at 7 minutes)

Compound	Concentration, Millimole/ Liter	Absorbance, 450 mμ
None (blank)	..	0.038
Vitamin D_2	0.05	1.060
Vitamin D_3	0.05	1.060
Vitamin A	0.10	0.130
Lumisterol	0.50	0.039
Ergosterol	0.50	0.039
7-Dehydrocholesterol	0.50	0.033
Cholesterol	0.50	0.037
Stigmasterol	0.50	0.037
Sitosterol	0.50	0.037

Table II. Constancy of Color Production of Reagent with 0.05m*M* Vitamin D_2 in Presence of Related Sterols

(Readings taken at 7 minutes)

Sterols Present	Concentration, Millimole/ Liter	Absorbance, 450 mμ
None	..	1.040
Lumisterol	0.25	1.045
Cholesterol	0.25	1.045
Ergosterol	0.25	1.035
Cholesterol and Ergosterol	0.25 0.25	1.040

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[1] Present address, The Procter & Gamble Co., Cincinnati 31, Ohio.

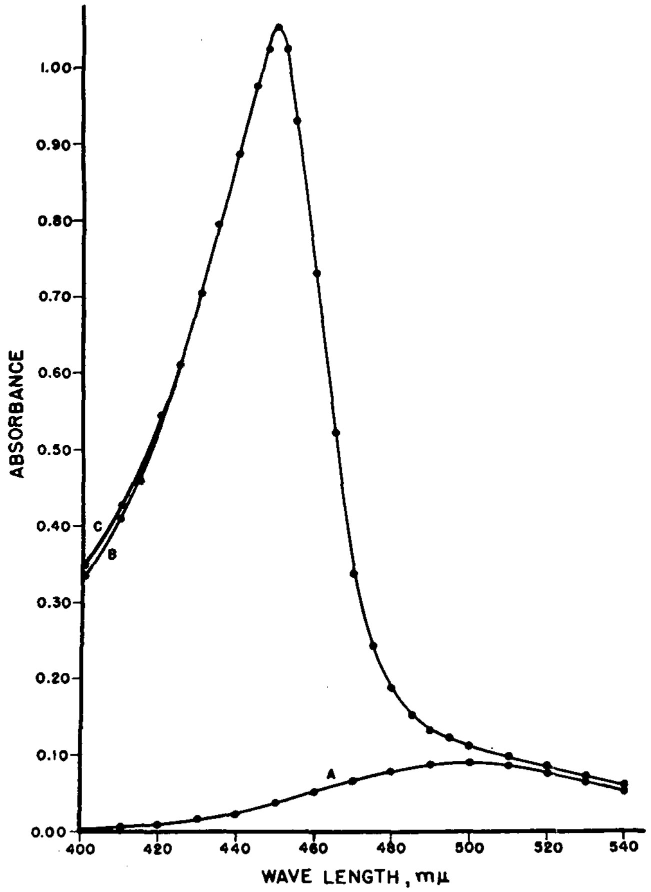

Figure 1. Spectral absorption of (*A*) iodine-ethylene dichloride reagent, (*B*) reagent with 0.05 mM vitamin D_2, and (*C*) reagent with 0.05 mM vitamin D_3

Reaction time 7 minutes

reagent. However, the reagent before reaction, but in the same dilution, showed an absorbance of only 0.038 at 450 mμ (Figure 1). Lower concentrations of iodine in the reagent did not produce maximal absorbance (450 mμ) of the vitamin D complex.

Specificity of Color Reaction. Six different sterols tested at 0.05 and 0.5mM concentrations showed no apparent reaction with the reagent (Table I) and produced no interference when present with vitamins D (Table II).

However, vitamin A (0.20mM solution) produced a medium blue color which changed after approximately 1 minute to medium violet. The solution showed a broad absorption band with a maximum at 555 mμ and some absorption at 450 mμ. In admixture with vitamins D, vitamin A interfered to give low values: Vitamin D_2 (0.10mM) with vitamin A (0.10mM) produced an absorbance of 0.990. Removal of the vitamin A would appear necessary for use of the reaction with natural materials such as fish liver oils. The chromatographic method of DeWitt and Sullivan (*3*) has been used satisfactorily for this purpose.

Polar compounds such as alcohol and acetone when present in large quantities reacted with the reagent to produce high absorption in the near ultraviolet with some overlapping into the 400- to 450-mμ region. However, no interference from such compounds was encountered when standard separation procedures were used prior to color development.

In specificity for vitamins D, the new reaction compared favorably with well-known reactions (Table III). However, the sensitivity attained in the study was below that of the improved (Nield) antimony trichloride reagent.

Factors Affecting Intensity of Color. When the mercuric *p*-chlorobenzoate was omitted from the reagent, color development was qualitatively the same but quantitatively about 10 to 15% as intense. An optimal concentration of the mercury salt was observed, above which increments in concentration caused a decrease rather than an increase in absorbance. Similar effects were observed with mercuric benzoate (Table IV) and other mercuric salts; however, mercuric *p*-chlorobenzoate was the most effective of the several mercuric salts tested (Table V). Other compounds which produced a similar, but weaker, effect included ferric, ceric, and ammonium benzoates, and commercial lots of ethylene chlorohydrin, trichloroacetic acid, and iodobenzene. Benzaldehyde produced as much color as the mercuric salts but the amounts required varied from 0.06 to 1.5% depending on the source of the material. Apparently an impurity was responsible for the effect. No definite relation was observed between the various compounds and their activity as color enhancers. The time required for maximum color development varied with the different compounds and their concentrations.

Interference with the color reaction was observed with some commercial samples of ethylene dichloride. The interfering substances were not removed completely by fractional distillation but were eliminated by treatment with sulfuric acid and silica gel by the procedure outlined above. Ethylene dichloride samples from five different manufacturers, when so treated, had excellent stability and gave uniform results in the color reaction. Other chlorinated solvents which were tested included carbon tetrachloride, chloroform, ethylidene chloride, the trichloroethanes, and 1,1,2,2-tetrachloroethane. While varying amounts of yellow color were observed in these solvents, the strong color with the characteristic sharp absorption peak was observed only in ethylene dichloride.

Temperature differences between 20° and 35° C. were shown to have no effect on the reaction. Light conditions—i.e., diffuse

Table III. Comparison of Absorptivities of Vitamins D and Certain Sterols in Three Color Reactions

	Absorptivity at Wave Length of Maximum Absorption		
Compound	Iodine reagent	Antimony trichloride[a]	Glycerol dichlorohydrin[b]
Vitamin D_2	530	1800	360
Vitamin D_3	545	1850	370
Ergosterol	0.0	5	14
7-Dehydrocholesterol	0.0	8	0.3
Cholesterol	0.0	25	0

[a] Improved reagent containing acetyl chloride; from data of Mueller (*6*).
[b] From data of Campbell (*2*).

Table IV. Effect of Mercuric Benzoate Additions on Intensity of Color Developed with 0.05mM Vitamin D_2

Mercuric Benzoate Added, P.P.M.	Maximum Absorbance, 450 mμ
None	0.132
6.3	0.640
11.5	0.915
12.7	0.970
13.5	0.990
14.3	0.970
19.0	0.900
38.0	No color formation

Table V. Effect of Various Mercuric Salts on Intensity of Color Developed with 0.05 mM Vitamin D_2

Mercuric Salt	Optimal Concn., P.P.M.	Maximum Absorbance, 450 mμ
Mercuric acetate	7	0.600
Mercuric trichloroacetate	11	0.990
Mercuric benzoate	14	0.990
Mercuric *p*-chlorobenzoate	40	1.060
Mercuric *p*-aminobenzoate	11	0.890

daylight compared to darkness—also showed no effect. A precision within 2% was obtained when the conditions prescribed for color development were adhered to strictly.

Nature of Colored Compound. Little has been learned about the nature of the color reaction. The literature is nearly devoid of mention of color formation between halogens and vitamins D or related sterols. Although the reaction of Tortelli and Jaffé (*11*) may be related in principle to the reaction disclosed here, the techniques used are different, and the resulting colors are dissimilar. A blue-green color observed by Green (*5*) between his iodine trichloride reagent and β-carotene may be related also. Green postulated a mesomeric change in the carotene molecule under the influence of ICl_3, Cl^-, or Cl_3^- similar to that produced by antimony trichloride on carotenoids and vitamin D. Although no direct evidence for a mechanism was observed in the authors' experiments, it is suggested that a loose union occurs between iodine and the unsaturated center of the D vitamins. More specifically, because traces of bromine increased both the intensity of color and the speed with which maximum color was attained, it appears that iodide ion may be the active form which unites with the vitamin to form the colored product. As the enhancing agents did not change qualitatively the spectral absorption properties of the colored vitamin D complex, their function may be to promote the reaction by increasing the concentration of the iodide ion.

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Determination of Urea in Blood and Urine with Diacetyl Monoxime

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The condensation of urea with acid diacetyl monoxime (Fearon reaction) with concomitant oxidation by arsenic acid has been extensively studied in an effort to improve reproducibility and the linearity of response of the reaction. The concentration of mineral acid and oxidizing arsenic acid was found to be critical. By performing the reaction in 3.8*N* hydrochloric acid and 0.08*N* arsenic acid maximum color is produced which conforms to Beer's law at urea concentrations up to 60 γ per 10-ml. reaction volume. Dilution of the reaction mixture results in a deviation from Beer's law, and the urea response curve no longer passes through the origin. By the study, a rapid and accurate method for the determination of urea in blood and urine has been developed. Comparative studies with existing methods and recovery studies have shown the suitability of the procedure. Analysis of a sample in duplicate requires less than 1 hour.

THE need for a direct, simple, and accurate method for determining urea in blood and biological fluids has resulted in the development of a variety of direct and indirect procedures. The indirect methods, which are the most widely used, depend on the hydrolysis of urea with the enzyme, urease, to form ammonia. The liberated ammonia is usually determined by direct nesslerization, by aeration and nesslerization, or by aeration and titration (*3, 7, 9, 11*). The methods for the direct determination of urea depend upon the condensation of urea with α-isonitrosopropiophenone (*1*) or diacetyl derivatives (*2, 5, 12, 13*) in the presence of strong acid solutions. The reaction with α-isonitrosopropiophenone requires special precautions because of the long heating time required for the production of color, and because of the photosensitivity of the color formed.

The reaction between diacetyl monoxime and urea [Fearon reaction (*4*)] to yield a yellow color appears to offer distinct advantages for the determination of urea. However, the various published modifications generally suffer from the fact that the color formed does not obey Beer's law and, at low concentrations, is not proportional to the concentration of urea. Preliminary studies using the Kawerau (*8*) modification indicated that both the concentration of hydrochloric acid and the concentration of arsenic acid greatly affected the production of the color. A more complete study of the conditions for the reaction was undertaken with the concomitant development of a reproducible and accurate procedure for the determination of urea in blood and biological fluids.

REAGENTS

Urea Stock Standard, 1 mg. of urea per ml. Dissolve 100 mg. of dried, reagent grade urea in 100 ml. of water, adding a few drops of chloroform as preservative. The solution is stable for at least 4 months when refrigerated.

Urea Working Standard, 0.05 mg. of urea per ml. Dilute 5 ml. of stock standard to 100 ml. with water. Add a few drops of chloroform as preservative.

Diacetyl Monoxime, 2.5% in 5% acetic acid. Dissolve 2.5 grams of diacetyl monoxime in 100 ml. of 5% acetic acid. The solution is stable at room temperature for at least 6 months. The appearance of a slight yellow color does not interfere.

Arsenic Acid, saturated stock solution. Suspend 50 grams of arsenic pentoxide (Baker's Analyzed reagent) in 1000 ml. of concentrated hydrochloric acid, and allow suspension to stand with occasional mixing one or more days. For use, decant the clear yellow supernatant or filter through sintered glass. The solution is standardized as follows. Dilute 2 ml. of stock solution with 50 ml. of 4*N* hydrochloric acid in a 250-ml. Erlenmeyer flask. Add 2 grams of iodate-free potassium iodide and 15 ml. of carbon tetrachloride. Mix, and after 10 to 30 minutes titrate the liberated iodine with 0.1*N* sodium thiosulfate using the disappearance of purple iodine color from the carbon tetrachloride as the end point. This solution is approximately 0.9 to 1.0*N* with respect to quinquevalent arsenic ion.

Arsenic Acid Working Solution. Dilute an appropriate amount of saturated stock solution with concentrated hydrochloric acid to yield a final solution which is 0.26 to 0.27*N* with respect to quinquevalent arsenic.

EXPERIMENTAL

The yellow color formed between diacetyl monoxime and urea has an absorption maximum at 480 to 485 mμ, as measured with a Beckman DU spectrophotometer. The Klett-Summerson filter

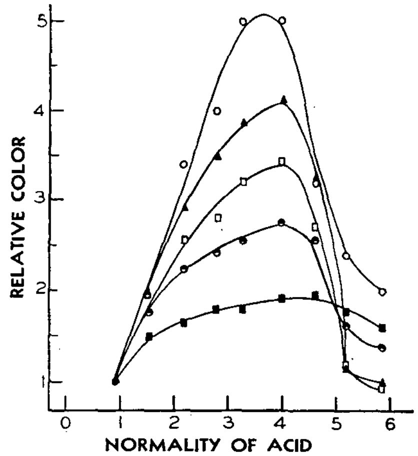

Figure 1. Effect of acid concentration on color formation at various concentrations of urea

○ 10 γ of urea per tube
▲ 30 γ of urea per tube
□ 45 γ of urea per tube
◓ 60 γ of urea per tube
■ 90 γ of urea per tube

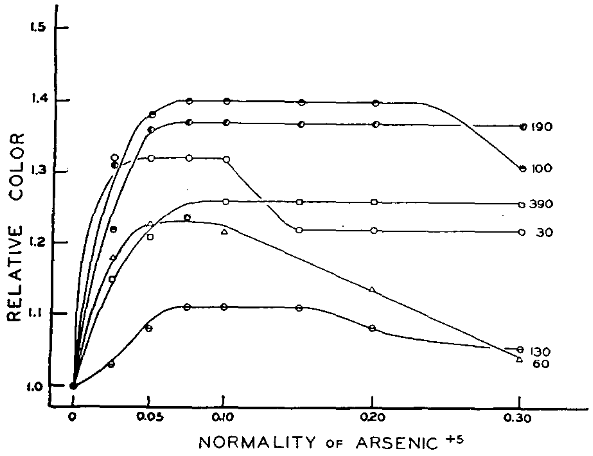

Figure 2. Effect of arsenic acid concentration on color formation at various concentrations of urea

Numerals on curves indicate micrograms of urea per reaction tube

No. 47 was, therefore, selected for use with the Klett-Summerson photometer.

Effect of Acid Concentration on Color Production. The concentration of hydrochloric acid necessary for maximum production of the yellow reaction product was determined by varying the concentration of hydrochloric acid in the reaction mixture. All other components of the reaction mixture were maintained at optimal concentrations as described under procedure. The concentrations of hydrochloric acid and urea are expressed in terms of the final 10-ml. reaction mixture. For absorbance measurements all reaction mixtures were diluted to a volume of 20 ml.

The optimum acid concentration (Figure 1) shifted from 3.6*N* for 10 γ of urea to 4.0*N* for 60 γ of urea and to 4.5*N* for 90 γ of urea. An average value of 3.8*N* acid was selected to cover the working range of concentration from 10 to 60 γ of urea. High concentrations of acid inhibit color formation to a great degree, and little, if any, color is produced when the acid concentration approaches 10*N*. The data of Figure 1 were obtained at optimal arsenic acid concentrations. Because the arsenic solution alone is sufficiently acid to produce some color, the data are presented on a comparative basis.

Effect of Arsenic Acid Concentration on Color Production. The concentration of arsenic acid for the maximum formation of the yellow reaction product was determined by varying the concentration of the oxidizing acid in the reaction mixture. All other components of the reaction mixture were maintained at optimal concentrations as described under procedure, and the concentrations of arsenic acid and urea are expressed in terms of the final 10-ml. reaction mixture. For absorbance measurements all reaction mixtures were diluted to a volume of 20 ml.

The optimum concentration of arsenic acid for the production of color is critical over a narrow range of concentrations and varies with the concentration of urea as shown in Figure 2. For all urea concentrations tested, maximum color formation is obtained with 0.08*N* quinquevalent arsenic. At greater concentrations of arsenic acid, color formation is inhibited at some, but not all, concentrations of urea. Since some color is produced in the presence of optimal hydrogen ion concentration in the absence of arsenic acid, the data are presented on a relative basis for comparative purpose.

Effect of Diacetyl Monoxime Concentration on Color Production. The concentration of diacetyl monoxime necessary for the production of maximum color was determined by varying the amount of the reagent in the reaction mixture. Maximum color production is obtained at a concentration of 0.25% diacetyl monoxime. At lower concentrations of reagent, color production falls off rapidly, and no color is produced in the absence of the reagent. At greater concentrations of diacetyl monoxime, color production is depressed.

Effect of Heat on Color Production. Color formation increases rapidly with heating in a boiling water bath for the first 25 minutes, in which time approximately 90% of the color is formed. Since continued heating for an additional 35 minutes increases the amount of color by only 10%, a 30-minute heating period was selected as the optimal time for color formation. The final color is somewhat photolabile and fades at a rate of approximately 5% per hour under fluorescent lighting in this laboratory.

Effect of Urea Concentration on Color Formation. With the information available for the optimal conditions of the condensation reaction, standard curves were prepared with graded quantities of urea. The reaction mixture, in a final volume of 10 ml., was read photometrically at 470 mμ, using a Klett-Summerson photometer. As shown in Figure 3, the color formed under these conditions (curve *A*) obeys Beer's law for concentrations of urea up to 60 γ per tube, and the curve passes through the origin. At concentrations above 60 γ per tube, the curve rapidly decreases in slope. When the final reaction mixture is diluted to 20 ml. (curve *B*), the curve is linear up to a concentration of 80 γ per tube, but no longer passes through the origin. On further dilution to 40 ml. (curve *C*) the curve remains linear for concentrations up to 160 γ per tube, but does not go through the origin. Each of the curves, however, is reproducible from day to day and the choice of standard curves for use depends on the sensitivity desired and on the concentration of urea to be measured.

PROCEDURE

On the basis of the above information, the procedure finally adopted is as follows.

Place 1 ml. of 1 to 10 protein-free filtrate of blood in the bottom of a test tube and dilute with approximately 4 ml. of water. A blank solution containing distilled water and a standard solution containing 0.05 mg. of urea are incorporated in each series of tests. To each tube are added 3 ml. of dilute arsenic acid solution and 1 ml. of 2.5% diacetyl monoxime solution. The volume is adjusted to 10 ml., and the solution is mixed by gentle shaking. The tubes are capped with small beakers and placed in a boiling water bath for 30 minutes. The level of the boiling water bath must at all times be above the level of the reaction mixture. At the end of 30 minutes the tubes are removed and are placed in tap water (5° to 15° C.) for 3 minutes. Absorbance is determined immediately with a Klett-Summerson photometer adjusted to zero with the blank. The concentration of urea of the unknown sample may then be determined by reference to a standard curve, or may be calculated over that portion of the standard curve which is linear. If desired, the blank, standard, and unknown solutions may be diluted with water to an appropriate volume before absorbance measurements are made.

For the determination of urea in urine or other fluids containing no protein, the samples are diluted with water to a suitable concentration and incorporated in the test. If proteinaceous material is present this may be removed by suitable methods before analysis.

RESULTS

Because the Fearon reaction is not specific for urea, but reacts with other substances containing the ureido group such as citrulline (5-ureidonorvaline), substituted ureas, and carbamyl amino acids, three samples of blood and three samples of urine were treated with an excess amount of urease by the Gentzkow procedure (*6*) as modified in this laboratory. Following precipitation of the proteins with tungstic acid, the filtrates were analyzed for residual urea using curve *B*, Figure 3. It was found that urease destroyed all of the chromogens in blood and 94 to 96% of the chromogens in urine. It would appear, therefore, that the proposed procedure primarily measures urea in this type of material.

The concentration of urea in blood and urine was determined by the diacetyl procedure and compared with the direct nesslerization modification of Gentzkow (*6*). As can be seen from Table I, the two methods are in good agreement for blood samples containing essentially normal amounts of urea. At high concentrations of urea, the diacetyl method yields values up to 8% higher than the urease method. It is probable that the diacetyl monoxime procedure for uremic blood samples yields more nearly correct values. Since many of the uremic samples were obtained from persons undergoing drug therapy, it is felt that some substances may inhibit the action of urease enzyme. This factor, however, requires further study. With urine samples, good agreement between the two methods was obtained.

Recovery experiments were performed by adding known amounts of urea to samples of blood and urine. The average recoveries and standard deviations are shown in Table II. Individual recoveries ranging from 94 to 103% in blood and 92 to 110% in urine were obtained.

DISCUSSION

The preceding experiments indicate that the condensation of diacetyl monoxime with urea to yield a yellow color is suitable for the estimation of urea in blood and biological fluids. The procedure is relatively simple, at least five times more sensitive than existing enzymatic procedures, and is more satisfactory for rapid, accurate estimations of urea.

Table I. Comparison of Diacetyl Monoxime and Direct Nesslerization Procedures

Sample	Blood, Mg./100 Ml.			Urine, Mg./Ml.		
	Diacetyl	Nessler's	Diff., %	Diacetyl	Nessler's	Diff., %
1	106.0	99.0	+6.6	15.0	15.6	−3.8
2	12.9	13.5	−4.4	26.2	25.1	+4.2
3	20.6	20.8	−0.8	14.5	13.9	+2.8
4	26.8	25.8	+3.8	26.2	25.2	+3.8
5	34.5	34.7	−0.6	21.8	20.4	+6.4
6	21.0	21.4	−1.9	14.0	15.6	−6.6
7	34.2	34.3	−0.3	13.5	12.9	+4.4
8	7.4	7.6	2.6	37.8	36.3	+4.0
9	90.0	88.5	+1.7	31.2	30.5	+2.2
10	96.9	90.0	+7.1	7.5	7.5	0.0
11	48.0	44.6	+7.1	14.8	14.5	+2.0
12	60.0	55.3	+7.8			

Table II. Recovery of Urea Added to Blood and Urine as Determined by Diacetyl Monoxime Procedure

Sample	No. of Detn.[a]	Urea Added, Mg./100 Ml.	Urea Recovered, %
Blood[b]	7	5	97 ± 3.9
	8	10	100 ± 4.5
	10	20	100 ± 3.7
	7	40	101 ± 3.3
Urine[c]	5	2.5	102 ± 6.5
	8	5.0	100 ± 3.9
	7	10.0	103 ± 4.4
	8	20.0	103 ± 4.8

[a] Each sample analyzed in duplicate or triplicate.
[b] Blood samples contained 17.9 to 45.4 mg. of urea per 100 ml.
[c] Urine samples contained 4.0 to 34.5 mg. of urea per ml.

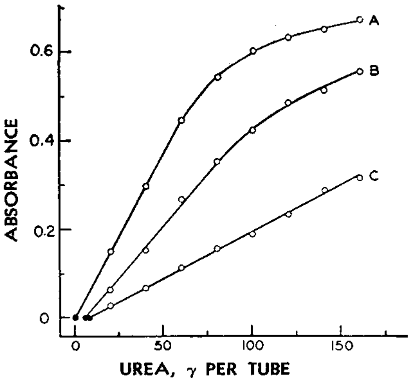

Figure 3. Effect of urea concentration on color formation

Although the procedure is not specific for urea and some color is given by other compounds containing the ureido grouping (*10*), only urea yields a yellow color with an absorption maximum at 480 to 485 mμ. Citrulline and other carbamyl amino acids react to form compounds with absorption maxima in the vicinity of 550 mμ. Moreover, Koritz and Cohen (*10*) have shown that only citrulline, carbamyl-glycine, and substituted ureas have chromogen equivalents greater than urea. All other substances tested by these authors have chromogenic equivalents less than 25% of urea. Citrulline does not interfere in our procedure, as the concentration in biological fluids is apparently too low to be measured except under unusual physiological conditions. For highly uremic blood samples, further dilution of the filtrates with water prior to incorporation in the test may be necessary. Dilution of the final colored reaction mixture to lesser absorbance is not recommended unless the standard solution is also diluted, because the absorbance is not reduced proportionately by dilution.

When the yellow reaction product is treated with alkali the color is markedly reduced at reactions more alkaline than pH 2. The effect of alkali and the deviation from Beer's law on dilution indicates that the colored reaction product may be in the form of a dissociable complex molecule.

Various types of deproteinating agents such as trichloroacetic acid, tungstic acid, or cadmium sulfate–sodium hydroxide may be used satisfactorily. When present at high concentrations, tungstic acid may, however, yield a cloudy reaction mixture. As much as 5 ml. of tungstic acid–blood filtrate may be used satisfactorily, but greater quantities of filtrate develop a precipitate.

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Determination of Hydrogen in Liquids and Suspensions by Nuclear Magnetic Absorption

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An investigation was made of the applicability of the modulation method of magnetic resonance absorption for the determination of hydrogen in various liquids and in aqueous suspensions of starch. The materials investigated contained from (1.1 to 6.7) × 10^{22} hydrogen atoms per cc. in the liquid phase. The thermal relaxation time, T_1, ranged from 0.3 to 3.7 seconds. The influence of T_1 on the measurements through radio-frequency saturation was eliminated by an extrapolation to zero radio-frequency magnetic field of a series of measurements at radio-frequency intensities in the range 10^{-4} to 10^{-3} gauss. The precision of the measurements was limited to about 2% by electrical noise in the spectrometer employed. The application of the magnetic absorption method for the determination of the water content of biological tissues on an absolute basis is briefly discussed.

WITH the development of techniques suitable for liquids and solids, the quantitative analytical aspects of magnetic resonance absorption spectroscopy have become of great interest. Unfortunately, of the various methods for measurement of magnetic absorption, none appears uniquely adapted for routine analysis. One method, widely used for the observation of nuclear magnetic resonance absorption, utilizes small amplitude sinusoidal modulation (*1*), with the detector followed by a narrow-band amplifier and lock-in amplifier. A small modulation of amplitude, H_m, and angular frequency, ω_m, is applied to the static field, H_0, as the frequency of the radio-frequency magnetic field is slowly varied through the resonance region. For a simple absorption line the observed signal, D, proportional to the slope of the absorption line, passes through the extreme values $D_{\max}$ and $D_{\min}$, corresponding to the inflection points of the absorption line. It is of interest in the analytical applications of magnetic absorption to consider the usefulness of the quantity $D_{\max}$ as a measure of N_0, the number per cubic centimeters of sample of magnetic nuclei of the species of interest. In the present paper the application of $D_{\max}$ is considered for the determination of hydrogen nuclei in liquids and in mixtures (suspensions) of liquids and solids in which the hydrogen nuclei of interest are contained in the liquid phase. A primary object was to determine whether N_0 can be determined accurately via $D_{\max}$ in systems in which radio-frequency saturation is appreciable under the conditions of measurement, and the extent of saturation is both unknown and variable with N_0. It was not the object of the present work to determine the ultimate precision of the method, but rather to explore the general performance in an analytical procedure in order to determine whether fundamental limitations exist.

THEORY

For a simple absorption line described by a shape function $g(\nu)$, the signal D observed in the absence of radio-frequency saturation effects (*3*) may be expressed as

$$D = C\zeta\chi_0\nu_0\left[\frac{\partial g(\nu)}{\partial\nu}\right] \tag{1}$$

Here C is a constant for an appropriate choice of instrument parameters, ζ is the filling factor of the radio-frequency coil containing the sample, ν_0 is the frequency at the center of the absorption line, and χ_0 the magnetic susceptibility given by the expression

$$\chi_0 = N_0\gamma^2\hbar^2 I(I + 1)/3kT \tag{2}$$

Here γ is the magnetogyric ratio, I is the nuclear spin, and T is the absolute temperature.

The maximum value of D may be expressed

$$D_{\max} = C\zeta\chi_0\nu_0\left[\frac{\partial g(\nu)}{\partial\nu}\right]_{\max} \tag{3}$$

From Equations 2 and 3 it is clear that if $D_{\max}$ is to be used in analysis as a measure of N_0, the other factors must either be constant or known. The only factors which require consideration here are $g(\nu)$ and ζ; ν_0, C, and T are either known or can be held constant, within desired limits, by a suitable choice of instrumentation and operating conditions. In general, however, $g(\nu)$ and ζ are subject to relatively wide variations depending upon the physical properties of the sample. In addition, $g(\nu)$, may be heavily influenced by the homogeneity of the static applied field. Thus the use of $D_{\max}$ as a measure of N_0 is unsatisfactory except under specialized conditions.

For liquids or certain other materials with relatively long T_1 and intrinsic line width of the absorption so small as to be negligible compared with the artificial broadening, δH, imposed by inhomogeneities in the static field applied to the sample, $g(\nu)$, may be held approximately constant by simply fixing the position of the sample in the static field and holding H_m constant. Similarly for liquids or other essentially homogeneous materials, ζ can be maintained constant by fixing the volume and position of the sample in the radio-frequency coil. Thus for liquids and other homogeneous materials it should be possible to utilize $D_{\max}$ as a measure of N_0.

A further complication which arises in any method of observing magnetic absorption is radio-frequency saturation. In the equations given above it was assumed that the applied radio-frequency magnetic field is so weak that the Boltzmann distribution of nuclei among the energy levels is not appreciably disturbed. Actually the radio-frequency effect may be considerable, especially for systems where T_1 is long, and the observed susceptibility is reduced from its maximum value. For analytical applications the effect is troublesome because in general T_1 varies with N_0. Moreover, for analytical work it is not considered feasible to determine T_1 for each specimen and thus apply a correction to the observed value of $D_{\max}$ to obtain a value $(D_{\max})_0$ in the absence of saturation. Thus for analytical applications a method for obtaining $(D_{\max})_0$ without a knowledge of T_1 would appear essential.

Saturation effects have been thoroughly examined by others (*1*) and utilizing their methods in the authors' experiments with water, ethyl ether, and a $0.002N$ aqueous solution of copper sulfate, it was found that the saturation followed a $(1 + S)^{-1.5 \pm 0.1}$ dependence. Bloembergen, Purcell, and Pound have shown that when

$$1/\gamma T_2 < H_m < \partial H \quad \text{and} \quad \omega_m T_1 > 1$$

the saturation parameter, S, equals $\gamma H_1^2 T_1/H_m$, where H_1 is the amplitude of the effective radio-frequency magnetic field.

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The effect of saturation may then be included by use of the approximate equation (*3*)

$$D_{\max} = C\zeta\chi_0\nu_0 \left[\frac{\partial g(\nu)}{\partial \nu}\right]_{\max} \times (1 + S)^{-3/2} \qquad (4)$$

From Equation 4 it appears that a possible method to allow for the effects of saturation is to plot $D_{\max}$ against H_1^2 for a few values of H_1^2 (near $H_1^2 = 0$) and obtain by a linear extrapolation the limiting value $(D_{\max})_0$ corresponding to $D_{\max}$ for $S = 0$. The simplicity of this procedure suggests that it may be satisfactory for routine analytical use.

EXPERIMENTAL

Proton magnetic resonance absorption was investigated for the following systems: a group of miscellaneous organic liquids, a series of solutions of dioxane in carbon tetrachloride, and a series of suspensions of starch in water. The materials were chosen to obtain a range of N_0 and T_1. Solutions and suspensions were prepared in volumetric flasks from weighed quantities of materials. The starch used was a commercial wheat starch containing less than 0.1% of water-soluble materials and 9.8% of absorbed water. Measurements were performed at room temperature (about 27° C.).

The magnetic absorption measurements were made in a static field of strength H_0, equal to 6380 gauss, provided by a permanent magnet with poles 6 inches in diameter and a 1.75-inch gap. The proton resonance was recorded by means of a radio-frequency spectrometer of the null-balance type (*2*) continuously standardized by a Watkins-Pound (*5*) calibrator. The radio-frequency coil was 22 mm. in inside diameter and 29 mm. in length and was designed to accept standard borosilicate glass test tubes which serve as containers for the test specimens. For those experiments where it was essential to maintain the filling factor constant, a single test tube was used.

The thermal relaxation time, T_1, of the various systems investigated was determined by the saturation method (*1*). The line width of the proton resonance was observed to be 1.7 ± 0.1 kc. in all samples for a modulation amplitude, H_m, of 0.23 gauss (0.98 kc.), and $\omega_m = 2\pi \times 50$ sec.$^{-1}$

For each sample $D_{\max}$ was determined for several values of H_1 in the range (2 to 6) $\times 10^{-4}$ gauss. Graphs of $D_{\max}$ *vs.* H_1^2 were extrapolated linearly to $H_1^2 = 0$ to obtain $(D_{\max})_0$.

At the lowest value of H_1 for which observations were made, the signal $D_{\max}$ for water was approximately 100 times the noise level. At higher levels of H_1, the noise decreased markedly. The effect of the noise is, of course, to decrease the precision with which $D_{\max}$ can be determined. This factor appears to be the principal limitation to the precision of the results obtained here since the procedure used requires the use of very low radio-frequency levels in order to be able to utilize a linear extrapolation as a means of correcting for saturation effects. It has not been determined to what extent improvement is possible through the use of longer time for observations and through improvements in spectrometer design.

RESULTS AND DISCUSSION

The results obtained for the organic liquids are summarized in Table I, which contains the values of $(D_{\max})_0$ obtained by extrapolation and values of N_0 for each sample as calculated from the theoretical composition and the density according to the relation

$$N_0 = n_m A\rho/M \qquad (5)$$

Here n_m is the number of hydrogen atoms per molecule, A is Avogadro's number, ρ is the density of the liquid at the temperature of the observations, and M is the gram-molecular weight. To obtain N_0 for the dioxane–carbon tetrachloride solutions, N_0 for dioxane was multiplied by the appropriate dilution factor. According to Equations 2 and 4 the values of $(D_{\max})_0$ should be proportional to the values of N_0. The data are in arrangement as shown by the fact that the ratio $(D_{\max})_0/N_0$ is approximately constant. The mean value of the ratio is 31.9×10^{-22} with a standard deviation of 0.8×10^{-22}. The indicated error appears consistent with the observed signal-to-noise ratio, so that it may be concluded that deviations observed in the ratio $(D_{\max})_0/N_0$ are primarily due to this source.

Table I. Results for Organic Liquids

Sample	$N_0 \times 10^{-22}$, Cc.$^{-1}$	T_1, Sec.	$(D_{\max})_0$, Arbitrary Units	$\frac{(D_{\max})_0}{N_0} \times 10^{22}$, Arbitrary Units
Iodobenzene	2.70	2.5[a]	86.5[b]	32.0
Ethylene bromide	2.79	..	88.5	31.7
Ethylene chloride	3.05	2.5	90.0	32.4
Ethyl malonate	4.76	1.1	150.0	31.5
n-Amyl bromide	5.42	..	166.5	30.7
Ethyl ether	5.77	3.7	182.0	31.5
Water	6.70	2.1	210.0	31.3
Dioxane, 20%, in CCl_4	1.13	..	37.5	33.3
Dioxane, 40%, in CCl_4	2.26	2.4	74.5	33.0
Dioxane, 60%, in CCl_4	3.38	..	104.5	30.9
Dioxane, 80%, in CCl_4	4.52	..	145.0	32.1
Dioxane, 100%	5.63	2.0	184.5	32.7
			Mean	31.9
			Standard deviation	±0.8

[a] Subject to estimated error of ±10%.
[b] Read only to nearest half unit.

Table II. Results for Starch Suspensions

Sample No.	Water Content, Wt. %	Density, Grams Cc.	$N_0 \times 10^{-22}$ Cc.$^{-1}$	T_1, Sec.	$(D_{\max})_0$, Arbitrary Units	$\frac{(D_{\max})_0}{N_0} \times 10^{22}$, Arbitrary Units
1	92.0	1.029	6.33	0.52[a]	206.5[b]	32.6
2	75.9	1.096	5.55	0.28	167.0	30.1
3	86.0	1.050	6.04	0.44	195.0	32.3
4	81.3	1.072	5.81	0.35	186.5	32.1
5	76.9	1.095	5.63	0.29	182.0	32.3
6	68.6	1.135	5.20	0.22	166.5	32.0
7	63.4	1.157	4.90	0.20	155.0	31.6
8	87.1	1.045	6.07	0.46	192.0	31.6
					Mean	31.8
					Standard deviation	±0.8

[a] Subject to estimated error of ±10%.
[b] Read only to nearest half unit.

The relaxation time, T_1, was not determined for all samples. As shown in Table I, the values found for T_1 range from 1.1 to 3.7 seconds. Over this range the quantity $(D_{\max})_0/N_0$ appears to be independent of T_1 and demonstrates that the extrapolation procedure used to obtain $(D_{\max})_0$ is satisfactory and that the intrinsic line width of the materials investigated is indeed negligible compared with the artificial width as set by magnet inhomogeneities.

Figure 1 shows the values of $D_{\max}/N_0$ for $H_1 = 5.6 \times 10^{-4}$

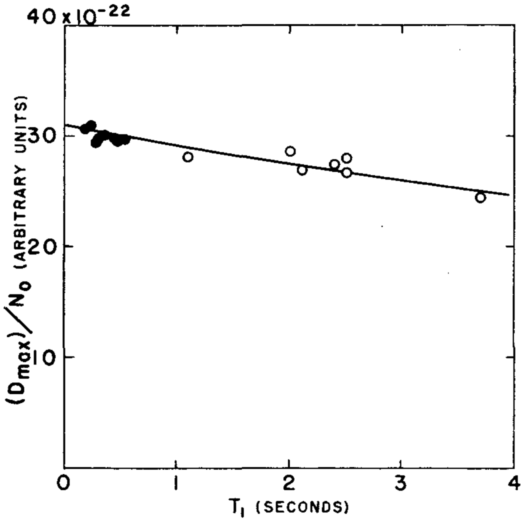

Figure 1. Relationship between experimentally observed values of $D_{\max}/N_0$ for $H_1 = 5.6 \times 10^{-4}$ gauss and T_1

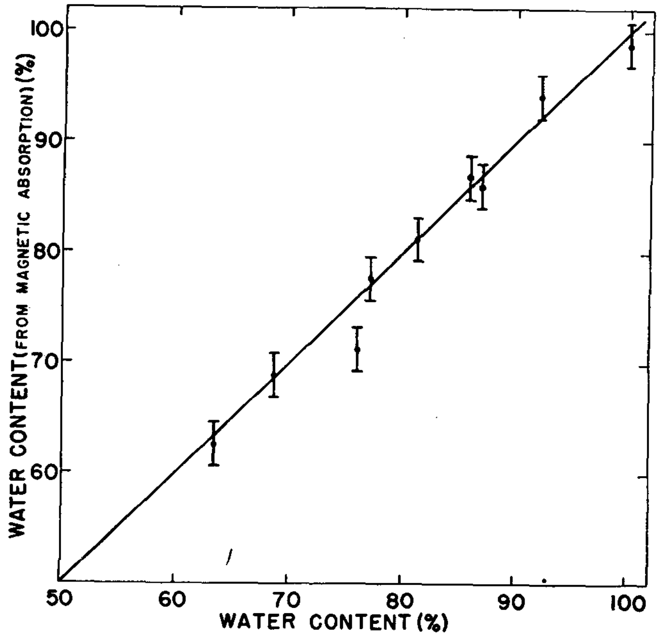

Figure 2. Comparison of water content calculated from magnetic absorption measurements with known water content of starch suspensions

gauss plotted as a function of T_1. The dependence on T_1 as calculated from Equations 2 and 4 is adjusted for best fit with the experimental points. The data are in general agreement with the approximate dependence on T_1 as expressed by Equation 4. The limiting value of D_{max}/N_0 for $T_1 = 0$ as obtained from Figure 1 is not significantly different from the mean value of $(D_{max})_0/N_0$ given in Table I.

Table II summarizes the data obtained for the aqueous starch suspensions. The water content of the suspensions ranged from 63 to 92%. As noted below, this range is of interest because it corresponds to the water content of some vegetable tissues and other biological materials. The table shows values of $(D_{max})_0$ determined for each suspension by the extrapolation procedure discussed above. The values of N_0 were calculated on the assumption that the hydrogen nuclei in the starch do not contribute significantly to the observed signal. This assumption is based on the fact, established earlier (*4*), that the magnetic resonance line for the hydrogen nuclei in starch is broad compared with the width of the line observed here for hydrogen nuclei in liquids. The correctness of this assumption is shown by the fact that the ratio $(D_{max})_0/N_0$ shows no systematic dependence on the moisture content. The average value of $(D_{max})_0/N_0$ of $31.8 \pm 0.8 \times 10^{-22}$ found for the starch suspensions agrees with the mean value $31.9 \pm 0.8 \times 10^{-22}$ found for the organic liquids.

The values of T_1 found for the starch suspensions are somewhat smaller than those for the organic liquids. As shown in Figure 1, the observed values of D_{max} for $H_1 = 5.6 \times 10^{-4}$ are consistent with the shorter T_1.

Aside from contributions from hydrogen nuclei in the starch, there are two other possible contributions to the magnitude of $(D_{max})_0$ that should be considered. The first factor, the hydrogen nuclei in any water-soluble portion of the starch, can be dismissed because of the small (<0.1%) amount of soluble material present. The second factor concerns the hydrogen nuclei of the water sorbed by the starch. In the earlier report (*4*) it was shown that the magnetic resonance line for the hydrogen nuclei of the water sorbed by starch is broad relative to that for the hydrogen nuclei of liquid water. It is clear that this broadening will result in a decrease in the contribution to $(D_{max})_0$ of the sorbed water relative to that of an equal quantity of liquid water. A calculation of the amount by which $(D_{max})_0$ is reduced is difficult because the amount of water which does not contribute cannot be precisely estimated.

To obtain an upper limit for the effects of sorption, however, it may be assumed that the contribution is zero for nuclei in water sorbed sufficiently strongly that the line width is greater than the line width (1.7 ± 0.1 kc.) observed here for hydrogen nuclei in liquids. For starch this water amounts to about 7% of the weight of the dry starch or about 0.03 gram of water per milliliter for the most concentrated suspension for which data are shown in Table II. Thus N_0 would be reduced from 4.90×10^{22} to about 4.7×10^{22} hydrogen nuclei per cc. and $(D_{max})_0/N_0$ would be increased from 31.6×10^{-22} as given in Table II to 33×10^{-22}. This increase is of the same order of magnitude as the standard deviation obtained for the values of $(D_{max})_0/N_0$ obtained for the organic liquids for which sorption effects are entirely absent. It seems reasonable to conclude that any systematic effects of sorption in the present experiments are masked by experimental errors.

Within the precision of the present experiments it appears that the starch makes no direct contribution and acts essentially as an inert (nonhydrogen-containing) diluent. Since the method responds essentially only to hydrogen nuclei in the aqueous phase, it may be used to determine the amount of water per unit volume in the suspension, and from a measurement of the density of the suspension one may also determine the per cent of water by weight.

This procedure has been applied to the data for starch. The average value of $(D_{max})_0/N_0$ as obtained for the organic liquids was assumed as a calibration factor. Then the per cent of water by weight was calculated for the starch suspensions from the experimental values of $(D_{max})_0$ and the density.

The results of the calculation are shown in Figure 2. The indicated error of ±2% in the water content is attributed to experimental errors in the magnetic absorption determinations. Although the precision of the measurements is only fair, it can be concluded that the calibration factor $[(D_{max})_0/N_0 = 31.9 \pm 0.8 \times 10^{-22}]$, which was established by measurements on organic liquids, yields accurate results when used to calculate the water content of starch suspensions from the experimentally observed magnetic absorption and density.

The results obtained for the starch suspensions suggest that a similar relation may exist between $(D_{max})_0$ and the water content of other hygroscopic materials such as vegetable tissues or other biological systems. Such a possibility is of interest because of its potential application for the determination of the moisture content of complex biological systems on an absolute basis. The principal factors which must be evaluated are: the contributions of water-soluble, hydrogen-containing constituents (sugars, etc.); and the contributions of fats, lipides, or other constituents which give rise to narrow proton resonances and which thus cannot be distinguished by the techniques used here from hydrogen nuclei in water. Procedures for obtaining satisfactory samples of tissue must also be developed. Work on these aspects of the problem has been undertaken and will be reported later.

ACKNOWLEDGMENT

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Determination of a Carbonyl Compound by Extraction of Its 2,4-Dinitrophenylhydrazone

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A spectrophotometric determination of butadiene-furfural condensation product, a carbonyl compound, can be made by reaction of the compound with 2,4-dinitrophenylhydrazine in a two-phase system composed of iso-octane and an alcohol-water-phosphoric acid mixture. The 2,4-dinitrophenylhydrazone is selectively extracted into the iso-octane phase and its concentration determined by absorption measurement at 340 mμ. The excess reagent remains in the aqueous phase and does not interfere.

THE use of 2,4-dinitrophenylhydrazine for the identification and determination of carbonyl compounds is well known. It was not until relatively recently, however, that this reagent was applied to the determination of parts per million quantities of carbonyl compounds. Two methods were published in 1951 based on the formation of 2,4-dinitrophenylhydrazones followed by the addition of alcoholic potassium hydroxide to produce a red color which could then be measured spectrophotometrically.

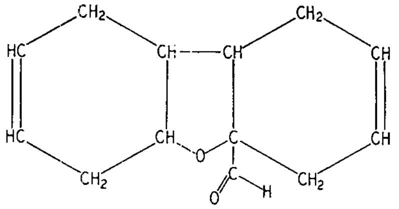

Figure 1. Butadiene-furfural condensation product (R-11)

In the method of Lappin and Clark (*2*) the 2,4-dinitrophenylhydrazone is formed by the direct reaction of the carbonyl compound with 2,4-dinitrophenylhydrazine in slightly acid methanol solution. The evaluation of this method by the authors of the work reported here indicated that the reaction conditions specified do not give reproducible results. After trying a number of modifications of the published procedure, including changes in reaction time, acid strength, reaction temperature, and solvent, they concluded that this method is not suitable for their purposes. In the procedure of Pool and Klose (*3*) the 2,4-dinitrophenylhydrazine reagent in benzene solution is adsorbed on an alumina column. The sample, also in benzene solution, is added to the column and the 2,4-dinitrophenylhydrazones formed are eluted by a further addition of benzene. Although it was found that the activity of the alumina column is critical, this procedure appeared to be reliable and could probably have been adapted to the use of the work reported. During the evaluation of these methods, however, another approach presented itself which was more readily applicable to the particular problem.

The red color formed by the addition of alkali to the 2,4-dinitrophenylhydrazone solutions in the methods described is not stable and fades appreciably within a few minutes after its formation. The formation of the red color is necessary in these methods, however, because the 2,4-dinitrophenylhydrazine reagent absorbs in the same region as the 2,4-dinitrophenylhydrazone product and, thus, interferes with the spectrophotometric measurement of 2,4-dinitrophenylhydrazone concentration. The addition of alkali shifts the absorbance of the 2,4-dinitrophenylhydrazone into the visible region and removes the spectral interference caused by the reagent. If a method for separating the product from the reagent could be found, the reagent would no longer interfere with the direct measurement of the 2,4-dinitrophenylhydrazone concentration, and the formation and measurement of the unstable red color would no longer be necessary.

During the evaluation of the published methods described, it was noticed that the 2,4-dinitrophenylhydrazone of the carbonyl compound being studied is much more soluble in iso-octane (2,2,4-trimethylpentane) than is the 2,4-dinitrophenylhydrazine reagent. This difference in solubility was utilized to develop an extraction procedure which permits the direct spectrophotometric measurement of 2,4-dinitrophenylhydrazone concentration without the addition of alkali.

This procedure was developed as a method for the determination of 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, a butadiene-furfural condensation product whose structure is shown in Figure 1 (*1*). This compound is an effective fly repellent known commercially as R-11. The procedure is discussed with reference to this particular compound; however, it is believed that the principle of the extraction procedure described is generally applicable to the determination of carbonyl compounds.

REAGENTS

Eastman 2,4-dinitrophenylhydrazine and Phillips Reference Fuel or Spectro Grade iso-octane are used without purification. Carbonyl free ethyl alcohol is prepared by refluxing 600 to 700 ml. of absolute alcohol for 2 hours with 5 grams of 2,4-dinitrophenylhydrazine and a few milliliters of concentrated hydro-

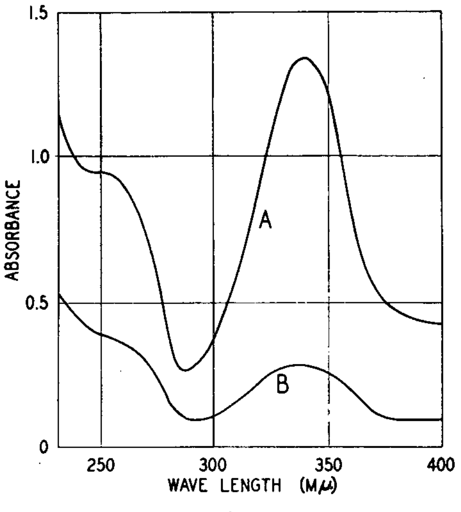

Figure 2. Spectra of typical R-11 solution and blank

A. Absorption spectrum of R-11–2,4-dinitrophenylhydrazone (approximately 6 × $10^{-5}M$)
B. Blank with same reagents

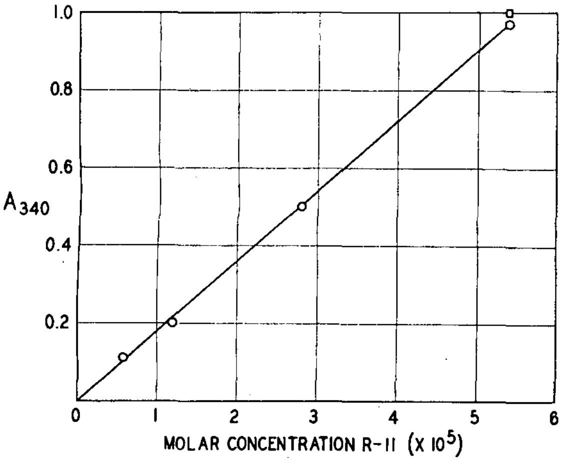

Figure 3. Relationship of absorbance (minus blank) of R-11-2,4-dinitrophenylhydrazone *vs.* concentration of R-11

○ Absorbance of blank = 0.27
□ Unpurified alcohol used in reagent, absorbance of blank = 1.03

chloric acid, and then distilling off the alcohol. The 2,4-dinitrophenylhydrazine reagent solution is prepared daily by mixing 1 volume of a fresh saturated solution of 2,4-dinitrophenylhydrazine in carbonyl-free alcohol with 3 volumes of 1 to 2 phosphoric acid (a solution of 1 volume 85% phosphoric acid in 2 volumes of water).

PROCEDURE

Add 10 ml. of iso-octane containing 1 to 20 p.p.m. of the carbonyl compound to a 40-ml. screw-cap vial containing 10 ml. of the 2,4-dinitrophenylhydrazine reagent solution. Run a duplicate procedure as a blank with 10 ml. of pure iso-octane in place of the sample. Allow the solutions to react for 30 minutes with continuous mixing. (In this laboratory a motor-driven rotator is used which turns the vials end over end at about 50 r.p.m.) Remove the reagent phase with a suction tube and wash the octane phase for 10 minutes with an equal volume of 1 to 2 phosphoric acid. Withdraw portions of the octane phases of the sample and the blank and measure the absorbance of the sample versus the blank at the wave length of maximum absorbance (340 mμ for R-11). Determine the carbonyl concentration from a calibration curve prepared from samples containing known amounts of the substance being determined.

RESULTS

Figure 2 shows the spectra of a typical R-11 solution and blank after treatment by the procedure described. The blank involved in this method is high, but it has been found to be constant in a series of determinations using the same reagent solutions. This is illustrated in Figure 3, which is a plot of the absorbance of the sample minus that of the blank versus carbonyl concentration. The linearity of the plot shows that the blank, although high, was constant over the entire series of measurements. The point on Figure 3 marked with a square was obtained from a measurement in which the reagent solution had been made up with unpurified alcohol. The blank had an absorbance (versus iso-octane) of more than 1.0 as compared with a normal blank of about 0.3, but the difference between the absorbances of sample and blank was such that the point fell on the same calibration line.

DISCUSSION

A number of solubility relationships are involved in this method, the first of which is that among the three solvents themselves. A qualitative phase diagram of the system iso-octane–ethyl alcohol–water was constructed (Figure 4) showing that in the presence of even a relatively small amount of water, the nonaqueous phase consists completely of iso-octane, all the alcohol being found in the aqueous phase. The diagram also shows that mixing an aqueous phase containing less than 50% alcohol with an iso-octane phase causes only a small decrease in the volume of the iso-octane.

The effect of the composition of the aqueous phase on the relative solubility of the 2,4-dinitrophenylhydrazone in the iso-octane was measured by determining the fraction of a known amount of 2,4-dinitrophenylhydrazone retained by the iso-octane in the presence of aqueous solutions containing different proportions of water and alcohol. The results of these measurements are shown in Figure 5. When the aqueous phase contains 70% alcohol, less than half of the 2,4-dinitrophenylhydrazone is retained by the iso-octane, with 50% alcohol the retention is about 80%, and when the alcohol content of the aqueous phase is 25% or less, essentially all of the 2,4-dinitrophenylhydrazone is retained in the iso-octane phase. Consequently, the aqueous phase used in this determination contains 25% ethyl alcohol.

With the Cary recording spectrophotometer used to obtain the

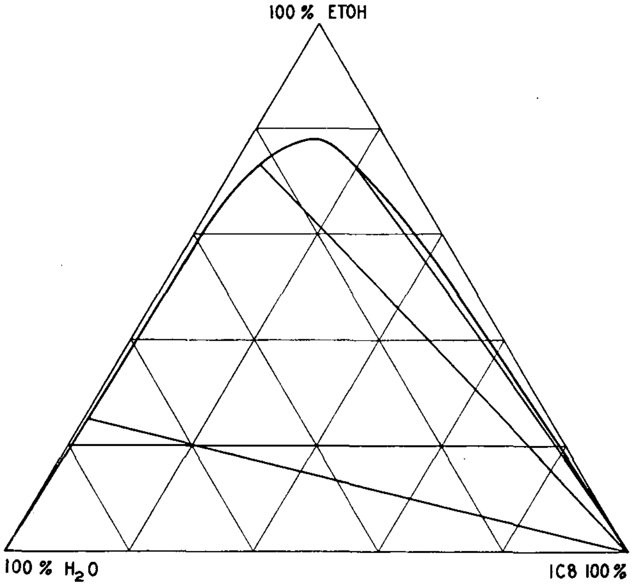

Figure 4. Phase diagram

Ethyl alcohol–iso-octane–water, temperature 30° C.

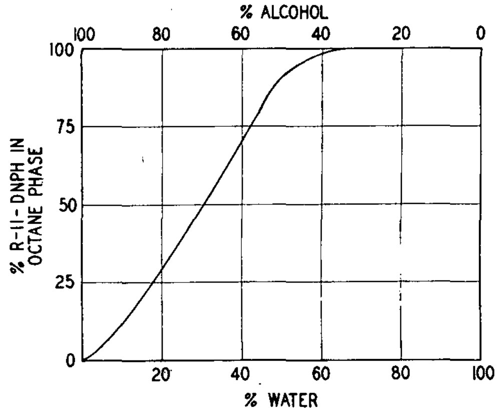

Figure 5. Effect of composition of aqueous phase on extraction of R-11-2,4-dinitrophenylhydrazone by iso-octane

results above, duplicate blanks from a given reagent solution are reproducible to about 0.02 absorbance units. For this reason, the maximum sensitivity claimed for this determination is about $5 \times 10^{-6}M$ R-11, which gives an absorbance of about 0.1. The sensitivity of the method can be increased by concentrating the carbonyl compound in the iso-octane solvent before performing the analysis. This procedure is being used to determine concentrations of $1 \times 10^{-6}M$ R-11 in milk by extracting 4 volumes of milk with one of iso-octane.

The data plotted in Figure 3 show that the calibration line for R-11 is independent of the reagent solution as long as the simultaneous blank determination is also made. Consequently, a single calibration is all that is required for the analysis of any number of samples over any period of time.

The work described is concerned with only one compound, and the general applicability of this procedure to all carbonyl compounds has not been experimentally verified. However, a number of semiquantitative experiments with other aldehydes and ketones indicate that extraction by iso-octane under the conditions of this method is probably a general property of 2,4-dinitrophenylhydrazones. In adapting this procedure to the determination of a different carbonyl compound, a new calibration is necessary, because the absorption maxima and molar absorptivities of the 2,4-dinitrophenylhydrazones vary with the structure of the carbonyl compound (*4*). The rate of reaction may also vary and the reaction time should be determined when calibrating for a different carbonyl compound.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Phillips Petroleum Co. for making possible the publication of this paper.

NOTE:

Since the presentation of this paper, it has been found that in some instances—for example, the determination of traces of acetone in hydrocarbons—it is possible to use 1 to 2 phosphoric acid alone in the aqueous phase, thus eliminating the need for repurified alcohol. The rate of the reaction may be decreased somewhat by this change, so the mixing time should be checked to be certain that the reaction is complete in the allotted time.

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Sensitivity of Bromine-Bromide Potentiometric End Point

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The sensitivity of the bromine-bromide potentiometric end point has been investigated with respect to the factors contributing to the reagent blank in a coulometric titration. The size of the blank often decreased with an increase in the surface area of the indicator electrode, an increase in the rate of stirring the solution, and a decrease in the current passed by the potentiometer, thereby indicating that polarization was taking place. The blank also depended upon other experimental factors such as the concentrations of reagents, the electrode material, and the electrode pretreatment. The size of the potentiometric blank could be diminished by proper choice of the experimental conditions so that it closely approximated the corresponding amperometric blank.

ALTHOUGH both potentiometric (*4*) and amperometric (*9*) methods have been employed in coulometric titrations, the former have the inherent advantage of being more readily adaptable to successive determinations of a number of substances in a mixture by observing in a single titration separate end points for each. On the other hand, potentiometry is usually limited to systems containing a reversible half-cell reaction whereas amperometry is not. The relative sensitivities for the two methods have not been compared using a reversible half-cell reaction, although it is interesting to note that one group of investigators switched from classical potentiometry (*4*), to a polarized potentiometric system when higher sensitivity was required (*3, 11, 12*).

It has long been known that potentials for solutions more dilute than about $10^{-7}M$ frequently do not correspond to those calculated with the aid of the Nernst equation (*5*). In fact, the observed potential has usually been found to be independent of the concentration of the substance in question. Though factors such as losses by adsorption onto walls of the container, hydrolysis, and colloid formation may contribute to the end result, control of the electrode potential may often pass over to competing reactions because polarization occurs during the measurement. The potential of a system, although presumably measured with no current flowing, is probably rarely ever measured under ideal conditions, because of the limited sensitivity of the galvanometer and the finite number of turns on the slide-wire used for balancing the bridge. In instruments of the vacuum-tube voltmeter type, a small grid current must be passed to obtain a signal.

The purpose of the present study, therefore, was to examine the factors that might influence the sensitivity of a classical potentiometric end point—i.e., the magnitude of the blank—on the assumption that such a potentiometric measurement would involve the passage of a small current. Then, under the same conditions as for the potentiometry, the amperometric sensitivity was determined for the same electrode, and the results were compared.

EXPERIMENTAL DETAILS

Reagents and Solutions. Stock solutions of $1M$ sulfuric acid were prepared by dilution of the 96% reagent. A stock solution of $2M$ sodium bromide was made by dissolving the weighed amount of sodium bromide in 1 liter of distilled water. These stock solutions were used in preparing the $0.1M$ sulfuric acid and $0.2M$ sodium bromide ordinarily used as the electrolyte.

A standard $0.05143M$ solution of trivalent arsenic was prepared by dissolving 1.2718 grams of arsenic trioxide in $1M$ sodium hydroxide, neutralizing with $1N$ sulfuric acid, and diluting to 250 ml.

Apparatus. A cell of the type reported by Myers and Swift

(*9*) was employed with the following changes: A 150-ml. beaker was substituted for the weighing bottle; an external cathode, in contact with 0.1*M* sulfuric acid, was used in the generating circuit; and an external saturated calomel electrode was used as the anode in the indicating circuit. Both the external generator cathode and the indicator anode were connected to the cell by means of potassium chloride–agar salt bridges after preliminary runs had shown no measurable effect in substituting them for sulfate bridges.

Platinum indicator electrodes of 11.54, 1.31, 0.201, and 0.0152 sq. cm. area (geometric) were employed at different times, but most of the work was done with the 0.201-sq. cm. wire. Electrodes of gold, graphite, tungsten, silver, palladium, tantalum, and platinized platinum of about the same size as the 0.201-sq. cm. platinum wire were also used.

Amperometric end points were detected by means of a Fisher Elecdropode; potentiometric end points, by means of a Rubicon potentiometer (Catalog No. 8702) having a 1.5-volt scale which could be read to the nearest 0.5 mv., a Leeds and Northrup pH meter (Model 7664), and a Beckman Model G pH meter. The sensitivity of the galvanometer on the Rubicon potentiometer was about 5 × 10^{-8} ampere per mm.; currents passed by the pH meters, according to each manufacturer, were 2 × 10^{-12} ampere for the Leeds and Northrup, and 1 × 10^{-12} ampere for the Beckman. Ordinarily, the Leeds and Northrup instrument was operated as a continuous indicator, whereas the others were actually in the circuit only for the few seconds required to make the measurement.

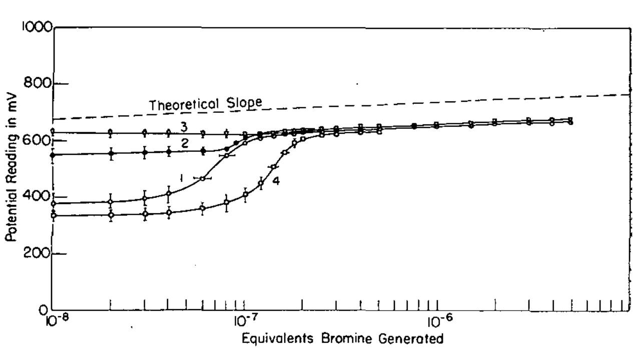

Figure 1. Effect of pretreatment on reproducibilities and shapes of curves

Obtained using Leeds and Northrup pH meter and a 0.201 sq. cm. platinum indicator electrode. Generation current, 100 μa. to 10^{-6} equivalent and 1.00 ma. beyond
Indicator electrode
1. Precathodized 5 minutes in 0.1*M* sulfuric acid
2. Pretreated with bromine solution from a prior blank run
3. Preanodized 5 minutes in 0.1*M* sulfuric acid
4. Precathodized 5 minutes in 0.1*M* sulfuric acid, and sulfuric acid–bromide solution deaerated 30 minutes with purified nitrogen before initial generation of bromine

Current for generating the bromine was fed from an electronic supply (*10*) through a parallel circuit so that currents from 1 μa. to 150 ma. could be selected. The current was determined by measuring the potential drop across a Leeds and Northrup decade box connected in series with the electrolytic cell.

Procedure. Ordinarily the generator anode and the indicator cathode were pretreated simultaneously by a 5-minute electrolysis in 0.1*M* sulfuric acid using a current of 3 ma. The electrodes were then washed by filling the cell with distilled water and stirring the solution vigorously for 45 seconds. Three successive washings were performed in this way. This washing procedure was always used regardless of the pretreatment employed. Additional washings had been found to produce little effect and were therefore abandoned early in the study.

A solution on which a blank was to be determined was prepared by adding an amount of distilled water to the empty cell so that addition of appropriate volumes of the stock solutions of sulfuric acid and sodium bromide brought the total volume to 100 ml. In each case a pipet was used to ensure reasonable reproducibility.

Bromine was usually generated at currents from 100 μa. to 2 ma., which, from polarographic studies, were known to be in the range where one could expect 100% current efficiency for the electrode in question (about 2-sq. cm. area). After a predetermined number of seconds, generation was stopped for 30 seconds and a measurement of potential or current then made. Ordinarily the solution was stirred vigorously during both the generation and the measurement.

Each curve was obtained at least three times in order to ensure the reliability of the result. In no case was a definite trend noted within a series though it was often observed that the first determination on a particular day did not agree with the others. Behavior of an electrode was checked each day by making one or more runs under known conditions before investigating new variables.

RESULTS

Pretreatment of Electrode. During the early stages of the study it was frequently observed that the curves could not be reproduced, especially in day-to-day comparisons. This led to an examination of the effect of various pretreatment procedures on the reproducibilities and shapes of the curves.

As shown in Figure 1, preanodized indicator electrodes, and those washed with concentrated nitric acid, produced flat lines until about 10^{-7} equivalent of bromine had been generated, following which the slope changed to a value corresponding to that for the bromine-bromide couple (0.03 volt per tenfold change in concentration of bromine). In several instances there was a slight decrease in potential between the initial flat portion of the curve and the beginning of the bromine-bromide slope. On the other hand, when the indicator electrode had been precathodized an S-shaped curve was obtained which, however, attained the bromine-bromide slope at the same point as the preanodized electrode. Deaeration of the bromide-sulfuric acid mixture prior to the generation of bromine produced an initial potential which was less noble than otherwise. In addition, a somewhat large number of equivalents of bromine had to be generated before attaining the bromine-bromide slope. Longer periods of precathodizing produced the same two effects as deaeration though the increase in the blank was very slight and may not have been significant.

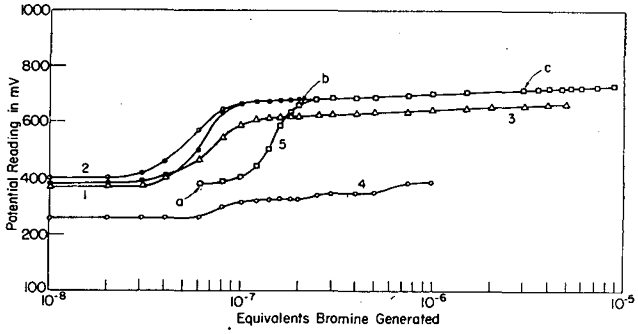

Figure 2. Effect of type of potentiometer and electrode area on potentiometric curves

Generation current, 100 μa. up to 10^{-6} equivalent and 1.00 ma. beyond
1–4. Leeds and Northrup instrument and platinum electrodes with geometric areas of 11.5, 1.31, 0.201, and 0.0152 sq. cm., respectively. Curve 1 also represents data obtained using Beckman instrument with the 11.5-sq. cm. electrode
5. Rubicon potentiometer and 11.5 sq. cm. electrode. *a*, *b*, and *c* are concentrations where large platinum foil, small platinum foil, and platinum wire, respectively, first gave "true" readings

When pretreatment consisted of the generation of 10^{-6} equivalent of bromine, followed by three regular washings with distilled water, an S-shaped curve was also obtained. However the initial potential was approximately halfway between those for the preanodized and the precathodized indicator electrodes.

Area of Electrode. As shown by the representative curves in Figure 2, the curves obtained with electrodes of different areas were found to depend upon the instrument employed for the measurement. For that reason, the results obtained with each instrument are discussed separately.

In using the Beckman Model G, the two large foils and the large wire electrode were found to require the same number of equivalents of bromine to reach the point where the bromine-bromide slope began. However, the smaller the electrode, the greater the drift in the readings at lower concentrations of bromine. Thus the larger foil was steady at the initial point, before any bromine had been generated. However, measurements with the small foil drifted slowly toward larger potentials until about 4 × 10^{-8} equivalent of bromine had been generated; those with the wire electrode, until 1.4 × 10^{-7} equivalent had been generated. The smaller wire (button) electrode produced readings which not only drifted but also moved erratically upward and downward in going from one concentration to the next; measurements did not stabilize until 5 × 10^{-6} equivalent of bromine had been generated.

In contrast to the drift usually obtained with the Beckman instrument, the Leeds and Northrup always produced comparatively steady readings. Figure 2 shows that the two-foil electrodes gave curves which were essentially identical and which agreed with those obtained with the Beckman instrument. On the other hand, the wire electrode produced a curve which started out the same as those for the two foils, but, although it reached the bromine-bromide slope at the same point as did the foils, its potentials beyond that point were about 0.06 volt lower than those for the two foils. This resulted in an apparent shift of the inflection point toward higher concentrations of bromine. The button electrode was distinctly different from the other three. It began about 150 mv. lower than the rest, showed a very slight increase (approximately 0.04 volt) in potential in the region where the other electrodes showed a large change, and at still higher concentrations of bromine showed an irregular trend toward higher potentials. It is obvious from this last example that the reliability of a potentiometric measurement should not be based upon its stability.

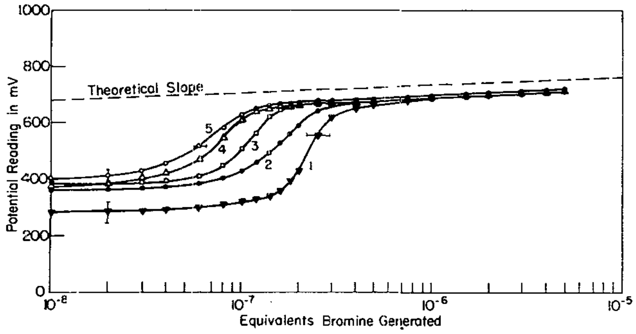

Figure 3. Effect of rate of stirring on potentiometric curve

Obtained using Beckman Model G pH meter, 0.201-sq. cm. platinum electrode, and generation current of 100 μa.

1. Solution stirred 1 minute after generation had been stopped; then stirring stopped for 1 minute before measuring potential

Stirring rates of

2. 150 r.p.m.
3. 300 r.p.m.
4. 450 r.p.m.
5. 600 and 900 r.p.m.

The Rubicon potentiometer appeared to be "dead" when attempts were made to measure solutions containing little or no bromine. This behavior was similar to that encountered when a high resistance was being measured, though, in this case, the resistance of the circuit was known to be less than 1000 ohms. As bromine was generated, the dead region gradually diminished until it finally disappeared. Hence, the decision was made to define arbitrarily a "true" reading as one in which a 1-mv. change in either direction from the null point on the scale of the potentiometer produced a 1-mm. deflection on the galvanometer. If more than a 1-mv. change was required to produce such a deflection, a potential was defined as "indeterminate."

The amounts of bromine which had to be generated in 100 ml. of solution in order to obtain the first true readings were 6 × 10^{-8}, 2 × 10^{-7}, 3 × 10^{-6}, and 2 × 10^{-5} equivalent for the large and small foils, the wire, and the button, respectively. At all lower concentrations for the respective electrodes the readings were indeterminate. Once a particular electrode had begun to produce true readings, its values fell on a curve shared by the larger electrodes. The curves for the large foil did not agree with the corresponding curves for the Beckman or Leeds and Northrup instruments either in the inflection point or in the point at which the bromine-bromide slope was first attained.

Rate of Stirring. Solutions were usually stirred with a glass paddle at 600 r.p.m. This represented the maximum rate at which one could stir without introducing large numbers of bubbles into the solution. It was recognized that stirring the solution might not lead to results as reproducible as those one might have obtained had the electrode itself been rotated. However, stirring was felt to be the better choice because measurements were to be made with large foils as well as with wires.

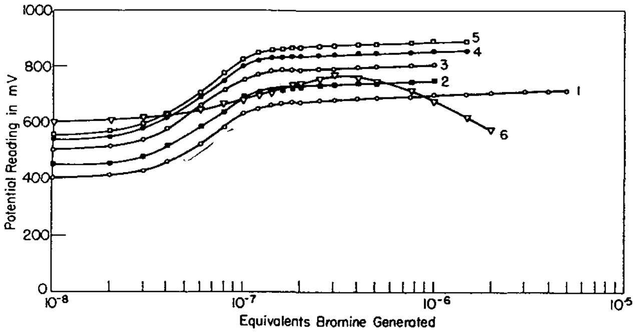

Figure 4. Effect of initial concentration of bromide on potentiometric curve

Obtained using Beckman Model G pH meter, 0.201-sq. cm. platinum electrode, and generation current of 100 μa. Electrolyte contained 0.1*M* sulfuric acid in addition to sodium bromide. Curves 1–6 represent tenfold decreases in bromide concentration from 0.2*M* to 0.000002*M*

Stirring rates of 600 and of 900 r.p.m. gave essentially identical results, despite the large number of air bubbles stirred into the solution at 900 r.p.m. However, as shown in Figure 3, rates of 450, 300, and 150 r.p.m. required progressively larger amounts of bromine to reach the bromine-bromide potential. The extreme is shown by curve 1, for which the solution was stirred 1 minute after generation of bromine had stopped, and was then allowed to stand another minute before the potential was read. Under such conditions the initial potential for the titration curve was always significantly lower, and the concentration of bromine required to reach the bromine-bromide slope was much higher. Hence, the rate of stirring had a marked effect on the apparent size of the blank.

Composition of Electrolyte. One of the main reasons for studying the effect of the composition of the electrolyte was to determine whether or not the presence of small amounts of impurities in the reagents was contributing to the blank. A study was first made using different concentrations of bromide in tenfold dilutions from 0.2*M* to 2 × 10^{-6}*M*. Figure 4 shows that between 0.2*M* and 2 × 10^{-4}*M* the predicted change of about 0.06 volt for each tenfold dilution was obtained in the region where the curves followed the theoretical bromine-bromide slope. However, the $2 \times 10^{-5}M$ solution produced only half the predicted change, while the $2 \times 10^{-6}M$ gave very irreproducible results from run to run. In the last case bromine may not have been generated at 100% current efficiency, but this factor was completely overshadowed by the effect of concentration polarization.

When the Leeds and Northrup instrument was operated continuously in the normal fashion, results similar to those reported above for the Beckman instrument were obtained, except that erratic curves were produced in $2 \times 10^{-5}M$ bromide rather than in $2 \times 10^{-6}M$. On the other hand, when the leads to the indicator electrodes were disconnected, except for the few seconds required to make a measurement, the polarization phenomena were decreased so that the curves were in essential agreement with those obtained using the Beckman instrument.

The blank was essentially constant regardless of the concentration of bromide. As a result, it was concluded that the impurity in the bromide reagent made an insignificant contribution to the blank.

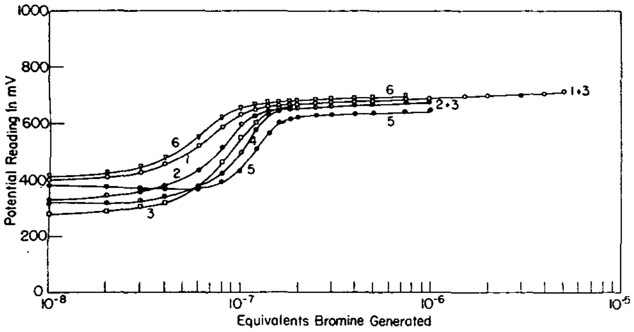

Figure 5. Effect of type of supporting acid and of concentration of sulfuric acid on potentiometric curve

Obtained using Beckman Model G pH meter, 0.201-sq. cm. platinum electrode, and generation current of 100 μa. Electrolyte contained $0.2M$ sodium bromide in addition to acid

1. $0.1M$ sulfuric acid
2. $0.1M$ hydrochloric acid
3. $0.1M$ perchloric acid
4. $0.1M$ phosphoric acid
5. $1.0M$ sulfuric acid
6. $0.01M$ sulfuric acid

In Figure 5 are shown the curves obtained with different acids and with different concentrations of sulfuric acid. Perchloric, hydrochloric, phosphoric, and sulfuric acids had essentially the same blank when present in comparable molar concentrations. More important, a tenfold increase in the concentration of sulfuric acid from 0.1 to $1.0M$ resulted in less than a twofold increase in the blank (and a decrease in the bromine-bromide potential of about 0.03 volt), whereas a tenfold decrease in the concentration produced no significant change in the blank. As a result, it can safely be concluded that the $0.1M$ concentration of sulfuric acid usually employed did not make a major contribution to the overall blank.

Electrode Material. Electrodes having about 0.2-sq. cm. geometric area were fashioned from platinized platinum (no lead in the platinizing solution), palladium, tantalum, tungsten, silver, gold, and carbon (pencil lead). The gold electrode produced a very interesting curve which had the same shape and the same size blank as that of the platinum, but had potentials 150 mv. more noble throughout the titration. This shift is in the direction expected if the gold were acting somewhat as an indicator electrode for its own ions in a solution of bromide. The silver electrode, which also acted as an indicator for its own ions in a bromide medium, produced a stable potential unaffected by the presence of bromine. The value of the potential (−0.125 volt) was very close to the formal value predicted for silver in $0.2M$ bromide solutions. The tantalum electrode also gave a constant potential at about 75 mv., but its value could not be interpreted in terms of known half-cell reactions. The tungsten electrode was apparently undergoing attack, because a measurable potential using the Leeds and Northrup instrument could not be obtained until a concentration of 6×10^{-7} equivalent of bromine had been generated. At higher concentrations of bromine the potential gradually increased, reaching 160 mv. after 9×10^{-6} equivalent had been generated.

A study was made with the platinized-platinum electrode, because it was felt that an increase in the microscopic or true area of the electrode might decrease the polarization effects. (The platinizing solution contained only chloroplatinate—i.e., ions such as lead, which are frequently added to improve the deposit of platinum black, were absent.) It was interesting to find, therefore, that such an electrode produced stable reading before bromine had been generated in the solution even when the Rubicon potentiometer was employed. However, its potential was comparatively insensitive to bromine, maintaining a relatively stable potential at about 400 mv., and showing only a small S-shaped increase in potential after 5×10^{-6} equivalent of bromine had been generated. If, instead of the usual precathodization, the platinized electrode had been anodized for 15 minutes, a flat line was obtained at 740 mv. over the entire range of bromine concentration that was usually examined.

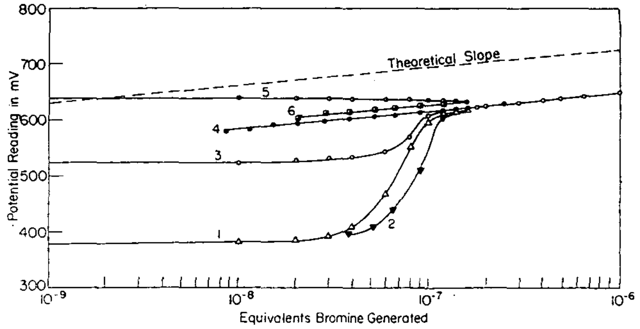

Figure 6. Effect of pretreatment upon potentiometric curves

Obtained during generation of bromine and upon subsequent curves obtained by dilution of those solutions, using 0.201-sq. cm. platinum indicator electrode and generation current of 100 μa.

1. Generation cycle with usual precathodizing procedure
2. Dilution cycle for curve 1 using $0.1M$ sulfuric acid, $0.1M$ sodium bromide, or a mixture of $0.1M$ sulfuric acid and $0.1M$ sodium bromide as diluent
3. Generation cycle using an electrode that had been "prebrominated"
4. Dilution cycle for Curve 3 using sulfuric acid–sodium bromide mixture

5 and 6. Generation and dilution (with sulfuric acid–sodium bromide mixture) cycles, respectively, using an indicator electrode that had been preanodized

1–4. Obtained with Leeds and Northrup instrument

5 and 6. Obtained with Beckman

Interesting results were also obtained with the palladium electrode. It was observed that hydrogen was not evolved during precathodization, even though oxygen was being evolved at the anode. When such an electrode was used to measure the potential of the bromine, a stable potential at −0.225 volt was obtained throughout the usual range of bromine concentrations. However, after prolonged anodizing, the electrode started out at about 200 mv. and maintained essentially a constant potential until the point where the gold and platinum electrodes had reached the bromine-bromide slope at which point it proceeded to increase slowly. Though it is obvious from these results that the palladium electrode cannot be used for measuring bromine, its avidity for hydrogen and the resulting stable potential lead to the belief that it could be used as an arbitrary, but very useful indicator electrode for acid-base titrations.

The carbon electrode gave a curve very similar to that for platinized platinum, except that its initial potential was about 0.05 volt higher, and the point at which the curve reached a slope approximating that of the bromine-bromide half-cell was closer to that obtained with smooth platinum, being about 3×10^{-7} equivalent. In this case, the larger blank may have been due to reaction of bromine with the electrode, particularly with any organic binders that might have been present.

Position of Generating Cathode. The question of the effect of the position of the generating cathode was investigated briefly, because it was felt that the hydrogen generated in the solution might be a factor contributing to the potential on the indicator electrode. Though curves for internal and external generator cathodes were essentially the same, the decision was made to use an external cathode to avoid the possibility of introducing variations as a result of the presence of hydrogen in the solution.

DISCUSSION

From polarographic studies, it is known that the presence of films on electrode surfaces requires the passage of a measurable number of microcoulombs in order to remove them, and thence to obtain a more nearly true value for the polarographic process alone (*6*, *8*). In addition, Wagner (*13*) has suggested that the current required to charge a double layer on the surface of a 1-sq. cm. electrode would also be appreciable when generating and measuring 10^{-7} equivalent or less. From those considerations it can be predicted that coulometric generations and detection of trace amounts of bromine would be more accurate using smaller areas for the generation and the indicator electrodes, because of the smaller amounts of current required to condition the electrode surfaces. Another reason for using small indicator electrodes in amperometry was encountered by Lothe (*7*) in preliminary studies on the bromine-bromide system using the exact setup of Myers and Swift (*9*) He observed that at generation rates of the order of 10 μa., the slope of the current versus the microequivalents of excess bromine was much smaller than when currents of 100 to 1000 μa. were employed. Furthermore, after generation had been stopped, a noticeable decrease in current occurred which could, however, be eliminated entirely by removing the externally imposed potential from the large amperometric indicator electrode. Apparently, appreciable consumption of the reagent by the indicator electrode (area of about 20 sq. cm.) was taking place. Therefore, the use of a smaller indicator electrode should, in principle, be desirable as a means of decreasing the blank for both potentiometric and amperometric end points. However, the present study showed that with present equipment, polarization effects became noticeable when small electrodes were employed in potentiometric measurements whereas amperometric currents obtained with the button electrode were too small to be measured accurately. The fact that the Rubicon, which draws a larger current than the other two instruments, was found to have larger blanks; that the blank decreased with an increase in electrode size in measurements made with the Leeds and Northrup instrument; that the results with the wire and with the button obtained with the Leeds and Northrup were in the direction of consumption of bromine; that stirring was effective in reducing the magnitude of the blank; and that the operation of the Leeds and Northrup as a continuous reading instrument, as opposed to an interrupted measurement, produced larger blanks—all point to the existence of polarization.

There is no question that polarization alone cannot account for the entire blank. It is likely that oxidizable impurities were present in the reagents. However, the experiments involving different concentrations of bromide and of sulfuric acid indicated that these sources together probably did not account for a major portion of the blank. Nevertheless, in an effort to obtain more information concerning this point, experiments were run on a solution in which 1.6×10^{-7} equivalent of bromine had been generated, following which 25 cc. of the solution had been removed by pipet, and 25 cc. of another solution substituted for it. As shown in Figure 6 repetitive dilutions, using an indicator electrode which had been preanodized, resulted in a continuation of the bromine-bromide slope to smaller concentrations than had been found to obey the Nernst relationship when bromine was being generated. The fact that the dilution was carried out with 25 cc. portions of a stock mixture of bromide in sulfuric acid indicated that the impurities in these two substances were not of major proportions. However, it is interesting to note that after an electrode had been precathodized or had simply been used to generate bromine in another aliquot of the stock solution of bromide-acid, the dilution curve tended to retrace the S-shaped generation curve. Dilution curves for the precathodized electrodes may indicate the presence of small amounts of hydrogen which diffused out of the platinum to establish a mixed potential, whereas those for the preanodized surfaces may indicate the presence of a film either of oxygen gas or a sorbed layer (*2*) with sufficient capacity to counteract the impurity added by the dilution.

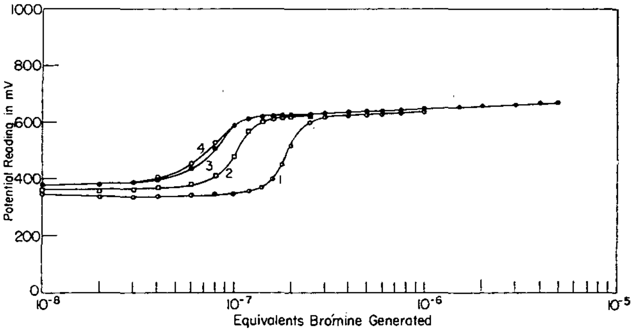

Figure 7. Effect of waiting different lengths of time before measuring potential of 0.201-sq. cm. platinum indicator electrode with Leeds and Northrup pH meter

Generation current was 100 μa.
1–4. 0, 15, 30, and 60 seconds of waiting, with generation current off, before measuring potential

From these dilution studies it would appear, by inference, that the chief source of impurities might be the distilled water. However, brief studies with water from different sources, including doubly-distilled water and distilled water stored for various times, did not result in significant differences which would justify additional experiments along this line.

Another factor considered as a possible contributor to the blank was that of incomplete stripping of bromine from the generator anode. As shown in Figure 7, the magnitude of the blank was indeed a function of the length of time that was waited before measuring the potential. However, a delay longer than 30 seconds before measuring the potential did not appear to produce a significantly smaller blank.

The question of whether or not the formation of a film and/or the charging of a double layer can account for the blank has already been mentioned briefly. One of the most important arguments, which would appear to indicate that the formation of such a film is not a major contributor to the blank in the present study, is the fact that in measurements with the Beckman instrument, where polarization effects were minimized, blanks for the wire and for the two foils were essentially identical. It must be concluded, therefore, that if film formation is indeed necessary, the amount of current required was too small to be detected by the technique employed in the present study.

A moderate amount of information has been accumulated which indicates that the initial portions of the titration curves, prior to the points beyond which the bromine-bromide slope is obeyed, are mixed potentials. Evidence for this is shown in the differences between the precathodized and the preanodized indicator electrodes. With each washing of a precathodized electrode, the potential progressively rose, whereas successive washings with a preanodized electrode gave progressively lower potentials; furthermore, the longer the precathodization, the lower the initial potential. Also, deaeration of the solution prior to generation of bromine resulted in lower potentials for precathodized electrodes. Other evidence may be found in the curves for electrode materials other than smooth platinum. The displacement of the curve of the gold electrode below that of smooth platinum is perhaps the most convincing, although the behavior of the other electrodes is also in accord with this conclusion. The fact that platinized platinum required a larger blank may have been because of the presence of more hydrogen on the surface, but it was more probably the result of the fact that the exchange current for the hydrogen reaction was higher on such a surface with the result that higher concentrations of bromine were required in order to make themselves felt by "taking over" control of the potential of the electrode. In general, slower reactions having smaller exchange currents would require higher concentrations of the species before they could control the potential of the electrode, or could minimize polarization effects to the same extent as a faster reaction (*1*).

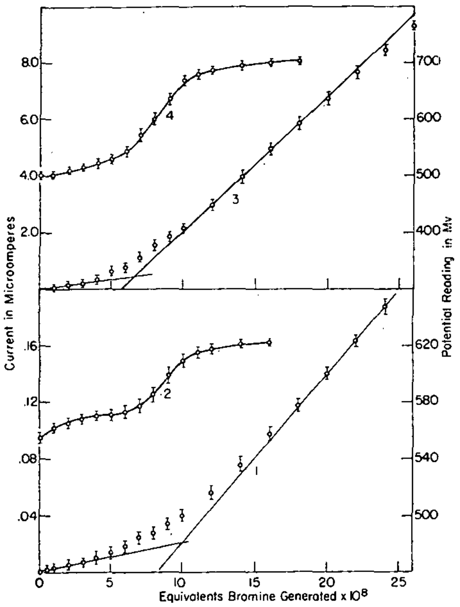

Figure 8. Variation of blanks with size of electrode using potentiometric and amperometric measurements

Potentiometric curves obtained using Leeds and Northrup pH meter. Generating current was 100 μa.

1 and 2. Amperometric and potentiometric curves for 0.201 sq. cm. wire

3 and 4. Amperometric and potentiometric curves for 11.54-sq. cm. platinum foil

In view of the fact that the literature often implies that the amperometric end point is more sensitive than the potentiometric, it appeared desirable to examine this conclusion using a single electrode under conditions that were maintained rigidly the same for both sets of measurements. As shown in Figure 8, this was carried out with two different sizes of electrodes, the potentiometry being carried out as described heretofore and the amperometry by imposition of a potential of +0.41 volt on the indicator electrode by means of a Fisher Elecdropode. The ordinates for the two sets of amperometric titrations were selected in such a way as to normalize them approximately in terms of the geometric areas of the electrodes.

The conclusions from these comparisons are most interesting. With both electrodes, amperometric indication of "excess bromine," if considered in terms of the first deviation from a linear extrapolation from the first two or three points of the amperometric curve, nearly coincides with the beginning of the rapid rise of the S-shaped portion of the potentiometric titration curve. For both electrodes, however, the first deviation on the amperometric curve appears to precede slightly the beginning of the rapid rise of the values on the potentiometric curve. The difference seems to be greater for the larger electrode, but it cannot really be said from the limited data on hand whether or not this difference is significant. Secondly, although the inflection point of the potentiometric curve (on a linear time-scale or on a logarithmic time-scale) cannot be determined with high precision, it appears to fall at the same concentration as the amperometric end point. Thirdly, the concentration at which the current first appears to fall on a straight line beyond the amperometric end point is the concentration at which the potentiometric curve first begins to follow the bromine-bromide slope. The close correspondence of the amperometric and the potentiometric curves would appear to leave little to choose between them. However, it is likely that the first indication of excess bromine might be detected less ambiguously by the amperometric method. Furthermore, excess reagent might be detected sooner under conditions where an appreciable portion of the blank was not contributed by the surface. The presence of an imposed potential should precondition the electrode surface, thereby decreasing the magnitude of the blank.

Figure 8 also shows the decrease in slope beyond the end point that was obtained when larger electrodes are employed. This decrease was attributed to the consumption of bromine by the amperometric indicator electrode. If still larger electrodes were used, the bromine was consumed faster (*7*) and produced noticeable curvature even though the potential was imposed on the indicator electrode only for the few seconds required to obtain a measurement of the current.

In view of the conclusions reached above, that the relative sensitivities of the amperometric and potentiometric end points were essentially the same for the electrodes used in the present study, it was of interest to compare the results of amperometric and potentiometric determinations of arsenic when they, too, were obtained under comparable conditions. As shown in Table I, the precision and accuracy of both potentiometric and amperometric determinations of 77.0 γ of arsenic(III) were in essential agreement. Trial calculations showed that it did not appear to matter which of the three corresponding pairs of points was selected as the end point, providing the comparable position on the curve for the other method was selected in accordance with the discussion given above. However, when the amount of arsenic was reduced tenfold, the results were high, by both methods although the amperometric method appeared to be somewhat more accurate and more precise than the potentiometric. The advantage was slight, but unambiguous at this level of concentration It seems likely that the advantage might have been increased at still lower concentrations of arsenic in accordance with the reasoning outlined above. However, the present study was not extended to smaller amounts of arsenic, because the precision even for the amperometric technique was rather poor even at a level of 7 γ of arsenic. It appears that such a study might best be carried out using a very small electrode for the reasons mentioned earlier. A more sensitive galvanometer would then be required for amperometric measurements and for detecting the null point in a potentiometric measurement. Alternatively, a vacuum tube voltmeter requiring the passage of a much smaller current might be useful in potentiometry. However, such measures will not decrease the apparent potentiometric blank if the relative magnitudes of the exchange currents for the desired reaction and for a competing one are such that the latter is already controlling the potential of the electrode.

Table I. Comparison of Potentiometric and Amperometric End Points for Coulometric Determination of Arsenic

(Using 0.201 sq. cm. platinum electrode in vigorously stirred solutions. After each period of bromine generation, intervals of 30 seconds for 77.0 γ and 45 seconds for 7.70 γ of arsenic were interposed before connecting the indicator leads for the few seconds required to obtain a reading)

Determination	Arsenic Taken, γ	Arsenic Found, γ	
		Amperometric	Potentiometric[a]
Single[b]	77.0	77.8 ± 0.6[c]	76.7 ± 0.9[c]
Successive[d]	77.0	77.3 ± 0.3	77.3 ± 0.5
Single[b]	7.70	8.51 ± 0.28	9.30 ± 0.38
Successive[d]	7.70	8.28 ± 0.18	8.92 ± 0.35
Single[e]	Blank	$9.8 \pm 0.2 \times 10^{-8}$ equiv.	$8.9 \pm 0.2 \times 10^{-8}$ equiv.

[a] Inflection point on linear-time basis used as end point; Leeds and Northrup instrument.

[b] Three determinations starting with fresh stock solution of bromide and acid, and with pretreatment consisting only of three regular washings to remove bromine from previous run.

[c] Av. dev.

[d] Three determinations run in series using a single portion of bromide-acid solution.

[e] Quadruplicates.

ACKNOWLEDGMENT

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Coulogravimetric Determination of Zinc and Cadmium

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The principle of coulogravimetric analysis, an example of double measurement, has been shown to be applicable with the mercury cathode. Mixtures of zinc and cadmium are deposited electrolytically and weighed. The number of coulombs required is also determined and results are obtained by solution of simultaneous equations.

COULOGRAVIMETRIC analysis or the simultaneous application of coulometric and gravimetric measurements was described earlier (*5*) in its application to the analysis of mixtures of chloride and bromide. Chloride and bromide, which cannot be separated by electrodeposition, were deposited together on a silver cathode and weighed. The number of coulombs required to deposit both was also determined, and the solution of simultaneous equations gave values for the amounts of chloride and bromide. At about the same time DuRose and Hutchison (*1*) reported use of the same principle in determining the composition of commercial lead-tin alloys plated from fluoborate electrolytes.

This paper reports the extension of the method to the mercury cathode, using zinc and cadmium as the pairs of metals. The mercury cathode in electrolytic analysis of individual metals has been studied by Lingane (*2*).

In the work described, the potentials suitable for depositing both metals on the mercury cathode were determined polarographically. The increase in weight of the cathode, w, and the total number of milliequivalents, m, of zinc and cadmium were determined from coulometric measurements. The weights of zinc and cadmium were obtained from the following considerations:

$$w = \text{weight of zinc} + \text{weight of cadmium} \tag{1}$$

$$m = \text{number of milliequivalents (Zn + Cd)} \tag{2}$$

$$= \frac{\text{coulombs used}}{96500} = \frac{\text{wt. of Zn}}{0.03269} + \frac{\text{wt. of Cd}}{0.05621}$$

$$m = \frac{\text{wt. of Zn}}{0.03269} + \frac{w - \text{wt. of Zn}}{0.05621} \tag{3}$$

$$\text{Wt. of Zn} = \frac{0.03269}{0.02352}(0.05621\,m - w) \tag{4}$$

$$\text{Wt. of Cd} = w - \text{wt. of Zn} \tag{5}$$

$$m = \frac{VP}{T}(2.141 \times 10^{-5}) \tag{6}$$

where

V = volume of gas in milliliters
P = corrected pressure in mm. of mercury
T = absolute temperature

APPARATUS

A Lingane-type potentiostat (*3*) was used and the contacts of the galvanometer relay were adjusted to give control to ±0.01 volt. The cell used is shown in Figure 1. It consisted of a 180-ml. tall-form electrolytic beaker which held the supporting electrolyte. The cathode support, *E*, was constructed from 15-ml. borosilicate glass tubing which insulated the platinum or tungsten contact and copper lead from the electrolyte. The anode, *A*, was a coil of silver wire or a coil of copper wire heavily plated with silver. Stirring was provided both by a magnetic stirrer, *H*, *J*, and a propellor-driven stirrer, *C*. Contact with the calomel electrode, *K*, was made with a saturated potassium chloride–4% agar salt bridge, *F*, and outgassing was done with nitrogen through the bubbler tube, *G*, *D*. The cell was covered in order to maintain an atmosphere of nitrogen over the electrolyte.

A 100-ml. hydrogen-oxygen coulometer of the Lingane type (*4*)

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Table I. Coulogravimetric Determination of Zinc and Cadmium

Approx. % Zn in Zn + Cd	Present, Mg.		Found, Mg.		Errors			
					Absolute, Mg.		Relative, %	
	Zn	Cd	Zn	Cd	Zn	Cd	Zn	Cd
100	130.8	...	131.2	...	0.4	...	0.3	...
100	130.8	...	131.3	...	0.5	...	0.4	...
100	130.8	...	131.2	...	0.4	...	0.3	...
84	117.7	22.5	117.5	23.0	−0.2	0.5	−0.2	2.2
67	91.6	45.0	91.3	45.8	−0.3	0.8	−0.3	1.8
51	91.6	89.9	91.1	90.9	−0.5	1.0	−0.6	1.1
37	65.4	112.4	66.3	111.5	+0.9	−0.9	1.4	−0.8
37	65.4	112.4	65.6	112.4	0.2	0.0	0.3	0.0
37	65.4	112.4	65.5	112.8	0.1	0.4	0.2	0.4
37	65.4	112.4	64.8	113.5	−0.6	0.9	−0.9	1.0
25	52.3	157.3	53.8	156.2	1.5	−1.1	2.9	−0.7
0	...	224.8	...	224.4	...	−0.4	...	−0.2
0	...	224.8	...	224.6	...	−0.2	...	−0.1

was used. The electrolyte was 0.25*M* potassium dichromate, for which the following vapor pressure corrections were used: 24° C., 22.2 mm.; 25°, 23.5 mm.; 26°, 25.0 mm.; 27°, 26.5 mm.; 28°, 28.1 mm.; 29°, 29.8 mm.; and 30°, 31.5 mm.

The value 0.1739 ml. per coulomb was used and the coulometer was found accurate to within ±0.2% upon comparison with a silver coulometer. Dichromate was used as coulometer electrolyte because it eliminated the mold growth which made potassium sulfate electrolyte unsuitable for more than 24 hours use.

PROCEDURE

The cathode, freshly filled with reagent mercury, was washed once with distilled water and twice with anhydrous acetone, and was allowed to stand 20 minutes at room temperature for the acetone to evaporate, and then weighed.

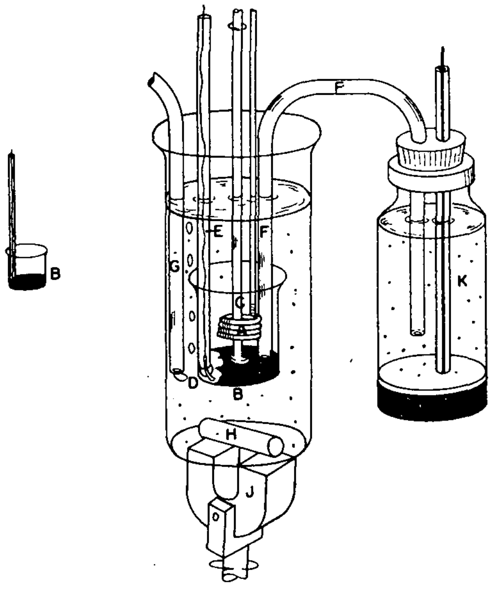

Figure 1. Electrolysis cell

The unknown zinc-cadmium solution was introduced into the cell with enough 2*M* potassium chloride and distilled water to make the resulting solution 1*M* in potassium chloride. The solution was outgassed with nitrogen for at least 5 minutes before introduction of the mercury cathode and the nitrogen was allowed to bubble throughout the electrolysis. The electrodes, mercury pool, stirrer, and salt bridge were lowered into place and the mercury pool stirrer was started at 250 r.p.m.

The electrolysis was begun at −1.45 volt *vs.* S.C.E. (saturated calomel electrode). (The sign of the electrode potentials used in this paper is in accord with the European convention, in which the calomel electrode is positive with respect to the normal hydrogen electrode.) When the current dropped to a constant value of 0.2 to 0.6 ma., the coulometer was read and the cathode was removed from the cell, washed, dried, and weighed just as was done prior to electrolysis. The cathode was quickly introduced into the cell again and a blank run was made to correct the original electrolysis for residual current. Care was taken to duplicate the conditions which existed for the actual run, particularly the rate of stirring and cathode potential. The blank was run for the same length of time, or some fraction of the same time, to determine the amount of gas liberated and gain in weight of the cathode which was due to the flow of the residual current. These blank corrections were subtracted from the original values and results were calculated from Equations 4, 5, and 6.

RESULTS AND DISCUSSION

Typical analyses of zinc and cadmium mixtures (Table I) show that the major constituent may be determined with acceptable accuracy for many purposes. The precision is not as good as for the chloride-bromide determination and this is attributed to variations in the rather large blank determination, which amounts to 2 to 6% of the total volume of gas liberated. The size of the blank correction was determined by measurements of the residual current which flows during the electrolysis.

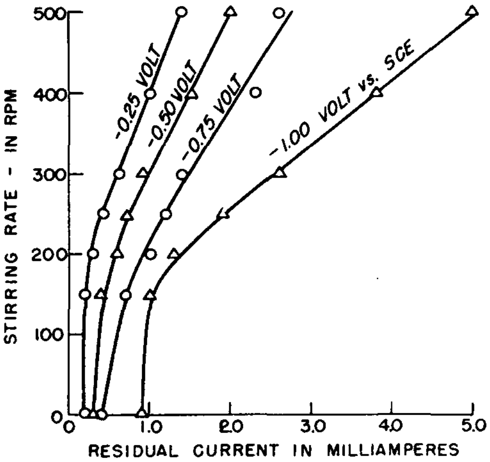

Figure 2. Effect of stirring rate and cathode potential on residual current

It was also observed that the residual current increases regularly with increased rate of stirring. Figure 2 shows this effect. Even at low stirring rates, the speed must be carefully controlled in order to obtain reproducibility. The residual current increases with increased cathode potential as shown in Figure 2, thus limiting the use of greater cathode potentials to hasten deposition. The residual current was not influenced by pH changes from 0.2 to 12.0 nor by changes in chloride ion concentration from 0.2 to 4.0*M*.

This work shows that the principle of simultaneous measurements, coulometric and gravimetric, can be successfully used with the mercury cathode and that mixtures of zinc and cadmium may be successfully analyzed electrolytically without separation, with accuracy sufficient for many purposes.

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Spectrophotometric Titration of Chromium and Vanadium

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Methods have been developed for the spectrophotometric titration of chromium(VI) with either iron(II) or arsenic(III) based on the change in absorbance observed at 350 mμ. Similarly, vanadium(V) has been titrated with iron(II). Solutions containing chromium and vanadium have been determined by oxidation with potassium bromate and spectrophotometric titration of the chromium(VI) with arsenic(III) followed by the titration of the vanadium(V) with iron(II). The method was tested on solutions containing 1.7 to 17.3 mg. of chromium and up to 4.8 mg. of vanadium and successfully applied to steel samples containing these elements.

DICHROMATE solutions have been shown (*2*) to possess two absorption peaks in the ultraviolet region while chromium(III) exhibits little absorption in the same range. The high molecular absorbance of the former (for potassium dichromate $\epsilon = 2400$ at 350 mμ) suggested the possibility of determination of chromium by photometric titration. It was found (*5*) that milligram amounts of chromium could be titrated with standard ferrous sulfate by observing the change in absorbance at 350 mμ. The iron(III) ion absorbs strongly at this point, but this interference may be overcome by addition of phosphoric acid which complexes the iron and shifts the absorption peak to the shorter wave-length region. The reverse titration, ferrous iron with dichromate, may be similarly performed. Quinquevalent vanadium is also reduced by ferrous sulfate and the titration can be followed spectrophotometrically, although the molecular extinction coefficient for vanadic acid ($\epsilon = 150$ at 350 mμ) is considerably lower than that of dichromate.

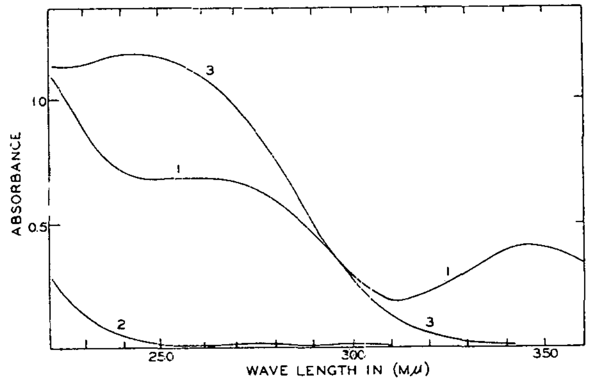

Figure 1. Absorption curves

1. 0.0010N $K_2Cr_2O_7$
2. 0.0010N $Cr_2(SO_4)_3$
3. 0.0002N Fe^{+++} in 5N H_3PO_4 and 1N H_2SO_4

It was thought that if solutions containing mixtures of chromium and vanadium were oxidized, then titrated with ferrous sulfate solution and the titration followed spectrophotometrically, breaks in the curve would indicate the relative amounts of the two ions present. In practice, good curves were obtained as predicted, but the results for chromium were consistently high and those for vanadium were consistently low. This indicated that some of the vanadium was being reduced along with the chromium. In addition, the slight slope of the curve, especially at the second end point, made readings difficult.

In the method of Kolthoff and Sandell (*4*), selective reduction of chromium in mixtures of chromium and vanadium is carried out using arsenious acid in acid medium. As neither arsenite nor arsenate ions absorb at 350 mμ, it seemed likely that the arsenite reagent could be substituted for the ferrous sulfate in the spectrophotometric titration of dichromate. It was found that dichromate could be titrated with arsenious acid, yielding curves very similar to those obtained with the ferrous sulfate method. When mixtures of dichromate and vanadate were titrated with arsenious acid, a sharp break was obtained at the chromium end point. The vanadium could then be titrated with the ferrous sulfate and a second end point obtained.

APPARATUS USED

A Model DU Beckman spectrophotometer equipped with the hydrogen lamp for operation in the ultraviolet range was used for the absorbance measurements. A Sorenson Isotronic power supply Model E was utilized instead of a storage battery.

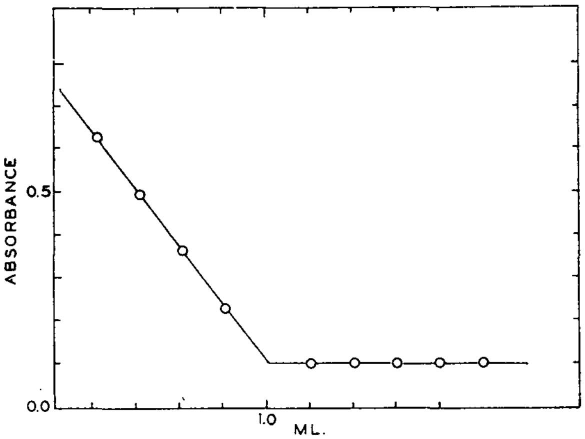

Figure 2. Titration of 0.0020N potassium dichromate with 0.1N ferrous sulfate in 5N phosphoric acid at 350 mμ

A special cell, similar to that described by Goddu and Hume (*3*), was constructed from quartz. The total capacity was about 150 ml. and the diameter of the part through which the light passed was about 2 cm. A special frame was constructed from aluminum stock which was designed to support the cell and fit into the 1-cm. cell compartment of the Beckman instrument. The only change required in the instrument was the removal of the carriage which normally supports the cell holder. This permitted the cell to extend well down below the light path. The cell was fitted with a two-holed rubber stopper to provide for a buret tip and a mechanical stirring device. All parts of the frame and cell, with the exception of the lower area through which the light must pass, were coated with a flat black paint. Titrations were made with a 5-ml. buret graduated in 0.01-ml. divisions.

METHOD FOR CHROMIUM

From Figure 1 it can be seen that addition of ferrous ions, which do not absorb in the ultraviolet region, would reduce the

Table I. Simultaneous Determination of Chromium and Vanadium by Titration with Arsenious Acid and Ferrous Sulfate

Cr Taken, Mg.	Cr Found, Mg.	Error, Mg. of Cr	Error, % Cr	V Taken, Mg.	V Found, Mg.	Error, Mg. of V	Error, % V
1.733	1.733	±0.00	±0.00	None	...	...	...
4.333	4.353	+0.020	+0.46	None	...	...	...
1.733	1.746	+0.013	+0.75	4.833	4.792	−0.041	−0.85
1.733	1.750	+0.017	+0.98	4.833	4.792	−0.041	−0.85
1.733	1.741	+0.008	+0.47	2.417	2.396	−0.021	−0.87
1.733	1.740	+0.007	+0.40	4.833	4.805	−0.028	−0.58
4.333	4.360	+0.027	+0.63	4.833	4.820	−0.013	−0.27
8.665	8.678	+0.013	+0.17	2.417	2.412	−0.005	−0.20
17.336	17.370	+0.20	+0.034	2.417	2.403	−0.014	−0.58

absorbance of the solution up to the removal of all chromium(VI) ions, provided the ferric ions were complexed with phosphoric acid. A plot of such a titration is shown in Figure 2.

Known samples of dichromate solutions were pipetted into the special titration cell and 3 ml. of 85% phosphoric acid and 25 ml. of 2*N* sulfuric acid were added. The rubber stopper was then placed in the top of the cell with the 5-ml. buret and stirrer in place. The tip of the buret was adjusted so that it extended just below the surface of the solution. The monochromator was set at 350 mμ and the absorbance at $A = 2$. The slit and sensitivity controls were then adjusted until the pointer registered zero. (For very dilute solutions of dichromate an initial setting of $A = 1$ was found to be more satisfactory.) After adjustment of the instrument, the 0.1*N* ferrous sulfate solution was added in 0.200-ml. increments. The solution was stirred and the absorbance was read after each addition. A graph was then plotted and the end point was established.

METHOD FOR MIXTURES OF CHROMIUM AND VANADIUM

The spectrophotometric method for mixtures of chromium and vanadium is based on the selective reduction of chromium(VI) by arsenious acid, followed by the reduction of the vanadium(V) with ferrous sulfate.

Known samples of mixtures of potassium dichromate and vanadic acid were placed in the titration cell and 6 ml. of 85% phosphoric acid and 2 ml. of concentrated sulfuric acid dissolved in 10 ml. of water were added. The volume was then made to approximately 50 ml. with water and stirred. The monochromator was set at 350 mμ and the absorbance at $A = 2.0$, and the slit control was adjusted until the needle rested at zero. Standard 0.1*N* arsenious acid solution was added in 0.200-ml. increments and absorbance readings were made after each addition. All absorbances were corrected for dilution of the titrant. The reaction was slow, especially near the end point, and care was taken to allow equilibrium to be reached before making readings. After the addition of about 1.0 ml. past the end point to establish the break in the curve, the buret containing arsenious acid was replaced by one containing 0.1*N* ferrous sulfate, and the titration was continued in the same manner. Good curves with sharp breaks at both end points were obtained by this method. A typical curve is shown in Figure 3.

Results by this method (Table I) were generally good with an average error of 0.44% for chromium and 0.60% for vanadium for quantities in the range of 1.7 to 17.3 mg. of chromium and up to 4.8 mg. of vanadium. The values from chromium are consistently a trifle high, while those for vanadium are consistently a trifle low. This may indicate that a small amount of the vanadium may have been reduced by the arsenite.

DETERMINATION OF CHROMIUM AND VANADIUM IN STEEL SAMPLES

Dissolve about 1 gram of sample in 10 ml. of dilute hydrochloric acid (1 ml. of water to 2 ml. of acid), warming slightly to hasten dissolution. As soon as evolution of hydrogen ceases, dilute to 200 ml. with boiling water and add sodium bicarbonate solution (80 grams per liter) from a buret until the first permanent precipitate forms, then add 3 ml. of excess. Boil for 1 minute and filter through asbestos. Transfer the precipitate along wit the asbestos into an Erlenmeyer flask, and add 25 ml. o water and 2 ml. of concentrate sulfuric acid. If a black precipitate of carbonaceous matter is observed upon the initia dissolution of the sample in hydrochloric acid, heat to boilin and add 1 ml. of concentrate nitric acid. Boil to expel th oxides of nitrogen and add gram of potassium bromate Boil for 5 minutes, then ad 3 grams of ammonium sulfat and continue boiling until n vapors of bromine are visible. Add 5 ml. of 1*N* hydrochloric aci and boil until the vapors give no test for bromine with starch iodide paper. Filter through sintered glass, add 5 ml. of 85 phosphoric acid, and make to approximately 50 ml. Transfe to a quartz titrating cell and titrate the dichromate with arsenious acid at 350 mμ as described above. After the end point ha been established, replace the buret with one containing ferrou sulfate and titrate the vanadate. The dichromate titration proceeds rather slowly, especially near the end point, and a fe minutes should be given for the solution to reach equilibriu before making absorbance readings in the final additions of arsenious acid.

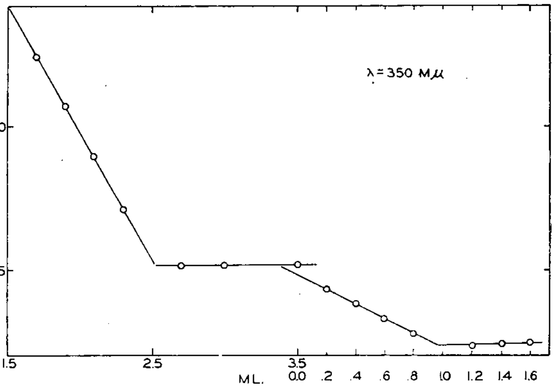

Figure 3. Titration of dichromate-vanadate mixture with arsenite and ferrous sulfate

The above method was tested on Bureau of Standards stee sample 30-d which contained in addition to chromium and vanadium, 0.363% of carbon, 0.78% of manganese, 0.031% of phosphorus, 0.031% of sulfur, 0.286% of silicon, 0.092% of copper 0.150% of nickel, and 0.034% of molybdenum. A preliminar separation of the iron was found to be necessary to prevent interference from the large excess of ferric ion which absorbed a 350 mμ even though complexed with phosphoric acid. Goo results were obtained after removing most of the iron employin the method proposed by Cain (*1*) and supplemented by Willar (*6*). By this process, the chromium hydroxide and vanadi

Table II. Determination of Chromium and Vanadium i Bureau of Standards Steel

(NBS sample 30-d, bromate oxidation and spectrophotometric titration wit arsenious acid and ferrous sulfate)

Chromium, %			Vanadium, %		
Bureau of Standards	Spectrophotometric	Error, %	Bureau of Standards	Spectrophotometric	Error, %
1.15	1.154	+0.004	0.19	0.180	−0.010
1.15	1.146	−0.004	0.19	0.187	−0.003
1.15	1.153	+0.003	0.19	0.188	−0.002
1.15	1.144	−0.006	0.19	0.192	+0.002

hydroxide were precipitated with sodium bicarbonate while the reduced iron remained in solution and was discarded in the filtrate.

The bromate oxidation method is somewhat more difficult than the usual persulfate oxidation. However, it was selected after repeated trials using persulfate gave erratic results for vanadium. Results are given in Table II.

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Microscopic Identification of Microgram Quantities of L-Arabinose and L-Fucose

Direct Synthesis of Crystalline 2,4-Dinitrophenylhydrazone and 1,1-Diphenylhydrazone Derivatives by Solvent Diffusion Technique

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The previously described solvent diffusion technique for the identification of D-fructose has been extended to the identification of L-arabinose using 2,4-dinitrophenylhydrazine and pyridine, and L-arabinose and L-fucose using 1,1-diphenylhydrazine and 70% ethyl alcohol. The sugar hydrazones are formed by direct synthesis at room temperature and are observed and identified microscopically. As little as 1 to 10 γ of the pure or 5 to 15 γ of the chromatographically separated pentose gives the test.

A TEST has been described for identifying microgram amounts of some pure and chromatographically separated pentoses and hexoses, particularly D-fructose, by direct synthesis and microscopic observation of their 2,4-dinitrophenylhydrazones (*3*). The test given by L-arabinose under these conditions was so insensitive that it was considered as an interference in the test for D-fructose. This paper reports an improvement in the sensitivity and specificity of the 2,4-dinitrophenylhydrazine test for L-arabinose and describes a similar test for identifying microgram amounts of pure and chromatographically separated L-arabinose and L-fucose as their crystalline 1,1-diphenylhydrazones. Information obtained by the combined use of these tests and the test previously published (*3*) can be a valuable aid in confirming the identity of an "unknown" sugar.

APPARATUS AND REAGENTS

Apparatus as previously listed (*3*).
Micropipet, approximately 0.5-μl. capacity.
Usual equipment for descending paper chromatography.
Equipment for eluting sugars from chromatograms (*3*).
Pure L-arabinose, L-fucose, D-ribose, and D-galactose.
2,4-Dinitrophenylhydrazine–ethyl acetate reagent (*3*).
Pyridine, reagent grade.

1,1-Diphenylhydrazine reagent. Add 0.25 millimole (21 mg.) of powdered anhydrous sodium acetate, reagent grade, to 0.25 millimole (55 mg.) of finely ground 1,1-diphenylhydrazine hydrochloride in a glass weighing bottle. Mix by gently rotating the container for a few minutes and keep in a desiccator when not in use. The reagent gradually becomes sticky, owing to absorption of moisture and liberation of the free base. It should be replaced with a fresh mixture when it becomes sticky.

Ethyl alcohol, 70%.
Mineral oil, heavy.
Filter paper, Whatman No. 1 sheets.
Ethyl ether, anhydrous, reagent grade.
n-Butyl alcohol–ethyl alcohol–water (10:1:2).
Duolite A-4 and Amberlite IR 120-H resins (*3*).

METHODS AND OBSERVATIONS

Using the reagents indicated below for the various tests, prepare and assemble the diffusion cell, and observe the progress of the diffusion as previously described (*3*). Compare the appearance of any reaction product with that of products formed under the same conditions by authentic sugars, with blanks prepared with the hydrazine reagents, and with the sugars only on the slide.

2,4-Dinitrophenylhydrazine–Pyridine Test for L-Arabinose. Wet the dried sugar spot with four or five applications of the 2,4-dinitrophenylhydrazine–ethyl acetate reagent, using a small glass rod. Apply sufficient reagent to leave some undissolved after diffusion is complete. Place 0.5 to 0.6 μl. of pyridine in the well of the culture slide and immediately assemble and seal the cell.

1,1-Diphenylhydrazine–Ethyl Alcohol Test for L-Arabinose and L-Fucose. Cover the dried sugar spot with an excess of the 1,1-diphenylhydrazine–sodium acetate reagent. Invert the slide and tap it to remove reagent not adhering to the sirup. Remove reagent remaining outside the sugar area with a fine camel's-hair brush. Place 1 μl. of 70% ethyl alcohol in the well of the culture slide. Assemble and seal the cell immediately.

Observations. Table I gives a brief description of the products formed in the various tests, the time required for the reaction products to appear, and the approximate amount of sugar required to give a visible test.

DISCUSSION

2,4-Dinitrophenylhydrazine–Pyridine Test for L-Arabinose. Under the conditions described for this test only three of the sugars listed in Table I—L-arabinose, L-fucose, and D-galactose—give a visible reaction product. L-Arabinose is readily separated from the other reacting sugars by chromatography with *n*-butyl alcohol–ethyl alcohol–water (10:1:2), so the test on an eluate from a chromatogram is specific for L-arabinose. The present test using pyridine is about ten times as sensitive for L-arabinose as was the previous one (*3*) using acidified moistened dioxane, and the product is characteristically crystalline instead of gel-like. However, the reaction product appears only after 16 to 24 hours instead of in a few minutes to 3 hours as in the previous test. The use of pyridine as the solvent also eliminates the possibility of acid hydrolysis of oligosaccharides, although this may occur in certain cases as a result of the resin treatment. L-Fucose and D-galactose do not give satisfactory tests with 2,4-dinitro-

Table I. Appearance of Reaction Products, Reaction Time, and Sensitivity of Sugars to Tests

	2,4-Dinitrophenylhydrazine–Pyridine Test				1,1-Diphenylhydrazine–Ethyl Alcohol Test			
			Sensitivity, γ				Sensitivity, γ	
Sugar[a]	Usual appearance of product	Time, hr.	Pure	Chromatographed[b]	Usual appearance of product	Time, hr.	Pure	Chromatographed[b]
L-Arabinose	Fine, trichitic, yellow needles in clusters or sheaves [Fig. 1, *F* (*3*)]	16–24	5	15–25	Small, white, dense clusters of radiating filaments–"moldlike" growth	0.02– 2	1–3	5–10
L-Fucose	Clusters of yellow, flat, narrow blades with ragged terminations	16–24	50	...	Segmented, dense, white clusters of fine needles	0.08– 2.5	10	15–25
D-Ribose	....	16–24	[c]	...	Loose clusters of blunt-ended, colorless bars or may be plumelike	0.1 – 2	75	...
D-Galactose	Yellow, clear, nondescript gel, may become crystalline on aging or with large amounts	16–24	25	...	....	16–24	[c]	...

[a] One hundred micrograms of each of the following sugars gave no visible reaction product after 16 hours with either test: D-xylose, D-xylose, L-rhamnose.-H_2O, D-glucose, D-mannose, D-fructose, and L-sorbose.

[b] Amount of sugar applied to paper.

[c] No visible reaction product with 100 γ of pure sugar.

phenylhydrazine when pyridine is the diffusing solvent. L-Fucose gives a crystalline product, but relatively large amounts of the sugar are required to give a test. D-Galactose reacts at moderate levels, but the product is usually an uncharacteristic gel. After 24 hours, or when a large amount of D-galactose is present, the field may show a mixture of the gel, sugar crystals, and well-defined clusters of fine, long yellow needles. These two sugars will react with 2,4-dinitrophenylhydrazine to give a visible product more rapidly, at much lower levels, and form more characteristic derivatives if dioxane reagent (*3*) rather than pyridine is the diffusing solvent.

The test for L-arbinose may be inhibited if other sugars are present, the extent depending on the sugar and relative amount. D-Fructose, D-glucose, L-sorbose, and D-xylose are only slightly inhibitory when present in an amount equal to or less than that of L-arabinose, whereas D-galactose and D-mannose are markedly inhibitory. D-Fructose and D-mannose would be the only sugars present with L-arabinose after chromatographic separation with the *n*-butyl alcohol–ethyl alcohol–water solvent.

The advantage of the 2,4-dinitrophenylhydrazine–pyridine test over the 1,1-diphenylhydrazine–ethyl alcohol test for L-arabinose is that the crystalline arabinose 2,4-dinitrophenylhydrazone formed is much more characteristic in appearance than is the arabinose 1,1-diphenylhydrazone. The disadvantages are that it is only approximately one third as sensitive and that it takes about 16 hours longer to obtain a positive test.

1,1-Diphenylhydrazine–Ethyl Alcohol Test for L-Arabinose and L-Fucose. 1,1-Diphenylhydrazine has been known for many years as a sensitive reagent for the identification and separation of L-arabinose (*1*, *2*) and L-fucose (*1*) from other sugars as their 1,1-diphenylhydrazones. The insolubility of L-arabinose and L-fucose 1,1-diphenylhydrazones and the slight influence of impurities on their crystallization make these derivatives well suited to the reaction conditions of the solvent diffusion test.

Under the conditions described for this test only three of the sugars listed in Table I—L-arabinose, L-fucose, and D-ribose—give a visible reaction product, although other sugars are known to react with this reagent under other conditions (*1*).

The L-arabinose and L-fucose 1,1-diphenylhydrazone crystals are similar in appearance and can be distinguished from one another only by careful comparison with tests made using authentic sugars. D-Ribose 1,1-diphenylhydrazone crystals are sufficiently characteristic in appearance to be easily differentiated from those of L-arabinose or L-fucose 1,1-diphenylhydrazone. Table I shows that the times for the appearance of the reaction products of the three sugars are short and essentially the same. The sensitivity of this test for L-arabinose and L-fucose is satisfactory; however, it is so poor for D-ribose that the test is of little value for this sugar, particularly in eluates from chromatograms.

The 1,1-diphenylhydrazine–ethyl alcohol test is specific for L-arabinose on eluates from chromatograms irrigated with *n*-butyl alcohol–ethyl alcohol–water (10:1:2), since L-fucose and D-ribose move much faster than does L-arabinose. L-Fucose and D-ribose move together in this solvent, so interference by D-ribose is encountered in the test for L-fucose. The extent of the interference depends on the relative amounts of the sugars. In general, the sensitivity of the L-fucose test is decreased about threefold and the time required for the product to appear is increased by large amounts of D-ribose.

Sugars that do not give a visible product with 1,1-diphenylhydrazine may prevent, delay the appearance of, or diminish the amount of the L-arabinose or L-fucose 1,1-diphenylhydrazone. The extent of the inhibition depends on the sugar and its relative amount.

The sugars giving visible products with 1,1-diphenylhydrazine react over a wide range of ethyl alcohol-water proportions. L-Arabinose and L-fucose gave the best tests at about 70% ethyl alcohol concentration, while the D-ribose gave the most sensitive test at 25 to 50% ethyl alcohol. The 70% ethyl alcohol concentration was chosen to eliminate interference from D-ribose as far as possible and to avoid atypical forms of L-arabinose and L-fucose 1,1-diphenylhydrazones.

The 1,1-diphenylhydrazine hydrochloride, Eastman Kodak No. 954 White Label, was used without further purification. Tests made with a purified crystalline preparation (*4*) showed less darkening and deterioration on long standing than did those made with the unpurified reagent; however, the purified reagent has the disadvantage that its crystal form superficially resembles that of D-ribose 1,1-diphenylhydrazone and it might be confused with this product.

Other hydrazine derivatives and solvents are being investigated for their utility in identifying microgram quantities of pentoses, hexoses, and other compounds containing carbonyl groups by the solvent diffusion technique.

ACKNOWLEDGMENT

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Molybdenum Determination in Soils and Rocks with Dithiol

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A simple colorimetric method is presented for the determination of submicrogram traces of molybdenum in soils and rocks. The procedure is developed on the extractable green complex formed between molybdenum and dithiol. Interference by ferric iron is eliminated by reduction with potassium iodide and sodium thiosulfate. Isoamyl acetate extracts of the molybdenum-dithiol complex are color-stable. Photometric measurements permit accurate determinations over the range of 0.02 to 10 γ of molybdenum in mineral materials.

MOLYBDENUM frequently occurs in soils and rocks in concentrations less than 1 γ per gram. The detection and estimation of these minute traces require sensitive and precise analytical procedures. Methods described in the literature either lack sensitivity or appear to yield test solutions generally susceptible to color fading and consequent uncertain precision (*15*). This investigation was undertaken therefore to develop a simple colorimetric procedure of requisite color stability for the determination of molybdenum in mineral materials.

Substituted dimercaptobenzenes were studied by Clark (*4*) as precipitants for metals of the hydrogen sulfide group. He gave to the compound 4-methyl-1,2-dimercaptobenzene (toluene-3,4-dithiol) the shortened name "dithiol" and recommended its use in the determination of tin. Later Hamence (*6*) discovered that molybdenum formed an extractable green precipitate with dithiol, and applied this reaction in a qualitative test for trace amounts of molybdenum. Miller (*10, 11*) studied the reactions of dithiol with molybdenum, tungsten, and rhenium. Wells and Pemberton (*18*), Bagshawe and Truman (*1*), and Short (*17*) used dithiol in microchemical determinations of molybdenum in metals. Bickford, Jones, and Keene (*2*) employed the delicate dithiol complexes for the simultaneous estimation of molybdenum and tungsten in organic materials. After systematic examination of reaction conditions, Piper and Beckwith (*13*) presented a dithiol method for the determination of small amounts of molybdenum in plants. The foregoing applications suggested the suitability of this reagent for the determination of molybdenum in mineral materials.

Soils and rocks, however, were found to possess constituents in concentrations sufficient to prevent the normal reaction between molybdenum and dithiol. Procedures were introduced to control interferences from silica, platinum, iron, tungsten, and copper. The modified method was then applied successfully to the determination of microgram quantities of molybdenum in a wide variety of inorganic materials.

REAGENTS

Water. Redistill in an all-borosilicate glass apparatus.

Dithiol, 0.2%. Dissolve a 1 gram vial of dithiol in 500 ml. of 1% sodium hydroxide solution. Stir the mixture occasionally during a period of 1 hour. Add thioglycolic acid (about 8 ml.) dropwise until a faint permanent opalescent turbidity begins to form. Transfer to 4-ounce narrow-mouthed bottles fitted with plastic screw caps. Store the reagent solution in a refrigerator at 5° C. at all times when not in actual use.

Isoamyl acetate, boiling range 135° to 142° C. The reagent grade product is sufficiently pure as received.

Potassium iodide, 50% (w./v.). Dissolve 10 grams of potassium iodide in 20 ml. of water. Prepare fresh solutions daily.

Sodium thiosulfate, 10%. Dissolve 10 grams of sodium thiosulfate ($Na_2S_2O_3.5H_2O$) in water and dilute to 100 ml. Store in a refrigerator at 5° C.

Standard molybdenum solution, 1 γ of molybdenum per ml. Dissolve 0.075 gram of molybdenum trioxide in 10 ml. of 0.1*N* sodium hydroxide. Dilute with water to 50 ml., acidify to litmus paper with 1*N* hydrochloric acid, and dilute to 500 ml. with water. Dilute 10 ml. of this stock solution (100 γ of molybdenum per ml.) to 1 liter with water acidified with 1 ml. of 1*N* hydrochloric acid. Store standard solutions in the dark.

Tartaric acid, 50%. Dissolve 50 grams of tartaric acid in water and dilute to 100 ml.

APPARATUS

Separatory funnels, Squibb-type, borosilicate glass, 125-ml. capacity. The stems should not exceed 9 mm. in outside diameter, so that the tips can be inserted into 10-mm. cuvettes.

Cuvettes, round, 12 × 75 mm., optically matched to 1% transmittance.

Photometer. Transmittances were measured with a Coleman Model 6A Junior spectrophotometer fitted with an accessory adapter for 12 × 75 mm. cuvettes. Satisfactory readings were also obtained with two filter photometers: the Fisher Model 7-101 electrophotometer and the Coleman Model No. 8 photoelectric colorimeter.

PREPARATION OF SAMPLES

The following procedure provides a sample solution from which several trace elements may be determined. In addition to molybdenum, the prepared solution is suitable for the separate colorimetric estimations of manganese, copper, and zinc.

Mix 2.000 grams of pulverized soil or rock sample with 4.00 grams of anhydrous sodium carbonate in a 35-ml. platinum crucible. Ignite for 30 minutes in the open over a Meker-type burner, gradually increasing the flame from low to high heat during the first 10 minutes. (Fusion in a muffle furnace may cause appreciable attack of the platinum crucible leading to subsequent colorimetric interference.) Cool the melt.

Detach the dry filter cake into a 250-ml. beaker. Wash the crucible with distilled water only, and combine the 30 to 40 ml. of washings with the fusion cake in the beaker. Pipet 20 ml. of concentrated hydrochloric acid into the beaker. (To avoid later colorimetric interference from dissolved platinum do not add hydrochloric acid to the fusion cake in the platinum crucible.) Digest the mixture on a steam bath at least 2 hours and preferably overnight. Crush any residual aggregates (chiefly silica) in the base angle of the tilted beaker with a flat-tipped stirring rod.

Evaporate the solution nearly but not completely to dryness on a steam bath. Stir occasionally to break the salt crust. Stir more frequently as dryness is approached and free liquid is no longer visible. Continue the evaporation beyond the gelatinous stage but not to complete dryness. Terminate evaporation when, on stirring, the nearly dry residue no longer adheres to the tip of the stirring rod. A distinct odor of hydrochloric acid should still be perceptible from the residue in the beaker.

Drench the cooled residue with 5 ml. of concentrated hydrochloric acid. Add 25 ml. of water, stir the mixture, and heat on a steam bath for 15 minutes. Filter while hot through a 9-cm. filter paper of medium texture into a 100-ml. volumetric flask. Wash the residue and paper with several small portions of hot dilute hydrochloric acid (2 to 98). Use water for the final two washings. Cool, and dilute to 100 ml. with water. The prepared sample solution should contain approximately 6 ml. of concentrated hydrochloric acid.

COLORIMETRIC PROCEDURE

Transfer a 25-ml. aliquot of the sample solution to a 125-ml. Squibb-type separatory funnel. This aliquot should be equivalent to 0.5 gram of sample in 6% by volume concentrated hydrochloric acid.

Add 10 ml. of concentrated hydrochloric acid to make the solution approximately 4*N* in that medium. Add 1 ml. of freshly prepared 50% (w./v.) potassium iodide solution, swirl, and let stand 10 minutes. The appearance of an amber to reddish brown color indicates the formation of iodine.

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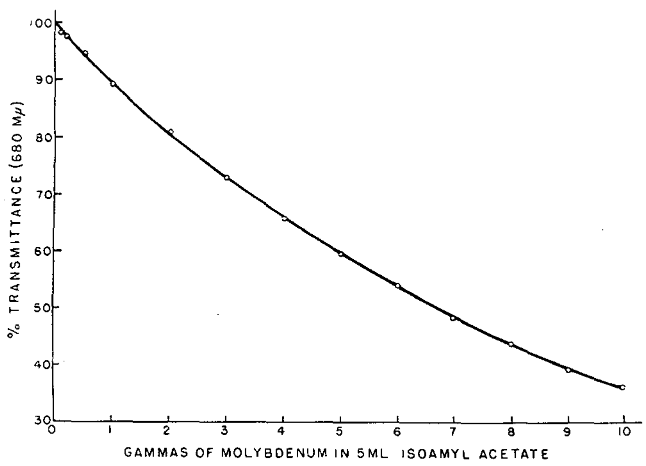

Figure 1. Transmittance of molybdenum-dithiol at 680 mμ

Add 10% sodium thiosulfate solution dropwise until the color of free iodine is just discharged. Add 1 ml. of 50% tartaric acid, stopper the funnel, and mix thoroughly by shaking. Add 2 ml. of 0.2% dithiol solution, shake 0.5 minute, and let stand 10 minutes.

Add 5 ml. of isoamyl acetate, shake the mixture 0.5 minute, and let stand until the two phases clear (normally 20 to 30 minutes).

Drain and discard the aqueous phase. Dry the stem of the separatory funnel with a spindle of filter paper. Insert a pledget of clinical absorbent cotton into the tip of the funnel and filter the acetate extract directly into a 12 × 75-mm. cuvette. Close the cuvette with a cork stopper.

Measure the transmittance at a wave length of 680 mμ using isoamyl acetate as the reference liquid. Determine the molybdenum content from a calibration curve based on the transmittances of a series of solutions containing from 0 to 10 γ of standard molybdenum treated according to the above procedure. Correct the result for any molybdenum found in a reagent blank.

Transmittance and absorbance curves are presented in Figures 1 and 2. It is probable that the determinable content of molybdenum in the test solution could be increased considerably beyond the upper 10-γ limit shown.

EXPERIMENTAL RESULTS

Sample Preparation. In accordance with the earlier work of Hoffman (*9*) it was found that 2 parts of sodium carbonate to 1 part of sample are sufficient for the fusion-decomposition of most rocks and minerals. The results in Table I show that molybdenum contents from 2-gram samples of rocks fused with 4 grams of sodium carbonate compare favorably with the contents determined when 2 grams of the samples were fused with 10 grams of flux. The smaller fusion mixture facilitates ignition and dehydration procedures; and, more importantly, it excludes unnecessary reagent contaminants.

Each of three different lots of sodium carbonate tested was found to contain an average of 0.1 γ of molybdenum per gram. Robinson and others (*16*) previously had detected and reported significant amounts of molybdenum in several lots of anhydrous sodium carbonate. The general presence of this element as a contaminant requires careful attention to reagent blanks.

When fused sodium carbonate was dissolved in dilute hydrochloric acid in the platinum ignition crucible, a blank value equivalent to 0.6 γ of molybdenum per gram of sodium carbonate was obtained. When the dilute acid was added to the fused sodium carbonate after transfer to a beaker, the blanks amounted to 0.1 γ of molybdenum, a value equal to that found when sodium carbonate was processed (without fusion) entirely in glassware. The colorimetric interference is caused by platinum brought into solution by attack of the crucible by hydrochloric acid solutions containing iron (*7*).

Table I. Molybdenum Found after Fusion of Rocks with Different Amounts of Sodium Carbonate

	Molybdenum Found, γ per Gram	
Rock (2 Grams)	After fusion with 10 grams of Na_2CO_3	After fusion with 4 grams of Na_2CO_3
Flint clay (NBS 97)	5.9	5.8
Plastic clay (NBS 98)	1.1	1.5
Gabbro	0.1	0.2
Serpentine	0.2	0.3

Table II. Recovery of Added Molybdenum from Hydrochloric Acid Digestates Evaporated on the Steam Bath

	Molybdenum, γ		
Digestate Evaporated	Added	Net recovered	Difference
Fusion cake from 2 grams of flint clay (NBS 97) + 50 ml. 2 to 3 HCl	5.0	5.0	0.0
	10.0	9.4	−0.6
50 ml. of 2 to 3 HCl, only	5.0	4.8	−0.2
	10.0	10.0	0.0

Traces of molybdenum may escape detection when samples high in silica are completely dehydrated with hydrochloric acid. Sample solutions of flint clay (NBS No. 97) containing 43% of silica were evaporated to dryness on a steam bath and then heated in an electric oven at 105° C. for 30 minutes. The resulting molybdenum contents were equal to or only about 1 γ higher than those of the reagent blanks. Other sample solutions of the flint clay, taken to proximate dehydration on a steam bath only, gave net values of 5.8 γ of molybdenum per gram. It is probable that complete dehydration renders the molybdenum insoluble through adsorption by colloidal silica. Any slight amounts of silica that entered the filtrates following proximate dehydration did not interfere with subsequent extractions with organic solvents.

Tests were conducted to determine possible loss of molybdenum by volatilization from hydrochloric acid solutions during evaporations on a steam bath. Known volumes of standard molybdenum solution were pipetted into 250-ml. beakers containing fusion cakes from 2-gram samples of flint clay in 50 ml. of 3 to 2 hydrochloric acid, and also into beakers containing 50 ml. of 3 to 2 hydrochloric acid only. All mixtures were covered and digested on a steam bath overnight. The digestates from the flint clay were then dehydrated to proximate dryness on a steam bath and the pure solutions of hydrochloric acid were evaporated to complete dryness. The results in Table II show that under both conditions 5.0 γ and 10.0 γ of added molybdenum were quantitatively recovered. Grigg (*5*) and Perrin (*12*) also reported the complete recovery of 5 γ of molybdenum which were added to soil extracts and then evaporated in 1 to 1 hydrochloric acid solution.

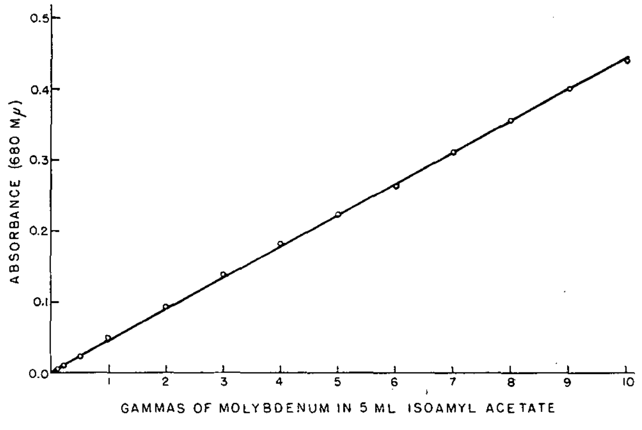

Figure 2. Absorbance of molybdenum-dithiol at 680 m

Color Development. The molybdenum-dithiol reaction proceeds effectively in concentrations of either 0.7 or $4N$ hydrochloric acid. The $4N$ acidity is preferred, since the phases separate more readily at the higher concentration. The $4N$ solution also produced extracts of slightly higher absorbance and consequent sensitivity.

Tests showed that 2 ml. of 0.2% dithiol solution were adequate for maximum color development over the range 0 to 10 γ of molybdenum. For 10 γ of molybdenum the mole ratio of reagent to metal is about 250 to 1.

The reaction is relatively rapid. A standing time of 10 minutes after shaking sample and dithiol permitted complete compound formation. A standing time of 30 minutes gave no increase in color intensity. The acetate extract and acidic phases cleared in about 20 minutes after shaking.

Interferences. Strong oxidants decompose dithiol. Addition of a few drops of added nitric acid or hydrogen peroxide to the test solution causes destructive oxidation of the reagent. Miller (*10*) states that perchloric acid behaves similarly and must be excluded.

Ferric iron interferes seriously. A synthetic solution containing 1 γ of standard molybdenum and 50 mg. of added iron (as ferric chloride) in $4N$ hydrochloric acid gave a dense black precipitate on the addition of 2 ml. of 0.2% dithiol. The black precipitate dissolved when shaken with 5 ml. of isoamyl acetate. The aqueous phase exhibited the characteristic greenish yellow color of ferric chloride; the green molybdenum-dithiolate acquired an atypical yellowish tinge and began to fade within minutes. The color was probably diminished through oxidation of the molybdenum-dithiol by the coextracted ferric chloride. Color interference from 50 mg. of iron(III) was not eliminated by addition of any of the following compounds: ammonium citrate, ammonium tartrate, citric acid, phosphoric acid, tartaric acid, thioglycolic acid, hydroxylamine hydrochloride, sodium fluoride, sodium sulfite, sodium thiosulfate, or stannous chloride.

Iron interference is, however, completely prevented by the reduction of iron(III) to iron(II) with potassium iodide followed by the decolorization of liberated iodine with sodium thiosulfate. The sequence of iodide followed by thiosulfate keeps the iron in the ferrous state, as shown by the complete elimination of ferric chloride color. Free sulfur, usually formed on prolonged standing, is caught on the pledget of cotton and hence does not disturb the colorimetric measurement of the extract.

The combination of potassium iodide and sodium thiosulfate evidently does not reduce molybdenum. The extract from a synthetic solution containing 1 γ of standard molybdenum plus the two reductants gave the same transmittance reading as the extract from a similar solution containing 1 γ of molybdenum but no potassium iodide or sodium thiosulfate.

The reduction procedure is applicable to samples of high iron content. With 5 ml. of 50% potassium iodide and correspondingly increased sodium thiosulfate solution it was possible to determine 2.9 γ of molybdenum in a lateritic soil containing 58% ferric oxide.

Arsenic, copper, platinum, and rhenium were tested as possible sources of interference in pure solutions containing 1 γ of molybdenum. Results in Table III show that 100 γ of arsenic have no measurable effect on 1 γ of molybdenum. Similarly, 100 γ of added copper are without effect. Subsequently 1.2 γ of molybdenum were determined very satisfactorily in an aliquot sample containing 50 mg. of copper. Platinum at 10 γ interferes slightly; and 100 γ of platinum produces an apparent increase nearly equal in magnitude to the 1 γ of molybdenum present. Tests show that 10 γ of rhenium cause only negligible interference; but 100 γ of rhenium produce an appreciable positive error. The latter quantity of rhenium imparts a visibly purer green color to the extract. Rhenium interference, for practical purposes, may be disregarded, since soils and rocks rarely contain as much as 10 γ per gram of that metal (*14*).

Table III. Effect of Added Elements on Determination of 1 γ of Molybdenum in Standard Solutions

Element Added, γ		Molybdenum, γ Present	Found	Difference
Arsenic	100	1.00	1.00	0.00
Copper	100	1.00	1.00	0.00
Platinum	10	1.00	1.09	+0.09
Platinum	100	1.00	1.90	+0.90
Rhenium	10	1.00	1.02	+0.02
Rhenium	100	1.00	1.47	+0.47

Table IV. Recovery of 1 γ of Molybdenum Added to Sample Solutions

Sample No.	Molybdenum, γ In sample	Added	Found	Recovered	Difference
C-A	0.42	1.00	1.43	1.01	+0.01
C-B	0.48	1.00	1.54	1.06	+0.06
C-C	0.40	1.00	1.36	0.96	−0.04
C-rock	0.22	1.00	1.22	1.00	0.00
H-A	1.00	1.00	2.15	1.15	+0.15
H-B	1.21	1.00	2.18	0.97	−0.03
H-C	1.22	1.00	2.23	1.01	+0.01
H-rock	0.37	1.00	1.35	0.98	−0.02

Tungsten or tin influence was not tested in this work. Presumably most of the tungsten is removed as precipitated tungstic oxide in the dehydration of silica (*8*). To ensure noninterference, however, tartaric acid is added to the sample aliquot to inhibit reaction between any remaining tungsten and dithiol as demonstrated by Hamence (*6*). Tin, according to Piper and Beckwith (*13*), has a spectral absorption below 470 mμ and, in trace amounts, does not introduce a significant error when the color of the molybdenum-dithiol extract is measured at 680 mμ.

Stability. Reagent solutions prepared as directed are stable. A standard solution containing 1 γ of molybdenum per ml. showed no change in transmittance readings point for point after standing in a dark cupboard for 6 months. Dithiol reagent solutions also retained full strength when stored in a refrigerator for 6 months.

Acetate extracts of the molybdenum-dithiol complex show excellent color stability. Extracts from 12 soil and rock samples were kept in stoppered cuvettes at room temperature and measured photometrically at five separate intervals during a period of 14 days. Complete stability was found; transmittance readings on individual samples remained the same throughout the 2-week test.

Recovery of added molybdenum confirms the reliability of the colorimetric dithiol procedure. Results in Table IV show that 1 γ of added molybdenum is fully recovered from solutions of seven different soil and rock samples. The average difference is computed to be 0.04 γ of molybdenum.

The precision of this dithiol method, measured by the standard deviation between determinations on a single sample, is calculated to be 0.02 γ of molybdenum per gram. This value approaches the sensitivity limit of the spectrophotometer used.

Applications. The modified dithiol method was used to determine molybdenum contents in 207 samples of soils and rocks from Maryland (*3*). The 181 soil samples examined had a range of 0.13 to 13.54 γ of molybdenum per gram with an average of 1.33 γ per gram. The 26 rock samples had a molybdenum range from 0.03 to 1.54 γ per gram and an average content of 0.39 γ of molybdenum per gram.

Although developed for soils and rocks, the colorimetric procedure has also been found satisfactory for determining trace amounts of molybdenum in sample solutions from activated sewage sludges and inorganic fertilizer materials.

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Photometric Determination of Copper in Molybdenum Products with Alpha-Benzoinoxime

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The α-benzoinoxime method of Dunleavy and others has been applied to a wide range of molybdenum compounds. Procedures for the solution of a variety of samples are described. Ratios of calcium to copper greater than 2 to 1 caused appreciable interference. The procedure is suitable for samples containing from 0.001 to 1.00% of copper. The technique is simple and results are reproducible under routine conditions.

A SPECTROPHOTOMETRIC method utilizing α-benzoinoxime has been found suitable for rapid and accurate determinations of copper on a wide range of molybdenum compounds.

Feigl (*4*) introduced α-benzoinoxime as a specific reagent for copper in ammoniacal solution. Kar (*6*) and Silverman (*8*) reported its use for gravimetric determinations of copper in steels. Although Feigl had stated previously that α-benzoinoxime was specific for copper, Jennings (*5*) reported that nickel, cobalt, platinum, and palladium also formed complexes with the reagent. Dunleavy, Wiberley, and Harley (*3*) reported a direct colorimetric method for the determination of copper in plain and low-alloy steels and cast irons. The effect of the variables—pH, amount of reagent (α-benzoinoxime), amount of Rochelle salt, stability of complex, interference of other ions (specifically nickel and cobalt)—are discussed thoroughly.

EXPERIMENTAL

An attempt was made to apply the α-benzoinoxime method in this laboratory. The general recommendations found in the literature (*3*) were followed and early efforts to obtain reproducible values on standard copper solutions were successful.

The pH range of 11.3 to 12.3 was obtained by using phenolphthalein indicator and a measured volume of a standard sodium hydroxide solution.

The sample solution was neutralized with 5*N* sodium hydroxide until 1 drop imparted a pink color to the solution. A measured volume of 2.5*N* sodium hydroxide was added in excess to give the desired pH range, as read on a pH meter.

The 2.5*N* sodium hydroxide solution was used as a standard reagent in routine analysis, eliminating the necessity for using a pH meter.

REAGENTS AND EQUIPMENT

α-Benzoinoxime (Eastman Kodak), 0.5% solution in 0.25*N* sodium hydroxide.
Sodium hydroxide, 5*N*.
Sodium hydroxide, 2.5*N* (use volumetric flask).
Rochelle salt, 300 grams in 500 ml. of water.
Chloroform, reagent grade.
Spectrophotometer (Beckman, Model DU was used).
Phenolphthalein indicator.

PREPARATION OF CALIBRATION CURVE

Prepare a calibration curve by adding aliquots of the standard copper solution containing 0.1 to 0.9 mg. of copper to 0.25-gram samples of pure molybdenum trioxide and follow the procedure described. Carry a reagent blank containing no added copper along with each set of samples and correct the spectrophotometric readings for all samples for the reagent blank. Plot the corrected readings against the concentration in the conventional manner. The readings are made at 440 mμ and 1-cm. Corex cells are used.

PROCEDURE FOR DETERMINATION OF COPPER

In all cases weigh a 0.25-gram sample into a 250-ml. borosilicate glass beaker.

A. For molybdenite concentrates and purified molybdenum disulfide, add 5 ml. of sulfuric acid and 10 ml. of nitric acid. Heat to fumes of sulfur trioxide, and continue to add nitric acid in 10-ml. portions until sample is in solution. On final addition, heat to strong fumes of sulfur trioxide. Cool, add 10 ml. of water and 3 ml. of nitric acid, and heat to dissolve soluble salts. Continue as for sample F.

B. For molybdic oxide (technical), add 5 ml. of hydrochloric acid and 5 ml. of nitric acid. Heat to decompose and evaporate to dryness. Bake, cool, and add 10 ml. of water and 10 ml. of 5*N* sodium hydroxide. Heat to boiling, and add 10 ml. of water and 10 ml. of nitric acid. Heat to dissolve soluble salts. Continue as for sample F.

C. For molybdic oxide (pure), weigh a 5-gram sample into a 250-ml. borosilicate glass beaker. Add 10 ml. of sulfuric acid and heat to strong fumes of sulfur trioxide. Cool, add 5 ml. of nitric acid cautiously, and heat to fumes of sulfur trioxide. Cool, add 10 ml. of water, and heat to dissolve soluble salts. Continue as for sample F.

D. For ferromolybdenum and molybdenum metal, add 50 ml. of water, 5 ml. of hydrochloric acid, and 5 ml. of nitric acid. Heat carefully to boiling until reaction ceases and the volume of the solution is reduced one half. (Digestion period is approximately 0.5 hour.) Continue as for sample F.

E. For molybdenum silicide, add 10 ml. of nitric acid and hydrofluoric acid a few drops at a time, until decomposition is complete. The solution may be heated gently between additions

Table I. Comparative Data from Thiocyanate and Photometric Methods of Analysis

Copper Present, Gram	Copper Found, Gram Thiocyanate-electrolytic method	Thiocyanate-photometric method	Photometric method
0.0050	0.0045	0.0048	0.0049
0.0050	0.0047	0.0048	0.0049
0.0050	0.0047	0.0047	0.0050
Av. 0.0050	Av. 0.0046	0.0048	0.0049

Standard copper nitrate solution—0.05 gram copper foil per liter of (1 to 99) nitric acid solution. 1 ml. = 0.00005 gram of copper.
Average copper found by thiocyanate method, 92.0%.
Average copper found by thiocyanate-photometric method, 96.0%.
Average copper found by photometric method, 98.0%.

Table II. Effect of Calcium on Standard Copper Solutions

Test	Calcium Added, Mg.	Copper Present, Mg.	Copper Found, Mg.
1	0.00	0.500	0.480
2	0.60	0.500	0.475
3	1.30	0.500	0.468
4	1.90	0.500	0.463
5	2.50	0.500	0.458
6	3.10	0.500	0.445
7	3.80	0.500	0.443

Standard copper solution, 1 ml. = 0.00005 gram of copper.
Standard calcium solution, 1 ml. = 0.000025 gram of calcium.

of hydrofluoric acid if necessary. After decomposition is complete, add 10 ml. of sulfuric acid (1 to 1) and evaporate to heavy fumes of sulfur trioxide. Cool, wash down sides of beaker, and again evaporate to heavy fumes of sulfur trioxide to eliminate hydrofluoric acid. Cool, and add 10 ml. of water and 3 ml. of nitric acid. Heat to dissolve soluble salts. Continue as for sample F.

F. After samples A, B, C, D, and E are in solution, cool slightly and add 25 ml. of Rochelle salt solution. Adjust the pH to range between 11.3 and 12.3 with sodium hydroxide using phenolphthalein indicator. Use the following procedure: Add 3 to 5 drops of phenolphthalein indicator and 5*N* sodium hydroxide from a buret until 1 drop imparts a pink color to the solution, which should persist for at least 30 seconds. Add 1 ml. of 2.5*N* sodium hydroxide in excess and 2 ml. of 0.5% α-benzoinoxime solution (use pipet).

Transfer to a 250-ml. separatory funnel, add 50 ml. of chloroform (buret) and shake vigorously for 5 minutes. Filter the chloroform through a retentive paper into a test cell.

Read the absorption of the solution, after having previously set the instrument at zero with a blank on reagents carried along simultaneously with the sample.

Measure the absorbance at 440 mμ. Slit width is 0.065 mm.

APPLICATION OF METHOD

Synthetic Samples. The reported specificity of α-benzoinoxime for copper was investigated by adding pure molybdic oxide to synthetic solutions. Different methods of analysis were used in order to compare the per cent recovery of copper. The results of these tests are found in Table I.

A straight-line curve was obtained for the copper-α-benzoinoxime complex when plotting "absorbance" as the ordinate and "concentration of copper" as the abscissa.

The calcium ion was found to interfere with the determination of copper in calcium molybdate. This was investigated by adding calcium ion to synthetic copper solutions. Results of these tests are found in Table II. A straight-line curve was obtained when plotting "per cent copper found" as the ordinate and "concentration of calcium" as abscissa.

Standard Samples of Roasted Concentrates and Ferromolybdenum. These samples were produced and values were determined several years ago by four laboratories using various methods. They are used in the author's laboratory for control purposes. In addition, a series of production samples of roaster concentrates, ferromolybdenum, and molybdenite concentrates were analyzed for copper. The samples were dissolved by standard acid treatment as indicated in the previous procedure. Comparative data are found in Tables III and IV.

RESULTS AND DISCUSSION

Copper values on standards and production samples compare favorably with the α-benzoinoxime and thiocyanate methods (Tables III and IV). Reproducibility of results was satisfactory on all samples analyzed.

Copper values on synthetic standards indicate that the α-benzoinoxime method of analysis is more sensitive in detecting minute amounts of copper than is the method of weighing an equivalent amount as is done in the thiocyanate-electrolytic method. Loss of copper by α-benzoinoxime is at a minimum in actual procedure.

The calcium ion interfered in the determination of copper in calcium molybdate. Ratios of calcium to copper greater than 2 to 1 caused definite interference in the recovery of copper (Table II). Separation of calcium is possible, but would lengthen the method. In other molybdenum compounds the calcium ion concentration is negligible and results are found to be favorable.

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Table III. Determination of Copper on Standard Molybdenum Products

Sample	% Copper Analyst 1	Analyst 2	Analyst 3	Analyst 4	Analyst 5 (thiocyanate method)	Analyst 5 (photometric method)
Molybdic oxide standard	0.26	0.24	0.25	0.25	0.22	0.24
Ferromolybdenum standard	0.22	0.21	0.20	0.23	0.22	0.23

Notes: Average per cent deviation on standard molybdic oxide technical (results from four laboratories) is −0.01% copper (photometric method of analysis).

Average per cent deviation on standard ferromolybdenum (results from four laboratories) is +0.01% copper (photometric method of analysis).

Analysts. 1. Climax Molybdenum Co., Detroit, Mich. 2. Climax Molybdenum Co., Climax, Colo. 3. Climax Molybdenum Co., Golden, Colo. 4. Ledoux and Co., Inc., Teaneck, N. J. 5. Climax Molybdenum Co., Langeloth, Pa.

Table IV. Determination of Copper on Molybdenum Products

Molybdenum Product	Sample No.	Copper, % Analyst 5 (thiocyanate method)	Analyst 5 (photometric method)
Molybdic oxide technical	1	0.15	0.16
	2	0.15	0.17
	3	0.17	0.20
	4	0.17	0.20
	5	0.16	0.18
	6	0.17	0.21
Ferromolybdenum	7	0.17	0.16
	8	0.22	0.20
	9	0.28	0.31
	10	0.28	0.28
	11	0.40	0.41
	12	0.40	0.43
Molybdenite concentrates	13	0.09	0.10
	14	0.03	0.04
	15	0.12	0.15
	16	0.10	0.13
	17	0.14	0.17

Average per cent deviation is +0.02% copper (photometric method of analysis).

Analyst 5, Climax Molybdenum Co., Langeloth, Pa.

Direct Volumetric Determination of Total Zinc in Mixed Paint Pigments with Ethylenediaminetetraacetic Acid

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The determination of total zinc in mixed paint pigments of unknown composition involves its separation from interfering cations with hydrogen sulfide, which is time-consuming. A more rapid, direct, and specific method can be used to replace the variety of methods in current use, without knowledge of the nature of the pigment mixture.

THE determination of certain cations by complex formation with chelating agents (*1*) is particularly well adapted to the determination of zinc in mixed paint pigments. A limited number of the metal ions that can be determined in this manner occur in mixed paint pigments: calcium, magnesium, barium, zinc, iron, and chromium. Zinc pigments, with the exception of zinc sulfide, have the distinction of dissolving in ammoniacal ammonium chloride which is used to buffer the solution at pH 10 during the titration. No other pigments are soluble in this solution, except certain chromium pigments which do not interfere with the titration of zinc. This provides a means for the rapid, specific, and direct determination of total zinc; a special modification can be made for pigments containing zinc sulfide, which is easily identified by the odor of hydrogen sulfide given off when acids are added.

Table I. Determination of Zinc Oxide and Zinc Chromate in Paint Pigments

Specification TT-E-00485b enamel containing lead chromate, red lead, zinc oxide, and siliceous extender

Zinc Oxide Present, %	Zinc Oxide Found, %
9.0	8.8
9.0	8.8
10.1	10.1

Specifications MIL-P-11414A and TT-P-664 primers containing iron oxide, zinc chromate, and siliceous extender

Zinc Chromate Present, %	Zinc Chromate Found, %
10.1	10.4
10.1	10.5
10.2	10.2

Specification TT-P-636b primer containing iron oxide, zinc chromate, zinc oxide, and siliceous extender

Zinc Chromate Present, %	Zinc Oxide Present, %	Total Zinc Calculated as Zinc Oxide, %	Zinc Oxide Found, %
9.6	9.9	14.2	14.1
9.7	9.9	14.3	14.2
8.6	10.4	14.3	14.3

The method in current use for determining zinc in mixed pigments of unknown composition involves separation of the zinc with hydrogen sulfide, followed by ferrocyanide titration or ignition of the sulfide to the oxide and weighing. Hydrogen sulfide separations are time-consuming. The accuracy of the method described here is equal but not superior to existing methods, although the absence of excessive handling and transfer of sample contributes to a high degree of reproducibility of analytical results.

In principle, the zinc pigment is dissolved in a buffered solution of ammonium chloride and ammonium hydroxide, and the insoluble pigments are filtered off. An indicator, Eriochrome Black T, is added which is a magnesium complex of a hydroxylated azo dye, purple in color. The sample is then titrated with a chelating reagent solution, ethylenediaminetetraacetic acid disodium salt [sodium(ethylenedinitrilo)tetraacetate], which complexes the zinc present. When all zinc has been complexed, the first excess of reagent breaks down the indicator by the formation of a stronger complex with the magnesium than the complex of the dye with magnesium, causing a color change to blue.

REAGENTS

Buffer solution (pH 10). Fifty-four grams of ammonium chloride and 350 ml. of concentrated ammonium hydroxide are diluted to 1 liter with water.

Eriochrome Black T (0.5%). Eriochrome Black T, 0.25 gram, and 2.2 grams of hydroxylamine hydrochloride (as stabilizer) are dissolved in 50 ml. of methanol.

Disodium ethylenediamine tetraacetate dihydrate (0.2*N*); 37.2 grams of the reagent are dissolved in water and diluted to 1 liter.

Standard zinc oxide solution. Approximately 3 grams of oven-dried zinc oxide of known purity are accurately weighed, dissolved in the buffer solution, then diluted to 500 ml. with buffer.

ANALYTICAL PROCEDURE

A small amount of pigment is tested for sulfides by the addition of dilute hydrochloric acid. If the odor of hydrogen sulfide is detected, the modified procedure is followed.

Procedure in Absence of Zinc Sulfide. Approximately 1 gram of the isolated paint pigment is accurately weighed into a 250-ml. Erlenmeyer flask having a 24/40 ground joint. If the sample is believed to contain more than 10% zinc oxide, proportionately smaller samples may be used. Twenty-five milliliters of the buffer solution are added, and the flask is stoppered and is shaken frequently and vigorously for 15 minutes. The solution is then filtered through paper of fine porosity into a 400-ml. beaker and diluted to 300 ml. with water. Exactly 25 ml. of the standard zinc oxide solution are transferred to a 400-ml. beaker and similarly diluted to 300 ml. Ten drops of the Eriochrome Black T indicator are added and the sample and standard are titrated with disodium ethylenediaminetetraacetate reagent. As it becomes apparent that the end point is near, 5 additional drops of indicator are added. In the absence of chromium, the color change at the end point is from wine-red to blue; if much chromium is present, the color change is from orange-red to green.

Procedure in Presence of Zinc Sulfide. Approximately 1 gram of the isolated pigment is accurately weighed into a 250-ml. beaker, 25 ml. of 6*N* hydrochloric acid are added, and the sample is heated for 30 minutes on a hot plate and finally boiled without cover for a few minutes to expel the hydrogen sulfide. It is then cooled and 2 ml. of 85% phosphoric acid are added, followed by 35 ml. of concentrated ammonium hydroxide and 25 ml. of the buffer solution. The resulting mixture should be alkaline (approximately pH 10). It is then filtered through paper of fine porosity and the filtrate is diluted and titrated as above. The 25-ml. aliquot of standard zinc oxide solution should be treated in a similar manner—i.e., 2 ml. of phosphoric acid, 25 ml. of 6*N* hydrochloric acid, and the 35 ml. of concentrated ammonium hydroxide are added before diluting and titrating the standard.

$$\%\ \text{zinc (as ZnO)} = \frac{\text{ml. of reagent} \times \text{normality of reagent} \times 4.069}{\text{weight of pigment sample}}$$

Some results of analysis are shown in Table I.

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Determination of Silica in Fluosilicates without Removal of Fluorine

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By the addition of aluminum(III) to hydrochloric acid before the evaporations, silica is recovered quantitatively by the ordinary gravimetric procedure without the removal of fluoride.

DURING the experimental work leading to a previous comprehensive report (*6*) on the chemical analysis of fluosilicates, the fact was noted that certain samples could contain surprisingly large amounts of fluorine and still give complete recovery of silica without fluoride removal. For example, a sample containing 2.25% of unremoved fluorine gave the same value for silica as a duplicate sample with fluoride removed. The presence of aluminum(III) appeared to be the only reasonable explanation.

Tests were made to determine the effect of added aluminum(III) on silica recovery with a variety of fluosilicates. The procedure used was an ordinary one for the gravimetric determination of silica (*3*). A 0.500- or 1.000-gram sample was fused with 5 grams of sodium carbonate; then the fusion was dissolved in 200 ml. of 1 to 3 hydrochloric acid containing the required amount of aluminum chloride. [Four and one half grams of aluminum chloride hexahydrate give about 500 mg. of aluminum(III).] A black glazed casserole, or preferably platinum, was the container. The solution was evaporated to dryness on the steam bath. With 1 gram of aluminum(III), in porcelain, a heat lamp was required to attain complete dryness. The lamp used was 150 watts 8 inches from the casserole. Acidification with hydrochloric acid, filtering, and washing were done as usual. A second evaporation to dryness and recovery of silica were made similar to the first except that dry pulped filter paper was added to help collect the small precipitate obtained in the second dehydration. Amount of pulp added was equivalent to one 11-cm. paper. Ignition, weighing, and sulfuric acid–hydrofluoric acid treatment were done as usual. The only significant variation was the introduction of aluminum(III) ions as aluminum chloride hexahydrate.

Results of the tests are in Table I. Silica found was corrected for silica content of the added aluminum(III). Recovery of silica was as complete as with the zinc oxide fusion procedure (*5*, *6*).

Recovery of silica was about 0.20% low, but this is normal for two hydrochloric acid dehydrations. Both the present work and that of others (*3*) have shown that complete recovery of silica with 0.5- to 1.0-gram samples is not attained with two hydrochloric acid evaporations. The loss ordinarily experienced is the same as that noted—0.20%. As stated in the previous report (*5*), a correction of 0.20% could be made, or the silica escaping could be recovered by standard methods. For nearly all work a known sample run concurrently, to indicate the correction factor required, should provide all the accuracy needed. One gram of aluminum(III) was no more beneficial than 500 mg. and was also more difficult to evaporate to dryness. The absolute minimum amount of aluminum(III) necessary for each type of sample was not determined. Zirconium(IV) and titanium(IV) were tried but did not prevent loss of silica.

A sodium silicate nonahydrate solution was analyzed for its silica content. To the first aliquot, 500 mg. of aluminum(III) plus 100 mg. of fluoride (as NaF) were added. No additions were made to the second aliquot. Recovered were 470.8 mg. silica for the first and 472.0 mg. for the second. This slight difference is believed to be caused by the fluffy nature of the first silica compared with the compact granularity of the second—hence, increased solubility in the wash solutions.

Boric acid or borates have been advocated as suitable for the prevention of silica loss in the presence of fluoride. One procedure (*4*), which gives as the proper reagent 20% perchloric acid saturated with boric acid at 50° C., has been used with varied success. Boron separates with the silica to an indeterminate extent and is counted as silica, for the hydrofluoric acid evaporation evolves all the boron contaminant as well as the silicon. Also, previous work (*6*) indicates that silicofluoride is more stable than fluoborate under acidic conditions and that therefore silica is preferentially lost during the dehydrations. With samples containing the silica in an already precipitated form (quartz), the rate of solution and therefore the loss are not serious; but with reactive, discrete silicate ions present the loss is much too large for accurate work. This combination of indeterminate plus and minus errors and the difficulty of complete perchloric acid removal from samples containing large amounts of silica make the borate additions suitable for special types of materials only.

Table I. Recovery of Silica in Presence of Aluminum(III) without Removal of Fluoride

Sample Type	Sample Size, Gram	Al (as $AlCl_3.6H_2O$), Mg.	SiO_2 Found, %	SiO_2 Present, %
Opal glass 91	1.000	None	66.17	67.53[a]
	1.000	None	65.93	67.53[a]
	1.000	500	67.24	67.53[a]
	1.000	500	67.28	67.53[a]
	1.000	500	67.25	67.53[a]
	1.000	1000	67.20	67.53[a]
USGS-G1[b]	1.000	1000	72.45	72.64
USGS-W1[c]	1.000	1000	52.61	52.66
BS No. 70[d]	1.000	1000	66.76	66.66[a]
Synthetic				
phlogopite	1.000	500	41.97	42.22
mica	1.000	1000	42.03	42.22
Topaz	1.000	1000	33.66	33.60
Phosphate rock				
56a, fused Na_2CO_3	1.000	500	11.08[e]	11.02[a]
Not fused	1.000	500	11.09[f]	11.02[a]
Phosphate rock 120, not fused	1.000	500	7.80[f]	7.6 to 7.7[a]

[a] National Bureau of Standards certificate value.
[b] U. S. Geological Survey sample G-1; 10% F^-, as NaF, added.
[c] U. S. Geological Survey sample W-1 with 10% F^- added.
[d] National Bureau of Standards feldspar 70 with 10% fluoride added.
[e] Correction of 0.20% made on this sample only.
[f] Correction of 0.10% made on this sample only

The method of analysis outlined [addition of aluminum(III)] should give satisfactory recovery of silica for most fluosilicates encountered in nature, such as micas, amphiboles, tourmaline, topaz, phosphate rock, or any rock sample containing <10% fluorine. The procedure has not been tried on high-fluorine, low-silica samples, as, for example, fluorspar. The only change involved in the standard procedure for silica is the addition of aluminum(III) to the hydrochloric acid used for dehydration.

Fluorine must, of course, be determined on a separate sample. This determination also is easy, for the zinc oxide–sodium carbonate fusion, with subsequent solution and filtration (*2*, *5*, *6*) lowers the residual silica to such an extent that it no longer interferes in the distillation of fluorine. Addition of a few milliliters of phosphoric acid eliminates the interference of aluminum(III) during the distillation of fluorine with perchloric acid (*1*, *2*, *6*).

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Determination of Neutral Equivalents by Titration in Alcohol

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Neutral equivalents of amino acids, peptides, and peptide derivatives can be determined on 2- to 10-micromole samples by titration in alcohol or aqueous alcohol. By means of the proper indicator mixtures, sharp end points are obtained. Although the method is useful only for virtually pure compounds and not applicable to mixtures, it is precise enough to serve as adjunct to the methods usually employed in establishing the chemical purity of these substances.

IN ORDER to study the kinetics of enzymic proteolysis, methods have been devised to determine amino acids by titration in alcohol (*1*) using thymolphthalein as indicator. The success of such methods is impaired by absorption of atmospheric carbon dioxide; the end point (colorless to light blue) is often masked by turbidity due to precipitation of enzyme and buffer salts, and comparison with a color standard at the end point is difficult. These disadvantages prompted a reinvestigation of the procedure, and resulted in the development of a method which is suitable for the titration of carboxyl groups and acid bound by the amino group of amino acids, peptides, and their derivatives. Atmospheric carbon dioxide is excluded and indicator mixtures, rather than single indicators, produce sharp end points even in slightly turbid or colored solutions.

Forty-five indicators and indicator mixtures have been studied in order to find some whose color change is sharp enough to be applicable to titration of weak acid and basic groups. The following groups have been titrated so far under the conditions described: carboxyl, α-amino.*X*, δ-amino.*X*, ε-amino.*X*, imidazol.*X*, phenol, and sulfonate (.*X* stands for bound HCl, HBr, HI, H_2SO_4, etc.). This method is not suitable for the determination of guanido.*X*, however.

The sharpest end points were obtained by the following indicator mixtures: (A) 3 ml. of 0.1% phenosafranine in 40% ethyl alcohol plus 4 ml. of 0.1% *m*-cresol purple in 95% ethyl alcohol; (B) 3 ml. of 0.1% ethylbis-(2,4-dinitrophenyl)-acetate in methanol and 1 ml. of 0.1% phenol red in 95% ethyl alcohol; (C) 3 ml. of 0.1% ethylbis-(2,4-dinitrophenyl)-acetate in methanol and 1 ml. of 0.1% *m*-cresol purple in 95% ethyl alcohol; (D) equal parts of 0.1% solutions of *o*-cresolphthalein and aminoazotoluene in 95% ethyl alcohol; (E) equal parts of 0.1% solutions of tropaolin 000 and *o*-cresolphthalein in 95% ethyl alcohol; (F) equal parts of 0.1% phenosafranine in 40% ethyl alcohol and 0.1% thymol blue in 95% ethyl alcohol; and (G) equal parts of 0.1% ethylbis-(2,4-dinitrophenyl)-acetate in methanol and 0.1% cresol red in 95% ethyl alcohol. The proper choice of indicator mixture will often depend upon an individual's color perception, and for this reason, mixture A was employed as most suitable in this study.

Table I. Neutral Equivalents of Amino Acids and Peptides and Their Derivatives

(Mixture A as indicator)

Compound	Groups Titrated	Neutral Equivalent		Recovery, %
		Calculated	Found	
Glycine	1(COOH)	75.05	74.7	100.5
L-Tyrosine	1(COOH)	181.09	179.8	100.7
L-Tyrosine amide	1(OH)	180.09	181.2	99.4
Diiodo-L-tyrosine	2(COOH, OH)	216.51	214.6	100.9
L-Aspartic acid	2(COOH)	66.53	67.1	99.2
L-Lysine.HCl	2(COOH), HCl)	91.29	91.6	99.7
L-Arginine.HCl	1(COOH)	210.59	209.3	100.6
Taurine	1(SO_3H)	125.14	125.4	99.8
L-Glutamic acid gamma ethyl ester.HCl	2(COOH, HCl)	105.32	105.8	99.5
Acetyl-L-alanine	1(COOH)	131.13	131.7	99.6
Benzoyl-L-lysine methyl ester.HCl	1(HCl)	300.77	301.0	99.9
Tricarbobenzoxylysyllysine (LD)	1(COOH)	677.4	677.5	100.0
Carbobenzoxyglycylalanylalanine (LD)	1(COOH)	351.3	350.3	100.3
Dicarbobenzoxyalanyllysine (DL)	1(COOH)	486.44	487.0	99.8
δ-Carbobenzoxy-L-ornithine methyl ester.HCl	1(HCl)	316.77	311.9	101.5
Lysyllysineamide.3HCl (LL)	3(HCl)	127.55	127.6	100.0
Alanylalanine (LD)	1(COOH)	160.18	161.0	99.5
Alanyllysine.HCl (DL)	2(COOH, HCl)	126.87	127.4	99.6
Lysyllysine.2HCl.H_2O (LL)	3(COOH, HCl)	121.77	120.6	100.9
Lysyllysyllysine.3HCl (LLL)	4(COOH, HCl)	128.22	129.1	99.3

One milliliter of solution containing 2 to 10 micromoles of substance is placed in a 25-ml. Erlenmeyer flask. Ten milliliters of absolute ethyl alcohol are added, followed by 5 drops of indicator, the flask is placed under manifold, and oil-pumped nitrogen is bubbled through the solution for about 3 minutes. The flask is then transferred to the titration rack. A buret, calibrated in 0.01 ml., is lowered into the flask, a bubbling tube is inserted, and nitrogen is passed through the solution during the titration in order to exclude atmospheric carbon dioxide. The buret is connected by means of a three-way stopper to a reservoir of 0.01*N* alcoholic potassium hydroxide. This is prepared by placing 0.6 gram of potassium hydroxide pellets into 100 ml. of absolute ethyl alcohol and stirring by means of a stream of dry nitrogen until dissolved. The solution is allowed to stand at room temperature for 2 days under nitrogen in order to allow all carbonate to settle and is then syphoned off under nitrogen through a porous plate and diluted with 9 volumes of absolute ethyl alcohol. The titer of the base is determined by titrating against either standard potassium acid phthalate or 0.01*N* hydrochloric acid, using the mixed indicator, and correcting for the indicator blank. Optimum results are obtained by chosing conditions such that the total volume of titrant does not exceed 5 ml.

In Table I are listed compounds representative of the different types.

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Photometric Determination of Chromium as Perchromic Acid in Ethyl Acetate Solution

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Chromium may be extracted with ethyl acetate and determined photometrically in the form of the blue perchromic acid. Large amounts of iron, manganese, vanadium, molybdenum, and tungsten do not interfere.

BROOKSHIER and Freund (*1*) proposed the separation of chromium from vanadium by extraction of perchromic acid with ethyl acetate, to be determined subsequently, by the diphenylcarbazide method (*2*). This procedure requires the re-extraction of the chromate with water, involving a number of manipulations that could be spared, if the perchromic acid were estimated directly in the ethyl acetate solution.

The optimum conditions for the extraction of perchromic acid were investigated by Brookshier and Freund, who found that the aqueous solution at equilibrium should have a pH of 1.7 ± 0.2, and that the ethyl acetate extractions are fairly stable up to 20° C. Their results were confirmed, and the conditions mentioned adhered to in the work described here.

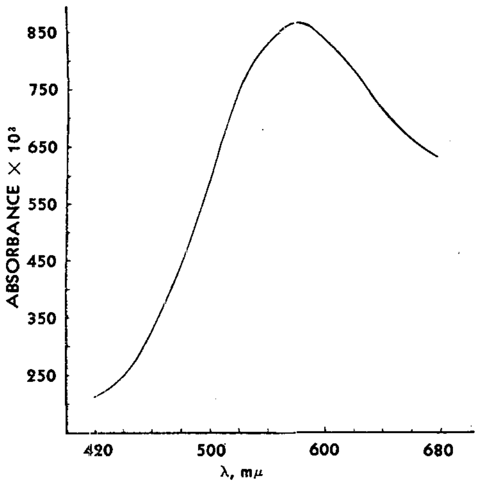

Figure 1. Absorption spectrum of a ethyl acetate solution of blue perchromic acid

Figure 1 shows the absorption spectrum of a perchromic acid solution in ethyl acetate as measured by a Unicam diffraction grating spectrophotometer (Model S.P. 350). There is a single absorption band in the visible region with a maximum at λ = 580 mμ. (The absorption spectrum obtained in a Beckman DU spectrophotometer exhibited two bands of lesser absorbance around 300 and 750 mμ, but no resolution of the main band.)

Figure 2 shows the absorbance of two series of solutions (at λ = 565 mμ) obtained by extraction from hydrochloric or sulfuric acid solution, containing varying initial amounts of potassium dichromate. The absorbances are seen to be a linear function of the concentration of the dichromate, up to and somewhat above the absorbance one, and independent of the mineral acid added. Hence, under the experimental conditions employed, the efficiency of the extraction is independent of the amount of dichromate and Beer-Lambert's law is observed.

In Table I are compared the results of a series of determinations made with known amounts of pure dichromate solutions: (1) by the direct photometric measurement of the ethyl acetate extract, and (2) by the diphenylcarbazide method, in an aliquot of the solution from a single extraction.

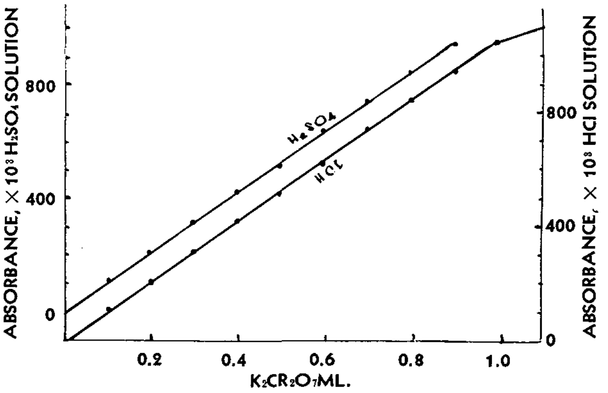

Figure 2. Absorbance of perchromic acid in ethyl acetate extracts from aqueous solutions, with varying initial concentration of dichromate

Composition of aqueous solution. *x* ml. 0.00512*M* $K_2Cr_2O_7$ + 1 ml. 0.082*M* H_2O_2 + 1 ml. 0.1*N* acid + (3-*x*) ml. H_2O

The satisfactory results of the direct method are further proof of the good reproducibility of this method. The results obtained by the diphenyl-carbazide method are a measure of the amount of chromium actually extracted by the ethyl acetate. This is in complete agreement with Brookshier and Freund's results; they obtained 96 to 97% of chromium in one extraction. Because of this, three extractions are advised for one quantitative analysis.

MATERIALS

The following solutions were used. Potassium dichromate 1.5070 grams, Baker's Analyzed, dried at 160° C. for 2 hours, and made up to 1000 ml.

Table I. Comparison of Determinations Made by Direct and Diphenylcarbazide Methods

Initial Amount of Cr, Mg.	Chromium by Direct Method		Chromium by Diphenylcarbazide Method	
	Mg.	%	Mg.	%
0.053_{26}	0.053_{34}	100.2	0.051_{54}	96.8
0.206_5	0.105_7	99.2	0.102_5	96.3
0.159_8	0.161_1	100.8	0.155_2	97.1
0.213_0	0.212_8	99.9	0.205_8	96.6
0.266_3	0.264_7	99.4	0.255_4	95.9

Hydrogen peroxide, 100 volume M. & B. "for analysis," diluted as required and determined daily by titration with standard $0.1N$ potassium permanganate.

Ethyl acetate, Baker's Analyzed.

PROCEDURE

The solutions were measured out from microburets into 50-ml. stoppered flasks. For each 5 ml. of the total aqueous solution, 6 ml. of ethyl acetate were added. The flasks were cooled to about 10° C., then hydrogen peroxide was introduced last, and the solutions were well mixed for 30 seconds. The liquid layers were allowed to separate for 2 minutes, whereupon an aliquot of the blue perchromate solution was transferred by pipetting into the spectrophotometer tube, and its absorbance was compared with that of the pure solvent.

One milliliter of the same sample solution was simultaneously taken for the diphenylcarbazide determination, decomposed by potassium hydroxide, extracted with water, and further treated as in (*2*).

Lastly, the direct method was tested by two analyses of alloy steel from Bureau of Analyzed Samples, Ltd. The samples were certified to have the percentage composition:

	C	Si	S	P	Mn	Ni	V	Mo	W	Cr
I	0.34	0.25	0.036	0.014	0.64	2.59	..	0.43	..	0.75
II	0.80	..	..	..	..	..	1.57	4.11	5.66	4.40

The percentage of chromium found by the photometric estimation of the blue perchromic acid was:

I	II
0.712 ± 0.008%	4.24 ± 0.04%

Identical results were obtained whether or not the iron was precipitated by ammonia before extraction.

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Radiochemical Separation of Actinium and Its Daughters by Means of Lead Sulfate

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A radiochemical separation of actinium-227 and its daughter elements from rat urine was required as an analytical method in the determination of these radioactive elements. A method is described in which actinium and its decay products are separated from urine salts on precipitated lead sulfate. Approximately 90% of the radioactivity in rat urine can be recovered by coprecipitation on lead sulfate in hot solutions. The lead is removed by precipitation with hydrogen sulfide, leaving the activity in solution. The actinium fraction is free of interfering salts except for a small quantity of urine salts coprecipitated with the lead sulfate. The method requires about 8 hours.

IN THE study of the effect of actinium-227 and its daughter elements on rats, a reliable radiochemical separation of these elements from rat urine was needed. Data obtained from such an analytical procedure would be of material assistance in ascertaining the ratio of radioactivity administered into a rat to that excreted daily through the kidneys.

Lead sulfate has been used as a carrier for radium in solution. Ames and others (*1*) described a method for the determination of small amounts of radium in uranium ores by the separation of the radioactivity on precipitated lead sulfate. This method was later adopted, with slight modifications, for the separation of radium from large volumes of human urine (*7*).

The technique of using lead sulfate as a carrier for actinium-227 was reported by McLane and Peterson (*5*). They stated that over 98% of a carrier-free actinium tracer is precipitated with 1 gram of lead per liter of $6M$ sulfuric acid.

Lead sulfate has been used as a carrier for actinium-227 only in relatively salt-free solutions. The presence of concentrated rat urine salts tends to contaminate the lead sulfate precipitate and makes it difficult to obtain quantitative separations.

The major components found in digested rat urine are sodium, potassium, and magnesium chlorides. As the rats are maintained on a controlled diet, the chemical composition of the urine remained relatively stable during the course of this work. Composition of a sample of typical rat urine is presented in Table I.

Table I. Composition of Digested Rat Urine Salts

Element	%
Potassium[a]	38.0
Sodium[a]	7.5
Chlorine	27.5
Magnesium	1.5
Iron	0.5
Calcium	0.1
Phosphates	5.5
Sulfates	1.5

[a] Flame photometer.

Silicon and boron, in moderate amounts, are also present in digested rat urine. These two elements are probably leached from the glassware during the digestion period.

PROCEDURE

To a 3-day sample of rat urine (approximately 120 ml.) in a 250-ml. boiling flask, add 60 ml. of concentrated nitric acid. Enclose the flask in an electric heating mantle and evaporate the contents nearly to dryness. Add small portions of nitric acid, with repeated evaporations, until the solution is clear and the residual urine salts are white. Finally, evaporate the contents of the flask to dryness.

Dissolve the dried urine salts in the flask with 10 to 15 ml. of dilute nitric acid and transfer the contents quantitatively to a clean 150-ml. beaker. Evaporate the salts to dryness and take up the residue in 100 ml. of $0.1N$ nitric acid. Slowly add 3 ml. of concentrated sulfuric acid to the solution. Heat the solution to 75° C. on a hot plate with mechanical stirring, and add dropwise 100 mg. of lead nitrate solution (0.5 ml. of a solution prepared by dissolving 32 grams of lead nitrate in 100 ml. of distilled water). Stir the lead sulfate precipitate for 30 minutes with the temperature maintained at 75° C. Rinse the stirring rod and thermometer with sufficient water to bring the volume of the solution to 100 ml. Cover the beaker with a watchglass and let the precipitate digest at 60° to 65° C. for at least 4 hours, or preferably, overnight. Stir occasionally.

Cool the beaker and contents to room temperature, transfer to a centrifuge tube, and centrifuge. Rinse the beaker thoroughly with portions of the supernatant liquid, add to the centrifuge tube, and centrifuge until the supernatant liquid is clear. If there are floating particles on top of the solution add a drop of 5% aerosol (5% aqueous solution of Aerosol-OT, dioctyl sulfosuccinate) and centrifuge again. Discard the supernatant liquid which contains the inactive urine salts. The major portion of the radioactivity has been carried with the lead sulfate. Set aside the original 150-ml. beaker for use in the next step.

Add 15 ml. of concentrated hydrochloric acid to the lead sulfate precipitate. Heat on a steam bath with occasional stirring until the precipitate is dissolved and then evaporate to dryness. Add dilute hydrochloric acid slowly to the residue until it dissolves and transfer to the original 150-ml. beaker with distilled water. The total volume of the solution in the beaker should be approximately 50 ml. Adjust the hydrogen ion concentration of the solution with dilute ammonium hydroxide so that it is weakly acid (pH 3.0 to 3.5) to alkacid paper. Heat almost to boiling and bubble hydrogen sulfide gas into the solution until it becomes saturated. Add 50 ml. of cold distilled water and continue to pass in hydrogen sulfide until the solution is clear and the precipitate settles to the bottom of the beaker. Centrifuge and then wash the precipitate twice with 5-ml. portions of hydrogen sulfide water. Dissolve the lead sulfide precipitate in a little hot concentrated hydrochloric acid, evaporate the resultant solution to dryness, redissolve in a small quantity of dilute hydrochloric acid, adjust the pH, and reprecipitate as before. After the reprecipitated lead sulfide has been washed with hydrogen sulfide water, discard the precipitate. Combine the two supernatant solutions, heat almost to boiling and test for completeness of precipitation by saturating the solution with hydrogen sulfide gas. Evaporate the combined solution to dryness.

Rinse the sides of the beaker containing the residue with about 10 ml. of concentrated nitric acid. Reduce the volume of the solution to 2 to 3 ml. by evaporation. Cool, and rinse the sides of the beaker again with nitric acid, followed by a second rinsing with distilled water. Again reduce the volume by evaporation to 2 to 3 ml. Transfer the solution quantitatively to a clean 10-ml. volumetric flask, using small portions of distilled water to wash the contents of the beaker into the flask.

Fill the volumetric flask to the mark and mount an appropriate aliquot on a stainless steel disk, the mount being spread over as large an area as possible to reduce self-absorption. For best results, the volume of the mounted aliquot should be selected so as to produce an alpha count within the range of 1000 to 50,000 counts per minute. When the mount is nearly dry, lower the heat lamp and heat strongly until all excess sulfuric acid has been volatilized. Carefully ignite the mounted sample in the flame of a Bunsen burner. Set the ignited sample aside for 3 to 4 hours to permit the short-lived daughters to attain equilibrium and then submit the sample for alpha counting.

EXPERIMENTAL DATA

In preliminary work, lanthanum-140 (40-hour half life) was used as a stand-in for actinium to determine whether lead sulfate could be used effectively to carry actinium and its daughter products quantitatively from the bulk of rat urine salts. In these experiments, the lead sulfate was precipitated at room temperature and digested in an ice bath as recommended by Russell, Lesko, and Schubert (*7*) in their work on the determination of radium in exposed humans. The results of these experiments, (Table II) as determined by beta counting, were low and inconsistent.

Table II. Adsorption of Lanthanum-140 on Precipitated Lead Sulfate[a]

Sample	Activity Found, %	
	Precipitate[b]	Filtrate
1	24	76
2	71	29
3	55	45
4	68	32

[a] Precipitated at 25° C., digested at 4° C.; 0.5 mg. Pb/ml. of solution.
[b] Calculated.

McLane and Peterson (*5*) reported that barium sulfate precipitates adsorbed 96% of an actinium tracer at 85° C. Because of the similarity of chemical properties of barium and lead sulfates it seemed very probable that lead would exhibit higher adsorptive qualities if precipitated in hot solutions. Accordingly, a number of experiments were conducted in which the lead sulfate was precipitated at 75° C. and digested for at least 4 hours at 60° to 65° C. The actinium and its daughter elements were carried quantitatively on precipitated lead sulfate from urine salts in hot solution. The lead sulfate precipitate was set aside for further work and aliquot samples of the filtrate were counted to determine the amount of radioactivity lost through removal of urine salts (Table III).

Table III. Loss of Activity in Filtrate Following Lead Sulfate Precipitation[a]

Sample	Activity Loss, %
1	0.52
2	2.30
3	0.18
4	0.31
5	0.21
6	0.60
7	0.15
8	2.00
9	0.38
10	1.20
Av.	0.77

[a] Precipitated at 75° C., digested at 60–65° C., 1 mg. Pb/ml. of solution.

The effect of varying the concentration of lead is shown in Table IV. The optimum quantity of lead for adsorption appears to be 1 mg. per ml. of solution. Increasing the amount of lead to 2 mg. per ml. does not appreciably alter the adsorption of the precipitated lead sulfate toward actinium. Decreasing it to 0.5 mg. per ml. does, however, correspondingly decrease the quantity of radioactivity carried. The data presented in Table IV record the loss of activity to the filtrate.

Table IV. Effect of Varying Concentration of Lead[a]

Concn. Lead, Mg./Ml.	Loss of Activity to Filtrate, %
0.5	12.10
0.5	9.75
1.0	0.52
1.0	0.15
1.0	0.38
2.0	0.18
2.0	0.31
2.0	0.21

[a] Precipitated in hot solution.

The lead may be separated from the radioactivity by electrolysis (*4*). In this method, lead is plated as the dioxide on the anode from a dilute nitric acid solution. The lead sulfide method is recommended, however, because in the authors' experience a more quantitative separation is obtained and the weight of the dissolved solids remaining in the final filtrate, which contains the activity, is considerably less. The weight of the salt content of the filtrate, after evaporation to dryness, was only 25 to 30 mg. in an average sample. By comparison, the total weight may run as high as 50 to 100 mg. per sample when the lead is removed electrolytically. In either case, the lead is almost completely removed and carries less than 2% of the radioactivity (Table V).

Table VI shows the total activity recovered from rat urine with a single lead sulfate precipitation. Varying amounts of carrier-free actinium-in-equilibrium were added to the urine to produce between 5700 and 4,800,000 alpha counts per minute. The actinium was added directly to the raw rat urine sample prior to digestion with concentrated nitric acid.

Table V. Loss of Activity by Removal of Lead Salts

Sample	Activity on Lead, %
Lead Sulfide Pptn.	
1	1.96
2	2.30
3	0.41
4	1.88
5	1.65
6	2.10
7	1.95
Av.	1.75
Electrolysis	
8	0.34
9	0.75
10	2.10
11	0.51
12	2.30
13	0.71
Av.	1.12

Table VI. Total Activity Recovered from Rat Urine with Lead Sulfate[a]

Total Activity, Counts per Minute		Activity Lost[b], %	Activity Recovered, %
Added	Recovered		
4,800,000	4,358,000	3.80	91
4,500,000	3,812,000	2.82	85
4,500,000	3,932,000	0.59	87
4,500,000	3,954,000	2.19	88
900,000	784,000	1.69	87
900,000	775,000	2.31	86
900,000	763,000	2.56	85
900,000	792,000	0.49	88
225,000	206,800	2.75	92
66,000	59,600	0.89	90
66,000	56,400	2.40	86
21,000	19,640	3.40	94
21,000	20,400	1.28	97
5,700	5,020	[c]	88
5,700	5,825	[c]	102
5,700	5,120	[c]	90

[a] Precipitated in hot solution.
[b] Combined losses of urine and lead salts.
[c] Represents alpha counts below accurate range of counting instruments used.

Approximately 90% of the actinium-in-equilibrium originally added to the rat urine was recovered in the final filtrate (Table VI). The combined losses of activity due to the separation of the urine and lead salts amounted to a little more than 2%. The discrepancy between the starting alpha counts and total alpha counts found (activity in the final filtrate plus the combined losses in the urine and lead salts) is due to sorption on glassware, inherent errors in the counting instruments, and self-absorption.

Self-absorption appeared to be of little significance except in the residual or discarded fractions.

In the case of separated urine salts, the solutions contained very small amounts of radioactivity and a high concentration of solids (approximately 3 grams per sample), making it necessary to prepare mounts of appreciable thickness. The weight of a typical mounted sample varied from 3 to 6 mg. per sq. cm., depending on the volume of the fraction required to obtain a mount of sufficient activity for accurate counting. Reference to Figure 1 (*2*) shows that 54 to 75% of the alpha radiation in these deposits would be counted by a proportional counter, the remainder being absorbed within the sample itself. While this may appear to be a significant error, actually it is of little importance if it is taken into consideration that only slightly more than 1% of the total activity is represented. The same is true of the separated lead fraction, only to a lesser degree, as in this case a mounted sample would weigh 0.5 mg. per sq. cm. or less.

In contrast, sample mounts of the final radioactive filtrate weigh less than 0.1 mg. per sq. cm., constituting a loss of not more than 2 to 3% due directly to self-absorption (Figure 1).

A study was made to determine which of the three major activities of actinium-in-equilibrium was being lost through chemical separations. Samples of both urine salts and separated lead carrier were counted at regular intervals for 30 or more days. A plot showing the growth and decay curves of the activity in both the urine salts and lead carrier tends to follow very closely pure thorium-227. This indicates that nearly all of the activity lost through chemical separations is thorium-227.

DISCUSSION

Actinium-227 is a beta emitter with a 22-year half life. Its principal decay products are thorium-227 (18.6-day half life), radium-223 (11.2-day half life), and a number of short-lived products including actinon or radon, bismuth, polonium, and lead isotopes. Since the beta rays are extremely weak, (0.02 m.e.v.), the alpha radiation is utilized for counting purposes although it comprises only 1.20% (*6*) of the total radiation. Both thorium-227 and radium-223 are alpha emitters.

Purified actinium grows rapidly for a number of weeks, owing to the formation of its daughter products, and then comes to equilibrium at the end of 185 days. At the end of this period, actinium decays according to its 22-year half life.

The use of specific carriers employed with precipitation methods for the determination of actinium-227 and its daughters gave incomplete separations due to the presence of urine salts. The removal of total activity from the bulk of these salts was found to be a practical method.

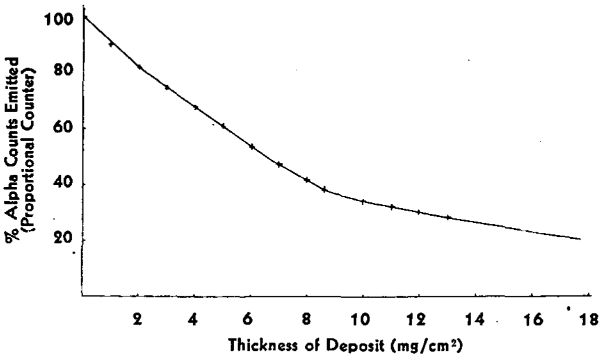

Figure 1. Alpha absorption of radioactive (actinium-227 in equilibrium) rat urine salts (dried at 700° C. on slide)

Individual activities may be determined by application of the data obtained from alpha counting. The authors found the technique of differential decay (*3*) to be an excellent method of determining individual activities using data obtained from counting procedures. Briefly, it consists of substituting known data in simultaneous equations and solving for the one possible combination of activities and the proportion of each present. In the work on actinium, the final filtrate was essentially in equilibrium with its daughters and it was found expedient to break the chain so that the counting period could be shortened. This was accomplished by the removal of thorium-227, the first important daughter, by homogeneous precipitation with a suitable carrier, as the iodate (*8*).

ACKNOWLEDGMENT

Special acknowledgment is made to J. J. Dauby, Mound Laboratory, who carried out the absorption studies reported in this paper. The authors also wish to acknowledge the assistance and many helpful suggestions of S. R. Barth, L. L. Bentz, and R. B. Smith.

The authors are indebted to French Hagemann, Argonne National Laboratory, who supplied the actinium-227 used in this work.

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Colorimetric Determination of Low Concentrations of Oxygen in Gases

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In order to ascertain the effects of small amounts of oxygen on the polymerization of various hydrocarbon gases, a rapid and accurate method was required for the determination of oxygen in these gases in the range of 0 to 100 p.p.m. The sodium anthraquinone-β-sulfonate colorimetric method has been applied successfully to the determination of traces of oxygen in gases including nitrogen, butadiene, butane, ethylene, and propylene. Analyses of synthetic samples of known oxygen content indicate an accuracy of ±7% in the range of 10 to 50 p.p.m. Hydrogen, carbon dioxide, and other acidic gases, and acetylene do not interfere. Carbon monoxide interferes if present in large amounts. The apparatus is simple and inexpensive and can be made portable. Six samples can be analyzed in an 8-hour period. The method is adaptable to continuous, automatic instrumentation.

In order to ascertain the effects of small concentrations of oxygen on the polymerization of various hydrocarbon gases, a rapid and accurate method was required for the determination of oxygen in these gases in the range of 0 to 100 p.p.m.

The Brady (*1*) method for determining small amounts of oxygen by spectrophotometric measurement of the bleaching effect of oxygen on the red color of the reduced form of sodium anthraquinone-β-sulfonate has been applied successfully in this laboratory. In using this method over the past 4 years several refinements have been made which greatly increase the accuracy, precision, and convenience of the determination: a more efficient electrolytic cell for calibration, inclusion of a caustic scrubber for prior removal of acid gases, and a more efficient packing for the gas scrubbers.

Although Brady (*1*) stated that hydrocarbons do not affect the analysis, he did not give any data for oxygen content of hydrocarbon gases. Data are presented in this paper for typical analyses of butadiene, butane, ethylene, and propylene, in which the oxygen content varied from less than 1 p.p.m. to several hundred parts per million. Accuracy of the procedure as applied to hydrocarbon gases is indicated by analyzing 10 known blends of oxygen in ethylene, ranging in oxygen content from 10 to 50 p.p.m.

Brady indicated that he successfully determined oxygen in mixtures of carbon monoxide, carbon dioxide, and nitrogen. In the present work it was found that either carbon monoxide or carbon dioxide in a hydrocarbon sample interferes if present in very large amounts. The interference of carbon dioxide was eliminated by inclusion of a caustic scrubber ahead of the oxygen reagent. Because interference by carbon monoxide could not be circumvented easily, several experiments were made to ascertain the amount that can be present in a hydrocarbon gas sample without causing serious interference.

APPARATUS

The assembly is shown in Figure 1. A second nitrogen scrubber was found to be necessary because some cylinders of commercial

Table I. Analyses of Ethylene of Known Oxygen Content

Sample	Oxygen, P.P.M. by Weight		Deviation	
	Contains[a]	Found	P.p.m.	%
A	12	13	+1	8.3
		12	0	0.0
		13	+1	8.3
B	13	12	−1	7.7
C	20	18	−2	10.0
		20	0	0.0
		19	−1	5.0
		18	−2	10.0
D	20	22	+2	10.0
		21	+1	5.0
		22	+2	10.0
		21	+1	5.0
E	21	20	−1	4.8
		20	−1	4.8
		24	+3	14.3
		23	+2	9.5
		23	+2	9.5
F	25	24	−1	4.0
G	25	25	0	0.0
H	32	37	+5	15.6
		35	+3	9.4
J	32	31	−1	3.1
		35	+3	9.4
		34	+2	6.2
K	53	55	+2	3.8
		53	0	0.0
		58	+5	9.4
		Av.	±1.7	6.8

[a] These values include 2.4 p.p.m. of oxygen which the ethylene contained originally.

"prepurified" nitrogen contain as much as 80 p.p.m. of oxygen. Nichrome Helipak packing was substituted for glass beads in the scrubbers.

PROCEDURE

The apparatus is assembled as shown in Figure 1 and calibrated by bringing known quantities of oxygen, generated electrolytically, into contact with a definite volume of reduced reagent and measuring the change in color of the reagent. Analysis of a sample is accomplished by bringing a measured volume of the sample gas into contact with the reduced reagent and measuring the change in color of the reagent. For samples high in oxygen content a flow of nitrogen through the system is used to dilute the sample before it contacts the reduced reagent.

Table II. Typical Analyses for Oxygen in Gases

Sample			Oxygen, P.P.M. by Weight	Deviation from Mean	
No.	Description			P.p.m.	%
A	*n*-Butane		0.50	+ 0.02	4.2
			0.43	− 0.05	10.4
			0.49	+ 0.01	2.1
			0.48	0.00	0.0
		Av.	0.48		
B	Butadiene		2.9	− 0.2	6.5
			3.3	+ 0.2	6.5
		Av.	3.1		
C	Butadiene		4.2	− 0.4	8.7
			4.6	0.0	0.0
			4.7	+ 0.1	2.2
			4.8	+ 0.2	4.3
		Av.	4.6		
D	Butadiene		8.9	− 0.2	2.2
			9.2	+ 0.1	1.1
		Av.	9.1		
E	Nitrogen		48	− 3	5.9
			49	− 2	3.9
			53	+ 2	3.9
			52	+ 1	2.0
		Av.	51		
F	Butadiene		70	− 4	5.4
			78	+ 4	5.4
		Av.	74		
G	Nitrogen		150	− 6	3.8
			164	+ 8	5.1
			155	− 1	0.6
		Av.	156		
H	Propylene		279	+ 5	1.8
			269	− 5	1.8
		Av.	274		
J	Ethylene		675	+31	4.8
			620	−24	3.7
			630	−14	2.2
			650	+ 6	0.9
		Av.	644		

ACCURACY AND PRECISION

Twenty-seven determinations on 10 samples of known oxygen content, as shown in Table I, indicate an accuracy of 6.8% relative in the range of 10 to 50 p.p.m. Samples of known oxygen content were prepared by blending measured volumes of air with ethylene containing 2.4 p.p.m. of oxygen.

The data in Table II give an indication of the precision of the method in analyzing several different gases in which the range of oxygen content varied from less than 1 p.p.m. to several hundred parts per million.

DISCUSSION

One of the authors' first observations in using this method was that the efficiency of the gas scrubbers was poor when a packing of glass beads was used, probably because of channeling effects. Consequently, the glass beads were replaced by Helipak packing. The scrubbing efficiency was improved, as indicated by a greater sensitivity of the reagent to small amounts of added oxygen.

Additional difficulty was experienced during the first several months in obtaining good calibration data. As bubbles of oxygen could be observed sticking to the walls of the electrolytic cell, a mechanical vibrator was used to help remove the bubbles. This technique facilitated the obtainment of apparent good calculation data, but the data were not reproducible on successive days. Consequently, the electrolytic cell was redesigned so that the nitrogen carrier gas could be made to flow through the cell (see Figure 1) and to sweep out the generated oxygen quickly and thoroughly. Calibration data obtained with the redesigned cell indicated that all of the generated oxygen was not being removed from the original cell, as shown by a greater change in the color of the reagent for a given quantity of oxygen. Using the redesigned cell the calibration data are reproducible from day to day.

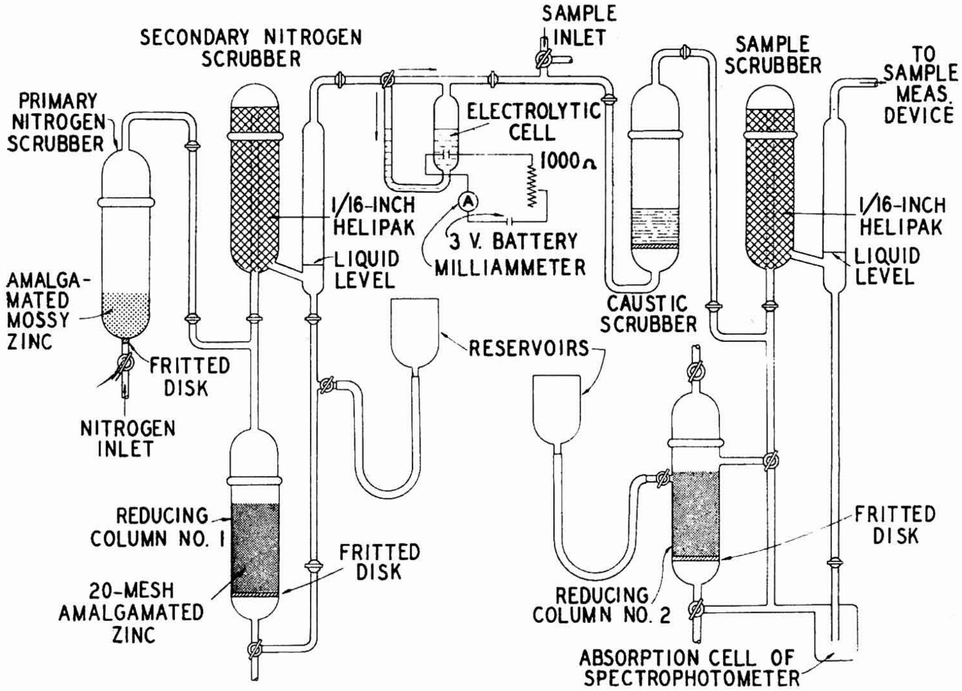

Figure 1. Diagram of apparatus

As shown in Table III, a large excess of acetylene or hydrogen does not decrease the accuracy of the oxygen determination by this method. Carbon monoxide does not interfere unless the ratio of carbon monoxide to oxygen is greater than about 10 to 1. When the carbon monoxide to oxygen ratio is 15 to 1, the oxygen results are high and erratic. When the carbon monoxide to oxygen ratio is raised to 20 to 1, the results are high and erratic and the reagent is soon destroyed. Data in Table III also show that a large amount of carbon dioxide interferes seriously unless it is removed from the sample gas prior to entering the oxygen scrubber. After three determinations of oxygen had been made in a sample containing 5% of carbon dioxide, the sodium anthraquinone-β-sulfonate reagent was destroyed.

Table III. Effects of Various Possible Interferences on the Determination of Oxygen in Ethylene

Substance	Amount Added	Oxygen, P.P.M. by Weight Present	Found	Deviation
Acetylene	Large excess	13	12	− 1
		25	24	− 1
Hydrogen	Large excess	25	25	0
Carbon monoxide	26 p.p.m.	20	19[a]	− 1
Carbon monoxide	80 p.p.m.	20	22[a]	+ 2
Carbon monoxide	200 p.p.m.	21	22[b]	+ 1
			43	+23
Carbon monoxide	290 p.p.m.	20	31	+11
			34	+14
			27	+ 7
Carbon dioxide (with caustic scrubber)	5%	13	13	0
Carbon dioxide (without caustic scrubber)	5%	13	70	+57
			75	+62
			99	+86
			[c]	..

[a] Average of four determinations.
[b] Average of five determinations.
[c] Reagent destroyed after three determinations.

The apparatus can be constructed easily by a competent glassblower, and can be made portable. Six samples can be analyzed by this method in an 8-hour day. The method has been adapted by Karasek and others (*2*) to continuous, automatic analyzer, for monitoring trace amounts (0 to 500 p.p.m.) of oxygen in gases.

ACKNOWLEDGMENT

The authors are grateful to Phillips Petroleum Co. for making this work possible, and to those members of the Research and Development Department who gave assistance during various phases of this work.

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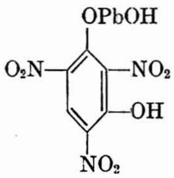
CRYSTALLOGRAPHIC DATA

101. Lead Styphnate (Normal)

Contributed by W. C. McCRONE and O. W. ADAMS, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula for Normal Lead Styphnate

Normal lead styphnate is best prepared by precipitation from a solution containing styphnic acid (2,4,6-trinitroresorcinol) and lead nitrate by adding sodium hydroxide. Excellent crystals of the normal salt (Figure 1) were obtained by preparing a solution of 1 × 10^{-3} mole of styphnic acid and 2 × 10^{-3} mole of lead nitrate in 200 ml. of 50% aqueous ethyl alcohol and adding dropwise with efficient agitation a 0.0917*N* solution of sodium hydroxide. Well formed crystals of the pure normal salt are precipitated throughout the pH range up to about 3.6. As the solution becomes more basic than pH 3.6, other lead salts begin to appear.

Principal Lines

d	I/I_1	Index[a]	*d*	I/I_1	Index[a]	*d*	I/I_1
7.82	10	110	2.76	3	321	1.872	1
6.80	1	011	2.69	1	003, 240	1.836	1
6.32	7	020, 101	2.63	1	330, 013	1.809	1
5.71	7	111	2.55	6	113, 050	1.773	3
5.33	1	120	2.52	1?	400, 312, 232, 241	1.748	1?
5.01	2	200, 021	2.48	1?	410, 331, 042, 023	1.723	1
4.67	2	210	2.41	7	401, 123, 142, 051	1.677	1
4.41	8	121	2.33	1?	420, 213, 151	1.609	2
4.17	4	030	2.29	4	340	1.562	1
4.00	8	002	2.25	7	250, 421, 033	1.507	1
3.86	1	130	2.21	1?	223, 133, 242	1.476	1
3.72	7	102, 031	2.16	3	430, 251	1.381	1
3.54	8	221	2.10	4	060, 303, 412, 152	1.318	3
3.37	7	300, 022	2.06	1	160, 313, 233	1.277	2
3.23	6	310, 230	2.02	4	004, 350, 422, 500	1.257	2
3.06	4	212	1.979	5	...	1.231	2
2.95	9	320, 041	1.932	2	...	1.206	1
2.81	5	222, 141, 132	1.905	1	...	1.109	1

[a] There are other reflections which might contribute to these lines and the indicated indices are suggested only as possible contributing reflections.

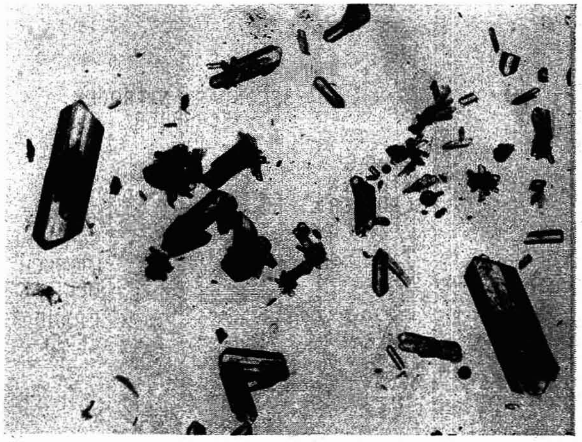


Figure 1. Typical crystals of normal lead styphnate precipitated from aqueous ethyl alcohol solution of styphnic acid and lead nitrate by sodium hydroxide

Normal lead styphnate is very insoluble in most of the usual solvents; hence is usually studied as first precipitated. Many solvents in which this compound dissolves hot—e.g., ethylene glycol—form crystalline solvates on cooling.

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. As precipitated from styphnic acid and lead nitrate by sodium hydroxide rods elongated parallel to *a* are usually formed. Slight flattening, so that many crystals lie on the 001 face, is usually observed. Other forms shown are: prism (110), clinodome (011), and the orthodome (101).

Axial Ratio. $a:b:c$ = 0.800:1:0.640. 0.799:1:0.638 (*2*).

Interfacial Angles (Polar). 110 Λ $1\bar{1}0$ = 102°. 011 Λ $01\bar{1}$ = 65.4°; 65° (*2*).

Beta Angle. 91.9°; 93 (*2*).

X-RAY DIFFRACTION DATA

Cell Dimensions. a = 10.06 A.; b = 12.58 A.; c = 8.05 A. a = 10.02A .; b = 12.54 A.; c = 8.00 A. (*2*).

Formula Weights per Cell. 4 (4.05 calculated from x-ray data).

Formula Weight. 468.31.

Density. 3.095 (flotation in CH_2I_2–CCl_4); 3.055 (x-ray).

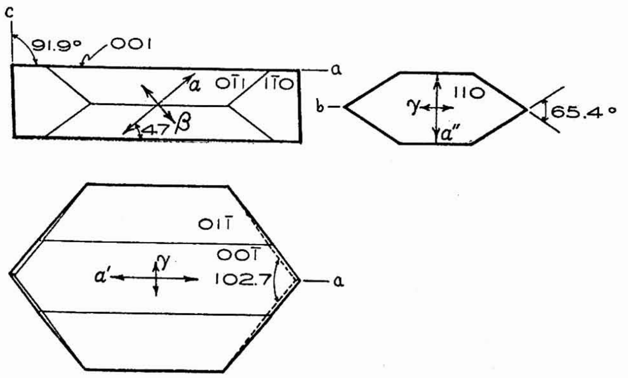


Figure 2. Orthographic projection of typical crystal of normal lead styphnate

OPTICAL PROPERTIES

Refractive Indices (5893 A.; 25° C.). $\alpha = 1.554 \pm 0.002$. $\beta = 2.20 \pm 0.03$. $\gamma = 2.22 \pm 0.03$. α' (in 001) = 1.833 ± 0.01.

Optic Axial Angles (5893 A.; 25° C). $2E = 57°$. $2V = 25°$.

Dispersion. $r > b$, very strong.

Optic Axial Plane. 010.

Sign of Double Refraction. Negative.

Acute Bisectrix. $\alpha \Lambda a = 47°$ in obtuse β.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.965$; R(obsd.) = 73.9. R(calcd.) cannot be calculated, since the molar refraction for lead is not known; the partial value not including the lead is 43.2, indicating that the molar refraction of lead is about 30.

ACKNOWLEDGMENT

Some work on normal lead styphnate by McCrone at Cornell in 1944 was reported under Contract OEMsr-193 between Cornell University and the Office of Scientific Research and Development. This work has now been reviewed and completed under Contract DAI-11-022-ORD-P-18 between The Armour Research Foundation and Army Ordnance.

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(2) Miles, F. D., *J. Chem. Soc.*, 1931, 2532.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

CORRESPONDENCE

Colorimetric Determination of *N*-Trichloromethylthiotetrahydrophthalimide

SIR: It has been brought to my attention by Arnold S. Roseman, University of Massachusetts, that the captions of Figures 1 and 2 of my article on "Colorimetric Determination of *N*-Trichloromethylthiotetrahydrophthalimide" [ANAL. CHEM., **24**, 1173–5 (1952)] are confusing.

The original work on this procedure was carried out using a manually operated spectrophotometer. With this instrument the absorbance peak at 425 mμ was missed and all the data in Table I and Figure 2 were obtained at 450 mμ. When a recording spectrophotometer was used, the curve shown in Figure 1 was obtained. This curve showed that a higher degree of accuracy would be realized by using 425 mμ instead of 450 mμ and therefore the lower wave length was suggested in the procedure. If an analyst were to apply this analytical procedure, he would make his own standard curve, in which case it would be best to use the 425 mμ wave length.

Possibly a more significant correction could be made regarding this article, which states: "The structure of the chromogenic reaction product has not been precisely determined, but is undoubtedly of the phthalein type that could be expected from the reaction of resorcinol with the dibasic acid portion of the SR-406 molecule." It has since been found that the color produced is the result of reaction between resorcinol and the $—SCCl_3$ group. This is substantiated by the fact that perchloromethyl mercaptan ($ClSCCl_3$) gives the same color reaction with resorcinol. Also, other compounds containing the $—SCCl_3$ group, such as *N*-phenyl *N*-trichloromethylthiomethylsulfonamide, give the same transmittance curve as SR-406 between 300 and 500 mμ when heated with resorcinol under the described conditions.

ALLEN R. KITTLESON

Esso Research and Engineering Co.
Linden, N. J.

CORRECTIONS

Far Ultraviolet Absorption Spectra of Unsaturated and Aromatic Hydrocarbons

In the article on "Far Ultraviolet Absorption Spectra of Unsaturated and Aromatic Hydrocarbons" [Jones, L. C., Jr., and Taylor, L. W., ANAL. CHEM., 27, 228 (1955)] in Figure 13 curve *A* should have been identified as *n*-decylcyclohexane.

Redox Determination of Tervalent and Total Cobalt in Presence of Excess Tungstate

In the article on "Redox Determination of Tervalent and Total Cobalt in Presence of Excess Tungstate" [Baker, L. C. W., and McCutcheon, T. P., ANAL. CHEM., 27, 1625 (1955)] in the last sentence in the first column the words "It was evident that such lower states exist in the cobalt determination" were printed twice in error. In the first paragraph on page 1626 (*2*, *6*) in the 17th line refers to Literature Cited, as do (*1*, *2*, *15*) in the 20th line, and (*1*, *2*, *6*, *15*) in the 22nd line. In reference (*6*) the volume number is **39**.

Simple Technique for Imprinting Scale of *d* Spacings on Powder Films

Stanley Ohlberg, Department of Research in Chemical Physics, Mellon Institute, Pittsburgh 13, Pa.

OTHER workers have described techniques for imprinting a scale on powder films with one of two purposes in mind. Hägg (*3*) and Barrett (*1*) have proposed techniques for imprinting millimeter scales, in which the scale is used to make film shrinkage corrections for precise determination of lattice constants. On the other hand, De Bretteville and Levin (*2*) have proposed a method for imprinting a scale of *d* spacings on powder films in order to facilitate identification. The simple technique described below is designed for this same purpose and can be set up with a minimum of time and materials.

Figure 1. Typical film with imprinted scale (*top*) and photographic template (*bottom*)

PREPARATION OF PHOTOGRAPHIC TEMPLATE

The printed side of one of the Nies scales (manufactured by N. P. Nies, 1495 Coolidge Ave., Pasadena 7, Calif., for use with cobalt, chromium, copper, iron, and molybdenum radiation with cameras of diameters of 57.3, 100, 114.6, and 143.2 mm.) is placed over a piece of unexposed 35-mm. x-ray film. In order to ensure close contact between the scale and the film, a glass plate is placed on top of the scale. A 5-second light exposure of the kind described below is sufficient to blacken that area of the film not protected by the printing on the Nies scale. The film is processed in the usual manner, care being taken to hang it horizontally while drying in order to get uniform shrinkage along the direction of the scale.

To calibrate the template for a given camera, a developed film is placed in the camera and over this an unexposed film, punched to accommodate the pinhole but not the beam stop, is fitted flush against the stationary film brace. The beam-stop hole is made light-tight, the camera is closed, and an instantaneous picture of the undeviated x-ray beam is taken. When the exposed film is removed from the camera, the approximate location of the image of the undeviated beam is marked. A drop of developer is placed over this area and when the black spot appears, the excess developer is wiped off. The distance from the center of the spot to the end of the film that fitted against the stationary film brace is measured, and is then marked off on the template from zero degrees two theta to the end that is subsequently fitted against the stationary film brace. As this measurement can be only approximately correct, a 1-mm. excess is allowed when the template is cut at this end.

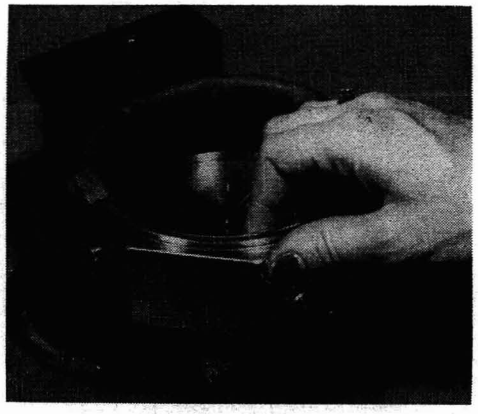

Figure 2. Template positioned in camera and two-part cover

Next a trial picture is made as described below. A 5-minute exposure using unfiltered copper radiation and a piece of copper wire as a standard sample was found satisfactory. The average

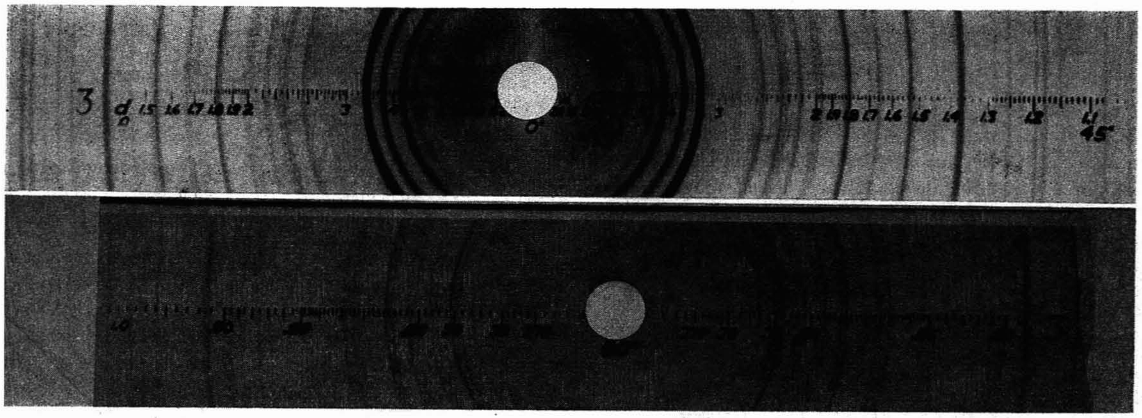

Figure 3. Typical film with imprinted scale

Cut in half for illustration

of the values for the *d* spacings read on either side of zero degrees two theta is compared with the known values for the standard sample. If the agreement is not satisfactory, the Nies scale selected was incorrect. The magnitude of this disagreement will indicate which of the remaining six scales should be selected for the preparation of a new template. The difference between two readings for the same *d* spacing in the front reflection region will indicate the length of film to be cut from the template, in order that the printed scale will be symmetrical with respect to zero degrees two theta. The dimensions of the template will change on standing unless it is stored in a desiccator. Hence, the dried template is equilibrated in a desiccator for a few days before calibration.

IMPRINTING TECHNIQUE

After the sample and film are placed in the camera, a photographic template (Figure 1, bottom) is fitted over the film with the calibrated end flush against the stationary film brace and the other end held firmly by the fingers, so that the template is pressed against the film. A two-part cover, made from any opaque material, is placed around the rim of the camera (Figure 2) to prevent blackening of the edge of the film. An overhead light (200-watt bulb, 6 feet above the camera) is flashed for about 1 second. The template is removed and the usual x-ray and photographic procedures are carried out.

Table I. Accepted (*4*) and Indicated *d* Spacings for Quartz

Front Reflection, A.		Back Reflection, A.	
Read from scale	Calcd.	Read from scale (α_1)	Calcd.
4.2+	4.25	0.915	0.915
3.35	3.34	0.839	0.840
2.12	2.13	0.804	0.804
1.66	1.67	0.786	0.786
1.37	1.37		
1.15	1.15		

A typical film with the imprinted scale is shown in Figure 1, top, and Figure 3. The sample is quartz, the *d* spacings of which are accurately known (*4*). Some of the accepted values are compared in Table I with those indicated by the scale.

ACKNOWLEDGMENT

H. P. Klug and L. E. Alexander, Department of Research in Chemical Physics, Mellon Institute, gave helpful advice and encouragement in this work. D. T. Pitman of this same department helped test the method.

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Device for Automatic Measurement of Integrated Absorbance

V. Z. Williams, V. J. Coates, and Frank Gaarde, The Perkin-Elmer Corp., Norwalk, Conn. ●

THERE is growing indication that the use of the integrated absorbance,

$$A_{\Delta\nu} = \int_{\nu_1}^{\nu_2} \ln \left(\frac{I_0}{I}\right)_\nu d\nu$$

where

ν_1, ν_2 = limits in cm.$^{-1}$
I_0 = incident radiation
I = transmitted radiation

from which can be obtained the absolute intensity coefficient,

$$\alpha_{\Delta\nu} = \frac{1}{cl} A_{\Delta\nu}$$

where

c = sample concentration
l = cell length

will be of value in the infrared for correlation of characteristic functional group bands (*1–3*) in addition to their present use for information on dipole moment (*4*). Their use for type compound analysis has been considered, and there have been two recent papers (*5, 6*) on type analysis where the use of $A_{\Delta\nu}$ as an ordinate function might have proved better than the one chosen. There has been little use of $\alpha_{\Delta\nu}$ as the basic ordinate term for multicomponent quantitative analysis, yet herein may be a major value. In comparison with the present use of absorptivity,

$$\alpha_\nu = \log_{10}\left(\frac{I_0}{I}\right)_{\nu_{\max}}$$

$\alpha_{\Delta\nu}$ would have the advantages of (1) better adherence to Beer's law where α_ν fails for lack of monochromaticity of the exit radiation beam, (2) higher accuracy and reproducibility, and (3) with proper instrumentation, a directly readable number eliminating operator errors of measurement and calculation, and the disadvantages of (1) lower ratio of diagonal to off-diagonal terms in the analytical matrix, and (2) more difficult correction for false radiation.

There are many analyses where the advantages outweigh the disadvantages.

Although the potential value of $A_{\Delta\nu}$ as an ordinate function has been recognized for some time, its study has been deterred by the extreme tedium of accurate manual measurement and calculation. Therefore, an attachment to the Perkin-Elmer Model 21 double-beam infrared spectrophotometer has been developed which will integrate the absorbance value continuously as the spectrum is being recorded over a given wave-length or frequency range and present the integrated value on a counter. A pen system is attached, so that on the abscissa border of the spectrum unit

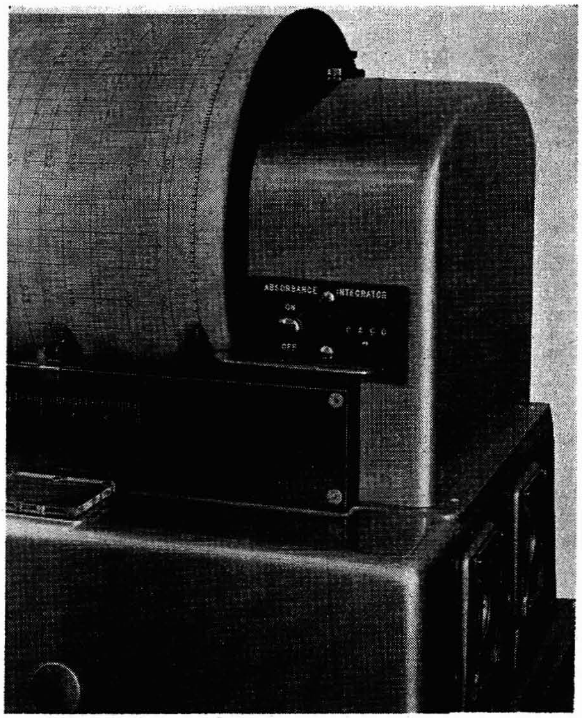

Figure 1. Absorbance integrator assembled on spectrophotometer

Four-digit reset counter reads integrated units and a separate pen marks abscissa

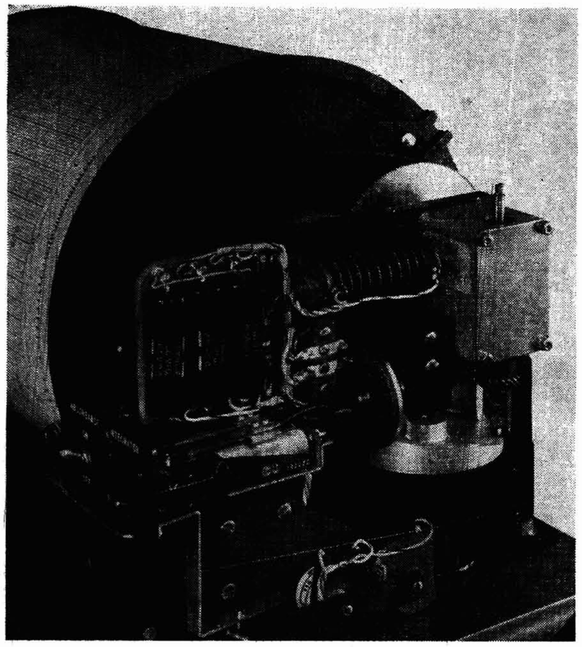

Figure 2. Integrator with cover removed to reveal mechanism

Barrel cam on pen cable drive pulley at lower right actuates ball and disk integrator, which is geared to recording drum. An electrical counter and pen marker are actuated by segmented commutator at integrated output

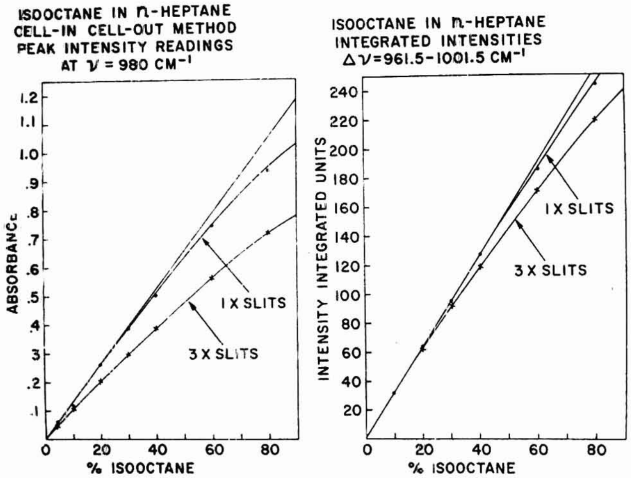

Figure 3. Beer's law adherence

Absorbance, A_ν, *vs.* integrated absorbance, $A_{\Delta\nu}$. On a typical band as of iso-octane deviation of $A_{\Delta\nu}$ is about one fourth that of A_ν

integrated values are marked by dashes. Thus, a reasonably accurate absolute intensity value can always be read from the standard spectrum. For most accurate work the difference in counter reading between ν_1 and ν_2 is used. For quantitative analysis a direct number for insertion in the calculation is available. The possible operator error in spectrum measurement, division, and log conversion is eliminated. The device is applicable to either linear wave-number or linear wave-length instruments, as $A_{\Delta\nu}$ can be converted to $A_{\Delta\lambda}$ by a known function.

The accessory is shown in Figure 1 with cover on, mounted on the Model 21. In Figure 2, the cover is removed to show the mechanism. A logarithmic barrel cam is mounted on the pulley, connected to the optical wedge whose rotation determines the transmittance position of the recording pen. A rider on this cam sets the radius position of a ball and disk integrator. The integration multiplier is geared to the recording drum rotation. The resultant integrated absorbance is presented on a four-digit high speed magnetic impulse counter actuated by a commutator. Electrical impulses from the counter actuate a marking pen on the margin of the recording paper at the proper abscissa position. The accuracy of the device (over its absorbance range of 0 to 1) is somewhat better than that of the direct measurement of peak absorbance, as a range of absorbance is involved and variations in the instrument linearity are averaged. Multiplying the counter reading by a predetermined integration constant provides intensity values in terms of absorbance × cm.$^{-1}$ or absorbance micron.

The reproducibility is excellent. Twelve readings over a 40-cm.$^{-1}$ range of a band with absorbance about 0.9 over a 24-hour period showed a spread of 1 part in 250 or ±1 part in 500. The accuracy is within 1%. These figures are based on absorbance. The accuracy and reproducibility of transmittance measurement would have to be roughly three times better to provide equivalent performance.

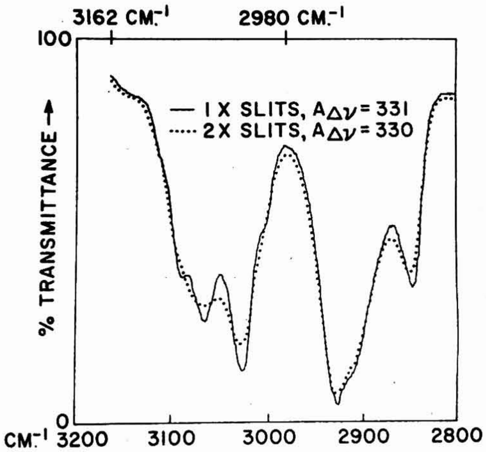

Figure 4. Effect of slit width on intensity

A_ν *vs.* $A_{\Delta\nu}$ in C—H stretching region of polystyrene. Although resolution and peak absorption values change appreciably, integrated absorption remains constant

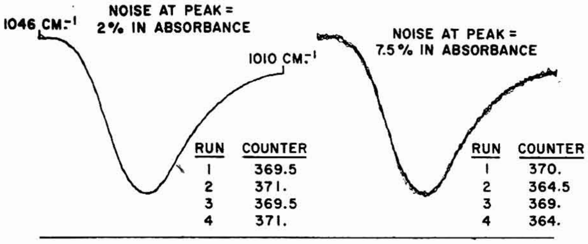

Figure 5. Reproducibility in presence of noise

Repeat runs of a polystyrene band varying speed of scan and signal-to-noise ratio. Although noise variation in A_ν changes appreciably, variation in $A_{\Delta\nu}$ is small

Figures 3, 4, and 5 illustrate the first two advantages listed above.

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Simple Countercurrent Distribution Apparatus

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The principle of the countercurrent distribution technique introduced by Craig (*2*) for the analysis and purification of complex mixtures has proved to be extremely useful in the analysis and separation of mixtures. The convenience of the method has been increased by the introduction of various designs of apparatus for mechanically effecting the required operations (*3, 4*). The apparatus described below has certain advantages in applications where emulsions are a source of difficulty: Each tube is separate from its neighbors, permitting removal of any tube at any time; emulsions can be centrifuged directly without transfer from one container to another; and the apparatus is simple in design and can be built in the usual laboratory shop. The glassblowing required is within the capability of any efficient amateur.

The essential features of the apparatus are: a series of equilibrating tubes with side arm through which the upper phase is decanted into an auxiliary series of test tubes, a frame for mechanically shifting the two series relative to each other, and a detachable section of each equilibrating tube which can be used for centrifuging the total contents of the tube. The operation consists briefly of decanting from one series of tubes to the other, then shifting the frame so as to decant back into the succeeding tube of the first series.

APPARATUS

Figure 1 gives a full length view of the apparatus when the upper phase in tubes *C* is being poured into tubes *B*. An end view is given in Figure 2.

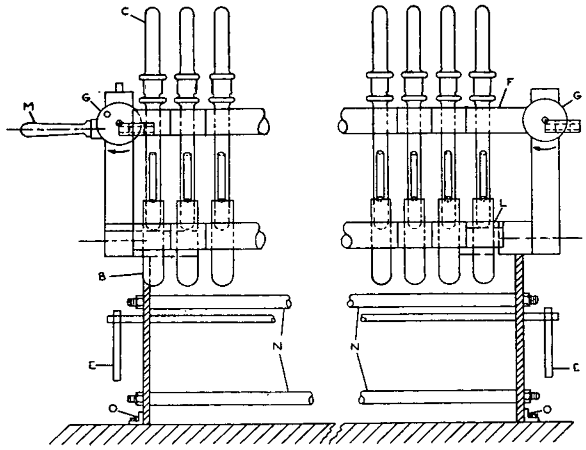

Figure 1. Countercurrent distribution apparatus

Stand *A* is a triangular support, 36 cm. wide, 29.5 cm. high (up to pivot *D*), and 122 cm. long. Three pipes, *N* (0.75 inch), running the whole length of the apparatus, hold the ends rigidly. *I* and *K* rotate independently about axis *D* and support bars *L* and *F*, respectively, carrying tubes *B* and *C*, numbered 1 to 25 in each series. These tubes are spaced regularly 25 mm. apart along the bars. The whole apparatus may be firmly bolted to a table at *O*. Bar *H* is bolted onto the triangle, so that when bar *I* rests on it, the whole bank of *B* tubes is exactly vertical. Support *E* in the up position holds *K* in a horizontal position suitable for shaking, and when in the down position allows *K* to rest on the small metal bar near the base of *E*.

The aluminum swivel, *G* (Figure 3), provides stops for the lateral movement for tubes *C* relative to tubes *B*. It consists of two swivels at right angles to each other. Swivel *X* is bolted onto bar *K* and held firmly by *Z*, such that it can revolve by movement of handle *R* if plug *S* is raised. *S* locks *X* in either of two positions on *K*. *W* is eccentrically mounted on *X*, such that it shifts to the left or right when *S* is in one position or the other. The dimensions are so arranged that, in one position, tube *C*-1 is opposite tube *B*-1. In the other position *F* moves one space to the right, so that *B*-1 is opposite *C*-2. The tubes *C* on bar *F* can be shaken by oscillation about axis *T*-*V* when clamp *T* is loosened. Handle *M* is for the convenience of the operator in shaking.

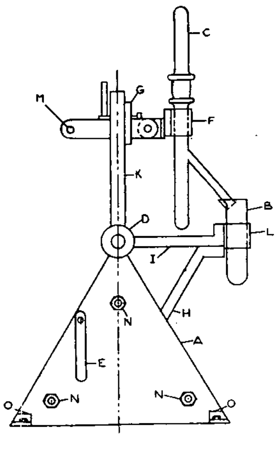

Figure 2. End view of apparatus

The glass parts of the apparatus, Figure 4, consist of 25 tubes *B* and 25 tubes *C*. The lower portion of tube *C* up to side arm *b* contains exactly 25 ml. *b* is the pouring spout for transferring the upper layer to tube *B*. Cap *a* is a standard-taper ground-glass joint (\$ 24/40), which is held in place by metal springs.

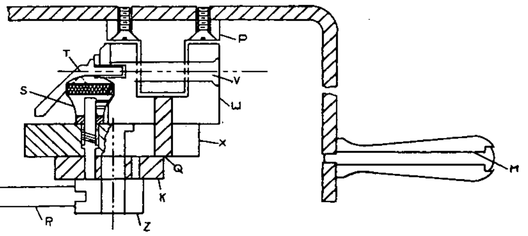

Figure 3. Aluminum swivel

The cap is large enough to contain the entire contents of the tube for centrifuging. For a tube with 25 ml. in its lower phase, the dimensions are as follows: diameter, 25 mm.; length, 260 mm.; and side arm, 15 × 70 mm. at a 60° angle from the tube. The length up to the side arm is approximately 70 mm.

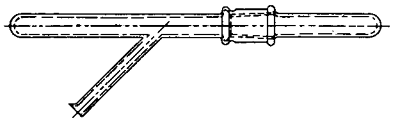

Figure 4. Detail of glass parts of countercurrent distribution apparatus

Tube *B* is an ordinary test tube of the same diameter as tube *C* and 140 mm. high. It has a small pouring spout.

PROCEDURE

Filling. With tubes *C* in vertical position, the lower phase is poured into each tube *C* until it overflows through side arm *b*, thereby introducing exactly 25 ml. of lower phase into each tube *C*. (Any excess which overflows into *B* is discarded.) Tubes *C* are then rotated to a horizontal position about axis *D* and supported on *E* in the up position. The required volume of upper phase is placed in tube *C*-1 through *b*, together with the substance to be treated.

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The operating cycle of this apparatus consists of five steps: equilibrating the two phases in tubes *C*, decanting upper phase into tubes *B*, shifting tubes *C* one position down the series relative to tubes *B*, emptying tubes *B* into tubes *C*, and returning tubes *C* to the original position relative to *B*.

Equilibrating. Clamp *T* is loosened and tubes *C* are equilibrated by oscillation about axis *TV*. Tubes *C* are then held in a horizontal position until the phases separate.

Decanting. With handle *R* in position 1, tubes *B* are raised to the side arms of tubes *C* by rotation about axis *D*. Minor adjustment at axis *TV* permits each side arm to enter the mouth of the corresponding tube. In this position tube *C*-1 is in relation with tube *B*-1. Both series of tubes are rotated forward together until *I* rests on *H*, bringing tubes *C* to the vertical position. In this operation the upper phase flows into *B* leaving the lower phase in *C*.

Shifting. Tubes *C* are raised slightly so that *b* will clear the rims of *B*. Handle *M* is shifted to position 2. With minor adjustments at *TV*, the pouring spouts of series *B* enter the mouths of *b*. In this position tube *B*-1 is in relation with tube *C*-2. Both series of tubes are rotated backward together until tubes *C* are horizontal. The upper phases flow back into tubes *C* displaced one position along the series.

Returning. Tubes *B* are returned to the rest position. Another volume of upper phase is placed in *C*-1. The cycle of operation is now repeated from the equilibrating step.

DISCUSSION

The above apparatus proved very satisfactory in the purification of the antifungal antibiotic, bacillomycin R (*1*). Troublesome emulsions developed in some tubes but these were immediately centrifuged, so that there was very little delay.

In order to avoid holdup due to surface tension effects, the various tubes (including centrifuge tubes) were coated on their inner surfaces with a liquid silicone lubricant.

At any stage in the procedure the contents of any tube *C* can be removed by rotating *C* backward past the horizontal position until the entire contents of the tube are contained in cap *a*. *a* can then be removed for centrifuging or other operation. This is particularly useful when the phases do not separate readily after shaking. The emulsion is removed and centrifuged at 500 times gravity to cause it to separate. The separated phases can then be replaced in the apparatus.

The addition of reagents to break any emulsions formed is unnecessary because contents of any tube may be centrifuged without difficulty. The choice of solvents is therefore not limited by the possibility of heavy emulsion formation.

The apparatus may be used in the single withdrawal procedure by the addition of a 26th *C* tube to collect the upper phase from tube *B*-25.

A motor may be attached to *F* to make the shaking mechanical. The conversion of the apparatus into a completely automatic one is not considered advisable because this would greatly increase the cost of the apparatus.

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Paper 62, Agricultural Research Station, Rehovot, Israel, 1953 Series.

Assignment of Wave Lengths on Spectra Recorded with Beckman Model DK-1 Spectrophotometer

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THE Beckman Model DK-1 recording spectrophotometer, which employs the same monochromator as the Beckman Model DU, has a nonlinear wave-length scale. The accurate assignment of wave lengths on spectra recorded by this instrument is therefore not a simple matter. The manufacturer supplies pressure-sensitive tapes on which are printed the wave-length scales appropriate to each of the scanning speeds of the instrument, but these tapes are intended only for the approximate location of wave lengths. They are somewhat elastic, and care should be taken when applying them to the chart paper not to stretch them to different extents in different regions. The tape provides wave-length markers only along a narrow strip of the chart, and the markers have to be extended to the absorption peaks either by eye or by the use of drafting equipment. Finally, they are a continuing source of expense, and a quantity of tapes must be kept on hand. For more accurate work, the manufacturer recommends that the wave-length scale of the monochromator be used directly.

A very rapid and convenient technique, as accurate as the direct use of the monochromator scale, consists in transferring the wave-length scroll markings of the monochromator to a sheet of transparent plastic, which thereafter serves as the wave-length standard, and the wave lengths of absorption peaks are read directly by placing it on top of the chart to be measured. The technique used for accurately inscribing the plastic sheet is as follows.

A spectrum is run with no specimen in either beam, so that a flat 100% line is traced out. The wave-length scroll of the monochromator is viewed with the aid of a magnifying glass, and as each graduation on the scroll passes underneath the hairline of the rider which sits on the scroll, the sample compartment shutter is quickly closed and opened. This places a sharp vertical line on the chart at a position corresponding to the wave length involved. (The term "horizontal" refers to the direction of feed of the strip chart, "vertical," to the direction of travel of the recorder pen.) With care, at scanning speeds of 10 or slower and 0.1-second time constant, these vertical lines can be placed on the chart paper in exact correspondence to every single marking on the monochromator wave-length scroll. This procedure should be repeated on several separate charts to establish the reproducibility of the marking technique.

The correct markers are then transferred to a 1-inch strip of paper which is pasted along the center, parallel to the long axis, of a 12 × 28 inch right rectangle of 0.25-inch Plexiglas (these dimensions are appropriate for the region 800 to 2850 mμ at speed 10). By means of a T-square and steel scriber, lines are scratched on the Plexiglas from top to bottom along the 12-inch dimension, leaving a gap corresponding to the width of the paper strip for the tens, and no gap for the hundreds of units. Wave-length designations are inscribed on the reverse side of the plastic sheet. In order to ensure that the plastic sheet is placed on the chart with its ruled lines parallel to the axis along which the recorder pen travels, it is convenient to inscribe a line perpendicular to the wave-length markers in the center of the plastic sheet. In using the sheet, this line is lined up with a grid line of the chart paper.

When running a spectrum, several wave-length markers are placed on the record by manipulating the sample compartment shutter as described above. The plastic sheet is then placed on the chart with the ruled side facing the paper, and is carefully lined up with the vertical wave-length markers and with the appropriate horizontal chart grid line. Absorption peak wave lengths are then read directly off the plastic scale, with visual interpolation between scale markings. If the plastic sheet is illuminated from one edge, the light scattered into the ruled lines makes these lines stand out sharply.

It has been suggested (by Lee Cahn, Beckman Instrument Corp., Pasadena, Calif., private communication) that a convenient procedure for comparisons of different spectral charts would be to inscribe the wave-length grid on a transparent sheet that is illuminated from below by means of fluorescent lamps, with the charts placed on top of this viewing fixture.

The use of the plastic wave-length scale described makes possible the greatest accuracy in comparisons of wave lengths of the absorption peaks of different specimens run on the same instrument. It is recommended that the markings on the plastic chart be calibrated by comparison with standard spectra, such as the emission of mercury, or absorption of water or chloroform.

Adapter for Suction Filtration Directly into a Volumetric Flask

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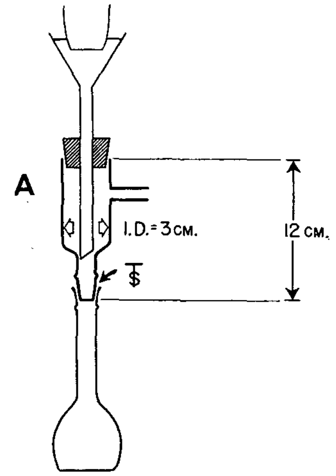
In precipitation reactions it is often desirable to transfer a filtrate, or the dissolution products of a precipitate, directly from a filtering crucible into a volumetric flask. The adapter indicated as *A* in Figure 1 facilitates such operations.



Three such adapters fitted with ₸ male joints, sizes 10/18, 14/20, and 19/38, will suffice for ₸ stoppered volumetric flasks of 500-ml. or less capacity. These filtering devices have been used in this laboratory with water aspirators for over a year without any implosion of either Pyrex or Exax brand volumetric flasks. However, a safety guard around the 500-ml. flask may be used in the interest of safety. Thin-walled flasks should not be used unless tests indicate their applicability.

Determination of Carbon and Hydrogen in Spontaneously Flammable Gases

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Attempts to determine carbon and hydrogen in silane-containing gas mixtures with conventional apparatus have frequently led to explosions or to low results. The mass spectrometer is probably adequate for analyzing most of these mixtures, but frequently a determination of total carbon and hydrogen is also desired. A method is described below for carrying out these determinations by a combustion technique on any gas mixtures in which there is danger of explosion.

In some cases, silane-containing gas mixtures can be analyzed in a standard Orsat. The silane reacts with the potassium hydroxide in the first pipet, liberating its hydrogen and leaving the silicon behind as soluble potassium silicate; the increase in volume is equal to three times the volume of silane in the original mixture. Hydrogen and hydrocarbons can then be burned in the Orsat combustion tubes in the usual way. However, the total carbon and hydrogen of some gas mixtures, such as those containing alkyl-substituted silanes, cannot be determined in an Orsat. In the case of ethyl silane, for example, the potassium hydroxide in the first pipet liberates the hydrogen which is bonded to the silicon, but retains the rest of the molecule without breaking the silicon-carbon bond; the ethyl group thus escapes detection.

Brunn and Faulconer (*1*) have described an apparatus for the combustion of highly volatile materials and for flammable liquids, in which oxygen is mixed with the combustible vapors before entering the ignition zone. As mixtures of silane and oxygen may explode spontaneously, this apparatus should not be used for this problem.

Zimmerman (*4*) described an apparatus for the combustion of materials whose vapors might form explosive mixtures with oxygen or air; his combustion tube and method are designed primarily for solids and would probably produce flashbacks and dangerous explosions if used with silanic gases.

The apparatus described below enables one to mix successive small portions of the gas sample with a large excess of oxygen and to burn it without uncontrolled explosions, which might rupture the combustion tube.

APPARATUS

The combustion apparatus is similar to that used for standard semimicrocombustions (*2*, *3*), except that the combustion tube is of clear vitreous silica, 10.5 mm. in outside diameter, 8.5 mm. in inside diameter, and 30 cm. long, with a side arm and three-way stopcock as shown in the sketch. The tube is packed with ignited asbestos, platinum gauze, platinum catalyst, copper oxide catalyst, silver catalyst, silver gauze, and ignited asbestos.

The silver–copper oxide can be heated by means of a Fischer microcombustion furnace (Catalog No. 20-286-1) and the temperature of this section should be maintained between 550° and 650° C.

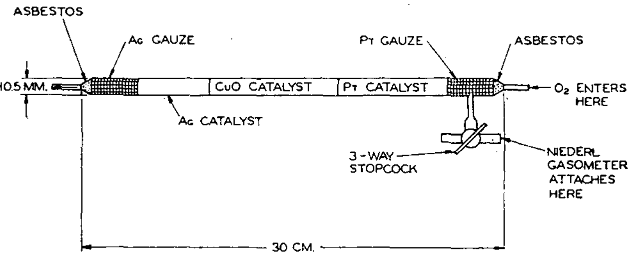
The platinum section of the train can be wrapped with nickel-chromium alloy micro gauze and heated with fishtail burners to a temperature between 850° and 1000° C.

The three-way stopcock on the side arm should be protected from radiated heat with an asbestos shield; a jet of air blowing on the glass surface close to the joint serves to dissipate conducted heat away from the ground-glass joint.

A Niederl gasometer (available from scientific apparatus supply companies) is used to hold the gas sample.

REAGENTS

Copper Oxide Catalyst. Dissolve 60 grams of copper nitrate in about 200 ml. of distilled water in a porcelain evaporating dish. Add 100 grams of Aloxite (Carborundum Co. brand aluminum oxide cylindrical pellets, $^3/_{16} \times {}^3/_{16}$ inch) and evacuate under a bell jar; this draws the air out of the pores of the pellets. When bubbles no longer erupt from the pellets, release the vacuum; the copper nitrate solution now rushes into the evacuated pores. Digest on a hot plate for one-half hour. Decant off excess liquid and dump pellets onto a wire gauze. Heat until dry, using a direct gas flame. Finally ignite in a muffle furnace at 800° C. for 1 hour.



Platinum Catalyst. Dissolve 5 grams of platinum chloride in about 75 ml. of distilled water and transfer to a 300-ml. porcelain evaporating dish. Add 100 grams of Aloxite pellets and complete the preparation as with the copper oxide catalyst.

Silver Catalyst. Prepare similarly to the platinum and copper pellets, using 40 grams of silver nitrate and about 80 grams of Aloxite pellets.

Silver gauze, 100-mesh pure silver gauze. Platinum gauze, 100-mesh platinum gauze.

PROCEDURE

Collect about 20 ml. of the gas to be analyzed in the Niederl gasometer and dilute with 40 to 50 ml. of argon. As the gasometer is calibrated, it is easy to determine how much of the gas mixture is burned. Connect the gasometer to the three-way stopcock on the combustion tube. Purge the train with oxygen in the customary way for 1 hour with the water and carbon dioxide absorbers attached. Weigh absorbers and very gently bleed the gas sample into the train.

Small flashes and explosions will probably occur at the junction of the side arm and the platinum gauze section; these explosions are controlled by pinching the rubber tube from the Niederl gasometer or the leveling bulb. The silicon-hydrogen bonds will be broken by the hot platinum catalyst. Organic compounds may reach the copper oxide catalyst, but will be oxidized there. Halides, if present, are trapped on the hot silver catalyst. The amount of sample to be burned should be governed by the appearance of the indicating Drierite.

This method has given good results with gases containing silane, SiH_4, and ethylsilane, $C_2H_5SiH_3$.

Sample	Theoretical		Determined	
	C, %	H, %	C, %	H, %
CH_3Cl	23.8	6.0	23.4	6.0
95% $C_2H_5SiH_3$ + 5% N_2	38.0	12.7	37.7	12.6
SiH_4	0	12.6	0	12.6

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Infrared Capillary Cell for Volatile Liquids

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For the production of infrared spectra, a capillary cell is sometimes preferred to a fixed space cell, because the capillary provides a thinner sample film and is easier to recondition if the salt windows become contaminated. A problem arises in using the capillary cell if the sample is volatile. Two simple modifications for overcoming this difficulty utilize reservoirs to provide replacement of material lost through volatilization at the edges of the salt windows, and are, therefore, applicable only to single component liquids and to qualitative work. Many workers (*1–6*) have proposed designs of infrared cells for handling volatile liquids. While the present suggestion offers only a partial solution to the general problem, it describes apparatus that can be made in a very short time from inexpensive and readily available materials using simple tools.

The simplest modification is accomplished by making a small depression in the salt windows themselves, as shown in the lower portion of Figure 1. (The Teflon and glass pieces are not utilized in this simplest modification.) Two salt plates held in a demountable cell frame are drilled at moderate speed either with a steel drill or with a spherical burring tool (rotary file). By using the latter tool a hemispherical depression is produced instead of the cylindrical one shown in the figure. A hypodermic syringe and needle are used to fill the cell and to keep the reservoir filled during production of a spectrum. A little loosening and retightening of the frame may occasionally be necessary for the elimination of air bubbles on first filling of the cell. For a moderately volatile liquid such as ethyl alcohol, less than 1 ml. of liquid will be required during a determination. The cell can be used with or without a spacer. When no spacer is used and it is desired to determine the average sample thickness, the capillary is best calibrated by measuring peaks of a suitable solvent in the capillary cell and in several fixed cells or a variable-space cell and calculating the capillary space from a plot of thickness against absorption.

The cell described is not suitable for more volatile substances such as carbon disulfide or acetone. A modification employing a Teflon and glass insert has been found adequate for these liquids and is also illustrated in Figure 1. Because the normal operating temperature at the cell is about 37° to 39° C., even this second modification is not suitable for liquids (such as ethyl ether) which have boiling points below 40° C. Although drilling is done in the vertical position, the windows are rotated in use, so that the reservoir is at about 10 o'clock. In this position there is an adequate slope for gravity feed of the sample, but the drill hole and insert are outside the path of the infrared beam. The Teflon portion of the insert is made by drilling a plug commonly furnished for closing Perkin-Elmer fixed cells. Drill sizes as indicated in the figure were found suitable but are not critical so long as reasonably snug fits are obtained. Teflon may be undesirable for some liquids, but otherwise its use as an adapter for the glass tube is advantageous (because of the softness and cold-flow properties of Teflon) over a direct glass-to-salt window connection. The total reservoir capacity of the cell shown is about 0.3 ml. For a 40-minute spectrum trace a total of five fillings (1.5 ml.) were required with carbon disulfide and one and one half fillings (about 0.5 ml.) with acetone.

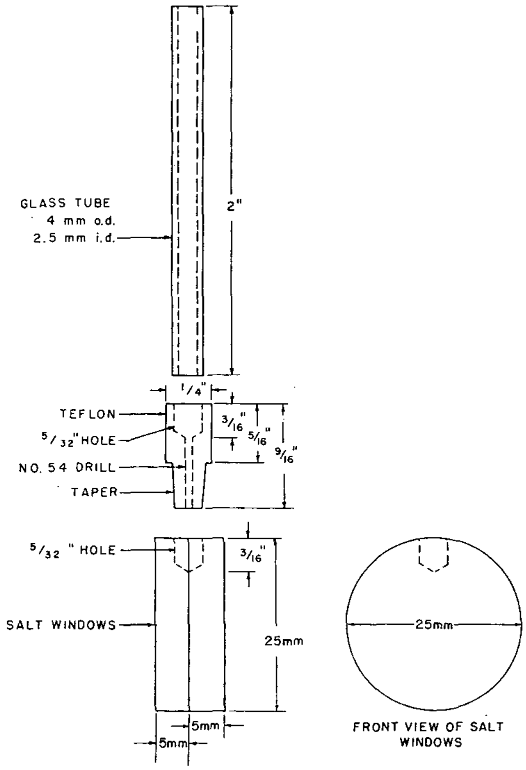

Figure 1

When reasonable care is taken in keeping the capillary filled during spectrum tracing, there should be no dripping of solvent onto the base of the instrument. Possibility of accidental solvent damage to the instrument finish may be guarded against in a number of ways—for example, by arranging several layers of cloth so as to intercept any spillage or droplets. If highly flammable liquids are examined in the cell, suitable precautions should be taken regarding fire or explosion hazards.

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