ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Hobart H. Willard Wins 1951 Fisher Award

THE announcement, at the general meeting of the AMERICAN CHEMICAL SOCIETY in Chicago, September 6, 1950, that Hobart H. Willard, of the University of Michigan, had been selected to receive the 1951 Fisher Award was received with wide acclaim. We are happy to congratulate Dr. Willard and the committee in its selection of him as the fourth to receive the Fisher Award in Analytical Chemistry. Dr. Willard joins an illustrious company. The first award was made in 1948 to N. Howell Furman, President-Elect of the AMERICAN CHEMICAL SOCIETY, the second to the late G. E. F. Lundell, of the National Bureau of Standards, and the third to I. M. Kolthoff, University of Minnesota.

We are especially proud to have had Dr. Willard on the advisory board of ANALYTICAL CHEMISTRY from 1935 through 1948. It was during some of these years when this journal was in its formative stage and suffering from growing pains that we had the benefit of his broad experience and drew heavily upon his knowledge, not only of analytical chemistry but of the men working in that field, for advice and counsel. ANALYTICAL CHEMISTRY has profited immeasurably from this.

Aluminum was the first element to engage Dr. Willard's attention, but he soon broadened his interests to include gallium, zirconium, titanium, thorium, and iron. He and his assistants were the first to discover the importance of slow precipitations, careful control of pH, and the presence of a suitable anion. His improved method for the determination of fluorine, reported in 1933, is in general use today, essentially as he devised it. By steam distillation of hydrofluosilicic acid he was able to make the separation from interfering elements and achieve a satisfactory volumetric method. He and his associates have recently completed two papers reporting refinements in this determination.

He has coauthored a series of papers which have established the use of periodate, perchlorates, ozone, and ceric sulfate as analytical reagents.

Dr. Willard has written several textbooks, and has published considerably over 100 papers which embrace a wide field of analytical chemistry. Many of these papers will be found in the pages of ANALYTICAL CHEMISTRY. His first book, "Elementary Quantitative Analysis," of which Dr. Furman was coauthor, has also been published in Spanish and German. His papers on the precipitation of various elements from homogeneous solutions are too well known for comment here.

Dr. Willard joined the staff of the University of Michigan as an instructor in 1905, became an assistant professor in 1912, an associate professor in 1918, and a full professor in 1922. He has taught there continuously, except for an interim in 1917–18 when he served as director of the chemical and metallurgical laboratory of the Bureau of Aircraft Production, Detroit district. He has no retired—so he says—after 45 years of teaching. But anyone who knows Dr. Willard and the active life he has always led questions this statement. With the announcement that he is retiring, comes the information that he is directing a number of research problems, has considerable consultant work on hand, and is actively engaged in revising the textbooks which will always be a monument to his name.

In addition to his invaluable service as a member of the advisory board of ANALYTICAL CHEMISTRY, Dr. Willard has been quite active in the affairs of the AMERICAN CHEMICAL SOCIETY for many years, having joined the Society in 1902. He has held various offices in the Society—national as well as local—having been a Director (1934–40), a Councilor-at-Large, served several terms as chairman, as secretary-treasurer, and as councilor of the University of Michigan Section, and as secretary and chairman of the Division of Physical and Inorganic Chemistry. At present he is chairman-elect of the Division of Analytical Chemistry, and since 1946 has been a member of the Committee on Predoctoral Fellowships and of the Committee on Standard Apparatus. He is a member of the American Association for the Advancement of Science, the Electrochemical Society, and Deutsche Chemische Gesellschaft.

The Willards have a cottage on a lake near Ann Arbor where Dr. Willard indulges in two of his favorite hobbies—swimming and hiking. Those of us who have seen him start out for an early morning swim when practically everyone else was sound asleep can give eloquent testimony to how much he enjoys this pastime. Another hobby is photography.

The influence which Hobart H. Willard has had upon his students during his 45 years of teaching reaches far beyond the confines of the United States. He has always enjoyed the zest of living to an unusual degree, has led an active, full, and useful life, and has entered into every task with an enthusiasm that has been contagious. We predict life has many more productive years in store for him.

RAMAN SPECTRA

Hydrocarbons and Oxygenated Compounds

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THE variety of analytical problems to which any spectroscopic method of analysis can be applied depends to a large measure on the number of calibration spectra of pure materials which are available. To a great extent the usefulness of a "library" of spectra is enhanced by graphically showing the intensity distribution of the lines or bands, as well as by listing such data in tabular form. Collections of this type have been useful in extending the applications of infrared absorption spectroscopy (*1*, *3*, *6*); however, comparatively few Raman spectral data which show this intensity distribution are available. Recently there has been an attempt to obtain such data and present them on a uniform basis (*1*, *5*).

This paper presents the Raman spectra of 119 different compounds in the form of graphs and tables. These data supplement the spectra of 172 compounds reported previously (*5*) and have been obtained with the same apparatus and procedure. The spectra of materials which are solid at 20° C. have been determined in the liquid state by circulating heated ethylene glycol solution through a jacket on the Raman sample tube. These samples and the temperatures used are: naphthalene (110° C.), 2-methylnaphthalene (110° C.), 2,3-dimethylnaphthalene (110° C.), and pentadecylcyclohexane (53° C.). All other samples have been run at 20° C.

The indexes of the spectra of the hydrocarbons are given in Tables I to V. These tables list, in addition to the name and spectrum number, the physical properties of the compounds examined, the best literature data on the properties (*2*, *4*, *7*), and, when known, the purities of the materials. Where purities have not been determined it is believed, from the method of preparation and the physical properties, that they are 98 mole % or better.

The indexes of the spectra of the oxygenated and miscellaneous compounds are given in Tables VI and VII. The compounds of this group have all been fractionated at reflux ratios of approximately 40 to 1 in columns having the equivalent of about 40 theoretical plates. The purities of these compounds are believed to be 95 mole % or better.

LITERATURE CITED

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Table I. Spectra Numbers and Properties of Pure Paraffin Hydrocarbons

Name of Compound	Spectrum No.	Boiling Point at 760 Mm. Hg, ° C. Determined	Boiling Point at 760 Mm. Hg, ° C. Literature values[a]	Refractive Index at 20° C., n_D^{20} Determined	Refractive Index at 20° C., n_D^{20} Literature values[a]	Estimated Purity, Mole %[b]	Source of Compound[c]
9-carbon atom							
2,3-Dimethylheptane	1	138.3	140.5	1.4087	1.4085	.....	B
2,4-Dimethylheptane	2	131.5	133.	1.4037	1.403	.....	B
2,5-Dimethylheptane	3	134.	136.	1.4038	1.4038	.....	B
2,6-Dimethylheptane	4	133.	135.21	1.4000	1.4007	.....	B
3,3-Diethylpentane	5	..	146.5	1.4205	1.4200	99.84 ± 0.02	C
2,2,3-Trimethylhexane	6	131.7	133.4	1.4100	1.4105	.....	B
2,2,3,3-Tetramethylpentane	7	140.27	140.23	1.4236	1.4234	99.936 ± 0.020	D
10-carbon atom							
2,2,4-Trimethylheptane	8	146.0	147.4 at 736 mm	1.4091	1.4097[d]	.....	B
2,2-Dimethyl-4-ethylhexane	9	147.	148.0 at 726 mm.[d]	1.4131	1.4120[d]	.....	B
11-carbon atom							
2,2,4-Trimethyloctane	10	168.5	169.9 at 736 mm.[d]	1.4155	1.4153[d]	.....	B
2,2,3,5,5-Pentamethylhexane	11	164.2	.....	1.4215	...	.....	B
12-carbon atom							
2,2,4,4,6-Pentamethylheptane	12	185.55	.....	1.4375	...	.....	F
16-carbon atom							
7-*n*-Propyltridecane	13	..	.....	1.4350	...	.....	E
19-carbon atom							
7-*n*-Hexyltridecane	14	..	.....	1.4406	...	.....	E
21-carbon atom							
8-*n*-Hexylpentadecane	15	..	.....	1.4439	...	.....	E
23-carbon atom							
9-*n*-Hexylheptadecane	16	..	.....	1.4461	...	.....	E
26-carbon atom							
11-*n*-Amylheneicosane	17	..	192.0 at 1 mm.[d]	1.4499	1.4497[d]	.....	E
28-carbon atom							
9-*n*-Octyleicosane	18	..	.....	1.4510	...	.....	E
34-carbon atom							
9-*n*-Octylhexacosane	19	..	.....	1.4552	...	.....	E

[a] All physical properties except those marked [d] are from (*7*).
[b] Purities listed were determined from freezing point measurements by donor. It is believed that all other materials had a purity of 98 mole % or better.
[c] Source of compounds: B. Organic Research Laboratory, School of Chemistry and Physics, Pennsylvania State College. C. American Petroleum Institute Research Project 45, Ohio State University. D. American Petroleum Institute Research Project 6, National Bureau of Standards. E. New York State Agricultural Experiment Station, Cornell University. F. Anglo-Iranian Oil Co., Sunbury-on-Thames, England.
[d] (*4*).

Table II. Spectra Numbers and Properties of Pure Olefin Hydrocarbons

Name of Compound	Spectrum No.	Boiling Point at 760 Mm. Hg, ° C. Determined	Boiling Point at 760 Mm. Hg, ° C. Literature values[a]	Refractive Index at 20° C., n_D^{20} Determined	Refractive Index at 20° C., n_D^{20} Literature values[a]	Estimated Purity, Mole %[b]	Source of Compound[c]
Olefins							
6-carbon atom							
1-Hexene	20	63.5	63.55	1.3876	1.3876	.....	B
cis-2-Hexene	21	68.7	68.6	1.3977	1.3954	.....	B
trans-2-Hexene	22	66.	67.9	1.3933	1.3935	.....	B
trans-3-Hexene	23	..	68.1	1.3944	1.3938	99.75 ± 0.03	C
2-Methyl-1-pentene	24	62.2	62.2	1.3921	1.3925	.....	B
2-Methyl-2-pentene	25	..	67.2	1.4003	1.4004	99.54 ± 0.05	C
3-Methyl-1-pentene	26	54.2	53.8	1.3840	1.384	.....	B
cis-3-Methyl-2-pentene	27	70.6	70.52	1.4044	1.4045	.....	B
trans-3-Methyl-2-pentene	28	67.7	67.8	1.4016	1.4016	.....	B
4-Methyl-1-pentene	29	53.9	54.0	1.3825	1.384	.....	B
cis-4-Methyl-2-pentene	30	56.5	55.	1.3881	1.388	.....	B
trans-4-Methyl-2-pentene	31	58.8	58.4	1.3888	1.389	.....	B
2-Ethyl-1-butene	32	..	64.95	1.3969	1.3969	99.64 ± 0.04	C
2,3-Dimethyl-2-butene	33	73.2	73.21	1.4121	1.4122	.....	B
7-carbon atom							
2-Methyl-1-hexene	34	91.8	91.3	1 4033	1.404	.....	B
3-Ethyl-2-pentene	35	95.	95.	1.4148	1.4143	.....	B
2,4-Dimethyl-1-pentene	36	..	81.	1.4038	1.397	.....	C
4,4-Dimethyl-1-pentene	37	72.50	72.2	1.3919	1.3918	.....	F
cis-4,4-Dimethyl-2-pentene	38	80.	76.	1.4022	1.399	.....	B
trans-4,4-Dimethyl-2-pentene	39	76.75	76.	1.3983	1.399	.....	F
8-carbon atom							
trans-3-Octene	40	..	123.3	1.4128	1.4126	.....	C
trans-4-Octene	41	..	122.4	1.4120	1.4118	99.65 ± 0.14	C
6-Methyl-1-heptene	42	113.2	113.2	1.4082	1.4070	.....	B
16-carbon atom							
2-Methyl-1-pentadecene	43		..	1.4440	1.4468 at 19.7° C	.....	F
Diolefins							
6-carbon atom							
1,3-Hexadiene (*cis* and *trans*)	44	72.	73.	1.4403	1.438	.....	A
2,4-Hexadiene (high boiling isomer)	45	83.	80.	1.4566	1.450	.....	A
2,3-Dimethyl-1,3-butadiene	46	..	68.5	1.4394	1.4391	.....	A
8-carbon atom							
2,5-Dimethyl-1,5-hexadiene	47	114.	114.3[d]	1.4290	1.4293[d]	.....	B
2,5-Dimethyl-2,4-hexadiene	48	..	134.5[d]	1.4778	1.4781[d]	99+	C

[a] All physical properties except those marked [d] are from (7).
[b] Purities listed were determined from freezing point measurements by donor. It is believed that all other materials had a purity of 98 mole % or better.
[c] Source of compounds: A. Petroleum Refining Laboratory, School of Chemistry and Physics, Pennsylvania State College. B. Organic Research Laboratory, School of Chemistry and Physics, Pennsylvania State College. C. American Petroleum Institute Research Project 45, Ohio State University. F. Anglo-Iranian Oil Co., Sunbury-on-Thames, England.
[d] (4).

Table III. Spectra Numbers and Properties of Pure Cycloparaffin Hydrocarbons

Name of Compound	Spectrum No.	Boiling Point at 760 Mm. Hg, ° C. Determined	Boiling Point at 760 Mm. Hg, ° C. Literature values[a]	Refractive Index at 20° C., n_D^{20} Determined	Refractive Index at 20° C., n_D^{20} Literature values[a]	Estimated Purity, Mole %[b]	Source of Compound[c]
Monocyclic							
Alkylcyclobutanes							
Ethylcyclobutane	49	..	70.7[d]	1.4021	1.4021	99.7 ± 0.1	C
Alkylcyclopentanes							
trans-1,2-Dimethylcyclopentane	50	91.87	91.87	1.4120	1.4120	99.81 ± 0.10	D
cis-1-Methyl-2-ethylcyclopentane	51	..	128.0	..	1.4295	.....	B
n-Butylcyclopentane	52	..	156.56[e]	1.4316	1.4316[e]	99.92 ± 0.03	C
Isobutylcyclopentane	53	..	...	..	...	99.84 ± 0.08	D
Alkylcyclohexanes							
cis-1,3-Dimethylcyclohexane	54	..	120.09	..	1.4229	99.91 ± 0.05	D
trans-1,3-Dimethylcyclohexane	55	..	124.45	..	1.4309	99.84 ± 0.07	D
1,1,3-Trimethylcyclohexane	56	136.63	...	1.4296	...	99.79 ± 0.05	D
n-Butylcyclohexane	57	180.95	180.90[e]	1.4408	1.4407[e]	99.92 ± 0.04	D
Isobutylcyclohexane	58	171.32	171.[d]	1.4386	1.4386[d]	99.83 ± 0.09	D
Pentadecylcyclohexane	59	..	366.	Melting pt. = 25.1° C.		99.4	F
Dicyclic							
cis-Hydrindane	60	..	...	1.4719	...	.....	C
1-Cyclohexyl-3(2-cyclohexylethyl)-hendecane	61	..	...	1.4754	...	.....	E

[a] All physical properties except those marked [d] and [e] are from (7).
[b] Purities listed were determined from freezing point measurements by donor. It is believed that all other materials had a purity of 98 mole % or better.
[c] Source of compounds: B. Organic Research Laboratory, School of Chemistry and Physics, Pennsylvania State College. C. American Petroleum Institute Research Project 45, Ohio State University. D. American Petroleum Institute Research Project 6, National Bureau of Standards. E. New York State Agricultural Experiment Station, Cornell University. F. Anglo-Iranian Oil Co., Sunbury-on-Thames, England.
[d] (4). [e] (2).

Table IV. Spectra Numbers and Properties of Pure Cyclo-olefin Hydrocarbons

Name of Compound	Spectrum No.	Boiling Point at 760 Mm. Hg, ° C. Determined	Refractive Index at 20° C., n_D^{20} Determined	Estimated Purity, Mole %[a]	Source of Compound[b]
2,4-Dimethyl-1-cyclopentene	62	93.2	1.4283	..	B
1,2,4-Trimethyl-1-cyclopentene	63	118.7	1.4391	..	B

[a] Purities are believed to have been 98 mole % or better.
[b] Source of compounds:
B. Organic Research Laboratory, School of Chemistry and Physics, Pennsylvania State College.

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Table V. Spectra Numbers and Properties of Pure Aromatic Hydrocarbons

Name of Compound	Spectrum No.	Boiling Point at 760 Mm. Hg, ° C. Determined	Boiling Point at 760 Mm. Hg, ° C. Literature values[a]	Refractive Index at 20° C., n_D^{20} Determined	Refractive Index at 20° C., n_D^{20} Literature values[a]	Estimated Purity, Mole %[b]	Source of Compound[c]
Monocyclic							
1-Methyl-2-*n*-propylbenzene	64	184.75	184.	1.4998	1.4993	99.4	F
1-Methyl-3-*n*-propylbenzene	65	181.75	182.	1.4936	1.4951	98+	F
1-Methyl-4-*n*-propylbenzene	66	183.10	183.45	1.4918	1.493	99.6	F
Dicyclic							
Naphthalene	67	..	..	Solid	..	..	A
1-Methylnaphthalene	68	..	..	1.6167	..	..	A
2-Methylnaphthalene	69	..	..	Solid	..	..	A
1,6-Dimethylnaphthalene	70	..	..	1.6073	..	..	F
2,3-Dimethylnaphthalene	71	..	..	Solid	..	..	F
2-*n*-Butylnaphthalene	72	..	..	1.5740	..	..	F
1-α-Naphthylhendecane	73	..	..	1.5400	..	..	E
1-Phenyl-3(2-phenylethyl) hendecane	74	..	..	1.5194	..	..	E

[a] All physical properties are from (*7*).
[b] Purities listed were determined from freezing point measurements by donor. It is believed that all other materials had a purity of 98 mole % or better.
[c] Source of compounds:
A. Petroleum Refining Laboratory, School of Chemistry and Physics, Pennsylvania State College.
E. New York State Agricultural Experiment Station, Cornell University.
F. Anglo-Iranian Oil Co., Sunbury-on-Thames, England.

Table VI. Spectra Numbers and Properties of Pure Oxygenated Compounds

Name of Compound	Spectrum No.	Refractive Index at 20° C., n_D^{20}, Determined	Source of Compound[a]
Acetals			
Dimethyl acetal (1,1-dimethoxyethane)	75	1.3666	A
Ethylal (diethoxymethane)	76	1.3730	A
Acetal (1,1-diethoxyethane)	77	1.3805	A
Alcohols			
Methanol	78	1.3286	A
Ethanol	79	1.3616	A
1-Propanol	80	1.3853	A
2-Propanol	81	1.3772	A
1-Butanol	82	1.3988	A
2-Butanol	83	1.3973	A
2-Methyl-2-propanol (*tert*-butyl alcohol)	84	Solid	A
3-Pentanol	85	1.4108	A
2-Methyl-1-butanol (active amyl alcohol)	86	1.4105	A
2-Methyl-2-butanol (*tert*-amyl alcohol)	87	1.4046	A
Aldehydes			
n-Butyraldehyde	88	1.3800	A
Isobutyraldehyde	89	1.3723	A
Isovaleraldehyde	90	1.3893	A
Esters			
Isobutyl formate	91	1.3852	A
Ethyl acetate	92	1.3721	A
Isopropyl acetate	93	1.3770	A
sec-Butyl acetate	94	1.3890	A
Isobutyl acetate	95	1.3900	A
Methyl propionate	96	1.3767	A
Isobutyl propionate	97	1.3972	A
Ethyl *n*-butyrate	98	1.3922	A
Ethyl isobutyrate	99	1.3873	A
Dimethyl carbonate	100	1.3686	A
Diethyl carbonate	101	1.3840	A
Methyl orthoacetate (1,1,1-trimethoxyethane)	102	1.3884	A
Ethers			
Diethyl ether	103	1.3523	A
Ethyl isopropyl ether	104	1.3627	A
Di-*n*-propyl ether	105	1.3803	A
Ethyl *n*-butyl ether	106	1.3816	A
Ethyl isobutyl ether	107	1.3759	A
Ethyl *tert*-butyl ether	108	1.3757	A
Ketones			
Acetone	109	1.3586	A
2-Butanone (methyl ethyl ketone)	110	1.3785	A
3-Methyl-2-butanone (methyl isopropyl ketone)	111	1.3870	A
3-Pentanone (diethyl ketone)	112	1.3919	A
2,4-Dimethyl-3-pentanone (diisopropyl ketone)	113	1.4000	A
4-Methyl-2-pentanone (methyl isobutyl ketone)	114	1.3956	A
Miscellaneous			
1,2-Epoxycyclohexane	115	1.4523	A
Benzofuran (coumarone)	116	1.5663	A

[a] Source of compounds:
A. Petroleum Refining Laboratory, School of Chemistry and Physics, Pennsylvania State College.

Table VII. Spectra Numbers and Properties of Pure Alkylhalide Compounds

Name of Compound	Spectrum No.	Refractive Index at 20° C., n_D^{20}, Determined	Source of Compound[a]
Isobutyl bromide	117	1.4360	B
sec-Butyl bromide	118	1.4361	B
tert-Butyl bromide	119	1.4277	B

[a] Source of compounds:
B. Organic Research Laboratory, School of Chemistry and Physics, Pennsylvania State College.

Raman Spectral Data for Hydrocarbons

No. 1. 2,3-Dimethylheptane

Wave No. Shift, $\Delta\tilde{\nu}$, cm.$^{-1}$	Scattering Coefficient[a]	Depolarization Factor, ρ
189	0.031	0.4
228	0.010	..
294	0.014	0.6
398	0.014	..
469	0.025	..
521	0.007	..
603	0.007	..
714	0.022	0.5
760	0.046	0.2
793	0.017	..
813	0.016	0.4
872	0.040	0.6
891	0.028	0.4
926	0.038	0.6
954	0.010	..
1006	0.012	0.7
1061	0.025	1.
1159	0.032	0.8
1208	0.013	0.7
1249	0.008	..
1273	0.018	0.7
1304	0.066	0.84
1352	0.034	1.
1391	0.032	0.9
1454	0.144	0.87

No. 2. 2,4-Dimethylheptane

186	0.030	0.3
233	0.010	0.9
294	0.040	0.3
430	0.025	0.3
611	0.007	0.9
732	0.009	..
809	0.067	0.2
889	0.024	0.5
956	0.024	1.
1033	0.025	0.8
1078	0.011	0.8
1158	0.051	0.73
1210	0.008	1.
1267	0.018	..
1334	0.052	0.6
1457	0.151	0.72

No. 3. 2,5-Dimethylheptane

190	0.020	..
257	0.014	..
432	0.027	..
615	0.006	..
768	0.036	0.3
801	0.039	0.5
830	0.069	0.2
907	0.023	0.9
954	0.035	1.
1045	0.023	0.6
1090	0.011	..
1152	0.051	0.7
1167	0.043	0.8
1298	0.034	0.7
1345	0.055	0.8
1455	0.158	0.86

No. 4. 2,6-Dimethylheptane

192	0.019	0.4
255	0.053	0.5
297	0.010	..
374	0.022	0.9
423	0.041	0.2
516	0.007	..
611	0.007	..
731	0.014	..
784	0.037	0.2
809	0.076	0.1
840	0.062	0.1
909	0.020	0.6
953	0.062	1.
987	0.015	..
1058	0.025	0.9
1095	0.009	0.7
1149	0.053	0.7
1171	0.046	0.7
1208	0.010	1.
1309	0.048	0.9
1341	0.066	0.6
1458	0.150	0.78

No. 5. 3,3-Diethylpentane

176	0.027	0.93
362	0.028	..
398	0.129	0.21
454	0.010	..
664	0.075	..
684	0.089	0.1
909	0.075	0.92
986	0.031	..
1015	0.119	0.62
1048	0.034	..
1087	0.072	..
1267	0.055	0.3
1355	0.027	..
1450	0.191	0.85

No. 6. 2,2,3-Trimethylhexane

191	0.027	0.4
310	0.064	0.6
375	0.021	..
459	0.026	..
492	0.023	..
528	0.044	0.2
606	0.006	..
667	0.011	..
720	0.099	0.2
765	0.012	..
843	0.031	1.
880	0.067	0.4
924	0.100	0.98
1000	0.022	0.6
1026	0.022	0.8
1050	0.035	0.9
1095	0.024	0.6
1113	0.014	0.5
1209	0.048	0.7
1223	0.052	0.82
1239	0.052	0.9
1272	0.009	..
1309	0.024	1.
1332	0.034	0.8
1402	0.025	1.
1453	0.157	0.84

No. 7. 2,2,3,3-Tetramethylpentane

363	0.105	0.40
467	0.041	0.80
511	0.024	0.3
557	0.008	..
601	0.031	0.3
658	0.290	0.09
860	0.107	0.71
919	0.174	0.95
1015	0.061	0.74
1055	0.050	0.66
1239	0.161	0.79
1308	0.011	1.
1460	0.180	0.82
1478	0.127	0.87

No. 8. 2,2,4-Trimethylheptane

293	0.038	0.5
363	0.012	..
425	0.009	..
480	0.020	..
524	0.011	..
750	0.092	1.
833	0.031	0.4
870	0.051	0.6
926	0.078	0.87
963	0.014	0.8
1018	0.022	0.5
1041	0.030	0.6
1107	0.023	0.5
1158	0.013	..
1202	0.034	0.7
1252	0.035	0.9
1294	0.029	0.7
1354	0.016	1.
1454	0.164	0.87

No. 9. 2,2-Dimethyl-4-ethylhexane

190	0.034	..
307	0.034	0.6
456	0.030	..
491	0.038	..
549	0.026	..
608	0.013	..
739	0.107	..
785	0.016	..
835	0.034	..
862	0.034	..
906	0.071	1.
926	0.088	0.95
983	0.017	..
1023	0.051	0.8
1033	0.054	0.7
1097	0.025	0.5
1150	0.015	..
1205	0.043	0.81
1245	0.052	0.98
1301	0.033	1.
1362	0.027	1.
1397	0.028	1.
1455	0.221	0.59

No. 10. 2,2,4-Trimethyloctane

240	0.014	..
484	0.014	0.7
520	0.009	..
690	0.009	..
744	0.084	0.1
878	0.044	0.5
898	0.063	0.6
926	0.068	1.
1065	0.025	0.7
1105	0.021	0.6
1147	0.011	1.
1204	0.042	0.8
1252	0.035	0.7
1292	0.029	0.9
1358	0.011	0.7
1453	0.153	0.87

No. 11 2,2,3,5,5-Pentamethylhexane

184	0.030	0.5
241	0.040	0.4
288	0.052	1.
364	0.016	..
385	0.020	..
469	0.020	0.7
537	0.077	0.2
613	0.010	..
657	0.012	..
701	0.026	..
720	0.037	..
753	0.205	1.
854	0.059	0.66
888	0.048	0.8
924	0.162	0.89
951	0.042	0.7
1019	0.012	..
1088	0.016	1.
1108	0.014	0.9
1188	0.060	0.90
1211	0.082	0.80
1255	0.080	0.85
1294	0.027	0.5
1354	0.018	0.8
1401	0.020	0.8
1455	0.177	0.86

No. 12 2,2,4,4,6-Pentamethylheptane

189	0.024	0.6
250	0.026	..
314	0.027	0.3
374	0.010	..
414	0.007	..
462	0.012	..
480	0.014	..
506	0.018	..
579	0.014	0.9
675	0.012	0.5
729	0.173	0.1
825	0.047	0.3
858	0.061	0.7
880	0.040	0.6
919	0.091	0.87
952	0.058	0.95
972	0.025	0.9
1032	0.012	0.8
1086	0.015	..
1105	0.014	..
1146	0.029	0.7
1171	0.047	0.6
1214	0.057	0.88
1245	0.062	1.
1275	0.027	0.8
1349	0.025	0.8
1393	0.025	0.6
1455	0.165	0.85

No. 13. 7-*n*-Propyltridecane

176	0.069	0.4
213	0.061	..
508	0.015	..
870	0.042	..
918	0.038	..
1096	0.061	0.7
1168	0.038	0.9
1239	0.034	0.7
1299	0.038	..
1327	0.099	1.
1469	0.175	0.69
1568	0.019	..

No. 14. 7-*n*-Hexyltridecane

218	0.040	0.4
875	0.040	0.6
918	0.040	0.6
1053	0.040	..
1101	0.079	0.6
1134	0.036	..
1172	0.040	..
1234	0.032	0.8
1275	0.016	..
1299	0.032	..
1321	0.103	0.51
1344	0.079	..
1399	0.024	..
1464	0.174	0.76
1572	0.024	..

No. 15. 8-*n*-Hexylpentadecane

197	0.055	..
493	0.016	..
860	0.040	0.5
909	0.040	0.6
1010	0.040	..
1072	0.079	0.3
1096	0.087	0.58
1153	0.040	..
1224	0.024	..
1286	0.032	..
1317	0.095	0.77
1455	0.198	0.73

[a] Scattering coefficient is defined as ratio of intensity of sample's Raman line to that of $\Delta\tilde{\nu}$ = 459 cm.$^{-1}$ line of carbon tetrachloride (δ).

Raman Spectral Data for Hydrocarbons (*Continued*)

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
No. 16. 9-*n*-Hexylheptadecane		
202	0.048	0.4
875	0.040	0.2
918	0.032	0.7
1053	0.032	..
1106	0.068	0.98
1125	0.024	..
1168	0.024	..
1229	0.032	..
1322	0.112	0.67
1459	0.184	0.93
No. 17. 11-*n*-Amylheneicosane		
207	0.046	0.7
865	0.038	..
913	0.042	..
986	0.042	..
1106	0.100	0.5
1163	0.046	..
1224	0.031	..
1327	0.123	1.
1455	0.208	0.81
No. 18. 9-*n*-Octyleicosane		
192	0.069	0.5
326	0.027	..
865	0.023	..
1048	0.038	0.9
1096	0.069	0.91
1149	0.031	0.3
1215	0.035	0.4
1317	0.115	0.80
1445	0.196	0.90
No. 19. 9-*n*-Octylhexacosane		
218	0.063	0.65
514	0.012	..
826	0.016	..
884	0.032	..
923	0.028	..
1063	0.039	0.6
1106	0.075	1.
1234	0.024	..
1327	0.118	0.87
1368	0.024	..
1464	0.212	0.99
No. 20. 1-Hexene		
310	0.019	..
361	0.042	0.3
399	0.020	..
456	0.010	..
552	0.007	..
627	0.024	0.8
731	0.005	..
816	0.042	0.4
877	0.026	0.6
897	0.044	0.7
911	0.054	0.5
990	0.022	1.
1058	0.051	0.6
1107	0.034	0.7
1224	0.031	0.6
1298	0.154	0.45
1365	0.023	..
1423	0.102	0.50
1448	0.113	0.77
1548	0.006	..
1590	0.015	..
1649	0.197	0.13
No. 21. *cis*-2-Hexene		
300	0.024	0.6
370	0.032	0.9
466	0.025	0.6
582	0.017	..
702	0.021	..
769	0.015	..
854	0.051	0.3
892	0.059	0.3
911	0.038	0.3
972	0.038	0.6
1045	0.067	0.4
1093	0.035	0.5
1208	0.019	1.
1260	0.115	0.75
1379	0.031	0.5
No. 21 *cis*-2-Hexene (*Continued*)		
1457	0.127	0.73
1564	0.008	..
1609	0.014	..
1666	0.199	0.16
No. 22. *trans*-2-Hexene		
319	0.014	0.5
388	0.050	0.4
447	0.020	0.5
491	0.029	0.3
530	0.018	0.8
608	0.012	..
704	0.006	..
766	0.022	0.7
858	0.032	0.4
889	0.039	0.7
1039	0.046	0.7
1099	0.034	0.5
1147	0.007	0.9
1216	0.035	0.7
1265	0.055	0.6
1308	0.125	0.45
1385	0.065	0.8
1452	0.150	0.80
1577	0.008	..
1623	0.016	0.5
1681	0.170	0.22
No. 23. *trans*-3-Hexene		
172	0.056	..
407	0.039	0.3
473	0.033	..
578	0.013	..
782	0.023	0.8
826	0.053	0.76
906	0.043	0.5
952	0.016	1.
986	0.046	0.5
1007	0.059	0.48
1034	0.030	0.5
1070	0.071	0.48
1210	0.019	0.8
1253	0.072	0.73
1311	0.092	0.42
1326	0.020	..
1367	0.026	0.4
1441	0.115	0.8
1605	0.018	0.4
1656	0.191	0.24
No. 24. 2-Methyl-1-pentene		
344	0.025	0.7
398	0.065	0.90
428	0.032	1.
527	0.044	0.5
702	0.046	0.2
738	0.058	0.1
821	0.116	0.2
890	0.079	0.85
964	0.016	0.3
997	0.042	0.5
1048	0.083	0.81
1102	0.090	0.51
1219	0.051	0.6
1265	0.046	0.4
1308	0.065	0.6
1395	0.162	0.60
1436	0.252	0.66
1555	0.008	..
1601	0.014	..
1663	0.266	0.17
No. 25. 2-Methyl-2-pentene		
176	0.036	0.4
259	0.010	..
305	0.020	0.9
356	0.043	0.60
407	0.020	0.2
468	0.035	0.2
483	0.030	..
513	0.037	0.3
752	0.033	0.5
821	0.070	0.48
No. 25. 2-Methyl-2-pentene (*Continued*)		
909	0.023	0.93
981	0.012	..
1015	0.023	0.8
1063	0.079	0.38
1206	0.020	0.7
1267	0.030	0.9
1303	0.046	0.5
1351	0.038	1.
1377	0.102	0.60
1445	0.142	0.94
1604	0.020	0.33
1662	0.218	0.26
No. 26. 3-Methyl-1-pentene		
252	0.020	0.7
316	0.046	0.7
416	0.028	..
496	0.059	0.2
653	0.013	..
762	0.055	0.2
813	0.074	0.2
899	0.034	0.8
984	0.034	0.9
1027	0.034	0.7
1093	0.039	0.8
1153	0.015	0.8
1294	0.133	0.44
1425	0.072	0.3
1459	0.123	0.83
1589	0.018	..
1647	0.167	1.
No. 27. *cis*-3-Methyl-2-pentene		
323	0.042	0.6
393	0.046	0.6
430	0.027	0.9
452	0.017	..
489	0.065	0.4
549	0.027	0.9
612	0.005	..
651	0.006	..
693	0.013	..
749	0.148	0.1
823	0.027	1.
925	0.031	1.
1006	0.064	0.4
1069	0.093	0.2
1116	0.033	0.9
1209	0.015	..
1256	0.049	0.5
1322	0.050	0.6
1352	0.052	0.7
1386	0.123	0.51
1451	0.180	0.79
1578	0.010	..
1622	0.024	0.3
1681	0.273	0.21
No. 28 *trans*-3-Methyl-2-pentene		
257	0.031	1.
390	0.046	0.6
523	0.070	0.4
551	0.051	0.4
607	0.013	..
638	0.006	..
681	0.023	0.3
739	0.155	0.2
799	0.026	0.7
837	0.013	..
919	0.032	1.
990	0.085	0.65
1028	0.071	0.54
1081	0.056	0.4
1111	0.037	0.11
1152	0.007	..
1170	0.012	..
1208	0.026	1.
1271	0.029	0.9
1315	0.036	1.
1361	0.114	0.63
1385	0.111	0.71
1456	0.198	0.83
1578	0.010	0.7
1625	0.021	0.3
1680	0.258	0.21
No. 29. 4-Methyl-1-pentene		
187	0.032	0.3
238	0.017	..
346	0.041	0.5
426	0.076	0.2
454	0.023	..
621	0.045	0.3
724	0.006	..
791	0.027	0.2
819	0.094	0.1
911	0.040	1.
960	0.044	0.8
990	0.014	0.6
1041	0.010	..
1117	0.044	0.8
1170	0.025	1.
1240	0.056	0.6
1281	0.055	0.3
1300	0.102	0.3
1338	0.046	0.8
1421	0.088	0.61
1457	0.089	0.91
1546	0.008	..
1591	0.017	..
1648	0.211	0.1
No. 30. *cis*-4-Methyl-2-pentene		
333	0.065	0.4
430	0.036	0.5
483	0.079	0.3
566	0.021	0.4
611	0.007	..
726	0.013	..
781	0.060	0.6
808	0.061	0.4
833	0.051	0.2
908	0.022	1.
953	0.025	0.6
983	0.019	0.3
1048	0.017	0.7
1103	0.048	0.8
1177	0.023	0.7
1203	0.011	0.6
1250	0.018	..
1302	0.174	0.41
1383	0.058	0.6
1456	0.147	0.76
1622	0.014	..
1679	0.183	0.17
No. 31 *trans*-4-Methyl-2-pentene		
253	0.085	0.9
351	0.033	0.6
417	0.024	..
493	0.036	0.6
592	0.048	0.3
720	0.016	0.3
764	0.017	..
818	0.155	0.1
844	0.011	..
902	0.024	0.6
953	0.073	0.5
1040	0.040	0.4
1105	0.047	0.8
1163	0.014	0.7
1205	0.008	0.4
1259	0.091	0.61
1308	0.042	0.7
1379	0.028	0.7
1412	0.034	0.9
1462	0.128	0.71
1563	0.007	..
1611	0.018	..
1672	0.191	0.1
No. 32. 2-Ethyl-1-butene		
176	0.038	..
300	0.018	0.9
402	0.039	0.5
433	0.024	0.8
468	0.028	..
523	0.011	..
608	0.014	..
658	0.009	..
708	0.031	0.4
757	0.073	0.3

Raman Spectral Data for Hydrocarbons (*Continued*)

No. 32. 2-Ethyl-1-butene (*Continued*)

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
782	0.024	..
801	0.024	..
894	0.054	0.4
943	0.021	1.
1005	0.070	0.68
1039	0.070	0.1
1092	0.081	0.2
1111	0.038	..
1206	0.018	..
1262	0.047	0.3
1358	0.028	..
1413	0.088	0.73
1427	0.105	1.
1445	0.088	0.73
1536	0.014	..
1582	0.017	..
1636	0.182	0.20
1662	0.019	..

No. 33. 2,3-Dimethyl-2-butene

409	0.059	0.4
502	0.119	0.7
633	0.032	..
690	0.250	0.15
947	0.018	0.6
1027	0.045	0.77
1071	0.039	0.7
1205	0.009	..
1269	0.027	0.8
1397	0.217	0.37
1456	0.199	0.78
1623	0.015	..
1683	0.237	0.24

No. 34. 2-Methyl-1-hexene

325	0.011	1.
367	0.011	..
398	0.038	1.
459	0.014	0.6
528	0.014	0.2
704	0.020	0.2
727	0.015	..
782	0.037	0.4
825	0.047	0.4
887	0.068	0.5
953	0.009	0.5
1004	0.020	0.8
1057	0.051	0.7
1110	0.058	0.7
1208	0.025	0.7
1305	0.063	0.82
1441	0.148	0.90
1603	0.014	0.2
1663	0.155	0.31

No. 35. 3-Ethyl-2-pentene

269	0.034	0.9
313	0.017	..
414	0.060	0.4
518	0.038	0.8
566	0.008	0.7
603	0.019	..
665	0.016	..
723	0.151	0.1
793	0.011	0.4
827	0.027	0.9
924	0.086	0.76
942	0.076	0.8
1012	0.126	0.54
1031	0.145	0.44
1052	0.158	0.3
1117	0.068	0.7
1205	0.017	0.8
1258	0.096	0.56
1329	0.072	0.4
1370	0.121	0.48
1458	0.311	0.74
1621	0.023	..
1678	0.343	0.20

No. 36. 2,4-Dimethyl-1-pentene

171	0.063	0.4
315	0.045	1.
387	0.042	1.
422	0.052	0.54
533	0.049	0.48

No. 36. 2,4-Dimethyl-1-pentene (*Continued*)

698	0.042	0.41
811	0.118	0.21
894	0.035	0.62
1111	0.049	0.92
1163	0.028	0.7
1224	0.065	0.63
1331	0.066	0.71
1377	0.059	0.82
1404	0.104	0.48
1445	0.104	0.71
1639	0.139	0.21

No. 37. 4,4-Dimethyl-1-pentene

182	0.052	0.6
231	0.036	..
325	0.063	0.7
339	0.057	0.9
376	0.029	0.9
459	0.036	0.5
522	0.009	..
561	0.008	..
619	0.089	0.2
654	0.013	0.8
703	0.020	0.5
755	0.154	0.08
885	0.063	0.85
925	0.088	1.
956	0.039	0.9
1029	0.026	0.7
1064	0.009	..
1092	0.010	..
1100	0.011	..
1122	0.011	..
1193	0.088	0.91
1199	0.088	0.81
1241	0.083	0.76
1293	0.161	0.46
1372	0.011	1.
1419	0.090	0.56
1450	0.122	0.83
1550	0.013	0.6
1588	0.012	..
1646	0.169	0.2

No. 38 *cis*-4,4-Dimethyl-2-pentene

256	0.098	0.91
308	0.047	0.73
382	0.020	0.7
497	0.038	0.9
625	0.075	0.2
688	0.021	..
744	0.239	0.12
877	0.021	1.
924	0.105	0.68
961	0.047	0.4
1027	0.037	0.5
1075	0.012	0.2
1209	0.109	0.89
1239	0.042	0.85
1304	0.035	0.4
1377	0.026	0.5
1457	0.144	0.78
1558	0.008	0.2
1605	0.016	0.2
1664	0.156	0.17

No. 39 *trans*-4,4-Dimethyl-2-pentene

191	0.032	0.88
233	0.019	..
342	0.148	0.53
454	0.015	1.
533	0.095	0.2
615	0.011	..
702	0.016	..
759	0.157	0.23
790	0.039	0.86
888	0.035	0.7
923	0.088	0.77
1028	0.035	0.9
1099	0.013	0.6
1123	0.014	..
1204	0.093	0.74

No. 39 *trans*-4,4-Dimethyl-2-pentene (*Continued*)

1271	0.031	0.5
1314	0.107	0.33
1384	0.048	0.8
1453	0.145	0.87
1625	0.012	0.8
1662	0.048	0.44
1680	0.138	0.27

No. 40. *trans*-3-Octene

181	0.020	0.4
323	0.030	0.4
475	0.015	1.
808	0.020	0.7
838	0.034	..
901	0.030	0.4
993	0.024	0.9
1055	0.044	0.4
1103	0.027	0.4
1151	0.008	..
1198	0.027	0.7
1245	0.042	1.
1300	0.085	0.76
1438	0.112	0.96
1552	0.014	..
1601	0.017	..
1655	0.189	0.31

No. 41. *trans*-4-Octene

176	0.027	..
307	0.027	0.4
384	0.017	..
460	0.017	..
774	0.031	..
847	0.027	0.3
896	0.072	0.42
1041	0.048	0.3
1098	0.051	0.4
1236	0.048	0.5
1291	0.068	0.48
1438	0.116	0.84
1597	0.015	..
1655	0.182	0.26

No. 42. 6-Methyl-1-heptene

291	0.030	0.5
343	0.005	..
406	0.030	0.3
570	0.023	..
644	0.021	0.6
745	0.012	0.7
811	0.052	0.1
911	0.030	0.4
952	0.032	0.8
1020	0.016	..
1053	0.012	0.5
1123	0.020	0.4
1173	0.014	0.9
1214	0.014	0.5
1299	0.102	0.52
1341	0.044	0.7
1425	0.080	0.70
1452	0.111	0.88
1550	0.007	..
1593	0.011	..
1651	0.146	0.1

No. 43. 2-Methyl-1-pentadecene

399	0.012	..
479	0.017	0.5
836	0.029	..
885	0.023	1.
1020	0.045	..
1076	0.076	0.9
1121	0.035	..
1211	0.041	0.3
1278	0.038	..
1304	0.092	0.67
1387	0.018	0.9
1446	0.163	0.88
1550	0.021	..
1595	0.011	..
1659	0.048	..
1689	0.024	..

No. 44. 1,3-Hexadiene (*cis* and *trans*)

380	0.076	0.4
470	0.065	0.5
499	0.083	0.5
611	0.024	1.
785	0.017	1.
843	0.081	0.87
898	0.138	0.89
933	0.062	0.7
960	0.028	1.
1022	0.079	0.4
1070	0.055	0.5
1110	0.029	0.4
1182	0.453	0.53
1245	0.299	0.46
1279	0.312	0.46
1292	0.345	0.36
1328	0.074	0.4
1372	0.039	0.5
1426	0.198	0.54
1440	0.190	0.60
1558	0.111	0.31
1608	0.402	0.33
1660	2.53	0.36

No. 45. 2,4-Hexadiene (High Boiling Isomer)

264	0.028	0.7
320	0.108	0.35
438	0.113	0.68
465	0.182	0.36
600	0.045	0.5
705	0.045	0.6
817	0.091	0.77
920	0.062	0.2
946	0.034	0.5
987	0.023	..
1034	0.113	0.70
1082	0.028	0.2
1153	0.346	0.53
1246	0.682	0.50
1281	0.402	0.51
1312	0.244	0.39
1382	0.238	0.47
1458	0.385	0.58
1563	0.158	0.35
1609	0.464	0.34
1668	3.65	0.35

No. 46 2,3-Dimethyl-1,3-butadiene

401	0.080	0.90
491	0.206	0.72
549	0.042	1.
668	0.023	..
727	0.222	0.16
891	0.222	0.90
964	0.168	0.24
1023	0.451	0.34
1207	0.011	..
1316	0.113	0.45
1345	0.187	0.53
1387	0.382	0.62
1414	0.688	0.45
1445	0.197	0.65
1471	0.126	0.38
1535	0.057	0.2
1580	0.195	0.24
1637	1.83	0.27

No. 47 2,5-Dimethyl-1,5-hexadiene

239	0.011	..
264	0.017	..
321	0.006	..
373	0.029	0.7
418	0.055	0.9
495	0.022	0.7
528	0.041	0.2
696	0.029	..
765	0.042	..
852	0.063	0.2
883	0.071	0.72
964	0.021	0.8
1017	0.056	0.4
1056	0.053	0.5
1168	0.018	..

Raman Spectral Data for Hydrocarbons (*Continued*)

No. 47
2,5-Dimethyl-1,5-hexadiene
(*Continued*)

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
1211	0.020	..
1310	0.045	0.4
1387	0.127	0.54
1419	0.242	0.58
1554	0.011	..
1600	0.032	..
1656	0.338	0.20

No. 48
2,5-Dimethyl-2,4-hexadiene

171	0.204	0.69
290	0.198	0.49
336	0.057	0.65
402	0.022	0.6
463	0.191	0.95
503	0.108	0.59
845	0.197	0.77
860	0.172	1.
1072	0.165	0.58
1101	0.134	0.46
1153	1.310	0.57
1229	0.535	0.58
1271	0.045	..
1322	0.134	0.36
1372	1.255	0.54
1445	0.688	0.62
1550	0.153	0.46
1595	0.465	0.41
1648	4.780	0.44
1707	0.089	0.2

No. 49. Ethylcyclobutane

176	0.017	0.7
356	0.024	..
412	0.086	0.67
618	0.021	0.4
742	0.034	..
933	0.188	0.57
986	0.079	0.38
1092	0.079	0.4
1115	0.031	0.9
1220	0.027	0.9
1313	0.010	1.
1358	0.027	0.3
1441	0.137	0.67

No. 50
***trans*-1,2-Dimethylcyclopentane**

267	0.021	0.7
438	0.006	..
497	0.086	0.2
526	0.032	0.9
605	0.018	0.5
768	0.089	0.2
854	0.025	0.3
896	0.089	0.1
947	0.026	0.9
955	0.021	0.8
1021	0.027	0.3
1082	0.044	0.7
1145	0.032	0.6
1207	0.021	0.3
1283	0.016	0.5
1348	0.029	0.9
1463	0.148	0.84

No. 51
***cis*-1-Methyl-2-ethylcyclopentane**

310	0.019	..
343	0.010	..
404	0.048	0.2
470	0.024	0.6
492	0.019	0.8
748	0.056	0.2
803	0.033	..
838	0.023	0.3
889	0.058	0.2
901	0.068	0.3
984	0.035	0.8
1026	0.048	0.4
1044	0.062	0.4
1087	0.028	0.4
1108	0.019	..

No. 51
***cis*-1-Methyl-2-ethylcyclopentane**
(*Continued*)

1158	0.009	..
1196	0.027	1.
1260	0.024	0.8
1292	0.028	0.8
1371	0.022	0.8
1455	0.172	0.78

No. 52. *n*-Butylcyclopentane

171	0.021	0.2
300	0.083	0.3
782	0.021	0.6
840	0.042	..
894	0.114	0.27
1024	0.048	0.5
1063	0.038	0.6
1130	0.026	..
1295	0.042	1.
1445	0.152	0.93

No. 53. Isobutylcyclopentane

308	0.075	0.3
423	0.046	0.3
817	0.077	0.2
889	0.089	0.1
953	0.039	0.9
1024	0.036	0.92
1041	0.029	0.8
1091	0.015	0.5
1143	0.016	..
1174	0.027	0.7
1314	0.027	1.
1350	0.046	1.
1460	0.173	0.89

No. 54
***cis*-1,3-Dimethylcyclohexane**

257	0.026	0.8
416	0.125	0.43
446	0.052	0.3
482	0.014	..
543	0.117	0.2
713	0.019	1.
769	0.147	0.32
846	0.052	0.48
935	0.016	0.7
984	0.060	0.88
1058	0.120	1.
1113	0.029	0.6
1170	0.088	0.85
1221	0.042	0.72
1271	0.051	1.
1305	0.035	1.
1348	0.094	1.
1465	0.154	0.94

No. 55
***trans*-1,3-Dimethylcyclohexane**

357	0.033	0.4
454	0.064	0.3
486	0.037	0.3
624	0.041	0.5
696	0.023	..
753	0.266	0.1
800	0.026	0.2
860	0.041	0.3
933	0.056	0.5
984	0.043	0.8
1006	0.055	0.3
1056	0.100	0.80
1101	0.043	0.7
1165	0.053	0.7
1213	0.036	0.7
1269	0.101	0.8
1316	0.034	1.
1339	0.040	0.8
1369	0.036	0.6
1451	0.157	0.78
1463	0.152	0.77

No. 56
1,1,3-Trimethylcyclohexane

329	0.062	0.70
402	0.041	0.8
465	0.062	0.5
499	0.017	..
557	0.109	0.1
669	0.014	..
728	0.163	0.1
796	0.032	0.6
853	0.033	0.6
912	0.017	0.8
937	0.047	0.61
979	0.047	0.88
1048	0.035	0.5
1080	0.057	0.75
1136	0.008	..
1195	0.071	0.66
1220	0.052	0.70
1252	0.040	0.6
1290	0.044	0.6
1313	0.041	0.6
1359	0.052	0.6
1452	0.156	0.80
1464	0.158	0.82

No. 57. *n*-Butylcyclohexane

284	0.033	0.38
443	0.030	0.4
471	0.024	..
605	0.020	..
773	0.048	0.3
793	0.041	0.3
845	0.033	0.3
880	0.020	..
1033	0.112	0.68
1057	0.056	0.63
1103	0.045	0.6
1160	0.021	0.8
1205	0.041	1.
1269	0.071	0.89
1299	0.033	1.
1351	0.032	0.6
1450	0.158	0.78

No. 58. Isobutylcyclohexane

290	0.081	0.48
451	0.053	0.3
573	0.011	..
721	0.008	..
778	0.110	0.29
812	0.074	0.2
847	0.077	0.1
955	0.028	0.6
979	0.015	0.6
1034	0.127	0.72
1100	0.013	0.5
1162	0.044	0.5
1198	0.024	0.8
1263	0.095	0.81
1312	0.027	0.5
1352	0.072	0.78
1452	0.213	0.80

No. 59. Pentadecylcyclohexane
(Observed at 53° C.)

317	0.033	0.3
470	0.030	0.6
613	0.040	0.5
790	0.040	0.5
845	0.043	..
1030	0.083	0.70
1081	0.059	0.72
1210	0.099	0.28
1273	0.086	0.65
1307	0.056	0.68
1450	0.155	0.97
1557	0.059	0.3
1578	0.030	

No. 60. *cis*-Hydrindane

178	0.036	0.2
297	0.026	0.9
323	0.041	0.4
353	0.026	..
409	0.053	0.65
470	0.020	0.4
575	0.053	0.2
764	0.192	0.14
798	0.053	0.5
852	0.033	0.7
886	0.105	0.28
925	0.034	0.4
983	0.069	0.4
1041	0.164	0.71
1079	0.049	0.5
1160	0.020	..
1203	0.058	0.4
1269	0.082	0.54
1338	0.043	0.87
1438	0.220	1.

No. 61
1-Cyclohexyl-3(2-cyclohexyl-ethyl)hendecane

336	0.045	..
463	0.045	0.95
508	0.030	..
638	0.023	..
772	0.042	..
806	0.129	0.44
860	0.102	0.3
1053	0.205	0.89
1072	0.089	0.75
1101	0.076	0.71
1220	0.076	0.28
1253	0.045	..
1286	0.162	0.64
1313	0.091	0.81
1359	0.072	0.71
1382	0.045	..
1450	0.394	0.79
1555	0.023	0.69

No. 62
2,4-Dimethyl-1-cyclopentene

309	0.014	..
376	0.049	1.
429	0.036	0.8
477	0.015	..
535	0.023	0.7
588	0.039	0.5
658	0.009	..
712	0.012	..
780	0.033	0.6
816	0.120	0.3
876	0.036	0.2
907	0.022	0.8
979	0.020	0.3
1012	0.064	0.69
1088	0.033	0.6
1213	0.029	0.9
1264	0.026	0.7
1334	0.050	0.7
1386	0.056	0.72
1455	0.287	0.84
1563	0.013	..
1608	0.019	..
1666	0.212	0.20

Raman Spectral Data for Hydrocarbons (*Continued*)

No. 63
1,2,4-Trimethyl-1-cyclopentene

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
281	0.052	0.9
324	0.013	..
382	0.018	..
422	0.025	0.3
488	0.032	1.
531	0.033	0.7
587	0.044	1.
624	0.033	0.4
740	0.030	0.2
766	0.037	0.2
812	0.053	0.1
836	0.053	0.1
891	0.013	0.5
945	0.017	1.
973	0.024	0.7
1031	0.025	1.
1095	0.039	0.7
1171	0.013	1.
1214	0.033	0.7
1283	0.020	0.4
1343	0.039	0.5
1389	0.140	0.48
1454	0.409	0.66
1590	0.003	..
1636	0.017	0.2
1693	0.203	0.23

No. 64
1-Methyl-2-*n*-propylbenzene

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
302	0.054	0.4
340	0.013	..
450	0.032	0.6
487	0.044	0.8
546	0.126	0.34
607	0.064	0.82
715	0.246	0.18
744	0.176	0.23
761	0.172	0.1
789	0.051	..
830	0.023	..
863	0.059	0.5
890	0.089	0.59
984	0.068	0.3
1036	0.353	0.19
1092	0.079	0.3
1160	0.123	0.69
1220	0.310	0.14
1295	0.037	0.8
1341	0.042	0.7
1385	0.089	0.57
1452	0.157	0.73
1555	0.035	0.6
1590	0.137	0.66
1612	0.256	0.89

No. 65
1-Methyl-3-*n*-propylbenzene

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
224	0.157	1.
320	0.054	0.2
525	0.183	0.45
601	0.034	0.95
715	0.123	0.18
739	0.135	0.19
770	0.011	..
800	0.007	0.5
855	0.028	0.4
942	0.039	0.3
1002	0.664	0.14
1035	0.100	0.63
1100	0.095	0.35
1172	0.034	0.90
1214	0.049	0.4
1253	0.170	0.1
1290	0.049	..
1345	0.040	0.7
1385	0.082	0.50
1453	0.113	0.82
1555	0.026	..
1600	0.136	0.71
1620	0.169	0.78

No. 66
1-Methyl-4-*n*-propylbenzene

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
310	0.156	0.54
358	0.074	0.78
390	0.024	0.4
409	0.036	..
466	0.050	0.4
538	0.017	0.9
641	0.171	0.78
708	0.028	..
743	0.032	..
802	0.382	0.09
834	0.470	0.15
871	0.034	..
892	0.046	0.52
1033	0.184	0.45
1099	0.139	0.15
1145	0.066	..
1205	0.534	0.14
1291	0.026	1.
1340	0.035	0.65
1385	0.097	0.50
1450	0.133	0.87
1521	0.013	..
1625	0.384	0.70

No. 67. Naphthalene
(Observed at 110° C.)

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
309	0.025	..
388	0.067	0.93
455	0.092	0.62
511	0.885	0.48
611	0.029	0.6
663	0.029	..
709	0.134	0.35
762	0.952	0.15
882	0.021	0.8
944	0.071	0.63
971	0.067	0.3
1028	0.611	0.17
1110	0.033	0.7
1151	0.121	0.78
1210	0.021	0.8
1244	0.059	0.63
1280	0.126	0.26
1327	0.314	0.24
1383	2.95	0.23
1466	0.612	0.36
1529	0.046	1.
1584	0.486	0.70
1637	0.017	1.

No. 68. 1-Methylnaphthalene

Wave Length Shift, $\Delta\nu$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor ρ
245	0.126	1.
410	0.105	0.50
438	0.147	0.48
477	0.266	0.78
513	0.449	0.48
567	0.323	0.25
603	0.028	..
647	0.049	..
703	0.997	0.19
739	0.091	0.63
799	0.048	..
862	0.175	..
979	0.204	0.30
1022	0.435	0.29
1077	0.281	0.40

No. 68. 1-Methylnaphthalene (*Continued*)

Wave Length Shift, $\Delta\nu$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor ρ
1144	0.126	1.
1168	0.084	1.
1210	0.035	..
1240	0.056	..
1269	0.168	0.31
1317	0.442	0.20
1371	4.490	0.29
1425	0.477	0.41
1456	0.448	0.51
1511	0.114	..
1571	1.107	0.65

No. 69. 2-Methylnaphthalene
(Observed at 110° C.)

Wave Length Shift, $\Delta\nu$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor ρ
395	0.117	0.87
446	0.299	0.41
512	0.293	0.62
620	0.033	0.4
666	0.024	..
705	0.091	0.2
765	0.654	0.15
882	0.027	1.
946	0.076	0.46
1017	0.260	0.25
1122	0.036	0.4
1170	0.092	0.51
1204	0.024	0.6
1275	0.113	0.2
1325	0.263	0.26
1380	2.035	0.29
1438	0.196	0.35
1468	0.348	0.37
1580	0.248	0.71
1640	0.018	0.5

No. 70. 1,6-Dimethylnaphthalene

Wave Length Shift, $\Delta\nu$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor ρ
329	0.052	0.43
418	0.090	0.46
450	0.202	0.33
491	0.078	0.68
518	0.078	0.55
573	0.172	0.31
613	0.032	..
661	0.022	..
716	0.461	0.15
833	0.067	
981	0.041	..
1058	0.135	0.29
1078	0.146	0.37
1110	0.029	0.76
1165	0.086	0.82
1208	0.033	0.65
1280	0.067	0.23
1329	0.187	0.24
1383	1.990	0.25
1432	0.236	0.21
1474	0.277	0.25
1522	0.042	..
1591	0.536	0.61
1642	0.052	0.70

No. 71. 2,3-Dimethylnaphthalene
(Observed at 110° C.)

Wave Length Shift, $\Delta\nu$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor ρ
308	0.045	..
414	0.200	0.80
443	0.518	0.32
517	0.067	1.
612	0.050	..
680	0.022	..
739	0.426	..
813	0.225	0.34
884	0.025	..
958	0.036	..

No. 71. 2,3-Dimethylnaphthalene (*Continued*)

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
1017	0.304	0.26
1112	0.031	..
1152	0.050	..
1182	0.031	..
1208	0.036	..
1280	0.090	..
1331	0.214	0.27
1386	2.170	0.30
1471	0.834	0.45
1580	0.233	0.72
1647	0.031	..

No. 72. 2-*n*-Butylnaphthalene

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
400	0.066	..
520	0.342	0.60
718	0.096	0.64
770	0.800	0.19
887	0.052	0.61
952	0.066	0.74
961	0.061	0.5
1020	0.386	0.33
1108	0.153	0.62
1175	0.145	0.35
1208	0.048	0.32
1293	0.171	0.24
1332	0.350	0.43
1390	2.780	0.35
1448	0.404	0.51
1476	0.588	0.44
1585	0.386	0.73
1644	0.175	0.88
1665	0.114	0.9

No. 73. 1-α-Naphthylhendecane

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
192	0.095	1.
453	0.095	0.67
528	0.315	0.53
573	0.091	0.55
628	0.059	0.45
708	0.118	0.42
732	0.284	0.28
894	0.102	0.39
1044	0.244	0.33
1096	0.264	0.31
1153	0.102	1.
1187	0.071	0.35
1224	0.071	0.45
1286	0.110	0.81
1327	0.236	0.49
1382	2.170	0.29
1445	0.590	0.52
1464	0.299	0.60
1524	0.079	0.97
1581	0.803	0.63

No. 74
1-Phenyl-3-(2-phenylethyl)-hendecane

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
503	0.055	..
638	0.133	0.65
782	0.055	0.33
855	0.063	0.33
913	0.023	..
962	0.039	..
1020	0.672	0.15
1048	0.258	0.16
1168	0.070	0.87
1191	0.070	0.65
1215	0.234	0.26
1317	0.109	1.
1349	0.070	0.5
1386	0.039	..
1445	0.227	0.85
1550	0.027	0.32
1586	0.086	0.52
1604	0.289	0.83
1657	0.031	..

Raman Spectral Data for Oxygenated Compounds

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
No. 75. Dimethyl Acetal (1,1-Dimethoxyethane)		
187	0.031	0.4
310	0.024	0.4
402	0.024	0.4
493	0.007	1.
543	0.083	0.52
643	0.021	0.4
816	0.131	0.1
840	0.048	0.2
879	0.052	1.
894	0.035	0.9
1000	0.062	0.54
1092	0.035	0.4
1130	0.059	0.54
1153	0.041	0.4
1210	0.021	0.9
1368	0.045	0.93
1450	0.117	1.
1478	0.052	1.
No. 76. Ethylal (Diethoxymethane)		
187	0.032	..
229	0.006	..
356	0.024	0.20
483	0.013	0.6
633	0.016	0.2
649	0.019	1.
796	0.021	0.6
855	0.175	0.35
991	0.019	0.7
1015	0.035	0.6
1053	0.029	0.6
1111	0.073	0.36
1215	0.014	0.6
1276	0.055	0.56
1364	0.013	..
1390	0.035	0.7
1459	0.119	0.72
1481	0.064	0.73
No. 77. Acetal (1,1-Diethoxyethane)		
346	0.029	0.7
361	0.036	0.5
468	0.015	..
538	0.062	0.3
663	0.011	..
816	0.069	0.2
860	0.062	0.87
923	0.029	0.7
962	0.018	..
1039	0.036	0.7
1101	0.062	0.3
1139	0.044	0.7
1215	0.020	..
1271	0.058	0.68
1396	0.033	0.7
1455	0.138	0.80
1478	0.044	1.
No. 78. Methanol		
302	0.016	1.
477	0.016	0.9
603	0.010	..
1031	0.160	0.34
1107	0.025	0.9
1203	0.033	0.5
1268	0.019	0.8
1447	0.064	0.9
1463	0.059	1.
1533	0.014	..
No. 79. Ethanol		
321	0.009	..
437	0.026	1.
490	0.012	..
615	0.012	..
777	0.009	..
819	0.015	..
881	0.150	0.29
1050	0.085	0.63
1094	0.065	0.3
1207	0.027	1.
1274	0.051	0.87
1406	0.022	0.8
1457	0.096	0.94
1552	0.014	..

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
No. 80. 1-Propanol		
412	0.007	..
468	0.058	0.4
777	0.014	0.9
811	0.014	0.9
865	0.119	0.24
889	0.072	0.38
972	0.040	0.84
1058	0.076	0.75
1106	0.040	0.8
1220	0.011	..
1271	0.043	0.6
1299	0.043	1.
1341	0.014	..
1409	0.029	..
1450	0.126	0.89
1478	0.061	0.83
No. 81. 2-Propanol		
188	0.050	0.3
311	0.009	..
373	0.017	0.9
425	0.023	1.
485	0.025	..
763	0.017	..
815	0.258	0.21
949	0.079	1.
1033	0.017	..
1127	0.064	0.68
1160	0.032	0.7
1207	0.022	1.
1340	0.032	..
1454	0.125	0.66
1548	0.012	..
No. 82. 1-Butanol		
342	0.018	0.2
390	0.036	0.4
478	0.017	..
517	0.011	..
604	0.009	..
824	0.070	0.35
877	0.018	0.4
898	0.015	0.2
946	0.030	0.5
955	0.027	0.5
1031	0.057	0.54
1064	0.064	0.76
1109	0.052	0.48
1202	0.019	0.7
1292	0.070	0.98
1437	0.138	0.77
1535	0.011	0.7
No. 83. 2-Butanol		
382	0.017	0.3
435	0.028	0.6
501	0.052	0.4
608	0.005	..
723	0.011	..
779	0.058	0.2
823	0.087	0.34
914	0.043	0.84
994	0.075	0.96
1034	0.040	0.6
1113	0.062	0.79
1155	0.026	1.
1210	0.012	0.3
1299	0.028	0.6
1357	0.038	0.5
1445	0.120	0.79
1537	0.008	..
No. 84. 2-Methyl-2-propanol (*tert*-Butyl Alcohol)		
213	0.032	0.2
382	0.075	0.67
508	0.036	1.
688	0.012	..
732	0.036	..
787	0.409	0.82
899	0.012	0.9
952	0.182	0.73
1058	0.024	0.4
1134	0.008	..

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
No. 84. 2-Methyl-2-propanol (*Continued*)		
1177	0.012	0.9
1239	0.097	0.83
1270	0.075	0.89
1422	0.038	0.5
1482	0.221	0.82
No. 85. 3-Pentanol		
246	0.007	..
298	0.009	..
398	0.039	0.2
473	0.043	0.4
498	0.022	0.4
530	0.008	..
598	0.012	..
765	0.041	0.1
838	0.022	0.2
860	0.033	0.2
921	0.025	1.
993	0.040	1.
1017	0.044	0.8
1042	0.051	0.3
1117	0.033	0.8
1151	0.023	0.4
1212	0.004	..
1278	0.039	0.7
1301	0.038	0.7
1355	0.047	1.
1445	0.152	0.91
1535	0.004	..
1640	0.010	..
1653	0.007	..
No. 86. 2-Methyl-1-butanol (Active Amyl Alcohol)		
269	0.007	..
387	0.023	0.4
444	0.037	0.3
498	0.014	0.5
765	0.058	0.2
801	0.042	0.1
823	0.037	0.2
901	0.037	0.9
977	0.037	0.6
1016	0.047	0.6
1037	0.047	0.5
1135	0.037	0.6
1276	0.023	0.7
1362	0.019	0.5
1463	0.124	0.92
No. 87. 2-Methyl-2-butanol (*tert*-Amyl Alcohol)		
267	0.007	..
309	0.006	..
343	0.025	0.7
367	0.029	0.7
468	0.024	0.8
528	0.016	..
623	0.012	..
675	0.024	..
731	0.207	0.10
784	0.005	..
833	0.006	..
883	0.062	0.83
940	0.076	0.81
1027	0.025	0.5
1058	0.035	0.3
1187	0.046	1.
1207	0.041	0.8
1272	0.019	0.3
1292	0.013	..
1393	0.025	0.8
1438	0.138	0.80
1455	0.104	0.86

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
No. 88. *n*-Butyraldehyde		
356	0.010	..
377	0.017	..
407	0.027	..
483	0.007	..
533	0.023	0.6
643	0.005	..
693	0.017	..
806	0.030	0.4
865	0.043	0.4
913	0.010	..
952	0.022	0.6
967	0.020	..
1063	0.050	0.65
1120	0.018	..
1139	0.027	..
1215	0.013	..
1308	0.013	0.4
1400	0.077	0.54
1459	0.070	0.82
1469	0.073	1.
1555	0.010	..
1721	0.087	0.44
No. 89. Isobutyraldehyde		
285	0.018	0.7
341	0.059	0.84
402	0.037	0.7
553	0.022	..
618	0.026	..
638	0.031	0.5
747	0.011	..
801	0.162	0.23
840	0.018	0.5
918	0.070	0.81
972	0.033	0.6
1106	0.033	0.7
1139	0.033	0.9
1220	0.015	..
1276	0.055	0.89
1399	0.044	0.6
1450	0.097	0.75
1464	0.084	0.92
1698	0.051	0.5
1716	0.077	0.46
No. 90. Isovaleraldehyde		
346	0.016	0.4
397	0.007	..
433	0.013	..
535	0.010	..
620	0.006	..
777	0.047	0.9
845	0.014	..
920	0.025	0.7
982	0.036	0.7
1022	0.028	0.5
1134	0.028	0.6
1243	0.008	..
1269	0.015	0.4
1333	0.024	0.9
1391	0.044	0.59
1449	0.104	0.59
1709	0.087	0.42
No. 91. Isobutyl Formate		
269	0.023	0.8
331	0.043	1.
458	0.030	0.2
488	0.019	0.3
762	0.047	0.2
828	0.070	0.2
845	0.048	..
904	0.038	0.3
933	0.043	0.2
967	0.075	0.50
1024	0.013	0.8
1134	0.040	0.8
1182	0.040	0.5
1206	0.025	0.4
1299	0.018	1.
1341	0.038	0.7
1377	0.050	0.4
1455	0.118	0.82
1703	0.080	0.3

Raman Spectral Data for Oxygenated Compounds (*Continued*)

No. 92. Ethyl Acetate

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
304	0.024	0.3
375	0.097	0.40
464	0.013	..
635	0.131	0.37
790	0.039	0.3
848	0.094	0.2
937	0.024	0.4
1006	0.031	0.3
1050	0.035	0.6
1107	0.048	0.3
1206	0.014	0.9
1271	0.028	0.9
1390	0.025	0.6
1445	0.098	0.92
1721	0.078	0.31

No. 93. Isopropyl Acetate

320	0.017	1.
336	0.081	0.76
433	0.027	1.
458	0.048	0.2
508	0.020	..
598	0.010	..
658	0.156	0.31
787	0.010	..
840	0.134	0.1
909	0.056	0.93
981	0.068	0.3
1053	0.017	0.3
1134	0.048	0.2
1168	0.037	0.9
1206	0.024	0.3
1224	0.014	0.9
1359	0.051	1.
1382	0.027	0.9
1404	0.034	0.9
1469	0.129	0.86
1510	0.010	..
1559	0.017	..
1739	0.061	0.3

No. 94. *sec*-Butyl Acetate

207	0.031	..
243	0.017	..
366	0.039	0.7
448	0.027	0.5
483	0.015	..
523	0.031	0.2
658	0.104	0.34
865	0.058	0.3
894	0.062	0.68
972	0.058	0.1
1015	0.042	0.5
1063	0.031	..
1120	0.023	..
1149	0.039	0.4
1206	0.023	..
1229	0.012	..
1299	0.027	0.5
1368	0.046	0.87
1395	0.035	1.
1469	0.116	0.76
1763	0.066	0.43

No. 95. Isobutyl Acetate

181	0.029	..
279	0.025	0.7
320	0.047	1.
407	0.036	0.7
508	0.018	0.7
583	0.011	..
613	0.025	0.5
638	0.079	0.4
831	0.086	0.1
913	0.036	0.4
928	0.036	0.3
967	0.032	1.
991	0.018	0.7
1034	0.022	..
1053	0.011	..

No. 95. Isobutyl Acetate (*Continued*)

1130	0.036	0.5
1182	0.025	1.
1206	0.011	..
1253	0.014	..
1299	0.018	..
1336	0.029	0.7
1450	0.111	0.62
1721	0.047	0.3

No. 96. Methyl Propionate

346	0.017	0.4
392	0.007	..
448	0.103	0.29
623	0.040	0.2
806	0.020	0.3
865	0.159	0.19
967	0.045	0.77
1034	0.045	0.4
1096	0.076	0.2
1191	0.016	0.6
1215	0.016	0.6
1267	0.010	0.5
1280	0.010	0.4
1399	0.030	0.4
1427	0.076	0.68
1459	0.102	0.60
1730	0.083	0.20

No. 97. Isobutyl Propionate

290	0.025	..
320	0.018	..
341	0.011	..
417	0.042	0.2
623	0.018	..
811	0.025	..
840	0.060	0.2
904	0.049	..
928	0.035	0.7
981	0.046	0.80
1034	0.021	0.5
1092	0.046	0.1
1144	0.030	0.7
1187	0.021	0.7
1220	0.018	0.7
1276	0.025	0.7
1308	0.025	0.7
1344	0.032	0.6
1399	0.025	0.7
1432	0.049	1.
1464	0.134	0.88
1730	0.053	0.2

No. 98. Ethyl *n*-Butyrate

213	0.030	0.5
356	0.066	0.3
402	0.031	0.7
498	0.013	1.
633	0.031	..
899	0.057	0.2
928	0.044	0.2
1058	0.057	0.69
1134	0.066	..
1239	0.013	..
1295	0.026	0.5
1478	0.122	0.8
1748	0.061	0.3

No. 99. Ethyl Isobutyrate

320	0.034	0.3
346	0.027	..
402	0.017	0.3
503	0.034	0.3
608	0.020	..
663	0.013	..
762	0.010	..
801	0.029	..
865	0.124	0.23
875	0.069	0.5
938	0.035	0.5
972	0.023	0.8
1044	0.042	0.7
1115	0.070	0.2
1134	0.080	0.3

No. 99. Ethyl Isobutyrate (*Continued*)

1220	0.013	..
1281	0.027	0.3
1303	0.042	0.7
1405	0.023	..
1464	0.148	0.83
1730	0.057	0.2

No. 100. Dimethyl Carbonate

249	0.021	0.7
315	0.007	..
377	0.014	0.9
417	0.007	..
468	0.019	0.5
518	0.250	0.39
693	0.018	..
865	0.035	..
918	0.317	0.22
1125	0.037	0.5
1163	0.018	..
1210	0.042	0.4
1271	0.011	..
1455	0.106	0.79
1739	0.053	0.1

No. 101. Diethyl Carbonate

181	0.034	0.3
361	0.100	0.33
523	0.045	0.5
806	0.017	..
855	0.027	..
904	0.144	0.17
981	0.021	0.4
1024	0.024	0.7
1092	0.041	0.1
1120	0.100	0.28
1172	0.007	..
1206	0.014	..
1276	0.031	0.7
1303	0.031	0.7
1345	0.007	..
1391	0.017	1.
1450	0.148	0.74
1730	0.034	0.1
1752	0.014	..

No. 102. Methyl Orthoacetate (1,1,1-Trimethoxyethane)

326	0.026	..
422	0.043	0.2
498	0.032	0.6
528	0.023	0.4
573	0.027	0.8
638	0.058	0.50
698	0.010	0.8
752	0.196	0.08
860	0.014	..
904	0.060	0.89
1063	0.050	0.68
1087	0.045	0.2
1134	0.037	0.7
1172	0.045	0.4
1210	0.026	..
1257	0.022	0.7
1445	0.129	0.75
1459	0.141	0.81

No. 103. Diethyl Ether

299	0.009	1.
374	0.028	0.2
439	0.112	0.37
483	0.019	..
609	0.012	..
796	0.018	0.5
843	0.078	0.48
922	0.021	1.
936	0.021	0.9
1026	0.030	0.6
1046	0.033	0.6
1074	0.033	0.7
1103	0.039	0.7
1151	0.059	0.4
1229	0.014	0.4
1269	0.048	0.77
1392	0.021	0.9
1452	0.130	0.92
1542	0.006	0.5

No. 104. Ethyl Isopropyl Ether

192	0.027	0.3
356	0.039	0.7
427	0.018	0.7
493	0.074	0.1
618	0.010	..
831	0.115	0.34
904	0.064	0.71
952	0.042	0.9
981	0.020	..
1034	0.013	..
1096	0.038	0.4
1125	0.044	0.4
1153	0.054	0.5
1201	0.040	..
1224	0.022	..
1285	0.035	0.7
1354	0.037	0.7
1400	0.030	0.3
1464	0.147	0.68

No. 105. Di-*n*-propyl Ether

213	0.020	..
320	0.010	..
356	0.033	..
443	0.036	0.2
498	0.016	0.4
904	0.056	0.2
923	0.063	0.1
938	0.049	0.2
962	0.026	0.9
1063	0.040	0.4
1087	0.030	..
1144	0.033	0.2
1182	0.026	..
1276	0.026	0.9
1308	0.063	0.85
1414	0.010	..
1478	0.138	0.81
1500	0.056	0.65

No. 106. Ethyl *n*-Butyl Ether

202	0.039	..
346	0.048	0.2
438	0.007	..
463	0.023	..
840	0.028	0.3
860	0.067	0.2
923	0.035	..
976	0.017	..
991	0.013	..
1044	0.027	..
1096	0.027	..
1125	0.033	0.3
1149	0.040	0.3
1168	0.037	0.3
1290	0.027	0.5
1313	0.050	0.65
1469	0.139	0.96
1496	0.053	1.

No. 107. Ethyl Isobutyl Ether

202	0.029	..
336	0.066	0.4
438	0.033	0.2
478	0.046	0.5
513	0.008	0.4
855	0.075	0.3
909	0.048	0.5
943	0.013	..
981	0.036	0.7
1134	0.033	0.5
1168	0.036	0.7
1206	0.026	0.9
1276	0.033	1.
1317	0.023	0.9
1358	0.025	0.9
1459	0.105	0.57
1473	0.137	0.70

Raman Spectral Data for Oxygenated Compounds (*Continued*)

No. 108. Ethyl *tert*-Butyl Ether

Wave No. Shift, $\Delta\tilde{\nu}$, Cm.$^{-1}$	Scattering Coefficient	Depolarization Factor, ρ
218	0.027	..
377	0.076	0.57
392	0.083	0.3
488	0.023	0.3
548	0.073	0.2
723	0.017	..
777	0.213	0.06
884	0.096	0.81
943	0.050	0.79
952	0.067	0.65
991	0.015	..
1058	0.020	..
1115	0.035	0.3
1158	0.030	..
1206	0.010	..
1234	0.050	0.5
1266	0.047	0.4
1299	0.053	0.76
1419	0.027	0.4
1441	0.025	..
1478	0.166	0.82
1486	0.163	0.69

No. 109. Acetone

295	0.007	0.4
311	0.007	..
389	0.019	0.9
493	0.024	0.9
530	0.052	1.
614	0.011	..
736	0.026	0.4
787	0.265	0.21
894	0.020	0.8
1039	0.020	..
1070	0.036	0.3
1111	0.010	0.8
1222	0.049	0.86
1262	0.006	..
1361	0.028	0.7
1425	0.100	0.75
1537	0.011	0.4
1642	0.009	..
1702	0.106	0.54

No. 110. 2-Butanone
(Methyl Ethyl Ketone)

406	0.041	0.7
473	0.014	..
593	0.052	0.59
714	0.016	0.6
762	0.170	0.20
947	0.022	0.35
1002	0.025	0.71
1035	0.009	..
1088	0.056	0.2
1202	0.028	0.4
1270	0.022	0.6
1414	0.092	1.
1456	0.070	0.88
1706	0.087	0.55

No. 111. 3-Methyl-2-butanone
(Methyl Isopropyl Ketone)

310	0.025	..
347	0.028	..
442	0.015	..
470	0.011	..
604	0.041	..
687	0.017	1.
723	0.157	0.21
882	0.053	0.46
957	0.035	0.5
1101	0.046	0.7
1107	0.042	0.6
1137	0.025	0.5
1202	0.033	0.81
1292	0.025	..
1347	0.017	0.8
1455	0.082	0.94
1715	0.074	0.63

No. 112. 3-Pentanone
(Diethyl Ketone)

314	0.014	..
407	0.084	0.41
752	0.045	0.2
783	0.062	0.1
951	0.025	..
1017	0.042	0.3
1097	0.082	0.2
1212	0.025	0.4
1425	0.092	0.79
1463	0.092	0.96
1715	0.076	0.37

No. 113
2,4-Dimethyl-3-pentanone
(Diisopropyl Ketone)

213	0.024	0.6
320	0.048	0.5
356	0.020	0.9
498	0.065	0.2
593	0.010	..
643	0.017	0.8
742	0.044	0.3
772	0.081	0.2
865	0.017	0.9
894	0.042	1.
923	0.145	0.31
991	0.037	0.77
1096	0.034	0.9
1144	0.061	0.67
1153	0.041	0.77
1201	0.020	0.65
1234	0.007	0.4
1303	0.041	0.5
1354	0.037	0.9
1409	0.020	0.8
1469	0.142	0.87
1721	0.064	0.4

No. 114. 4-Methyl-2-pentanone
(Methyl Isobutyl Ketone)

310	0.063	0.72
339	0.021	0.7
413	0.025	0.2
472	0.011	0.4
524	0.013	0.9
592	0.088	0.36
790	0.069	0.1
817	0.064	0.3
953	0.053	0.73
1121	0.066	0.67
1170	0.040	1.
1199	0.034	0.7
1329	0.049	0.7
1407	0.070	0.68
1443	0.110	0.79
1534	0.017	0.9
1698	0.080	0.49

No. 115. 1,2-Epoxycyclohexane

370	0.042	0.6
439	0.037	0.8
534	0.016	0.1
638	0.042	0.9
684	0.009	..
747	0.095	0.58
781	0.379	0.27
834	0.174	0.09
882	0.063	0.77
966	0.093	0.43
1035	0.194	0.82
1088	0.049	0.8
1189	0.063	0.55
1264	0.200	0.47
1349	0.033	0.7
1449	0.146	0.71

No. 116. Benzofuran
(Coumarone)

227	0.048	0.99
259	0.056	0.94
427	0.035	0.2
483	0.013	..
538	0.169	0.61
588	0.101	1.
613	0.308	1.
665	0.025	..
707	0.051	0.3
763	0.814	0.21
803	0.018	0.5
861	0.286	0.63
901	0.121	0.80
955	0.033	..
1014	0.634	0.15
1034	0.303	0.24
1112	0.136	0.98
1134	0.318	0.46
1151	0.111	0.89
1195	0.043	0.3
1255	1.04	0.46
1336	0.399	0.34
1354	0.364	0.61
1443	0.111	0.25
1458	0.242	0.37
1482	0.141	0.33
1541	1.55	0.26
1603	0.332	0.47
1619	0.824	0.51

No. 117. Isobutyl Bromide
(1-Bromo-2-methylpropane)

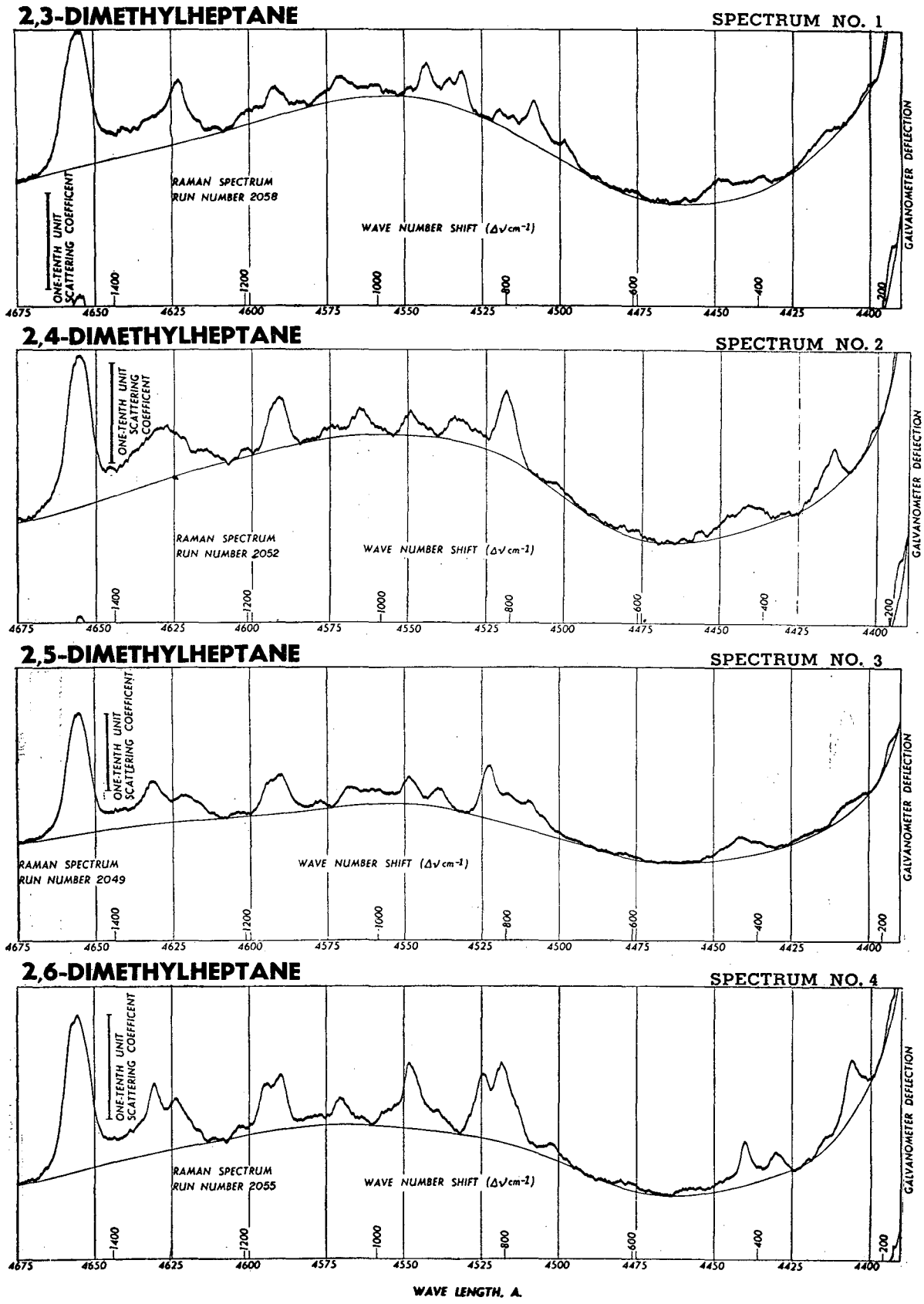
194	0.078	0.49
309	0.167	0.39
396	0.067	0.44
419	0.065	0.2
466	0.110	0.43
566	0.024	..
623	0.267	0.23
652	0.457	0.33
807	0.088	0.43
924	0.053	0.54
941	0.076	0.49
1060	0.026	0.9
1113	0.040	0.6
1178	0.031	0.8
1200	0.053	0.72
1237	0.077	0.56
1320	0.072	0.61
1345	0.028	0.7
1459	0.128	0.93

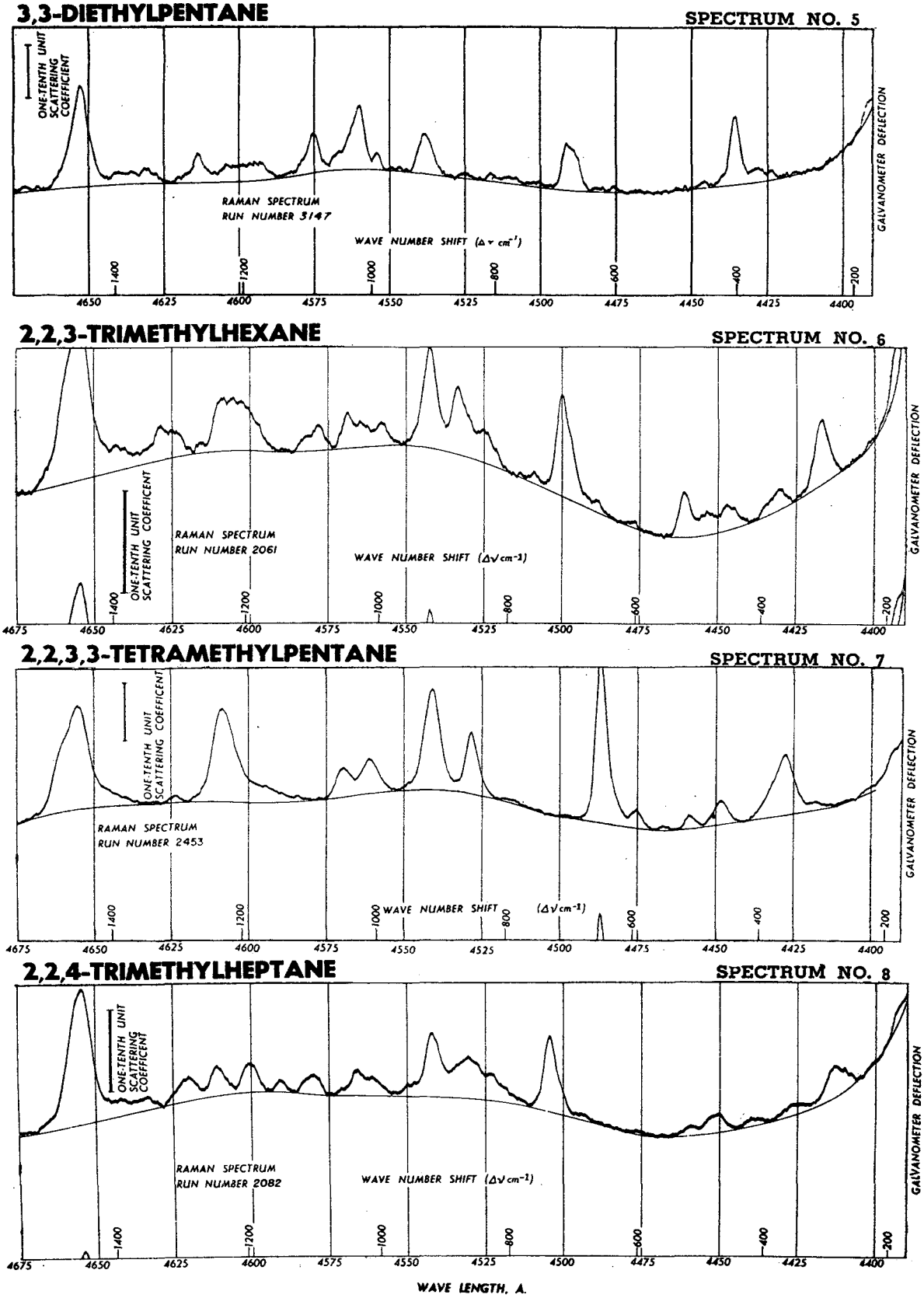
No. 118. *sec*-Butyl Bromide
(2-Bromobutane)

213	0.027	1.
297	0.262	0.41
314	0.166	0.37
352	0.100	0.34
458	0.121	0.30
479	0.158	0.35
530	0.444	0.33
580	0.104	0.31
609	0.141	0.39
786	0.078	0.65
838	0.092	0.74
951	0.049	0.56
997	0.036	0.4
1049	0.053	0.3
1114	0.040	0.5
1152	0.053	0.53
1211	0.104	0.37
1281	0.037	0.6
1388	0.022	0.9
1454	0.154	0.88

No. 119. *tert*-Butyl Bromide
(2-Bromo-2-methylpropane)

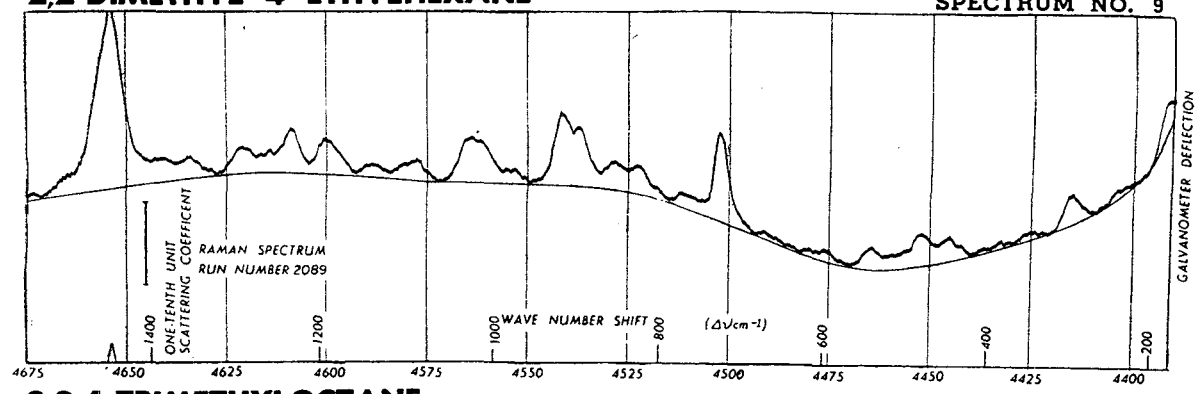
252	0.035	0.66
306	0.537	0.36
397	0.025	0.5
458	0.046	0.2
514	0.514	0.23
801	0.127	0.70
926	0.015	0.9
1031	0.018	0.5
1085	0.023	0.2
1145	0.223	0.33
1242	0.033	0.5
1462	0.081	0.55


2,3-DIMETHYLHEPTANE
SPECTRUM NO. 1
RAMAN SPECTRUM
RUN NUMBER 2058
ONE-TENTH UNIT
SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
2,4-DIMETHYLHEPTANE
SPECTRUM NO. 2
RAMAN SPECTRUM
RUN NUMBER 2052
ONE-TENTH UNIT
SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
2,5-DIMETHYLHEPTANE
SPECTRUM NO. 3
RAMAN SPECTRUM
RUN NUMBER 2049
ONE-TENTH UNIT
SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
2,6-DIMETHYLHEPTANE
SPECTRUM NO. 4
RAMAN SPECTRUM
RUN NUMBER 2055
ONE-TENTH UNIT
SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
1400 1200 1000 800 600 400 200
WAVE LENGTH, A.


3,3-DIETHYLPENTANE
SPECTRUM NO. 5
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3147
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
2,2,3-TRIMETHYLHEXANE
SPECTRUM NO. 6
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2061
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
2,2,3,3-TETRAMETHYLPENTANE
SPECTRUM NO. 7
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2453
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
2,2,4-TRIMETHYLHEPTANE
SPECTRUM NO. 8
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2082
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
1400 1200 1000 800 600 400 200
4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
WAVE LENGTH, A.

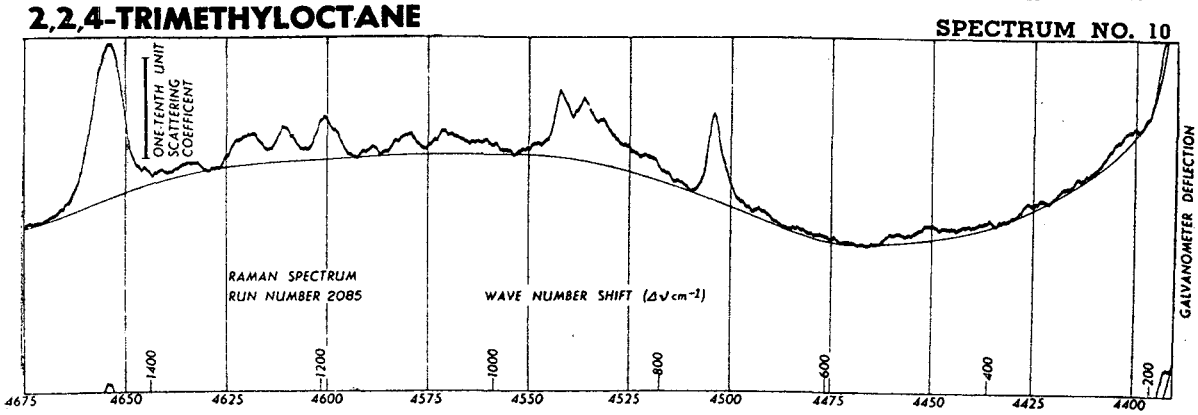
2,2-DIMETHYL- 4- ETHYLHEXANE

SPECTRUM NO. 9



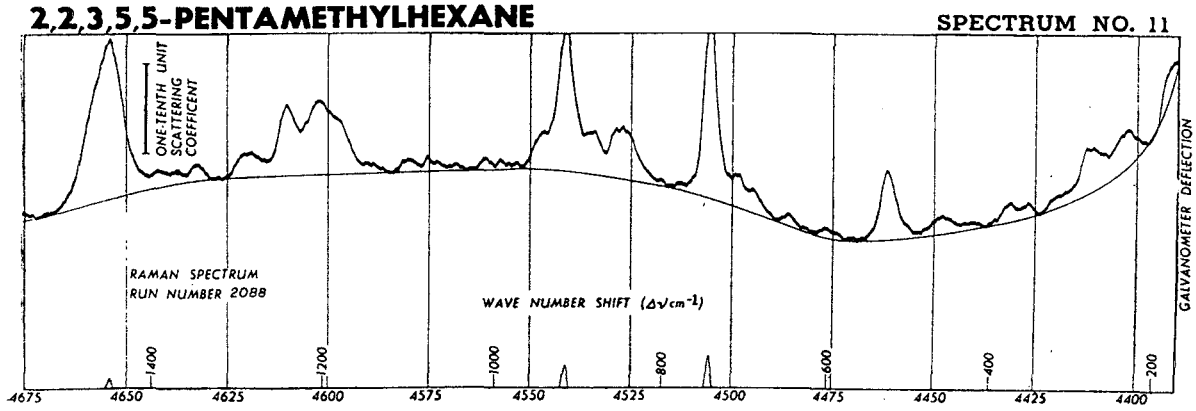
2,2,4-TRIMETHYLOCTANE

SPECTRUM NO. 10



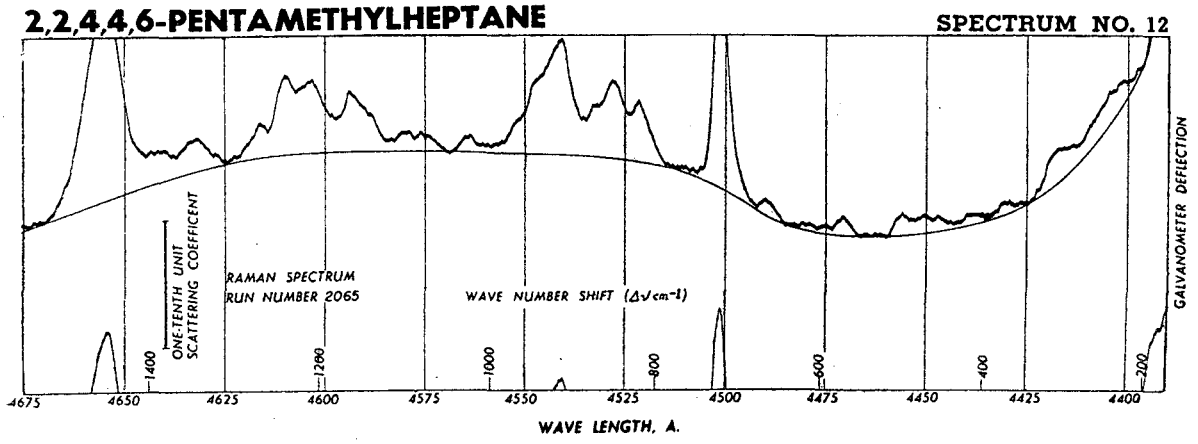
2,2,3,5,5-PENTAMETHYLHEXANE

SPECTRUM NO. 11



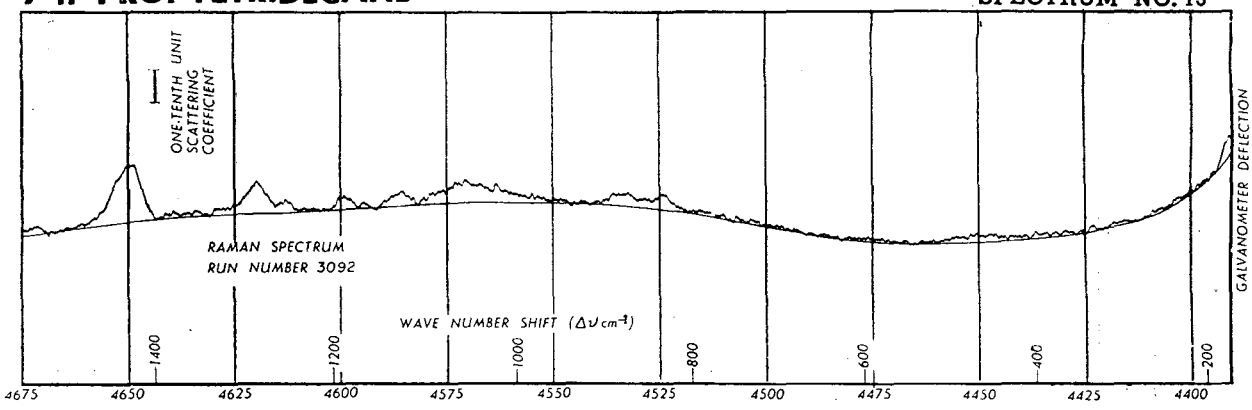
2,2,4,4,6-PENTAMETHYLHEPTANE

SPECTRUM NO. 12



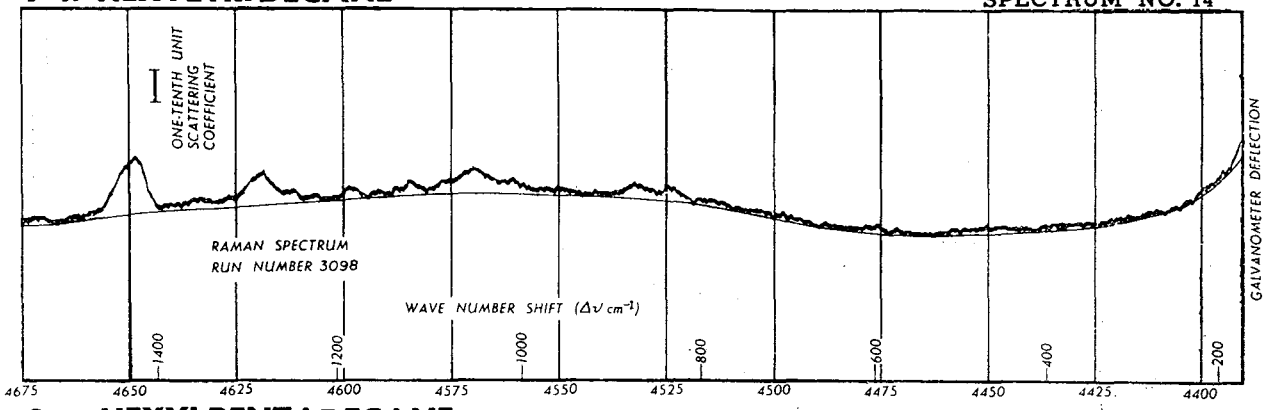
7-n-PROPYLTRIDECANE

SPECTRUM NO. 13



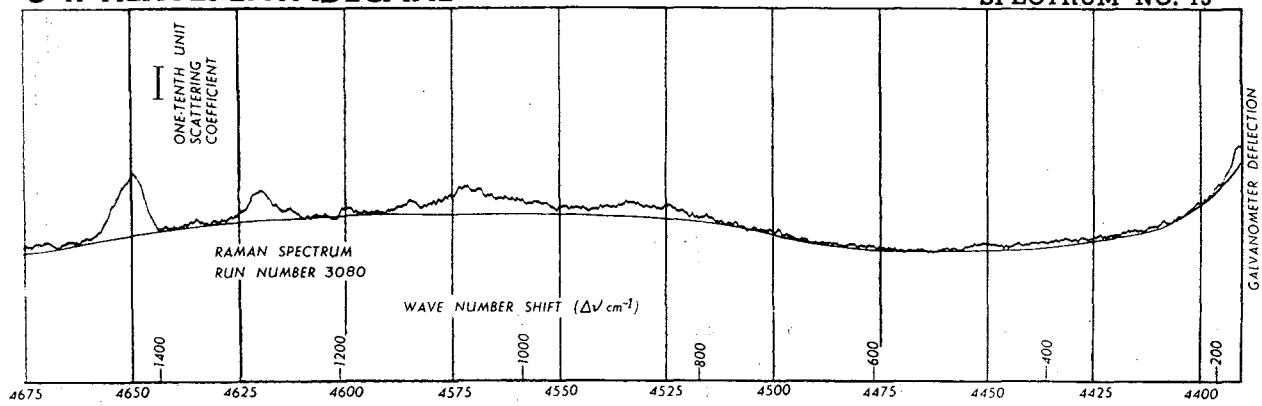
7-n-HEXYLTRIDECANE

SPECTRUM NO. 14



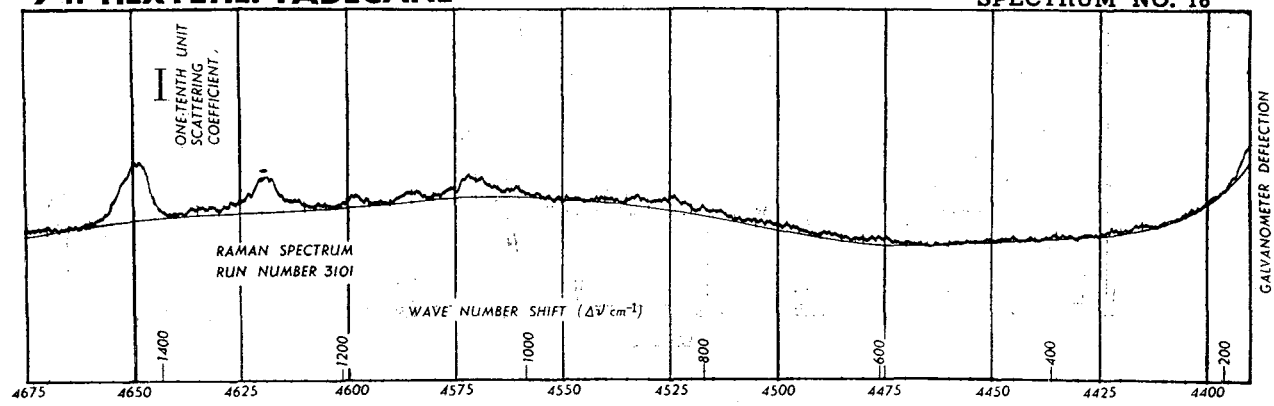
8-n-HEXYLPENTADECANE

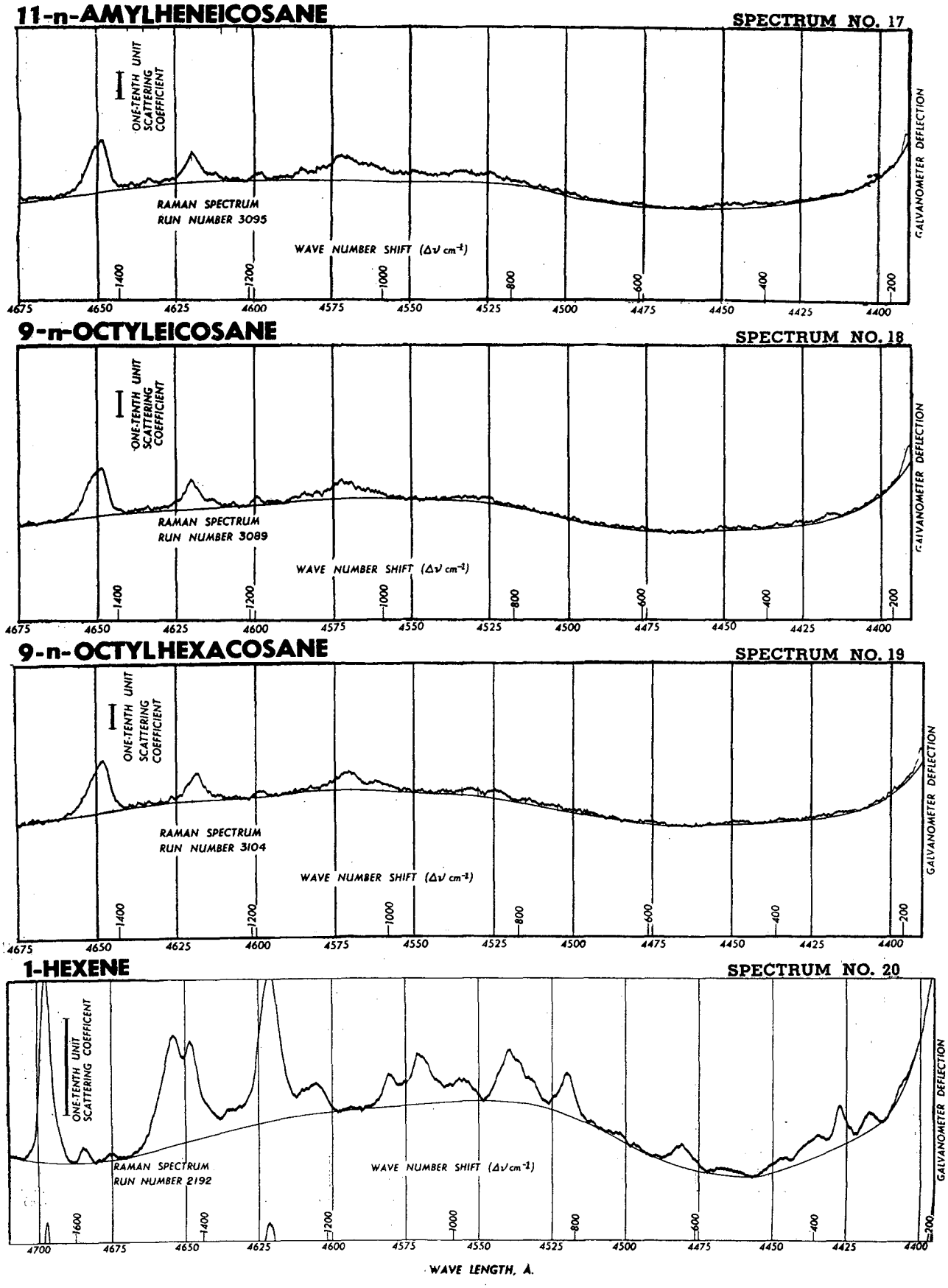
SPECTRUM NO. 15



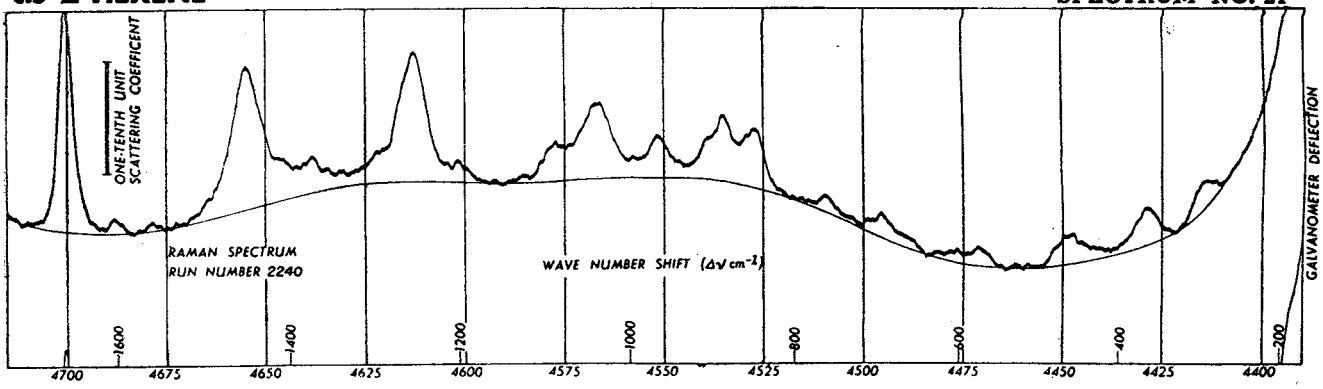
9-n-HEXYLHEPTADECANE

SPECTRUM NO. 16

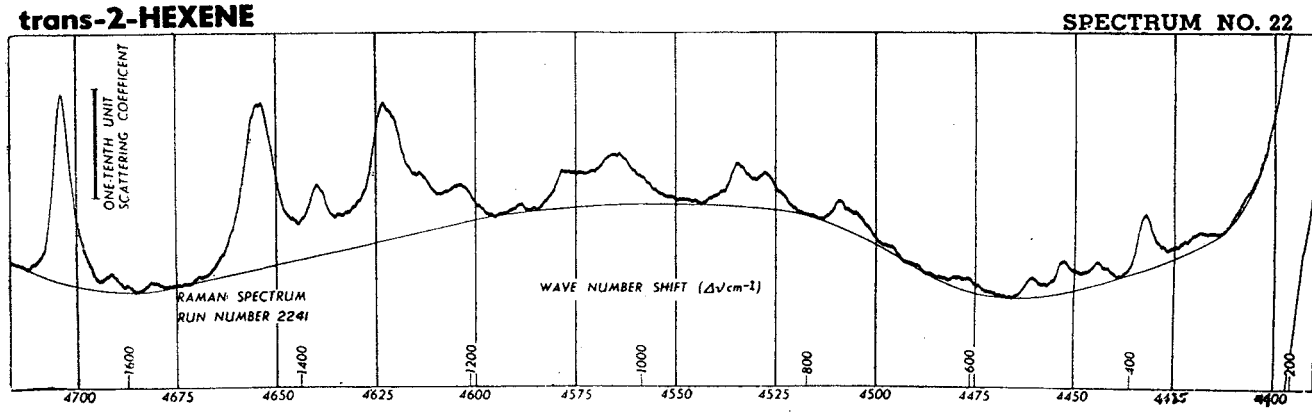



11-n-AMYLHENEICOSANE
SPECTRUM NO. 17
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3095
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
9-n-OCTYLEICOSANE
SPECTRUM NO. 18
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3089
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
9-n-OCTYLHEXACOSANE
SPECTRUM NO. 19
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3104
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
1-HEXENE
SPECTRUM NO. 20
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 2192
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
WAVE LENGTH, Å.

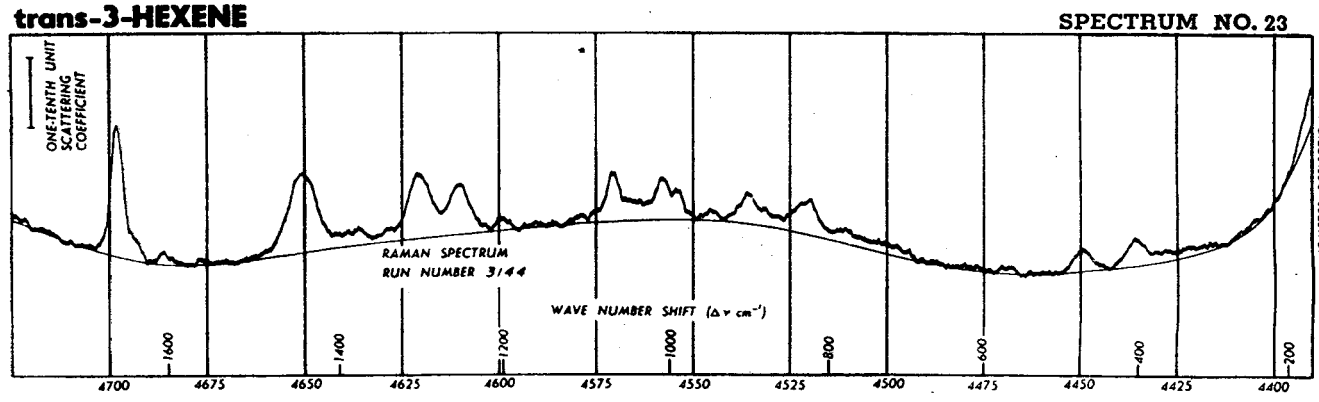
cis-2-HEXENE SPECTRUM NO. 21



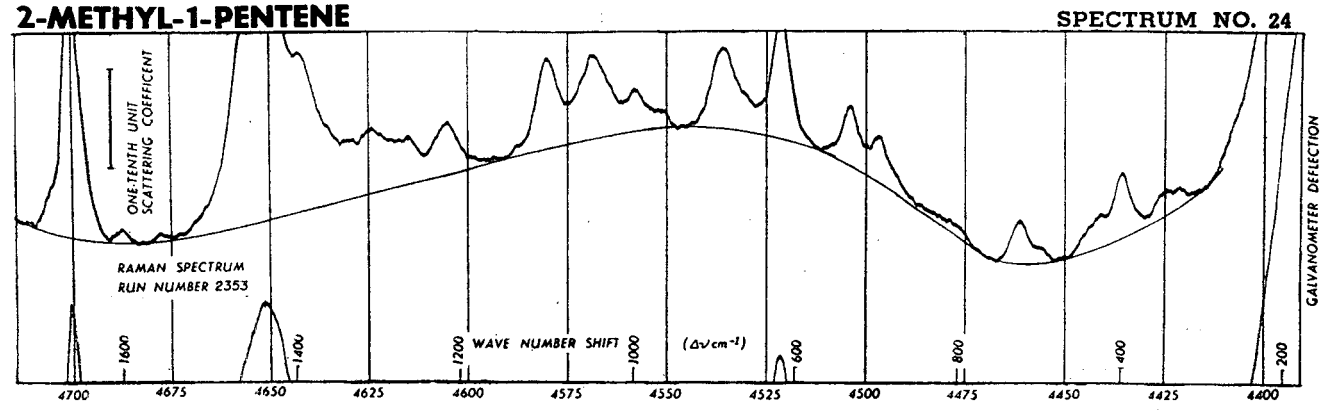
trans-2-HEXENE SPECTRUM NO. 22



trans-3-HEXENE SPECTRUM NO. 23



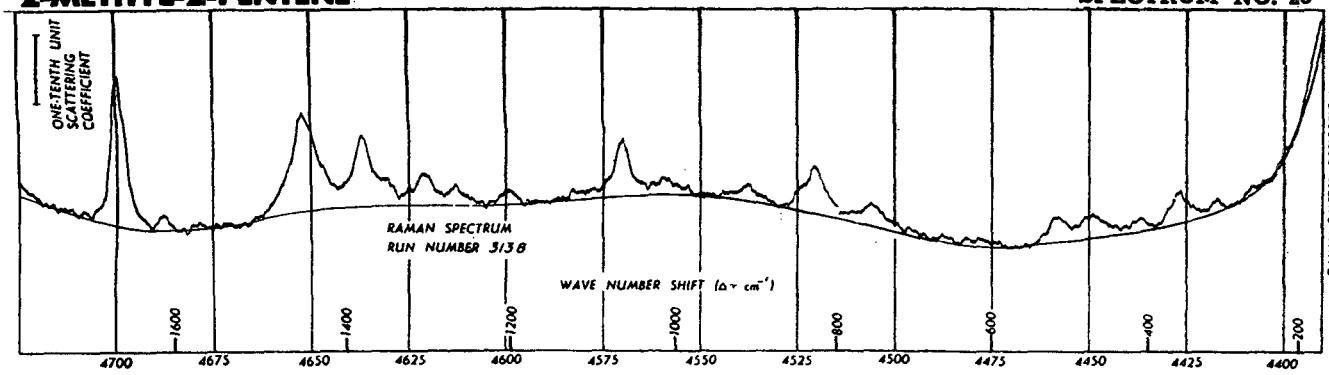
2-METHYL-1-PENTENE SPECTRUM NO. 24



WAVE LENGTH, A.

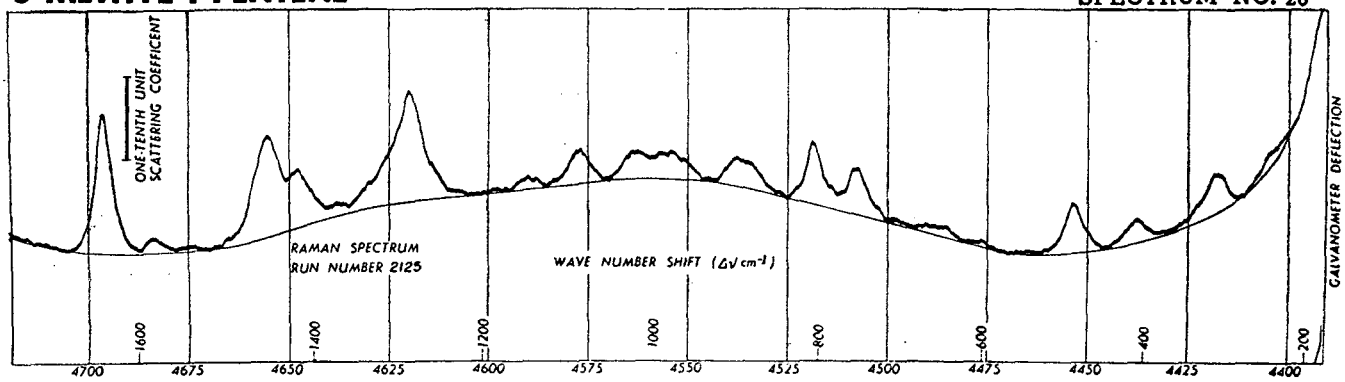
2-METHYL-2-PENTENE

SPECTRUM NO. 25



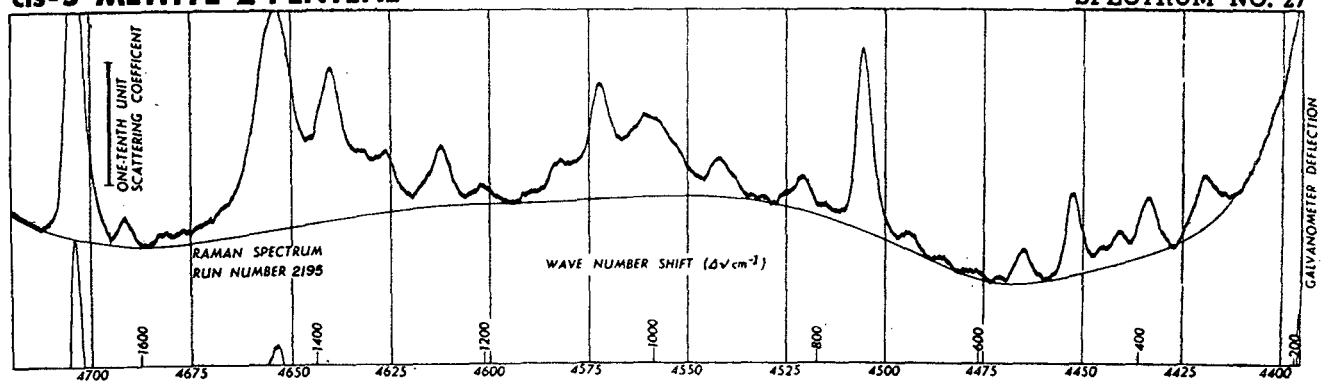
3-METHYL-1-PENTENE

SPECTRUM NO. 26



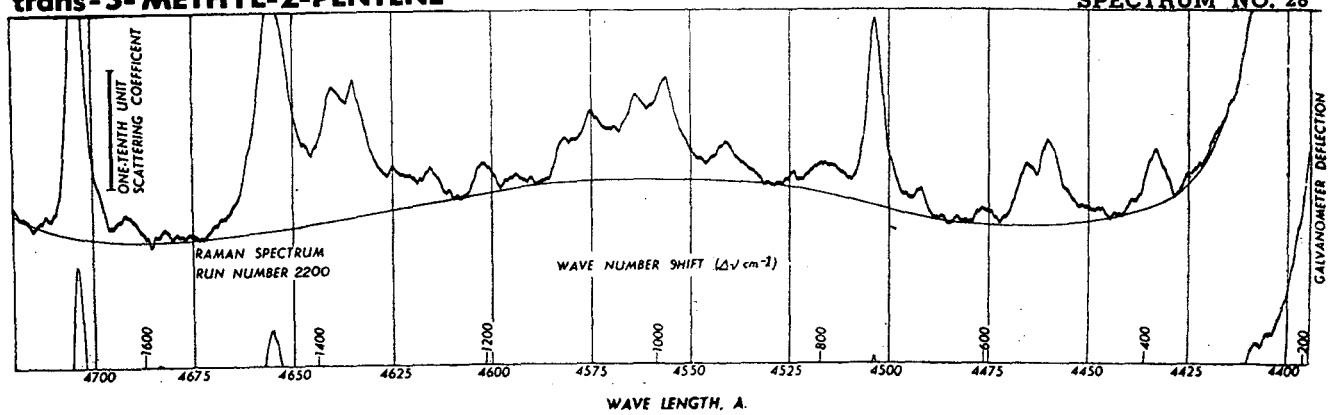
cis-3-METHYL-2-PENTENE

SPECTRUM NO. 27



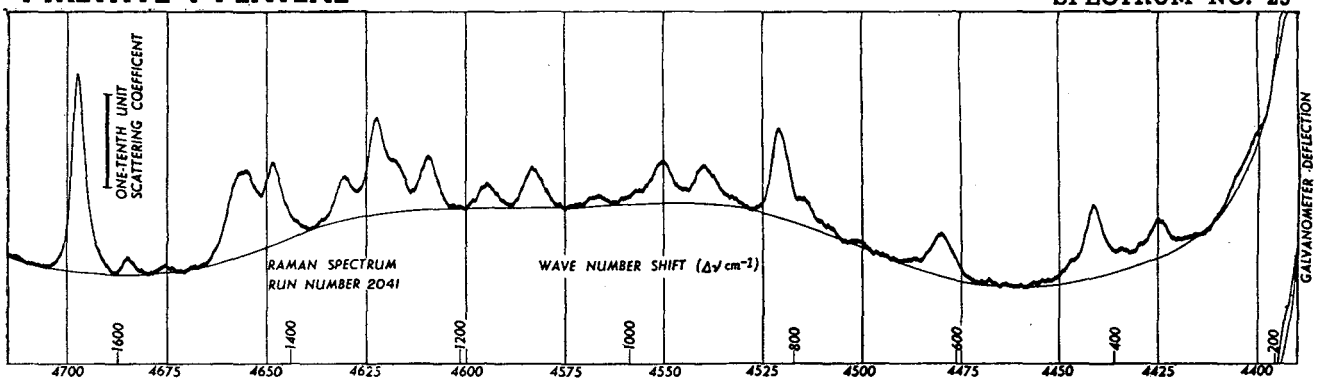
trans-3-METHYL-2-PENTENE

SPECTRUM NO. 28



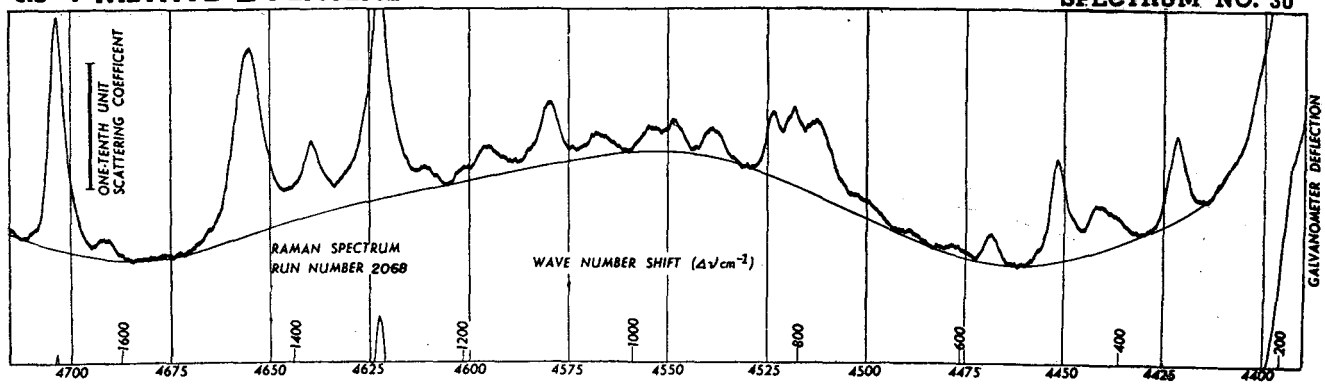
4-METHYL-1-PENTENE

SPECTRUM NO. 29



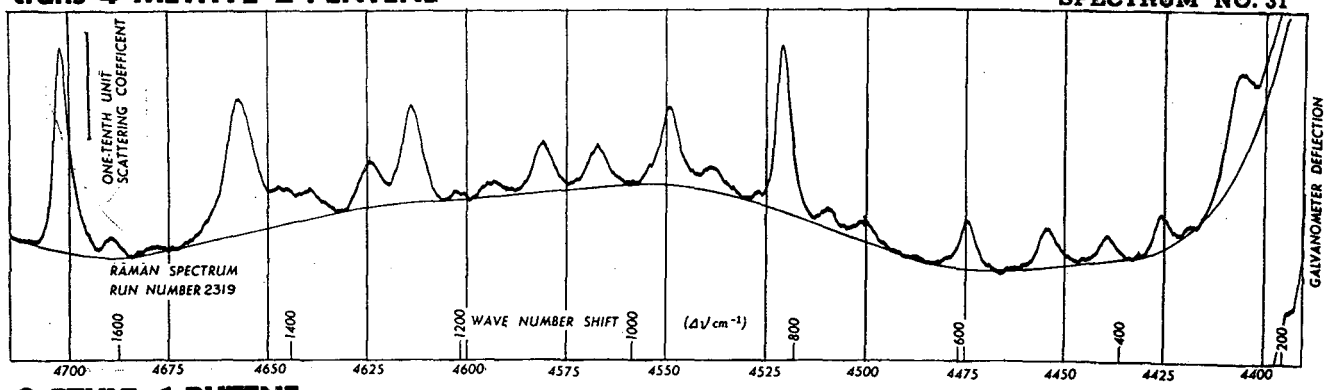
cis-4-METHYL-2-PENTENE

SPECTRUM NO. 30



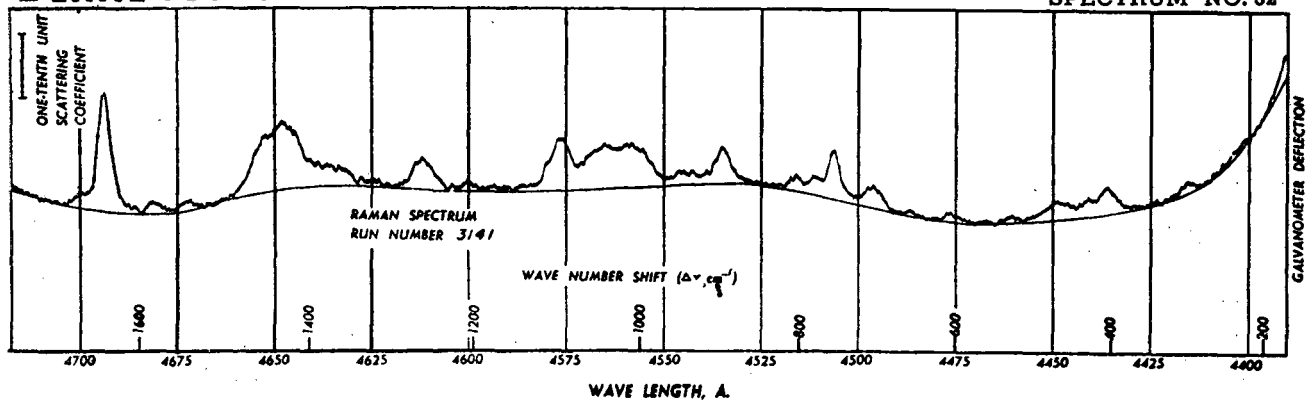
trans-4-METHYL-2-PENTENE

SPECTRUM NO. 31



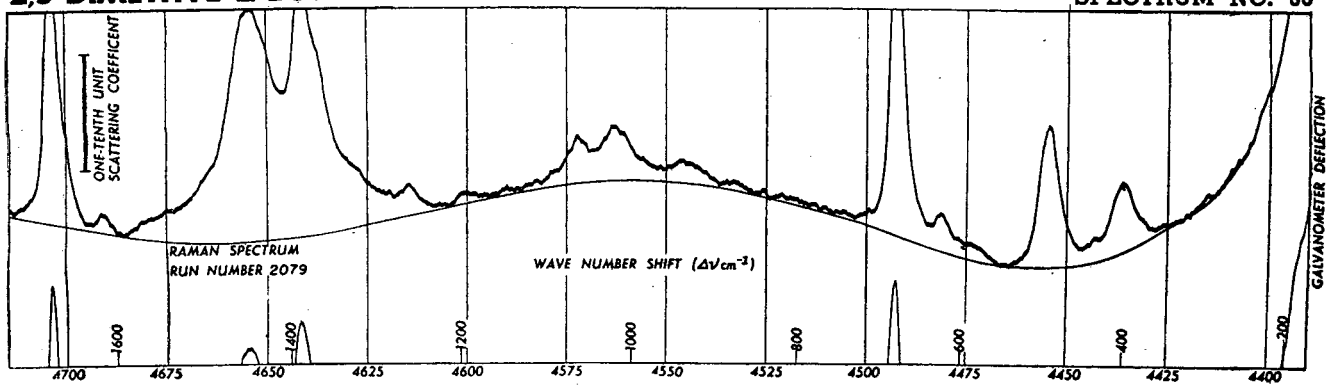
2-ETHYL-1-BUTENE

SPECTRUM NO. 32



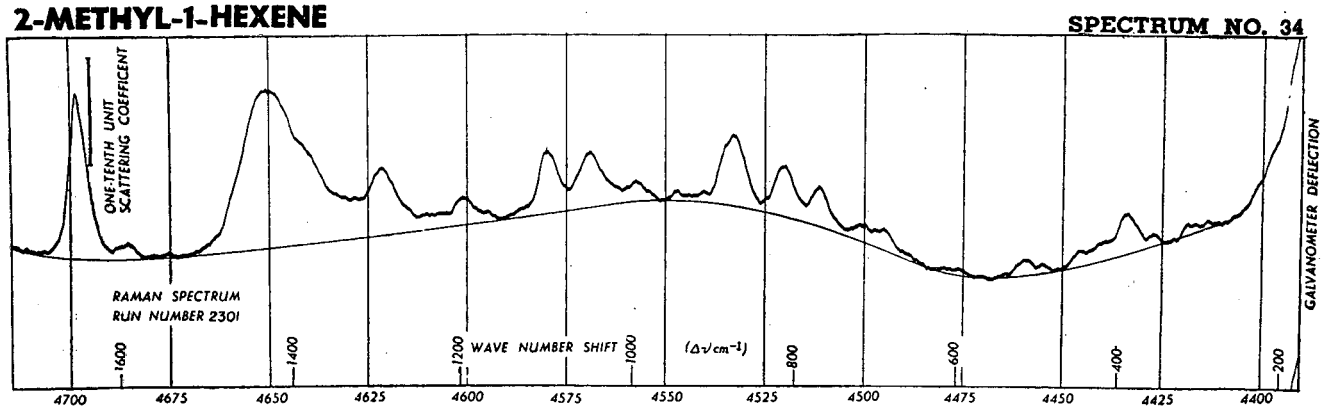
2,3-DIMETHYL-2-BUTENE

SPECTRUM NO. 33



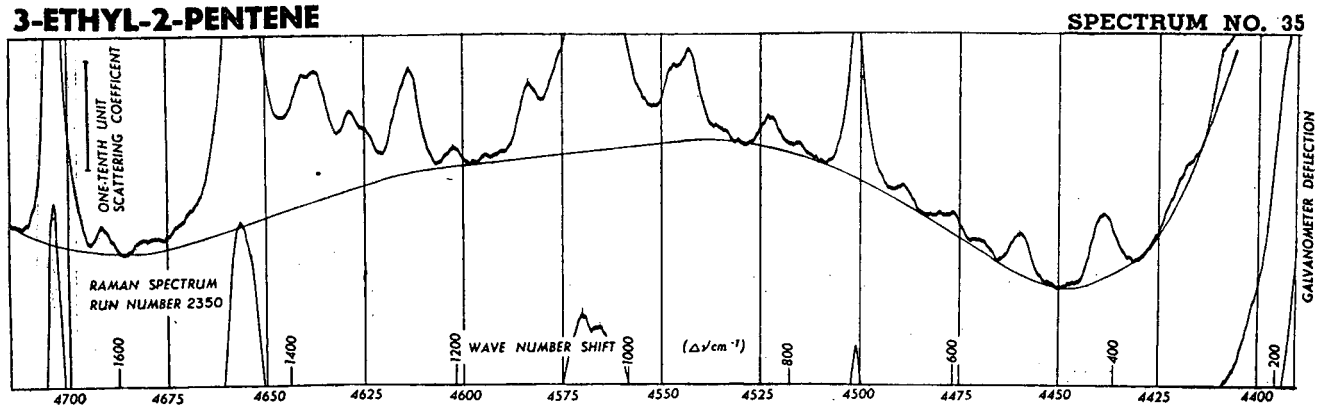
2-METHYL-1-HEXENE

SPECTRUM NO. 34



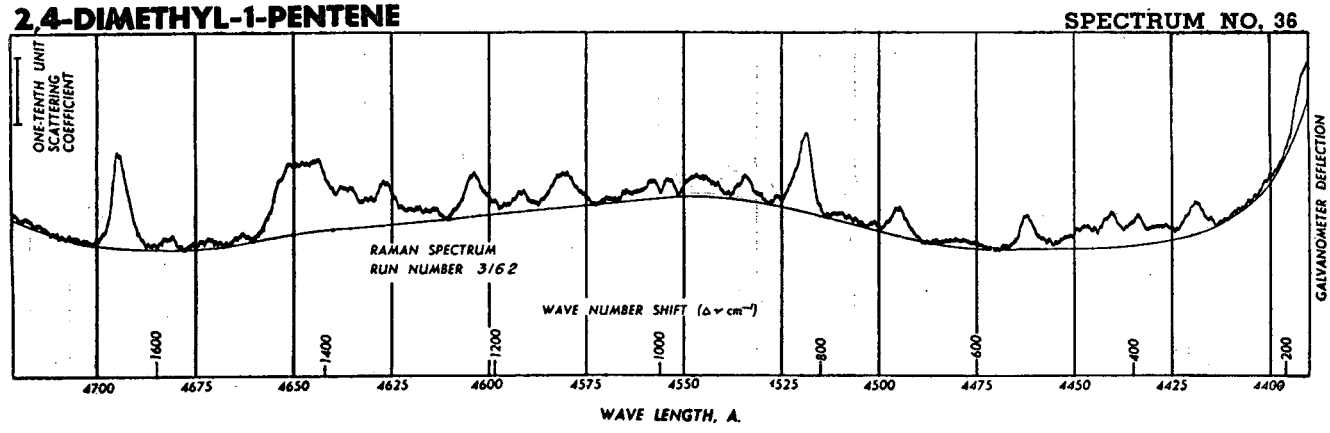
3-ETHYL-2-PENTENE

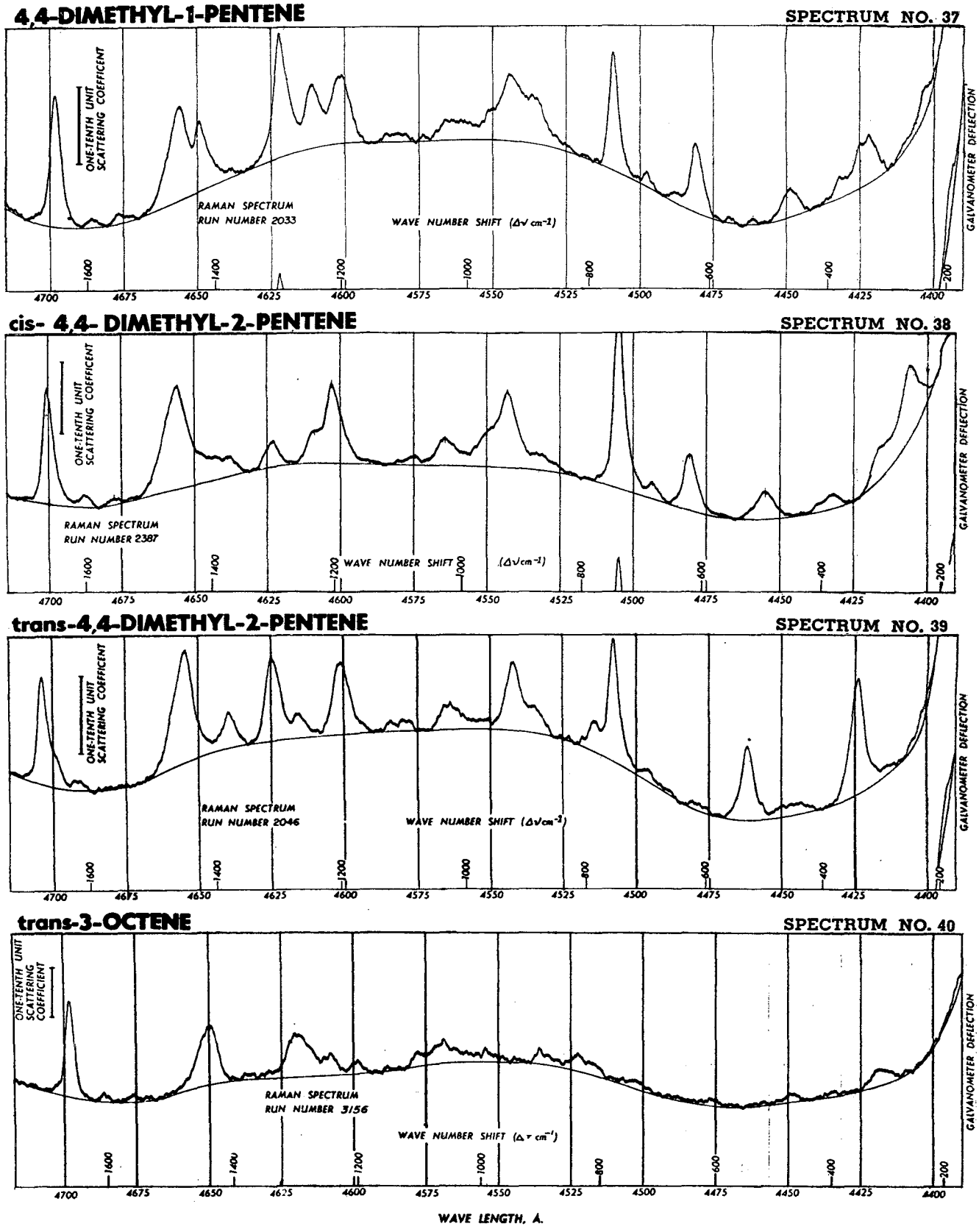
SPECTRUM NO. 35

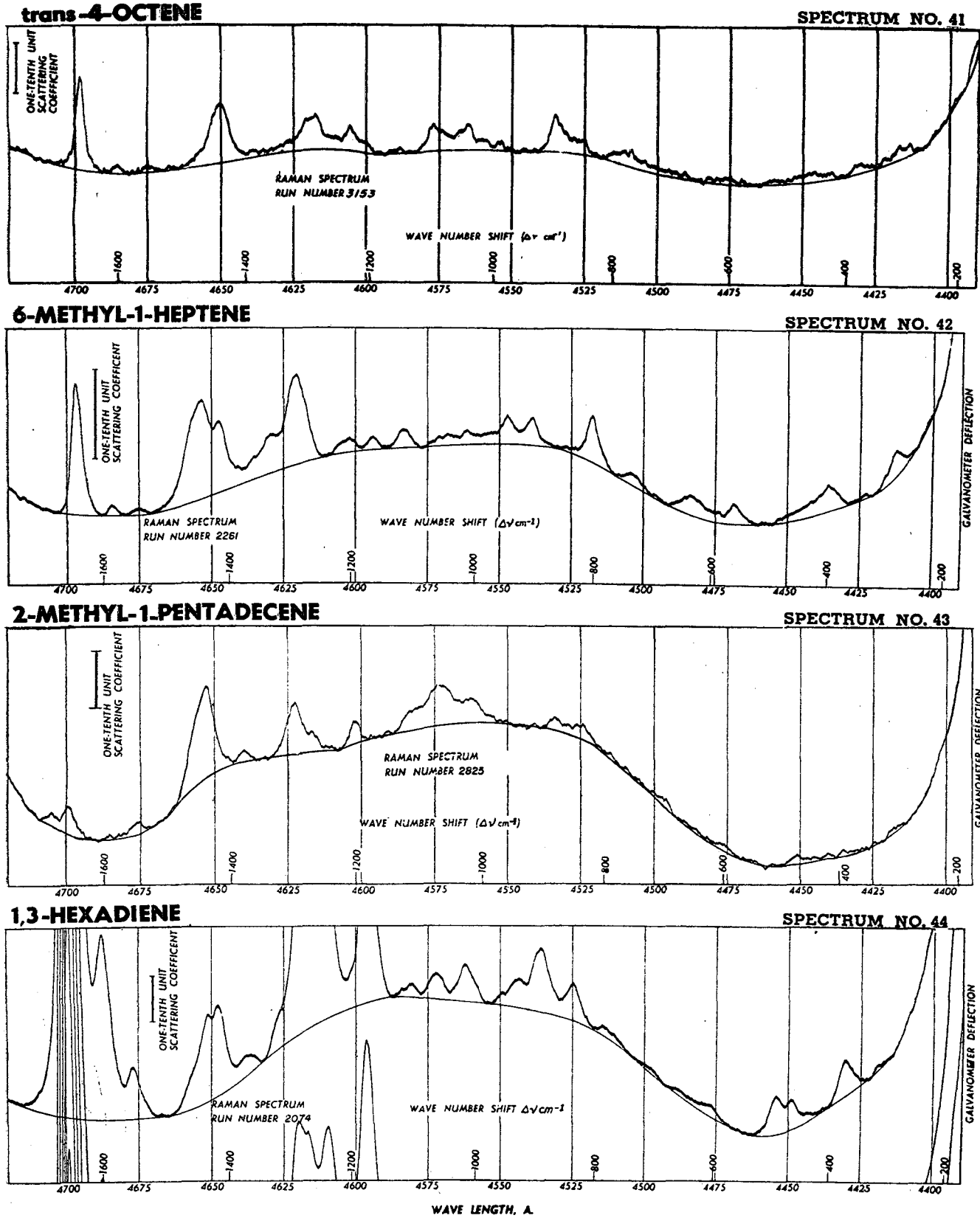


2,4-DIMETHYL-1-PENTENE

SPECTRUM NO. 36

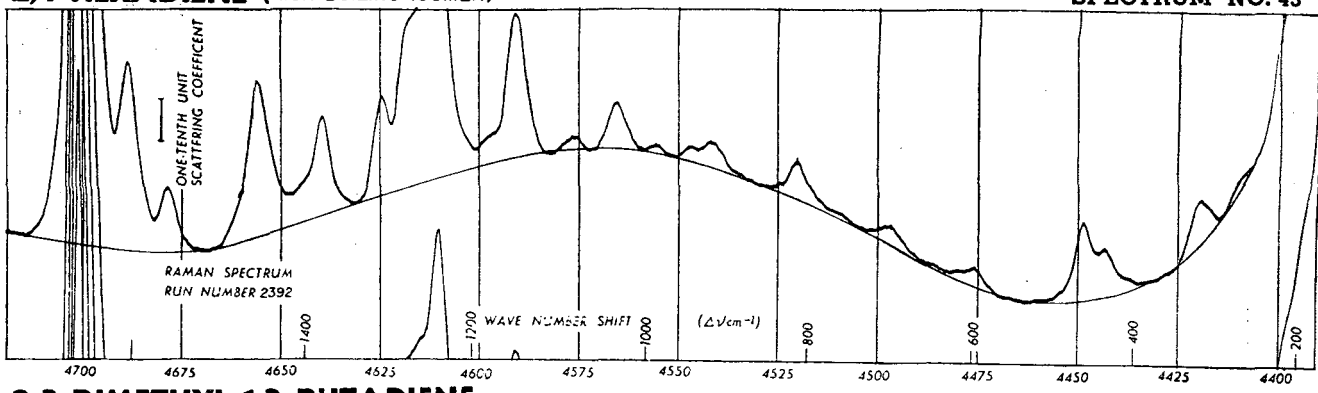



4,4-DIMETHYL-1-PENTENE
SPECTRUM NO. 37
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2033
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
cis- 4,4- DIMETHYL-2-PENTENE
SPECTRUM NO. 38
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2387
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
trans-4,4-DIMETHYL-2-PENTENE
SPECTRUM NO. 39
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2046
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
trans-3-OCTENE
SPECTRUM NO. 40
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 3156
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
1600 1400 1200 1000 800 600 400 200
4700 4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
WAVE LENGTH, A.


trans-4-OCTENE
SPECTRUM NO. 41
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 3153
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
6-METHYL-1-HEPTENE
SPECTRUM NO. 42
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM RUN NUMBER 2261
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
2-METHYL-1-PENTADECENE
SPECTRUM NO. 43
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2825
WAVE NUMBER SHIFT (Δν cm-1)
GALVANOMETER DEFLECTION
1,3-HEXADIENE
SPECTRUM NO. 44
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2074
WAVE NUMBER SHIFT Δν cm-1
GALVANOMETER DEFLECTION
1600 1400 1200 1000 800 600 400 200
4700 4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
WAVE LENGTH, A.

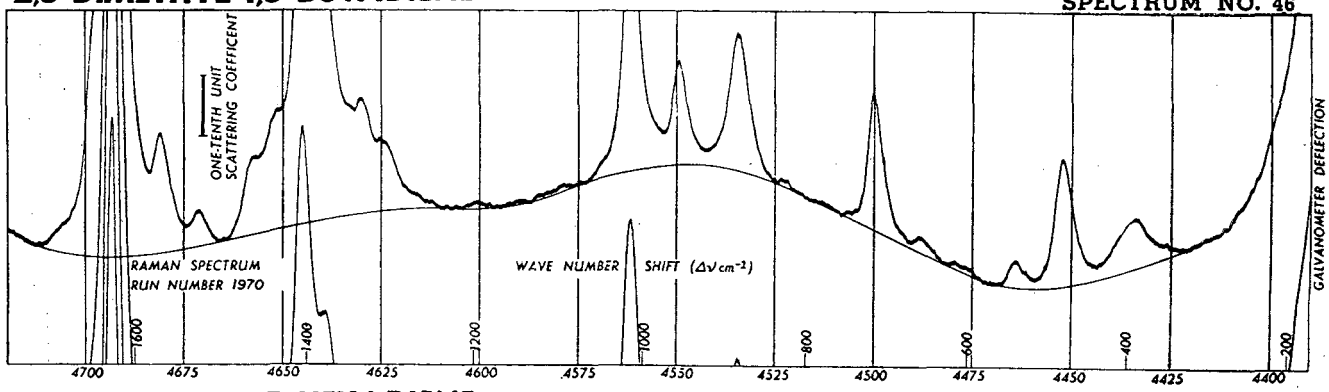
2,4-HEXADIENE (HIGH BOILING ISOMER)

SPECTRUM NO. 45



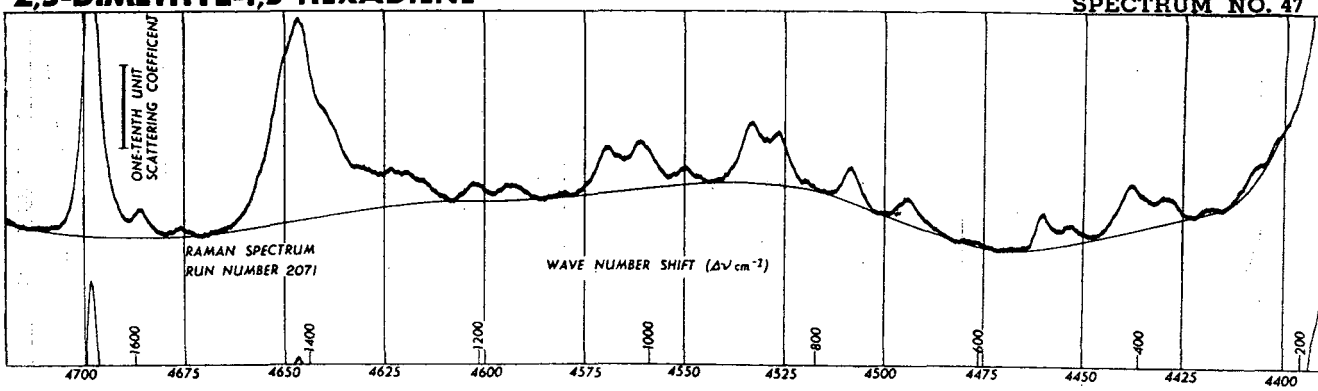
2,3-DIMETHYL-1,3-BUTADIENE

SPECTRUM NO. 46



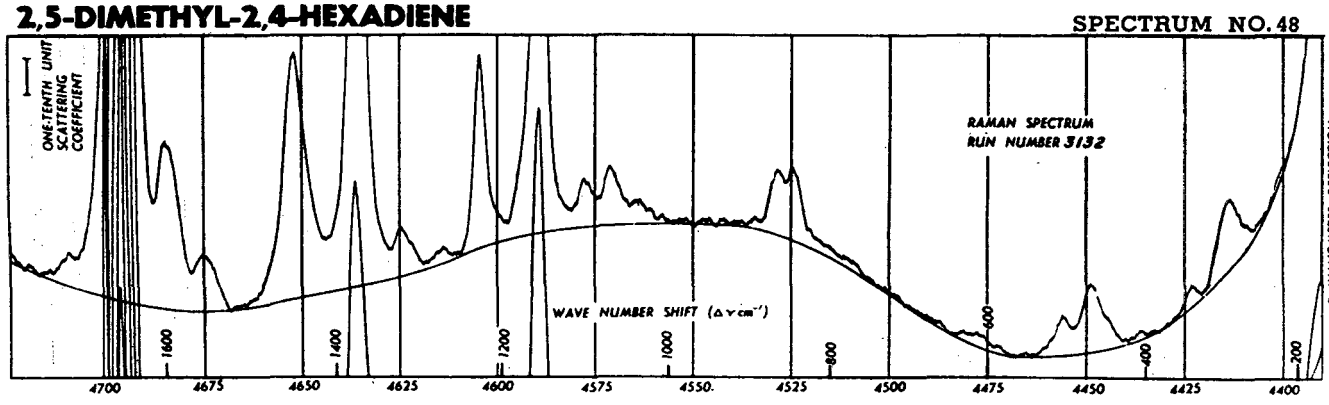
2,5-DIMETHYL-1,5-HEXADIENE

SPECTRUM NO. 47



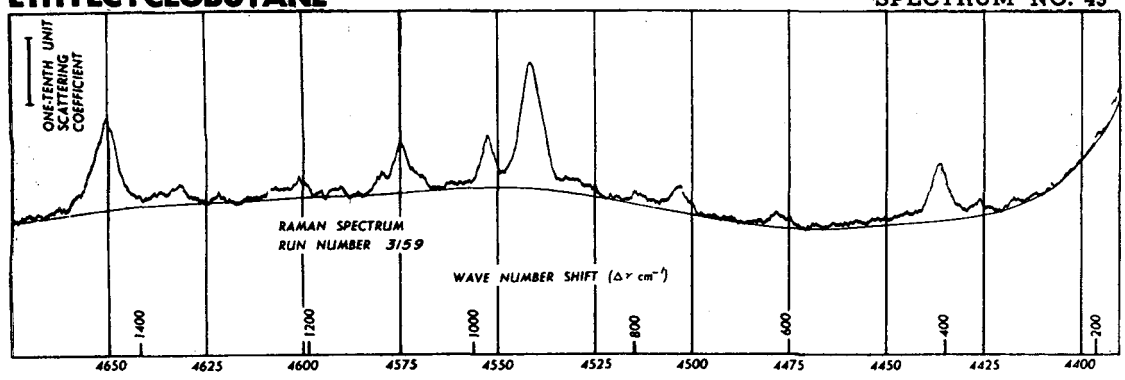
2,5-DIMETHYL-2,4-HEXADIENE

SPECTRUM NO. 48

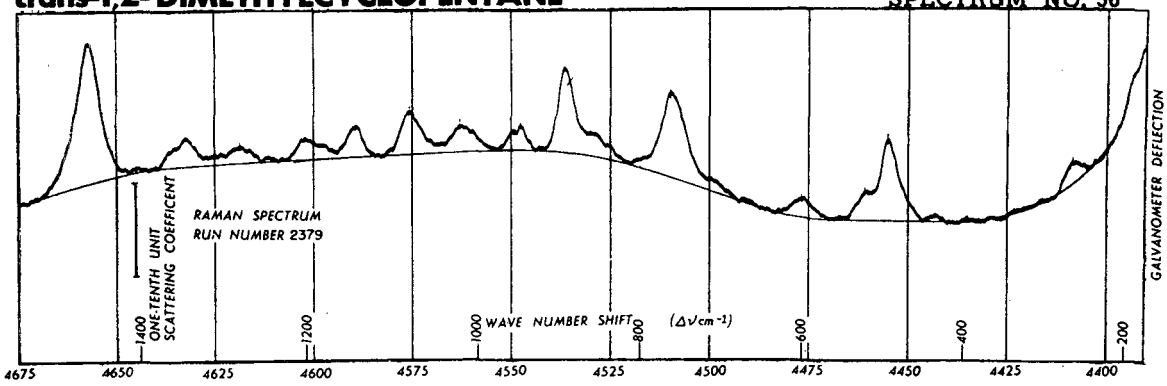


WAVE LENGTH, Å.

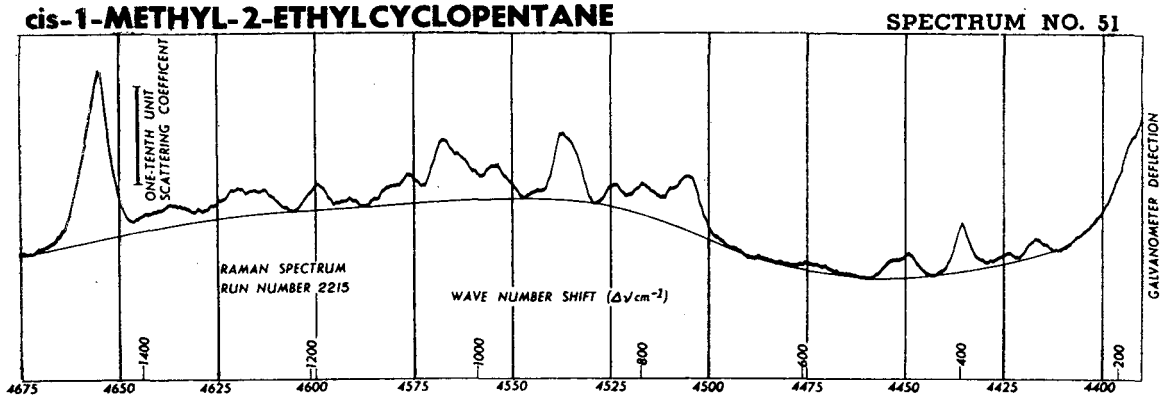
ETHYLCYCLOBUTANE SPECTRUM NO. 49



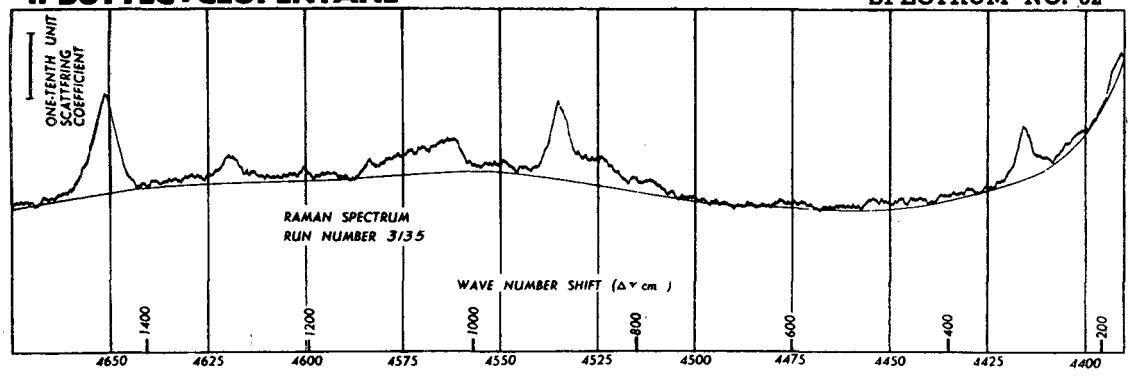
trans-1,2-DIMETHYLCYCLOPENTANE SPECTRUM NO. 50



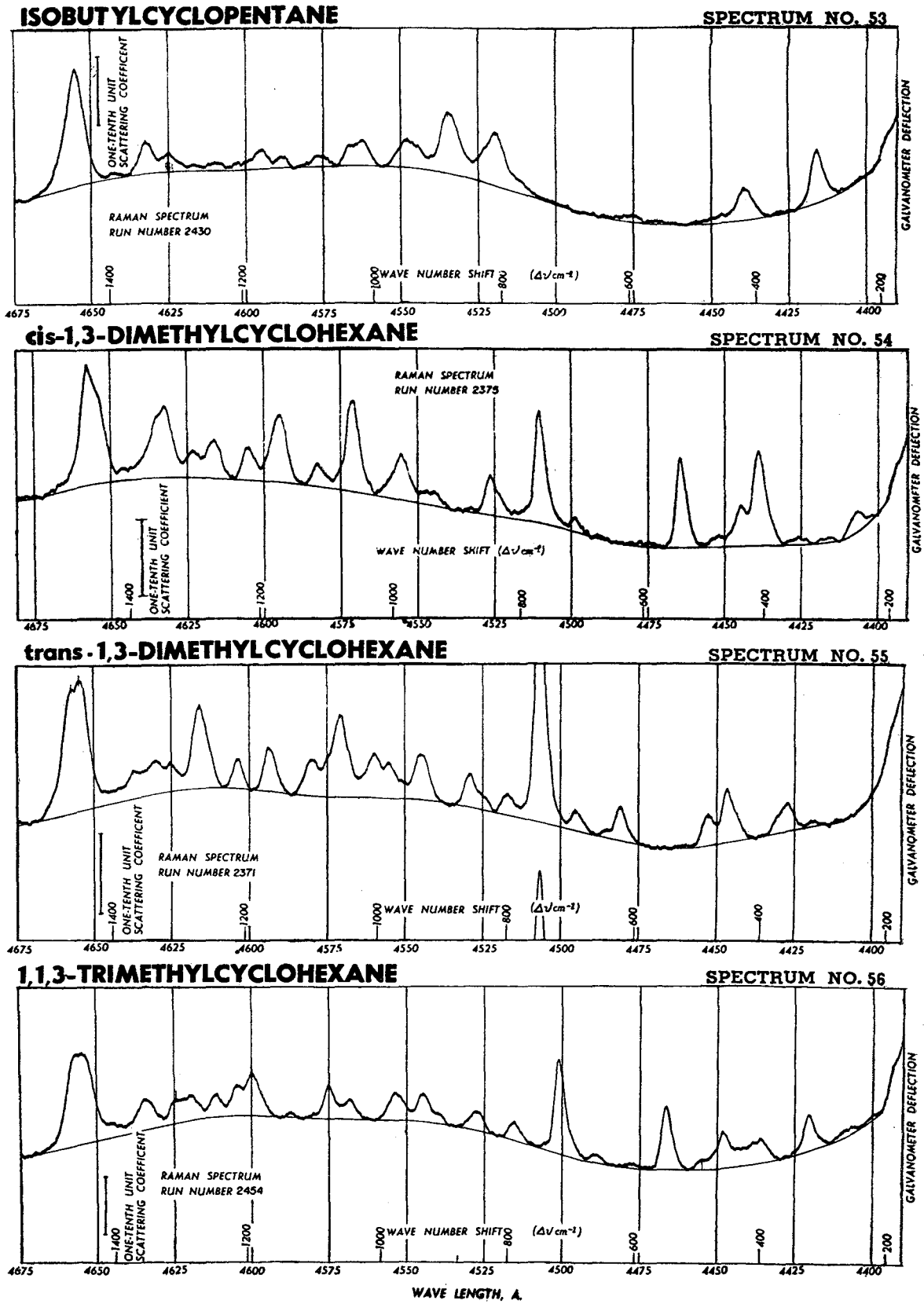
cis-1-METHYL-2-ETHYLCYCLOPENTANE SPECTRUM NO. 51

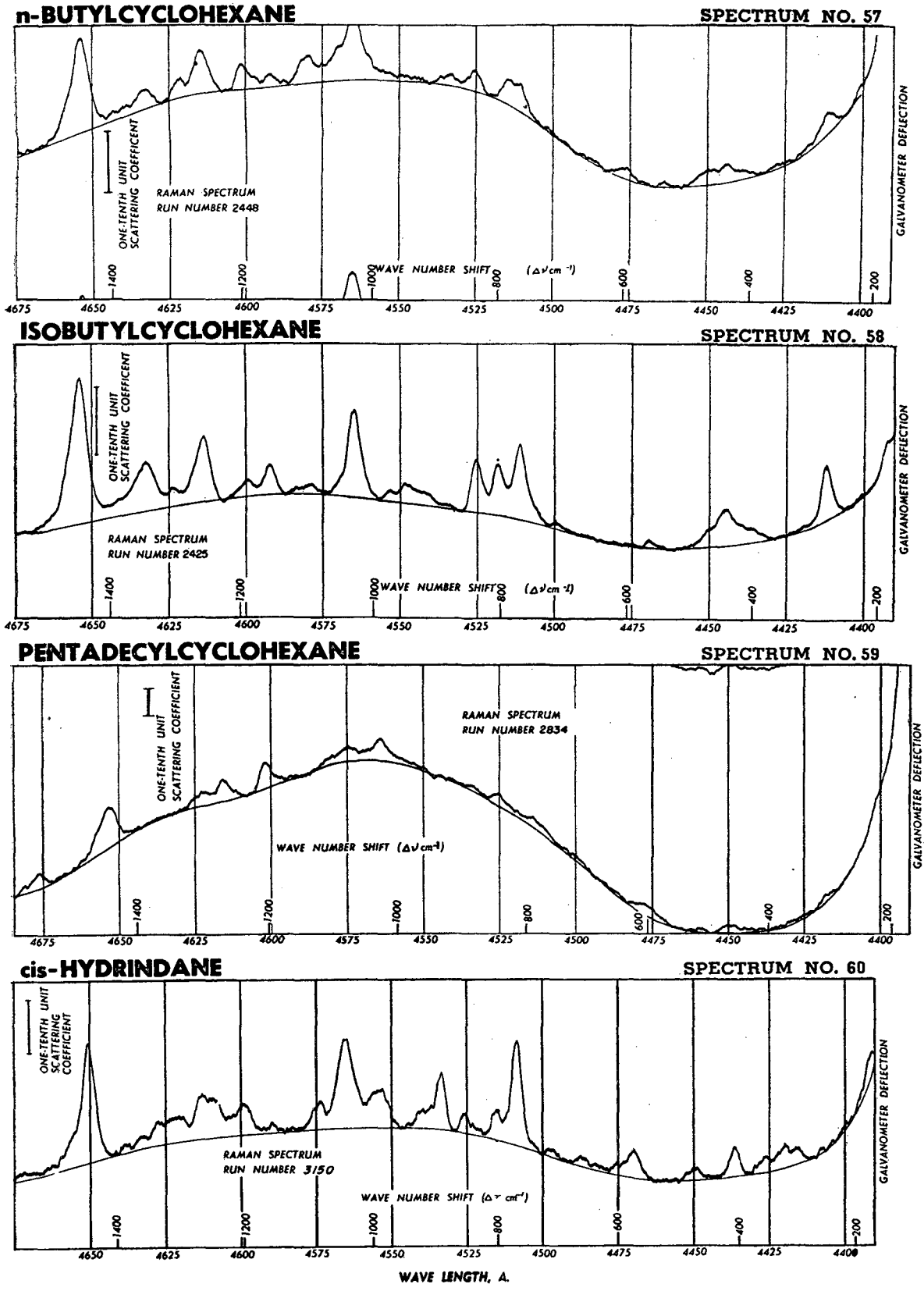


n-BUTYLCYCLOPENTANE SPECTRUM NO. 52



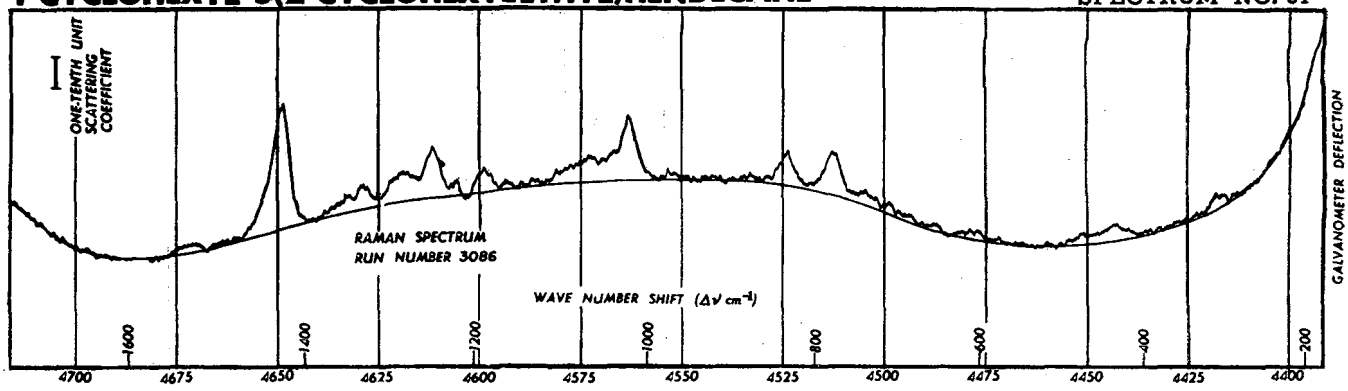
WAVE LENGTH, A.


ISOBUTYLCYCLOPENTANE
SPECTRUM NO. 53
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2430
WAVE NUMBER SHIFT (Δν/cm⁻¹)
GALVANOMETER DEFLECTION
cis-1,3-DIMETHYLCYCLOHEXANE
SPECTRUM NO. 54
RAMAN SPECTRUM
RUN NUMBER 2375
ONE-TENTH UNIT SCATTERING COEFFICIENT
WAVE NUMBER SHIFT (Δν/cm⁻¹)
GALVANOMETER DEFLECTION
trans-1,3-DIMETHYLCYCLOHEXANE
SPECTRUM NO. 55
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2371
WAVE NUMBER SHIFT (Δν/cm⁻¹)
GALVANOMETER DEFLECTION
1,1,3-TRIMETHYLCYCLOHEXANE
SPECTRUM NO. 56
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2454
WAVE NUMBER SHIFT (Δν/cm⁻¹)
GALVANOMETER DEFLECTION
1400 1200 1000 800 600 400 200
4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
WAVE LENGTH, A.


n-BUTYLCYCLOHEXANE
SPECTRUM NO. 57
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2448
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
ISOBUTYLCYCLOHEXANE
SPECTRUM NO. 58
ONE-TENTH UNIT SCATTERING COEFFICENT
RAMAN SPECTRUM
RUN NUMBER 2425
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
PENTADECYLCYCLOHEXANE
SPECTRUM NO. 59
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 2834
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
cis-HYDRINDANE
SPECTRUM NO. 60
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3150
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
WAVE LENGTH, A.

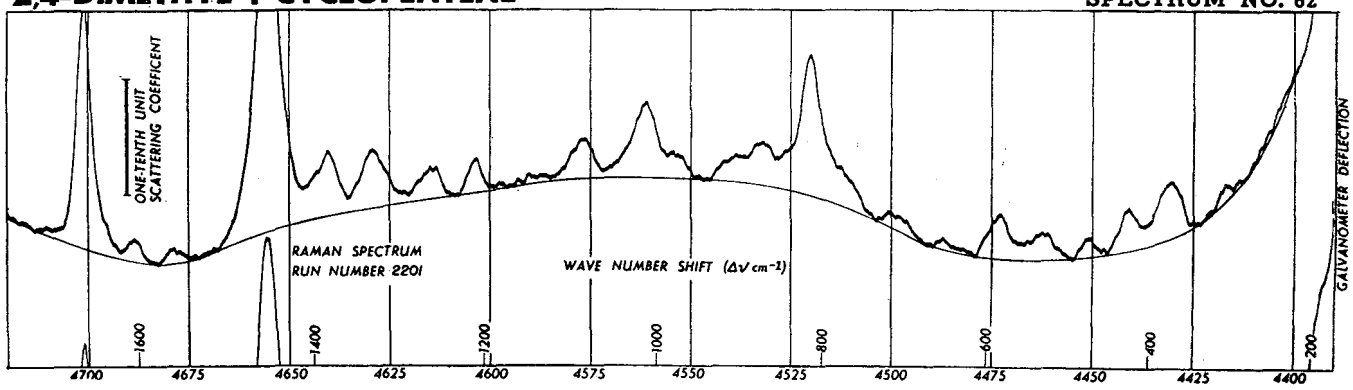
1-CYCLOHEXYL-3(2-CYCLOHEXYLETHYL)HENDECANE

SPECTRUM NO. 61



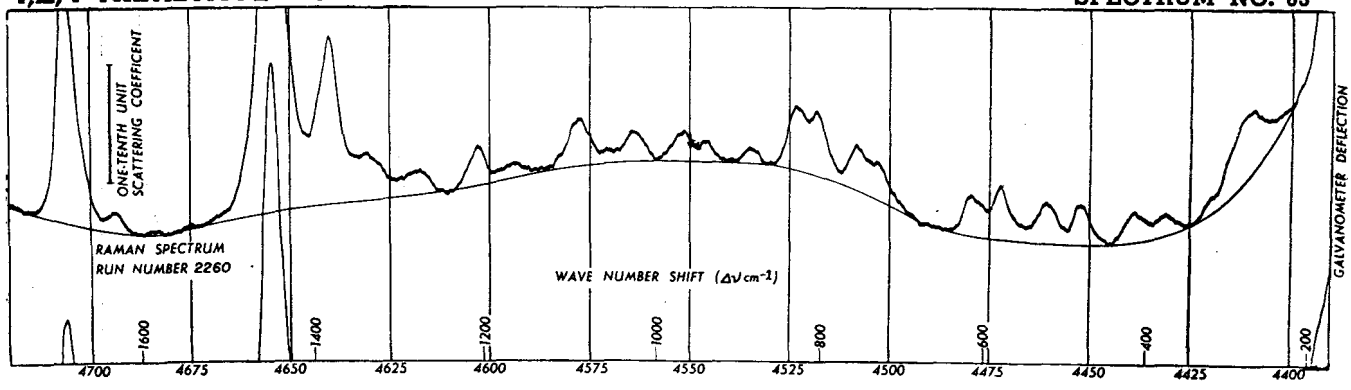
2,4-DIMETHYL-1-CYCLOPENTENE

SPECTRUM NO. 62



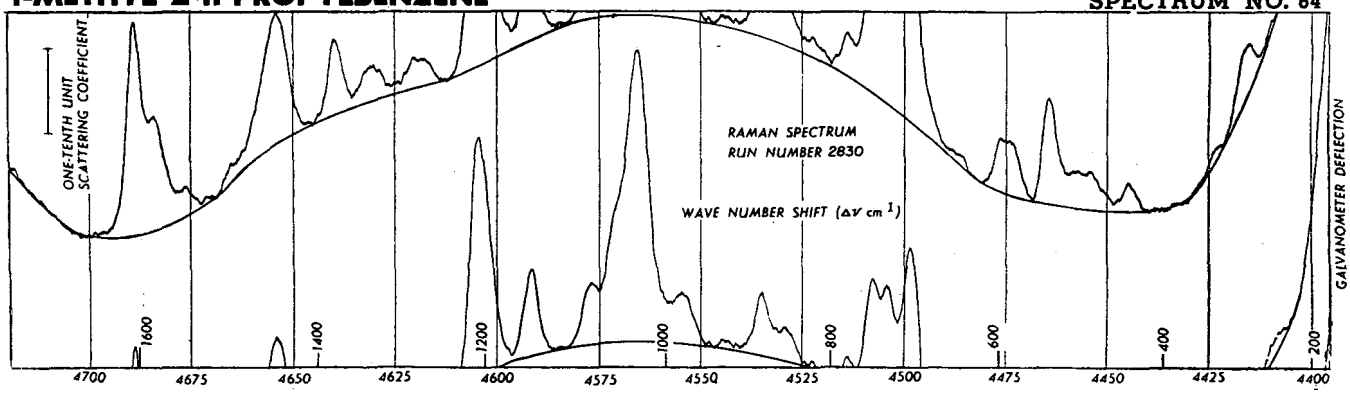
1,2,4-TRIMETHYL-1-CYCLOPENTENE

SPECTRUM NO. 63



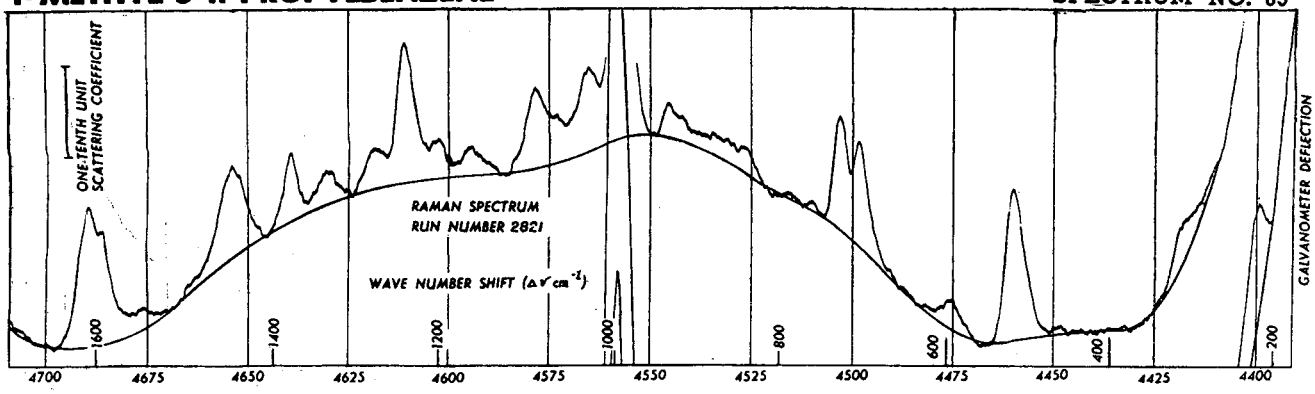
1-METHYL-2-n-PROPYLBENZENE

SPECTRUM NO. 64



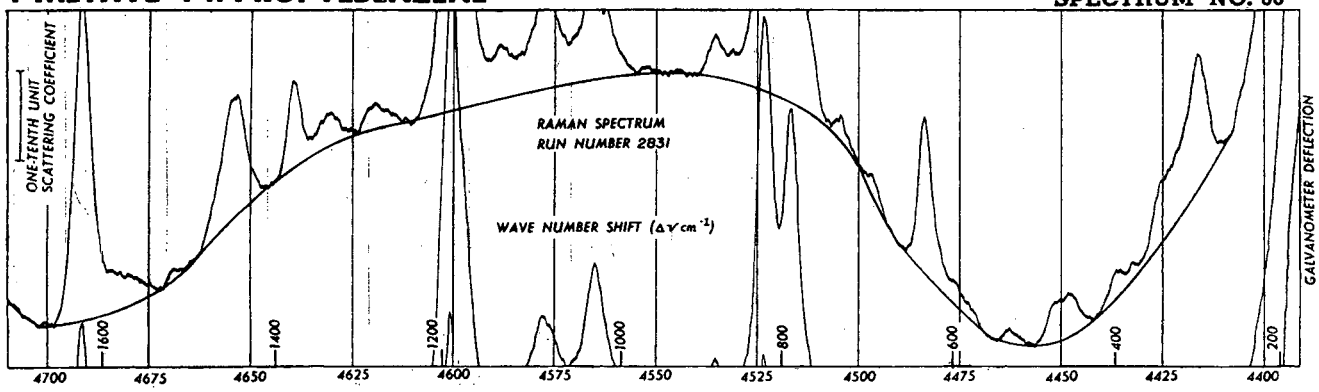
1-METHYL-3-n-PROPYLBENZENE

SPECTRUM NO. 65



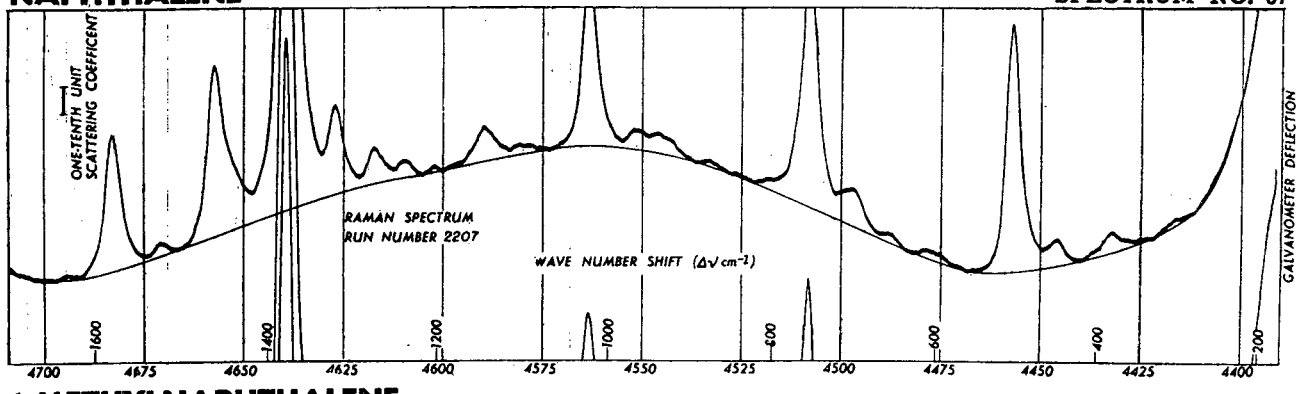
1-METHYL-4-n-PROPYLBENZENE

SPECTRUM NO. 66



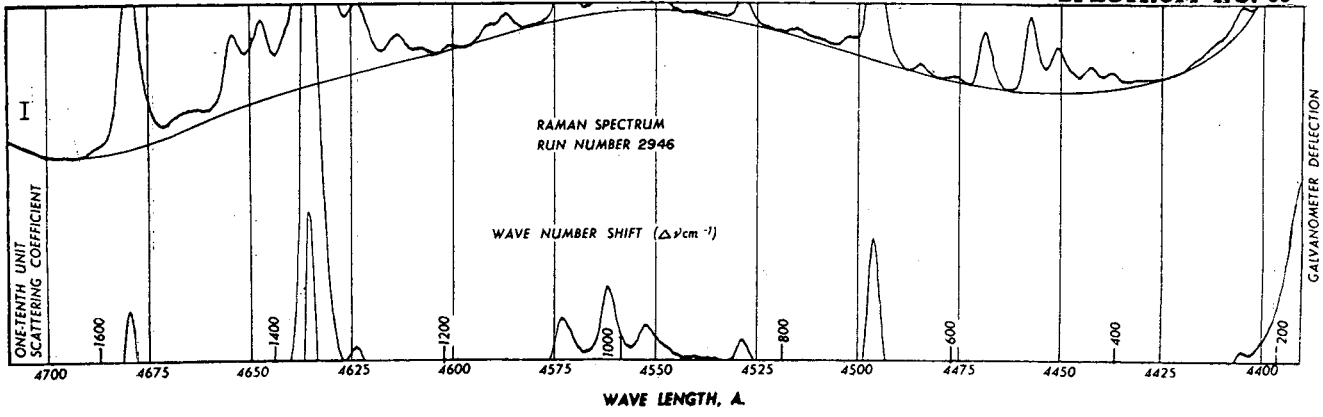
NAPHTHALENE

SPECTRUM NO. 67



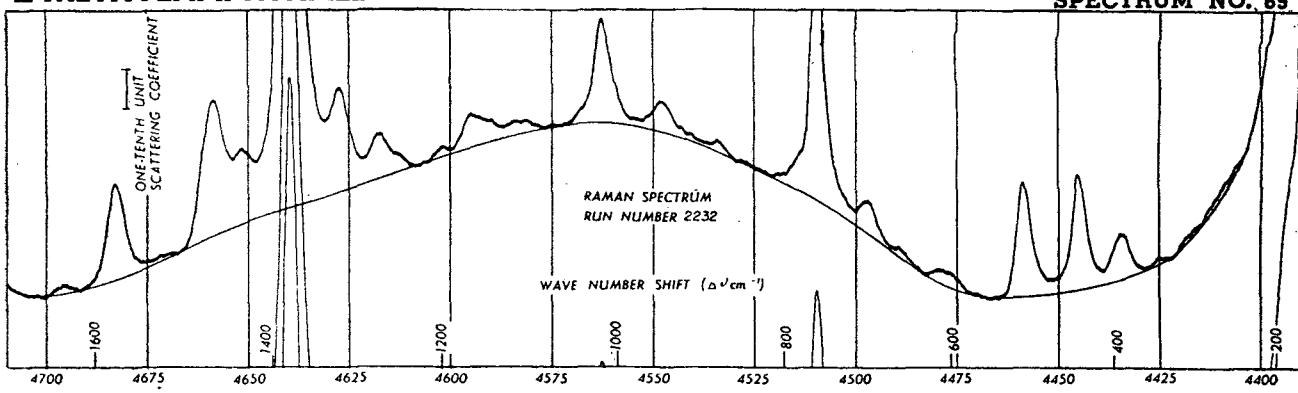
1-METHYLNAPHTHALENE

SPECTRUM NO. 68



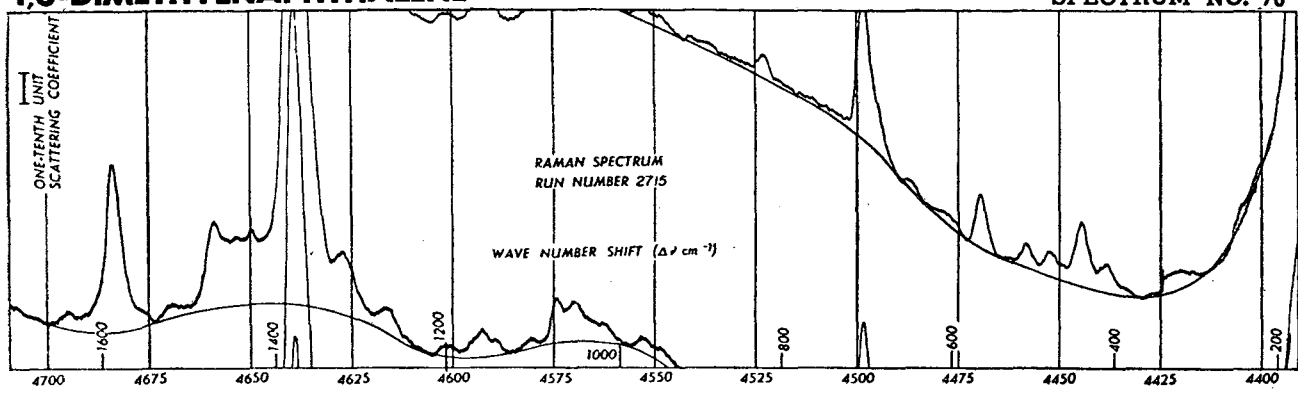
2-METHYLNAPHTHALENE

SPECTRUM NO. 69



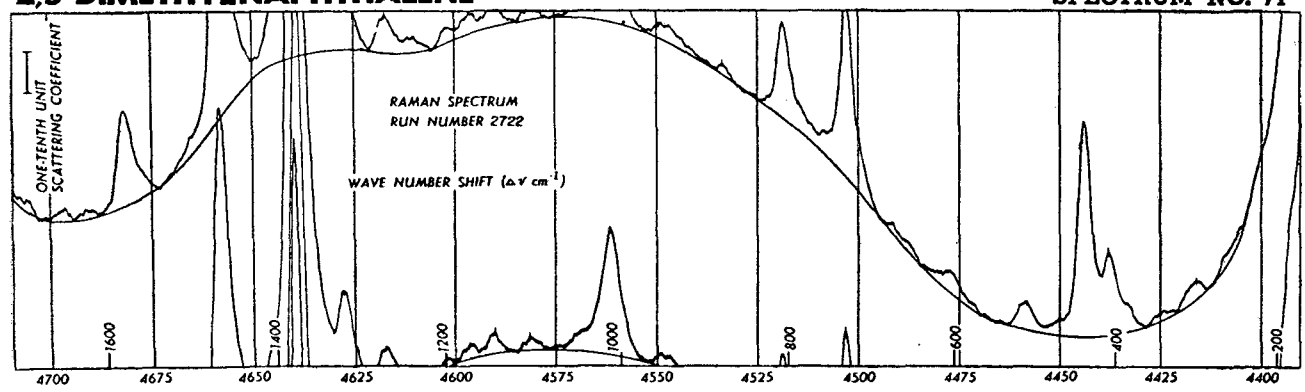
1,6-DIMETHYLNAPHTHALENE

SPECTRUM NO. 70



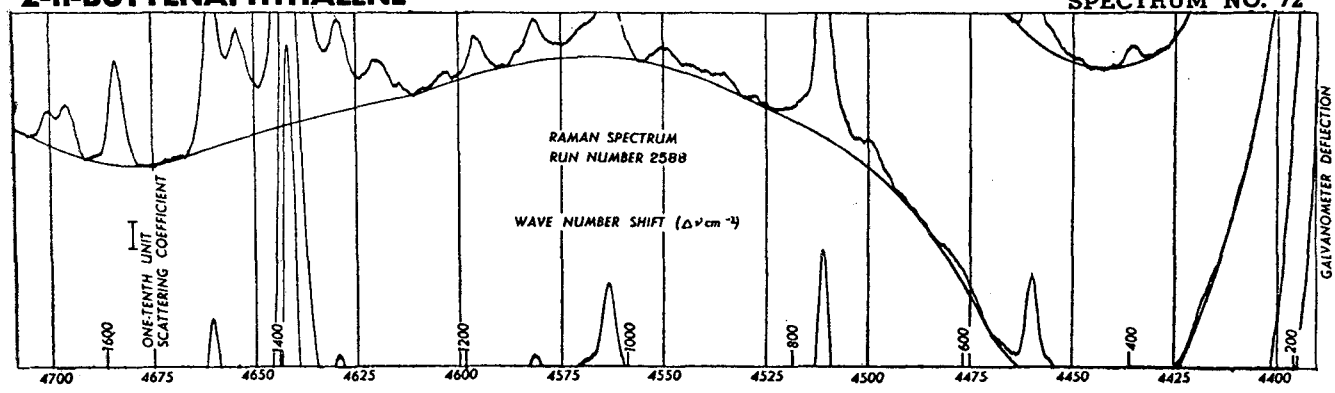
2,3-DIMETHYLNAPHTHALENE

SPECTRUM NO. 71



2-n-BUTYLNAPHTHALENE

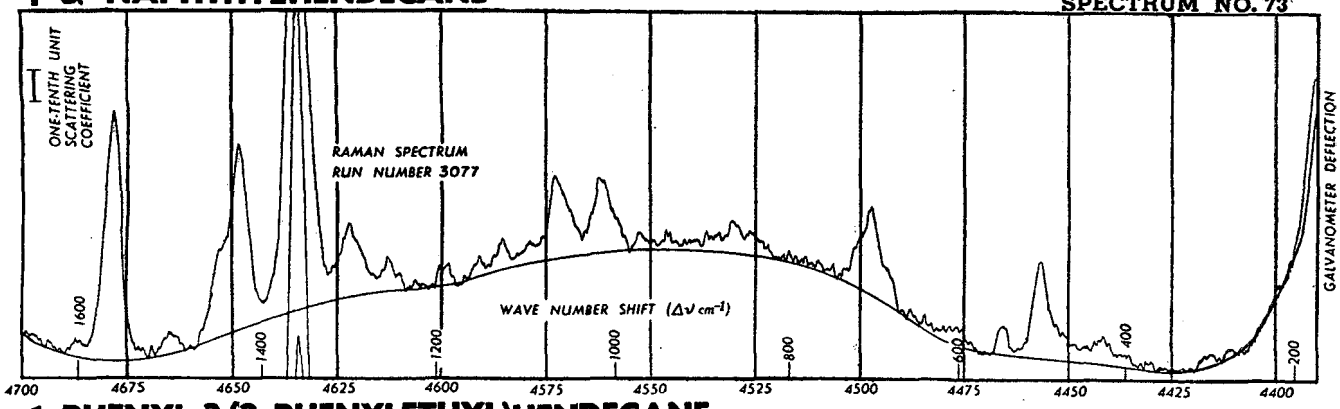
SPECTRUM NO. 72



WAVE LENGTH, A.

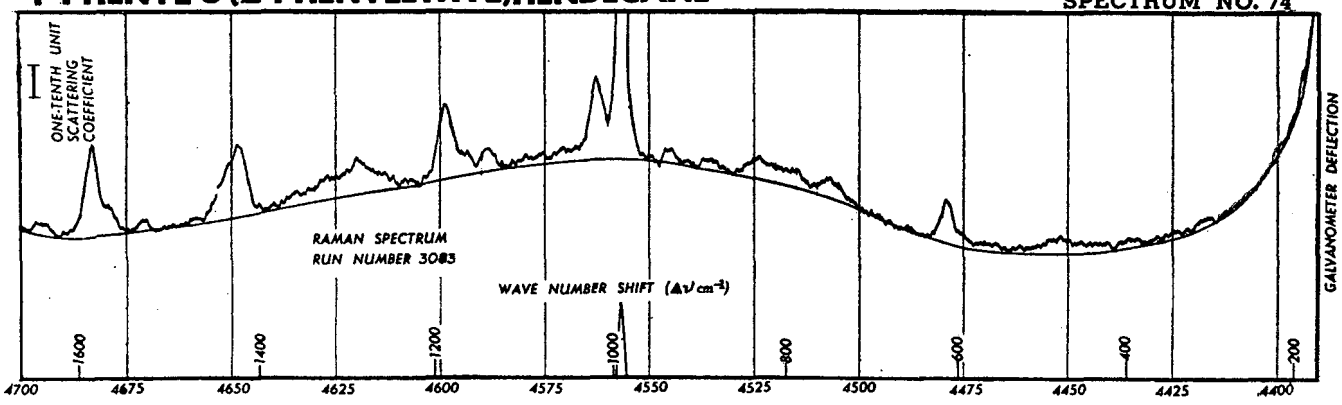
1-α-NAPHTHYLHENDECANE

SPECTRUM NO. 73



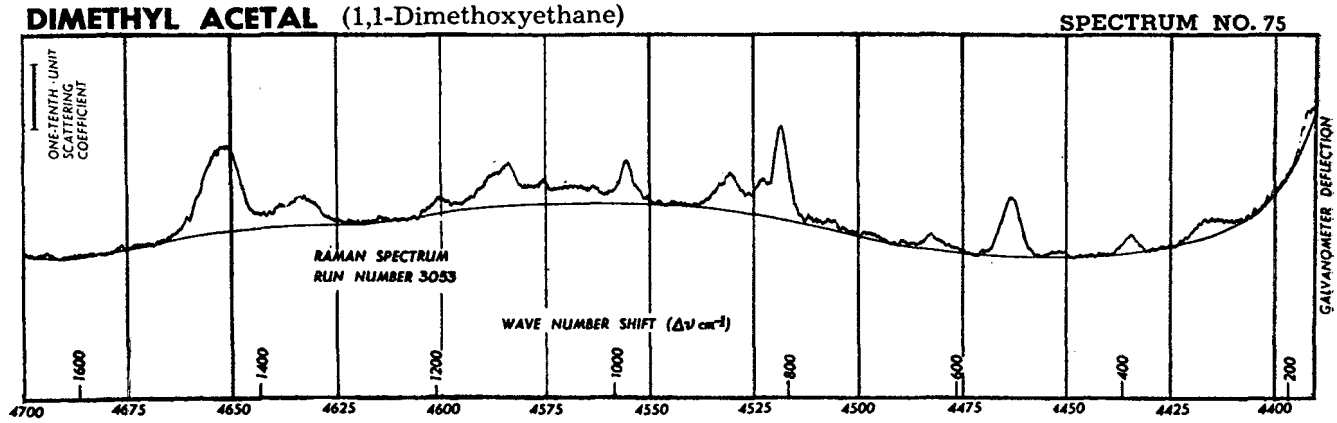
1-PHENYL-3 (2-PHENYLETHYL)HENDECANE

SPECTRUM NO. 74



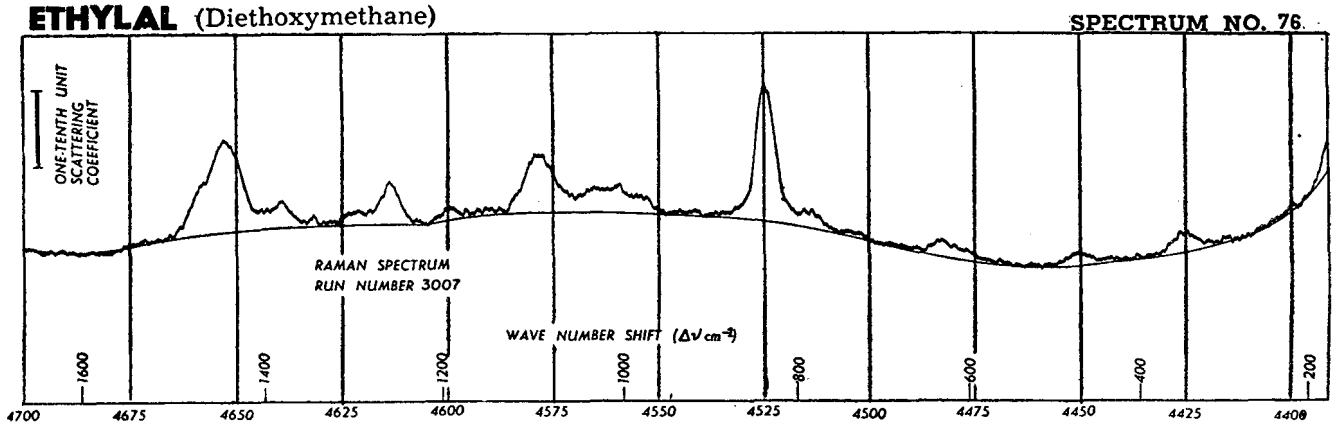
DIMETHYL ACETAL (1,1-Dimethoxyethane)

SPECTRUM NO. 75

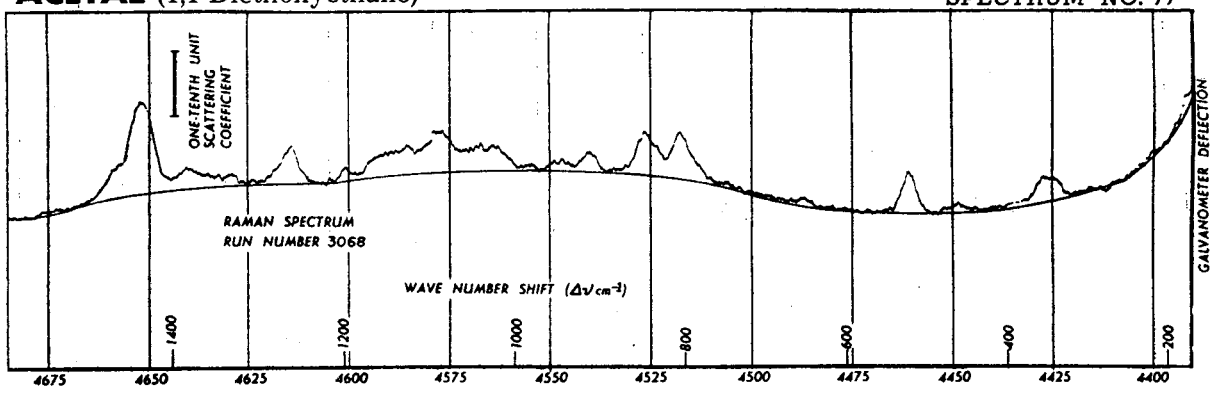


ETHYLAL (Diethoxymethane)

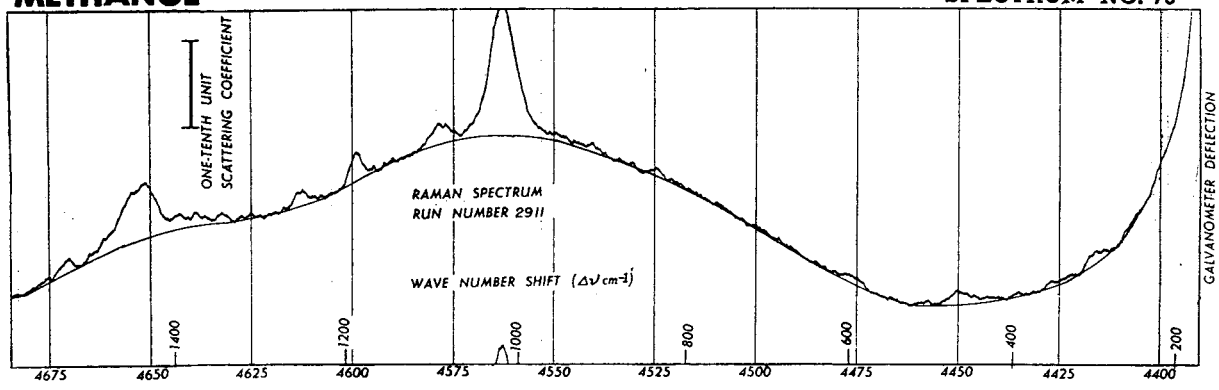
SPECTRUM NO. 76



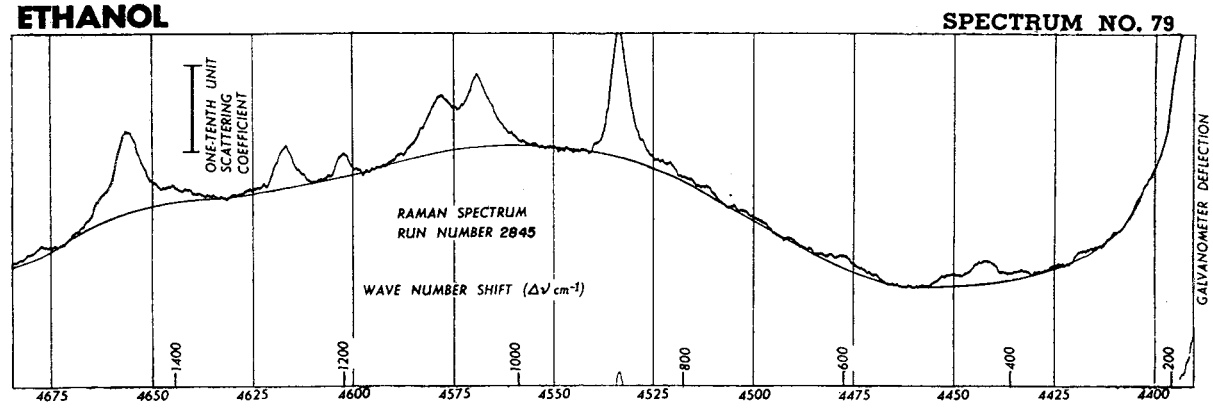
ACETAL (1,1-Diethoxyethane) SPECTRUM NO. 77



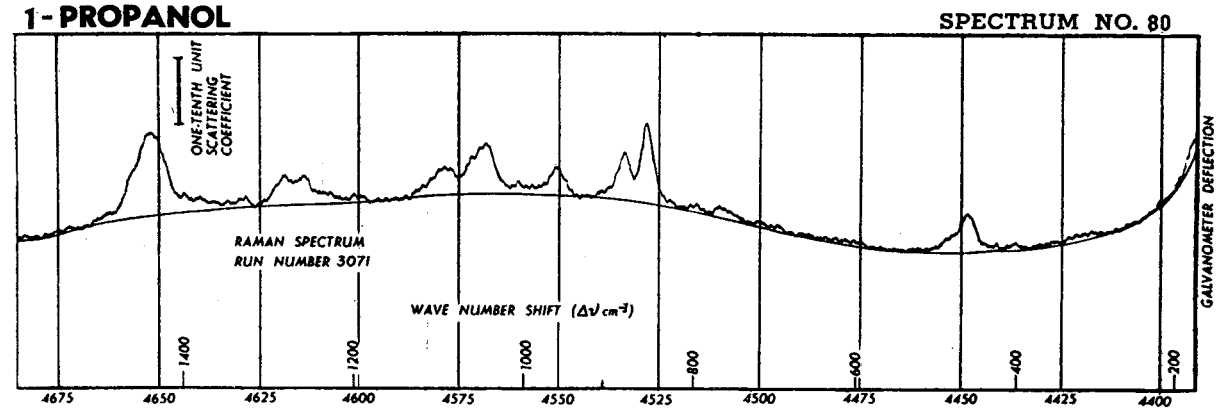
METHANOL SPECTRUM NO. 78



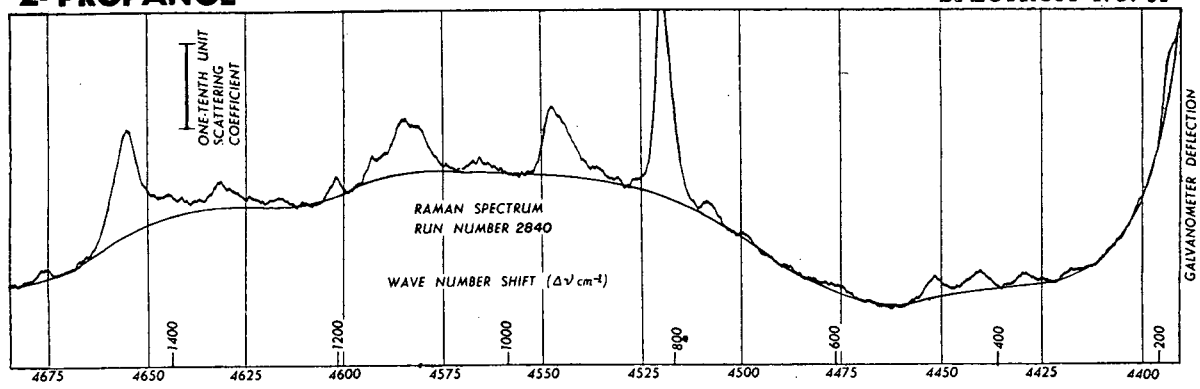
ETHANOL SPECTRUM NO. 79



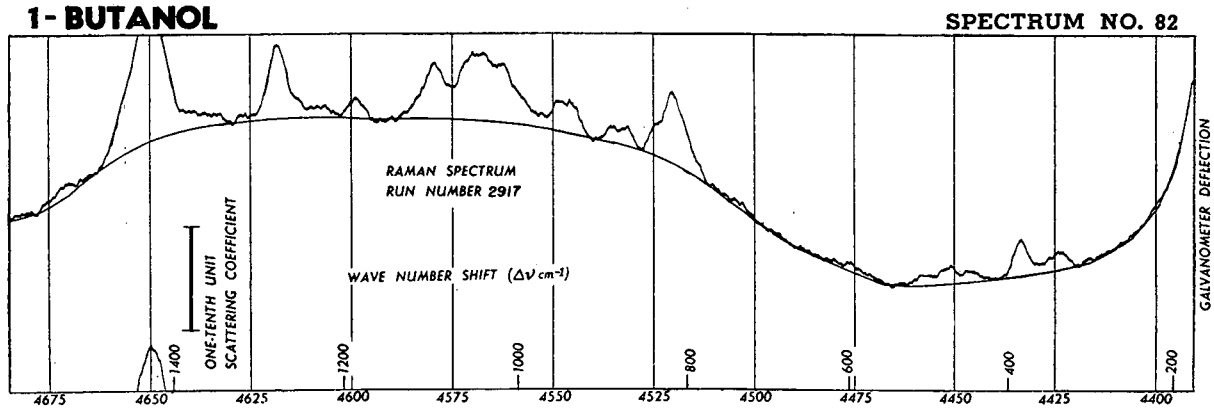
1-PROPANOL SPECTRUM NO. 80



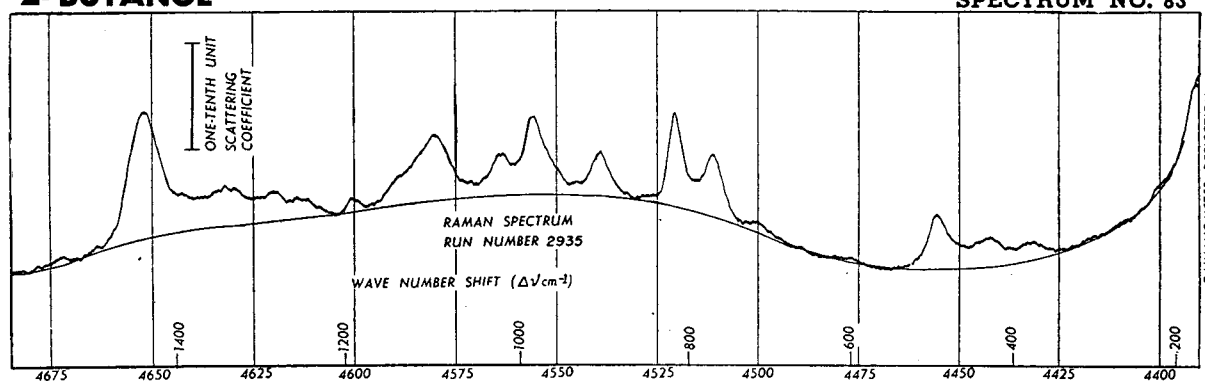
2- PROPANOL SPECTRUM NO. 81



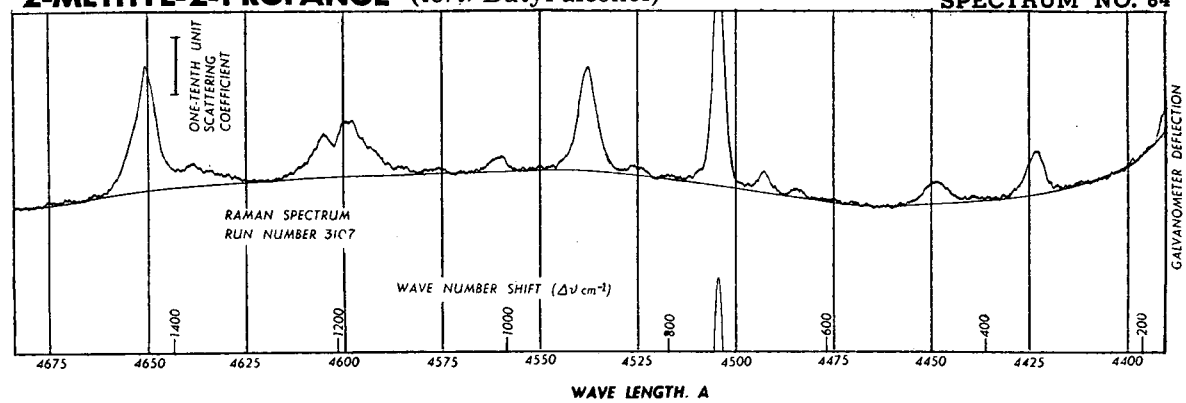
1- BUTANOL SPECTRUM NO. 82



2- BUTANOL SPECTRUM NO. 83

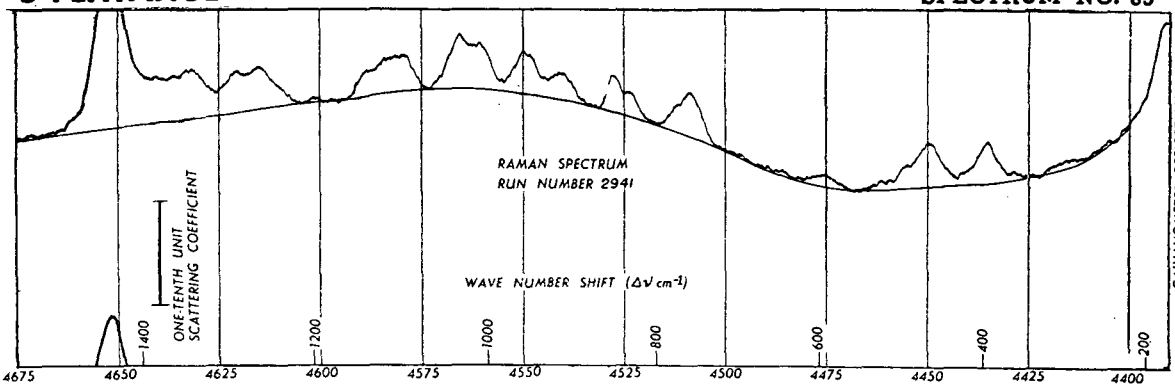


2-METHYL-2-PROPANOL (*tert.*-Butyl alcohol) SPECTRUM NO. 84

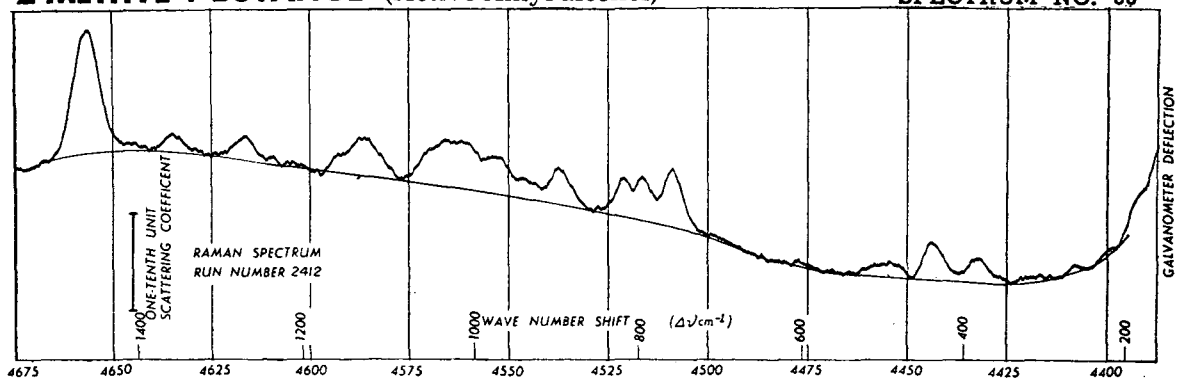


WAVE LENGTH. A

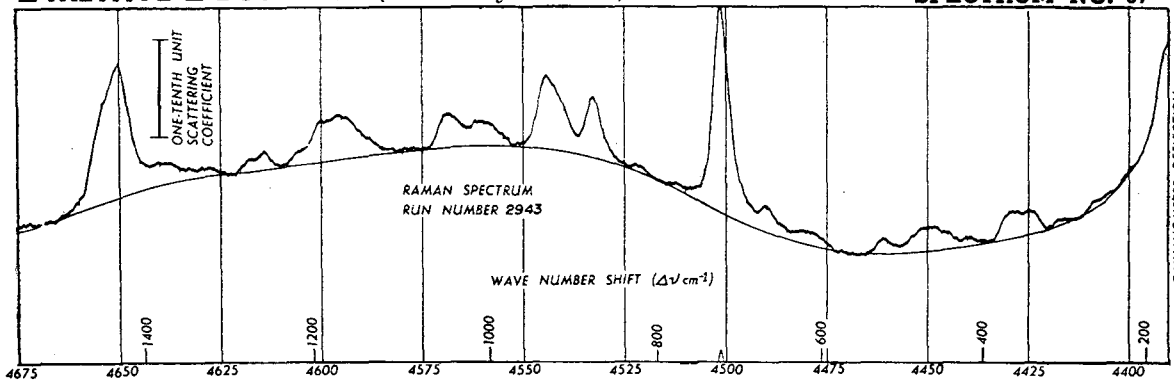
3-PENTANOL SPECTRUM NO. 85



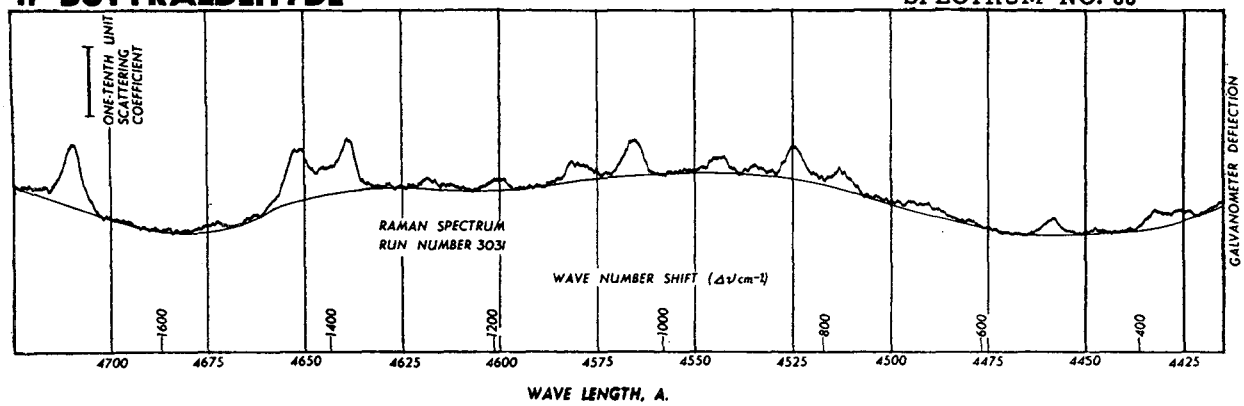
2-METHYL-1-BUTANOL (Active Amyl alcohol) SPECTRUM NO. 86



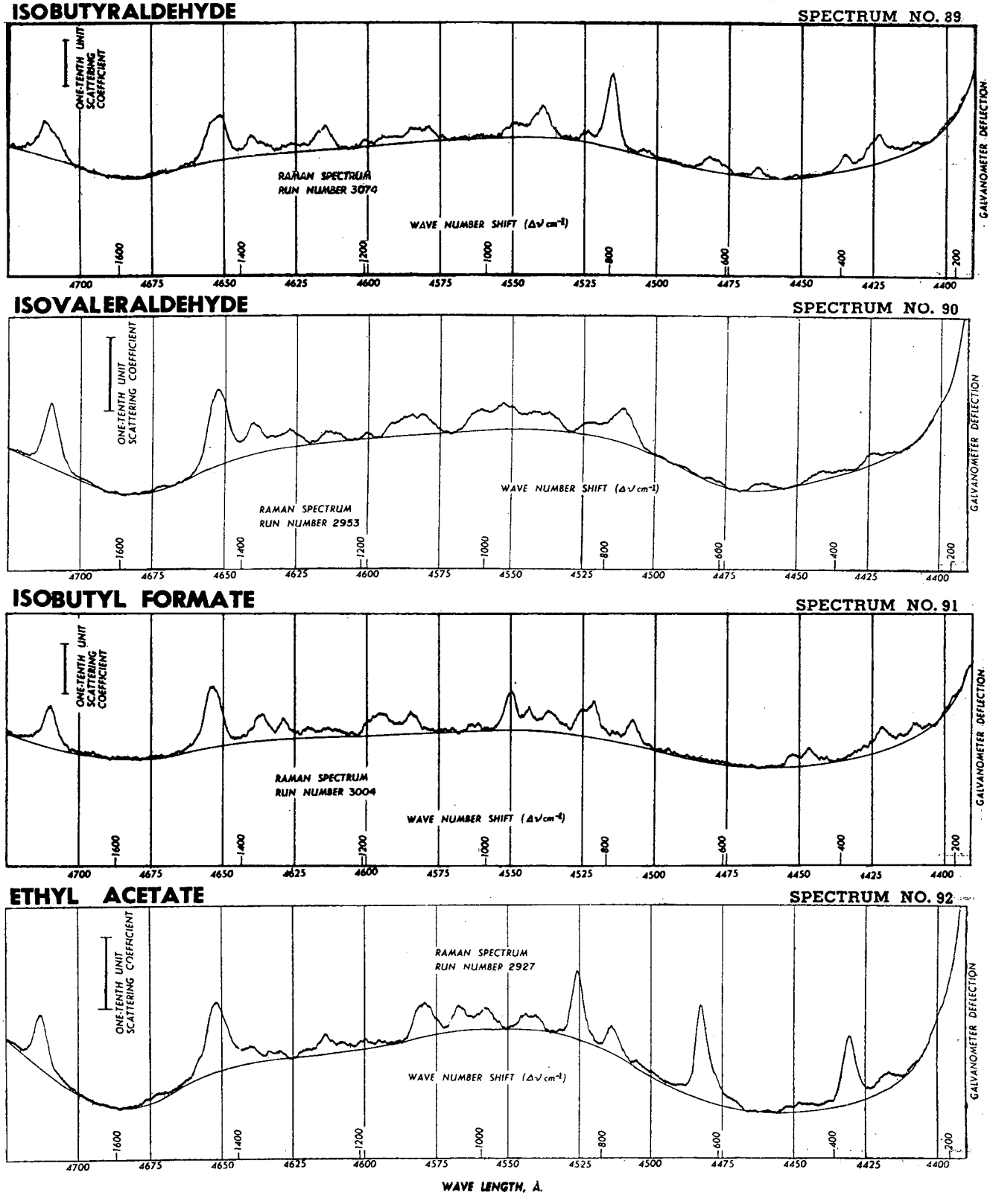
2-METHYL-2-BUTANOL (*tert* -Amyl alcohol) SPECTRUM NO. 87



n-BUTYRALDEHYDE SPECTRUM NO. 88

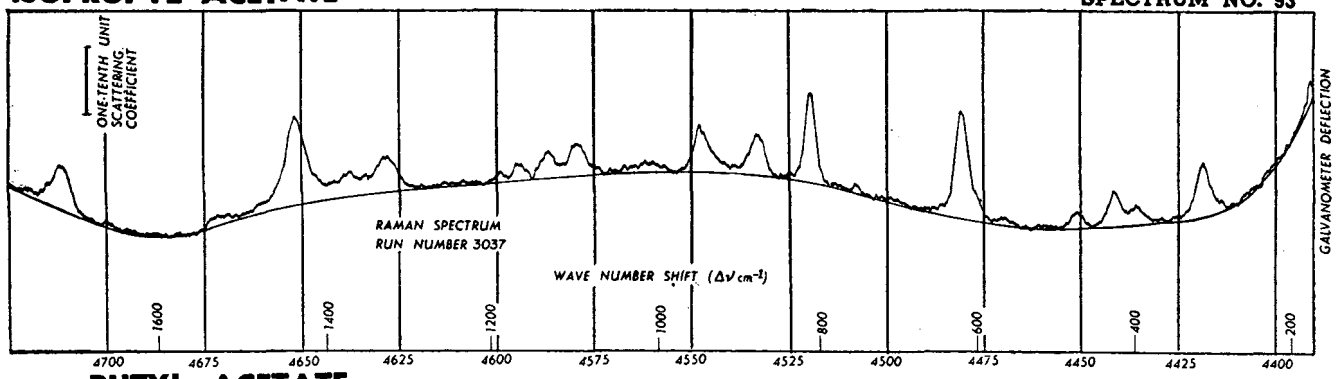


WAVE LENGTH, A.


ISOBUTYRALDEHYDE
SPECTRUM NO. 89
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 3074
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
ISOVALERALDEHYDE
SPECTRUM NO. 90
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2953
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
ISOBUTYL FORMATE
SPECTRUM NO. 91
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 3004
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
ETHYL ACETATE
SPECTRUM NO. 92
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM RUN NUMBER 2927
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
1600 1400 1200 1000 800 600 400 200
4700 4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
WAVE LENGTH, Å.

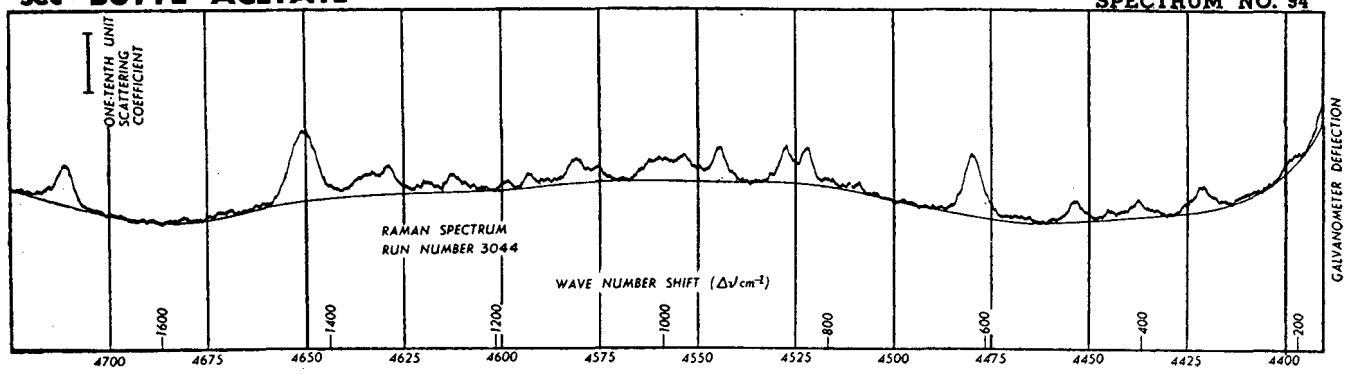
ISOPROPYL ACETATE

SPECTRUM NO. 93



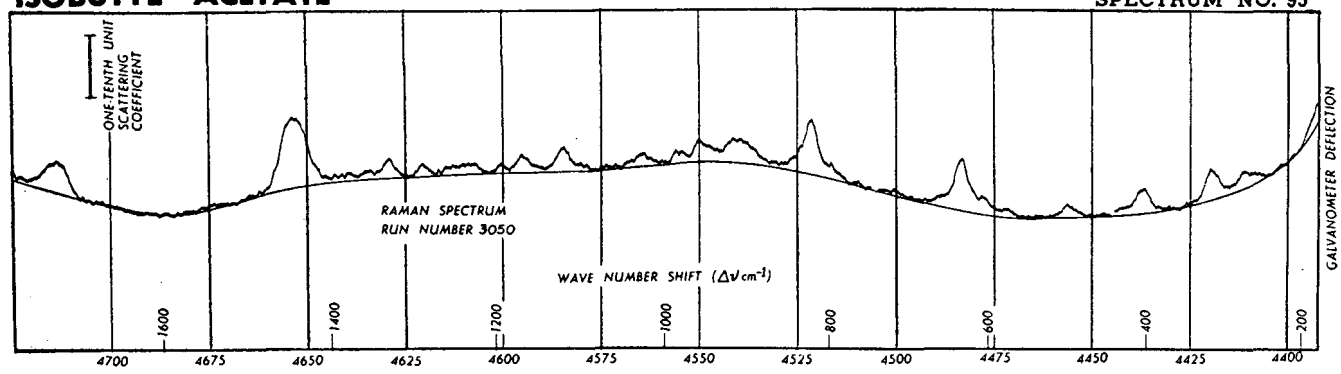
sec-BUTYL ACETATE

SPECTRUM NO. 94



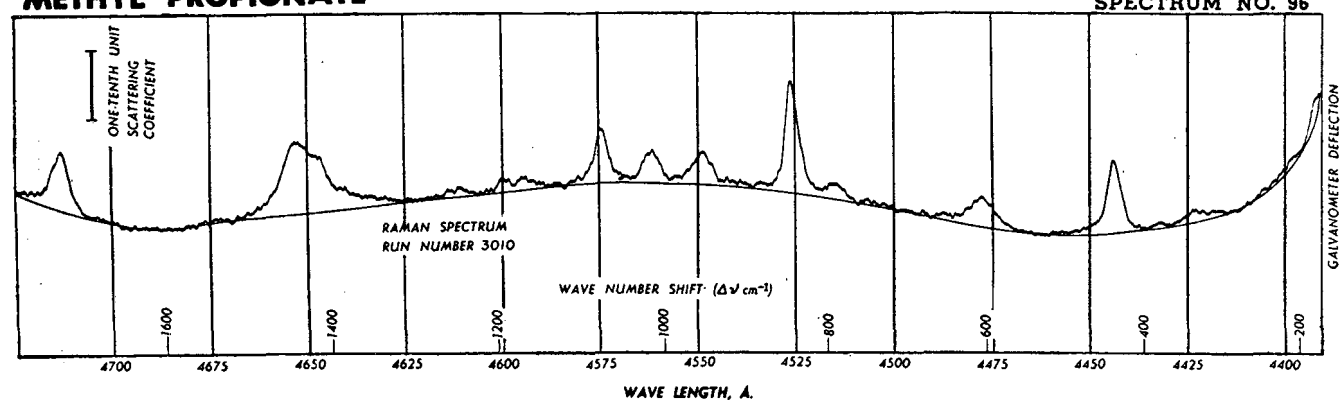
ISOBUTYL ACETATE

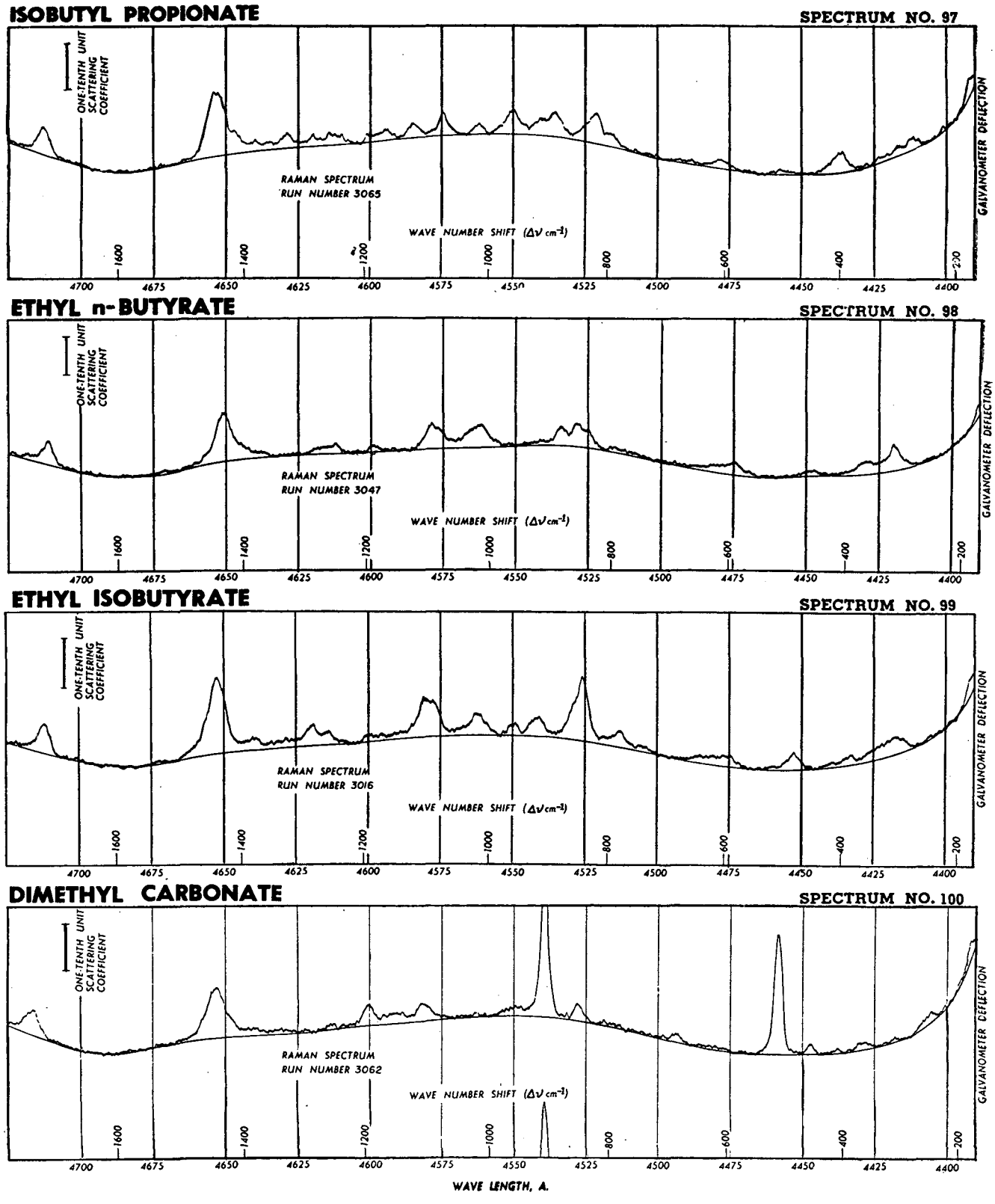
SPECTRUM NO. 95



METHYL PROPIONATE

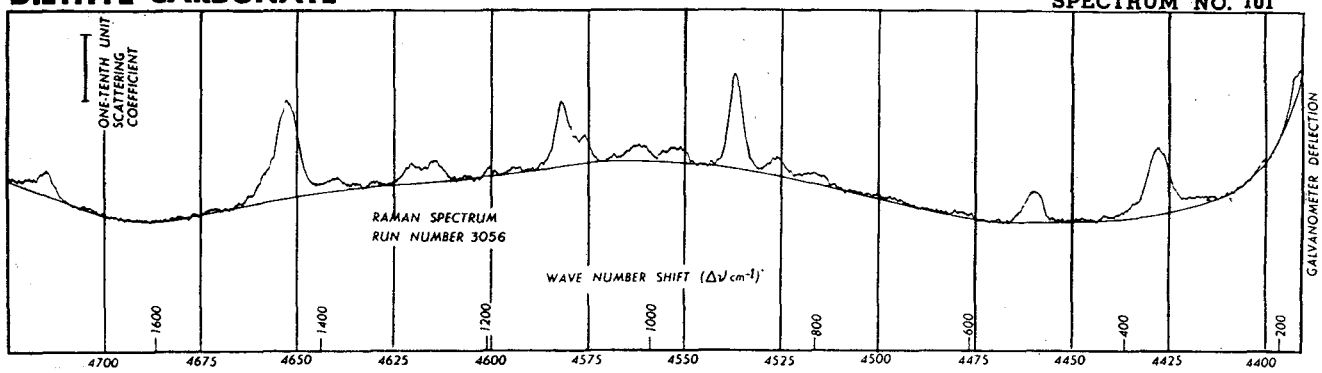
SPECTRUM NO. 96




ISOBUTYL PROPIONATE
SPECTRUM NO. 97
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3065
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
ETHYL n-BUTYRATE
SPECTRUM NO. 98
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3047
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
ETHYL ISOBUTYRATE
SPECTRUM NO. 99
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3016
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
DIMETHYL CARBONATE
SPECTRUM NO. 100
ONE-TENTH UNIT SCATTERING COEFFICIENT
RAMAN SPECTRUM
RUN NUMBER 3062
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
1600 1400 1200 1000 800 600 400 200
4700 4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
WAVE LENGTH, A.

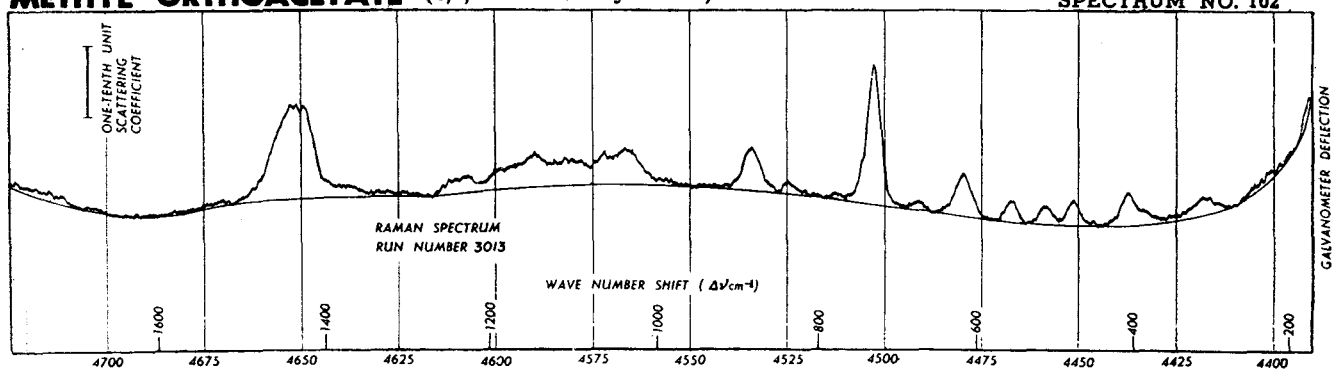
DIETHYL CARBONATE

SPECTRUM NO. 101



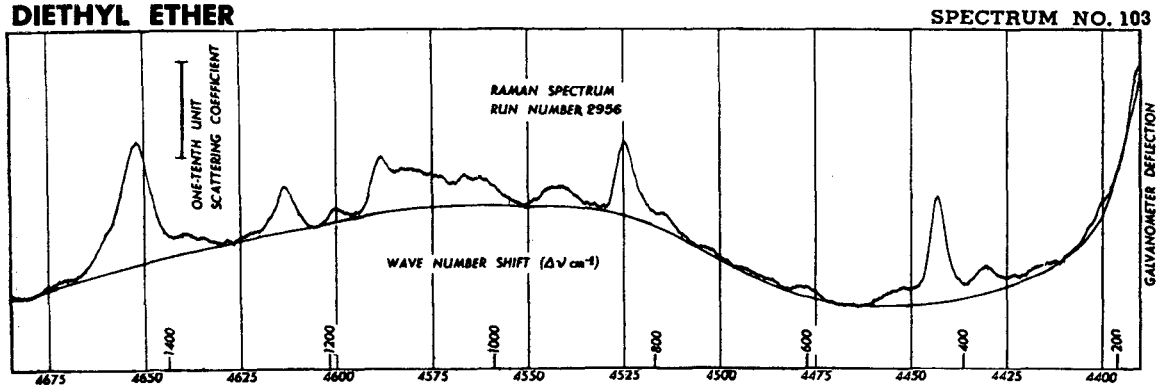
METHYL ORTHOACETATE (1,1,1-Trimethoxyethane)

SPECTRUM NO. 102



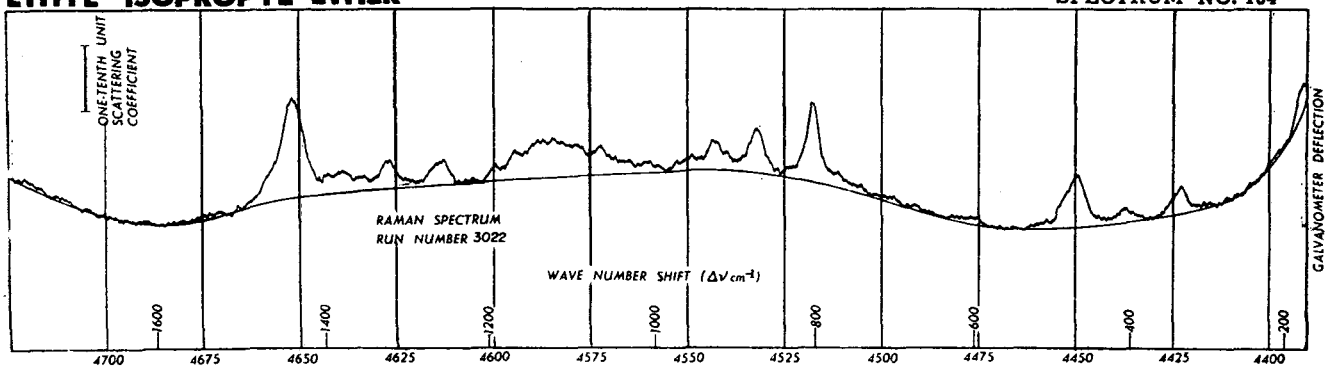
DIETHYL ETHER

SPECTRUM NO. 103



ETHYL ISOPROPYL ETHER

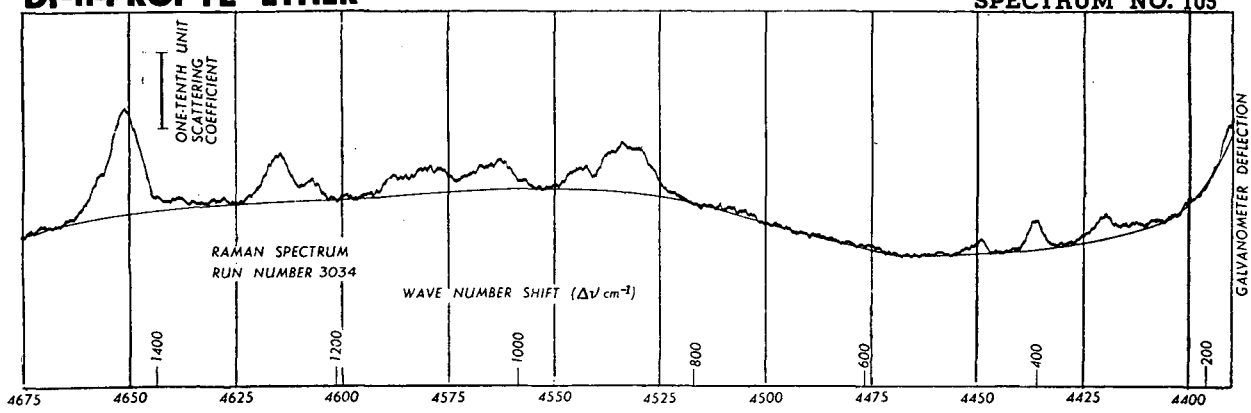
SPECTRUM NO. 104



WAVE LENGTH, A.

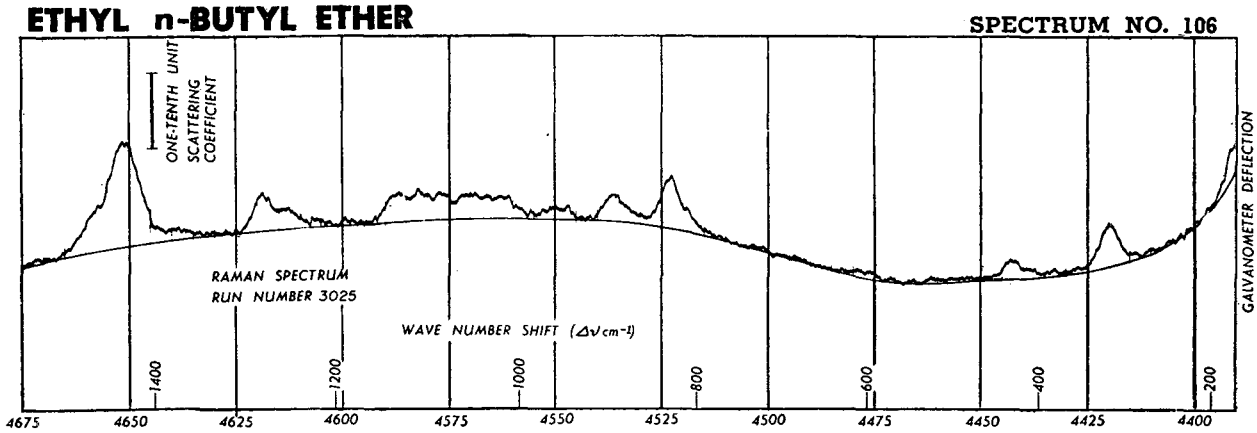
DI-n-PROPYL ETHER

SPECTRUM NO. 105



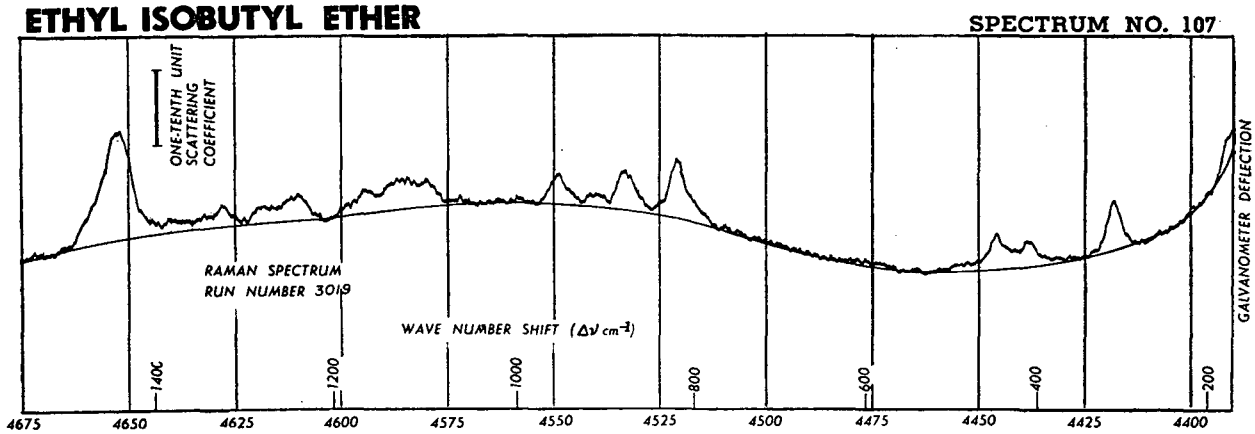
ETHYL n-BUTYL ETHER

SPECTRUM NO. 106



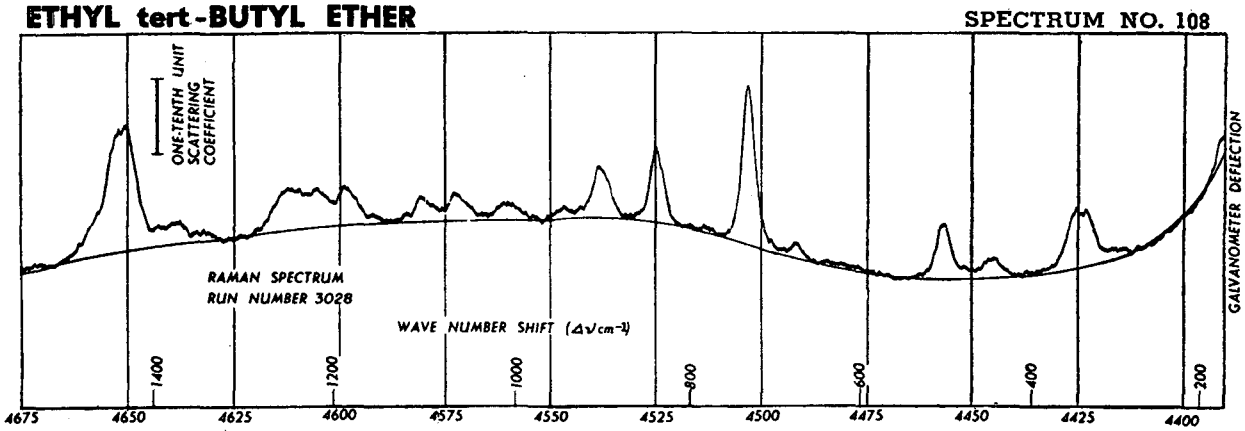
ETHYL ISOBUTYL ETHER

SPECTRUM NO. 107



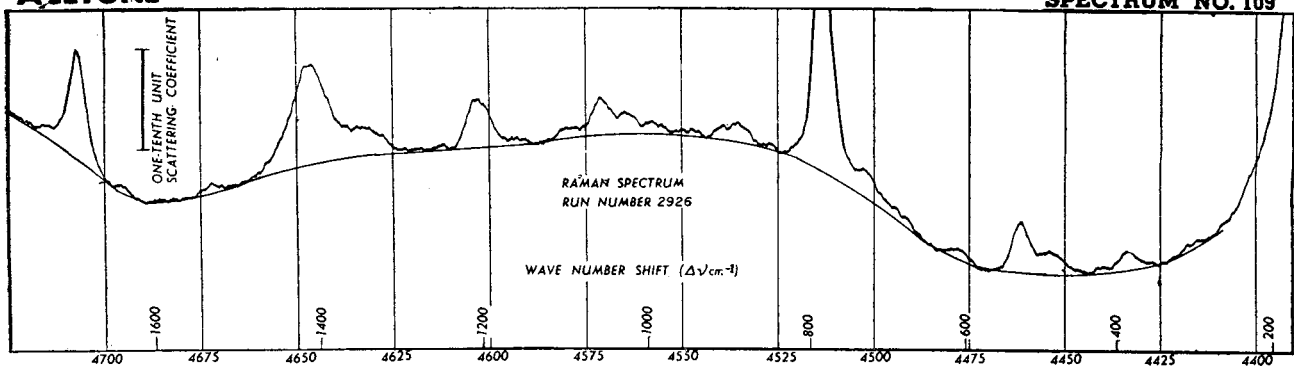
ETHYL tert-BUTYL ETHER

SPECTRUM NO. 108

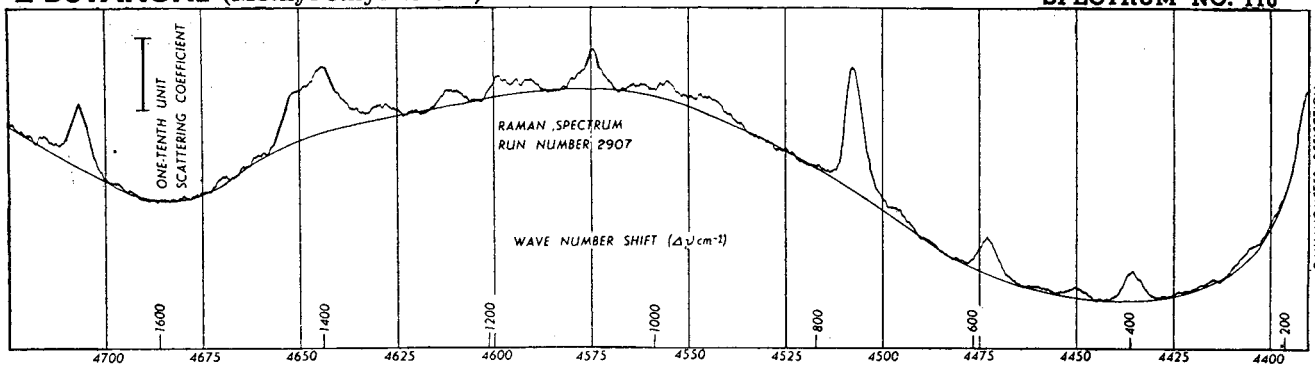


WAVE LENGTH, A.

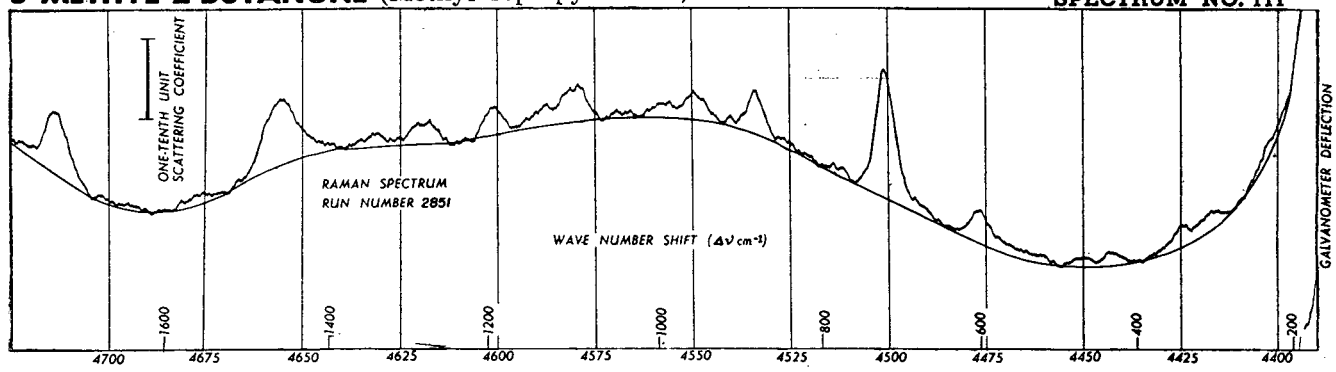
ACETONE SPECTRUM NO. 109



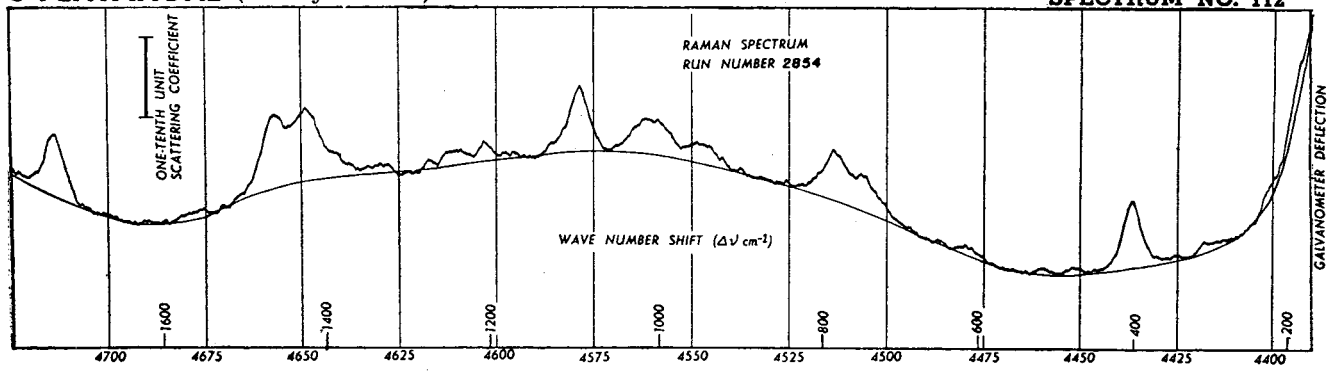
2-BUTANONE (Methyl ethyl ketone) SPECTRUM NO. 110



3-METHYL-2-BUTANONE (Methyl isopropyl ketone) SPECTRUM NO. 111

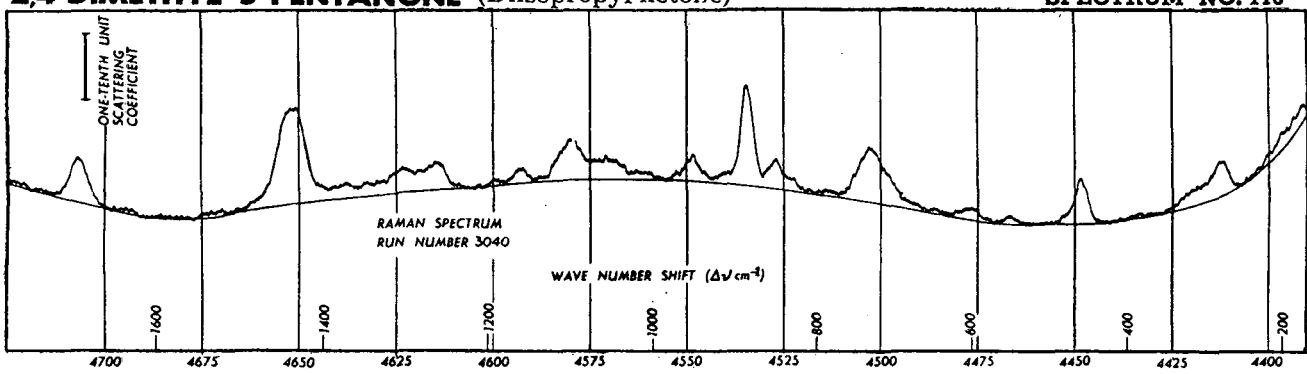


3-PENTANONE (Diethyl ketone) SPECTRUM NO. 112



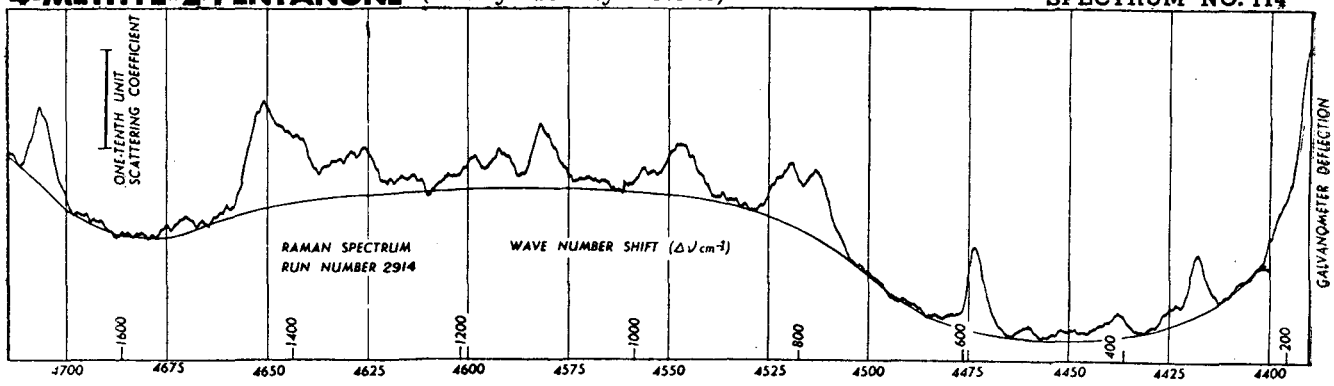
2,4-DIMETHYL-3-PENTANONE (Diisopropyl ketone)

SPECTRUM NO. 113



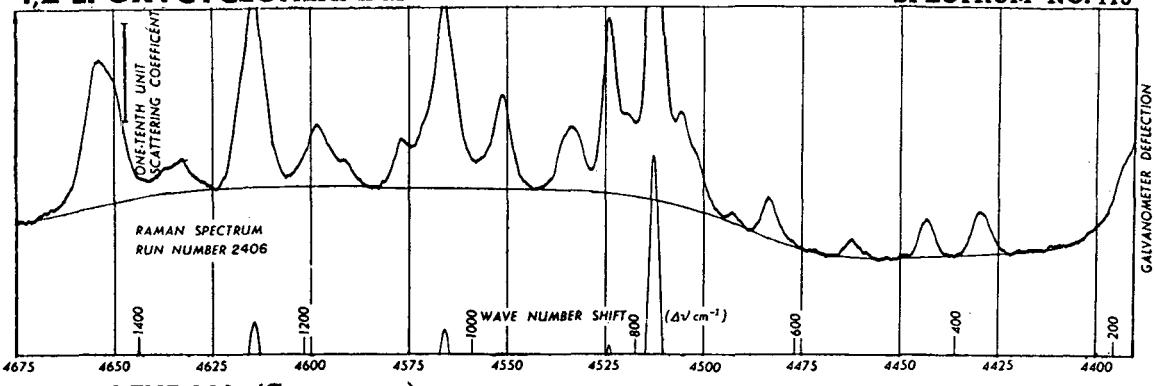
4-METHYL-2-PENTANONE (Methyl isobutyl ketone)

SPECTRUM NO. 114



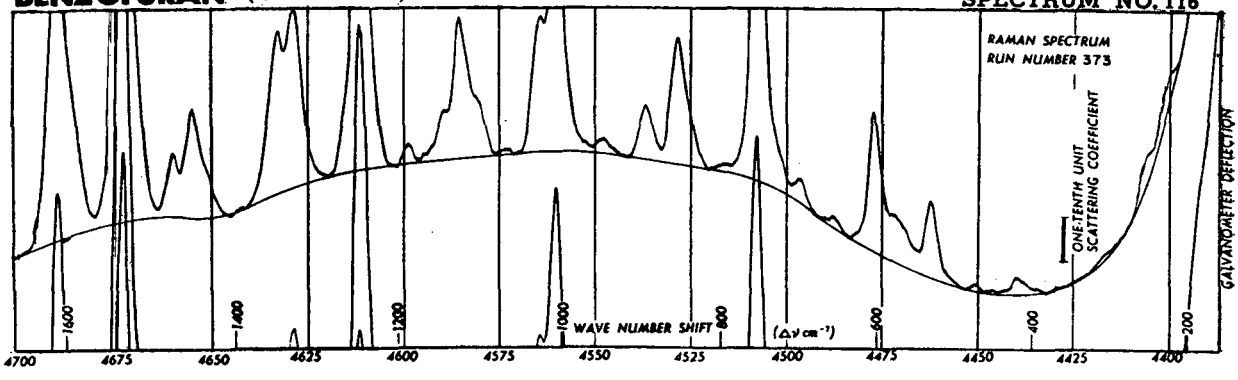
1,2-EPOXYCYCLOHEXANE

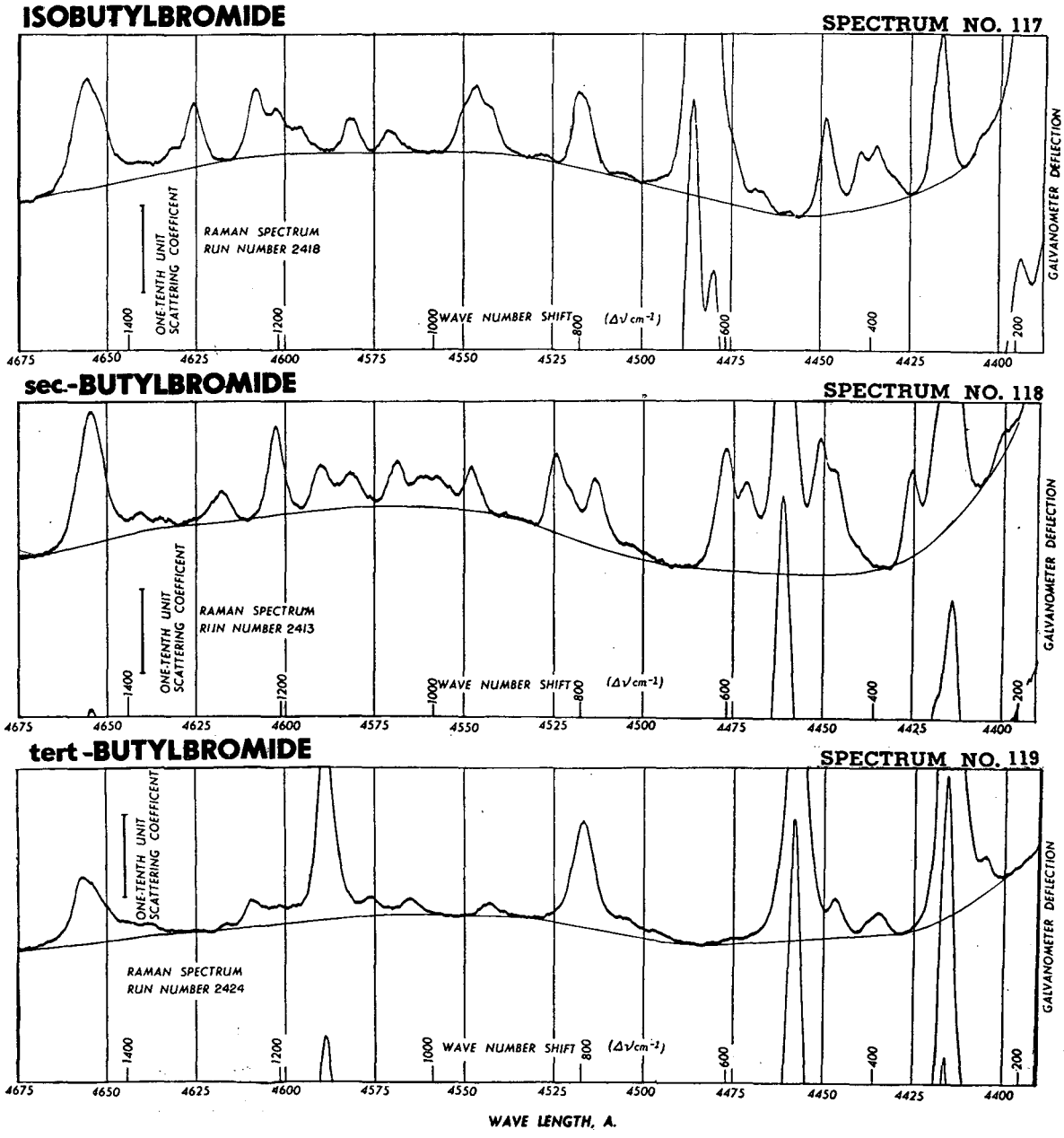
SPECTRUM NO. 115



BENZOFURAN (Coumarone)

SPECTRUM NO. 116




ISOBUTYLBROMIDE
SPECTRUM NO. 117
RAMAN SPECTRUM
RUN NUMBER 2418
ONE-TENTH UNIT SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
sec.-BUTYLBROMIDE
SPECTRUM NO. 118
RAMAN SPECTRUM
RUN NUMBER 2413
ONE-TENTH UNIT SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
tert.-BUTYLBROMIDE
SPECTRUM NO. 119
RAMAN SPECTRUM
RUN NUMBER 2424
ONE-TENTH UNIT SCATTERING COEFFICENT
WAVE NUMBER SHIFT (Δν cm⁻¹)
GALVANOMETER DEFLECTION
4675 4650 4625 4600 4575 4550 4525 4500 4475 4450 4425 4400
1400 1200 1000 800 600 400 200
WAVE LENGTH, A.

Quantitative Spectrochemical Analysis of Ashes, Deposits, Liquids, and Miscellaneous Samples

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A general technique is described which is applicable to the quantitative spectrochemical analysis of a wide variety of materials. Sample preparation, the incorporation of spectrochemical buffers, and excitation procedures are discussed for typical cases which illustrate the scope and possibilities of the method. Examples include the analysis of the ashes of rubber, plastics, paper, and cloth; deposits on walls of vacuum tubes and other surfaces; water, oils, and other liquids; and miscellaneous solid materials.

MANY spectrochemical laboratories, particularly those that are engaged in a general research program, are faced with the necessity of analyzing quantitatively by spectrochemical techniques a wide variety of materials with moderate dispatch and precision. In addition to metals and alloys, many miscellaneous samples must be analyzed, such as water, reagents, deposits of thin films in vacuum tubes, and ashes of a variety of organic compounds.

DISCUSSION

The spectrograph, an extremely versatile analytical tool, is adaptable to the quantitative analytical requirements in all the above cases, provided that the samples can be converted into a form or state in which corresponding standards are available, or can be readily made. The amount of work involved in making a separate set of standards for each type of sample encountered is prohibitive. For this reason, a technique has been devised in the author's laboratory for utilizing a single set of standards for the analysis of many types of materials. This is accomplished by buffering miscellaneous samples with a common base material to such a degree that the resultant sample behaves, during excitation, in a manner similar to that of the buffer used. The elements to be determined in the sample are thus in the concentration range of impurities with respect to the buffer, and the buffer in turn dominates their behavior. This and other functions of a spectrochemical buffer have been discussed by the author (*5–7*) and others (*3, 4, 8, 9*).

Because this method is broad in scope, covering the analysis of a wide variety of materials involving a large number of elements, this scheme of analysis is presented in a comprehensive manner rather than as a detailed step-by-step procedure. In this way it is possible to cover the many aspects of the method, such as alternative means of preparing samples, the utilization of different forms of samples, the choice of buffers, the variation in ratio of weights of buffer to sample, standards, and other factors, without obscuring the presentation with many details which may not be applicable in some cases.

This system of analysis is equally applicable to solution or powdered samples. Dry powders are preferred for most applications, particularly for the analysis of ashes, because the solubility of an unknown ash in a simple reagent is not always predictable. Experience has indicated that more reproducible results can be obtained with dry powders. If solutions are used, it is essential that they be stable, without the danger of precipitation of some of the constituents, and that the concentration of acids be matched in standards and samples. Inasmuch as the principles of analysis involved are common to both solutions and powders, to simplify the presentation the remainder of this discourse deals only with the use of dry powdered samples which are more universally applicable.

The standards employed are made to contain known amounts of the elements to be determined, in a range of 0.01 to 1.0% with respect to the metal or compound used as buffer. The number of elements contained in a single series of standards is generally limited to five or six to minimize the complexity of the system, particularly in the upper range, with regard to the interinfluence of constituent elements upon each other in the excitation source.

The major factors which govern the choice of buffer and standard base are:

1. High purity, at least with respect to the elements to be determined
2. Compatibility in the excitation source, with respect to both spectral emission and physical evaporation characteristics
3. Simplicity of spectra to minimize spectral line interference
4. Availability in relative abundance

Cupric oxide and lead sulfate satisfactorily meet the above requirements. Very pure copper and lead metals are obtainable from the American Smelting and Refining Company. National Bureau of Standards melting point standards, copper No. 45B and lead No. 49C, are also of satisfactory purity. Nickel is also an excellent buffer, but because of its relatively rich spectrum, its use is restricted to large spectrographs where spectral line interference is at a minimum. Zinc is satisfactory for the determination of elements in the lower boiling point range. Special consideration might favor the choice of other buffers in dealing with a limited group of elements, such as the alkalies or the alkali earth elements. Further discussion is limited to the use of copper or lead as the buffer element, although it is recognized that other buffers are as good or better in certain applications.

The chosen ratio of sample to buffer weights depends upon the expected order of magnitude of the element that is being determined, and should be such that the expected concentration of the element sought is between 0.003 and 0.3% by weight with respect to total weight of buffer used. As an example, in the analysis of distilled water, where the amounts of solids are very low, a ratio of weights of sample to buffer of 2000 to 1 is employed, whereas for the analysis of tap water or a somewhat impure reagent, the ratio is reduced to 100 to 1, or lower. In the case of an ash where the concentration of the element to be determined is 10% with respect to the weight of the unashed sample, the amount of buffer used is increased so that the ratio of sample to buffer weights is 1 to 100. Thus it is possible, by the judicious handling of the sample and buffer, to cover an almost unlimited range of concentrations. When the concentration of the element sought is completely unknown, a preliminary qualitative spectrochemical analysis is run, or the sample is prepared in two or more ratios. This latter scheme is generally employed, because it supplies a duplicate sample and is useful if several elements of different orders of magnitude are to be determined. The standards, which cover a range of a factor of 300 to 1000, will then usually be found to embrace the amount of elements present in the prepared samples.

Samples dealt with in this presentation fall into one of three general classifications:

1. Inorganic solutions whose constituents can be concentrated by simple evaporation
2. Organic liquids or solids which require ashing
3. Deposits, segregates, or other samples which require a special sampling technique

Each of these classes of samples is handled in the manner described below.

There are, of course, limitations to this scheme of analysis. In the case of some samples falling within classification 3, where it is not possible to concentrate the element to be determined and where it is diluted heavily with the buffer material, a low limit of determination of concentration of element of several tenths of 1% with respect to the weight of the sample is imposed.

A single buffer material may not always be adequate to serve as an internal standard for all elements to be determined in a particular sample. However, volatile elements on the one hand, such as the alkalies, and on the other, highly refractory materials, such as tantalum, tungsten, uranium, or zirconium, can be determined without reference to an internal standard with some sacrifice in precision or, if warranted, a more compatible buffer may be used in the determination of a particular element.

Under "Procedure" Pyrex glass No. 7740 or Vycor glass No. 7900 laboratory ware is specified, but where silicon, boron, or aluminum is to be determined in low concentrations, platinum should be used or blanks determined. Blanks should also be determined on all reagents used if the analysis is critical.

APPARATUS

Excitation Source. A direct current arc source is employed with open circuit voltage of 250 volts, maximum arc current 15 amperes. Series resistance is used to control and limit the current.

Spectrograph. A spectrograph is employed which has sufficient resolving power and linear dispersion to separate clearly the analytical lines from other lines in the spectrum of the sample in the spectral region 2100 to 6000 A. These conditions are satisfied by commercial grating and quartz prism instruments having a linear dispersion of the order of 0.2 mm. per A. at 2800 A. for the determination of all elements covered by this procedure.

Recording Equipment. The spectra are recorded on Eastman Process or Eastman Process Panchromatic emulsion, or equivalent.

Densitometer. The absorbance or transmittance of the analytical lines is measured on a densitometer having a precision of ±1.0% for absorbances between 0.2 and 1.5.

Developing Equipment. Emulsions are developed in an open tray with temperature control.

PROCEDURE

Preparation of Samples. Samples in the first classification include water, solutions of deposits and films, acids, and other inorganic reagents, excepting concentrated solutions of metal salts or hydroxides. Weighed samples, or measured volumes thereof, are concentrated by simple evaporation. The buffer metal is added from a nitric acid solution during this concentrating stage in an estimated amount such that the concentration of the element sought will be in the range of 0.003 to 0.3% with respect to the weight of buffer. From 0.2 to 0.5 gram of buffer element, calculated as the metal, is used and the calculated weight of the sample is chosen to obtain the desired ratio.

If copper is the buffer element, the solution of sample and buffer is evaporated to dryness. (If large volumes, 0.5 to 2.0 liters, are involved, the major part of the evaporating process is done in a Pyrex beaker of appropriate size on a hot plate until the residual volume is about 20 to 30 ml. The sample is then transferred to a Pyrex glass No. 7740 or Vycor glass No. 7900 evaporating dish for drying.) The sample is transferred to a muffle oven and baked at 400° C. for 10 minutes to convert the copper nitrate to cupric oxide. The sample incorporated in the cupric oxide is ground, mixed thoroughly in an agate mortar, then mixed with purified graphite powder in a ratio of 1 to 1 by weight.

If lead is the buffer element, the solution of sample and buffer is evaporated to about 20 ml. Two milliliters of 1 to 1 sulfuric acid are added to precipitate the lead as lead sulfate. This is dried, baked, ground, and mixed with graphite powder in the ratio of 1 to 1, as for copper.

Samples thus prepared are ready for excitation.

Organic samples require ashing. The ashing technique employed depends to a large degree upon the nature of the sample, and should be carried out in a manner designed to prevent losses due to foaming, blowing away of light particles, or evaporation of wanted constituents at high temperatures.

Oils, greases, waxes, and other petroleum or similar products, plastics, and rubbers are handled in the following manner. A weighed sample is placed in a Vycor glass No. 7900 or Pyrex glass No. 7740 evaporating dish and heated on a hot plate in a fume hood until charred. At this stage, the dish is removed from the hot plate and a measured quantity of buffer is added from a nitric acid solution. If lead is the buffer, 2.0 ml. of 1 to 1 sulfuric acid are also added to convert the lead to the sulfate. The sample is dried on the hot plate, then transferred to a muffle furnace at a temperature of 600° C., maximum, until ashing is complete.

Other samples requiring ashing, such as paper or cloth, which are difficult to confine to a small volume on dry ashing, are first partially wet-ashed to prevent loss of light floating particles. A weighed sample is placed in a Vycor or Pyrex evaporating dish with sufficient concentrated nitric acid to cover the sample. Buffer is added. If lead is used, 2.0 ml. of 1 to 1 sulfuric acid are also added. The sample is digested, charred, and evaporated to dryness, after which ashing is completed in a muffle furnace at 600° C. maximum.

Other ashing techniques are given by the Association of Official Agricultural Chemists (*1*).

The ashed sample with its incorporated buffer is thoroughly ground and mixed in an agate mortar, then mixed 1 to 1 with purified graphite powder.

Deposits and segregates sometimes require the application of special techniques in order to isolate the samples. Deposits on the surfaces of vacuum tube parts and other surfaces are usually removed with an appropriate reagent. Once in solution, they are handled in the same manner as samples in the first classification above.

Insoluble segregates in ores, ceramics, or other materials are removed and isolated by mechanical means. Once isolated, they are crushed to a fine powder in an appropriate mortar, mixed with a powdered buffer (cupric oxide), and analyzed in the same manner as ceramics, for which the method was described by the author in 1946 (*7*).

It is important in the case of ashes that the buffer be added while the sample is in a charred state, to incorporate the buffer thoroughly with the sample and to prevent light particles of ash from being blown away by any slight draft that might be present. The addition of the buffer to solution samples before drying also ensures better incorporation of buffer and sample. The early addition of the buffer becomes more important as the size of residue of solution or ash becomes smaller.

Preparation of Standards. A standard base solution of pure copper or pure lead in dilute nitric acid is made; concentration of copper or lead is 100.0 mg. per ml.

Standards whose constituents are normally soluble in nitric acid are made as follows:

Measured amounts of the elements to be included in each standard are added to a 40.0-ml. aliquot (4.0 grams as metals) of the standard copper- or lead-base solution, from standardized nitrate solutions of each element. A series of standards to cover the range of 0.001 to 1.0% of each element with respect to copper or lead in steps of factors of about three (0.001, 0.003, 0.01%, etc.) is made in nitric acid solutions.

For copper-base standard, these solutions are evaporated to dryness in Pyrex glass No. 7740 or Vycor glass No. 7900 evaporating dishes on a hot plate, transferred to a muffle oven, and baked at 600° C. maximum for 15 minutes to convert the copper to black cupric oxide. The oxide is ground, mixed thoroughly in an agate mortar, then mixed 1 to 1 with purified graphite powder. Standards are stored in screw-capped vials.

For lead-base solutions, 10 ml. of 1 to 1 sulfuric acid are added to each solution to precipitate the lead as the sulfate. This is dried, baked at 600° C. maximum for 15 minutes, thoroughly ground and mixed in an agate mortar, then mixed 1 to 1 with purified graphite powder. Standards are stored in screw-capped vials.

Standards for determining elements that are insoluble in nitric acid, such as silicon, titanium, or zirconium, are added to the

standard base solution as oxides calculated as per cent of metal. These oxides do not go into solution but are incorporated with the dried cupric oxide or lead sulfate after drying and baking as above.

If elements other than copper or lead are used as buffer, standards can be made in a manner similar to that described above.

Electrode System. The lower sample-carrying electrode (anode) is a high purity graphite rod, 0.25 inch in diameter, and 1 inch long, with a cup cut into the end with a No. 15 drill to a depth of 0.125 inch. Each cup is packed level full with its designated sample or standard. A water-cooled electrode holder is used for this electrode.

For the upper counter electrode (cathode) a purified graphite rod $^3/_{16}$ inch in diameter and 1.125 inches long with a flat end is used.

Arc gap width is maintained at 0.25 inch.

Excitation. The samples are arced in triplicate, along with a series of standards in the direct current arc at an initial current of 12 amperes, limited by resistance in series. After initial setting, no further adjustments of current are made.

Exposure Conditions

Spectral region, A.	2100–6000
Slit width, mm.	0.02–0.08[a]
Slit length, mm.	1.0–3.0[a]
Filters	Step, 100%, 20%, 4%[b]
Preburn period	None
Exposure period, seconds	60[c]

[a] Slit width and slit length used depend upon particular spectrograph and densitometer employed.

[b] A step sector may be used, or if spectrograph is not stigmatic, appropriate filters may be placed at camera to filter particular wave-length regions. Exact transmission values are not critical.

[c] For determination of a few highly refractory elements such as Ta, Zr, W, or U, sample should be completely volatilized and time adjusted accordingly

The spectrograph is illuminated in such a manner that the resulting spectral lines represent radiant energy from the entire arc column except for about $^1/_{64}$ inch from either electrode, which is screened out.

Photography. EMULSION CALIBRATION. Where emulsion calibration in terms of relative intensities is required, it is calibrated by means of a step sector, step filter, or the two-line method of Churchill (*2*).

Where the spectra of the standards are placed on the same emulsion, it is calibrated directly in terms of per cent concentration of the element sought versus the ratio of the absorbances or transmittances of the line pairs employed.

Photographic Processing. EMULSION. Eastman Process or equivalent

DEVELOPMENT. Eastman Dektol, open-tray, 4 minutes, 68° C.

STOP BATH. Flowing water, 15 seconds.

FIXING. Eastman acid fixing powder with hardener, 3 minutes.

WASHING. Flowing water, 5 minutes.

DRYING. Blower and heater.

Photometry. EMULSION CALIBRATED. The absorbances or transmittances of the analytical and internal standard line pairs listed in Table I are determined and converted to relative intensities using an emulsion calibration curve obtained as above. From the data thus obtained, a plot is made relating log concentration to log intensity ratios from the spectra of the standards for each element for which a control line is listed in Table I. For the elements for which no control line is listed in Table I, a plot is made of intensity versus log concentration. The concentration of the sought-for element in the samples is determined by reference to the appropriate analytical curve. The average of triplicate determinations is reported.

EMULSION NOT CALIBRATED. The absorbance or transmittance is determined of the analytical and internal standard line pairs listed in Table I. An analytical curve is plotted relating log concentration to absorbance differences (or log transmittance ratios) from the data obtained on the standards for each element for which a control line is listed in Table I. For the elements for which no control line is listed in Table I, absorbance (or log transmittance) versus log concentration is plotted. The concentration of the element sought is determined by reference to the appropriate analytical curve. The average of triplicate determinations is reported.

This procedure is permissible if the spectra of standards are registered on the same emulsion as the spectra of the samples and if the lines used have absorbances between 0.20 and 1.5.

Calculations. Because a single set of standards is employed for the analysis of samples in various degrees of concentration or dilution, the results as normally obtained in terms of the standards must be multiplied by a concentration or dilution factor as determined by the ratio of sample to buffer weights employed.

Table I. Analytical Line Pairs for Spectrochemical Analysis of Miscellaneous Samples

Element to Be Determined		Wave Length, A. Element line	Copper internal standard line	Lead internal standard line
Aluminum		3082.2	2768.9	3220.5
		3092.7	2768.9	3220.5
Antimony		2311.5	...	2399.6
		2598.1	2768.9	...
Arsenic		2349.8	...	2399.6
Barium	II	4934.1	...	5005.4
		5853.7	5782.1	...
		3071.6	3126.1, 3292.8	...
Bismuth		2898.0	2768.9	3220.5
		3067.7	2768.9	3220.5
Boron		2496.8	2768.9	...
Cadmium		3261.1	2768.9	3220.5
Calcium		4226.7	5105.5	...
	II	3968.5, 3933.7	3292.8	3220.5
		3158.9, 3179.3	3292.8	3220.5
Copper		3247.5, 3274.0	...	3220.5
		2961.2	...	3220.5
Germanium		2709.6	2768.9	(Zn2712.5)
Iron		2719.0	2768.9	2657.1
	II	2599.6	2768.9	2657.1
Lead		2833.1	2768.9	...
		2873.3	2768.9	...
Magnesium		2852.1	2768.9	2657.1
	II	2795.5	2768.9	2657.1
Manganese		2798.3	2768.9	...
		2603.7	2768.9	...
Nickel		3134.1	2768.9	3220.5
		3101.6	2768.9	3220.5
		3101.9	2768.9	3220.5
Phosphorus		2535.6	2768.9	2657.1
Silicon		2506.9	2768.9	2657.1
		2516.1	2768.9	2657.1
		2881.6	2768.9	2657.1
Silver		3382.9	3292.8	3220.5
		3280.7	3292.8	3220.5
Strontium		4607.3	5105.5	5005.4
		4077.7	3292.8	...
		3351.2	3126.1, 3292.8	...
Tin		2863.3	2768.9	3220.5
		2354.8	...	2399.6
Titanium	II	3349.4	...	3220.5
		3354.6	2961.2	3220.5
Tungsten		2944.4	...	...
Zinc		3345.0	3126.1, 3292.8	3220.5
		2138.6	...	...

EXPERIMENTAL

This procedure has been applied with good success to a long list of miscellaneous materials for the determination of most of the common elements. The critical part of the procedure is the preparation of the samples. Once the sample has been incorporated with its powdered buffer, the spectrochemical part of the procedure has become thoroughly established. Reproducibility or precision on a single prepared sample is of the order of ±5 to 6% of the amount present for most elements. The accuracy has not been generally established because of the variety and nature of many of the samples. Standard samples are not available for most of the materials covered by this procedure and only in a few cases have chemical checks been made. Those checks that have been made indicate the method to be sound and capable of an accuracy of the order of ±10 to 15% or better.

Reasonable precautions must always be taken in the preparation of samples to guard against the loss of volatile compounds. For this reason the use of chlorides is generally avoided, because so many of the chlorides are volatile at ashing temperatures. A few nitrates and sulfates are also volatile. Sample preparation must therefore be tailored to each particular problem. Checks must be made to determine whether any losses occur during processing, and if so, to modify the techniques to prevent such loss. The analyst must rely, to a large degree, upon his basic knowledge

Table II. Applications of Procedure

Material	Elements Determined	Remarks
Water	Al, B, Ca, Cu, Fe, Mg, Na, Si, Zn	Concentration by evaporation
Reagents	Al, B, Ca, Cu, Fe, Mg, Na, Si, Zn	Concentration by evaporation
Extracts, deposits	Al, B, Ba, Cd, Cu, Fe, Mg, Mo, Na, Ni, Si, Sr, Sn, Pb, W, Zn	Concentration by evaporation
Paper	Al, Cu, Fe, Mg, Si	Ashing
Rubber	Ca, Cu, Na, Mg	Ashing
Cloth	Zn	Ashing
Tar	Zn	Ashing
Ethyldiamine tartrate	Ba, Ca, Cr, Fe, Mg, Ni, Si	Ashing
Polyethylene	Pb	Ashing
Polyvinyl chloride	Al, Pb, Si, Ti	Ashing
Oils and greases	Cu, Fe, Pb, Sb, Sn, Zn	Ashing
Segregates	Ge	Mechanical separation, pulverization or solution
Cathode coating	Ba, Ca, Sr	Solution in reagent concentration or dilution

of the behavior of the chemical elements and compounds to avoid gross errors in some instances. If a volatile compound is encountered, steps should be taken to convert it to one that is less volatile. As an example, boron as the chloride or sulfate is extremely volatile and will be completely lost from a sample at ashing temperatures, but is nonvolatile as the nitrate or oxide. For this reason when boron is to be determined, the copper buffer, added as a nitrate solution or as dry cupric oxide, is used, and never lead sulfate. In handling many miscellaneous samples, situations similar to the case of boron are certain to be encountered, and it is imperative that the analyst prepare the sample in a manner which will retain all of any constituent to be determined.

Some of the applications of this procedure that have been made in the author's laboratory are listed in Table II, to indicate the wide scope of the method. The elements listed for one material are seldom all run on a single sample. Many elements not listed can be run if required. Ranges of concentration of the elements have been omitted because, in the cases of solutions and ashes, the amount of concentration possible is limited only by sample size, and the ranges can be further modified by variations in ratio of amounts of buffer to sample.

SUMMARY AND CONCLUSIONS

A procedure for the spectrochemical analysis of a wide variety of materials has been developed. With an understanding of the fundamental principles involved, it is possible to apply the procedure to the many analytical problems that are likely to confront the staff of a spectrochemical laboratory. The preparation of the samples and their conversion to a form suitable for excitation in the source are the important part of the procedure. The concentration or dilution of samples and the judicious use of buffers reduce the problem of the subsequent excitation, and recording and interpretation of the spectra, to the basis of the simple spectrochemical analysis of a powder or solution. Although details of the procedure have been presented only for the case of powdered samples, the fundamental concepts are equally applicable to solutions.

ACKNOWLEDGMENT

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Determination of Trace Metallic Components in Petroleum Oils

By Means of the Emission Spectrograph

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THE determination of inorganic components in oils and residua is among the frequent and necessary analyses required in the processing and manufacture of petroleum products. As a single example, periodic control analyses of oil feed stocks to catalytic cracking are required to indicate the amounts of potential catalyst contaminants which may be introduced into the reactor from this source. Conventionally, the determination of inorganic content consists of burning the oil sample freely in an open tared dish with subsequent ignition of the residual coke, and, if individual components are to be determined, chemical analysis of the weighed ash. This procedure is somewhat tedious and time-consuming, especially where large amounts of oil must be burned to obtain an adequate amount of ash for chemical analysis; furthermore, for special experimental oils of low inorganic content that are available only in limited sample amounts, the conventional method may be very difficult, if not impossible, to perform.

Although the sensitivity of the emission spectrograph for detecting trace elements in various substances is well known, there is, to the authors' knowledge, no record of its application to the direct determination of metals in oils in concentrations below 0.01% or 100 p.p.m., a scope that may be clearly defined as "trace" (*3*, *4*). A method for the determination of oil additives has been described by Calkins and White (*1*), in which spark excitation of an electrode impregnated with the oil sample is employed in direct spectrographic analysis. This method provides results of satisfactory precision and accuracy in the concentration ranges of 0.01 to 0.1% of certain elements, but it does not appear to be

applicable to the detection of ash component elements in oils in concentrations of about two orders of magnitude less than this range.

It therefore appeared desirable to investigate the possibilities of developing a spectrographic method capable of determining trace metals in oils in concentrations as low as a few parts per million. The carbon arc cathode layer technique, because of its high sensitivity, has been employed extensively in the analyses of various nonconducting substances such as geological rocks (*9*) and soils (*6–8*) for trace elements. Its high sensitivity is due to the concentration of positively charged ions, formed upon volatilization of the sample from the cathode of the direct current arc, in a layer 1 to 2 mm. above this electrode. By employing the cathode layer region as the optical source, the sensitivity for certain elements of low ionization potential may thus be enhanced by as much as 100-fold over that obtainable in the mid-portion of the arc (*9*). Although a method affording high sensitivity often is accompanied by a sacrifice in relative precision, this method of excitation appeared to be the most applicable for the direct detection and determination of trace elements in oils.

As objectives in directing development of a spectrographic method the following requirements were considered essential: (1) that direct inspection of the oil be made without a preliminary ashing step; (2) that the technique be simple and the time of analysis short; (3) that the method possess adequate sensitivity and accuracy for the determination of elements in trace concentrations. A procedure has been developed which to a large measure meets these requirements, although it has the disadvantages of not detecting the nonmetallic elements or large particles of inorganic substance in an oil not absorbable by the electrodes.

A method is presented whereby trace metals occurring in oils in concentration ranges of 2 to 100 parts per million may be determined by direct spectrographic inspection of carbon electrodes impregnated with the oil sample. The method employs the cathode layer principle with the use of added internal standards. The method is rapid and provides results of better than semiquantitative accuracy over the established calibration ranges. Calibration procedures for the determination of iron, nickel, chromium, vanadium, calcium, sodium, silicon, and aluminum are described and applications of the technique are discussed.

EQUIPMENT

The source of excitation used in developing this method is a direct current full-wave rectifier unit supplying current at 250 volts with induction control of the amperage. The spectrograph is an Applied Research Laboratories 1.5-meter instrument with a grating of 24,000 lines per inch and affording a dispersion of about 7 A. per mm. over the spectral range of 2480 to 4500 A. Photometry measurements are performed with the comparator-densitometer also supplied by Applied Research Laboratories.

The electrodes used are 1/8-inch special spectroscopic graphite rods, each carton of which is furnished with a statement of purity, supplied by the National Carbon Company, Niagara Falls, N. Y. Spectrum analysis No. 1 film is used.

STANDARDS

The necessity of using base stocks and reagents of the utmost purity available for preparing oil standard blends is emphasized. Among the most satisfactory base stocks used in this laboratory are silica gel percolated mineral oil, *n*-hexadecane, and C.P. distilled oleic acid. A preliminary spectrographic examination by the technique described should be made to establish the freedom from metal contaminants of each stock contemplated for use. A concentrated base stock is prepared by adding a C.P. metal compound, the metal content of which has been predetermined by chemical analysis, to oleic or naphthenic acid and heating at 150° C. for 2 hours or longer (*2*).

Internal Standard Blend. The blend containing the internal standards is prepared by adding cobalt acetate and lithium hydroxide to oleic acid, so that the resulting mixture contains 10,000 p.p.m. of cobalt and 250 p.p.m. of lithium.

Calibration Standard Blends. Concentrated standard oleic (or naphthenic) acid blends of iron, calcium, nickel, and chromium are prepared from the metal acetates; vanadium from ammonium vanadate; aluminum from aluminum stearate; silicon from a Dow-Corning Silicone; and sodium from sodium hydroxide.

Preparation of Sample Electrodes. The method of preparing oils for analysis is the same for calibration blends as for unknown samples. A 10.00 ± 0.05-gram portion of the sample to be analyzed is weighed into a 50-ml. beaker. Exactly 1.000 ± 0.005 gram of the prepared cobalt-lithium internal standard blend is added to the beaker. If the oil is solid or viscous, it should be warmed sufficiently so that the standard can be mixed with it rapidly and thoroughly.

For a number of years this laboratory has employed absorption by a hot porous carbon rod as a satisfactory method of electrode sampling for the direct inspection of either aqueous solutions or oils. This sampling technique is essentially the same as described by Calkins and White (*1*), differing only in the size and preshape of the electrode used and in the use of added internal standards.

Three 1/8-inch special carbon rods 2 inches (5 cm.) in length are employed as sample electrodes in this method of analysis. Each electrode is pre-arced with 10-ampere current for a 10-second period and, while it still glows, is added to the previously prepared oil sample. (A beaker cover glass should be kept convenient to extinguish the sample should it ignite; only in rare instances, however, has the sample caught fire in the impregnation of thousands of electrodes with oils and naphthas of various gravities by this procedure.) The electrodes are allowed to remain with the pre-arced portion immersed in the oil for 10 minutes and are then removed from the oil by means of tongs. After the electrode is wiped with clean cheesecloth it is placed in the electrode holder for arcing. Caution must be exercised to avoid extraneous contamination of the electrodes at any time.

SPECTROGRAPHIC TECHNIQUE

The prepared sample electrode is placed in the lower (negative) holder, so that its tip is at the predetermined optical axis of the instrument. The upper (positive) electrode, also 1/8-inch special purity carbon, is placed so that the arc gap between the two electrodes is 7 mm. The slit width of the instrument is adjusted to 20 microns. After the electrodes are properly spaced they are arced for 30 seconds with an excitation current of 10.0 ± 0.1 amperes, and no adjustment in electrode positions other than the initial spacing is made in the arcing procedure. In the event the initial arc is extinguished by the momentary expulsion and burning of oil, the arc is quickly restruck and normal arcing period is completed. A relatively small amount of soot is formed, which is readily removed by an aspirator-blower placed above the arc stand. A rotating sector (four steps, factor of 2) is operated in the optical path 17 cm. from the slit. The arc-slit distance is 42 cm., and a cylindrical quartz lens of 5-inch focal length is used in front of the arc to obtain focus at the slit. Three arcing exposures or spectra are recorded on each plate.

The film is developed for 3 minutes in D-19 developer at 70° F., fixed, washed, and dried in preparation for microphotometer density measurements. The analytical line pairs measured are listed in Table I. Intensity values are obtained by reference to an H and D blackening curve based on the iron spectrum over the spectral region of measurement.

In establishing calibration working curves similar to those of Figure 1, the foregoing procedure is applied to oil synthetic blends to obtain data relating the concentration of each metallic element to the appropriate intensity ratios.

From 2 to 3 hours' working time normally is required for the analyst to complete analysis of an oil sample containing eight elements in concentration ranges for which calibrations are established.

Table I. Analytical Line Pairs Used in Determining Trace Metals in Oils

Internal Standard, A.	Sought Element, A.	Calibration Range, P.P.M.
Co 3049	Fe 3021	2–100
Co 3049	Fe 3048	30–300
Li 3233	Na 3303	2–100
Li 3233	Ca 3179	2–100
Co 3088	Al 3093	2–100
Co 2887	Si 2881	2–100
Co 3261	V 3184	1.5–100
Co 3261	Ni 3134	2–100
Co 3261	Cr 3131	2–100

Table II. Spectrographic Analysis of Mineral Oil Synthetic Blends

Component	Blend 1, P.P.M.			Blend 2, P.P.M.			Blend 3, P.P.M.		
		Found			Found			Found	
	Added	A	B	Added	A	B	Added	A	B
Fe	25	26	31	79	78	78	12	12	12
Na	14	19	15	5	4	6	66	52	56
Ca	65	71	46	5	6	5	5	6	6
V	3	3	4	5	5	5	9	15	17
Ni	9	8	9	9	5	5	6	4	4
Cr	6	5	6	10	10	11	6	7	5
Al	7	11	19	6	8	11	4	5	7
Si	4	4	8	13	18	12	5	4	8

EVALUATION OF THE METHOD

Estimates of the accuracy and precision of the present spectrographic method are provided by the data of Tables II to VI. Although the variance noted between spectrographic and synthetic or chemically determined values are in some instances relatively large, extent of agreement on an absolute basis indicates accuracies of the spectrographic determinations are considerably better than semiquantitative or order of magnitude estimates.

Table III. Analyses of Heavy and Light Metal Synthetic Blends

Component	Added, P.P.M.	Found, P.P.M. Spectrograph A	Spectrograph B	Ignition ash
	Heavy Metals			
NiO	5.0	5.7	5.1	
Fe_2O_3	17.9	15.8	17.0	
V_2O_5	8.6	6.8	6.6	
Cr_2O_3	3.2	4.4	2.6	
Na_2CO_3	10.1	7.8	6.9	
Total	44.8	40.5	38.2	46.7 50.0 51.6
	Light Metals			
Na_2CO_3	20.7	13.3	14.0	
CaO	6.5	12.6	9.0	
Al_2O_3	8.5	11.4	8.9	
SiO_2	14.1	11.6	10.0	
Total	49.8	48.9	43.9	38.7 39.8 42.7

In Table II are presented results for analysis of three mineral oil blends (viscosity of 79 cs. at 100° F.) containing eight added metals. The reproducibility of determinations of the "heavy" metals, iron, vanadium, nickel, and chromium, appear to be better than for the "light" metals—for instance, a variation from synthesis by a factor of almost 3 is noted for one aluminum determination, whereas the maximum variation noted for iron is 24%.

Table III presents results of spectrographic and ash (determined by burning and ignition) analyses of heavy and light metal synthetic blends. The component concentrations are calculated as metal oxides, except sodium, which is calculated as the carbonate, to obtain comparisons on an ash basis. Again it is noted

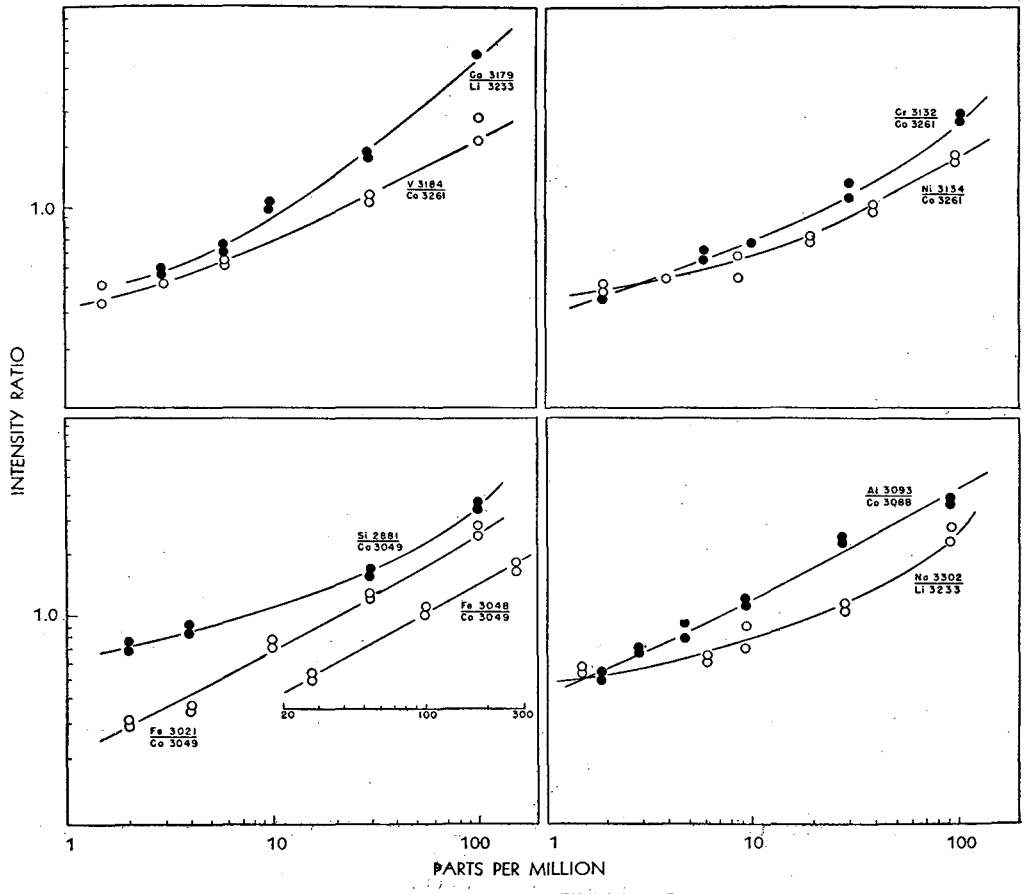

Figure 1. Calibration Working Curves

Table IV. Influence of Viscosity on Determination of Metals in Oils

Component	Acryloid Blend[a], P.P.M. Added	Found A	Found B	Iso-octane Blend, P.P.M. Added	Found A	Found B
Fe	42	37	37	27	27	25
Al	18	27	24	4	2	3
V	12	14	15	4	4	5
Ni	17	17	17	4	3	4
Cr	7	11	11	3	2	2
Na	35	20	26	10	7	6
Ca	..	..		5	8	9
Si	..	..		3	<2	<2
Viscosity at 100° F., cs.	2379			1.4		

[a] Acryloid initially contained undetermined trace amounts of calcium and silicon.

Table V. Sodium and Iron in Light Petroleum Oils

Sample No.	Sodium, P.P.M. Chemical	Sodium, P.P.M. Spectrographic	Iron, P.P.M. Chemical	Iron, P.P.M. Spectrographic
1[a]	11	11	...	...
2	7	2	8	6
3	14	12	8	10
4[b]	..	..	2800	3000[b]
5	..	...	11	15
6	..	...	9	6
7	..	...	16	16
8	28	22	...	...
9	30	24	...	...
10	28	25	...	...
11	26	22	...	...
12	5	2	...	...
13	6	2	...	...

[a] Sample contained 12 p.p.m. sodium in mineral oil by synthesis.
[b] Fischer synthesis oil diluted 20-fold in oleic acid prior to spectrographic analysis.

ferences in rates of volatilization and excitation between it and the sought elements constitute major points for consideration. Interval exposures made during the arcing period showed that the alkaline metals volatilized more quickly than either the other sought elements or cobalt. Lithium was introduced for determination of the alkaline metals, in conjunction with cobalt for determining the other elements, and this resulted in observable improvement in accuracy for the alkaline metals over that obtained by the use of cobalt standard alone.

Film background, initial contamination of the standards or electrodes, and a spectral line of an interfering element all are factors which reduce sensitivity of detection of a given element and cause a decrease in slope of the calibration curve of the element for lower concentrations. Measurements corrected for background resulted in no detectable improvement in reproducibility. The use of a masking aperture near the arc to allow passage of radiation chiefly from the highly centralized cathode layer region provided very little decrease in background. However, the use of a selective filter consisting of aqueous nickel sulfate hexahydrate (500 grams per liter) in a 1-cm. quartz cell at the slit results in a marked reduction in background over the spectral region of density measurements (*5*). A slit opening of 40 microns may be used with the selective filter.

In addition to the quantitative applications which have been discussed, the spectrographic method also has been used extensively in rapid qualitative screening tests of oils. Examples of such applications for "go–no-go" type tests are detection of calcium, barium, and phosphorus additives in oils and traces of lead and copper in naphthas.

Table VI. Iron, Vanadium, and Chromium in Treated Heavy Petroleum Residua

Sample No.	Total Ash, P.P.M., Chemical	Iron, P.P.M. Chemical	Iron, P.P.M. Spectrographic A	Iron, P.P.M. Spectrographic B	Vanadium, P.P.M. Chemical	Vanadium, P.P.M. Spectrographic A	Vanadium, P.P.M. Spectrographic B	Nickel, P.P.M. Chemical	Nickel, P.P.M. Spectrographic A	Nickel, P.P.M. Spectrographic B
1	3960	65	45	42	1	<2	<2	3	15	10
2	781	6	11	13	4	5	5	11	17	17
3	3396	59	30	30	15	18	20	13	13	16
4	4833	87	60	52	6	0	10	10	23	18
5	800	28	15	15	<1	<1	<1	12	16	11
6	15	0.3	No detection		No detection			0.3	No detection	
7	383	6	6	7	4	3	4	11	15	16
8	795	20	18	18	12	11	13	25	27	24
9	68	2	<3	..	3	<2	...	1	<2	<2

that the accuracy and reproducibility of spectrographic determinations are better for the heavy than for the light metals.

Table IV presents results of analyses of two oil blends of greatly different viscosities, the base being an acryloid with mineral oil in one case and iso-octane with oleic acid in the other. The accuracy and precision obtained are comparable with those obtained for the mineral oil blends of Tables II and III, in which cases the viscosity was 79 cs. at 100° F. Thus, no differences definitely attributable to extremes in viscosity are reflected in these results.

In Tables V and VI are presented results for analysis of petroleum light oils and of residua, respectively. The chief metallic contaminants in the light oils were the sought elements, and it may be observed in Table V that good agreement between chemical and spectrographic methods was obtained. For the heavy residua of Table VI the agreement between the methods is not as good in some instances as was found for the light oils. The variance in these cases may be attributable to nonhomogeneous electrode sampling of the heavy stocks or to interference from the large quantities of inorganic substances present other than the determined elements. The cathode layer effect is reduced or even eliminated by high ionic conductivity in the arc (*9*).

During progress of the present problem, the choice of the most suitable internal standard for determining each of the several elements was considered. For cobalt the broad distribution of spectral lines is favorable, although other factors such as dif-

Although the spectrographic technique has thus far been limited to analyses of petroleum oils, it should apply equally well to determinations of organo-metals in vegetable and animal oils or in other organic substances.

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The Ratio Method in Spectrophotometric Analyses

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A method has been developed for normalized spectrophotometric analyses of multicomponent mixtures in which only the mutual relationships of absorption coefficients are determined rather than the absolute values. The method is intrinsically rapid and accurate, and has been shown to effect a considerable saving in time and materials relative to older methods.

ANY system of analysis of a mixture depends on differences among the constituents. To determine the component distribution when all components are practically identical chemically, it is necessary to resort to physical methods of analysis such as mass spectrometry and absorption spectrophotometry.

The practice of making spectrophotometric analysis depends on the accurate and precise knowledge of the coefficients of optical absorption involved in the analysis. These absorption coefficients are usually obtained by measuring several solutions of accurately known strengths for optical absorption at the wave lengths that have previously been selected for the analysis. In order to know the strengths of these solutions, the operator weighs in the pure component separately for each solution. If Beer's law is under test, several different strengths must be made up. Once these data have been acquired, measurements on synthetics and unknowns may commence.

Table I. Ratios for *m*-Xylene

2613 A.	2709 A.	2723 A.	2742 A.
7.28	4.88	9.72	4.50
7.64	5.16	10.32	4.60
6.52	4.48	9.00	4.00
0.74897	0.50206	1.00000	0.46296
0.74031	0.50000	1.00000	0.44574
0.72444	0.49778	1.00000	0.44444
0.73791	0.49995	1.00000	0.45105

This standard approach to obtaining absorption coefficients has several disadvantages:

It is time-consuming.
It involves a good deal of handling, which permits errors to be introduced.
It requires, for assurance of accuracy, preparation of two or several standard solutions.
The absorption coefficients determined are unrelated except by the consistent accuracy and precision of the operator as he proceeds through a variety of independent operations.

The ratio method permits calibration data to be obtained not only more quickly and with less reliance on technique than more usual methods, but also with intrinsic accuracy.

The basis of the method described depends on the fact that the absolute values of the absorption coefficients need not be known in order to effect a normalized multicomponent analysis. What is desired in such an analysis is to establish the concentration relationships of the various components making up the mixture to be analyzed. This can be done merely by establishing the correct relationships of the absorption coefficients, without regard to absolute values.

SETTING UP THE ANALYSIS

The First Object. OBTAINING ABSORPTION RATIOS. An analysis for the xylenes and ethylbenzene, which was set up in this laboratory as a demonstration, can serve to illustrate the ratio technique.

The first object is to measure quantitatively the relationships of the optical densities of each pure component at the wave lengths selected for the analysis. Table I reproduces, for instance, the data sheet obtained for *m*-xylene. (Five significant figures are usually carried in order not to lose data in calculations on matrices.)

The column headings (Table I) are the wave lengths in Ångström units. The first three rows correspond to chart readings (which are ten times the optical densities) obtained from measurements on *m*-xylene. The solution of *m*-xylene is made up to a desired concentration as follows:

The drawn-out end of a capillary tube is dipped into the *m*-xylene, and subsequently dipped into the absorption cell which is already filled with solvent (*n*-heptane). The concentration of the resulting solution is then adjusted (by dilution with solvent, or by addition of more *m*-xylene) until the optical densities at the measured wave lengths are between 0.4 and 1.0. This procedure is repeated until enough data to furnish a good average are obtained; here, three separate entries of sample are generally used.

The second three rows show the readings in the first three rows expressed as ratios to the readings for one arbitrary wave length. The last row, being the average of the preceding three rows of ratios, is the object of the first set of operations.

This method is used to obtain all the optical data. There are no standard solutions to make up; only a minute amount of the material is used; there is no call for highly skilled technique; the data are related; and the time required is about 10 to 15 minutes per component or per mixture of components.

Data similar to those shown in Table I are obtained for all the components in the analysis, and for as many synthetic samples as are desired to set up and check the analysis. These data are then arranged into a "raw" matrix, as in Table II. For this demonstration, one synthetic was used as an "adjustment" synthetic.

The Second Object. ARRIVING AT CORRECT COLUMN RELATIONSHIPS. The raw matrix shown in Table II is then solved, using the method of Crout (*4*), and the answers are normalized. The percentages found and known are shown in Table III, and the percentage deviations of the found from the known based on the known.

The percentage deviations are used to change each figure in the corresponding columns by the amount and in the direction indicated. This correction will tend to establish the correct relation-

Table II. Raw Matrix

A.	Ethylbenzene	1,2-Xylene	1,3-Xylene	1,4-Xylene	Syn. L
2613	1.00000	1.18083	0.73791	0.46791	1.59427
2709	0.15988	1.00000	0.49995	0.35648	1.00000
2723	0.080349	0.71189	1.00000	0.28759	1.03323
2742	0.037748	0.25457	0.45105	1.00000	1.37168

Table III. Results from Raw Matrix

Compound	Found	Known	% Deviation
Ethylbenzene	16.38	24.87	−34.14
1,2-Xylene	14.90	24.23	−38.51
1,3-Xylene	20.78	24.90	−16.55
1,4-Xylene	47.94	26.00	+84.38

ships between the columns. The result of the first correction to the raw matrix is shown in Table IV. This matrix, which converged with one adjustment, is used to treat the data from synthetics L and M. Table V presents these results.

A perfect match was obtained for synthetic L, which was used as an adjustment synthetic; synthetic M was analyzed as an unknown, with an average absolute error of 0.5%. Had results from both synthetics been used in the adjustment process, no perfect match would have been obtained, but the errors would have been halved.

Table IV. Adjusted Matrix

A.	Ethylbenzene	1,2-Xylene	1,3-Xylene	1,4-Xylene	Syn. L	Syn. M
2613	0.65860	0.72609	0.61579	0.86273	1.59427	1.59445
2709	0.10530	0.61490	0.41721	0.65728	1.00000	1.00000
2723	0.052918	0.43774	0.83450	0.53026	1.03323	1.05075
2742	0.024861	0.15654	0.37640	1.84380	1.37168	1.34030

Table V. Results

Compound	Synthetic L		Synthetic M	
	Found	Known	Found	Known
Ethylbenzene	24.87	24.87	24.59	23.94
1,2-Xylene	24.23	24.23	24.25	24.67
1,3-Xylene	24.90	24.90	26.38	26.92
1,4-Xylene	26.00	26.00	24.79	24.47

The ratio method can be summarized symbolically, using matrix algebra notation. Let a matrix be expressed by A, a column in that matrix by $\{A_i\}$, and a row by $[A_i]$.

Let a matrix be formed with elements in the rows corresponding to optical densities at analytical wave lengths, and elements in the columns corresponding to optical densities for components. In the raw matrix and in the final matrix, the internal relationships of the column elements remain unchanged:

$$\left[\frac{A_i}{A_j}\right]_{\text{raw}} = \left[\frac{A_i}{A_j}\right]_{\text{final}}$$

Let a proportionality constant, K_i, be defined as

$$K_i = f_{fi}/f_{kni}$$

where f_{fi} = fraction of component i found from normalization of answers from solution of raw matrix and f_{kni} = known fraction of component i. Then the final matrix is related to the raw matrix by the relationship

$$\{A_i\}_{\text{final}} = K_i \{A_i\}_{\text{raw}}$$

APPLICABILITY OF THE RATIO METHOD

The criteria which are used to judge whether or not a given analysis can be done by the ratio method are general and, for the most part, apply equally to any spectrophotometric multicomponent analysis.

Obviously, the first requirement is that the system to be analyzed absorb radiation. The ratio method, however, requires that all components obey Beer's law at least to within experimental error, while other methods can be set up which require only that components exhibit reproducible behavior.

Secondly, absorption curve shapes of the components to be determined must show mutual differences sufficient to allow the desired precision in results.

Thirdly, the general level of light absorption of the various components at their analytical wave lengths should be as nearly alike as possible.

These last two criteria are matters of choice and of degree; they can be abused or ignored to the extent that the recipient of the results is willing to accept lower precision. Obviously, if two components have nearly identical absorption curves, no measurement depending on differences between the curves is going to differentiate between these components. If the uncertainty of a measurement is a substantial fraction of the total absorption of one of the components, that component can be determined only with very poor precision.

ERROR *vs.* COMPONENT DISTRIBUTION

It is a general characteristic of any measurement that for a given absolute error, the relative error increases inversely as the magnitude of the measurement. It follows that relative errors increase as the region near zero is approached.

At times, for one reason or another, such as poor leverage or relatively weak absorption of the component considered, an analysis will fail when a component approaches zero concentration, or will show very poor results. Specific application of more general iteration methods can be brought to bear on such a situation. The first analysis of the unknown can be taken merely as an indication of component distribution. If one component seems near zero, then the analyst can add a weighed amount of that component to the unknown and reanalyze.

The addition has put the concentration range back in a region of much better precision, and the new results can be calculated back to the original unknown. The first results will have the usual absolute errors multiplied by the dilution factor used, the relative error will still be high for the low component, but the absolute answers will be much nearer the truth.

Two xylene synthetics, A+ and B+, shown in Table VI, were analyzed in this fashion, illustrating the results of intentional alteration of the sample, reanalysis, and recalculation. Components present at 0% were found to be zero or negative (negative results are taken as zero), and the average absolute error of 1.4% for A and B was reduced to 0.5% for A+ and B+.

It has been preferred in this approach to assume that components in general obey Beer's law, unless there is good reason to think otherwise—e.g., suspected chemical reaction or association in the mixture. Special cases, such as mixtures in which some concentrations approach zero with known large resulting errors, are treated as such. This is in contrast to systems in which all unknowns are treated as special cases, and the routine treatment is to employ some form of iteration method to arrive at the final answer (*3*, *5*).

Table VI. Xylene Analysis

(Negative results recorded as zero, positive results normalized)

Syn.	*o*-Xylene		*m*-Xylene		*p*-Xylene	
	Found	Known	Found	Known	Found	Known
A	61.4	63.5	1.8	0.0	36.8	36.5
A+	62.8	63.5	0.0	0.0	37.2	36.5
B	43.7	42.4	55.6	57.6	0.7	0.0
B+	43.0	42.4	57.0	57.6	0.0	0.0
C	62.1	63.0	18.3	17.7	19.7	19.3
D	42.8	43.0	42.0	43.1	15.2	13.8
E	9.5	8.2	8.2	8.0	82.3	83.7
F	0.9	0.0	27.4	26.5	71.7	73.5
G	15.1	14.3	65.8	66.3	19.1	19.5

Average relative error 3.6%
Average absolute error 1.05%
Slit width 0.05 mm.

OPTICAL DENSITY RANGE USED

If a variety of circumstances exists under which some physical measurement can be obtained, the measurement is always made where experimental conditions are most favorable to accuracy and precision. It has been pointed out (*1*, *2*) that photometric error is least when the transmission is 37%—that is, when the optical density is 0.43. From the standpoint of accuracy, it has been noted (*6*) that measurements of optical density are consistently too low at high density values, and too high at density values less than 0.4. Extensive observations in this laboratory confirm these reports.

Therefore, all measurements of optical density fall between 0.4 and 1.0, the concentration of the measured substance being ad-

Table VII. Raw Data for Ethylbenzene

(10 × optical densities)

2613 A.	2695 A.	2709 A.	2723 A.	2742 A.
10.32	4.42	....	....	....
10.58	4.45	....	....	....
10.08	4.28	....	....	....
....	10.82	3.98	....	....
....	11.00	4.26	....	....
....	10.94	4.10	....	....
....	....	10.36	5.22	2.58
....	....	12.54	6.25	2.76
....	....	11.08	5.60	2.65
		Calculation of Ratios		
1.00000	0.42829	....	....	....
1.00000	0.42060	....	....	....
1.00000	0.42460	....	....	....
1.00000	0.42450	....	....	....
....	0.42450	0.15615	....	.....
....	0.42450	0.16440	....	....
....	0.42450	0.15909	....	....
....	0.42450	0.15988	....	....
....	....	0.15988	0.080557	0.039816
....	....	0.15988	0.079685	0.035189
....	....	0.15988	0.080306	0.038238
....	....	0.15988	0.080349	0.037748
1.00000	....	0.15988	0.080349	0.037748

justed to achieve this. Below 0.4, the measurements are subject to excessive uncertainty; above 1.0 they tend to become too low. The variation in absolute extinction coefficient is less than 1% between 0.4 and 1.0, so that a factor approaching 2.5 is usually available to the operator in adjusting the concentration of the substance being measured.

MEASUREMENTS ON SAMPLES HAVING RATIOS GREATER THAN 2.5

If one of the pure components absorbs at one or two of the analytical wave lengths, but not appreciably at the others, no adjustment of concentration will suffice to fix the optical densities for all wave lengths within the required optical density range, 0.4 to 1.0, for a given concentration of the component.

In this case, some wave length is chosen, preferably not on the side of an absorption peak, such that it can be measured first with one of the analytical wave lengths and subsequently with the other or others. In this way, a quantitative relationship can be established between the densities at the analytical wave lengths.

Table VIII. Diethylbenzenes and *sec*-Butylbenzene

(Cases where mixtures contain *o*- and *m*-DEB and SBB)

o- plus *m*-DEB		*p*-DEB		SBB	
Found	Known	Found	Known	Found	Known
47.4	46.4	30.6	30.0	22.0	23.6
54.6	52.9	29.7	29.3	15.7	17.8
39.1	39.3	23.6	23.6	37.4	37.2
37.5	39.0	30.1	30.4	32.4	30.6
51.5	52.7	15.8	17.0	32.8	30.4
51.4	51.9	35.9	35.7	12.7	12.4
45.5	45.6	9.1	9.0	45.3	45.4
60.7	60.2	0.0	0.0	39.3	39.9

Average relative error 2.6%
Average absolute error 0.8%
Slit width 0.18 mm.

Ethylbenzene exhibits such behavior at the wave lengths 2613, 2709, 2723, and 2742 A. Therefore, three separate sets of measurements were made on ethylbenzene, as shown in Table VII. The wave length 2695 was chosen as an intermediate, to which the values both for 2613, and for 2709, 2723, and for 2742 could be related. The result is that an accurate relationship is established between the optical density for 2613, and that for 2742, even though ethylbenzene absorbs only 3.8% as strongly at the latter wave length as at the former.

EXPERIMENTAL

Spectrophotometer. The Cary recording spectrophotometer was used for all work referred to in this report.

Light Source. Both a hydrogen lamp, which came with the instrument, and a modified G.E. AH mercury lamp, obtainable with mounting attachments from the Applied Physics Corporation, have been used in the course of this work. The mercury source is preferred in this laboratory because its emissivity is higher than the hydrogen lamp throughout the ultraviolet; it supplies occasional emission lines over the continuum which can be used as precise wave-length markers; it has a rated life of several thousand hours, which exceeds the rated life (200 hours) of the hydrogen discharge lamp; and it is no more expensive than the hydrogen discharge lamp.

Slit Widths. The slit width in the Cary instrument ranges from 0 to 3 mm. The order of magnitude of slit width is controlled by a servomechanism acting to hold constant the light flux on the standard photocell, and by a manual control of smaller range.

General practice is to use as narrow a slit as possible. With a mercury arc as source, the Cary optics allow a customary half-intensity band width of 1 to 2 A.

Absorption Cells. There is no inherent preference, relative to the ratio method, for a given length of cell. However, experimental data have indicated that measurements are more reproducible for a shorter cell length. Separate data also indicate that the optimum place for the cell is in the center of the absorption cell compartment, where the diameter of the light beam is at a minimum.

Solvent. *n*-Heptane, purified by adsorption through silica gel, was used as solvent for all analyses discussed. Purification was considered adequate when the solvent matched the transmission of distilled water at and above 2400 A.

Pure Components. Hydrocarbons used for analyses cited in this report were obtained from Phillips Petroleum Company (Research grade), the National Bureau of Standards, or the American Petroleum Institute.

Table IX. Benzene-Toluene Analysis

Benzene		Toluene	
Known	Found	Known	Found
89.2	89.4	10.8	10.6
44.8	45.7	55.2	54.3
5.8	5.7	94.2	94.3
15.1	14.9	84.9	85.1

Average relative error 0.67%
Average absolute error 0.38%
Slit width 0.03 mm.

At times, it is necessary to use as standards chemicals which are known to contain impurities. The nature of such impurities should be as well known as possible, for they may exhibit much higher absorptivity than the standard and therefore introduce large errors. For the same reason, unknowns to be analyzed should contain only components included in the calibration. If other components are known to be present, the effects of their presence should be thoroughly investigated.

SOME APPLICATIONS OF RATIO METHOD

The ratio method has been used to analyze mixtures of the xylene isomers (Table VI), of the diethylbenzene isomers and *sec*-butylbenzene (Table VIII), and of benzene and toluene (Table IX). The ultraviolet absorption spectra of the *o*- and *m*-diethylbenzene isomers are practically identical, so that they were treated as a unit in the analysis, with good success (2.7% average relative error), when both ortho and meta isomers were present. If the two were treated as individuals, the precision of the analysis suffered badly, rising to an average relative error for all synthetics of 16%. All diethylbenzene–*sec*-butylbenzene samples were checked qualitatively in the infrared if necessary, for the presence of all components, because synthetics showed that the total absence of either the ortho or the meta isomer led to high error. Finally, mixtures in which the *sec*-butylbenzene approached zero could not be analyzed with any accuracy by the usual straightforward analysis; such mixtures were treated by adding *sec*-butylbenzene in known quantity, reanalyzing, and calculating back, in the manner previously discussed.

Since this paper was first presented, one of the authors has set

up the diethylbenzene–*sec*-butylbenzene analysis in the infrared, with an average absolute error of 0.5% for all components. The infrared is thus the region to be preferred for this analysis.

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Infrared Analysis of Certain C_1–C_4 Hydrocarbon Mixtures

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Infrared analytical methods are developed for the direct determination of small amounts of ethane, propane, *n*-butane, and isobutane in natural and purified methane streams. Accuracies of ±0.1% are obtained with the permanent gas absorption cell of the Beckman IR-2 spectrophotometer. An additional comparison cell set permits the direct determination of methane to within ±0.5%. The methods have proved satisfactory in routine application for the past 2 years.

IT HAS been necessary to provide accurate, rapid, routine determinations of very small amounts of ethane, propane, *n*-butane, and isobutane in methane streams both direct from the natural gas field and after continuous purification. Although the mutual interference situation suggested difficulties in the development of infrared methods for the analysis of such mixtures, the experience of others (*4*) in related problems encouraged sufficient exploratory work with a Perkin-Elmer 12-B infrared spectrophotometer to prove that it is possible to determine the above contaminants in methane to within ±0.1%. On the basis of the preliminary results, a Beckman IR-2 spectrophotometer was specially adapted and calibrated and has performed satisfactorily in daily routine operation for the past 2 years.

Success in this application of the methods of infrared analysis was possible because the compositions of normal "before and after" purification streams are such as to require few large interference corrections. Good instrumental stability and careful calibration and operation permitted this favorable circumstance to be exploited to obtain high accuracy. It was expected and found that the composition of natural gas direct from the field varied only slightly day to day—the average methane stream containing about 4% ethane, 1.5% propane, 0.3% *n*-butane, and 0.3% isobutane (plus pentanes). The purification process preferentially removes the contaminants of higher molecular weight, leaving only traces of propane and small amounts of ethane. From the standpoint of the subsequent chemical processes propane is the critical component. Reference to Table I shows considerable *n*-butane interference at both useful propane peaks; however, the concentration of *n*-butane is never more than a fraction of the propane content and can be most accurately determined, permitting appropriate correction of the propane data. Mutual interference was not a serious problem in the case of any of the other contaminants, but the high relative concentration of methane necessitated particular care in the determination of its contribution at all analytical wave lengths.

Except for methane, the analytical methods were developed in the following way:

From the 2.5 to 15μ absorption spectra of the several components characteristic analytical wave lengths were selected and a program of appropriate slit widths and background filters was set up to permit straightforward determination of each component from separate samples loaded into the permanent (24.8-cm.) Beckman gas cell at a working pressure of 740 mm. Accurate calibration data were obtained by expanding small, known, high pressure volumes of the pure components into the evacuated absorption cell. In the case of ethane, the pressure-broadening effect was empirically taken into account by externally preparing mixtures of ethane with nitrogen. Data were recorded in per cent transmittance, corrected for the effects of stray radiation, and calculated to absorbances which were then plotted against concentrations to give calibration charts permitting the direct graphical analysis of similarly handled unknowns by the method of successive approximations—the components being determined in the order methane, *n*-butane, isobutane, ethane, and propane.

The determination of methane was handled differently. In this case the usual difficulties of determining the predominating component of a mixture were further complicated by the high intensity of the 7.646μ methane peak, its "sharpness," and its marked pressure broadening (*2*). In such a situation all experimental variables involved in single-cell methods become critical. In practice it has usually been satisfactory to obtain the methane concentration by difference, but it has also been possible to construct a comparison cell set—fitting into the liquid cell compartment of the Beckman IR-2 spectrophotometer—which permits the direct determination of methane to within ±0.5%.

Table I. Equivalent Parts Interference Data

	Propane	Ethane	*n*-Butane	Isobutane	Methane
Propane at 13.345μ	1	28.3	1.12	73.4	607
Ethane at 12.260μ	22.6	1	13.8	8.8	146
n-Butane at 10.305μ	50.5	1170	1	57.5	2520
Isobutane at 8.490μ	22.5	288	51.1	1	186
Methane at 7.646μ	39.7	61.9	9.7	20.7	1
Propane at 9.345μ	1	240	11.0	17.2	397

APPARATUS AND MATERIALS

The long wave-length infrared absorption peaks which distinguish ethane, propane, *n*-butane, and isobutane from each other and from methane are not of inherently high absorptivity nor are they entirely free of serious mutual interference. To reach analytical accuracies of the order of ±0.1% with commercially available spectrophotometers requires that a long path length and high operating pressure be combined with precise meas-

urements and suitable means for the application of interference corrections.

The original work on the system was carried out in the 1-meter absorption cell of a Perkin-Elmer 12-B spectrophotometer. The methods were later adapted to make most efficient use of the greater electronic and thermal stability, operating speed, and accuracy of the Beckman IR-2 spectrophotometer. In the latter instrument the longest available path length is that of the permanent cell, which is an integral part of the optical system (24.8 cm.). The other gains more than compensated for the ~fourfold loss in sensitivity due to the shorter path length and except for the addition of a set of comparison cells for the determination of methane and the mounting of hairlined magnifying lenses over the per cent transmittance and wave-length scales to eliminate parallax and obtain greater reading accuracy, no changes in the commercially available equipment were found necessary. The wave-length scale was checked against the known spectra of carbon dioxide, ammonia, and water vapor.

The component gases were obtained from the Matheson Company. Their 2.5 to 15μ spectra were compared with the spectra of high purity standards (*1*). No identifiable contamination was detected in the stocks of *n*-butane and isobutane, claimed to be of ~99 mole % purity. One of two tanks of propane contained at least the 0.2 mole % limit of ethane and isobutane. A trace of carbon dioxide was noted in the methane, and strong ethylene absorption was observed at 10.5μ in the ~95 mole % ethane. This impurity was removed by a bromine water–sodium bisulfite–phosphorus pentoxide absorption train. With the exceptions noted above, the gas stocks of contaminants were assumed to be pure, because at the very low concentrations used the effects of small traces of undetected impurities would be negligible.

ANALYTICAL METHODS AND THEIR CALIBRATION

Expressed in the recommended (*3*) terminology, the general law of optical absorption states that the radiant power, P, of a monochromatic infrared beam leaving a sample of concentration c and path length b is related to the radiant power, P_o, incident upon the sample in the following way.

$P = P_o e^{-kbc} = P_o 10^{-abc}$ where k is the familiar extinction coefficient and a is a molecular constant called the absorptivity. The fraction of the incident radiant power which is transmitted by the sample—called t, the transmittance—is equal to P/P_o. For analytical work it is most convenient to handle the latter relationship in terms of absorbance, A, defined as $A = \log_{10} \frac{1}{t} = abc$. It is apparent that at constant path length, b, the absorptivity, a, for any single absorber at any single wave length is the slope of a plot of observed absorbances, A, versus known concentrations, c. Such measurements make up the necessary calibration data for this system.

Selection of Analytical Wave Lengths. Characteristic spectra of the components indicated the following analytical wave lengths to be both relatively free from interference and sufficiently intense to permit high accuracy of determination at a fixed level of instrumental sensitivity. Little interfering absorption was found at the strong peaks 7.646μ for methane, 12.260μ for ethane, 10.305μ for *n*-butane, and 8.490μ for isobutane. In the case of propane, serious *n*-butane interference was noted at both the strongest useful peak at 13.345μ and the alternative 9.345μ wave length. Fortunately, the composition of the natural gas from the field and of the purified stream is such that the *n*-butane content is never more than a fraction of the propane present. There is practically no interference at the sensitive *n*-butane position; therefore its concentration can be accurately determined and appropriate corrections applied to observed absorbances at the two propane peaks. Because the routine analytical application of the methods has normally been concerned with the purified gas stream which contains even lower concentrations of C_5 hydrocarbons than of C_4's, no attempt has been made to set up calibration curves for the independent determination of *n*- and isopentane. These components, when present, are almost quantitatively determined as (apparent) isobutane because of their comparable absorption at 8.49μ. All other nonidentified interfering contaminants are assumed to be absent.

Table II. Operating Conditions

Component	Wave Length, μ	Slit Width,[a] Mm.	Shutter	Stray Radiation, %	Path Length, Cm.[b]
Propane	13.345	~0.920	Glass	1.24	24.8
Ethane	12.260	~0.659	Glass	0.94	24.8
n-Butane	10.305	~0.408	Glass	0.70	24.8
Propane	9.345	~0.330	Glass	0.71	24.8
Isobutane	8.490	~0.255	Glass	0.72	24.8
Methane	7.646	~0.340	Solid	..	1.00

[a] In routine operation corresponding vernier scale settings were used.
[b] Real optical path length of permanent cell of Beckman IR-2 spectrophotometer used was found to be only 24.8 cm.

Operating Conditions. A set of slit widths and amplifier gains was selected to afford a satisfactory compromise between the requirement that the slits be sufficiently small to prevent departure from linearity of the absorbance versus concentration calibration curves on the one hand and the desirability of operating at nearly constant gain levels low enough to minimize troublesome instru-

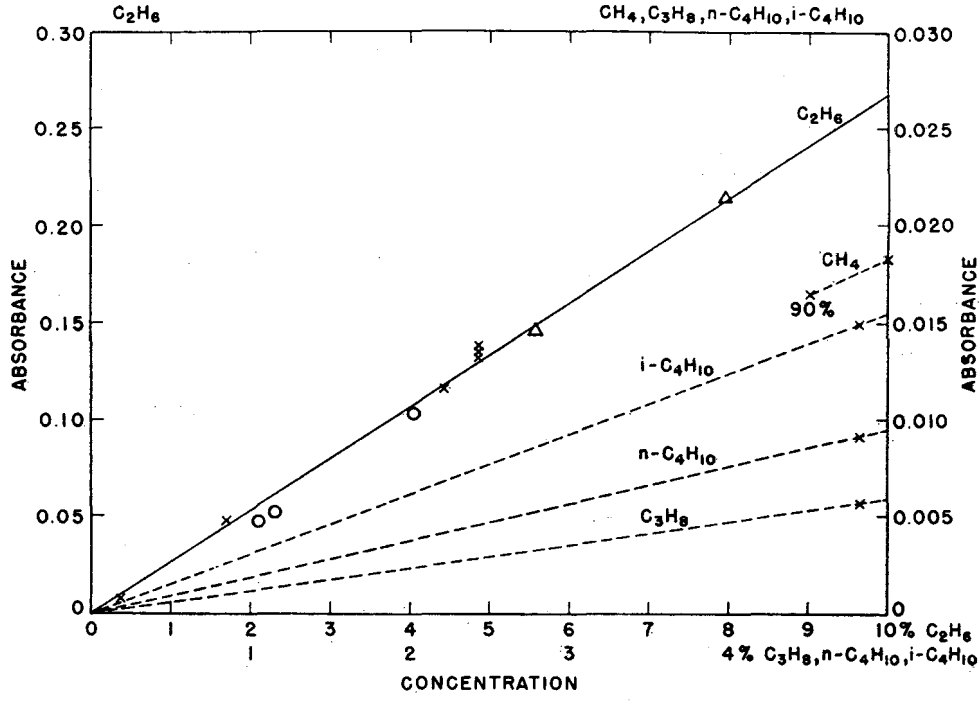

Figure 1. Working Curves for Ethane at 12.260μ

× Direct calibration points with N_2
△ Carbon equivalent (combustion) mixtures
○ Mass spectrometer mixtures

mental noise on the other. That the slits listed in Table II satisfied the first condition was proved by direct check of linearity at double their widths and it was found that the random noise vibration of the null meter at the 2-second period did not seriously hamper the per cent transmittance readings made at about one-half full gain setting (~"5").

Employing the glass shutter at all wave lengths except 7.646μ, the minimized effects of stray radiation were directly determined at 8.490, 10.305, and 13.345μ by the approximate method of Nielsen and Smith (*5*) using the respective gases as complete absorbers. The permanent cell path length was not sufficient to cause complete absorption of propane at 9.345μ nor of ethane at 12.260μ. Rough stray radiation corrections at the latter positions were satisfactorily interpolated from measurements made at comparable slit widths at bracketing wave lengths. Table II summarizes the operating conditions.

Preparation of Calibration Mixtures. It was troublesome to measure accurately small pressures of the contaminant gases directly into the absorption cell for calibration work. This difficulty can be circumvented by externally preparing mixtures with nitrogen in a simplified train with provision for adequate mechanical mixing. Pressure-broadening effects were empirically taken into account by methane and ethane mixtures with nitrogen prepared in this way. For the other components it was found both handy and adequate to accomplish the same end by expanding very small known volumes of each gas directly into the much larger known volume of the absorption cell. In this way it was possible to work with the calibration gases in the sample bulbs at high pressures (corrected for departures from ideality) with minimum relative pressure reading errors and, by the expansion process, rapidly and certainly to obtain satisfactory, homogeneous, low concentration, calibration mixtures for all components showing no pressure broadening.

Calibration Procedure. The Beckman IR-2 spectrophotometer is designed to measure the transmittance of the sample, $t = \frac{P_s}{P_o}$, directly as per cent transmittance, $T = t \times 100$, by comparing the radiant power transmitted by the absorption cell plus contents, P_s, to that passed by the evacuated cell, P_c, under identical conditions. Schematically

$$P_o \longrightarrow \text{(evacuated cell)} \longrightarrow P_c \longrightarrow \text{[sample]} \longrightarrow P_s$$

The experimental measurement is made in the usual way: With the appropriate setting of wave length and slit for any analytical peak, one first checks the null meter to read zero with the beam cut off by a solid shutter, then with the evacuated cell in the beam path the potentiometer is set to 100% transmittance—"Check"—by slight adjustment of the gain level. The calibration (or unknown) sample is loaded into the cell to the required pressure and its per cent transmittance is read directly from the "Read" scale attached to the potentiometer slide-wire indicating a position of exact restoration of null balance. Single per cent transmittance readings made in this way normally deviate from their average by no more than ±0.05%. The average of several observations is finally corrected for stray radiation and calculated to effective absorbance as indicated below.

$$A = \log_{10} \frac{100 - \text{stray radiation}}{T - \text{stray radiation}}$$

Contaminant Calibration Data. For all contaminants except ethane, calibration points at several concentrations showed no systematic departure from linearity and very little experimental scatter—thus leading to reasonable confidence in the single-point observations made in cases of minor interference. Figure 1, showing the ethane calibration data discussed below, is an example of the form in which the calibration data proved most useful. Here are plotted the absorbances of ethane and of each of the other components at the ethane analytical wave length. From the similarly obtained absorbance of an unknown and knowledge of approximate concentrations of the interfering components their corrections can be determined and applied to give an absorbance which directly indicates the concentration of ethane in the sample.

For the sake of brevity in presentation all the contaminant calibration data have been calculated to absorptivities = absorbance per centimeter path length per unit mole per cent (see Table III). The respective absorptivities $\times 10^5$ obtained directly from the linear, low concentration calibration charts are given a relative representation in the equivalent parts listings of Table I. In the cases of methane and ethane, only empirical account was taken of the effect of pressure broadening, so that it is necessary to add the qualification that all data were obtained at total effective pressures of 740 mm.

Table III. Absorptivities $\times 10^5$

	Propane	Ethane	*n*-Butane	Isobutane	Methane
Propane at 13.345μ	115.2	4.07	102.9	1.57	0.19
Ethane at 12.260μ	4.77	108.0	7.83	12.30	0.74
n-Butane at 10.305μ	3.49	0.15	176.0	3.06	0.07
Isobutane at 8.490μ	13.10	1.02	5.79	294.0	1.59
Methane at 7.646μ	13.50	8.67	55.30	25.85	536.0
Propane at 9.345μ	43.65	0.18	3.97	2.55	0.11

Methane Calibration. It has usually been satisfactory to estimate methane by difference. Its concentration can be determined in the usual way at sufficiently low pressures or high dilution in the permanent cell or at high pressures in a cell of shorter path length; however, the resulting accuracy has been found to depend primarily upon the precision of the handling steps rather than upon the limitations of the measuring instrument. To circumvent the experimental uncertainties, a set of comparison cells was designed and calibrated. The determination was made both simple—by shortening the path length to the point where normal interference from other components could no longer be detected—and accurate by reference to a methane standard at the same pressure.

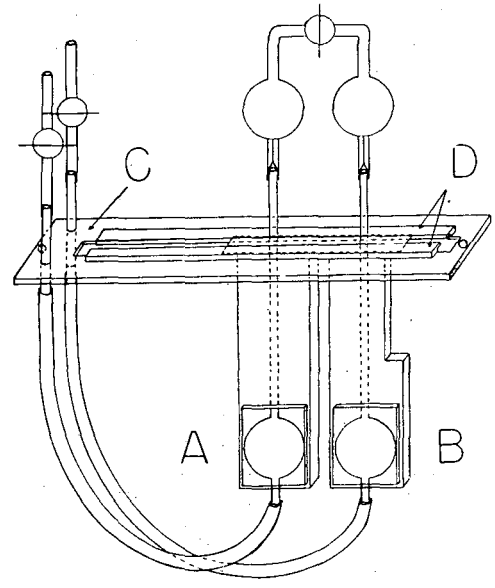

Figure 2. Methane Comparison Cell Set

A. Sample cell
B. Comparison cell
C. Mounting cover fitting liquid cell compartment of Beckman IR-2 spectrophotometer
D. Ways guiding sliding cell set

The device (Figure 2) consists of a pair of 1-cm. cells mounted to be positioned in the liquid cell compartment. Each cell has a gas inlet port and is connected to the other by a short line containing a stopcock. One cell is loaded to ~740 mm. with pure methane; the other contains the sample at ~740 mm. By momentary opening of the connecting stopcock any small pressure difference is equalized without appreciable transfer of gas into either absorbing path.

With the filled comparison cell in the beam, "Check" (100% transmittance) slits are obtained by gain adjustment. Changing nothing but the cell positioned in the beam, one records the per cent transmittance of the sample. Calibration data were found to be relatively insensitive to the exact pressure within the range

Table IV. Check Analyses

Components	Sample 1, %		Sample 2, %		Sample 3, %		Sample 4, %		Sample 5, %		Sample 6, %	
	MS	IR	MS	IR	MS	IR	MS	IR	MS	IR	MS	IR
CH_4	96.2	96.6	95.9	96.6	93.0	92.4	89.2	...	93.4	...	95.5	...
C_2H_6	2.1	1.83	2.3	2.00	4.0	3.83	6.5	6.4	4.3	4.0	2.2	1.8
C_3H_8	0.05	−0.07	0.4	0.37	1.0	1.02	2.2	2.4	1.2	1.3	0.8	0.6
n-C_4H_{10}	0.03	0.00	0.3	0.38	0.2	0.25	0.6	0.6	0.4	0.3	0.5	0.5
i-C_4H_{10} + C_5's	0.0	−0.02	0.2	0.24	0.2	0.21	0.4	0.4	0.1	0.3	0.4	0.3

740 ± 10 mm. because the critical pressure broadening of the methane contents cancels out, leaving only the difference in methane concentrations to be determined.

ANALYSIS OF TEST MIXTURES

Several typical natural gas mixtures were analyzed by this method and by mass spectrometer. A discussion of their handling and of the results obtained will serve to illustrate the application of the method and to permit an estimate of the absolute accuracy and reliability of the calibration data.

Experimentally the analysis of an unknown mixture is carried out in exactly the same way as is the determination of the absorbance of any calibration mixture—the permanent cell being reloaded for independent per cent transmittance measurements at each analytical wave length, methane being determined in the manner detailed above.

Ideally each component of an unknown mixture absorbs independently of the others and because each component will normally be the chief absorber at its characteristic wave length and all calibration and unknown mixtures are handled at the same pressure and path length, component concentrations can be readily calculated from the absorbance data by the method of successive graphical approximations. For example, at the 13.345μ propane peak,

$$A_{C_3H_8} = A_{mix.} - A_{CH_4} - A_{C_2H_6} - A_{n\text{-}C_4H_{10}} - A_{i\text{-}C_4H_{10}}$$

$$a'_{C_3H_8}C_{C_3H_8} = A_{mix.} - a'_{CH_4}C_{CH_4} - a'_{C_2H_6}C_{C_2H_6} - a'_{n\text{-}C_4H_{10}}C_{n\text{-}C_4H_{10}} - a'_{i\text{-}C_4H_{10}}C_{i\text{-}C_4H_{10}}$$

where the a' values—the respective "calibration coefficients" measured at 13.345μ at 740 mm. in the permanent cell ($a' = a \times 24.8$)—are known and approximate knowledge of the concentration of each interfering component is available to permit its contribution to be subtracted from that of the mixture. The concentration of propane is then obtained directly from the calibration plot at 13.345μ.

From the similar relations holding at each of the other peaks the approximation treatment proceeds through the following steps: first, a determination of the concentration of methane from its direct calibration data, then appropriate correction for methane interference made at each peak, starting with n-butane and picking up additional approximate interference corrections for each component as determined in the order n-butane, isobutane, ethane, and finally propane. A second series of similar graphical calculations, using in each case the above preliminary concentration values for each interfering component, permits sufficient accuracy to make a third approximation step unnecessary. The process is simple and takes only a few minutes at most.

In Table IV the results of typical routine infrared analysis of a half dozen representative hydrocarbon samples are compared with mass spectrometer data thought to be accurate to within ±0.5% for methane and ethane and ±0.1% for propane, n-butane, and isobutane plus C_5's. The first three are laboratory samples used to test the original calibration work; the others were obtained as checks at intervals during the routine operation of the equipment.

DISCUSSION

In general, the two independent analytical methods appear to give closely comparable results, except for the case of ethane, where the mass spectrometer values are systematically higher. On an absolute basis the calibration data are felt to be at least adequate to reach accuracies of ±0.1% in the determination of propane, n-butane, and isobutane. Routine experimental uncertainties have been shown not to cause variations greater than ±0.02% for these components and there appears to be little likelihood of serious undetected interference; therefore relative infrared results are thought to be satisfactory to limits of ±0.05%. Experimentally the direct comparison cell infrared determination of methane should be accurate to within ±0.2% methane on a relative basis. Because the absolute standard for this work was a methane supply found by mass spectrometer analysis to contain ~1.5% nonhydrocarbon impurities, there is a greater uncertainty in the absolute values.

The infrared results for small concentrations of ethane are consistently lower than the mass spectrometer values. Laboratory mixtures 1, 2, and 3 were made up from the same stocks used in the calibration work and it is unlikely that unidentified contamination is responsible for the higher mass spectrometer results. The infrared calibration data provided by direct external mixtures of ethane with nitrogen and with methane and by similar mixtures for which independent combustion (carbon equivalent) analyses were available (Figure 1) must and does adequately satisfy the linear absorption law after straightforward corrections are made for stray radiation and pressure-broadening effects. If the mass spectrometer points are correct, an empirical calibration curve would have to depart considerably from linearity in the region close to the origin where any real effects are known to be at least significant. It would appear either that the ±0.5% limits of accuracy for mass spectrometer measurements of ethane are consistently positive in the low concentration range or that ethane is not completely absent from the methane used in calibrating its significant interference at 12.260μ. This discrepancy is unimportant in the application for which the methods were developed. Unfortunately, it is within the limits of uncertainty of alternative independent analytical methods. Relative ethane determinations by infrared absorption have not varied more than ±0.05% owing to purely experimental factors; therefore it does not seem unreasonable to assume that on an absolute basis the values approach error limits of ±0.1% of the total sample.

ACKNOWLEDGMENT

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Analysis of Sodium Carboxymethylcellulose

Copper Salt Precipitation Method for Determining Assay Value and Degree of Substitution

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A method has been developed for determining the assay value and degree of substitution of sodium carboxymethylcellulose, also known as cellulose gum and CMC, and by other trade names. The copper salt of carboxymethylcellulose is precipitated, weighed, and analyzed for copper content iodometrically. From the weight of the precipitate and the copper content the assay value—i.e., the sodium carboxymethylcellulose content—and the degree of substitution may be readily calculated. The method is primarily applicable to purified grades of sodium carboxymethylcellulose over the commonly encountered ranges of viscosity and degree of substitution (0.6 to 1.2). The salt impurities most frequently present, with the exception of phosphates, do not interfere. The method has been applied to a number of purified-grade commercial samples with an average recovery of 98% or better, and with a degree of substitution accuracy of within 0.03 unit.

IN RECENT years the extensive development of new and broader fields of application for sodium carboxymethylcellulose, also known as cellulose gum and CMC, and by other trade names (*6, 7*), has made increasingly evident the need for suitable methods of analyzing this material. The entrance of a number of manufacturers into this field and their subsequent marketing of a wide variety of types and grades of sodium carboxymethylcellulose have, however, complicated the development of analytical methods to the extent that little on this subject has appeared in the literature.

Problems involving the determination of various salt impurities in sodium carboxymethylcellulose have been readily solved by the use of more or less standard procedures modified for application to cellulosic materials. Despite the fact that little or none of this work has been published, because of its general nature it need not be emphasized until a greater standardization of such methods is required.

The physical and physicochemical properties of sodium carboxymethylcellulose have been described (*1, 13*). Considerable attention has likewise been directed toward the development of methods for the determination of the degree of substitution—i.e., the average number of sodium carboxymethyl groups substituted per anhydroglucose unit (*1, 2, 4, 9, 11*). Methods for determining the metal content of the sodium, potassium, aluminum, and zinc salts of carboxymethylcellulose have also been devised (*12*), as has a procedure by which paper may be analyzed for its sodium carboxymethylcellulose content (*3*). However, to the authors' knowledge, no method for determining the assay value—i.e., the sodium carboxymethylcellulose content—of various types and grades of sodium carboxymethylcellulose has been published. Currently, a considerable variety of assay methods are in use in the trade. Although each of these methods can be applied with reasonable accuracy to certain types of sodium carboxymethylcellulose, none of the methods which have come to the authors' attention is generally applicable to the variety of commercial products now on the market. Thus, it is in the interest of stimulating further work in this field, and in an attempt to encourage standardization of methods of sodium carboxymethylcellulose analysis, that one of the more versatile methods developed in this laboratory for determining the assay value and degree of substitution of sodium carboxymethylcellulose is presented in this paper.

Several investigators (*1, 2, 6, 13*) have reported that a number of the metallic salts of carboxymethylcellulose are essentially insoluble in water. These include the salts of copper, lead, aluminum, silver, mercury, and zirconium. Chowdhury (*2*), Brown and Houghton (*1*), and others have suggested determining the degree of substitution of carboxymethylcellulose by precipitation of one of the insoluble metallic salts, and subsequent analysis of the precipitate to determine its metal content. However, some of these insoluble metallic salts do not contain a theoretical amount of metal and would thus be of little value for analytical purposes. On the other hand, it has been found that the copper salt of carboxymethylcellulose contains very close to the theoretical amount of copper—i.e., one mole per two carboxyl groups substituted. Reid and Daul (*9, 10*) have employed this principle in the determination of the degree of substitution of the carboxymethylcellulose resulting from the carboxymethylation of cotton.

The emphasis in all these cases has been on determining the degree of substitution. It is apparent that, if the copper salt is precipitated in a quantitative manner, the method may also be used to determine the amount of sodium carboxymethylcellulose present in a given commercial sample. The method described is based on this principle.

DEVELOPMENT OF THE METHOD

Initially, it was thought that a satisfactory approach might lie in precipitating the copper salt of carboxymethylcellulose by simply mixing aqueous solutions of sodium carboxymethylcellulose and an inorganic copper salt under essentially neutral conditions. The resulting precipitate could then be filtered off, washed free of excess copper solution, dried, and weighed. The copper content of this precipitate could be determined by any one of a number of standard techniques—e.g., iodometrically, by electrodeposition, or by ignition.

As the preliminary work progressed, it became increasingly evident that such a procedure would not produce a precipitate of satisfactory physical properties. In an effort to prevent the precipitation of the copper carboxymethylcellulose as a stringy, fibrous, nonuniform mass, a number of factors affecting the precipitation were studied: effects of salt impurities, sample size, concentration of precipitating solutions, and agitation. In the course of this work, several precipitates of copper carboxymethylcellulose, derived from a purified commercial material of known degree of substitution, were analyzed for copper content by the volumetric method of Hoffpauir and O'Connor (*5*). The data in Table I show that the precipitates contained very close to the theoretical amount of copper as calculated from the known degree of substitution. The method thus appeared to be suitable as a means of determining degree of substitution, but in order to

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serve as an assay method, it was essential that the physical form of the precipitate be improved. Toward this end, experimental factors such as pH during precipitation, variation of precipitating media, and precipitate washing techniques were considered.

The ultimate result of these investigations was a procedure consisting of (1) introducing an aqueous solution of the sample into a well agitated precipitating mixture of 1% cupric sulfate pentahydrate solution and methanol under acid conditions (pH 2 to 3), (2) adjusting the pH upward with dilute ammonium hydroxide to a value of 4.0 to 4.1 to obtain essentially complete precipitation of the copper carboxymethylcellulose, and (3) filtering the precipitate, washing with methanol-water solution, then with anhydrous methanol, and drying to constant weight. Excellent precipitates, light, fluffy, and uniform, were obtained in this way along with an appreciable increase in recovery.

The volumetric copper determination method of Hoffpauir and O'Connor (*5*), which at first had been used to analyze the copper salt precipitate, was modified to give a better end point and consequently more consistent results. The procedure resulting from these modifications was essentially that outlined by Pierce and Haenisch (*8*). As a matter of preference, the nitric acid used to dissolve the copper carboxymethylcellulose was removed by boiling in the presence of concentrated sulfuric acid rather than by the addition of urea. It is believed that slightly more consistent results are obtained by the former technique. Alternate procedures for determining the copper content of the copper carboxymethylcellulose precipitate are by electrodeposition or by ignition to the metallic oxide.

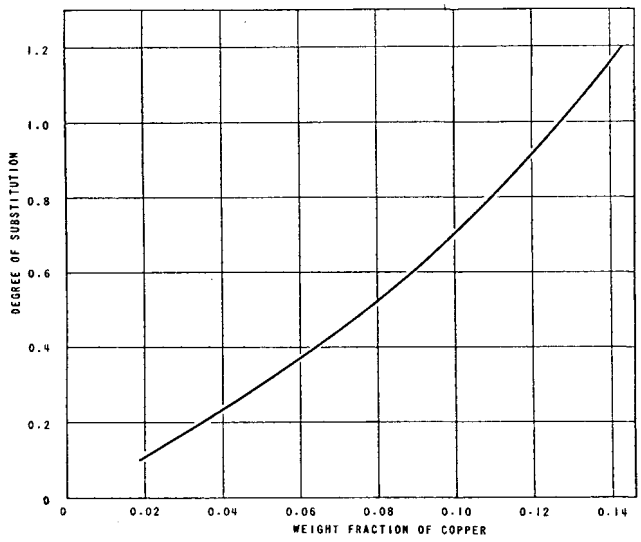

Figure 1. Graph for Calculation of Degree of Substitution

Table I. Analysis of Copper Salt of Carboxymethylcellulose

Sample No.	% Copper (Theory 11.16%)	Degree of Substitution (Actual 0.82)
1	11.01	0.81
2	10.92	0.80
3	11.10	0.82
4	11.31	0.83
	Av. 11.08	0.82

APPLICATIONS AND LIMITATIONS

The copper salt method is primarily applicable to purified grades of sodium carboxymethylcellulose over the commonly encountered ranges of viscosity and degree of substitution (0.6 to 1.2). In some cases it has also been successfully applied to unpurified grades free of interfering substances. However, in such cases the variable factors involved must be further investigated.

Table II. Analysis of Purified Sodium Carboxymethylcellulose by Copper Salt Precipitation Method

Sample No.	Viscosity Type[a]	% Sodium Carboxymethylcellulose	Degree of Substitution: Copper method	Degree of Substitution: Other[b]
1	High	100.9	0.56	
		100.1	0.55	
		100.4	0.55	
		Av. 100.5	0.55	0.55
2	Medium	100.0	0.64	
		99.4	0.64	
		Av. 99.7	0.64	0.65
3	Medium	98.6	0.80	
		99.3	0.81	
		Av. 99.0	0.80	0.77
4	Medium	97.5	0.82	
		99.6	0.82	
		99.6	0.82	
		100.0	0.82	
		Av. 99.2	0.82	0.83
5	Medium	100.3	0.85	
		100.0	0.85	
		Av. 100.2	0.85	..
6	Medium	98.2	1.24	
		97.8	1.38	
		Av. 98.0	1.31	1.34
7	Low	99.1	0.83	
		98.0	0.83	
		Av. 98.6	0.83	0.81
8	Extra low	98.5	0.71	
		100.3	0.70	
		99.6	0.71	
		Av. 99.5	0.71	0.75
9	Extra low	99.7	0.74	
		99.7	0.74	
		100.3	0.74	
		Av. 99.9	0.74	0.75
10	Extra low	99.6	0.76	
		99.6	0.79	
		99.7	0.78	
		Av. 99.6	0.78	0.79
11	Extra low	98.7	0.97	
		98.7	0.96	
		Av. 98.7	0.96	0.90
12[c]	Medium	98.1	0.86	
		98.1	0.84	
		Av. 98.1	0.85	0.85
13[c]	Low	97.8	0.81	
		98.1	0.84	
		Av. 98.0	0.82	0.84

[a] Viscosity ranges indicated are: High, 1300–2200 cp. (1% solution). Medium, 300–600 cp. (2% solution). Low, 25–50 cp. (2% solution). Extra low, <25 cp. (2% solution).
[b] Values in this column are average of not less than two determinations made by one or more standard methods—conductometric, colorimetric, or acid-wash.
[c] All samples are products of Hercules Powder Co. except these, which are from another manufacturer.

The type of cellulosic raw material used, the manufacturing process employed, and the subsequent treatment of the product all appear to have some influence on the recoveries obtained. Because unpurified samples of known composition are not generally available, it is believed that the best approach in determining the applicability of the method is first to analyze the material for all possible salt impurities, and then apply the copper salt method. The sum of all the analyses should closely approach 100%. The presence of any organic or inorganic salts that will form a precipitate with copper under the conditions of the test will interfere. The most commonly encountered salt impurities in commercial sodium carboxymethylcellulose are sodium chloride, sodium glycolate, and in some cases, sodium phosphates. Of these, only phosphates interfere.

METHOD AND DATA

The developed procedure is presented below along with Table II, in which are recorded the results of applying this method to a number of purified grades of sodium carboxymethylcellulose. All these data were obtained on materials having a theoretical assay value of 99.5+% sodium carboxymethylcellulose—i.e., less than 0.5% salt impurities. All samples were also analyzed for degree of substitution by at least one other standard procedure—e.g., conductometric, colorimetric, or acid-wash. Accompanying the method are two plots which have been found useful in calculating the results. Figure 1 is a plot of weight fraction of copper

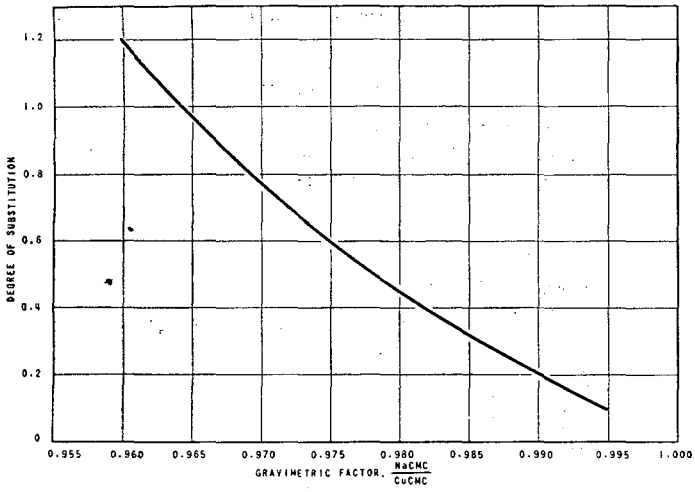

Figure 2. Graph for Calculation of Gravimetric Factor

in copper carboxymethylcellulose versus the degree of substitution; Figure 2 is a plot of the gravimetric factor $\frac{\text{NaCMC}}{\text{CuCMC}}$ versus the degree of substitution.

Reagents and Apparatus. Copper sulfate solution, 1% $CuSO_4.5H_2O$ in distilled water.

Standard copper solution. Weigh about 1 gram of electrolytic or other high-purity copper to the nearest 0.2 mg. and dissolve in 25 ml. of concentrated nitric acid in an Erlenmeyer flask. Add 10 ml. of concentrated sulfuric acid and heat to white fumes. Continue heating for about 10 minutes, cool, and cautiously add 50 ml. of distilled water. Boil for 5 minutes, cool, and dilute to 1 liter.

Sodium thiosulfate solution, 0.02 *N*, accurately standardized against the above copper solution.

Sodium hydroxide solution, approximately 0.5 *N*.

Starch indicator solution, 1%.

Methyl red indicator solution, 1%.

Laboratory model pH meter. Any commercially available laboratory model meter with glass-calomel electrodes similar to Leeds & Northrup assembly No. 7663-A1.

Procedure. Grind the sample, if necessary, in a micro Wiley mill or equivalent to pass a 20-mesh screen. Weigh roughly about 0.25 gram of sodium carboxymethylcellulose into a glass-stoppered weighing bottle and dry in an oven for 1 to 2 hours at 100° to 105° C. Remove, stopper, cool in a desiccator, and weigh. Transfer the dry sample to a 125-ml. glass-stoppered Erlenmeyer flask, reweigh bottle, and obtain sample weight by difference. Thoroughly moisten the sample with methanol and allow to stand for a few minutes. Slowly add 50 ml. of distilled water, shaking vigorously after each addition. Add 3 ml. of 0.5 *N* sodium hydroxide, stopper, and shake by hand or mechanical shaker until the sample is dissolved. Wash down the sides of the flask with water.

To the above solution, add 2 drops of methyl red indicator and then sufficient concentrated hydrochloric acid to cause the indicator to turn red. Add 2 to 3 drops of concentrated hydrochloric acid in excess. Introduce a variable-speed stirrer with glass paddle and shaft, and the electrodes of a laboratory model pH meter into a 400-ml. beaker containing 75 ml. of 1% cupric sulfate pentahydrate solution and 25 ml. of absolute methanol acidified with 2 drops of concentrated hydrochloric acid. Slowly pour in the sample solution with vigorous agitation and rinse flask with distilled water. The pH of the final mixture should be about 2.5 corrected for temperature. Mixing the solutions at too high a pH produces precipitates of copper carboxymethylcellulose which are stringy, fibrous, and rubbery, and which cannot be washed satisfactorily. Mixing at too low a pH may cause precipitation of the free acid rather than the desired copper salt.

When the solutions are thoroughly mixed (some initial precipitation may occur), adjust the pH of the mixture of 4.0 to 4.1 by dropwise addition of 3% ammonium hydroxide solution. Neutralization to this pH is critical and is necessary to give essentially complete precipitation of the copper salt. If the pH is taken appreciably higher, copper hydroxide may precipitate, leading to serious errors. If the 4.0 to 4.1 value is inadvertently exceeded, reacidify to a pH of 2.5 with concentrated hydrochloric acid and readjust pH to 4.0 to 4.1. The solution at the final pH should be purplish in color and contain a fluffy, bluish-white precipitate. Allow the precipitate to settle completely (20 to 30 minutes), and decant as much of the supernatant liquid as possible through a dried, weighed, sintered-glass crucible (medium porosity or equivalent) under aspirator suction from a filter flask. Wash the precipitate by decantation with one or two 50-ml. portions of 50% methanol solution. If the precipitate tends to form a mat, break up the particles with a glass stirring rod. Transfer the precipitate to the crucible with the aid of 50% methanol and thorough policing, and wash with the same solution until free of red or purple color. Washing and transfer should require about 150 ml. of wash solution. During washing, the precipitate should be frequently stirred in the crucible with a short stirring rod. When the copper carboxymethylcellulose is free of red or purple color, wash with anhydrous methanol (75 to 100 ml.). As the washing proceeds, the precipitate will become light green in color and fluffy in appearance. Aspirate to dryness and dry in an oven for 2 hours at 100° to 105° C. Cool in a desiccator, weigh, and obtain the weight of the copper carboxymethylcellulose precipitate. If the degree of substitution (D.S.) has been determined by other means, the assay value (per cent sodium carboxymethylcellulose) may be calculated at this point. If the degree of substitution value is not known, the procedure must be continued.

Degree of Substitution. Transfer as much of the copper salt precipitate as possible to a 150-ml. beaker and crush to a fine powder with a small spatula or stirring rod. Set the crucible in the beaker and add 15 ml. of hot concentrated nitric acid. Allow to stand until practically all the precipitate is in solution. Warming on a steam bath may aid. Transfer the nitric acid solution to a 250-ml. Erlenmeyer flask and rinse the beaker, using as little water as possible. Add 10 ml. of concentrated sulfuric acid and a few boiling chips and clamp the flask to a ring stand in a hood at about a 45° angle. Heat the flask with a Bunsen or Tirrell burner to white fumes. If charring occurs, add 5 ml. of concentrated nitric acid and again heat to white fumes. Repeat, if necessary, until the solution is clear green in color. After initial emission of white fumes, continue heating for 15 minutes. Allow to cool and very cautiously add 50 ml. of distilled water. Boil for 5 minutes being careful to avoid bumping, and cool to room temperature in a water bath. Add 30% ammonium hydroxide solution until the deep blue of the copper complex is not quite permanent (60 to 70 ml.). Cool slightly and add 5 ml. of glacial acetic acid. Stopper and cool to room temperature. Solution should be at a pH of 3.5 to 4.0 to obtain a satisfactory end point. Add 5 to 7 grams of potassium iodide in 10 ml. of water, stopper flask, and allow to stand 1 to 2 minutes. Titrate with 0.02 *N* sodium thiosulfate which has been previously standardized against a standard copper solution, utilizing the same procedure as above. Add thiosulfate until the yellow color of the solution just disappears or is very faint. Then add 2 grams of potassium thiocyanate in 10 ml. of water and swirl for 15 seconds. Add 3 to 5 ml. of starch solution and continue titration dropwise until 1 drop removes the color from the solution. The end point should be fairly sharp, and with practice is readily detectable.

Calculation. Determine the weight fraction of copper, *C*, in the copper carboxymethylcellulose precipitate from the thiosulfate titration as follows:

$$\text{Wt. fraction of copper, } C = \frac{\text{ml. of thiosulfate} \times \text{grams of Cu/ml.}}{\text{grams of CuCMC}}$$

From this value the degree of substitution may be readily calculated:

$$\text{Degree of substitution, D.S.} = \frac{162C}{32 - 89C}$$

Knowing the degree of substitution, the purity or assay value is determined as follows:

$$\% \text{ NaCMC} = \frac{\text{wt. of CuCMC} \times \frac{\text{NaCMC}}{\text{CuCMC}} \times 100}{\text{wt. of sample}}$$

where

$$\frac{\text{NaCMC}}{\text{CuCMC}} = \frac{162 + 80\,(\text{D.S.})}{162 + 89\,(\text{D.S.})}$$

DISCUSSION AND CONCLUSIONS

The data resulting from the application of the copper salt precipitation method to thirteen representative samples of purified sodium carboxymethylcellulose are given in Table II. In all cases an average recovery of 98% or better was effected, and the

degree of substitution values checked closely those obtained by other standard methods. It may also be concluded that duplicate determinations should agree within 0.5 to 1.0% sodium carboxymethylcellulose for assay value, and within about 0.03 unit for degree of substitution.

The method is not particularly suited for control work because of its length. It is estimated that approximately 6 to 8 hours are required to run a set of up to four determinations. However, it has a definite use as a referee method or in other analytical work not of the control type.

The assay values obtained by the copper salt precipitation method on purified grades of sodium carboxymethylcellulose agree reasonably well with those found by an alcohol-washing procedure currently employed in the trade. The chief source of error in this method is an appreciable loss of sodium carboxymethylcellulose through solubility in the wash liquor. The extent of this loss varies and is mainly dependent on the degree of substitution, degree of degradation, and uniformity of the product. Salt impurities insoluble in the alcohol will, of course, interfere.

Further evaluation of the copper salt method may result in improvement in time required and accuracy. If an organic base, which does not give a precipitate with copper ions, could be found for the adjustment of the final pH, the danger of precipitating copper hydroxide could be obviated and the solution made more basic to ensure complete precipitation. It may also be possible to precipitate the copper salt in a suitable physical form in some organic solvent–aqueous copper salt solution which would eliminate pH adjustment. These possibilities will be considered in future investigations.

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Spectrophotometric Determination of Anthracene in Crude Anthracene Cakes

Method of Correcting for Extraneous Background Absorption

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An ultraviolet method for determining anthracene in anthracene cakes is described that is at least as accurate as any known chemical method and much more convenient. The results are within about 1% of the known concentration in synthetic cakes. Spectrophotometry has also been applied to the analysis of mixtures of anthracene, phenanthrene, and carbazole. A method of correcting for extraneous background absorption is outlined which assumes only that the background is linear. In particular, it is unnecessary to know the intensity of the extraneous absorption. The method is similar to that of Banes and Eby.

ANTHRACENE occurs in coal tar in concentrations of less than 1%. When coal tar is distilled, the anthracene concentrates in the anthracene oil fraction distilling between 300° and 400° C. This fraction on cooling deposits a mixture of solids which may be centrifuged to yield an anthracene cake containing from 10 to 25% by weight of anthracene (the so-called "crude cake"). Other major constituents of this cake are carbazole and phenanthrene.

To evaluate properly a commercial method for the recovery of anthracene, the analysis of the starting material, the treated material at intermediate stages, and the purified product must be known. The chemical properties of the many compounds present are so similar that a chemical analysis is difficult.

Two chemical procedures are available for the determination of anthracene. In the Höchst test the anthracene is oxidized to anthraquinone and the amount of anthraquinone is determined gravimetrically (*9*). In the dienometric or maleic anhydride method anthracene reacts with a known amount of maleic anhydride and the excess anhydride is then determined by titration (*4*, *5*, *8*). Both procedures require experienced technicians and a considerable amount of time. Furthermore, their absolute accuracy is open to question when they are used in the analysis of crude anthracene cakes containing less than 40% anthracene. In the maleic anhydride method, for example, low results obtained are apparently due to acidic components of the cake.

There is no good chemical procedure available for the determination of phenanthrene. Carbazole in anthracene cakes may be estimated by a Kjeldahl nitrogen analysis.

The use of ultraviolet absorption spectrophotometry for the analysis of anthracene overcomes the disadvantages of the chemical procedures and is of equal or greater accuracy. In addition to the determination of anthracene, the ultraviolet method permits

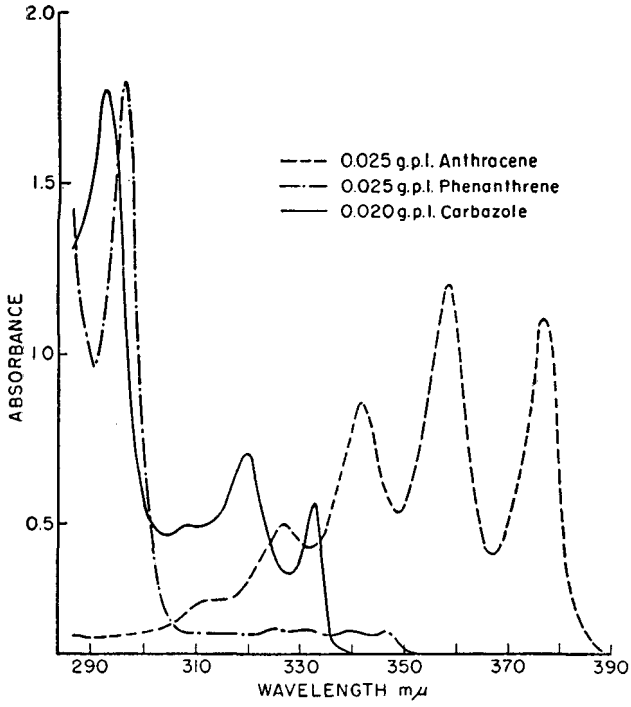

Figure 1. Absorption Spectra of Anthracene, Phenanthrene, and Carbazole

Chloroform Solutions

the identification and in many cases the determination of the impurities present. The progress of the separation of anthracene from crude anthracene cakes in an industrial process can be followed quickly and routinely by means of the general picture of the constitution contained in the ultraviolet spectrogram.

APPARATUS AND SPECTROSCOPIC PROCEDURES

A Cary double-beam ultraviolet spectrophotometer was used throughout this investigation. This instrument records absorbance *vs.* wave length. Tests on standard solutions of potassium dichromate gave extinction coefficients which agree within 1% with those given by Hogness, Zscheile, and Sidwell (*3*). Absorbance readings are reproducible within ±0.01 unit. At the absorbances used in this work the error in the analysis due to photometry is estimated to be within ±2% of the amount present.

The spectra of anthracene, phenanthrene, and carbazole in chloroform solution are shown in Figure 1. It will be observed that the spectra of carbazole and phenantherene interfere seriously with one another in this limited spectral region, but not with that of anthracene. Extinction coefficients for highly purified samples of these compounds were determined from the Lambert-Beer law:

$$A = \log_{10} I_0/I = Ecl \qquad (1)$$

where A = absorbance, I_0/I = ratio of radiant intensity incident on sample to that transmitted by the sample, E = extinction coefficient, c = concentration in grams per liter, and l = cell length (1.000 cm. in all cases). Table I shows these extinction coefficients at various wave lengths for this instrument.

The validity of the Lambert-Beer law for anthracene was tested by plotting concentration against absorbances for the 358 and 376 mμ bands. There is a slight deviation from linearity when $A > 0.9$, which is almost certainly due to too wide slit widths. Consequently, all analyses were made at absorbances below this value.

Table I. Extinction Coefficients of Pure Compounds (in Chloroform Solution)

(Liters per gram-cm.)

Wave Length, Mμ	Anthracene, E	Carbazole, E	Phenanthrene, E
376	39.0	0.0	0.0
358	42.7	0.0	0.0
346	18.4	0.4	1.5
338	22.9	16.7	1.3
333	12.4	19.6	0.8
330	11.9	16.7	1.7
320	8.8	22.5	0.9
296	2.3	67.0	70.0
292	1.9	101.0	39.9
284	2.9	62.7	58.8
277	6.0	23.7	78.9

PURIFICATION OF REFERENCE COMPOUNDS

Anthracene. A Reilly Tar and Chemical Company sample of anthracene was recrystallized from pyridine and made to react with maleic anhydride, and the anhydride adduct was hydrolyzed to the potassium salt. The alkaline-water solution of the acid adduct was washed with xylene at 90° C., and the adduct was recovered in the form of the anhydride. This step was repeated until the ultraviolet spectrum of the adduct was transparent in the 270 to 400 mμ range. The adduct was then heated under vacuum to recover the anthracene. The anthracene was chromatographed on alumina and the eluent evaporated to yield the purified anthracene.

Phenanthrene. A Reilly Tar and Chemical Company sample of phenanthrene was recrystallized from dichloroethane and made to react with maleic anhydride. The anthracene–maleic anhydride adduct was removed by repeatedly washing the benzene solution of the reaction mixture with 5% aqueous potassium hydroxide at 90° C. The benzene was then evaporated and the phenanthrene chromatographed on alumina.

Carbazole. A Reilly Tar and Chemical Company sample of 97% carbazole was recrystallized from a benzene-acetone mixture (6 to 1) and the 9-acetyl carbazole compound formed. The 9-acetyl compound was repeatedly recrystallized from acetic acid and hydrolyzed to regenerate carbazole.

ANALYSIS OF CRUDE ANTHRACENE CAKE

The crude anthracene cake obtained by centrifuging anthracene oil is a mushy mass containing 10 to 25% anthracene and 35 to 50% of oily matter in addition to carbazole and phenanthrene. The spectrum of a typical crude cake (Figure 2) shows the presence of background absorption in the 350 to 390 mμ range. This absorption, which is caused by substances other than carbazole and phenanthrene, necessitates correction of the spectral data before analyzing for anthracene. Because the background absorption increases sharply at shorter wave lengths, it is impossible to determine carbazole or phenanthrene in the crude cake.

Base-Line Method of Background Correction. The simplest type of background correction is to assume that the extraneous absorbance is linear. In what is often termed the "base-line" method, illustrated in Figure 3, a straight line is drawn between points on the absorbance curve at two fixed wave lengths (*1*, *2*, *10*), which are conveniently taken as the positions of minima for the pure compound on each side of the band to be used. It will now be shown that the height, A_z, from this base line to the band maximum is directly proportional to the concentration of the material present. Furthermore, the concentration of the material can be obtained without knowing anything about the background except that it is linear—in particular, without knowing its absolute amount.

These results have appeared in a paper by Banes and Eby (*1*), which seems to have escaped general notice. The present authors first derived them in a slightly different form that is somewhat easier to use with their instrument. In view of this fact and of the utility of the method, it seems worth while to give the mathematical proof. A paper by Morton and Stubbs (*6*, *7*) also describes a method that is similar in principle, but much more complicated in practice.

Consider first the case of one pure component (substance D). From Figure 3 it can be seen that the slope of the base line may be expressed as

$$\frac{\Delta A}{\Delta \lambda} = \frac{A_1 - A_3}{\lambda_1 - \lambda_3} = \frac{A_1 - (A_2 - A_x)}{\lambda_1 - \lambda_2}$$

Rearranging:

$$A_x = \frac{(A_1 - A_3)(\lambda_1 - \lambda_2)}{\lambda_1 - \lambda_3} - A_1 + A_2$$

Let

$$n = \frac{\lambda_1 - \lambda_2}{\lambda_1 - \lambda_3}$$

Then

$$A_x = (n - 1)A_1 + A_2 - nA_3 \qquad (2)$$

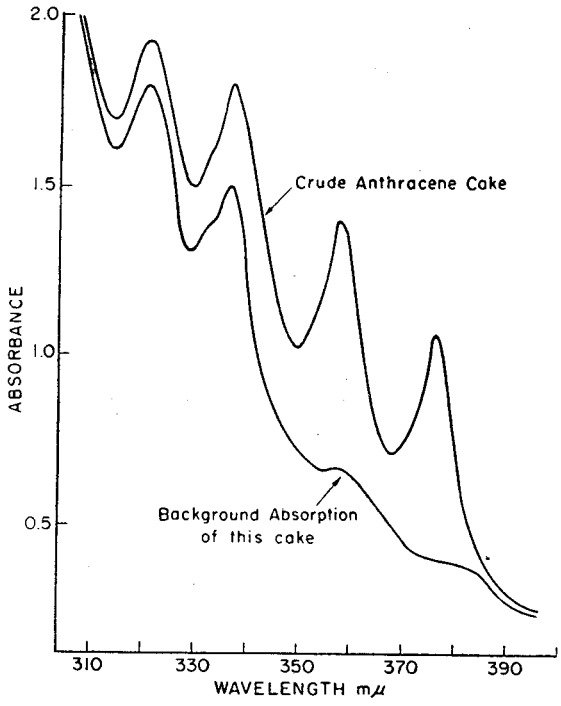

Figure 2. Absorption of Crude Cake Before and After Removal of Anthracene

Assuming that the Lambert-Beer law is obeyed, and setting $k = El$, there is had at each wave length:

$$A_1 = E_1cl = k_1c$$

$$A_2 = k_2c$$

$$A_3 = k_3c$$

Substituting in Equation 2,

$$A_x = [(n - 1)k_1 + k_2 - nk_3]c \qquad (3)$$

Now consider the case of this same component plus a linear background absorption. The background absorbance can be expressed by the equation for a straight line,

$$A_B = g\lambda + h$$

where g and h are constants. The absorbance at any wave length will now be the sum of the absorbances of the sample (substance D) and the background. Therefore

$$A_1 = k_1c_D + g\lambda_1 + h$$

$$A_2 = k_2c_D + g\lambda_2 + h$$

and

$$A_3 = k_3c_D + g\lambda_3 + h$$

Substituting these expressions in Equation 2:

$$A_x = (n - 1)(k_1c_D + g\lambda_1 + h) + (k_2c_D + g\lambda_2 + h) - n(k_3c_D + g\lambda_3 + h)$$

Separating terms:

$$A_x = [(n - 1)k_1c_D + k_2c_D - nk_3c_D] + [(n - 1)(g\lambda_1 + h) + g\lambda_2 + h - n(g\lambda_3 + h)]$$

$$A_x = [(n - 1)k_1 + k_2 - nk_3]\,c_D + (0)$$

But this equation is identical with Equation 3. Because n and the k's are constants, this shows that the value of A_x is independent of the background as long as the background is linear. One can also write:

$$k_{\text{eff.}} = (n - 1)k_1 + k_2 - nk_3 \qquad (4)$$

and

$$c_D = \frac{A_x}{(n - 1)k + k_2 - nk_3} = \frac{A_x}{k_{\text{eff.}}} \qquad (5)$$

where $k_{\text{eff.}}$ is an effective extinction coefficient. It follows from Equation 5 that the concentration of the desired component (substance D) can be determined by the measurement of the absorbance of the mixture at three fixed wave lengths, without knowing anything about the background except that it is linear. (Although only one absorbance appears explicitly in Equation 5, two more are needed to fix the base line for the measurement of A_x. See also Equation 2.)

From the foregoing considerations the following points may be noted:

1. Only two assumptions have beem made—namely, that the Lambert-Beer law is valid and that the background is linear.
2. Measurements are always made at the same wave lengths, even though in a mixture the absorption minima may no longer occur at these wave lengths. Furthermore, it is not necessary that the two extreme wave lengths be at absorption minima. The absorbance measurements will usually be more accurate if they are at minima; but if the minima are far apart the chance of the background's being linear between them is lessened. Consequently, with a broad band it may be advantageous to choose wave lengths that are on the sides of the band. One of the wave lengths may also be on the tail-off portion of an absorption curve, such as point III of Figure 3.
3. It is possible to evaluate the effective extinction coefficient, $k_{\text{eff.}}$, in two different ways. (1) Determine k_1, k_2, and k_3 for pure D at the three wave lengths, and insert them in Equation 4. (2) Determine $k_{\text{eff.}}$ directly for pure D by measuring the height, A_x, above the base line and using this datum in Equation 5. The latter method was followed in this work.
4. Banes and Eby's results, in the present authors' notation, are:

$$c_D = \frac{(n - 1)A_1 + A_2 - nA_3}{(n - 1)k_1 + k_2 - nk_3} = \frac{(n - 1)A_1 + A_2 - nA_3}{k_{\text{eff.}}} \qquad (6)$$

The numerator is an analytical expression for A_x (Equation 2). The results may be easier to use in this form for a nonrecording instrument, such as the Beckman Model DU spectrophotometer. With the Cary spectrophotometer, Equation 5 is simpler, as the base line can be ruled on the recorded curve and height A_x above this read directly.

In Table II results obtained by the base-line method are compared with chemical analyses by the maleic anhydride procedure. The results agree fairly well for cakes containing more than about 25% anthracene, but are not so satisfactory for lower concentrations. The chemical results are probably low for these crude cakes because of the presence of acidic components such as phenols. This table therefore does not constitute a critical test of either method. More disturbing is the fact that the ultraviolet analysis based on the 358 mμ band is always higher than that based on 376 mμ, indicating that the background may not be linear as assumed. The reason for this discrepancy was shown by removing the anthracene from several crude cakes.

Direct Determination of Background Absorption. Anthracene was removed from four typical cakes by reacting them individually with maleic anhydride in benzene to form 9,10-dihydro-

Table II. Anthracene in Crude Anthracene Cakes

(Weight %)

Test No.	Ultraviolet (Base-Line Method) 358 mμ (A)	376 mμ (B)	Diff. (A − B)	Chemical
6	13.5	10.9	3.6	9.2
7	12.1	11.1	1.0	9.4
8	13.4	11.3	2.1	10.4
9	17.9	16.3	1.6	13.4
10	19.8	18.8	1.0	19.0
11	25.0	24.1	0.9	23.3
12	36.2	35.4	0.8	35.0
13	40.7	40.0	0.7	38.4
14	42.1	41.8	0.3	41.1
15	71.5	71.0	0.5	70.6
16	91.1	90.6	0.5	91.1
17	96.0	95.0	1.0	93.0

anthracene-9,10-endo-α,β-succinic anhydride, which was extracted from the reaction mixture by washing the benzene solution at 70° C. with aqueous potassium hydroxide. The alkaline extracts were combined and acidified, and the precipitated adduct of anthracene and maleic anhydride was removed by filtration. The acidified extracts were then washed with benzene and these benzene extracts were added to the solution of anthracene-free cake. After the benzene was completely evaporated on the steam bath, the absorption spectrum for the anthracene-free cake was obtained, which is compared with the spectrum of the original crude cake in Figure 2. The concentration of the material contributing the background absorption is nearly identical in these two curves. This same type of background was obtained with all four cakes.

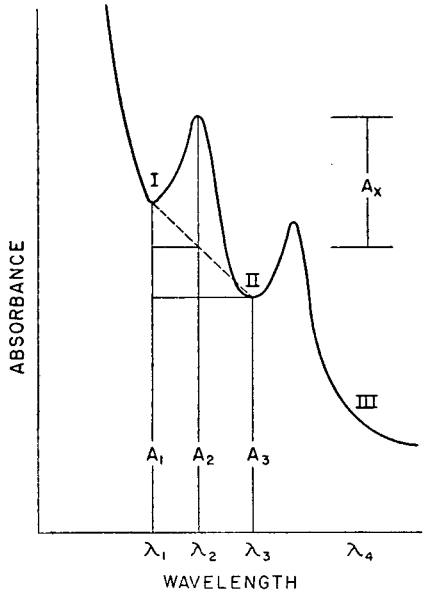

Figure 3. Base-Line Method of Background Correction

The background absorbance is not linear, but, on the contrary exhibits two small maxima in the general region of the anthracene maxima. It is now apparent why a higher analysis was obtained with the 358 mμ band than with the 376 mμ band by the base-line method of calculation.

Tests of Spectrophotometric Analysis. The possession of these deanthracened cakes enabled a critical test of the accuracy of the analysis.

The spectrum of the deanthracened cake was matched with that of the crude cake in the wave-length region where anthracene is relatively transparent. Subtraction of these curves yields analyses at each of the two maxima which are almost the same, as brought out in Table III. These data show that a true background absorption spectrum was subtracted. The magnitude of these analyses may not be correct.

Critical tests were performed in which the deanthracened materials were used to make up synthetic crude cakes by adding known amounts of anthracene. The spectra of the synthetic cakes were then analyzed by the base-line method. The results on four cakes are summarized in Table IV.

In the latter tests the base-line results obtained with either band are, with one exception, correct within 2% of the known concentration, and their average is correct within about 1% of the known concentration. After the nature of the background was known, it was observed that the results could be slightly refined by drawing the base line between points I and III (Figure 3) rather than between I and II and between II and III. The two bands then gave results which always bracketed the correct value, and their averages were a little more accurate. These results are included in Table IV under "modified base-line method." This latter procedure is advantageous only for the cruder cakes (up to about 35% anthracene), where the nonlinear background

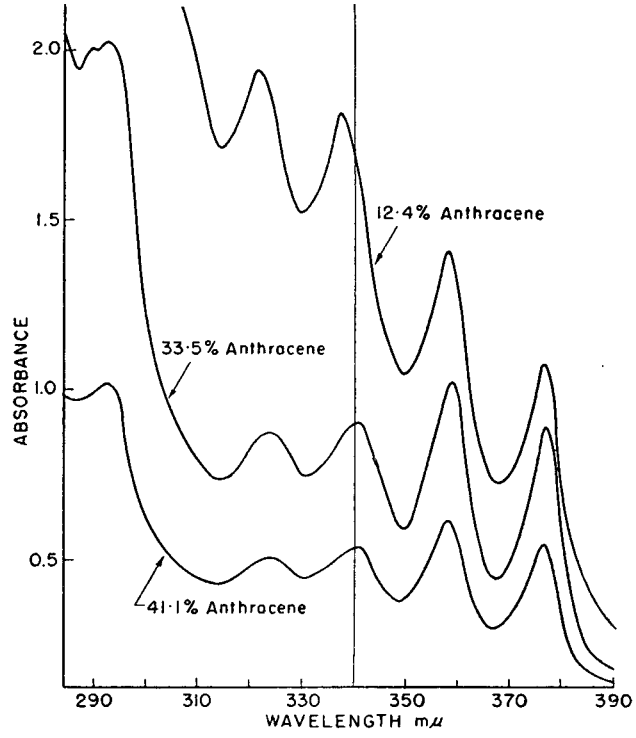

Figure 4. Absorption Spectra of Crude Anthracene Cakes

Illustrating successive stages of purification

Table III. Experimental Tests of Spectrophotometric Analyses in Analysis of Crude Cakes

Sample No.	Subtracting Approximate Background, Wt. % 376 mμ	358 mμ	Av.
1	17.1	16.9	17.0
2	22.5	22.8	22.6
3	17.7	17.7	17.7
4	34.9	35.0	35.0

Table IV. Experimental Tests of Spectrophotometric Analyses in Analysis of Synthetic Cakes

Sample No.	Known Addition (A), Wt. %	Usual Base-Line Method, Wt. % 376 mμ	358 mμ	Av. (B)	Diff. (A − B)	Modified Base-Line Method, Wt. % 376 mμ	358 mμ	Av. (C)	Diff. (C − A)
1	12.3	12.0	14.0	13.0	+0.7	11.3	13.0	12.1	−0.2
2	22.9	22.8	25.2	24.0	+1.1	21.8	23.8	22.8	−0.1
3	12.4	12.1	14.1	13.1	+0.7	11.6	13.2	12.4	0.0
4	35.6	36.2	37.0	36.6	+1.0	35.3	35.6	35.4	−0.2

absorption could cause large errors if not taken into account. For purer cakes the usual base-line method using the 376 mμ band is satisfactory.

A rather valuable by-product of the ultraviolet analysis of crude anthracene cakes is illustrated in Figure 4. Along with the determination of anthracene there is obtained a general picture of the constitution of the crude anthracene cakes. As the purification process proceeds, the ultraviolet spectrum loses its sharp upward trend at shorter wave lengths, and the carbazole peak at 292 mμ becomes evident. By noting the change in the spectrum at 340 mμ, the removal of an impurity can be followed. The peak at 337 mμ gradually shifts to 342 mμ, where it coincides with a characteristic anthracene peak.

ANALYSIS OF MIXTURES OF ANTHRACENE, CARBAZOLE, AND PHENANTHRENE

Some preliminary work has been done on the analysis of mixtures of anthracene, phenanthrene, and carbazole. An examination of the ultraviolet spectra of the three compounds shows that there are suitable analytical wave lengths for each of them. In this work the best band for carbazole could not be employed because the studies were made in chloroform solution, and this band occurs at a wave length which is below the cutoff in the transmittance of chloroform. (Chloroform was chosen because other conventional solvents did not completely dissolve the crude cakes that were the object of the original studies.) In spite of this handicap, results were obtained which compare favorably with chemical analysis.

Figure 5 demonstrates that small amounts of carbazole (1.5%) in anthracene and anthracene (0.18%) in carbazole, respectively, can be determined. Similarly, traces of phenanthrene in anthracene, and the converse, can be determined. These analyses are difficult or impossible by chemical means.

It may be seen from Figure 1 or Table I that the determination of anthracene is independent of the amounts of the other two compounds, inasmuch as they do not absorb at the 376 mμ anthracene band. Therefore when all three substances are present in the same sample, the anthracene content is readily and accurately obtained. In chloroform solutions, however, the bands that must be used for carbazole and phenanthrene interfere with each other to such an extent that their analysis is somewhat uncertain.

Table V contains the results of several of these three-component analyses which are typical of the experience in this laboratory. The chemical analysis given for comparison is based on the maleic anhydride method for anthracene and a Kjeldahl nitrogen determination for carbazole. Everything else is assumed to be phenanthrene. It is evident that even in chloroform solution the ultraviolet analysis compares favorably with the chemical analysis, and it is much more easily conducted. It also has the advantage of giving an independent check on all three components.

Table V. Analysis of Mixtures of Anthracene, Carbazole, and Phenanthrene

Test No.	Mixture	Ultraviolet	Chemical
18	Anthracene	100.2	96.0
	Carbazole	1.8	1.6
	Phenanthrene	0.1	2.4
		102.1	
19	Anthracene	4.2	3.6
	Carbazole	2.8	1.6
	Phenanthrene	91.5	94.8
		98.5	
20	Anthracene	0.2	0.5
	Carbazole	100.0	96.5
	Phenanthrene	2.2	3.0
		102.4	
21	Anthracene	2.1	2.0
	Carbazole	52.1	49.1
	Phenanthrene	46.2	48.9
		100.4	
22	Anthracene	53.2	51.0
	Carbazole	3.4	3.1
	Phenanthrene	44.2	45.9
		100.8	

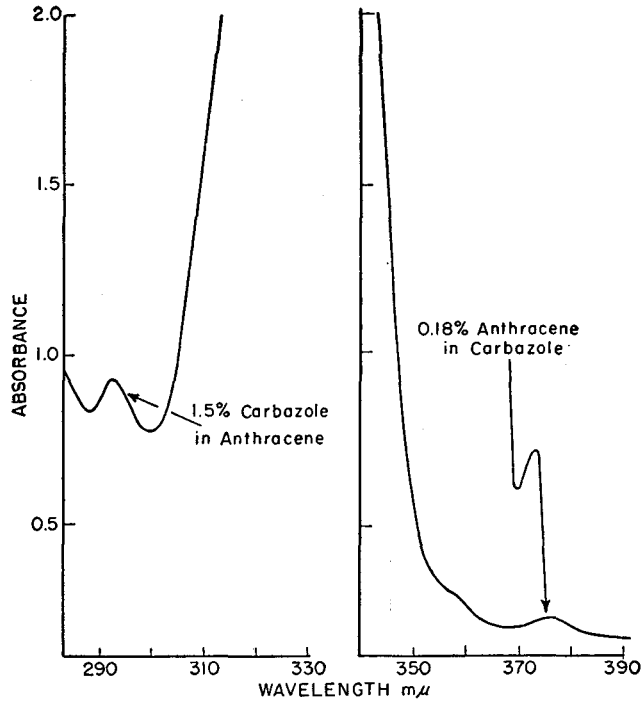

Figure 5. Absorption Spectra of Anthracene-Carbazole Mixtures

SUMMARY

An ultraviolet determination of anthracene in anthracene cakes is at least as accurate as any available chemical method, and is much more convenient. The results are within about 1% of the known concentration in synthetic cakes. Spectrophotometry is also suitable for mixtures of anthracene, carbazole, and phenanthrene.

Accurate analyses can be made when there is extraneous background absorption, if this can be assumed to be linear. The amount of the background need not be known. The method is theoretically sound.

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Polarographic Determination of Elemental Sulfur in Petroleum Fractions

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A polarographic procedure is described for the determination of elemental sulfur in petroleum fractions. Evaluation with synthetic mixtures has shown the method to be accurate to ±2% of the elemental sulfur content in the range of 1 to 100 p.p.m. The method is rapid (about 20 minutes per sample), sensitive, and free from interference from organic sulfides, disulfides, and thiophene.

THE corrosiveness of elemental sulfur to automobile engine parts is well known, and a great deal of care is exercised by petroleum refiners to minimize free sulfur in their products. In doctor sweetening careful control of free sulfur addition must be maintained to avoid production of a corrosive gasoline. A number of analytical methods have been proposed for determining the free sulfur content of gasoline and other hydrocarbons, but a survey of publications on the determination of elemental sulfur indicates that there is no recognized quantitative method for the direct determination of small quantities of elemental sulfur in hydrocarbons. The polarographic procedure described in this report was developed for the determination of free sulfur in the gasoline fractions of petroleum, although it is expected that it could be applied to the measurement of elemental sulfur in liquefied petroleum gases or heavy hydrocarbons. The method is rapid (about 20 minutes per sample), sensitive, and free of interference from organic sulfides, disulfides, and thiophene in gasolines. Evaluation with synthetic mixtures has shown the method to be accurate to ±2% of the elemental sulfur content over the range from 1 to 100 p.p.m.

In surveying the literature for information pertaining to the qualitative and quantitative estimation of elemental sulfur, it was found that several A.S.T.M. methods (*1*, *2*, *4*) make use of the copper strip corrosion test for detecting elemental sulfur. Another A.S.T.M. method (*3*) employs the mercury corrosion test to detect free sulfur. Mapstone (*7*) in a study of the Sommer test, the inverse doctor test, and the mercury corrosion test for the qualitative detection of free sulfur in gasoline, for plant control work, found that an approximate estimate of the free sulfur content of gasolines could be obtained by any of the three tests, but much more rapidly and easily by the Sommer test. Probably the most often used method for the quantitative determination of free sulfur in appreciable amounts is the butyl mercaptan (butanethiol) or inverse doctor test described by Wirth and Strong (*10*). In a study of the methods for determining elemental sulfur, Ball (*5*) found the butyl mercaptan test to be the best of the existing methods, but concluded that even this method was subject to inaccuracy when employed for low concentrations of elemental sulfur in hydrocarbons. In a recent paper Morris, Lacombe, and Lane (*8*) describe a method for the quantitative determination of elemental sulfur in aromatic hydrocarbons. The method is based upon the reaction

$$S + Na_2SO_3 \longrightarrow Na_2S_2O_3$$

but is satisfactory only for free sulfur in the range of 0.1 to 20%. Morris, Lacombe, and Lane studied both the sodium sulfite and the butyl mercaptan methods for determining very small amounts of free sulfur and found both methods unsatisfactory for determining elemental sulfur in the range 0 to 100 p.p.m. They concluded that no reliable method appears to exist for the determination of elemental sulfur in this low concentration range.

Proske (*9*) published a polarographic procedure for the quantitative determination of free sulfur that he used in connection with studies on the vulcanization of rubber. Proske extracted the free sulfur from the rubber with pyridine and ran a polarogram on the pyridine extract. The electrolyte solvent consisted of acetic acid, sodium acetate, and tylose. The procedure developed independently and discussed herein differs from Proske's method mainly in the nature of electrolyte-solvent employed. Inasmuch as free sulfur is not readily extracted quantitatively from petroleum fractions, an electrolyte-solvent consisting of methanol and pyridinium hydrochloride was chosen because of its miscibility with hydrocarbons.

APPARATUS AND REAGENTS

A Sargent Model XXI recording polarograph was used in developing the method. An H-type electrolysis cell was employed, and a saturated calomel electrode was used as the reference electrode. The capillary constants for an open circuit were

$$t = 4.0 \text{ seconds (drop time)}$$
$$M = 1.76 \text{ mg. per second (rate of flow of mercury)}$$
$$M^{2/3}t^{1/6} = 1.64 \text{ mg.}^{2/3} \text{ sec.}^{1/6}$$

The mercury column, h, was 67.0 cm. All experimental measurements were made in an air-conditioned room with the temperature held constant to 25° ± 0.5° C.

One hundred milliliter flasks were used to dilute the unknown sample to volume with the electrolyte-solvent.

Baker's C.P. methanol, pyridine, and concentrated hydrochloric acid were used to prepare the solvent and none of the reagents required any further purification.

Powdered monoclinic sulfur of better than 99.9% purity was used for the preparation of standard solutions.

The electrolyte-solvent is prepared by mixing 90 ml. of methanol, 9.5 ml. of pyridine, and 0.5 ml. of concentrated hydrochloric acid. The pyridine and pyridinium hydrochloride form a highly buffered solution that has a pH of 6 as measured by a Beckman pH meter.

A standard solution of known sulfur content is prepared by dissolving weighed amounts of the monoclinic sulfur in the solvent. The monoclinic form of sulfur is used because it is more soluble than other forms of sulfur, and also because the monoclinic form is used in doctor sweetening processes for gasolines.

METHOD

Calibration. Because it is doubtful that the present chemical methods are reliable for accurate determinations of free sulfur in very low concentrations, synthetic solutions of known amounts of free sulfur were used as standards for plotting diffusion current against sulfur concentration as shown in Figure 1. (In refinery laboratories it is often desirable to express concentration of sulfur as milligrams per unit volume, and by expressing concentration of sulfur in such a way the density of the liquid in question does not have to be determined.)

Analysis of Samples. The size of sample required for analysis depends on the free sulfur concentration; however, for petroleum fractions containing 5 to 10 p.p.m. of free sulfur, 20 ml. of the sample to be analyzed are added to a 100-ml. volumetric flask and diluted to volume with the solvent. If the elemental sulfur content of the sample is approximately 1 or 2 p.p.m., the size of the sample should be increased to produce a diffusion current large enough to provide a determination that is ±2% accurate. However, in the case of motor gasoline, the maximum amount of sample that can be analyzed is usually about 40 ml. because this is the limit of miscibility of the gasoline and solvent. The solutions in the flask are well mixed and then added to the electrolysis cell. The solution is bubbled for 5 minutes with an inert gas before the reduction wave is determined. The inert gas should be bubbled through methanol prior to passing through the sample to prevent excessive evaporation of the sample solution. The amount of free sulfur present in the sample will determine what damping position and sensitivity setting should be used. The wave height multiplied by the sensitivity setting gives the diffusion current in microamperes. A calibration curve, such as Figure 1, is then used to obtain the concentration of free sulfur in the sample.

DISCUSSION OF EXPERIMENTAL WORK

The principle of the polarographic method is based upon the measurement of the diffusion current that is produced when elemental sulfur is reduced at the dropping mercury electrode. The number of electrons, N, involved in the electrode reaction was determined by the equation:

$$E_{de} = E_{1/2} - \frac{0.0591}{N} \log \frac{i}{i_d - i} \quad (6)$$

which is the fundamental equation of the polarographic wave. It can be seen from the above equation that a plot of log $i/(i_d - i)$ versus E_{de} should be a straight line with a slope of $0.0591/N$ volt.

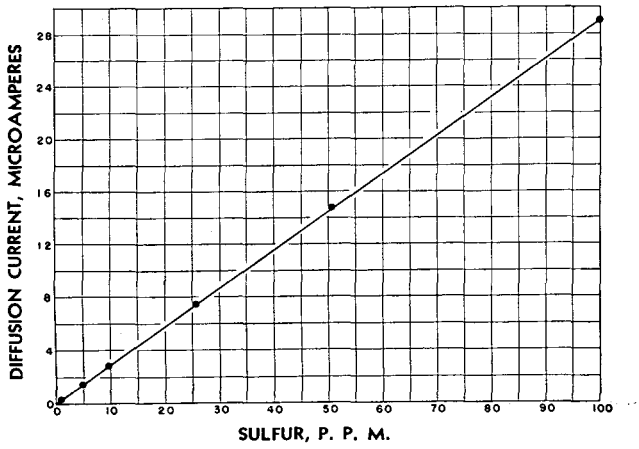

Figure 1. Diffusion Current *vs.* Parts per Million Sulfur in Methanol-Pyridine Solution

Such a plot was made from the polarogram obtained with a Model XXI Sargent recording polarograph of a 10 p.p.m. sulfur solution; the points of the log plot formed a straight line with a slope of 0.031 volt which is in good agreement with the theoretical value, 0.0296 volt, for $N = 2$. On the basis of the determination that 2 electrons are involved in the electrode reaction, it is believed that the reduction of sulfur occurs according to the following equation:

$$S + 2H^+ + 2e^- \longrightarrow H_2S$$

From the magnitude of the diffusion current and by using the Ilkovič equation ($i_d = 605\ ND^{1/2}Cm^{2/3}t^{1/6}$), the diffusion coefficient of elemental sulfur was calculated to be 2.2×10^{-5} cm.^{2} sec.$^{-1}$ at 25° C. in the methanol-pyridine solution.

Because the method was to be applied mainly to petroleum samples, one of the major problems was to find a satisfactory solvent for the gasoline, sulfur, and a supporting electrolyte. At first, mixtures of various ratios of pyridine, water, and concentrated hydrochloric acid were tried, but were found unsatisfactory mainly because not enough gasoline would blend with the electrolyte solvent to permit an accurate determination of sulfur in the concentration range of 1 to 3 p.p.m. in the gasoline; at least 30 ml. of the gasoline sample in a 100-ml. blend are necessary for most accurate work in this concentration range. Mixtures of various amounts of the low molecular weight alcohols with pyridine and concentrated hydrochloric acid were investigated as solvents, and the most suitable combination found for mixing with gasoline was the mixture of methanol, pyridine, and concentrated hydrochloric acid already described. The methanol serves as a solvent for gasoline and the electrolyte; the pyridine serves as a solvent for free sulfur. The hydrochloric acid reacts with part of the pyridine to form pyridinium chloride which is the supporting electrolyte, and also helps form a highly buffered solution with a pH of 6. Using an electrolyte-solvent of this composition, as much as 40 ml. of gasoline will blend with 60 ml. of the solvent.

Table I. Free Sulfur in Synthetic Solutions

Blend No.	Sulfur Taken, P.P.M.	Sulfur Found, P.P.M.	% Error
1	1.00	1.02	+2.0
2	5.00	4.93	−1.4
3	10.0	9.8	−2.0
4	25.0	25.6	+2.4
5	50.0	50.5	±1.0
6	100.0	99.3	−0.7
		Average % error	±1.6

A brief study of the effect of changes in pH revealed that the electrode reaction should be carried out below a pH of 7. Above pH 7 the reduction wave spread out over a range of 1 volt or more and was unsatisfactory to use for calculating the sulfur concentration. At a pH of 6 or lower, well defined waves were obtained with a half-wave potential of −0.50 volt versus the saturated calomel electrode.

Table II. Reproducibility of Polarographic Method for Determining Elemental Sulfur in Petroleum Fractions

Detn. No.	2-Methylheptane Sulfur Found, P.P.M.	Kerosene Sulfur Found, P.P.M.
1	10.1	100
2	10.0	103
3	10.0	100
4	9.9	101
	Av. 10.0 ± 0.05	101 ± 1

Because there appear to be no reliable chemical methods for the analysis of free sulfur in gasoline on which to base calibrations in very low concentration ranges, synthetic standard solutions were used for plotting diffusion current versus concentration of sulfur as shown in Figure 1. The diffusion current is directly proportional to the concentration of sulfur over a range of 0 to 100 p.p.m. Experimental data showing the accuracy and precision of the method are given in Tables I and II.

Several organic sulfur compounds were added to synthetic and plant gasoline samples to determine if such compounds interfered with the analysis of the free sulfur. Butyl sulfide, propyl di-

sulfide, and thiophene offered no interference. When a mercaptan was added, the sulfur was consumed in forming a disulfide. The rate of consumption of the free sulfur by a mercaptan can be followed with the polarograph. Hydrogen sulfide, if present, will be removed upon bubbling.

Table III. Elemental Sulfur Content of Gasoline Fractions and Kerosene after Doctor Sweetening

(Determined by polarographic procedure)

Sample	S, P.P.M.
Light crude naphtha	7
Heavy crude naphtha	10
Light cracked naphtha, high octane	4
High sulfur refined oil (kerosene)	101

APPLICATIONS

The polarographic method for elemental sulfur has been applied in connection with studies of different gasoline sweetening processes. Table III shows the concentration of sulfur found in various gasolines and kerosene after doctor sweetening. The materials of higher molecular weight appear to retain more sulfur. This is probably a solubility effect rather than a variation in plant process. The method should be applicable to aqueous solutions as well as to hydrocarbons.

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Copper Contamination and Ascorbic Acid Loss in Waring Blendor

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The Waring Blendor, which has extensive usage in food analysis, vitamin assays, and biochemical preparations, is regarded with disfavor by some workers. Loss in ascorbic acid and loss in enzyme activity during blending have been cited. Adverse results may arise from use of containers having worn chrome plating on their blending assemblies. Copper dissolving from exposed brass parts may catalyze the loss of ascorbic acid in solutions during blending or create false high results in the determination of the copper content of certain foodstuffs. Inhibition of the destructive effect of copper dissolved from the blending assembly on ascorbic acid is discussed.

THE Waring Blendor is an exceedingly popular and useful tool for comminuting or homogenizing material in the laboratory, especially for food analysis, vitamin assays, and biochemical preparations (*6*).

There are few reports concerning limitations on its use. The Blendor has been recommended for ascorbic acid determinations in tissues (*1, 5, 8, 12*), although Roe *et al.* (*11*) advise against use of any homogenizer that would introduce increased amounts of oxygen into the slurry. Stern and Bird (*13*) found that treatment of wheat germ and mill stream suspensions in the Waring Blendor caused oxidation of sulfhydryl groups and inactivation of enzyme systems.

Divergent results and conclusions in work involving use of the Waring Blendor in some instances may be traced to the indiscriminate use of containers. This study was initiated as a result of discrepant data on the ascorbic acid content of identical solutions blended in different containers. Inspection of a container in which significant losses of ascorbic acid occurred showed that the chrome plate on parts of the metal blending assembly was worn, exposing the brass undersurface. Brass contains a high percentage of copper, which is a known catalyst for the oxidation of ascorbic acid (*2, 3, 7*). This paper shows that blending ascorbic acid solutions in different containers resulted in widely varying losses in ascrobic acid, which can be attributed to the dissolution of copper during the blending procedure.

EXPERIMENTAL PROCEDURE AND DISCUSSION

Two hundred milliliter portions of a solution of ascorbic acid (20 micrograms per ml.) in 5% metaphosphoric acid (HPO_3) initially at room temperature (23° C.), were blended for 3 minutes in triplicate in each container. The average temperature rise in the solutions at the end of the blending was 8.7° C. with a low of 6.0° C. and a high of 12.0° C. The small differences in temperature rise were not reflected in the results. Ascorbic acid was determined by the 2,4-dinitrophenylhydrazine method (*11*) on aliquots of all blended solutions and nonblended controls. The copper content was determined by a dithizone method not subject to interference by other metals (*9*).

The results from triplicate runs with a single Waring Blendor container were not in agreement where the loss in the first blending was high. Instead, these triplicate runs seemed to form a pattern in which there was progressive lowering of ascorbic acid loss with the two succeeding blendings. This phenomenon of lowered ascorbic acid loss with successive blendings was further investigated. Using only one container (No. 6) nine consecutive 3-minute blendings of 200-ml. portions of ascorbic acid (20 micrograms per ml.) in 5% metaphosphoric acid were carried out. The ascorbic acid and copper contents were determined in each solution immediately after blending, as well as in an unblended control. This experiment was then repeated in the same container scrupulously cleaned, after an interval in which the container had general laboratory use in blending various materials.

The values determined for ascorbic acid loss and copper concentration in all experiments are shown in Table I, and the correla-

Table I. Loss of Ascorbic Acid in Different Blendor Containers

[Ascorbic acid (20γ/ml.) in 5% HPO_3 solutions blended for 3 minutes]

Appearance of Blending Assembly	Blendor Container	Blending Run 1	2	3	4	5	6	7	8	9
Bright plating	No. 1									
	Cu, p.p.m.	0.0	0.24	0.02	..	..	..	..	..	..
	ASA[a] lost, %	1.5	2.0	1.2	..	..	..	..	..	..
Bright plating	No. 2									
	Cu, p.p.m.	0.89	0.43	0.54	..	..	..	..	..	..
	ASA lost, %	5.0	3.0	2.5	..	..	..	..	..	..
Plating somewhat dull	No. 3									
	Cu, p.p.m.	2.66	0.21	0.08	..	..	..	..	..	..
	ASA lost, %	5.0	3.0	3.5	..	..	..	..	..	..
Dull plating	No. 4									
	Cu, p.p.m.	1.50	0.74	1.36	..	..	..	..	..	..
	ASA lost, %	13.0	13.0	11.0	..	..	..	..	..	..
Plating worn, brass exposed	No. 5									
	Cu, p.p.m.	4.00	2.48	0.88	..	..	..	..	..	..
	ASA lost, %	21.0	19.0	13.0	..	..	..	..	..	..
Plating worn, brass exposed	No. 6									
	Cu, p.p.m.	6.96	3.44	1.60	..	..	..	..	..	..
	ASA lost, %	36.5	22.5	13.0	..	..	..	..	..	..
	Cu, p.p.m.	12.80	2.68	3.04	1.48	0.80	1.12	1.40	..	1.06
	ASA lost, %	52.1	14.9	17.6	9.4	7.1	7.9	8.6	6.1	6.6
	Cu, p.p.m.	8.27	2.20	1.86	..	1.28	2.64	1.62	..	1.92
	ASA lost, %	45.6	11.6	10.1	13.6	7.8	14.9	8.1	10.1	9.6

[a] Reduced ascorbic acid.

tion between the amount of copper dissolved during blending and the percentage loss of ascorbic acid is shown in Figure 1. The relationship is expressed by the straight-line equation $y = 0.0221 \times -0.051$. The coefficient of correlation between copper concentration and per cent ascorbic acid lost in this case is 0.97; a perfect correlation coefficient is 1.0. When tested, the correlation coefficient 0.97 was found to be highly significant. When the values for copper concentration and ascorbic acid loss that were obtained with solutions blended in a single container, No. 6, are plotted (not shown), similar results are obtained. The straight-line equation is $y = 0.0223 \times -0.064$ and the correlation coefficient is 0.98. Actually, adding increasing amounts of copper (as cupric sulfate) to ascorbic acid solutions resulted in losses of ascorbic acid during blending in a container of excellent condition (No. 1) that were almost identical to those found in solutions containing dissolved copper which originated from blending assemblies having worn plate. The results make it evident that, under the conditions of these experiments, copper dissolved from the blending assembly is responsible for the loss of ascorbic acid in the solutions blended.

The extent of loss of ascorbic acid correlates rather well with the physical appearance of the blending assembly (Table I). Solutions in contact with well plated blending assemblies showed small losses in ascorbic acid, whereas with blending assemblies having worn plating or exposed brass parts losses were considerably higher. However, the loss of ascorbic acid, as well as the amount of dissolved copper, decreases and finally remains fairly constant with consecutive blendings in the same container. No attempt was made to study the mechanism for the progressively smaller amount of copper dissolved with successive blendings. Possibly an insoluble copper metaphosphate forms and deposits at worn surfaces of the plated metal parts of the blending assembly and retards further dissolving of copper. It appears that a blending assembly in which copper causes large ascorbic acid losses could be improved by preblending with a metaphosphoric acid solution. However, such a procedure would seem inadvisable unless it results in very low losses of ascorbic acid.

There is the possibility that about 1 ml. of a solution could come in contact with the sealed bronze bearing (90% copper, 10% tin) of the blending assembly when the blades are not in motion (*14*). For this reason an experiment was carried out to determine whether allowing ascorbic acid (20 micrograms per ml.) in 5% metaphosphoric acid solution to stand in a container of excellent condition (No. 2) for increasing lengths of time, immediately followed by a 3-minute blending, would result in increased loss in ascorbic acid. The times of standing in the nonagitating container prior to blending were 0, 0.5, 1, 2, 3, 5, 7, 10, and 15 minutes. There was no increase in the amount of ascorbic acid lost with increased time of standing of the solution. The average loss was 1.5%.

The effectiveness of stannous chloride and thiourea, known inhibitors of ascorbic acid oxidation, in lowering the ascorbic acid loss during blending of a solution in a container in poor condition (No. 5) were tested by incorporating them into the ascorbic acid (10 micrograms per ml.) in 5% metaphosphoric acid solution before blending for 3 minutes. As shown in Table II, 0.5% stannous chloride (higher concentrations were not used because of precipitation of stannous metaphosphate) reduces the loss of ascorbic acid. It probably retards oxidation by combining with molecular oxygen. Thiourea (1%) markedly reduces ascorbic acid loss by combining with copper and inhibiting its catalytic action on the oxidation of ascorbic acid.

Indiscriminate use of containers may also cause trouble in the determination of trace metals in foodstuffs. In one laboratory, contamination of the food by biologically important heavy

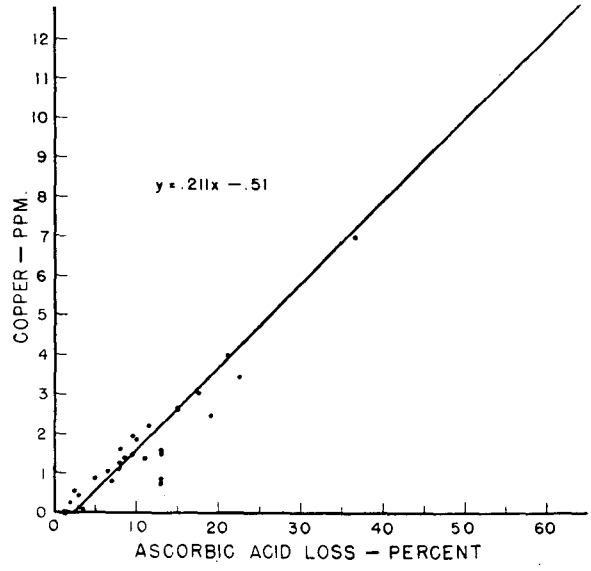

Figure 1. Correlation between Concentration of Copper Dissolved during Blending and Percentage Loss of Ascorbic Acid

200-ml. portions of ascorbic acid (20μ/ml.) in 5% HPO_3 blended for 3 minutes in various containers

Table II. Effect of Adding Stannous Chloride or Thiourea to Ascorbic Acid on Loss of Ascorbic Acid during Blending

Container	Ascorbic Acid Lost, % Control[a]	Control + 0.5% $SnCl_2$	Control + 1% thiourea
No. 5 (av. of 3 runs)	40.2	13.8	3.0
No. 1 (av. of 3 runs)	1.3	1.2	0.0

[a] Ascorbic acid (10γ/ml.) in 5% HPO_3.

Table III. Determination of Concentration of Copper in Canned Tomato Juice before and after Blending

Tomato Juice Sample	Copper Concn., P.P.M.
Unblended control	1.52
Blended in container 2	2.04
Blended in container 6	8.52

metals, which may be present in the metal blending assembly, is prevented by rhodium-platinum plating these parts (*10*).

Use of the Waring Blendor for comminution of foods prior to the determination of their copper content has been mentioned in the literature (*4*). If such a procedure is carried out with little regard for the plating of the blending assembly, false results are likely, as shown in the following brief experiment:

A determination was made on the copper content of a sample of thoroughly mixed canned tomato juice immediately removed from the can after opening. A 200-ml. portion of this same tomato juice was blended for 3 minutes in container 2 (in excellent condition) and another 200-ml. portion was similarly treated in container 6 (in poor condition, brass exposed).

It is evident from Table III that a container with worn plating on its metal parts can cause false high results in the copper content of a food subjected to blending during the determination.

CONCLUSIONS

The Waring Blendor is a valuable tool; however, judgment must be exercised in its use. The condition of the metal parts of the container should be noted at all times. Where dissolved trace heavy metals may affect enzyme systems, vitamin assays, or the determination of trace metals, special plating, such as rhodium-platinum, of metal parts may be desirable. Furthermore, oxidation during blending may be retarded by replacing air over the blended material with inert gas (*1*), removing oxygen by a chemical agent such as stannous chloride, and inhibiting the catalytic action of trace metals as in the binding of copper by thiourea.

ACKNOWLEDGMENT

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Analysis of Colloidal Electrolytes by Dye Titration

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The determination of total amount of surface-active material present in a system can be obtained by titration with a suitable dye solution. Anionic soaps can best be determined with cationic dyes, and cationic soaps with anionic dyes. Total amount of surface-active components as well as the amount of each component can be determined in soap mixtures, in soap-electrolyte systems, and in soap solutions containing other materials such as hydrocarbons, polar compounds, latices, and various adsorbants if the proper experimental techniques are employed. In the case of determinations in turbid or slightly colored media, reproducible results were obtained only with dyes that show changes in fluorescence intensity. Experimentally, the most consistent data were obtained by observations made in a darkened room using a narrow beam of light falling on a thin layer of the soap solution.

A NUMBER of methods have been used to measure the concentrations of soaps and detergents. These include various chemical methods (*5*), a turbidimetric method (*12*) which is a modification of one used by Preston (*16*) and by Hartley and Runnicles (*4*), and a direct titration of sulfonates with *p*-toluidine hydrochloride (*14*). Some of these methods have been critically evaluated recently (*2*). For the determination of critical micelle concentration (CMC), a relatively simple method involving titration with various dyes based in part on earlier qualitative work of Hartley (*3*) and Sheppard and Geddes (*18*) has been reported recently (*1*) and application of this technique to various systems has been demonstrated (*9*, *15*).

This method depends essentially on the fact that a color or intensity of fluorescence of a dye solution changes markedly in the region of the critical micelle concentration (the amount of free nonmicellar soap). This makes it possible to follow these changes spectrophotometrically or visually, for the changes in most instances are marked enough to obtain reproducibility of results to better than 2 to 3%. The usual procedure is to titrate a volume or weight of soap solution or a known amount of dry soap with a freshly prepared dye solution, the concentration of which is usually about 1 to $5 \times 10^{-5} M$. The amount of added dye solution at the point of color change in the dye-soap solution is that necessary to bring the concentration of the solution to the critical micelle concentration. It has been found that, to be most effective, the dye used must carry a different charge than that of the colloidal electrolyte. For anionic soaps dyes like pinacyanol chloride or anisoline are suitable, whereas acidified indophenol, eosin, sky blue FF, and benzopurpurine can be used with cationic detergents. With those dyes which exhibit a monomer $\rightleftharpoons$ polymer equilibrium, it has been found that the color changes at the critical micelle concentration are much greater than the

Table I. Determination of Soap and Detergent Concentrations by Dye Titration

(At 25° C.)

Soap	CMC, %	Concentration, % Weighed	Concentration, % Determined
		Pinacyanol chloride (5×10^{-5} *M*)	
Potassium laurate	0.565	1.92	1.89
		3.43	3.48
		0.358	0.356[a]
		0.102	0.104[a]
Potassium myristate	0.160	1.57	1.52
		0.110	0.112[a]
Sodium dodecyl sulfate	0.150	0.712	0.708
		0.035	0.037[a]
		Acidified indophenol (10^{-4} *M*)	
Dodecyl amine hydrochloride	0.286	0.942	0.951
		0.364	0.358

[a] Determined by titration with soap-dye mixture.

gradual changes observed with dyes that have the same spectra in both polar and nonpolar media.

APPLICATION OF METHOD

The application of this titration method to the determination of the concentration of pure soaps is straightforward. It is, of course, necessary to know the critical micelle concentration, because this value must be used in the calculation of the total amount of soap present. Essentially, the procedure involves titration of a known amount of soap or a soap solution with a solution of a suitable dye to the point where a color change or a change in fluorescent intensity occurs, depending, of course, on the particular soap-dye system being used. The amount of soap is determined by use of the fact that the critical micelle concentration equals x grams of soap per total milliliters (volume of dye solution plus water in original soap solution). Often, it has been found that insufficient soap is present (below the critical micelle concentration) and it has then been necessary to titrate with a dye-soap solution (soap concentrations above the critical micelle concentration) to the desired end point. Data showing typical results are included in Table I. The agreement between the prepared concentrations and the determined values indicates that this rapid titration technique is readily applicable to the direct analysis of the amount of surface-active material present if the critical micelle concentration is known.

When the surface-active material is a mixture of two or more soaps, or is a salt-soap mixture, certain modifications must be made in the method as well as in the calculations, for the critical micelle concentration of a soap is decreased by the addition of electrolytes or of a less soluble soap (*7*, *15*). In the case of a soap mixture where the identity of each soap in the mixture is known, it is possible to determine not only the total amount of surface-active material present but also the amount of each constituent. By use of calibration curves of soap mixtures such as those seen in Figure 1, and by addition of soap with the higher critical micelle concentration in certain cases to bring the critical micelle concentration of the mixture to the portion of the curve with a steep slope, the data in Table II were obtained.

The actual procedure involved in these determinations is to titrate the weighed soap mixture of known ingredients to an end point (critical micelle concentration). One obtains a total amount of surface-active material, say a grams of A plus b grams of B. Referral to a calibration curve such as Figure 1 gives the weight fraction of one constituent B—i.e., $b/(a+b)$. It is then possible to calculate not only the total amount, $a+b$, present but the actual concentration of each constituent in the mixture. This procedure has been applied many times to mixtures and some typical data are shown in Table II. When the critical micelle

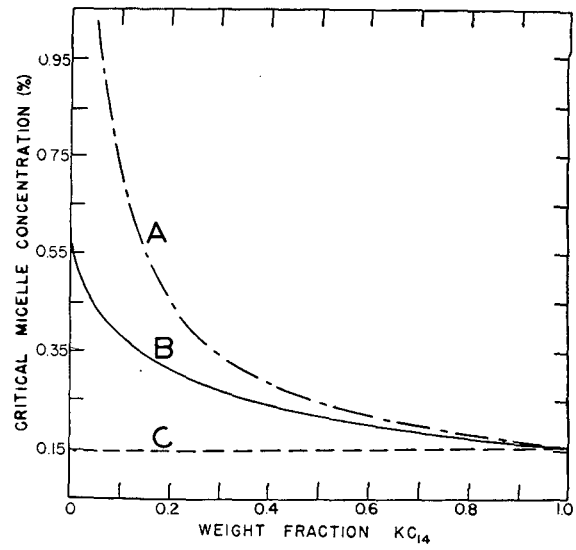

Figure 1. Type of Standardization Curves Suitable for Soap Mixtures

A. **Potassium myristate and potassium caprate**
B. **Potassium myristate and potassium laurate**
C. **Potassium myristate and sodium lauryl sulfate**

Table II. Determination of Total Soap Concentration and Concentration of Each Constituent in Mixtures

Soap	Concn. of Sample, Gram	CMC, %	Soap Added, Gram	Concn. Determined, Gram
KC_{14}	0.020	0.315	...	0.023
KC_{12}	0.102			0.105
KC_{14}	0.084	0.175[a]	...	0.087
KC_{12}	0.019		0.051	0.017
KC_{14}	0.052	0.225	...	0.055
KC_{12}	0.070		...	0.066
KC_{14}	0.055	0.158	...	0.148[b]
NaC_{12} sulfate	0.091			
$C_{12}NH_3Cl$	0.037	0.491	...	0.035
$C_{10}NH_3Cl$	0.152			0.153

[a] Concentrations not determinable without added soap because slope in curve is almost zero.
[b] Not determinable as individual constituents because CMC of soaps in mixture are about equal.

concentration values are about equal, as in the case of soaps and detergents of the same chain lengths (*10*), it is not possible to determine the actual concentration of each constituent in a mixture. An example of this is the mixture of potassium myristate and sodium dodecyl sulfate. Mixtures of anionic and cationic soaps are also not determinable by this method but may, in certain instances, be obtained by a modification of the turbidimetric method.

Similar techniques may be applied to the determination of soap-salt mixtures by means of calibration curves such as the one shown in Figure 2. For example, 0.090 gram of a mixture of potassium laurate, KC_{12}, and potassium chloride required 18.1 ml. of 5×10^{-4} *M* pinacyanol chloride, indicating a critical micelle concentration of 0.497%. This corresponds to a weight fraction of 0.84 and thus the mixture is composed of 0.076 gram of KC_{12} and 0.014 gram of potassium chloride.

This type of curve may be used to calculate the concentration of the surface-active component in the presence of any added electrolyte, for it has been shown that the critical micelle concentration is affected only by the number of equivalents of added electrolytes. A direct-reading percentage curve such as that in Figure 2 is thus slightly in error, but, as can be seen in Table III, the errors involved in the use of potassium chloride, potassium bromide, potassium nitrate, potassium sulfate, and potassium

phosphate trihydrate are about the same order of magnitude as those generally observed in the use of this dye titration technique.

The presence of hydrocarbons has been shown to cause discontinuities in interfacial tension–concentration curves which occur at concentrations somewhat lower than the surface tension–concentration curves of the pure soaps (*13*). Similarly, changes in critical micelle concentration have been noted from conductivity studies of alkyl amine hydrochloride–hydrocarbon systems (*17*). Small, but definite, decreases in critical micelle concentration have been observed when increment additions of benzene to soap solutions have been made (*10*).

Table III. Determination of Total Soap Concentration in Soap-Salt Mixtures

Soap-Salt	Concn. of Sample, Gram	CMC, %	Concn. Determined, Gram
KC_{12}	0.074	0.475	0.070
KCl	0.016		0.020
KC_{12}	0.105	0.410	0.101
KCl	0.050		0.054
KC_{12}	0.061	0.375	0.060
KBr	0.049		0.050
KC_{12}	0.011	0.205	0.012
KNO_3	0.075		0.076
KC_{12}	0.068	0.365	0.066
KNO_3	0.057		0.059
KC_{12}	0.074	0.338	0.070
K_2SO_4	0.076		0.079
KC_{12}	0.015	0.225	0.016
K_2SO_4	0.071		0.070
KC_{12} ·	0.009	0.165	0.009
$K_4P_2O_7.3H_2O$	0.096		0.096
KC_{12}	0.035	0.252	0.029
$K_4P_2O_7.3H_2O$	0.078		0.084

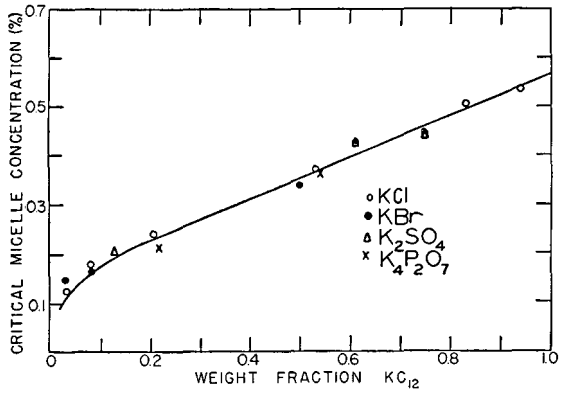

Figure 2. Type of Standardization Curve Suitable for Soap-Electrolyte Systems

Curve is for added KCl. Other added electrolytes such as K_2SO_4, KBr, KNO_3, and $K_4P_2O_7$ fluctuate somewhat about this curve

When the amount of hydrocarbon present is less than that amount which would cause a separation into layers or in which no emulsion droplets are present, a direct titration using a fluorescing dye as indicator yields results which are not in error by more than about 5%. Most dyes suitable for these titrations are insoluble in hydrocarbons such as benzene or *n*-heptane, so that their presence will not affect the color or fluorescence changes.

Polar-type compounds will form a type of mixed micelle similar to that observed in the case of soap-soap mixtures (*6*, *17*). The presence of short-chain alcohols will cause a decrease in critical micelle concentration at low alcohol concentrations, followed by a subsequent increase as shown by the conductivity studies of soap-alcohol mixtures of Ralston and Eggenberger (*17*). Thus, in the presence of short-chain alcohols, a direct titration of the soap-alcohol mixture with a suitable dye solution will result in concentration values of surface-active material which will not be in error by more than about 5%, if the original soap concentration is much above the critical micelle concentration, and if the soap-alcohol ratio is large.

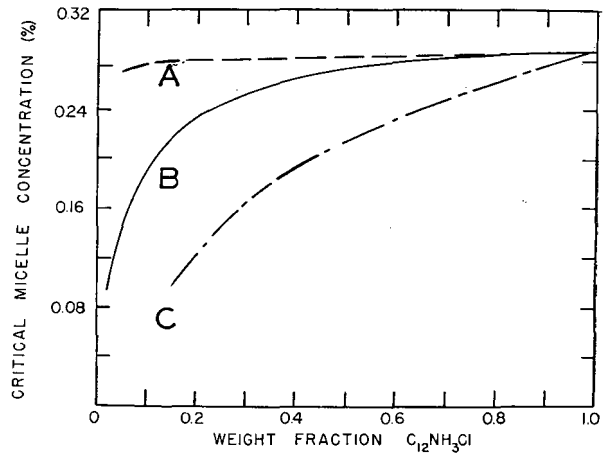

Figure 3. Type of Standardization Curves Suitable for Soap-Alcohol Mixtures

Lauryl amine hydrochloride with (*A*) ethyl alcohol, (*B*) *n*-butyl alcohol, and (*C*) *n*-heptyl alcohol

In the case of soap-alcohol mixtures, in which the alcohol is a longer chain one, the determination of surface-active materials would involve use of calibration curves such as those in Figure 3. The critical micelle concentration of a particular soap decreases more rapidly as the chain length of the added alcohol is increased, owing to the enhancement of the energy of the attraction of the long alcohol and soap hydrophobic tails. Recent experiments in this laboratory on enhancement of solubilization of hydrocarbons by the use of long-chain alcohols as additives supports this concept of increase in the total energy of attraction when soap-alcohol micelles are used as solubilizers (*8*). A set of typical data illustrating the application of the dye titration method to such mixtures is presented in Table IV. The smaller variation in critical micelle concentration upon the addition of short-chain alcohols can be seen to result in concentrations determined by titration which are not so accurate as those obtained in the soap–long-chain alcohol systems.

Table IV. Determination of Detergent Concentration in Lauryl Amine Hydrochloride–Alcohol Mixtures

Soap-Alcohol	Concn. of Sample, Gram	CMC Determined, %	Concn. Determined, Gram
$C_{12}H_{25}NH_3Cl$	0.055	0.231	0.053
n-$C_7H_{15}OH$	0.038		0.040
$C_{12}H_{25}NH_3Cl$	0.028	0.252	0.025
n-C_4H_9OH	0.061		0.064
$C_{12}H_{25}NH_3Cl$	0.020	0.230	0.019
n-C_4H_9OH	0.086		0.087
$C_{12}H_{25}NH_3Cl$	0.042	0.282	0.037–0.048
C_2H_5OH	0.046		0.051–0.040

DISCUSSION

It is evident that the determination of the total amount of surface-active materials by dye titration is practical under the conditions specified above. In the case of determinations in turbid or slightly colored media, reproducible results were obtained only with dyes that showed fluorescent effects. Experimentally, the most consistent data were obtained by observations

made in a darkened room using a narrow beam of light falling on a thin layer of the soap solution. It has been possible to obtain results indicating the amount of residual detergent present in various systems such as latices, textile dips, emulsions, and colored soap solutions under these experimental conditions. For example, the use of changes in dye fluorescence to determine the total area of polymer particles and, from this, particle diameters was found to give results in agreement with those diameters determined by light scattering (*9*). Similarly, determinations of total areas of polymer particles using conductivity, surface tension, and changes in dye spectra resulted in polymer particle diameters which were in good agreement with those calculated from electron microscopic data on the same samples (*11*).

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Determination of Chlorine in Silicate Rocks

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A suitable photometric method for the determination of small amounts of chlorine in silicate rocks may be based on the formation of a colloidal suspension of silver sulfide by treatment of the ammoniacal solution of isolated silver chloride with sulfide.

THE method commonly used for determining total chlorine in silicate rocks involves precipitation as silver chloride and weighing as such after sodium carbonate fusion of the sample. Although the gravimetric method gives satisfactory results with higher chlorine contents if a double precipitation is made (Table I) it is not very suitable for the small amounts (usually less than 0.05%) ordinarily encountered in the common igneous rocks, when results of the greatest accuracy are desired. Moreover, the gravimetric method is inadequate when the amount of sample is limited, as when chlorine is to be determined in the component minerals of a rock. For geochemical studies it is desirable to have available a method that will allow the determination of a few hundredths of 1% of chlorine in a 0.1-gram sample within 0.005%.

Two methods for the determination of small amounts of chlorine were investigated. The first was based on the potentiometric titration of chloride with silver nitrate solution to the equivalence potential existing under the conditions, as empirically determined. Although this method was not studied as fully as desirable, it appeared to be a feasible one. However, it seemed to offer no advantages over the second method studied, in which silver chloride is precipitated with an excess of silver nitrate, the washed precipitate is dissolved in ammonium hydroxide, and silver in the solution is then determined photometrically with sodium sulfide as reagent (yellow-brown coloration).

The determination of silver, and indirectly of chloride, with sulfide as photometric reagent is an old procedure, but it has been applied more in biochemical than in inorganic analysis. In ammoniacal medium a stable silver sulfide sol is obtained, and in the authors' experience it is not necessary to use a protective colloid when the silver concentration is less than 0.1 mg. per milliliter. The solution appears clear as long as this limit is not exceeded, and no appreciable change in optical density is shown on long standing (Table II). The relation between silver concentration and extinction is linear up to 100 p.p.m. of silver when a suitable filter is used.

The use of potassium dithio-oxalate and thioacetamide as reagents in place of sulfide was investigated, but they were found to have no general advantages. These substances furnish sulfide ion by hydrolysis and give colloidal suspensions of silver sulfide. Dithio-oxalate behaves practically the same as sulfide, the only difference being that clear sols can be obtained at higher silver concentrations than in the case of sulfide. Thioacetamide produces turbid solutions unless the silver concentration is less than 25 to 30 p.p.m., but the optical density is almost twice that which is obtained with sulfide or dithio-oxalate as reagent.

Results obtained by the proposed sulfide method according to the directions given in the procedure following are listed in Table III. It is believed that in the majority of cases, the error in the determination of 0.01 to 0.02 and 0.05% of chlorine will not exceed 20 and 10% relative, respectively, when a 0.5-gram sample is taken. Even with a 0.1-gram sample the error will not be much greater than this if the volume at the time of precipitation is

Table I. Gravimetric Determination of Chlorine in Silicate Rocks[a]

Sample	Chlorine Present[b], %	Chlorine Found, % Single pptn.	Double pptn.[c]
Quartz monzonite	0.019	0.03	..
	0.032	0.04+	..
	0.038	..	0.04
	0.046	0.06	..
	0.069	0.08+	0.07
	0.13	0.18	0.15
	0.19	0.19	0.18
Diabase	0.020	0.03	..
	0.033	0.04+	..
	0.070	0.08+	0.07
	0.125	0.14	..
	0.185	0.21	..
	0.26	0.28	0.26[d]
	0.51	0.50	0.51

[a] 0.5-gram sample fused with 2.5 grams of Na_2CO_3. AgCl precipitated from 100 ml. of solution containing 0.5 ml. of 1 to 1 HNO_3 in excess.
[b] Sum of chlorine originally present and that added.
[c] Silver chloride precipitate dissolved in 2 ml. of 1 to 1 NH_4OH, solution diluted to 10 ml., and slight excess nitric acid added.
[d] Refusion of residue from leach of first sodium carbonate melt showed 3% of total chlorine remaining in this residue.

reduced proportionately for the smaller sample. The volume of the final solution in which the silver sulfide sol is developed is maintained constant at 10 ml. independent of the sample size, so that the photometric error may become significant when a 0.1-gram sample is taken. Under the authors' conditions, 1 p.p.m. of silver in solution gives an extinction ($\log I_0/I$) of approximately 0.003 in a 1-cm. cell. Inasmuch as 1 p.p.m. of silver is equivalent to about 0.3 p.p.m. of chlorine 0.1 p.p.m. of chlorine will give an extinction of 0.001, which is the average deviation in the determination of low extinctions with a moderately good photometer. We will assume that a photometric error greater than 0.002 in extinction (about 0.4% in transmittancy) is unlikely at low extinctions (up to 0.05 or somewhat more). This means that the photometric error will usually be less than 0.002% chlorine when a 0.1-gram sample is used.

Table II. Stability of Silver Sulfide Sols in Ammoniacal Solution

(Final solution 1.5 *M* in NH_4OH and 0.001 *M* in Na_2S)

Concentration of Silver, P.P.M.	Log I_0/I (1 Cm., Blue Filter)		
	10 minutes	1 day	1 week
0	0.000	0.000	0.000
5.4	0.024	0.022	0.021
10.8	0.045	0.047	0.042
27.0	0.097	0.102	0.100
54	0.188	0.187	0.192
81	0.275	0.274	0.280
108	0.347	0.364	0.361

The error due to the solubility of silver chloride is largely compensated by applying the blank correction obtained by running a blank on the sodium carbonate under the same conditions as in the determination itself. The use of specially purified sodium carbonate very low in chloride is inadvisable in most cases, for it may be expected to result in less complete cancellation of the solubility error. On the other hand, the chloride content of the sodium carbonate should be sufficiently low so that the error in the determination of the blank will be small. The following blank values were obtained with various amounts of sodium carbonate used in the present work:

Weight of Na_2CO_3, g.	0.5	0.5	1.0	1.0	2.5	2.5	5	5	5
Cl found, γ	5	3	13	10	35	30	59	65	53
Equivalent % Cl in rock sample 1/5 weight of Na_2CO_3	0.005	0.003	0.0065	0.005	0.007	0.006	0.006	0.0065	0.0055

In these blank runs the volume of the solution in which the precipitation of silver chloride was carried out was adjusted to correspond to the amount of sodium carbonate, so that the ratio was constant. However, proportionately more wash solution was used for the smaller samples, particularly the 0.5-gram, which is reflected in the smaller apparent chloride content found for the latter weight of sample.

The advantage in the use of the photometric method over the gravimetric method is especially marked in the case of samples of 0.1-gram weight. If the error in weighing the silver chloride precipitate may amount to 0.2 mg., the resulting error in the chlorine percentage is 0.05 compared to about 0.005 in the photometric method.

Bromine is counted with chlorine in the proposed method, but because bromine is almost always very small compared to chlorine in igneous rocks (average bromine-chlorine ratio believed to be of the order 0.01), the analytical result may be taken to represent the chlorine content without appreciable error. The effect of phosphate was investigated by adding the equivalent of 1% phosphorus pentoxide to a sample of granite (0.01% chlorine) and of diabase (0.02% chlorine). The chlorine values found agreed within 0.002% with those obtained with the omission of phosphate.

Table III. Determination of Chlorine in Silicate Rocks by Photometric Sulfide Method

Sample	Chlorine Present, %	Chlorine Found, %
Quartz monzonite[a]	0.019	0.019[b]
	0.031	0.031
	0.038	0.040
	0.069	0.062
	0.069	0.069
	0.069	0.077
	0.100	0.100
	0.127	0.129
	0.127	0.110
Diabase[c]	0.020	0.022
	0.020	0.021[b]
	0.039	0.042
	0.070	0.065
	0.070	0.065
	0.101	0.113
	0.101	0.108
	0.128	0.133
	0.128	0.132
	0.188	0.20

[a] Chlorine content 0.007%. 0.5-gram samples. Cl added as NaCl before Na_2CO_3 fusion.
[b] K dithio-oxalate as photometric reagent.
[c] Chlorine content 0.008%. 0.5-gram samples.

PROCEDURE

When ample material is available, mix 0.5 gram of 100-mesh sample (0.005 to 0.07% chlorine) with 2.5 grams of sodium carbonate (low in chlorine) in a platinum crucible and fuse in the customary manner. Digest the cooled melt with about 20 ml. of water, adding a drop or two of alcohol to reduce any manganate present. Filter through a small retentive filter paper (previously washed with water to remove any foreign chloride) and wash with small portions of hot water totaling about 75 ml. Neutralize the cold filtrate and washings by careful addition of 1 to 1 nitric acid, using methyl orange as indicator, and add an excess of 0.5 ml.

Add 2 ml. of 0.2 *N* silver nitrate solution, heat almost to the boiling point, and allow the solution to stand overnight. Collect the precipitate in a small glass filter crucible (Jena 1G4) or porous porcelain crucible. Wash the beaker, crucible, and precipitate carefully with five 2-ml. portions of 0.02 *N* nitric acid. Dissolve the precipitate in 2 or 3 ml. of 1 to 1 ammonium hydroxide and wash the crucible with a few milliliters of water. A bell-jar type of filtration apparatus is preferably used in these operations.

Transfer the solution to a 10-ml. volumetric flask, dilute to about 8 ml. with water, add 1.0 ml. of 0.01 *M* (0.25 gram of sodium sulfide nonahydrate in 100 ml.) sodium sulfide solution, mix, dilute to the mark, and again mix. Obtain the transmittancy of the solution with the aid of a blue filter (a Wratten No. 47, C5, filter is suitable).

Establish the standard curve by taking 0, 0.25, 0.50, 0.75, and 1.0 mg. of silver as silver nitrate in 10-ml. volumetric flasks, treating with 2 ml. of 1 to 1 ammonia and 1 ml. of sodium sulfide solution, and obtaining the transmittancy as described above. Find the amount of chlorine from the theoretical ratio Cl/Ag = 0.329.

Run a blank by taking 2.5 grams of sodium carbonate and treating as described above.

Table IV. Determination of Chlorine in Diabase by Photometric Sulfide Method[a]

(0.1-gram samples)

Cl present[b], %	0.008	0.020	0.022	0.022	0.038	0.040
Cl found, %	0.012	0.023	0.019	0.023	0.041	0.046

[a] Similar results obtained with 0.1-gram samples of granitic rocks.
[b] Sum of original chlorine (determined on 0.5-gram sample) and added chlorine.

If the amount of sample is limited, fuse 0.1 gram or more with five times its weight of sodium carbonate. Carry out the succeeding operations as described above, but reduce the volumes in proportion to the reduction in the size of sample. Use a small porous porcelain or sintered-glass filter crucible of about 10-ml. volume for collecting the silver chloride precipitate. Wash carefully with five 1-ml. portions of 0.02 *N* nitric acid. Dissolve the precipitate in 2 ml. of ammonium hydroxide and proceed as directed.

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Buffer for High pH Measurements

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A calcium chloride-hydroxide buffer system has been devised having a reproducible pH in the range of 11 to 12.65, depending on the concentration of calcium chloride. Such buffers are useful in checking the performance of pH meters and electrodes in this pH range. A study of the precision of glass electrode measurements in this range indicates that their precision is largely dependent on the variability between electrodes.

IN INDUSTRIAL processes, many solutions are maintained at pH values as high as 12 to 13. The routine measurement of pH provides one analytical control for these solutions. In the past, these pH measurements have been made in this laboratory by standardizing with a borax buffer at pH 9.18 and checking the electrodes with another buffer at 4.01. The pH measurements in the region 11 to 13, therefore, represented considerable extrapolation from the standardizing value, and this caused uncertainty at times as to whether the solution measured or the electrode of the pH meter was in error. It was felt that a standard buffer in this pH range would assist in removing these uncertainties. A good buffer should be easily prepared, stable for long periods, and capable of precise reproduction of pH, and should have a good buffer capacity.

The buffer system investigated was a calcium chloride solution saturated with calcium hydroxide. This system functions in the following manner: Addition of acid in small amounts results in neutralization of some of the calcium hydroxide. The latter is present as a slurry and as the dissolved calcium hydroxide is neutralized, more solid calcium hydroxide goes into solution, maintaining an approximately constant pH. On the other hand, addition of a base precipitates calcium hydroxide, thus also maintaining the pH at an approximately constant value.

The effect of carbon dioxide is to precipitate calcium carbonate. Again calcium hydroxide goes into solution to maintain the concentration of calcium ions constant and so the pH does not change appreciably until a large fraction of the calcium hydroxide has been converted to calcium carbonate.

The solutions measured in this laboratory are in the neighborhood of 2 molar in sodium ion, and in order to minimize any variation in pH meter readings due to sodium ion error, one of the buffer solutions was made 2 molar in sodium chloride.

It is reasonable to believe that systems such as strontium chloride-hydroxide, barium chloride-hydroxide, and magnesium chloride-hydroxide would act in much the same manner, the pH range covered being largely dependent upon the concentration of the chloride and the solubility of the hydroxide in question. Preliminary experiments showed that the calcium chloride-hydroxide buffer had pH values in the range of primary interest to this laboratory; therefore it was chosen for investigation.

APPARATUS AND REAGENTS

Three types of instrument assemblies were used for pH measurements of the buffers described. One was a Leeds & Northrup, catalog No. 7673, thermionic amplifier, and a Rubicon Type B potentiometer. Most of the pH values, however, were obtained with Beckman Model G pH meters. A few readings were taken with a Beckman Model H pH meter. The assemblies were standardized using borax buffer having a pH of 9.18 at 25° C. Beckman Type E glass electrodes and saturated calomel reference electrodes were used throughout the entire series of measurements.

Reagent quality chemicals were used throughout the study.

PREPARATION OF BUFFER WITH pH OF 11.97 AT 20° C.

The buffers were prepared by adding an excess of calcium hydroxide to a predetermined volume of standard hydrochloric acid. Several concentrations of calcium chloride were tried. Figure 1 shows the calculated dependence of pH on molarity of calcium chloride. Three experimental values are represented by circles. The difference between measured and calculated values is greatest in the more concentrated solutions. The solution prepared to contain 0.2 mole of calcium chloride and 2 moles of sodium chloride per liter gave a pH of 11.97 at 20° C. and was considered most suitable for the intended purpose. This was called buffer 1. One or 2 days were required for the solutions to come to equilibrium.

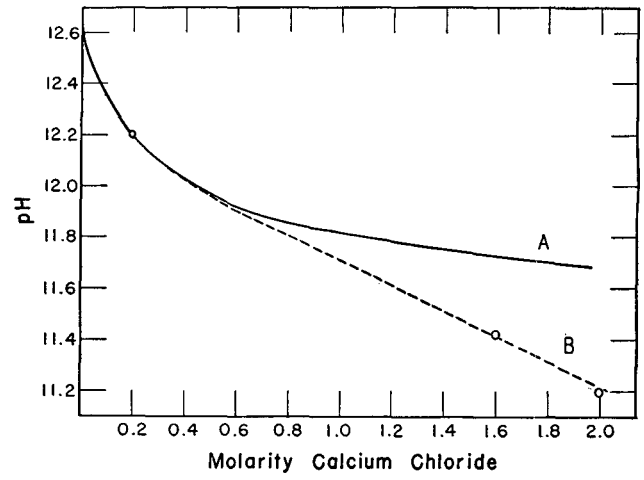

Figure 1. Effect of Calcium Chloride Concentration on pH

Solution of calcium chloride saturated with calcium hydroxide
A. Calculated curve
B. Curve based on experimental values

Calcium Hydroxide Buffer, 0.2 Molar in Calcium Chloride and 2 Molar in Sodium Chloride. Pipet 25 ml. of 8.00 *N* hydrochloric acid into a 500-ml. volumetric flask. Add about 300 ml. of water. Add 7.5 grams of reagent quality calcium hydroxide. Add 58 grams of sodium chloride and dissolve by swirling the stoppered flask. Dilute to the mark with water. Transfer to a glass-stoppered reagent bottle. Add 5 grams of calcium hydroxide and shake solution several minutes. Allow the solution to stand for 2 days before using.

By varying the concentration of hydrochloric acid or omitting the sodium chloride, other pH values can be obtained.

PROPERTIES

Reproducibility. BUFFER 1 (2 MOLAR IN SODIUM CHLORIDE). The pH values obtained for several mixes prepared to contain 0.2 mole of calcium chloride and 2 moles of sodium chloride per liter of saturated calcium hydroxide solution are given in Table I.

These solutions were prepared at different times by different people and the pH of each solution was measured one or more times, as shown. In all cases, excess calcium hydroxide was present as a slurry. The electrodes used were those in routine use with the respective instruments. The average of 24 values was 11.852 with a standard deviation of 0.030. The measure-

Table I. pH Values, Buffer 1

(Obtained at 23.8° ± 0.4° C. for solutions 0.2 molar in calcium chloride, 2 molar in sodium chloride, and saturated with calcium hydroxide)

Solution No.	Potentiometer		Model G pH Meters		Model H pH Meter	
	Solution age, days	pH	Solution age, months	pH	Solution age, days	pH
1-1	1	11.810	5 days	11.83	5	11.86
			4	11.83		
			7	11.81		
2-1	Fresh	11.896	4	11.85	Fresh	11.88
			7	11.78		
3-1	3	11.848	...	...	..	...
4-1	3	11.849	..	...	..	...
5-1	3	11.848	..	...	..	...
6-1	3	11.846	..	...	..	...
7-1	..	..	4 days	11.93	..	...
			5 days	11.88		
			7 days	11.85		
			3	11.87		
8-1	...	..	4 days	11.87	..	...
			5 days	11.86		
			7 days	11.84		
			3	11.85		
9-1	..	..	4 days	11.88	..	...
			7 days	11.85		
			3	11.84		

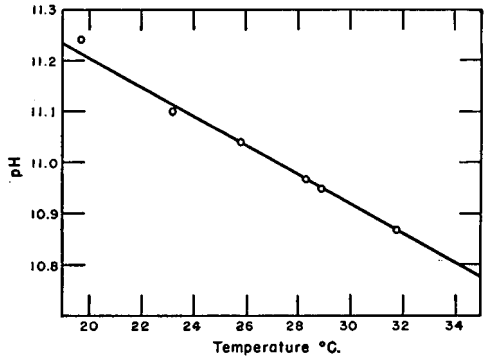

Figure 2. pH *vs.* Temperature

2 *M* calcium chloride saturated with calcium hydroxide

ments were made at room temperature, which was maintained at 23.8° ± 0.4° C.

Part of the standard deviation represents the reproducibility of preparing the solutions and part is contributed by the use of different glass and reference electrodes in these measurements. To give a better indication of the reproducibility, independent of a glass electrode, hydrogen electrode measurements were made on solutions 3-1, 4-1, 5-1, and 6-1. The cell used included a 3.5 *N* potassium chloride salt bridge. The hydrogen and calomel electrodes were standardized with 0.01 *M* borax buffer. Because of the several liquid junctions involved, some constant error may have been introduced into these measurements. Because of this and other possible sources of error, the values obtained are reported merely to indicate the reproducibility of preparing buffer solutions of this type. These data, shown in Table II, indicate that the buffers in question are reproducible as to pH.

Buffer 2 (No Sodium Chloride). The data obtained for saturated calcium hydroxide solutions that were 0.2 molar in calcium chloride but contained no sodium chloride are given in Table III.

Here again solid calcium hydroxide was present in the samples tested. The average of 17 pH values at 23.8° ± 0.5° C. was 12.063. The standard deviation of these data is 0.038.

Hydrogen electrode measurements of solutions 3-2, 4-2, 5-2, and 6-2 are shown in Table IV.

Effect of Temperature on pH. Figure 2 is a plot of pH versus temperature for a third buffer, 2 molar calcium chloride saturated with calcium hydroxide, containing no sodium chloride. The data for this plot were obtained using the potentiometer and the thermionic amplifying unit. The temperature coefficient for buffer plus electrodes in the range 20° to 35° C. is about 0.03 pH unit per degree. The temperature coefficients for the other buffers tested appeared to be of the same order of magnitude. If any of these buffers is to be used at a temperature greatly different than 24° C., its pH value should be determined at that temperature.

Inasmuch as the pH at any temperature is dependent upon the solubility of calcium hydroxide, one must be sure that the equilibrium condition has been realized at the time the solution is used. As far as possible, these buffers should be used only at the temperature at which they are stored.

Buffering Capacity. The buffer capacity was checked by titrating the buffers with 0.1 *N* sulfuric acid and with 0.1 *N* sodium hydroxide. In doing this, care was taken to shake the buffer so that the sample titrated contained some of the solid calcium hydroxide.

The acid titration of buffer 1 represented by curve *A*, Figure 3, resulted in a pH rise that agreed well with the calculated rise due to dilution alone. Addition of sulfuric acid to the buffer dissolves calcium hydroxide and precipitates calcium sulfate. The solubility of calcium sulfate is low enough ($S_{CaSO_4} = 2.25 \times 10^{-4}$ at 25° C.) (*4*) that one can assume virtually complete precipitation. This leaves only the effect of dilution.

Dilution with distilled water changes the molarity of the calcium chloride, with the greatest effect on pH at high concentrations. The pH change resulting when a solution 2 molar in calcium chloride and saturated with calcium hydroxide is diluted 1 to 1 is about 0.5 pH unit as determined from curve *B*, Figure 1. If a 0.2 molar solution is diluted 1 to 1, the pH change should be about 0.15 unit.

If one adds an acid, such as hydrochloric, that will not cause precipitation of calcium ions, a different effect is encountered. Calculation shows that the results of addition of hydrochloric acid will depend upon the ratio of the concentration of acid used to the original molarity of calcium chloride. Taking activity

Table II. Hydrogen Electrode Measurements of High pH Buffer Solutions at 25° C., Buffer 1

[0.2 *M* $CaCl_2$, 2 *M* NaCl, saturated with $Ca(OH)_2$]

Solution No.	pH
3-1	11.73
4-1	11.74
5-1	11.74
6-1	11.74

Table III. pH Values, Buffer 2

(Obtained at 23.8° ± 0.5° C. 0.2 molar in calcium chloride and saturated with calcium hydroxide)

Solution No.	Potentiometer		Model G pH Meters		Model H pH Meter	
	Solution age, days	pH	Solution age, months	pH	Solution age, days	pH
1-2	1	12.08	5 days	12.10	5	12.10
			4	12.08		
			7	12.02		
2-2	Fresh	12.118	4	12.08	Fresh	12.10
			7	12.03		
3-2	3	12.015	..	...	..	...
4-2	3	12.015	..	...	..	...
5-2	3	12.009	..	...	..	
6-2	3	12.019	..	...	..	...
7-2	..	...	4 days	12.12	..	...
			5 days	12.06		
			7 days	12.04		
			3	12.08		

Table IV. Hydrogen Electrode Measurements of High pH Buffer Solutions at 25° C., Buffer 2

[0.2 *M* $CaCl_2$ saturated with $Ca(OH)_2$]

Solution No.	pH
3-2	11.88
4-2	11.86
5-2	11.88
6-2	11.88

coefficients equal to unity, if the normality of the acid is greater than twice the molarity of the calcium chloride, the pH should drop upon addition of acid in the presence of excess calcium hydroxide. If it is just twice the concentration of the calcium chloride, the pH should remain constant. If the normality of the acid is less than twice the molarity of the calcium chloride, the pH should rise. Experiment showed that the titration of a buffer that was approximately 0.5 molar in calcium chloride with 0.1 *N* hydrochloric acid resulted in an initial rise in pH, as expected.

The addition of sodium hydroxide to these buffers precipitates calcium hydroxide and dilutes the solution. Both effects tend to raise the pH. As is seen in curve *B*, Figure 3, the pH change for buffer 1 is greater in the early stages of the alkaline titration than in the acid titration.

For use as a standardizing medium for high pH measurements, a buffer ought to have enough buffering capacity to ensure that the glass electrode attains its equilibrium condition rapidly. Perley has shown that the response time of glass electrodes in poorly buffered solutions is slow (*3*). The curves of Figure 3 show that the buffer capacity is adequate in this respect and in addition the buffer is capable of reacting, without an appreciable change in pH, with traces of previous solutions that may remain in the sample container or on the electrodes.

Table V. Duplicate pH Values

[Solution 0.2 molar $CaCl_2$, 2 molar NaCl and saturated with $Ca(OH)_2$, obtained with eight combinations of four glass and two calomel electrodes]

	Glass Electrode				Reference Electrode Averages	Glass Electrode Range
	A	B	C	D		
Reference electrode I	11.92	11.95	11.93	11.91		0.04
	11.90	11.94	11.94	11.90		0.04
Av.	11.91	11.94	11.94	11.90	11.92	
Reference electrode II	11.93	11.95	11.93	11.92		0.03
	11.93	11.96	11.95	11.93		0.03
Av.	11.93	11.96	11.94	11.92	11.94	
Glass electrode averages	11.92	11.95	11.94	11.92	11.93	
Reference electrode range	0.03	0.02	0.02	0.03		
					Total range	0.06

The data were analyzed (*1*) and are summarized as follows:

Source of Variation	Degree of Freedom	Mean Square
Glass electrodes	3	0.0010396
Reference electrodes	1	0.0006563
GE × RE	3	0.0000896
Duplicates	8	0.0000812

In order to guard against any lowering of pH due to the presence of acid, some calcium hydroxide should be present as a slurry. No difficulty has been experienced with small amounts of the precipitate adhering to the electrodes. It is recommended, however, that the slurry be allowed to settle before the electrodes are immersed in the solution, and that the electrodes be carefully washed and wiped after each use.

GLASS ELECTRODE MEASUREMENTS IN HIGH pH SOLUTIONS

The pH of buffer 1 (2 molar in sodium chloride) was found to be 11.85 with a standard deviation of 0.03 at 23.8° C. It was stated that part of the standard deviation represents the reproducibility of preparing the solutions and part is contributed by the use of different glass and reference electrodes. With the high pH buffer, an attempt was made to determine the precision of glass electrode measurements at this high pH. Duplicate measurements were made on buffer 1 using eight combinations of four glass and two calomel electrodes. The measured values are shown in Table V.

The standard deviation of duplicate determinations is about 0.009. The fact that the mean square for GE × RE is of the same magnitude indicates that there are no appreciable interactions among the electrodes tested. The much larger magnitude of the mean square for electrodes suggests that the variation between electrodes is the most important source of reduced precision in measurements of high pH solutions.

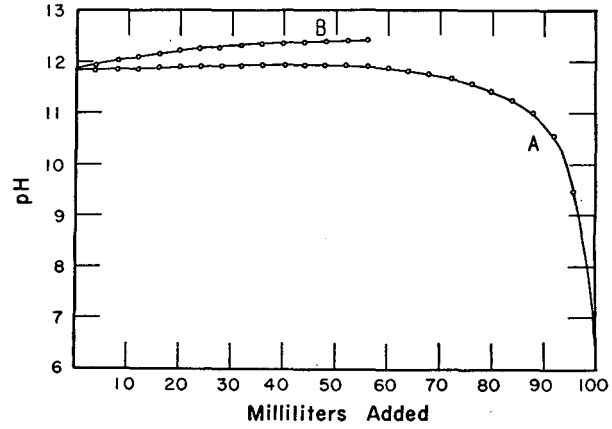

Figure 3. Buffer Capacity of Buffer 1

***A*. 50-ml. sample titrated with 0.1 *N* sulfuric acid**
***B*. 25-ml. sample titrated with 0.1 *N* sodium hydroxide**

The accuracy of glass electrode measurements at high pH values is also limited, especially in solutions containing sodium chloride. If both the standardization medium and the solutions to be measured have approximately the same sodium ion concentration, this error should be minimized (*2*).

CONCLUSION

Calcium chloride-hydroxide buffer systems may be prepared covering the pH range from 11 to 12.65, depending on the concentration of calcium chloride. Although the exact pH values of the buffers studied have not been determined, it is felt that the data presented are adequate to show the potential value of these buffers as standardizing media. The reproducibility and stability of the solutions are considered to be acceptable for such use. The addition of sodium chloride does not appear to affect the pH reproducibility or stability of the solutions to any extent. Although the pH values obtained with the glass electrode are probably somewhat in error, it is believed that they are of value as arbitrary standards.

The precision of glass electrode measurements in the pH range 11.0 to 12.0 is largely dependent upon the variability between electrodes.

ACKNOWLEDGMENT

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An Electronic Image Converter and Its Use in Chromatography

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An electronic image converter and its accessories, enabling observation of textures and color reactions of adsorption columns illuminated by an infrared light source, are described. The converter employs an image tube, Type CV-147, which converts infrared into visible light. The color sensitivity of the tube also permits observation of ultraviolet light. The color range of this device is 300 to 1400 millimicrons and its magnification power is 2.5×. It is suggested that the converter be applied to medical, criminological, and industrial investigations as well as problems in analytical chemistry. The addition of this device to chromatographic techniques necessitates a new or broader definition of the term "ultrachromatography," and the introduction of the terms "infra-" and "fluorochromatography."

COLORLESS substances adsorbed in a chromatographic column are difficult to locate. Karrer and Schöpp (*8*) and Winterstein and Schön (*17*) used filtered ultraviolet light for identification of some colorless substances because of their fluorescence. This method of identification, erroneously called ultrachromatography, is limited in its use because not all colorless substances fluoresce in the region of visible light when activated with ultraviolet radiation.

Some colorless substances form fluorescent compounds after their adsorption in the chromatographic column when chemical reagents are added (*3, 14–16*). Strain (*13*) suggested forming fluorescent compounds of colorless samples before they are adsorbed in the chromatographic column, and identifying them after their adsorption.

Some adsorbents interfere with the fluorescence of the compounds; they fluoresce and make identification of chromatographic layers difficult.

The author investigated reflection, absorption, and fluorescence in the infrared region of the spectrum of some colorless compounds to study the possibility of chromatographic identification using infrared as a light source. Infrared light is invisible to the human eye, and can be observed only by devices for converting infrared to visible light. Electronic image converters permit the observation of infrared absorption and reflections from specimens illuminated by various light sources.

Electronic image converters use image tubes as elements for converting infrared to visible light. As early as 1934, image tubes were used to study the infrared-reflecting and absorbing properties of materials. An image tube was attached to a microscope or telescope and objects illuminated with infrared light were observed (*2, 12, 18*). The image tube was improved in its physical and electronic performance during World War II (*9, 10*). An electronic eyepiece attached to a spectroscope, permitting direct observation of the infrared and ultraviolet portion of the spectrum, was described by the author at the Chicago meeting of the American Physical Society in 1947 (*6, 7*). The use of the image tube attached to a microscope (previously reported, *18*) was reported and applied to petrographic research in 1948 by Bailly (*1*). The importance of electronic image converters in analytical chemistry, and possible uses in criminology, medical sciences, and industry were pointed out by the author at the meeting of the AMERICAN CHEMICAL SOCIETY, September 1948 (*5*).

The transformation of infrared light into visible light by the use of the image tube is explained as follows:

The chromatographic column (Figure 1, center), illuminated by an infrared light source (150-watt projector bulb inserted into a light-tight box and equipped with an infrared filter, Corning No. 2540, Figure 1, left), reflects and absorbs a certain amount of infrared light. Infrared light reaches the electronic image converter (Figure 1, center), where an optical, infrared image is focused upon the photocathode of the image tube (also Figure 2, *P*). The electrons released by the photocathode of the image tube, *P*, fall upon a fluorescent screen, *W*, covered with a phosphor of a composition similar to that of cathode-ray tubes. Electrons activate the phosphor, *S*, to a greenish luminescence and the electron image formed on the screen, *S*, is observed through a magnifying eyepiece, *EP*. The image on the screen can be photographed on any green-sensitive film.

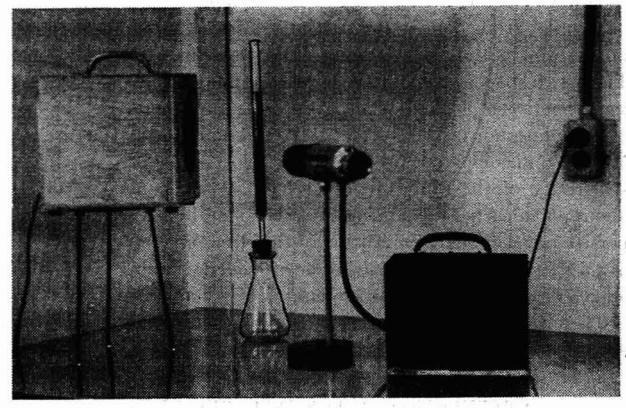

Figure 1

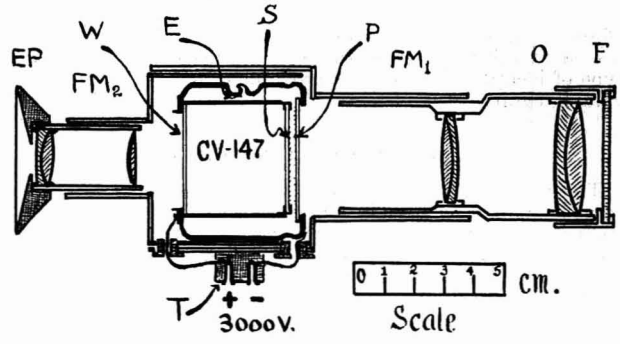

Figure 2

The electronic image converter, because of its simple construction, can be built by any individual familiar with electronic circuits. It (Figure 2) consists of the image tube *CV*-147 (*11*) (Figure 2, *CV*-147), the objective lens system, *O*, and the magnifying eyepiece, *EP*. The image tube is mounted in a brass shielding tube, the inside of which is lined with mica. An outer tube is slipped over the shielding tube. As a focusing mount, FM_1, for the objective lens system, *O*, a tube of somewhat smaller diameter is attached to the outer tube. The objective lens system, *O*, has

a focal length of 5.0 cm., and is used to focus the object on the photocathode, *P*, of the image tube. To prevent visible light from reaching the photocathode, a filter, *F*, can be slipped over the objective lens system (*15*). The magnifying eyepiece, *EP*, Kellner, with a focal length of approximately 6.0 cm., is placed in the focusing mount end of the image tube, FM_1. The image tube is connected to the power supply by a socket, *T*.

The power supply (Figure 1, right, and Figure 3) consists of a high voltage transformer (1800 to 2000 volts secondary), a filament transformer for the rectifier tube (2.5 volts secondary), a filter condenser of 0.25 microfarad having an insulation of 3000 volts, and a resistor of 5 megohms and one resistor of 10 megohms, both of 2-watt type. A resistor of 40 megohms shunts the 0.25-microfarad condenser. This shunt resistor consists of four 10-megohm resistors (2-watt type in series connection).

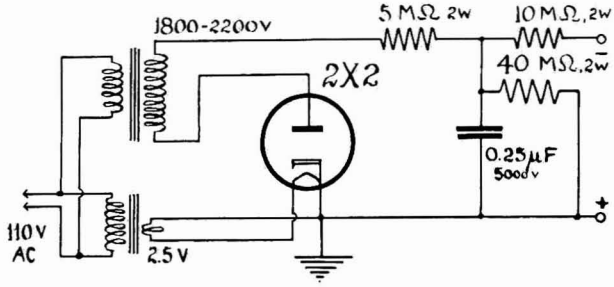

Figure 3

The electronic image converter enables the observation of ultraviolet from 300 to 400 mμ, and infrared from 760 to 1400 mμ, as well as the visible range of the spectrum (400 to 760 mμ). The greatest sensitivity of the electronic image converter is in the ranges between 350 and 450 mμ, and 560 and 900 mμ.

The phosphor of the screen has its maximum radiation around 525 mμ, and coincides closely with the color range of the maximum sensitivity of the human eye; it has a resolution definition of about 350 lines per inch, using television standards, and a medium persistency. The magnification of the electronic image converter is 2.5×, and can be increased or decreased, depending on the focal length of the objective lens system.

The electronic image converter can be used in different ways to aid the discrimination of layers in the chromatographic column.

OBSERVATION IN THE INFRARED REGION

The illumination of the chromatographic column with infrared light is achieved by placing a Corning No. 2540 infrared transmitting filter, polished, 2 mm. thick, in front of the light source. Observation is made in a dark room. If any infrared light is reflected, it will be detected through the image converter. This type of identification of chromatographic zones is termed infrachromatography.

OBSERVATION IN THE ULTRAVIOLET REGION

The illumination of the chromatographic column by filtered ultraviolet light is achieved by a mercury arc as light source in connection with a Corning No. 5860 ultraviolet transmitting filter, polished, 3 mm. thick. At the same time, a Corning No. 5860 ultraviolet transmitting filter, polished, 3 mm. thick, is attached to an electronic image converter to prevent any visible (fluorescent) light from reaching the photocathode of the image tube. The chromatographic column of borosilicate or quartz glass ensures maximum ultraviolet transmission. Individual chromatographic layers reflect ultraviolet light, and are observed by the electronic image converter. This method differs in principle from the method Karrer and Schöpp described in 1934 (*8*). This type of identification of chromatographic zones is termed ultrachromatography.

OBSERVATION OF FLUORESCENCE IN THE INFRARED REGION

To observe fluorescence in the infrared region of the chromatographic column, the electronic image converter is covered with a Corning No. 2540 infrared transmitting filter, polished, 2 mm. thick (Figure 2, *F*), which prevents all ultraviolet and visible light from reaching the image tube. The incandescent light source described above is used, and the Corning 2540 is replaced by a filter consisting of Corning Nos. 3389 and 4407, polished, standard thickness (*4*). This filter combination transmits visible light only.

Some substances require higher activation energies than the energy transmitted through the Corning No. 3389 and 4407 filter combination. When the former is omitted, a more bluish light will be transmitted, and this includes some ultraviolet. High activation energies can be obtained by using a Corning No. 5860 ultraviolet-transmitting filter in connection with a mercury vapor lamp.

Light reaches the chromatographic column and activates the adsorbed substances to fluorescence. Because only infrared light is transmitted to the image tube, and no infrared was present in the light source, any infrared observed through the electronic image converter originates in the adsorbed compound. This method of observation using a light source emitting shorter wave-length light than the light observed through the electronic magnifier is called fluorochromatography. This term is also applicable to the fluorescence produced in adsorbed layers when they are exposed to filtered ultraviolet light, and is synonymous with Karrer and Schopp's definition of ultrachromatography (*8*).

The above-mentioned observation methods suggest a more precise terminology: Ultrachromatography uses an ultraviolet light source, and the light observed is reflected from the chromatographic column, and is in the ultraviolet region of the spectrum. Infrachromatography uses an infrared light source, and the light reflected from the chromatographic column is in the infrared region of the spectrum. Fluorochromatography uses any convenient light source, and the light emitted from the chromatographic column is of longer wave length than the longest wave length emitted by the light source.

PREPARATION OF SAMPLES

Selected leaves of the mulberry tree, *Morus rubra* (200 grams), were boiled in 2000 ml. of distilled water for 3 hours and stored at 4° C. for several days. The extract was filtered, and a small portion of it was diluted with distilled water to ten times the original volume. This diluted extract, of brown-greenish color, was used in the adsorption tests.

IDENTIFICATION OF ZONES USING CHROMATOGRAPHIC COLUMNS

Activated charcoal, silica gel, alumina, magnesium oxide, and fuller's earth were used as adsorbants. Each adsorbant was placed in a borosilicate glass tubing 1.1 cm. in diameter and 35 cm. in length. Six columns were prepared for each type of adsorbant and the diluted extract was permitted to flow through the columns. Observations of the resulting chromatographic zones were made in visible light and by illumination of the chromatographic column with infrared light, using the electronic image converter (Figure 1). Table I shows the result of these observations.

Table I. Number of Zones Separated in Adsorption Column

		Eluant					
Adsorbant	Before Elution	Water	Ethyl alcohol	Ethyl ether	Acetone	CCl_4	Benzene
Charcoal							
Visible	?	0	0	0	0	0	0
Infrared	?	0	0	0	0	0	0
Silica gel							
Visible	4	3	4	4	4	4	4
Infrared	6	4	5	5	5	5	5
Alumina							
Visible	4	3	4	4	4	4	4
Infrared	5	4	5?	5	5?	5	5
Magnesia							
Visible	3	2	3	3	3	3	3
Infrared	5	3?	4	4	5	5	5
Fuller's earth							
Visible	4	3	4	4	4	4	4
Infrared	6	4	5	5	5?	6	6?

Table II. Number of Zones Separated by Paper Chromatogram

	Eluant					
	Water	Ethyl alcohol	Ethyl ether	Acetone	CCl_4	Benzene
Visible light	3	4	3	2	2	2
Infrared light	7	6	6	5	6	2

IDENTIFICATION OF ZONES USING PAPER CHROMATOGRAPHY

Filter paper strips, 6 × 200 mm., were cut and a small drop of the undiluted extract was placed on the strip 25 mm. from the lower end. The paper strip was immersed in the eluant. Six strips were prepared, one for each eluant (water, ethyl alcohol, ethyl ether, acetone, carbon tetrachloride, benzene). After 2 hours' elution the chromatographic zones were observed in visible and infrared light. Table II gives the results of the observations.

CONCLUSION

The experimental data indicate that the use of an infrared light source in connection with the electronic image converter increases discrimination of chromatographic layers.

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Reduction of Nitroammonocarbonic Acids with Titanous Ion in Acid Media

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The behavior of a number of compounds containing the —$NHNO_2$ group, when one attempts to reduce them with titanous chloride, has been studied. Such nitroammonocarbonic acids as nitroguanidine, nitroaminoguanidine, and their alkyl and acyl derivatives do not give reproducible values in reduction by titanous chloride. In such cases ferrous ions have a marked effect, in that the amount of titanous ion consumed varies with the amount of ferrous ion added. By adjusting the concentration of ferrous ion to a value dependent on the type of nitroammonocarbonic acid concerned, it is possible to attain a reduction equivalent of 6 titanous ions per nitro group. Both nitroguanidine and nitroaminoguanidine require about 0.75 to 0.85 equivalent of ferrous ion for this purpose; on the other hand, nitrourea, in either the presence or absence of ferrous ions, consumed only 2 equivalents. The use of suitably chosen concentrations of ferrous ion permits an assay of nitroguanidine and nitroaminoguanidine between 1.3 and 2.2%, comparable with that often obtained in assaying them by the Dumas nitrogen method. However, the reaction should not be used as a quantitative procedure unless one has an understanding of the pitfalls involved. Mechanisms are suggested to account for the consumption of 4 or more equivalents of titanous chloride in the reduction of nitro- and nitroaminoguanidine. The stoichiometric mechanisms assume that reductive cleavage may occur during the reduction.

IN THE synthesis of compounds of high nitrogen content intermediates containing the "nitramide" group, —$NHNO_2$, are of frequent occurrence. Nitroguanidine, nitrourea, nitroaminoguanidine, and their alkyl and aryl derivatives are typical examples of compounds which are widely used in this field. As a group they may be termed "nitroammonocarbonic acids" and at present no rapid and accurate methods are available for their assay. It is rational to approach this problem by utilizing the reactivity of the nitro group, which is the characteristic functional group common to them all. The present paper summarizes the results of an investigation of the reduction of nitroammonocarbonic acids by titration in acid media and the effect of ferrous ions on this reduction, together with suggestions as to the mechanism involved in the reduction.

PREVIOUS WORK

The titanous ion, in a high concentration of hydrogen ion, has long been used for the estimation of the aromatic nitro group (*10*):

$$6Ti^{+++} + C_6H_5NO_2 + 6H^+ \rightarrow C_6H_5NH_2 + 6Ti^{++++} + 2H_2O$$

An examination of the extensive literature indicates that this reaction is characteristic of the nitro group, 6 equivalents of titanous ion being required for each nitro group. The first work of any importance was done by Knecht and Hibbert (*9*), and has since been incorporated in their book (*10*). They found that titanous chloride in acid media reduced aromatic nitro groups to the amine, consuming 6 equivalents of the titanous ion for each nitro group. Their method was to dissolve the sample in water, acid, or alcohol, add an excess of acidic titanous chloride, boil the solution, and then titrate the unreacted titanous chloride with standard ferric alum solution, using potassium thiocyanate as the indicator. Kolthoff (*11*) found that the reduction potential of titanous chloride increased with decreasing acid content. Thus, titanous chloride is a much more powerful reducing agent in basic solution than in acid solution. Van Duin (*22*) had previously reported that the titer of titanous chloride solutions decreased as acid concentration increased. Kolthoff and Robinson (*12*) sug-

Table I. Summary of Preparation and Analysis by Dumas Nitrogen of Nitroammonocarbonic Acids

Name	Structure	M.P., ° C.	Analysis, %N Calcd.	Found	Preparation Reference
Nitroguanidine	$H_2NC(:NH)NHNO_2$	237–39	53.79	53.18	(4)
Nitrourea	$H_2NC(:O)NHNO_2$	164	39.95	39.95	(8)
Nitroaminoguanidine	$H_2NNHC(:NH)NHNO_2$	189[a]	58.81	59.16	(21)
N-Ethyl-*N'*-nitroguanidine	$C_2H_5NHC(:NH)NHNO_2$	147–8	42.41	42.70	(16)
N-*n*-Butyl-*N'*-nitroguanidine	$CH_3(CH_2)_3NHC(:NH)NHNO_2$	84.5	34.99	34.72	(16)
N-Phenyl-*N'*-nitroguanidine	$C_6H_5NHC(:NH)NHNO_2$	152	...	...	(16) [b]
N-(*m*-Methylphenyl)-*N'*-nitroguanidine	$(m)CH_3C_6H_4NHC(:NH)NHNO_2$	125.5-6.5	...	...	(16) [b]
Acetylnitroaminoguanidine	$CH_3CONHNHC(:NH)NHNO_2$	194	...	...	(16) [b]
Benzoylnitroaminoguanidine	$C_6H_5CONHNHC(:NH)NHNO_2$	196.5	...	...	(16) [b]
Benzaldehyde nitroguanylhydrazone	$C_6H_5CH:NNHC(:NH)NHNO_2$	186–7	...	...	(16) [b]
Nitrosoguanidine	$H_2NC(:NH)NHNO$	160[a]	63.04	63.64	(14)

[a] Explosion point.
[b] From U. S. Naval Ordnance Test Station, China Lake, Calif.

gested using a buffer in the titanous chloride reduction, recommending sodium citrate. The reduction of nitro aromatics proceeded rapidly at room temperature. This method is still used in the dye and explosive industry.

Evenson (*6*), in a recent paper, recommended the use of mono- or disodium tartrate as the buffer in the titanous chloride reduction. Robinson (*20*) first reported the use of 8 equivalents of titanous chloride for the reduction of a mononitro aromatic compound, *p*-nitrophenylhydrazine. He proved that the hydrazine was reductively cleaved, and the products were *p*-phenylenediamine and ammonia. This was corroborated by MacBeth and Price (*15*). Robinson suggested that the reductive cleavage was the first step in the reduction of *p*-nitrophenylhydrazine. He cited the work of Hyde (*7*), who had found that this compound decomposes when heated in a sealed tube with hydrochloric acid. Titanous chloride reduction is used largely in the explosives and dye field. Knecht and Hibbert (*10*), Callan and Henderson (*3*), English (*5*), Becker (*1*), and Butts *et al.* (*2*) have all reported the use of titanous chloride to reduce nitro nitrogen in explosives. Rathsburg (*18*) reported the reduction of the "nitration product of hexamethylenetetramine" with titanous chloride, but did not give very complete data. He apparently found that 12 equivalents of titanous chloride (4 equivalents for each nitro group) were required. Oldham (*17*) found that only 7.5 equivalents of titanous chloride were required to reduce a nitrate group in aliphatic nitrates. He attributed the low results, as compared to the theoretical 8 equivalents, to the loss of nitric oxide.

No fundamental work on the titanous ion reduction of the nitroammonocarbonic acids, as a group, has been reported in the literature. However, Kouba, Kicklighter, and Becker (*13*) have recently presented data on the titanous chloride reduction of nitroguanidine, which were used as a basis for an assay of that substance. They reported that 1 mole of nitroguanidine required 4 moles of titanous chloride and inferred that similarly constituted nitramines should require 4 equivalents of titanous ion per nitro group. The procedure they used was essentially that of Knecht and Hibbert (*10*).

EXPERIMENTAL

Preparation and Purity of Nitroammonocarbonic Acids. The nitroammonocarbonic acids used in this investigation were prepared by methods described in the literature. A number of these were obtained through the courtesy of Ronald Henry, U. S. Naval Ordnance Test Station, China Lake, Calif. These are summarized in Table I, all compounds being assayed for purity by melting point and elementary nitrogen analysis.

The additional compounds used for orientation purpose comprised Eastman Kodak White Label reagents, used without additional purification.

Reagents. The reagents required for the reduction of nitroammonocarbonic acids with titanous chloride were prepared and standardized as described by Butts, Meikle, Shovers, Kouba, and Becker (*2*).

Procedure. The sample (0.0500 to 0.3000 gram) was accurately weighed in a 50-ml. beaker and transferred quantitatively to a 300-ml. Erlenmeyer flask with 50 ml. of 1 to 1 hydrochloric acid. Nitrogen, previously scrubbed with Fieser's solution to remove oxygen, was passed through the flask by means of the inlet tube for 5 minutes. Fifty milliliters of standard titanous chloride were added to the solution from the automatic buret. A condenser was filled with cracked ice and placed on the flask. The solution was then refluxed for 15 minutes on an electric hot plate. A slow stream of nitrogen was continuously passed through the solution. After the reflux period, the solution was cooled and titrated with standard ferric alum solution, using 5 ml. of the ammonium thiocyanate as the indicator.

This procedure was used for the majority of the experiments. In later experiments, ferrous sulfate solution was added by buret or pipet to the solutions before the reflux period.

In all cases, a blank determination was run at the same time and in the same manner as the sample. The blank was made up of all the reagents used for reducing the sample, but contained no sample.

Calculations. The number of equivalents of titanous chloride consumed during the reduction was calculated by substitution in the equation

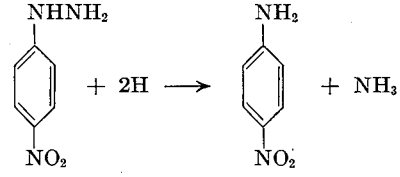
$$\frac{\text{Equivalents of } TiCl_3 \text{ consumed}}{\text{mole}} = \frac{(B - A) \times N \times M}{1000 \times W}$$

where

A = ml. of ferric alum solution used to titrate sample
B = ml. of ferric alum solution used to titrate blank
N = normality of ferric alum solution
M = molecular weight of sample
W = weight of sample

EXPERIMENTAL RESULTS AND DISCUSSION

The initial phase of this investigation consisted in checking the titanous chloride reagent, using the procedure of Kouba, Kicklighter, and Becker (*13*), for the reduction of the aromatic nitro group and in determining whether reductive cleavages occurred in certain hydrazine and nitro-substituted hydrazine derivatives. 3-Nitrophthalic acid and *p*-nitroaniline were found to consume exactly 6 equivalents of titanous chloride. Various hydrazine compounds—e.g., hydrazine sulfate, phenylhydrazine, aminoguanidine bicarbonate, and semicarbazide hydrochloride—were found to be entirely inert to the reagent. However, *p*-nitrophenylhydrazine required approximately 8 equivalents of titanous chloride, while 2,4-dinitrophenylhydrazine approached 14 equivalents for complete reduction, indicating that reductive cleavage of the hydrazine group had occurred:



the resulting nitro compounds then consuming the normal equivalents of reagent. This had been previously observed by Robinson (*20*) and MacBeth and Price (*15*). On the other hand, *m*-nitrophenylhydrazine was found to consume exactly 6 equivalents of titanous chloride for complete reduction. These data

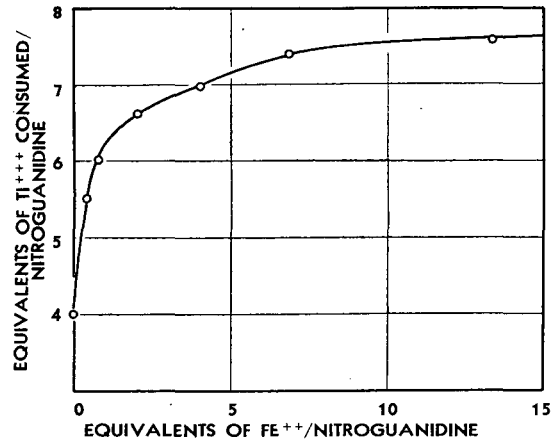


Figure 1. Effect of Concentration of Ferrous Ion on Titanium-Nitroguanidine Ratio

1 to 1 hydrochloric acid, 15-minute reflux

indicate that the reductive cleavage of the hydrazine group is influenced by the orientation of the nitro group in the nucleus, the lack of reductive cleavage in meta-orientation being consistent with modern electronic theories (*19*) of the effect of substituents on aromatic nucleus reactivity. These observations were considered to have some bearing on possible nitrogen-nitrogen bond cleavages in the titanous chloride reduction of the nitroammonocarbonic acids. The inertness of the hydrazine to the titanous chloride reagent also indicates that if the nitroammonocarbonic acids are reduced to hydrazines—e.g., aminoguanidines, diaminoguanidine, etc.—they would not undergo reductive cleavage under the conditions used, and any postulated mechanism must take this into account.

The work of Kouba, Kicklighter, and Becker (*13*) on the reduction of nitroguanidine was now investigated. The procedure of Kouba *et al.* involves the use of a 200 to 300% excess of titanous chloride in 1 to 1 hydrochloric acid, using a reflux period of 15 minutes. The work of Kouba *et al.* was substantially confirmed, but the data demonstrated that the application of this reagent was very erratic, especially when extended to other nitroammonocarbonic acids such as nitrourea, nitroaminoguanidine, and substituted nitroguanidines. The variation in the equivalents of titanous chloride consumed per mole of nitroguanidine varied from 3.75 to 4.21 and in the case of nitroaminoguanidine the maximum equivalents of titanous chloride consumed was 3.4. The lack of reproducibility in the reduction of nitroguanidine and the low values obtained for nitroaminoguanidine indicated that the reaction was probably not quantitative. It seemed likely that slight changes in the procedure described by Kouba *et al.* (*13*) could increase or decrease the amount of reduction.

A study of the effect of time and acid concentration on the reduction of nitro- and nitroaminoguanidine was carried out. Substantially similar results were obtained, the variation in equivalents of titanous chloride consumed per mole of nitroguanidine being from 3.5 to 4.6, while for nitroaminoguanidine it was from 3.4 to 4.4. Dissolving the samples in glacial acetic acid had the greatest single effect in increasing the equivalents of titanous chloride consumed. The time of reflux did not affect the extent of reduction. For nitroguanidine the reduction was found to have proceeded to its limits after 7 minutes.

Kouba, Kicklighter, and Becker (*13*) attempted to assay cyclotrimethylenetrinitramine, popularly known as RDX, by the method described above. They found that only 60% consumption of titanous chloride was obtained, based on 4 equivalents per nitro group. The addition of small amounts of ferrous ion was found to have a powerful effect on the reductive capacity of titanous chloride. However, Kouba, Kicklighter, and Becker did not investigate the effect of the ferrous ion on the reduction of nitroguanidine with titanous chloride.

Table II is a compilation of the data obtained for the reduction of nitroguanidine with titanous chloride in the presence of the ferrous ion as conducted in this investigation. It was found that nitroguanidine not only can consume the theoretical 6 equivalents of titanous chloride, but also approaches the value of 8 equivalents as found for *p*-nitrophenylhydrazine. As the concentration of the ferrous ion approaches very large values (Table II) the consumption of titanous chloride appears to drop from this maximum of 8 equivalents per mole of nitroguanidine. Figure 1 illustrates the very marked effect of the ferrous ion on the extent of reduction by titanous chloride, particularly at the very low concentrations of ferrous ion. These data indicate that if the combination of ferrous ion–titanous ion is used for the reduction of a nitroammonocarbonic acid, the concentration of the ferrous ion must be explicitly controlled in order to maintain a given stoichiometry. This is discussed further below.

The use of the ferrous ion to enhance reduction with titanous chloride was extended to nitroaminoguanidine and nitrourea, as shown in Tables III and IV. The reduction of nitroaminoguanidine under these conditions closely parallels that of nitroguanidine, and again it will be observed that very small concentrations of ferrous ions have a marked effect on the extent of reduction. The maximum consumption of titanous chloride does not reach as high a value as in the case of nitroguanidine.

Again, as in the case of nitroguanidine, a ferrous ion–nitroaminoguanidine ratio of 0.75 to 0.85 causes a consumption of approximately 6 equivalents of titanous ion per nitro group. However, the extent of reduction of nitrourea (Table III) by titanous chloride was unaffected by the presence of ferrous ion. Nitrourea is reduced with consumption of only 2 equivalents of titanous chloride, regardless of the presence of the ferrous ion.

Table V is a compilation of data obtained for substituted nitro- and nitroaminoguanidines. The data indicate that these compounds are reduced in a manner similar to their homologs. The

Table II. Reduction of Nitroguanidine with Titanous Chloride in Presence of Ferrous Ion

Expt. No.	Sample Weight, G.	Reduction Conditions[a] Equiv. $TiCl_3$ per mole	Reduction Conditions[a] Equiv. Fe^{++} per mole	Equiv. $TiCl_3$ Consumed per Mole
Control	0.1254	11.7	None	4.1
1	0.1025	11.0	0.35	5.5
2	0.1146	9.8	0.32	5.4
3	0.1034	10.9	0.70	6.0
4	0.1046	10.8	0.70	5.9
5	0.1123	9.7	1.9	6.6
6	0.1089	10.0	2.0	6.6
7	0.1044	10.4	4.4	6.9
8	0.1130	9.7	3.9	6.9
9	0.1032	10.9	7.0	7.4
10	0.1065	10.6	6.8	7.4
11	0.1052	10.7	13.8	7.5
12	0.1113	10.1	13.1	7.6
13	0.1183	9.5	30.7	7.3
14	0.1126	10.0	32.3	7.2

[a] 50 ml. of 1:1 HCl and 15-minute reflux time used in all cases.

Table III. Reduction of Nitroaminoguanidine with Titanous Chloride in Presence of Ferrous Ion

Expt. No.	Sample, Weight, G.	Reduction Conditions[a] Equiv. $TiCl_3$ per mole	Reduction Conditions[a] Equiv. Fe^{++} per mole	Equiv. $TiCl_3$ Consumed per Mole
Control	0.1089	11.7	None	3.4
1	0.1332	9.4	0.31	5.3
2	0.1084	11.5	0.38	5.3
3	0.1048	11.8	0.79	5.8
4	0.1067	11.6	0.78	5.8
5	0.1033	12.0	2.5	6.4
6	0.1121	11.0	2.2	6.5
7	0.1178	10.5	4.2	6.9
8	0.1072	11.5	4.6	6.8
9	0.1097	11.3	7.7	7.1
10	0.1056	11.7	7.9	7.2
11	0.1037	12.5	16.1	7.2
12	0.1035	12.5	16.1	7.1

[a] 50 ml. of 1:1 HCl and 15-minute reflux time used in all cases.

Table IV. Reduction of Nitrourea with Titanous Chloride

Expt. No.	Sample Weight, G.	Reduction Conditions: 1:1 HCl, ml.	Reflux time, min.	Equiv. $TiCl_3$ per mole	Equiv. Fe^{++} per mole	Equiv. $TiCl_3$ Consumed per Mole
1	0.1047	75	15	10.3	0	2.0
2	0.1100	75	15	10.3	0	2.0
3	0.1095	50	15	10.4	13.4	2.1
4	0.1013	50	15	11.2	14.5	2.1

Table V. Reduction of Nitroguanidines with Titanous Chloride

Expt. No.	Compound	Sample Weight, G.	Reduction Conditions[a]: Equiv. $TiCl_3$ per mole	Equiv. Fe^{++} per mole	Equiv. $TiCl_3$ Consumed per Mole
	Reduction of Aromatic Substituted Nitroguanidines				
1	Phenylnitroguanidine	0.1025	18.8	1.2	6.1
2		0.1081	17.8	1.2	5.9
3	*m*-Methylphenylnitroguanidine	0.1007	20.6	1.4	6.0
4		0.1030	20.1	1.3	6.0
5	Nitroguanylhydrazone of benzaldehyde	0.1185	17.3	1.1	6.0
6		0.1186	17.3	1.1	6.0
	Reduction of Alkylnitroguanidines				
1	*N*-Ethyl-*N'*-nitroguanidine	0.1043	13.3	0	4.2
2		0.1051	13.2	0	4.3
3		0.1048	14.7	0.88	5.4
4		0.1030	15.0	0.89	5.4
5		0.1035	13.4	1.8	6.2
6		0.1024	13.5	1.8	6.1
7		0.1154	13.5	16.0	7.3
8		0.1017	15.4	18.2	7.2
9	*N*-*n*-Butyl-*N'*-nitroguanidine	0.1046	16.1	0	4.3
10		0.1031	16.3	0	4.3
11		0.1249	13.7	0.90	5.3
12		0.1058	16.2	1.06	5.3
13		0.1010	16.6	2.2	6.0
14		0.1126	14.9	2.0	6.0
	Reduction of Substituted Nitroaminoguanidines				
1	Acetylnitroaminoguanidine	0.1249	13.8	0.90	6.0
2		0.1186	14.5	0.95	6.1
3	Benzoylnitroaminoguanidine	0.1083	22.0	1.44	6.2
4		0.1036	23.0	1.51	6.2

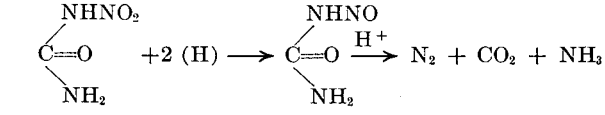
[a] 50 ml. of 1:1 HCl and 15-minute reflux time.

quantity of ferrous ion used in the titanous chloride reduction of these compounds can be precisely determined so as to cause the consumption of 6 equivalents of titanous chloride per nitro group. It will be observed from the data summarized in Table V that a somewhat higher ratio of ferrous ion to substrate is required to reach 6 equivalents of titanous chloride than in the case of nitroguanidine and nitroaminoguanidine. This is particularly noticeable in the case of the *N*-alkyl-*N'*-nitroguanidines, in which 1.8 to 2.2 equivalents of ferrous ion are required per mole of substrate.

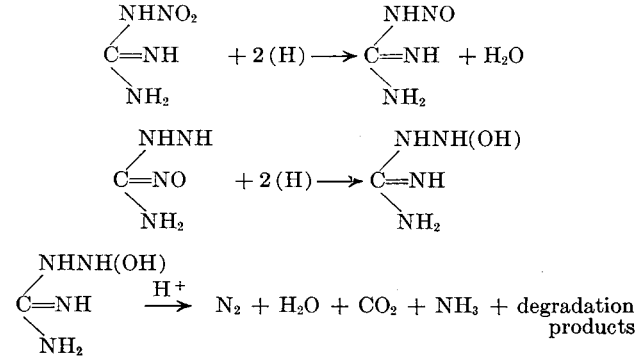
The variation on the effect of ferrous ion with type of nitroammonocarbonic acid under consideration detracts considerably from the value of titanous ion reductions as an analytical tool in this field, because it is necessary to determine precisely the specific ferrous ion concentration for 6 equivalent titanous ion consumption for each particular type of compound. One is even unable to generalize within a particular class, as marked differences exist between closely related homologs—e.g., compare *N*-ethyl- and *N*-*n*-butyl-*N'*-nitroguanidine. However, for both nitroguanidine and nitroaminoguanidine the critical concentration of ferrous ion for a 6-equivalent titanous ion consumption is between 0.70 and 0.80 equivalent per mole of substrate. This permits an assay of these substances with an accuracy between 1.3 and 2.2% as summarized in Table VI. This is the order of magnitude that is often obtained in a Dumas nitrogen assay of these substances. It is evident that although the adjustment of ferrous ion concentration is a troublesome feature of the titanous-reduction assay procedure, as described in this report, it does enable one to exercise a much more precise control on the equivalents of titanous ion consumed per nitro group, because in the absence of ferrous ion the consumption of titanous ion is much more erratic.

The data obtained for the reduction of nitroguanidine and nitroaminoguanidine indicate that the amount of titanous chloride consumed varies from 4 to a probable maximum of 8 equivalents. The reduction does not proceed in precise steps of 4, 6, and 8 equivalents of titanous chloride absorbed, the final amount of reduction often falling in between these values. However, mechanisms can be proposed for the consumption of various amounts of titanous chloride, depending upon the reduction conditions. The actual reduction obtained could then be considered as the net reduction, accomplished by different mechanisms. The data obtained are too incomplete to point out a definite mechanism, as no reduction products were isolated. However, the following mechanisms are proposed, keeping in mind that all the samples were boiled in moderately strong acid solution which could readily cause hydrolysis. It is assumed that the presence of the ferrous ion increases the amount of reduction that occurs. The consumption of 8 equivalents of titanous chloride requires the assumption that the reductive cleavage occurs as in the reduction of *p*-nitrophenylhydrazine.

The reduction of nitrourea, in either the presence or absence of ferrous ion, proceeds with the consumption of 2 equivalents of titanous ion. This is interpreted to mean that the nitrourea is reduced only to the nitroso stage, immediate decomposition setting in:



That nitrosourea has never been isolated is indicative of its unstable nature. On the other hand, nitrosoguanidine is stable enough to survive the conditions of a titanous chloride reduction consuming 2 equivalents of titanous ion, as summarized in Table VII. This presents some clue as to the mechanism operable to the 4 equivalent titanous reductions of nitro- and nitroaminoguanidine:



The products (nitrogen, carbon dioxide, etc.) arise from the hydrolysis of the "hydroxylamine"-type reduction product. A similar set of equations can be written for nitroaminoguanidine.

The consumption of more than 4 equivalents of titanous ion per nitro group requires the presence of ferrous ion. The consumption of 6 equivalents must proceed with the formation of the hydrazine derivatives.

Table VI. Assay of Nitro- and Nitroaminoguanidine

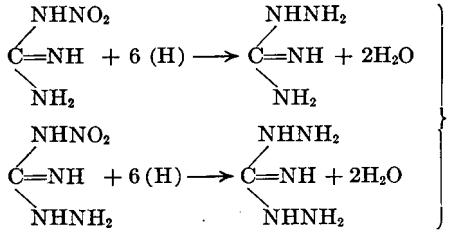
(In presence of 0.7 to 0.8 equivalent of Fe^{++}. Stoichiometry based on 6 equivalents of Ti^{+++})

Substance	Sought, G.	Found, G.	Error, Weight, G.	Error, %
Nitroguanidine	0.1034	0.1034	−0.0000	0.00
	0.1046	0.1029	−0.0017	1.63
	0.1065	0.1047	−0.0018	1.68
	0.1085	0.1067	−0.0018	1.65
	0.1027	0.1010	−0.0017	1.64
			Av.	1.32
Nitroaminoguanidine	0.1020	0.1003	−0.0017	1.67
	0.1120	0.1095	−0.0025	2.23
	0.1037	0.1022	−0.0015	1.45
	0.1105	0.1087	−0.0018	1.61
	0.1048	0.1014	−0.0034	3.24
	0.1067	0.1033	−0.0034	3.18
			Av.	2.23

Table VII. Reduction and Hydrolysis of Nitrosoguanidine with Titanous Chloride Reduction Procedure

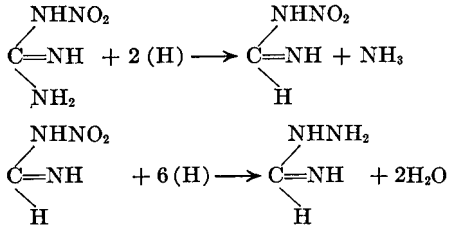
Expt. No.	Sample Weight, G.	Reduction Conditions[a] Equiv. $TiCl_3$ per mole	Equiv. $TiCl_3$ Consumed per Mole
1	0.1072	7.4	2.1
2	0.1004	8.0	2.1
3	0.1041	7.7[b]	0.14
4	0.1031	7.7[b]	0.07

[a] 50 ml. of 1:1 HCl and 15-minute reflux periods used in all cases.
[b] Titanous chloride added after sample had refluxed with acid. Thus, these samples were hydrolyzed, then reduced.

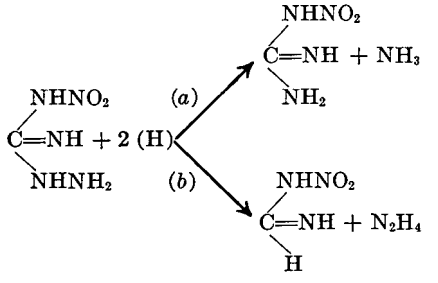


which are stable to further reductive cleavage

The consumption of 8 equivalents of titanous ion, requiring the presence of a high concentration of ferrous ion, must involve a reductive cleavage, probably as an initiating step. In the case of nitroguanidine this may proceed as follows:

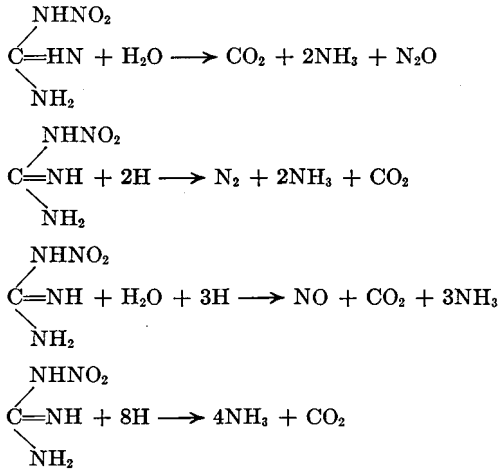


Two types of reductive cleavages may be postulated for nitroaminoguanidine



Route (*b*) is favored, because in route (*a*) an additional 2-electron cleavage is possible as already outlined for nitroguanidine. Route (*a*), in effect, would then require 10 equivalents of titanous ion as the limiting stage in the reduction of nitroaminoguanidine. However, in all cases studied thus far, a limiting value of only 8 equivalents has been approached.

The difficulty of postulating stoichiometric mechanisms for the types of reductions observed arises from the fact that it is not possible to isolate the products of these reductions from the strongly destructive media in which the reactions are carried out. On the basis of preliminary experiments with known hydrazides such as aminoguanidine, semicarbazide, etc., in which no titanous chloride was consumed, it was logical to select those conditions of ferrous ion concentration which led to 6 electron reduction as the analytical procedure to be used for estimation purposes. This would lead to reduction products which were not susceptible to further attack by the reagent. However, it will be noted from Figure 1 that the "6-equivalents" region is in the rapidly rising portion of the curve. One of the reviewers of this paper has postulated the reasonable assumption that the effects of ferrous ion, acetic acid, and other conditions are really the net result of shifts in the relative extents of processes such as



similar equations being possible for the other nitroammonocarbonic acids studied. No explanation can be offered at present as to why the presence of ferrous ion should stave off these breakdown reactions and induce further reduction, why a similar effect is not found with nitrourea, or why the amount of ferrous ion needed varies with the nature of the nitramine.

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Determination of Starch and Amylose in Vegetables

Application to Peas

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Sugars are extracted from peas with 80% ethyl alcohol and the starch is solubilized with dilute perchloric acid. Starch is then determined colorimetrically, without previous acid hydrolysis, by means of the sugar-anthrone–sulfuric acid reaction. Amylose is estimated colorimetrically with iodine. Both total starch and amylose can be determined in about 30 minutes in sugar-free solutions. The accuracy and precision are at least equal to those of standard methods involving acid hydrolysis. Analyses of smooth and wrinkled dried peas and fresh wrinkled peas are presented.

IN CONNECTION with work on the starch and amylose content of peas, it was necessary to analyze many samples, some of which were available in only small amounts.

Various methods for starch were examined. Those based upon optical rotation were unsatisfactory because of turbidity and the relatively large quantities of material required. Enzymatic hydrolyses are unreliable with starches of varying amylose-to-amylopectin ratios, while methods involving acid hydrolysis followed by reducing-sugar determinations are slow and require much of the analyst's working time.

A more expeditious method for starch and amylose in peas, applicable to small amounts of material, was required. Morse (*13*), Morris (*12*), Viles and Silverman (*19*), and Seifter *et al.* (*18*) used Dreywood's (*2*) color reaction of sugars with anthrone in sulfuric acid to determine sucrose, glycogen, and mixtures of pure dry starch and cellulose. None of these methods is directly applicable to the determination of starch and amylose in vegetables, but they offer a means of solving the problem.

This paper describes a method based upon the anthrone reaction, after removal of sugars with alcohol and solubilization of starch with dilute perchloric acid (*14, 15, 17*). Amylose is separately determined on an aliquot of the perchloric acid solution by its formation of an iodine-blue complex.

REAGENTS

Glucose Standard. Dissolve 0.100 gram of anhydrous glucose in 100 ml. of water. Preserve with 0.1% benzoate. Dilute 10 ml. of the stock solution to 1000 ml., and use 5 ml. for the standardization at 50 micrograms of glucose. Prepare the dilute standard daily.

Anthrone–Sulfuric Acid. Dissolve 2 grams of anthrone in 1 liter of cold 95% sulfuric acid. Store near 0° C. and prepare fresh every 2 days. This reagent is unstable and gives high blanks and variable results when too old. [Anthrone can be synthesized (*4*) or purchased. The Paragon Division, Matheson Company, Inc., Joliet, Ill.; the Panrone Chemical Company, Farmington, Conn.; and the National Biochemical Company, 3106 West Lake St., Chicago 12, Ill., are sources of anthrone known to the authors.]

Ethyl Alcohol–Water. Dilute 1680 ml. of 95% ethyl alcohol to make 2 liters of 80% alcohol.

Iodine–Potassium Iodide. Dissolve 2 grams of iodine in 20 ml. of a solution containing 20 grams of potassium iodide and dilute to 1 liter.

Perchloric Acid, 52%. Add 270 ml. of 72% perchloric acid to 100 ml. of water. Store in glass-stoppered containers.

PROCEDURE

Extraction of Sugars and Starch. DRIED PEAS. After grinding a composite sample of peas to pass a 60- to 80-mesh screen, weigh 0.200 gram of the flour into a 50-ml. centrifuge tube. Add a few drops of 80% alcohol to wet the flour and prevent clumping, add 5 ml. of water, and stir thoroughly. Add 25 ml. of hot 80% ethyl alcohol, stir thoroughly, and centrifuge after 5 minutes' standing. Decant and discard the alcoholic solution. Add 30 ml. of fresh hot 80% ethyl alcohol, stir, and centrifuge as before. Discard the alcoholic solution. Repeat this washing treatment twice more for a total of four washing treatments or until a test with anthrone is negative.

To the residue after the final centrifugation add 5 ml. of water, cool in ice water, and while stirring add 6.5 ml. of diluted perchloric acid reagent. Stir for about 5 minutes with a glass rod and occasionally thereafter for 15 minutes, keeping the mixture cold. Add 20 ml. of water and centrifuge. Pour the aqueous starch solution into a 100-ml. volumetric flask, add 5 ml. of water to the residue, cool in ice water, and stir while adding 6.5 ml. of diluted perchloric acid reagent. Solubilize as before for 30 minutes at 0° C. with occasional stirring and wash the contents of the tube into the 100-ml. flask containing the first extract. Dilute the combined solutions to 100 ml. and filter, discarding the first 5 ml. of solution. Cooling during solubilization is unnecessary if amylose is not determined.

FRESH PEAS. Blend 100 grams of fresh peas, or an amount sufficient to prevent sampling errors, with an equal weight of water in a mechanical blender for 5 minutes. Weigh a 5.00-gram sample of this slurry into a 50-ml. centrifuge tube and extract four times with 30-ml. portions of hot 80% alcohol by centrifugation and decantation or until a qualitative test with anthrone is negative.

After the final extraction add water to the sugar-free residue to make 10 ml. Cool the tubes and contents in an ice water bath and stir while adding 13 ml. of perchloric acid reagent. Stir occasionally for 15 minutes. Add 20 ml. of water, stir, and centrifuge. Pour the solution into a 100-ml. volumetric flask. To the residue add water to 5 ml., cool as before, and add 6.5 ml. of perchloric acid reagent. Solubilize as before for 15 minutes and wash the contents of the tube into the 100-ml. flask. Dilute the combined solutions to 100 ml. and filter, discarding the first 5 ml. of solution.

Determination of Starch. Dilute 5 to 10 ml. of the filtered starch solution to 500 ml. or to contain 25 to 100 micrograms of starch per 5 ml. of solution. Pipet 5 ml. of the diluted solution into a 25 × 250 mm. borosilicate glass tube, cool in a water bath, and add 10 ml. of fresh anthrone reagent. After the anthrone has been added to all of a series of sample tubes cooled in water, mix each one thoroughly and heat them together for 7.5 minutes at 100° C. Cool the tubes rapidly to 25° C. in a water bath and determine the color intensities, using light of wave lengths near 6300 A. (*12, 18*). Prepare a daily standard curve, using 0, 50, and 100 micrograms of glucose containing the same amount of perchloric acid as that in the starch aliquots, and use this calibration curve to obtain the yield of glucose from starch. Using the Klett-Summerson colorimeter, the K-64 filter, and the 12.5-mm. tube, scale readings of color intensities from the anthrone-sugar reaction, with 0 to 100 micrograms or more of glucose, fall on a line passing through the origin. Multiply glucose found by 0.90 to convert to starch.

Determination of Amylose. Dilute 5 ml. of the sugar-free starch solution containing about 5 mg. of starch with about 400 ml. of water. Add 5 ml. of iodine–potassium iodide reagent, mix, and dilute to 500 ml. After at least 15 minutes, determine the intensity of the blue colors with light of wave lengths near 6600 A. Use an iodine blank containing the same amount of perchloric acid as that in the samples to be analyzed. The Klett-Summerson colorimeter scale reading with 5 mg. of pea amylopectin was 25 (iodine "sorption" 0.2%) and that with purified pea amylose was 330 (iodine "sorption" 18.4%), using the K-66 filter and 20-mm. glass cell. (The iodine sorptive values were determined by plotting the e.m.f. against milliliters of iodine solution. The end point was taken at the point of inflection.) Scale readings of mixtures of these components totaling 5 mg. fall on a straight line

connecting these points. Other starch fractions behave similarly (*7, 8, 11*). Correct the observed scale reading to 5 mg. of starch material and read the per cent amylose from the graph.

VARIABLES AND LIMITATIONS

In carrying out the procedure it is necessary to extract the sugars from the pea flour or slurry, inasmuch as they react with anthrone. Care must be exercised to prevent contamination of solutions with lint, saliva, or other carbohydrate-containing materials. Pea proteins in the concentrations encountered here do not interfere; they may contribute to turbidity but do not yield an appreciable color with anthrone.

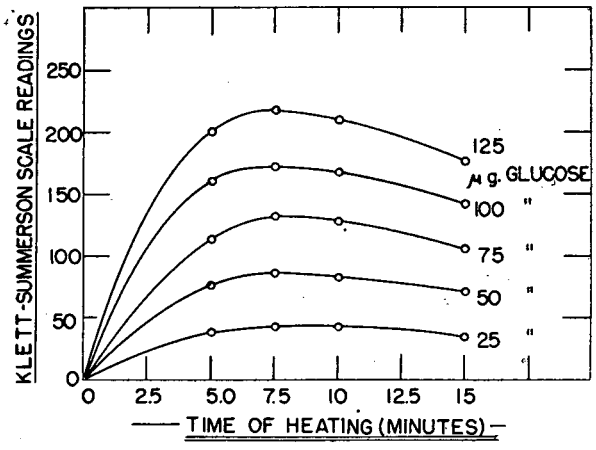

Figure 1. Effect of Time of Heating Glucose with Anthrone–Sulfuric Acid Reagent.

Modification of the proposed method along the line of the procedure of Pucher *et al.* (*17*) will extend the scope of the method to other starchy vegetables or fruits.

It is necessary to carry out two extractions with dilute perchloric acid (*17*), because 5% of the starch is extracted in the second treatment. When amylose is to be determined, the starch extraction should be carried out in an ice bath. Significant decreases in intensity of the iodine-amylose blue solution results when amylose extracts are in contact with dilute perchloric acid (4.8 *M*) for even 30 minutes at 25° C. No measurable decreases occur at 0° C. for an hour or more. For the most precise measurements of the "blue value" it is advisable to discharge the iodine color with a crystal of thiosulfate and determine a possible turbidity blank. This blank, sometimes 2 divisions on the Klett-Summerson scale, should be subtracted from the gross reading of the blue values.

In the determination of starch with anthrone, the samples of sugar-free starch solution in test tubes are cooled in a water bath during the addition of the anthrone reagent. This precaution ensures a similar heating treatment for all samples in a given series and prevents erratic results from the heat developed by the water–sulfuric acid reaction. Figure 1 shows the results of heating various quantities of glucose in anthrone–sulfuric acid for different times. The heating period of 7.5 minutes produced the maximum color intensities measured with light of 6300 A. wave length.

Starch, sucrose, pentoses, and pectic acid were heated with the anthrone reagent. The intensities of the colors compared to a glucose standard are presented in Table I. Starch and sucrose yield the calculated quantities of glucose as if they were first hydrolyzed to simple sugars. Pentoses and the uronic acids, both of which yield furfural among their dehydration and degradation products, did not interfere in the concentration encountered here.

Air-dried starches, starch fractions, and some fresh wrinkled peas were analyzed by the proposed method and by acid hydrolysis, as suggested by the association of Official Agricultural Chemists (*1*), followed by Hassid's method (*5, 6*) for determining reducing sugars. A factor of 0.9 was used, although Etheredge (*3*) and others (*10*) reported factors varying from 0.90 to 0.94 to convert glucose to starch. A summary of these results is presented in Table II. The results are averages of two or more determinations and variations in replicate analyses agree within ±1.5%. The precision of anthrone values is equal to the method using acid hydrolysis. Close agreement may be obtained between the two methods if a factor of 0.92 is used for the conversion of acid hydrolysis–glucose values to starch.

Table I. Direct Analyses by Anthrone-Sugar–Sulfuric Acid Reaction

Substance, 100 γ	Glucose Equivalent	
	Found, γ	Theory, γ
Glucose	100	100
Sucrose	106[b]	105.2
Starch[a]	110	111
Arabinose	2	...
Xylose	2	...
Pectic acid	3	...

[a] Soluble starch, reagent grade, according to Lintner.
[b] Fructose yields same quantitative color reaction as glucose.

RESULTS AND DISCUSSION

Single samples (about 20 grams of dried peas) of several varieties of smooth and wrinkled dried peas were analyzed for starch and amylose. Starch was also isolated from several samples of

Table II. Analyses of Starches, Starch Fractions, and Fresh Wrinkled Peas[a] by Direct Acid Hydrolysis (Factor 0.90) and Proposed Anthrone Reaction

Sample	Starch, %		Anthrone/ Acid Hydrolysis Ratio
	Acid hydrolysis	Anthrone	
Wisconsin sweet pea amylopectin	87.2	90.0	1.03
Wisconsin sweet pea amylose	87.2	89.1	1.02
Alaska pea amylopectin	88.0	88.2	1.00
Alaska pea amylose	85.6	88.7	1.03
World Record pea starch	84.1	86.5	1.03
Thomas Laxton pea starch	74.8	75.6	1.01
Mammoth White pea amylopectin	84.4	88.2	1.045
Mammoth White pea amylose	87.8	90.0	1.02
Alah pea amylose	86.3	90.0	1.025
Alah pea amylopectin	85.0	87.5	1.03
Fresh wrinkled peas (sieve size 3)	15.3[b]	15.8	1.03
Fresh wrinkled peas (sieve size 8)	25.8[b]	26.1	1.01

[a] Ferry Morse No. 9, Thomas Laxton variety.
[b] Results presented on dry-solids basis.

Table III. Determination of Starch and Amylose in Dried Peas and Pea Starch

Variety and Sample	Seed Type	Granule Type	Starch, %	Amylose in Starch, %
Alderman peas	Wrinkled	Compound	33.9	68
Alderman starch	Wrinkled	Compound	..	65[a]
Stratagem peas, 1	Wrinkled	Compound	33.8	69
Stratagem peas, 2	Wrinkled	Compound	32.8	65
Stratagem starch, ?	Wrinkled	Compound	..	69[a]
Stratagem starch, 1	Wrinkled	Compound	92.8	72
Thomas Laxton peas, 1	Wrinkled	Compound	33.1	71
Thomas Laxton starch, 1	Wrinkled	Compound	94.0	72
Perfection peas, 1	Wrinkled	Compound	33.3	69
Perfection starch, 1	Wrinkled	Compound	92.2	70
Perfection starch, ?	Wrinkled	Compound	..	69[a]
Steadfast peas, 1	Wrinkled	Compound	36.8	70
Steadfast starch, 1	Wrinkled	Compound	..	98[b]
First and Best peas, 1	Smooth	Simple	42.8	35
First and Best starch, 1	Smooth	Simple	94.6	36
Alaska peas, 1	Smooth	Simple	45.1	37
Alaska starch, 1	Smooth	Simple	93.6	37
Early Bird starch	Smooth	Simple	..	29[b]

[a] Calculated from iodine "sorptive" capacity of starch (*9*).
[b] Calculated from iodine-starch "blue value" of starch (*16*).

Table IV. Starch and Amylose in Fresh Wrinkled-Seeded Peas[a]

Sample	Sieve Size	Solids, %	Starch, %	Amylose in Starch, %	Amylose per Pea, Mg.
Lot 1	3	20.0	15.8	44	22
	4	21.6	15.8	47	34
	5	22.0	17.1	59	56
	6	23.1	20.1	63	82
	8	25.7	26.1	67	150
Lot 2	3	19.8	15.1	44	21
	4	20.6	14.6	55	36
	5	21.6	15.4	65	55
	6	23.0	20.6	69	92
	8	26.2	26.4	69	157

[a] Ferry Morse No. 9, Thomas Laxton variety.

these peas and analyzed for amylose. The results, presented in Table III, are compared with amylose determinations conducted by others. The samples were not the same in all cases. The results, however, are in general agreement with those of Nielsen (*14, 15*), Hilbert and MacMasters (*9*), and Peat, Bourne, and Nicholls (*16*) in the conclusion that starch of wrinkled peas is high in amylose. The highest amylose content encountered here is 72%; the authors' analyses of Steadfast peas indicate 70% of amylose in the starch rather than 98% as previously reported (*16*). It is concluded that wrinkled-seeded peas with compound starch granules contain about 34% starch of amylose content near 70%, and smooth-seeded peas 44% starch of amylose content near 36%.

Two lots (different harvest dates) of several sieve sizes of fresh peas (Ferry Morse No. 9, a wrinkled-seeded garden variety) were analyzed for starch and amylose (Table IV). The dry weight, total starch, and amylose in both the peas and the starch increase with sieve size. Peas are graded by size and there is a definite correlation between size and tenderness in any one variety grown in the same locality and harvested on the same date. There is nothing to refute the contention that a No. 4 size pea in one pod might be as mature as a No. 6 pea in a different pod. These data, however, seem to indicate that size did correlate with amylose content of the starch. This method of measuring both starch and amylose in peas may offer a means whereby maturity can be correlated with a chemical property.

CONCLUSION

A rapid method for the determination of starch and amylose in peas is described, by which samples as small as a single pea can easily be analyzed. Starch is estimated by the glucose-anthrone–sulfuric acid reaction. Amylose is determined by its iodine color reaction. The accuracy is at least equal to that of other methods and a great saving of time is realized.

Analyses of both dried and fresh peas are presented. Wrinkled peas contain about 34% starch of 70% amylose content. Smooth peas contain 44% starch of 36% amylose content.

The ratio of amylose to amylopectin in wrinkled pea starches increases as the peas mature.

ACKNOWLEDGMENT

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Determination of Pectic Substances in Cotton

Colorimetric Reaction with Carbazole

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A colorimetric method for the estimation of pectic materials in cotton is described. It involves the reaction of the extracted pectic acid with carbazole to give a colored reaction product.

PECTIC substances account for an appreciable portion of the noncellulosic constituents in cotton fiber (*3*). In studying the distribution of pectic acid and its correlation with the growth and properties of the fibers, a number of investigators have developed methods for its determination in cotton. Several (*1, 4, 5*) are based on the preliminary extraction with ethyl alcohol (4 to 24 hours), followed by extraction of the pectic acid and its precipitation as calcium pectate. The precipitates are difficult to filter and wash, and require long periods of drying to attain constant weight (*1*). In addition, they must be purified (*5*). Other methods (*4–6*), based on decarboxylation with hydrochloric acid require special apparatus and close attention over extended periods of time, frequently longer than the normal working day. Calculations of the results obtained by all these methods are based on empirical factors.

A colorimetric method is now presented, which is based on the evaluation of the extracted pectic acid by the reaction of carbazole with hexuronic acids (*2*). The values obtained are expressed in terms of anhydrogalacturonic acid, which is the fundamental unit of the pectic acid chain. This method uses samples

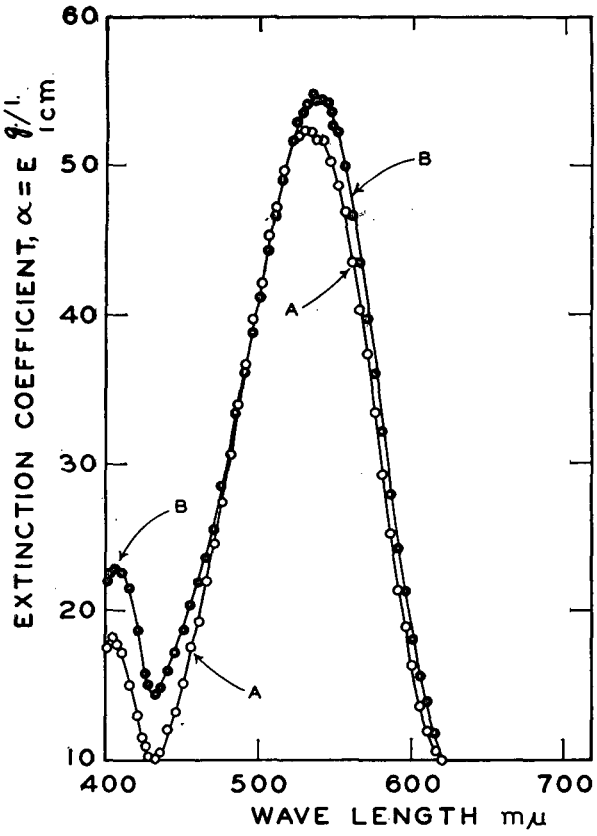

Figure 1. Absorption Spectrum of Galacturonic Acid-Alcoholic Carbazole System

A. **Galacturonic acid, sulfuric acid, alcoholic carbazole**
B. **Same with added dextrose**
Against blank without carbazole

about one seventh the weight of those required by the methods mentioned above, and can be adapted to even smaller samples. Only apparatus and equipment usually found available in the laboratory are required in carrying out the procedure specified.

REAGENTS

Acetic acid, 10 *N*, 60 ml. of glacial acetic acid diluted to 100 ml. with distilled water.

Ethyl alcohol, 95%.

Ethyl Alcohol, Purified. Reflux 1 liter of reagent grade, 95 to 100% ethyl alcohol with 4 grams of zinc dust and 4 ml. of 1 to 1 sulfuric acid at least overnight (preferably 24 hours). Distill, using all-glass apparatus. Redistill from zinc dust and potassium hydroxide, using 4 grams of each to 1 liter of alcohol.

Carbazole, reagent grade recrystallized from toluene.

Carbazole, 0.1% Solution. Dissolve 100 mg. of the recrystallized carbazole in purified alcohol and dilute to 100 ml. A blank of 2 ml. of water, 12 ml. of sulfuric acid, and 1 ml. of carbazole solution carried through the entire procedure should be water-white or nearly so. When compared with a blank in which water is substituted for the carbazole solution (3 ml. of water, 12 ml. of sulfuric acid), it should have a transmittance of about 95%.

Galacturonic acid, monohydrate, reagent grade.

Hydrochloric acid, dilute, approximately 0.03 *N*.

Salt Solution. Mix 800 ml. of 0.4% sodium hydroxide and 200 ml. of dilute hydrochloric acid, neutralize with 10 *N* acetic acid (ca. 7 ml.), and dilute to 2000 ml. (This corresponds, in ionic strength, to the sample solution, exclusive of the plant extractives from the cotton.)

Sodium hydroxide, 0.4%.

Sulfuric Acid, C.P. Reagent grade, free from contamination. Prepare standards with each different lot of sulfuric acid. Reject lots of acid that give erratic results.

METHOD

Weigh accurately about 1.5 grams of cotton which has been ground to pass a 20-mesh screen. Place sample in a suitable Soxhlet extractor, using a double-thickness paper thimble. Dewax by extracting overnight with 95% alcohol. Siphon alcohol off, rinse several times with ether, and then add fresh ether to the apparatus and extract for a short time to free the cotton completely of the alcohol. Remove the thimble from the Soxhlet and allow the sample to air-dry.

Transfer the cotton to a 100-ml. centrifuge tube, add 50 ml. of hot dilute hydrochloric acid, and heat in a boiling water bath for 15 minutes, with occasional stirring. Centrifuge at about 2000 r.p.m. (1000 × gravity) for 10 minutes. Decant the aqueous extract into a 500-ml. volumetric flask. Extract the residue in a similar manner, but with 10-minute heatings, with 50-ml. portions of the following hot reagents in the order: distilled water; twice with 0.4% sodium hydroxide; distilled water; twice with 0.4% sodium hydroxide; and three times with distilled water. Decant each extraction, after centrifuging, into the same volumetric flask. Add 1.7 to 1.8 ml. of the dilute acetic acid (ca. 10 *N*) in order approximately to neutralize the excess sodium hydroxide. Dilute to volume. (Solution may stand overnight before proceeding with the colorimetry.)

Filter through dry paper, discarding the first 50 ml. of filtrate. Pipet 2 ml. into each of two large test tubes (25 × 180 mm.), and slowly add exactly 12 ml. of concentrated sulfuric acid to each while the tubes are $^1/_4$ to $^1/_3$ immersed in ice water. (It is well to agitate the tubes constantly during the addition of the first 3 ml. and then after the addition of each 3-ml. increment.) Mix thoroughly and then heat for 20 minutes in a boiling water bath. Cool to room temperature, or slightly below, and add 1 ml. of 0.1% alcoholic solution of carbazole to one tube and 1 ml. of purified alcohol to the other tube. Mix thoroughly and allow to stand exactly 2 hours for complete development of color. Determine the per cent transmittance of the tube containing the carbazole by means of a photoelectric colorimeter equipped with a filter with maximum transmittance at 520 mμ, after having set the instrument with the blank tube. Compare this value with the standard curve to obtain the concentration of anhydrogalacturonic acid in the sample solution. Calculate the per cent anhydrogalacturonic acid in the cotton using the formula,

$$\% = \frac{\text{volume of solution} \times \text{concentration (in micrograms)}}{\text{sample weight (in grams)} \times 1{,}000{,}000} \times 100$$

STANDARD CURVE

Weigh accurately about 100 ± 0.3 mg. of galacturonic acid monohydrate (vacuum-dried over phosphorus pentoxide at room temperature), add 5 ml. of 0.1 *N* sodium hydroxide, and dilute to 1000 ml. with salt solution. Allow this standard solution to stand several hours or overnight before using. Prepare a series of working standards covering the range of 10 to 70 micrograms of galacturonic acid monohydrate per milliliter, by diluting aliquots of the standard solution with the salt solution. Calculate the concentrations of anhydrogalacturonic acid by multiplying the respective concentrations of the monohydrate in the series by their molecular weight ratio, 176/212. Develop color standards by treating 2-ml. aliquots of each dilute standard solution as described for sample solutions and record the percentages of transmittance. For the standard curve, plot log per cent transmittance against concentration of anhydrogalacturonic acid—this will be a straight line, or nearly so, if proper reagents have been used. It is preferable to obtain several sets of standard data and plot all points.

INTERFERENCE

Sugars will react with carbazole, but the color intensity is weak. Spectrophotometric curves of the color developed by a solution containing 50 micrograms of galacturonic acid per milliliter and that developed by a solution containing 50 micrograms of galacturonic acid and 50 micrograms of dextrose per milliliter are shown in Figure 1. The former shows a maximum at 528 mμ with an extinction coefficient of 52.3, while the latter shows the maximum at 534 to 538 mμ with an extinction coefficient of 54.7. With transmittance measured at 540 mμ, the presence of an equal amount of dextrose would introduce very little error in the determination of galacturonic acid. When a filter with maximum transmittance at 520 mμ is used, the error is even smaller because the curves are superimposed in this region.

The interference by amygdalin, arbutin, arabinose, xylose, sucrose, raffinose, dextrin, soluble starch, gum arabic, and agar was investigated and found to be of the same order of magnitude as that of dextrose.

Table I. Comparison of Methods for Pectin in Cotton

Variety	Anhydrogalacturonic Acid	
	Decarboxylation method	Colorimetric method
	%	%
Unknown	0.78	0.69
Wilds	1.13	1.07
Stoneville 2B	0.81	0.78
Stoneville 2B	0.79	0.71
Empire	0.91	0.83
Deltapine	0.71	0.66
Unknown immature	1.17	1.04
Wilds 17	0.81	0.73

Traces of impurities in the alcohol used to dissolve the carbazole interfered seriously by reacting under the conditions of the test to give highly colored products. This interference is eliminated by purification of the alcohol as specified.

COMPARISON OF METHODS

Eight different cotton samples were analyzed by the decarboxylation procedure of Whistler, Martin, and Harris (*6*). The linear regression equations for the straight-line portion of the carbon dioxide rate curves were calculated by the method of least squares. To determine the amount of carbon dioxide due to the uronic acid content of a cotton, the rate curves of the sample and the cellulose blank were extrapolated to zero time and the intercept of the latter was subtracted from that of the sample. The amount of pectic acid was calculated as anhydrogalacturonic acid using the factor 4.0, based on the assumption that there is 1 mole of carbon dioxide evolved for each anhydrogalacturonic acid unit of the polyuronide.

These samples were analyzed by the proposed colorimetric method described in this paper. Each result in Table I is the average of two or more determinations.

These data indicate that the colorimetric method gives values which are slightly lower than those obtained by the decarboxylation method. The values obtained by both methods are, however, in fair agreement for this type of determination.

Three of the cotton samples reported in Table I were used to check recovery of added pectin by the colorimetric method. The samples were dewaxed and transferred to centrifuge tubes. Aliquots of a standardized solution of low methoxyl pectin were added to the cotton samples, which were then analyzed by the method. The values shown in Table II indicate that the method gives satisfactory recovery of pectin. The colorimetric procedure, therefore, offers a rapid and convenient method for the determination of pectic materials in cotton.

Table II. Recovery of Added Pectin

Variety	Sample Weight	Anhydrogalacturonic Acid			
		Native	Added[a]	Found	Calculated
	Grams	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Wilds 17	1.5039	10.9	14.1	24.2	25.0
	1.5033	10.9	14.1	24.3	25.0
	1.5225	11.1	14.1	24.8	25.2
Stoneville 2B	1.5030	11.8	14.1	25.1	25.9
	1.5019	11.8	14.1	24.2	25.9
	1.5003	11.8	14.1	24.2	25.9
Wilds	1.5013	16.1	14.1	29.0	30.2
	1.5037	16.1	14.1	29.5	30.2
	1.5106	16.2	14.1	29.9	30.3

[a] Added as solution of low-methoxyl pectin.

ACKNOWLEDGMENT

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Determination of Long-Chain Hydroxamic Acids

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A procedure for determining long-chain hydroxamic acids consists in hydrolysis to carboxylic acid and hydroxylamine hydrochloride with a known excess of aqueous, alcoholic hydrochloric acid, followed by titration of either the excess hydrochloric acid or the hydroxylamine hydrochloride formed. The former technique gives slightly low results; the latter, slightly high. Hydroxylamine hydrochloride cannot be titrated in the presence of fatty acids containing ten or less carbon atoms.

IN A study of the reaction of glycerides and other esters of long-chain fatty acids with hydroxylamine, it was necessary to determine hydroxamic acids in the presence of unconverted esters in order to calculate the degree of conversion, and also to determine the purity of the hydroxamic acids isolated by crystallization. A method was desired which was rapid and accurate, did not require specialized apparatus, and could be employed as a routine method.

Direct titration of hydroxamic acids with alkali using phenolphthalein as indicator (*7*) was unsatisfactory because of the extremely low acid strength of long-chain hydroxamic acids. Reaction of hydroxamic acids with bromine (*6*) could not be employed because of interference by double bonds. Bromic acid oxidation (*4*) was unsatisfactory because of the difficulty in obtaining a suitable reaction system for the water-insoluble hydroxamic acids and aqueous potassium bromate (the source of bromic acid). Reaction systems containing ethyl alcohol, acetic acid, chloroform, and dioxane as cosolvents were investigated. It was thought that a reliable iodine number could be obtained on unsaturated hydroxamic acids, and the bromine reaction method (*6*) could be corrected for absorption of bromine by the double bonds present. This failed because even pure saturated hydroxamic

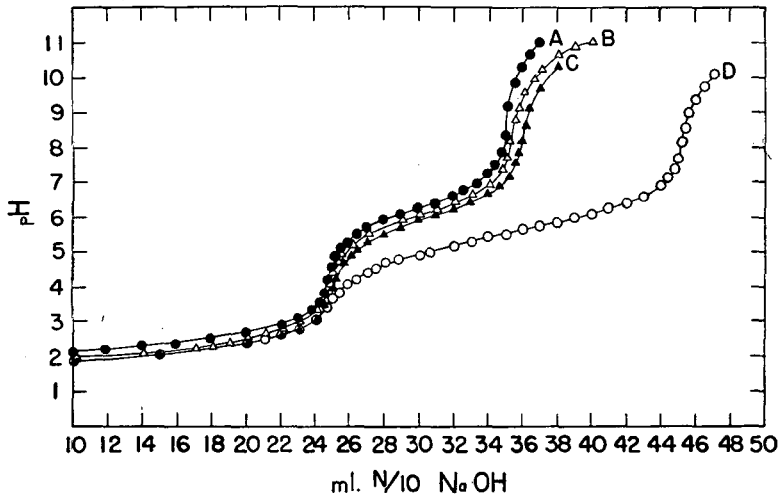
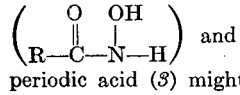


Figure 1. Titration Curves of Synthetic Mixtures

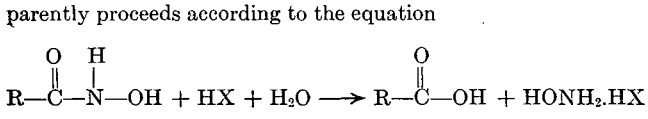
A. 0.00100 mole of hydroxylamine hydrochloride, 25 ml. of 0.1 *N* HCl, 50 ml. of H_2O
B. Same as *A* + 0.00106 mole of oleic acid
C. Same as *A* + 0.00100 mole of lauric acid + 10 ml. of 95% ethyl alcohol
D. Same as *A* + 0.00102 mole of caprylic acid + 10 ml. of 95% ethyl alcohol

acids consumed significant but variable quantities of Wijs solution.

The formal structural similarity between hydroxamic acids  and α-ketols suggested the possibility that periodic acid (*3*) might be a useful reagent for the analysis of hydroxamic acids. Investigation of the usual experimental variables (time, temperature, excess of periodic acid, etc.), as well as various solvent systems, indicated that the equivalent weight of the hydroxamic acids was approximately one third the molecular weight, when 80% acetic acid was employed as the solvent for the periodic acid and dioxane as the solvent for the hydroxamic acid. Results were erratic, however, and it was soon discovered that when peroxide-free dioxane was employed, no consumption of periodic acid occurred. It was demonstrated by a series of control experiments, in which dioxane-containing peroxide was employed but the periodic acid was omitted, that the peroxides in the dioxane were not reacting with the hydroxamic acids.

METHOD EMPLOYED

The observation by Inoue and Yukawa (*1*, *2*) that hydroxamic acids are quantitatively converted to the parent carboxylic acids by heating with dilute aqueous alcoholic sulfuric acid suggested that a simple hydrolytic reaction might be employed for the determination of hydroxamic acids. Because acid hydrolysis apparently proceeds according to the equation



is was evident that for each mole of hydroxamic acid hydrolyzed, one mole of hydroxylamine salt should be formed, consuming one mole of mineral acid. A simple approach, therefore, would be to employ a known excess of mineral acid in the hydrolysis, titrate unconsumed acid, and by difference obtain the quantity of mineral acid equivalent to hydroxylamine liberated, which in turn is equivalent to hydroxamic acid. Alternatively, by a differential technique it might also be possible to titrate liberated hydroxylamine salt directly. Consideration was also given to the direct determination of hydroxylamine liberated by the ferric-ferrous reaction, but it was eliminated from study because of the insolubility of iron salts of long-chain fatty acids.

It was necessary to determine whether free mineral acid and hydroxylamine salt could be determined in the presence of each other and free fatty acid.

In a control experiment, 0.0696 gram (0.00100 mole) of hydroxylamine hydrochloride, 0.2992 gram (0.00106 mole) of oleic acid, and 25 ml. of 0.1 *N* aqueous hydrochloric acid were mixed and titrated potentiometrically. At the first point of inflection in the titration curve, 24.8 ml. of 0.1 *N* aqueous sodium hydroxide had been consumed and at the second inflection an additional 10.4 ml. had been consumed. These titrations correspond to hydrochloric acid and hydroxylamine hydrochloride added, respectively (calculated, 25.0 and 10.0 ml.). Repetition of this experiment with synthetic mixtures containing equivalent quantities of myristic, lauric, capric, or caprylic acid instead of oleic acid gave satisfactory results in the titration of free hydrochloric acid in all cases (25.0 to 25.2 ml. of 0.1 *N* sodium hydroxide consumed), slightly high results (10.6 to 10.9 ml. of 0.1 *N* sodium hydroxide consumed) in the titration of hydroxylamine hydrochloride when lauric or myristic acid was present, and extremely high results in the titration of hydroxylamine hydrochloride in the presence of capric or caprylic acids. These last two compounds are stronger acids than the longer-chain acids and are titrated in large part before all of the hydroxylamine hydrochloride is neutralized. An additional control mixture containing only the hydrochloric acid and hydroxylamine hydrochloride was also titrated.

The titration curves for this mixture and three other mixtures containing a fatty acid are shown in Figure 1.

A systematic study was made of conditions necessary to achieve complete hydrolysis of hydroxamic acids and the following procedures given in detail were adopted.

HYDROXAMIC ACID ANALYSIS (ACID HYDROLYSIS)

Reagents. Ethyl alcohol, U.S.P., 95%. Hydrochloric acid of normality preferably between 0.9 and 1.0 *N*. Sodium hydroxide, 0.1 *N*.

Procedure. Weigh sample into small glass weighing cup, in accordance with the accompanying table, and place in special iodine flask with side arms to accommodate electrodes (*5*). (When the sample is known to contain less than 100% hydroxamic acid, its weight is increased proportionally.)

Approximate Equivalent Weight of Hydroxamic Acid	Weight of Sample
300	0.45–0.55
270	0.40–0.50
240	0.35–0.45
215	0.30–0.40
190	0.30–0.35
160	0.25–0.30

Add exactly 5 ml. of 1 *N* hydrochloric acid (a 200% excess of hydrochloric acid is employed) from a pipet and 10 ml. of 95% ethyl alcohol. Reflux the sample on the steam bath for 2 hours (4-hour reflux time is suggested when hydroxamic acid content is below 50%) and wash the stoppers and sides of the flask with 50 ml. of distilled water.

METHOD I. Titrate with 0.1 *N* sodium hydroxide to a pH of 4, using a pH meter with external electrodes. Run blank determinations.

$$\text{Hydroxamic acid, \%} = \frac{A \times N \times M.E.}{\text{weight of sample}} \times 100$$

where A = difference between blank and sample titration, ml.
N = normality of sodium hydroxide
$M.E.$ = milliequivalent weight of hydroxamic acid

$$\text{Equivalent weight of hydroxamic acid} = \frac{(\text{weight of sample})(1000)}{A \times N}$$

METHOD II. When it is known that acids containing ten or less carbon atoms are absent, hydroxylamine hydrochloride can be titrated, instead of or in addition to unconsumed hydrochloric acid, although higher results must be anticipated. The difference between titration to pH 4 and pH 8 is equivalent to hydroxylamine hydrochloride and to hydroxamic acid originally

present. The calculations shown above are used, except that A is the difference between the titrations at pH 4 and pH 8.

Several indicators were tried but were found unsatisfactory, probably because the change in pH was not sufficiently abrupt to give a distinct color change.

RESULTS

Table I shows the results obtained in the analysis of pure and crude hydroxamic acids, some synthetic mixtures, and a reaction product obtained from tallow and hydroxylamine.

The results obtained by Method I are usually a few percentage units low; those by Method II are a few percentage units high, except when acids containing ten or less carbon atoms are formed. Either method of titration can be used when the chain length of the hydroxamic acids is in the proper range, although Method I is more convenient because only one end point is required and knowledge of the chain length is not necessary. Although the absolute accuracy of the method is not so good as is usually desired, the results are sufficiently accurate for most purposes. Precision of duplicate determinations is usually within 1%. The main advantage of the method is its simplicity.

ACKNOWLEDGMENT

The authors thank Jean Idell and Jeanne Stutzman for technical assistance.

Table I. Hydroxamic Acid Analyses

Material Analyzed	Hydroxamic Acid Found, % Method I[a]	Method II[b]
Oleohydroxamic acid, m.p. 64.1–64.7° [c,d]	95.9–98.5	99.2–103.2
Stearohydroxamic acid, m.p. 106.2–106.8° [c,e]	95.3–96.9	101.3–101.5
Caprylohydroxamic acid, m.p. 77.9–78.4° [c,f]	95.0–96.8	135–195
Stearohydroxamic acid, crude reaction product[c]	76.2–79.2	..
Oleohydroxamic acid, crude reaction product[c]	81.7–82.0	..
Oleohydroxamic acid, 77.2%; beef tallow, 22.8%	71.0	80.8
Oleohydroxamic acid, 60.2%; beef tallow, 39.8%	53.3	62.4
Oleohydroxamic acid, 0%; beef tallow, 100%	0–0.6	0–5.8
Stearohydroxamic acid, 75.0%; methyl stearate, 25.0%	68.1	72.6
Stearohydroxamic acid, 25.0%; methyl stearate, 75.0%	24[g]	31.1[g]
Reaction product of tallow with hydroxylamine[h]	50.1	..

[a] Difference between blank and back-titration to pH 4 employed in calculation.
[b] Difference between titrations at pH 4 and 8 employed in calculation.
[c] Prepared by mixing a solution of 0.1 mole of the methyl ester of the corresponding carboxylic acid in 180 ml. of absolute alcohol with a solution of 0.15 mole of hydroxylamine hydrochloride and 0.250 mole of NaOH in 180 ml. of absolute alcohol, and refluxing for 1 hour. Dilute aqueous HCl was then added to the cooled reaction mixture until the pH was 4 and the mixture was diluted with a large volume of water. The crude reaction product was filtered, washed free of salt, and dried. It was recrystallized from petroleum naphtha, hexane fraction, boiling range 63° to 70° (10 to 15 ml. of solvent per gram of solute) at 0° to constant melting point.
[d] Calcd. C, 72.7; H, 11.9; N, 4.71. Found. C, 72.8; H, 11.7; N, 4.80.
[e] Calcd. C, 72.2; H, 12.5; N, 4.68. Found. C, 72.1; H, 12.4; N, 4.73.
[f] Calcd. C, 60.3; H, 10.8; N, 8.80. Found. C, 60.3; H, 10.7; N, 8.74.
[g] 4-hour reflux time during analysis.
[h] Prepared as described in [c], but 0.03 mole of tallow used instead of 0.1 mole of methyl ester.

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Separation of Iron as Basic Formate from Homogeneous Solution with Urea

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A study has been made of the separation of ferric iron from manganese, cobalt, nickel, copper, zinc, cadmium, magnesium, calcium, and barium. Ferric basic formate, precipitated from homogeneous solution by the hydrolysis of urea in acid solution, is denser, is more readily filtered and washed, and adsorbs fewer impurities than the precipitate obtained by any other hydrolytic procedure. A modified fractional precipitation process called two-stage precipitation gives still better separation.

THE technique of precipitation from homogeneous solution has been employed by Willard and co-workers (*6, 10–12, 14, 15*) and by Gordon and Caley (*5*) to produce dense precipitates, easily filtered and washed, which carry down relatively few impurities.

This paper extends the work involving hydrolytic precipitation employing urea to the separation of iron as ferric basic formate from manganese, cobalt, nickel, copper, zinc, cadmium, magnesium, calcium, and barium.

The experimental portion of the work comprises three parts. First, a study was made of the effectiveness of the separation of iron from a second metal by precipitating a definite quantity of iron from a solution containing the second metal in known concentration, then determining the latter in the washed, but not ignited, precipitate. In the second part the iron was precipitated from a solution containing the second metal, and this precipitate was ignited and weighed as ferric oxide. In the third part the second metal in the filtrate was determined.

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SEPARATION OF FERRIC IRON FROM OTHER METALS

Materials Used. All the metals used were in the form of reagent grade chlorides. All other chemicals were either reagent grade or C.P.

Method of Precipitation. Except where otherwise stated, each solution contained in 400 ml. the following substances in addition to the metal chlorides: 2 ml. of formic acid (specific gravity

Table I. Relation between Initial Concentration of Iron and Optimum Initial pH Value

Initial Concentration of Iron, G./400 Ml.	Optimum Initial pH
0.022	2.00
0.110	1.80
0.220	1.70
0.330	1.65
0.660	1.55

Table II. Iron Unprecipitated at Different Final pH Values

	Fe_2O_3 Unprecipitated, Mg./400 Ml.	
Final pH	H_2O_2 present	No oxidizing agent
3	0.05–0.1	0.9
4	0.01	0.5

Table III. Relation between Quantity of Iron Precipitated and Urea Required

Iron Precipitated, G.	Urea, G./400 Ml.	Time, Minutes, from Appearance of Turbidity to: pH 2.50	pH 3.0	pH 4.0
0.110	2.5	50–55	90	...
	4.0	...	50–55	135
0.220	5.0	...	55–60	120
0.330	3.5	100	...	...
	5.5	...	75	135
0.660	5.0	100–120	...	...
	7.5	...	75	135

1.20), 10 grams of ammonium chloride, 2.0 to 7.5 grams of urea (from a freshly prepared, 10% solution), and hydrochloric acid sufficient to give the desired initial pH. Each solution was heated to boiling and then transferred to an air bath heater designed to enclose most of the liquid portion of the beaker. A stirring rod with a 1-mm. carpet tack indentation was inserted in the beaker to prevent bumping. The solutions were then boiled for various periods of time. Except in the case of manganese, hydrogen peroxide was added 10 minutes before the desired pH was reached to ensure the complete oxidation of all the iron, because chloride ion reduces some of the iron in this formate solution.

The precipitates were filtered on a loose-texture paper and washed 15 times with hot 1% ammonium nitrate solution, usually adjusted to pH 4. With 0.336 and 0.672 gram of iron a small amount of filter paper pulp was added with stirring just before filtering the solution.

In all cases the precipitates were dissolved in concentrated hydrochloric acid and the adsorbed metals were then determined as described below.

Optimum Initial pH Value. Each solution was adjusted to a pH value just below the pH at which ferric basic formate began to precipitate. An optimum pH value must be selected in order to avoid a large excess of free acid which would have to be neutralized by urea, and yet there must be present sufficient acid to prevent the occurrence of a gelatinous precipitate prior to boiling. The ideal situation occurs when a turbidity appears 5 to 10 minutes after the solution has begun to boil.

Table I shows the optimum initial pH as a function of the initial iron concentration.

Final pH. Because chloride ion reduced ferric iron slightly, it was necessary to add an oxidizing agent near the end of the precipitation. Hydrogen peroxide was satisfactory. In Table II are given results comparing the residual iron found under varying conditions.

For practical purposes precipitation can be considered complete at pH 3 to 3.2

Quantity of Urea Required. The quantities of urea and the times required to reach certain pH values are given in Table III. These times are applicable where steady boiling conditions are maintained.

The amount of impurity in the precipitate was determined by the following methods.

Determination of Manganese. The hydrochloric acid solution was evaporated with sulfuric and nitric acids to fumes and diluted, and the manganese was determined by the colorimetric periodate method of Willard and Greathouse (*13*).

Determination of Cobalt. The hydrochloric acid solution was diluted to a definite volume with additional acid, the ferric iron in an aliquot portion reduced with dry stannous chloride, and the blue color (*8*) compared with standards similarly prepared.

Determination of Nickel. The nickel was precipitated with dimethyl glyoxime from a slightly ammoniacal solution containing tartrate. The larger precipitates were weighed; the smaller quantities were determined colorimetrically.

Determination of Copper. The hydrochloric acid solution was diluted to a definite volume and the copper in an aliquot portion was determined colorimetrically as copper pyridine thiocyanate in chloroform solution by the method of Chalk (*3*).

Determination of Zinc. After removal of the iron with cupferron, zinc was determined turbidimetrically as zinc ferrocyanide according to the method of Bodansky (*2, 9*).

Determination of Cadmium. After removal of the iron with cupferron, cadmium was determined turbidimetrically in slightly ammoniacal solution as cadmium sulfide (*4*).

Separation of Ferric Iron from Bivalent Manganese. Because bivalent manganese can be oxidized to higher valence states, the hydrogen peroxide added to effect complete iron recovery might be expected to introduce a complication. The effect of this oxidizing agent is clearly seen in the manganese curves of Figure 2. The addition of hydrogen peroxide increases the adsorption of manganese approximately 0.3 mg. in the case where 0.112 gram of iron is separated from 1.0 gram of manganese.

Separation of Ferric Iron from Cobalt, Nickel, Zinc, and Cadmium. The results are presented graphically in Figures 1 and 2. No cadmium was found in the precipitate.

Separation of Ferric Iron from Copper. The separation of ferric iron from copper was complicated by the formation of a basic copper chloride under certain conditions. Ferric basic formate normally is brownish, but under certain conditions it was greenish in color owing to its admixture with the basic copper chloride. Washing this greenish precipitate with 2 *N* ammonium hydroxide produced a very blue solution.

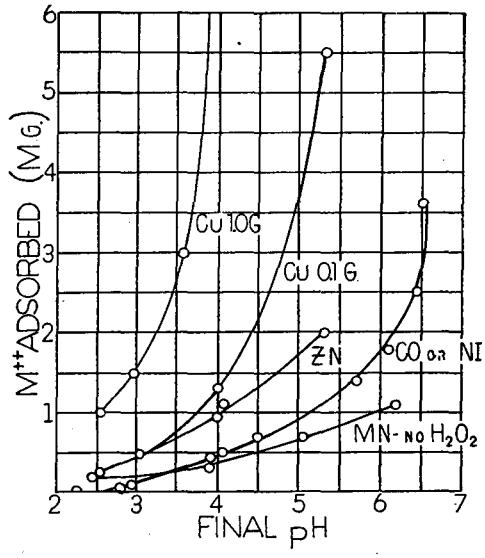

Figure 1. Relation between Final pH and Amount of Metallic Ion Adsorbed

Weight of precipitate corresponds to 0.112 gram of iron
Concentration of M^{++} 1.0 gram per 400 ml. except where noted

Basic copper chloride was precipitated at pH 4.2 and 3.8 when the concentration of copper was 0.02 and 0.04 molar, respectively. In the absence of iron, the basic chloride was precipitated at pH 4.2 for a 0.04 molar copper solution.

In Figures 1 and 2 can be seen the influence of the higher pH on the amount of adsorbed copper, which increased markedly owing to the formation of basic copper chloride.

The addition of hydrogen peroxide to hot solutions containing

cupric ion resulted in vigorous decomposition of the peroxide. Loss of some of the solution sometimes occurred. To avoid this difficulty it was necessary to allow the solution to cool for 2 or 3 minutes, before adding the peroxide, then to continue heating for a few more minutes.

Another effect, found only in the case of copper, and which may be intimately connected with this vigorous decomposition of the peroxide, is the influence of copper concentration on the amount of unprecipitated iron. The amount of iron remaining unprecipitated appeared to increase slightly with increased copper concentration.

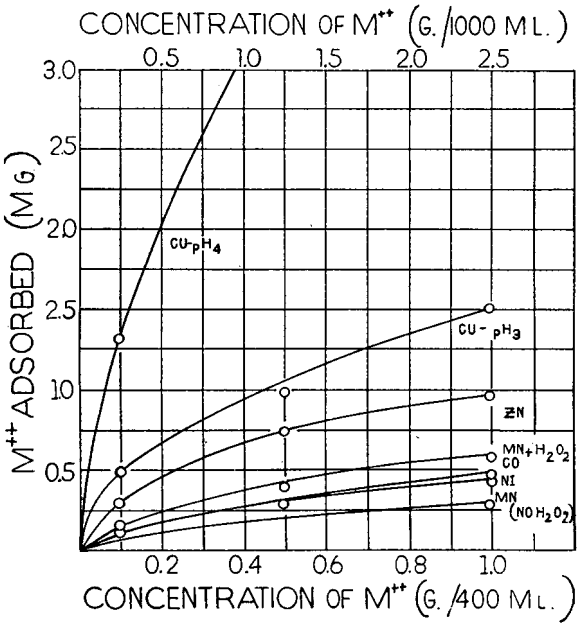

Figure 2. Relation between Concentration of Metallic Ion and Amount Adsorbed

Weight of precipitate corresponds to 0.112 gram of iron Final pH 4 except where noted

Separation of Ferric Iron from Trivalent Chromium and Chromate. The separation of ferric iron from trivalent chromium was not so sharp as from copper and zinc, even though the precipitation pH of trivalent chromium is the same as the latter two. The trivalent ion is adsorbed in larger amount. In separating 0.1 gram of iron from 0.1 gram of chromium, about 7.5 mg. of chromium were adsorbed. This amount was increased to 9.4 mg. when hydrogen peroxide was added, owing to partial oxidation to chromate which is more strongly adsorbed, as indicated by experiment.

Separation of Ferric Iron from Magnesium, Calcium, and Barium. Quantitative data for these metals are given in Table IV.

Relation between Initial Concentration of Iron and Amount of Second Metal Adsorbed. At constant pH and concentration of the second metal, the amount of the second metal adsorbed increased as the amount of iron precipitated was increased. The relationship between these two is not linear and is shown in Figure 3.

Effect of pH upon Adsorption of Cations by Ferric Basic Formate. In Figure 1 can be seen the effect of pH upon the adsorption of cations. Because the charge on the precipitate becomes less positive with increase in pH, as migration velocity studies have shown (*7*), the precipitate becomes a better adsorbent for cations as pH increases. Close examination of Figure 2 also shows that at pH 2.5 where only about 1 mg. or less of ferric oxide remains unprecipitated, the adsorption of the second metal is very slight. This has led to a new and efficient technique known as two-stage precipitation.

TWO-STAGE PRECIPITATION

The classical method of purifying an analytical precipitate which is contaminated with some component of the solution in which the precipitation occurred is to redissolve and then reprecipitate the entire quantity.

Two-stage precipitation takes advantage of the phenomenon of diminished adsorption at low pH, as shown in Figure 1.

In this method the pH of the solution during precipitation is allowed to rise to about 2.5, and the precipitate is then filtered off. Additional urea is added to the filtrate, the solution is heated until the pH has increased to 4, the small amount of iron thus obtained is filtered, and both precipitates are then combined.

The first precipitate obtained at the lower pH is obtained under conditions such that the contamination is slight. The second precipitate obtained at the higher pH will contain relatively more contaminant, but the size of the precipitate is so small that the contamination is negligible.

An alternative two-stage precipitation which could also be used and which would save time would employ the urea method for the first precipitation, followed by the addition of ammonia using Blum's procedure (*2*) for the second precipitation. In general, a single urea precipitation effects a satisfactory separation without recourse to either a two-stage or double precipitation.

In Table III are given the quantities of urea and the times required to reach pH 2.5 for given quantities of iron. When this pH is reached, additional urea is added, so that the total added equals the larger quantity of urea given in the table, and the filtrate is then boiled until pH 4 is reached.

DETERMINATION OF FERRIC IRON AFTER SEPARATION FROM OTHER METALS BY PRECIPITATION WITH UREA

Materials Used. Pure electrolytic iron was dissolved in hydrochloric acid and oxidized with hydrogen peroxide. This ferric chloride solution was standardized gravimetrically by precipitation with urea. Other metals used were reagent grade chlorides.

Method of Precipitation. The precipitations were carried out as described above with but slight modification. After the addition of 5 ml. of 3% hydrogen peroxide and a few minutes' additional boiling, 10 ml. of 0.02% gelatin solution were added to improve the filtering and washing characteristics of the precipitate. These solutions, 400 ml. in volume, could be filtered in about 3 minutes.

The film of adherent precipitate in the beaker, usually 25 to 30 mg. of ferric oxide, was washed after the dense precipitate was transferred to the filter, then dissolved by boiling 5 ml. of concentrated hydrochloric acid in the covered beaker. The procedure of Blum (*1*) was used to reprecipitate the iron, which was then filtered and washed on a separate paper. Both precipitates

Table IV. Determination of Iron after Separation from Various Metals

Element Added	Time of Boiling[a]	Final pH	Fe_2O_3 Taken	Fe_2O_3 Found	Difference
Gram	*Min.*		*Gram*	*Gram*	*Mg.*
1.0 Ba	120	4.02	0.2876	0.2885	+0.9
1.0 Ba	120	4.00	0.2876	0.2882	+0.6
1.0 Ca	120	3.93	0.2876	0.2881	+0.5
1.0 Ca	120	3.70	0.2876	0.2883	+0.7
1.0 Mg	120	4.00	0.2876	0.2877	+0.1
1.0 Mg	120	3.94	0.2876	0.2875	−0.1
1.0 Zn	55	3.15	0.1437	0.1440	+0.3
0.1 Zn	135	4.15	0.1437	0.1443	+0.6
1.0 Co	55	3.00	0.1440	0.1440	0
1.0 Co	55	2.97	0.1440	0.1438	−0.2
1.0 Co	55	2.98	0.1440	0.1442	+0.2
1.0 Ni	55	3.20	0.1437	0.1442	+0.5
1.0 Ni	55	3.10	0.1437	0.1443	+0.6
1.0 Cd	55	3.20	0.1437	0.1441	+0.4
1.0 Cd	135	4.10	0.1437	0.1441	+0.4
1.0 Mn	55	3.05	0.1437	0.1443	+0.6
0.1 Mn	55	3.08	0.1437	0.1441	+0.4
0.1 Cu[b]	55	3.12	0.1437	0.1442	+0.5
0.1 Cu[b]	60	3.10	0.1437	0.1442	+0.5
1.0 Cu[b]	60	2.95	0.1437	0.1456	+1.9

[a] After appearance of turbidity.
[b] 15 grams of ammonium nitrate substituted for 10 grams of ammonium chloride.

Table V. Two-Stage Precipitations

Iron Taken *Gram*	Element Added *Gram*	pH 1st Stage	Iron Pptd. 2nd Stage *Mg.*	Final pH 2nd Stage	Element in 1st Ppt. *Mg.*	Element in 2nd Ppt. *Mg.*	Element in Ppt. Formed at Same Final pH in One Ppt. *Mg.*
0.112	1.0 Co	2.25	3.5	4.10	Negligible	Negligible	0.5
0.112	1.0 Co	2.50	0.5	3.90	Negligible	Negligible	0.5
0.672	1.0 Co	2.35	0.7	3.85	0.1	Negligible	1.3
0.672	1.0 Co	2.55	0.7	4.20	0.3	Negligible	1.8
0.112[a]	1.0 Mn	2.55	0.7	4.10	0.2	0.01	0.6
0.672[a]	1.0 Mn	2.50	0.5	3.90	0.7	0.01	2.3
0.112[b]	1.0 Mn	2.45	3.5	3.90	0.2	0.01	0.3
0.672[b]	1.0 Mn	2.50	4.9	3.90	0.6	0.02	1.1
0.112	0.5 Cu	2.62	0.7	[c]	0.8	0.2	...
0.112	0.1 Cu	2.55	0.5	[c]	0.3	0.02	...

[a] Hydrogen peroxide used for both stages.
[b] Hydrogen peroxide used for second stage only.
[c] Ammonium hydroxide used for second precipitation.

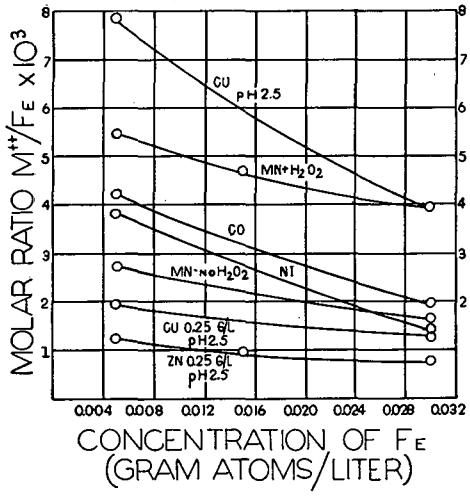

Figure 3. Relation between Concentration of Fe^{++} and Adsorption of Various Metal Ions

Concentration of M^{++} 2.5 grams per liter, final pH 4 except where noted

were ignited in a porcelain crucible for 1 hour at 850° C. in an electric muffle.

Iron in Filtrates. In some instances the iron in the filtrate and washings was determined. At pH 2.9 to 3.2, the filtrates contained 0.05 to 0.1 mg. of ferric oxide; at pH 4, 0.01 to 0.05 mg. of ferric oxide. The washings (250 to 350 ml.) contained 0.02 to 0.05 mg. of ferric oxide.

Final pH. A final pH of 3 was used for these separations, with the exception of barium, calcium, and magnesium. The very slight solubility of ferric basic formate at pH 3 is more than compensated for by the reduced contamination (Figure 1).

Results Obtained. The data obtained are given in Table IV. Acceptable separations were obtained of 0.2 gram of iron from 1.0 gram each of barium, calcium, and magnesium; of 0.1 gram of iron from 1.0 gram each of manganese, cobalt, nickel, zinc, and cadmium; and 0.1 gram of iron from 0.1 gram of copper.

The slightly high results obtained are probably due to the silica which is usually present when precipitation is made in glass vessels.

The use of a two-stage precipitation would undoubtedly improve the sharpness of these separations, as is evidenced by Table V, and would lead to even better results in the quantitative determination of ferric iron by precipitation with urea.

The method is not recommended for the gravimetric determination of iron but only as a separation from other metals, which are determined in the filtrate.

DETERMINATION OF OTHER METALS IN FILTRATE AFTER PRECIPITATION OF IRON AS BASIC FORMATE

The efficiency of this method is shown in Table VI, in which are given the results obtained by filtering the precipitate and determining the other metal in the filtrate by a standard method. The precipitation was performed as previously described. The final pH varied from about 3.0 to 3.2.

In the analyses shown in Table VI the nickel was determined by precipitation as nickel dimethylglyoxime and weighed as such. Manganese was determined volumetrically by the bismuthate method; cobalt was weighed as sulfate after evaporating the filtrate with sulfuric acid and igniting at 500°C., a blank on the reagents was run and subtracted. Zinc was precipitated as sulfide, which was converted into sulfate and weighed in this form. Cadmium was deposited electrolytically. In all cases the stock solution from which the samples were taken was standardized by the same procedure.

Table VI. Determination of Various Metals after Precipitation of Basic Ferric Formate

Iron Present *Gram*	Other Metal Added *Gram*	Other Metal Found *Gram*	Error *Mg.*
0.60	0.1016 Ni	0.1017	+0.1
0.60	0.1016 Ni	0.1017	+0.1
0.10	0.0973 Mn	0.0975	+0.2[a]
0.10	0.0973 Mn	0.0973	0 [a]
0.10	0.0973 Mn	0.0972	−0.1[a]
0.60	0.0973 Mn	0.0969	−0.4[b]
0.60	0.0973 Mn	0.0971	−0.2[c]
0.60	0.0973 Mn	0.0969	−0.4[c]
0.10	0.1087 Co	0.1089	+0.2
0.10	0.1087 Co	0.1086	−0.1
0.60	0.1087 Co	0.1070	+0.3
0.60	0.1087 Co	0.1087	0
0.10	0.1004 Zn	0.1005	+0.1
0.10	0.1004 Zn	0.1005	+0.1
0.10	0.1004 Zn	0.1004	+0.1
0.60	0.1004 Zn	0.1006	+0.2
0.10	0.1005 Zn	0.1005	0
0.10	0.1005 Zn	0.1005	0
0.60	0.1005 Zn	0.1005	0
0.60	0.1005 Zn	0.1005	0
0.10	0.1065 Cd	0.1059	−0.6
0.10	0.1065 Cd	0.1070	+0.5
0.60	0.1065 Cd	0.1073	+0.8
0.60	0.1065 Cd	0.1069	+0.4
0.60	0.2130 Cd	0.2124	−0.6
0.60	0.2130 Cd	0.2127	−0.3

[a] 0.1 mg. of Mn in precipitate.
[b] 0.6 mg. of Mn in precipitate.
[c] 0.5 mg. of Mn in precipitate.

SUMMARY

The precipitation of ferric iron in a dense form, readily filtered and washed, has been effected by the hydrolysis of urea in hydrochloric acid solution containing formic acid. This method of precipitation, particularly the modified two-stage precipitation, effects an excellent separation from bivalent metals and is pro-

posed primarily as a method for the separation of iron, preparatory to the determination of other metals in the filtrate.

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Colorimetric Determination of Copper with Pyridine and Salicylic Acid

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If an aqueous solution of pyridine containing a large amount of salicylic acid is added to a solution of copper salt, a complex precipitate is formed. This precipitate dissolves readily in most organic solvents. The intensely blue color of the extract may be used for the colorimetric determination of copper. The method may be applied to a variety of metallurgical samples, and is suitable for routine determinations. A microprocedure may be adopted for samples where copper is present in extremely low concentrations.

IT WAS found during a study of some organic copper complexes that copper could be readily removed from an aqueous solution of its salts if both an organic acid, such as benzoic or salicylic acid, and pyridine were present. The reaction was similar to that between copper and pyridine in the presence of potassium thiocyanate, applied analytically by Spacu (*9*) and Biazzo (*2*), and improved by Elvehjem and Lindow (*3*) and Goethals (*4*, *10*).

The purpose of this investigation was to study the combined effect of salicylic acid and pyridine upon aqueous solutions of copper salts and its analytical possibilities.

When an excess of pyridine and salicylic acid is added to a solution containing even minute amounts of copper ions, a voluminous greenish white precipitate is formed. This precipitate dissolves readily in chloroform, carbon tetrachloride, ether, and other organic solvents with the formation of a brilliant and intense blue color. The composition of this precipitate was investigated by Ley and Erler (*1*, *6*), who defined it as a cupric salicylate coordinated with two pyridine rings $[Cu(Py)_2(C_6H_4OHCOO)_2]$. The author is inclined to believe that when the precipitate is formed in the presence of a very large excess of pyridine this formula should be corrected by addition of two more pyridine rings.

The color of the solvent extract is specific for copper, its intensity is proportional to the concentration of copper, and any interference due to the presence of other colored ions may be easily prevented or limited.

APPARATUS AND REAGENTS

A Beckman quartz spectrophotometer (Model DU) with its standard equipment (10-mm. absorption cells) was used for all measurements. No appreciable difference was recorded when cells of a different type were used. Changing the slit width or using different phototubes (red or blue light-sensitive) did not affect the accuracy of measurements.

Basic Reagent. It was found that optimum sensitivity is obtained with the reagent prepared by dissolving 22 to 25 grams of C.P. salicylic acid in 32 to 35 ml. of copper-free pyridine. This solution should be diluted to a volume of 100 ml. with distilled water. The reagent is stable for many weeks if protected from bright light and excessive heat. One milliliter of the reagent provides enough excess to precipitate up to 20 mg. of copper ions.

Solvent. C.P. chloroform is the best extraction solvent.

Standard Copper Solution. A solution containing 1 to 2 mg. of copper per ml. (preferably as sulfate) is the best standard, either for the preparation of a calibration curve or as a comparative solution. This solution may be prepared by dissolving 1 to 2 grams of pure (hydrogen-reduced) copper shot in 10 to 15 ml. of concentrated nitric acid. It is diluted with distilled water to about 30 ml. and evaporated almost to dryness. Then 20 to 30 ml. of water and 4 to 6 ml. of concentrated sulfuric acid are added to the cooled residue. The solution is evaporated to dense white fumes of sulfuric acid, cooled, diluted with about 200 ml. of water, transferred quantitatively to a volumetric flask, and diluted to 1000 ml. The copper content of the solution may be checked, if necessary, either electrolytically or iodometrically.

GENERAL PROCEDURE

Weigh a sample containing 0.100 to 5.0 mg. of copper and, depending on the nature of the sample, char or digest with a mixture of oxidizing acids. The amount of acids may be adjusted according to the weight of the sample and its copper content. In most cases 3 to 5 ml. of concentrated nitric acid mixed with an equal volume of concentrated perchloric or sulfuric acid provide a satisfactory excess for digestion. After the digestion is completed, cool the solution, dilute it carefully with a few milliliters of distilled water, and filter off any residue, washing it repeatedly with small portions (1 to 2 ml.) of a hot 2% solution of nitric acid. The final volume should not be larger than 40 to 50 ml. Evaporate if a larger volume has been obtained. Remove the excess of acids either by evaporation or by adding 8 *M* ammonium hydroxide to pH 4.5 to 7.0. Transfer the solution to a separatory funnel (about 60-ml. capacity), and add 3 to 5 ml. of chloroform, followed by 1 ml. of the pyridine–salicylic acid reagent. Shake vigorously, allow the chloroform phase to settle, and withdraw

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it into a volumetric flask (10- to 25-ml. capacity). Repeat the extraction with small portions of the solvent (2 to 3 ml.) until no more blue color is visible in the solvent phase. Test for completeness of extraction by adding 1 to 2 droplets of the reagent before each extraction. Dilute the extract to the mark. If the extract is cloudy, transfer it quantitatively to a larger volumetric flask and add more solvent. Measure the transmittance in a suitable spectrophotometer or colorimeter, using the range of 590 to 620 mμ.

If the sample contains less than 0.150 to 0.100 mg. of copper, proceed as above, using smaller volumes of acids, reagent, and solvent (about 50% of the amount given above). Collect the extract in a microcrucible and evaporate it to dryness over a hot plate. Dissolve the residue in the smallest carefully measured volume of the solvent to which one droplet of the reagent has been added. Transfer 0.05 to 0.1 ml. to a microabsorption cell [described for the Beckman spectrophotometer by Lowry and Bessey (7) and recently by Kinsey (5)] and measure the transmittance in the usual way. Using this technique it was possible to analyze as little as 50 to 100 cu. mm. of the extract without decreasing the length of the light path (1 cm. for Beckman spectrophotometer).

The calibration curve may be prepared by recording readings obtained with chloroform extracts containing a known amount of copper.

Most common ions do not interfere with the color of the complex. There is no interference from silver, aluminum, arsenic (as arsenite and arsenate), bismuth, chromium (ic), mercury (ous) and (ic), magnesium, manganese (ous), lead, antimony, tin (ic) (stannous tin should be oxidized), vanadium (as vanadate), wolfram, zinc, alkali and alkaline earth metals, and ammonium compounds (if the solution is acid or neutral), even if these ions are present in concentrations many times exceeding that of copper.

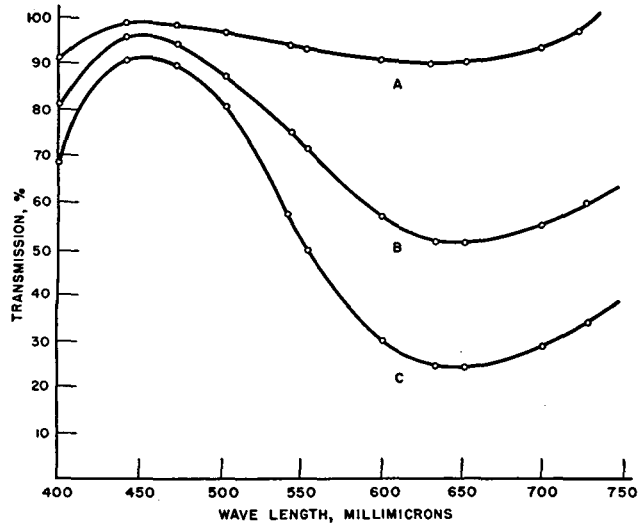

Figure 1. Concentration of Copper

A. **0.040 mg. per ml.**
B. **0.250 mg. per ml.**
C. **0.500 mg. per ml.**

A serious interference is caused by iron, nickel, cobalt, gold, and uranyl ions. This interference may be easily eliminated in the case of iron, cobalt, nickel, and uranyl by addition of a very large excess of ammonium bifluoride previous to extraction (before addition of the color reagent); 1.5 to 2.0 grams of ammonium bifluoride dissolved in 40 ml. of solution make it possible to determine copper accurately, although the amount of copper may be only 4 to 5% of iron, 8 to 10% of nickel or cobalt, and 15 to 20% of uranium. Larger concentrations of interfering elements cause a decrease in the accuracy of determinations, due to coprecipitation.

The interference of gold may be removed by addition of an excess of ammonium bifluoride ions, if the pH of the aqueous phase has also been adjusted (before the extraction) to the strictly neutral range (6.8 to 7.2). Most common anions may be present in an almost unlimited concentration. Anions precipitating copper—e.g., sulfides, ferrocyanides, etc.—or forming stable complexes with it—e.g., cyanide, tartrate—must be excluded. Chromate, dichromate, and permanganate do not interfere if reduced to the chromic or manganous form. Sulfurous acid added in a considerable excess was found to be the most convenient reducing agent in all three cases.

Table I. Determination of Copper in Samples of Medium and High Concentrations

Weight of Sample	Copper		
	Present	Found	Error
Mg.	*Mg.*	*Mg.*	%
454.6[a]	8.2	8.20	0.0
456.1[a]	10.4	10.35	0.5
449.8[a]	12.3	12.35	0.4
459.2[b]	20.1	20.05	0.2
455.0[b]	35.6	35.60	0.0
448.3[b]	65.6	65.50	0.2
461.3[c]	95.5	95.55	<0.1
494.5[c]	108.1	108.10	0.0
465.3[c]	120.4	120.30	<0.1
460.8[c]	145.6	145.70	<0.1
456.7[d]	196.7	196.70	0.0
461.4[e]	426.7	426.50	<0.1
458.9[e]	432.1	432.00	<0.1

[a] Copper furnace slag (commercial).
[b] Copper ore, low grade (commercial).
[c] Copper ore, higher grade.
[d] Nonferrous alloy.
[e] Brass and bronze samples.

Table II. Determination of Copper in Samples of Low Concentrations

Weight of Sample	Copper		Difference
	Present	Found	
Mg.	*Mg.*	*Mg.*	*Mg.*
205.1[a]	2.40	2.45[b]	0.050
152.1[a]	1.55	1.53[b]	0.020
100.4[a]	1.15	1.15[b]	0.000
78.4[a]	0.65	0.66[b]	0.010
75.3[a]	0.55	9.54[b]	0.010
54.5[a]	0.42	0.42[b]	0.000
104.3[c]	0.25	0.20[b]	0.050
101.5[c]	0.20	0.21[b]	0.010
25.4[c]	0.05	0.050[d]	0.000
22.7[c]	0.01	0.015[d]	0.005

[a] Food samples contaminated with analyzed copper sulfate solution.
[b] Normal macroprocedure followed.
[c] Biological specimens contaminated with analyzed copper sulfate solution.
[d] Microprocedure (microadsorption cells) applied.

DISCUSSION

The absorption of the complex formed by copper with the pyridine–salicylic acid reagent was investigated throughout the entire range of visible light (Figure 1). The absorption is at its maximum in the vicinity of 650 mμ, but the best agreement between the observed transmittance and the values derived from the Beer-Lambert law was found in the range of 590 to 620 mμ. At this range, the transmittance plotted logarithmically as a function of the concentration (expressed in micrograms of copper per ml.) gives a straight line. This relation remained unchanged even at the range of high transmittance (over 90%).

The color of the complex is very stable. Measurements were repeated on a series of samples during several (6 to 7) weeks. No difference in the intensity of the color was observed as compared with the intensity originally measured. Evaporation to dryness followed by a dilution with fresh solvent back to the original volume does not affect the intensity of the color.

The sensitivity of the reaction is rather low as compared to some other methods—e.g., sodium diethyldithiocarbamate. Although the theoretical sensitivity may be assumed to be about 20 micrograms per ml. of the solvent extract, the actual working sensitivity should be accepted as 100 to 150 micrograms per ml.

Although it was possible to determine as little as 2 micrograms of copper (0.01% of the total sample) by means of the described microprocedure, the working range of the method should be limited to concentrations of 0.100 to 0.600 mg. of copper per milliliter of extract.

The method was applied to a variety of samples (biological specimens, food samples, ores, slags, and ferrous and nonferrous alloys). Following the suggestion of Mehlig and Durst (*8*) concerning the application of colorimetric methods to determination of major constituents, samples of copper alloys (brass and bronze) were analyzed for copper in routine runs. Good and quick results were obtained with samples containing as much as 80% of copper. Generally the best results were obtained with samples containing 0.1 to 5% of copper by weight.

The completeness of extraction of copper from the aqueous phase was tested in a twofold manner. First, a negative qualitative test was obtained on checking the aqueous phase after the copper was extracted. For this purpose several highly sensitive spot tests were applied. Then the chloroform phase was "re-extracted" by shaking with 20 to 25 ml. of 1.5 to 3 *M* ammonium hydroxide. Under these conditions copper leaves the solvent medium and is found as copper-ammonia complex in the water phase. The excess of ammonia was then evaporated by boiling the solution and the copper content was rechecked by analyzing the solution iodometrically or electrolytically. The average recovery of copper was 99.99 to 100%.

Although some determinations were carried out successfully at pH as low as 2.2 and as high as 8.5, the optimum pH range for the described method lies between pH 4.5 and 7.0. At this range the rate of extraction is greatly increased, whereas the extraction at lower or higher pH values requires a larger volume of solvent and more extracts.

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Determination of Oil in Paraffin Waxes

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A rapid method for the determination of oil in petroleum waxes has been developed, in which the sample is chilled with dyed methyl ethyl ketone to −25° F., a portion of the solvent-oil mixture is filtered off, and an aliquot of the filtrate reacts with a sodium bisulfite solution in a skim milk test bottle. The oil is separated from the mixture by centrifuging, and the volume is read. Results by this method check very closely with A.S.T.M. method D 721–47. This method is particularly suitable for samples of low oil content (under 5.0%) but with modifications has been used up to 75% successfully.

SEVERAL methods for determining oil in wax are in use in the petroleum industry. The standard procedure is A.S.T.M. D 721–47 (*1*) or its semimicromodification (*2*). A.S.T.M. D 721–47 gives reliable results but requires too much elapsed time to be of value for plant control. The semimicroprocedure is a somewhat delicate operation because of the small quantities involved on sampling and testing, and considerable laboratory experience is required to obtain consistent, reliable results.

The method described in this article was developed for testing deoiled wax in conjunction with plant operation where test results were needed as quickly as possible in the interest of plant efficiency. It was also desired to have a method which required no elaborate or delicate equipment, and which was as simple as possible, so that plant operators could obtain reliable results. Another requirement was to avoid the evaporation of solvent in the interest of safety.

The method described, which provides for the separation of oil from solvent by chemical means, meets the above conditions better than the previously mentioned methods.

APPARATUS

Beaker, 250-ml.; aluminum or copper.

Allihn filter tube, length approximately 100 mm., capacity 30 ml.; fritted-glass filter disk of No. 4 porosity and 20-mm. diameter.

Cooling bath, an insulated bath which is divided by an insulated partition such that the temperature in one side can be maintained at −50° to −100° F., while the other side is maintained at −25° ± 1° F. Dry ice and alcohol are suitable cooling media. Dimensions and design of the box may vary according to the volume of work to be done.

Skim milk test bottle, 6-inch, with double neck, one neck graduated in 50 divisions; each division equals 0.002 ml.

Thermometers. Two A.S.T.M. cloud and pour, one A.S.T.M. low cloud and pour.

Laboratory centrifuge, capable of turning approximately 1500 r.p.m. with cups of dimensions suitable for holding the 6-inch skim milk test bottle.

SOLVENT AND REAGENT

Dyed methyl ethyl ketone, commercial grade, refractive index at 68° F. 1.378 ± 0.002, and containing approximately 2 mg. per gallon of a red oil-soluble dye. Ten milliliters treated with a solution of sodium bisulfite as described in the method should not show more than 0.0005 ml. of oil.

Sodium bisulfite, saturated solution, filtered just before using.

PROCEDURE

Precool a filter tube by inserting it in a beaker submerged in the −25° F. bath.

Weigh 36 ± 0.5 grams of melted wax into a 250-ml. metal beaker, and add 82 ± 1 grams of warm (120° to 160° F.) dyed methyl ethyl ketone. Place on A.S.T.M. cloud and pour ther-

Table I. Filtering Temperatures *vs.* Pour Point

Filtering Temperature, ° F.	Per Cent Oil Found: 125/130° F. AMP, Sample 1	125/130° F. AMP, Sample 2	143/150° F. AMP, sample 3	Micro Solid Point on Oil, Av., ° F.
−12	0.43	0.54	0.76	25
−20	0.42	0.53	0.73	12
−25	0.41	0.51	0.71	6
−30	0.38	0.49	0.69	−8

Table II. Oil Content of Wax Samples

A.S.T.M. D 721-47 AMP, ° F.	% oil	Deviation	Bisulfite Method % oil	Deviation	Deviation of Bisulfite from A.S.T.M.
125/130	0.26, 0.22	±0.02	0.30, 0.30	0	+0.06
125/130	0.21, 0.17	±0.02	0.20, 0.20	0	+0.01
125/130	0.48, 0.58	±0.05	0.55, 0.58	±0.02	+0.04
143/150	0.29, 0.22	±0.04	0.30, 0.30	0	+0.04
143/150	0.63, 0.70	±0.04	0.73, 0.73	0	+0.06
143/150	0.45, 0.49	±0.02	0.53, 0.55	±0.01	+0.07
		Average deviation between methods			+0.05

mometer in the beaker, and, if the sample is not completely dissolved, heat on a steam hot plate (or equivalent), while stirring, until it just dissolves. Transfer the beaker to the −50° to −100° F. bath, and stir with the thermometer, scraping off the wax coat which is formed on the sides and bottom as completely and rapidly as possible. Continue stirring until the mixture has a slushy consistency, after which stirring may be intermittent. When the temperature drops to −20° F., stir continuously until it drops to −25° F. Transfer the beaker to the −25° F. bath, place the precooled filter tube in the mixture nearly to the bottom of the beaker, and attach a vacuum line to the filter tube. Turn the vacuum on slightly, and when an estimated 15 ml. of filtrate are in the filter tube, detach the vacuum line, remove the filter tube from the beaker, and transfer the filtrate to a test tube or other suitable container.

Pipet 5 ml. of the filtrate, if it is estimated the oil content is over 2.5%, or 10 ml. if under 2.5%, into a skim milk test bottle. Add 5 ml. of distilled water and 20 ml. of a saturated solution of sodium bisulfite to the skim milk test bottle. Press the end of a rubber tube attached to the vacuum line against the open end of the capillary neck, and adjust the vacuum so that maximum agitation occurs without loss of liquid from the bottle. Continue the agitation for 3 minutes, then detach the vacuum line. Add water to the bottle until the surface is in the upper one third of the calibrated portion of the capillary neck. Centrifuge the bottle at approximately 1500 r.p.m. for 5 minutes and read the number of divisions of separated oil to the nearest half-division (smallest division = 0.002 ml.).

Calculate the oil content by means of the following formula:

$$\text{Oil, \% by weight} = \frac{0.002 \times A \times B \times D \times 100}{W \times (C - 0.002A) \times 0.805}$$

where A = number of 0.002-ml. divisions of separated oil, B = specific gravity of separated oil, C = ml. of aliquot treated with sodium bisulfite solution, D = grams of methyl ethyl ketone added to the sample, and W = weight of sample. (0.805 is the specific gravity of methyl ethyl ketone at 20° C.)

For routine control work the above formula may be simplified without serious error as follows:

Specific gravity of the separated oil may be assumed to be that of the sample—e.g., 125/130° F. American melting point = 0.900 specific gravity.

Grams of methyl ethyl ketone in the mixture at the time of filtration may be assumed to be 80.5 and grams of sample 36.0.

The correction of the aliquot for the volume of oil it contains ($0.002A$) may be disregarded.

The formula then simplifies to:

When C = 10. Oil, % by weight = $0.05A$

When C = 5. Oil, % by weight = $0.10A$

EXPERIMENTAL DATA

For the purpose of this investigation, oil is considered as "zero pour point oil," determined by filtering three samples of various melting point wax at four different temperatures, with the results shown in Table I.

A wire is constructed with about a 0.5-inch (1.25 cm.) length at right angles to a small loop. Microsolid point is determined by placing a small amount of oil on the end of a thermometer bulb and embedding the small loop of wire in the oil. By chilling the embedded wire in the vertical position and allowing it to warm slowly in the horizontal position, the temperature at which the wire starts to fall from the horizontal position is recorded as the micro solid point, and is correlated with the pour point of the oil. With the wire used zero pour oil has a micro solid point of 6° F. Each wire must be standardized against zero pour oil before use.

It was concluded from the data in Table I that a filtering temperature of −25° F. would give zero pour point oil.

In order to obtain data on the accuracy of the outlined method, a number of samples were run in duplicate by A.S.T.M. D 721-47 and this method.

The duplicate results in Table II were determined by different operators. The average deviation between methods is better than the repeatability of the A.S.T.M. method.

No experimental work was done with microcrystalline or high melting point waxes.

CONCLUSIONS

The oil content of paraffin wax, as determined by this method, agrees closely with results obtained by A.S.T.M. D 721-47 on commercial waxes of the usual low oil content. The advantages of the method are simplicity, reliability, and speed. Approximately 0.5 hour is required for completing the test. Another advantage is that it avoids evaporation of solvent, and thus includes light oils if present in the determination.

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Improved Potentiostat for Controlled Potential Electrolysis

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POTENTIOSTATS, which automatically perform the function of maintaining the potential of an electrode constant during an electrolysis, have not yet become commercially available, so that those who wish to exploit the manifold analytical applications (*4*) of the controlled potential electrolysis technique must construct for themselves the necessary apparatus. In recent years a number of different types of potentiostat have been described (*1-5*), whose relative merits are best assessed by reference to the original papers.

The instrument described herein, whose operating principle is indicated schematically in Figure 1, is an improved version of an instrument previously described (*4*). The chief improvement is the use of a rectifier and filter circuit to enable operation from an ordinary 110-volt alternating current line and the concomitant employment of Variac autotransformers to control the alternating current input and hence the output direct current voltage applied to the electrolysis cell.

An improved potentiostat is described, with the following characteristics: complete operation from the 110-volt alternating current line, except for one ordinary 1.5-volt dry battery; either cathodic or anodic potential control with sensitivity of ±0.01 volt; output capacity up to 5 amperes at 6 volts, and voltage output up to 25 volts with smaller electrolysis currents; and no preliminary calibration or adjustments required before use. Because all components have long life expectancy, long periods of use without servicing may be anticipated.

The first Variac transformer provides a convenient means of manually controlling the input to the automatically operated Variac to provide the optimum direct current output range for a particular electrolysis experiment. The motor-operated Variac is followed by a stepdown transformer, a full-wave selenium rectifier, and a conventional inductance-capacitance filter circuit to smooth the rectified direct current. A voltmeter across the output indicates the total voltage applied to the cell and the electrolysis current is read on a multirange ammeter. High precision is not required of either of these meters, and ordinary panel-type instruments with an accuracy of the order of ±2% are adequate.

The control circuit comprises an ordinary radio-type potentiometer powered by a 1.5-volt dry cell to provide the reference voltage, which is read directly on a voltmeter, a Weston Model 30 galvanometer relay, and a dual electronic relay to control the reversible shaded-pole motor which drives the Variac autotransformer.

When the potential of the working electrode against the reference electrode (usually a saturated calomel electrode) differs from the opposing reference voltage, the galvanometer relay makes contact right or left and activates the double electronic relay, which in turn causes the motor to operate the Variac in the appropriate direction either to increase or decrease the total voltage applied to the cell until the potential of the working electrode returns to the value of the reference voltage. Because the reference voltage is read directly on a meter, the control circuit requires no preliminary adjustment or calibration before use, and the reference voltage can be changed instantly during the course of an electrolysis by merely readjusting the potentiometer.

The complete circuit of the instrument, with specifications of all components, is shown in Figures 2 and 3, and Figures 4 and 5 show the chassis arrangement and completely assembled instrument. To put the instrument into service it is only necessary to close switches *S*-1, *S*-2, and *S*-5, adjust *R*-6 to the desired reference voltage, adjust Variac *T*-1 to provide the desired direct current output range, and finally close the output circuit and select the optimum range of ammeter M-1 by switch *S*-3. None of the components is required to function critically, and all are conservatively rated to provide long life expectancy, so that extended periods of use without servicing may be anticipated.

The control sensitivity depends primarily on the sensitivity of the Weston galvanometer relay (extreme top right on panel in Figure 5). This instrument has a rated sensitivity of ±15 microamperes per mm., and an internal resistance of 1100 ohms, corresponding to a voltage sensitivity of approximately ±16 mv. per mm. By adjusting the contacts to minimal clearance the net sensitivity can be adjusted to somewhat better than ±10 mv. when the resistance in the control circuit does not exceed about 1000 ohms. Most of the resistance in the control circuit occurs in the salt bridge between the reference electrode (usually a saturated calomel electrode) and the electrolysis solution, and it is a simple matter so to design the salt bridge that the total resistance in the control circuit amounts to only a few hundred ohms. A control sensitivity smaller than about ±10 mv. usually is of no practical use, and control to ±50 mv. is adequate for many purposes. The potential of a working electrode usually undergoes rapid erratic fluctuations which may vary from a few to as much as 10 or 20 mv., depending on the types of electrode, conditions of stirring, and similar factors. The use of a control sensitivity smaller than these natural fluctuations only leads to objectionable "hunting" and decreased control efficiency.

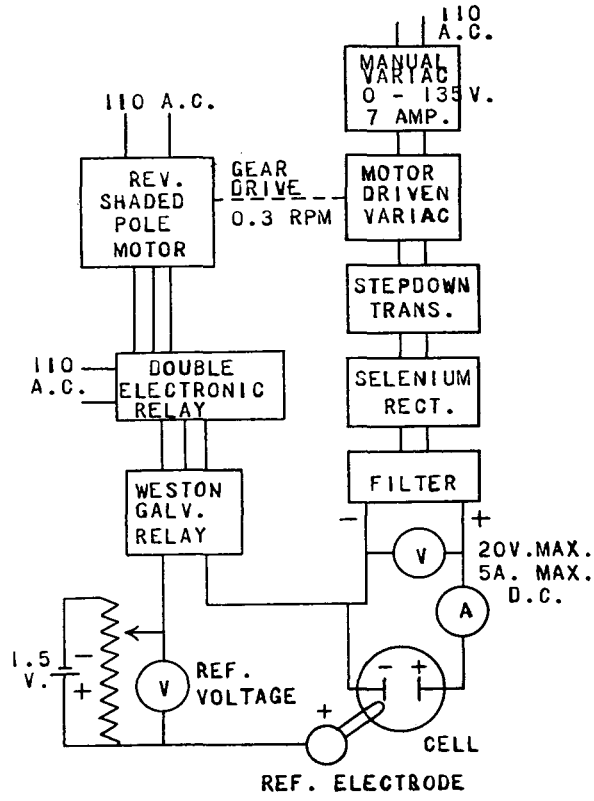

Figure 1. Schematic Circuit

The rate of rotation of Variac *T*-2 is an important factor for optimum control action. It should be great enough so that the voltage applied to the cell is promptly corrected and yet not so great that overshooting and consequent hunting occur. A rate of rotation of approximately 0.3 r.p.m. is optimum for most purposes.

The shaft of Variac *T*-2 is extended to the rear and provided with a large spur gear which is driven via a reducing spur gear train by the drive motor. Two normally closed microswitches in the shading coil circuit of the drive motor, actuated by a peg on the spur gear attached to the shaft of Variac *T*-2, serve as limit switches to prevent damage to *T*-2.

Pilot lights *I*-2 and *I*-3 (right-hand side of panel below Weston relay) in the shading coil circuit of the drive motor indicate the direction of operation of the motor. Because these 6-volt pilot lamps operate on a current (0.15 ampere) approximately equal to that in the motor shading coils, they do not affect the operation of the motor.

The function of the dual electronic relay (Figure 3) is to reduce to an infinitesimal magnitude the current handled by the contacts of the galvanometer relay. The electronic relay was designed to operate on an input current (2 microamperes) which is so small that any contact resistance in the galvanometer relay is unimportant and feather-light contacting is sufficient for positive operation. The input terminals, 1, 2, and 3 (Figure 3), are connected to the three output terminals of the galvanometer relay. When the galvanometer relay makes contact in one direction terminal 1 is shorted to the common terminal 2, and the consequent abrupt increase in the left-hand plate current of the 6SN7 twin triode

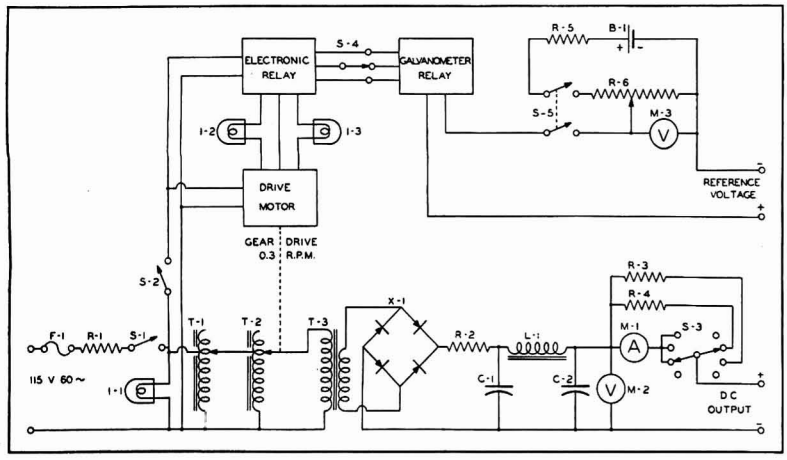

Figure 2. Complete Circuit

B-1. 1.5-volt dry battery
C-1, *C*-2. 6000-μfd. electrolytic capacitors (50 volts)
F-1. 3-ampere fuse (250 volts)
L-1. Choke (0.015 henry, 5 amperes)
M-1. Milliammeter (0–100 ma. d.c.)
M-2. Voltmeter (0–30 volts d.c.)
M-3. Voltmeter (0–1 volt, ±1% or better; Weston Model 741)
I-1. Pilot lamp (115 volt a.c.)
I-2, *I*-3. Pilot lamps (G.E. No. 47, 6.3 volt, 0.15 ampere)
R-1. 5 ohms, 50 watts, wire wound (±5%)
R-2. 1 ohm, 100 watts, wire wound (±5%)
R-3, *R*-4. Empirically adjusted shunts
R-5. 40 ohms, 5 watts, wire wound (±5%)
R-6. 100-ohm potentiometer (General Radio No. 214)
S-1, *S*-2. S.p.s.t. toggle switches
S-3. 4-position rotary switch (low resistance contacts)
S-4. 3-position single pole switch
S-5. D.p.s.t. toggle switch
T-1, *T*-2. General Radio Co. Type V-5 Variac autotransformers (0–135 volts, 7.5 amperes)
T-3. Power transformer (U.T.C. Special Series Type S-63, stepdown ratio 5 to 1, 10 amperes)
X-1. Selenium rectifier (48 volts, 5 amperes)

Drive motor. Barber-Coleman Model No. gYAz 804 (110-volt a.c., shaded pole, reversible)
Galvanometer relay. Weston Model 30 (±15 microamp. per mm., 1100 ohms internal resistance)

causes relay *E*-2 to close motor leads 1 and 2 and operate the motor in one direction. When the galvanometer relay makes contact in the opposite direction input terminal 3 is shorted to 2 and relay *E*-1 connects motor terminals 3 and 2 to reverse the direction of rotation of the motor. When the galvanometer relay is balanced, both plate currents of the 6SN7 tube are much smaller than required to operate relays *E*-1 and *E*-2, so these relays both remain open (inactivated) and the motor remains at rest. Relays *E*-1 and *E*-2 were chosen to operate on a current (3 ma.) only about half as large as the plate currents (6 ma.) of the 6SN7 tube, so that positive snap action is obtained, which does not depend on critical functioning of either the 6SN7 tube or the relays.

Switch *S*-4 (between pilot lights on right side of panel) is provided for manual operation of the motor to preposition Variac *T*-2, or drive it to either end of its range, before an experiment.

The desired reference voltage (control potential) is set by potentiometer *R*-6 (directly below middle meter) and read on the 0- to 1-volt voltmeter, *M*-3 (large middle meter on panel). The fixed resistor, *R*-5, in series with the 1.5-volt dry cell, *B*-1, has a resistance somewhat less than one half that of *R*-6, and serves to reduce the voltage across *R*-6 to slightly over 1 volt. The voltmeter, *M*-3, should have a range of 0 to 1 volt and should be capable of being read with a precision and accuracy of ±0.01 volt or better. The accuracy of the meter at several points over its scale should be checked with a precision potentiometer. A range of reference voltage from 0 to 2 volts can be provided, if desired, by powering *R*-6 with two dry cells and using a 0- to 2-volt voltmeter, but this is seldom if ever required.

The reference voltage terminals are connected in opposed polarity to the reference electrode and to the cell electrode whose potential is to be controlled. Separate leads should be provided from the reference voltage terminals to the electrolysis cell, rather than connecting one lead to the direct current output terminal on the panel, to avoid including in the apparent control potential the *iR* drop in the output leads between the panel and the cell.

The rectified direct current power supply is designed to provide a maximum output of approximately 25 volts when Variacs *T*-1 and *T*-2 are both at their maximal 135-volt settings, and hence the stepdown transformer, *T*-3, has a ratio of approximately 5 to 1. A larger output voltage range (up to 135 volts) can, of course, be

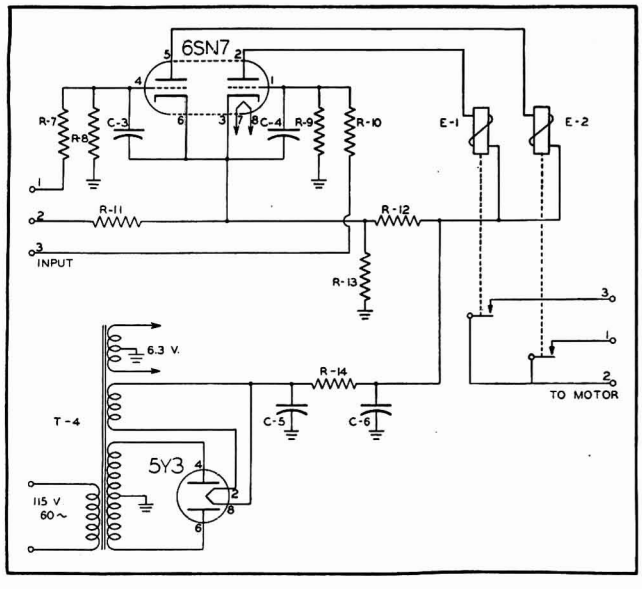

Figure 3. Electronic Relay Circuit

C-3, *C*-4. 0.003 μfd. mica capacitors (200 volts)
C-5, *C*-6. 8 μfd. electrolytic capacitors (450 volts)
E-1, *E*-2. S.p.s.t. relays (10,000 ohms, 30 volts d.c.)
R-7, *R*-10. 4 megohms, 1 watt, carbon (±10%)
R-8, *R*-9. 10 megohms, 1 watt, carbon (±10%)
R-11. 100,000 ohms, 1 watt, carbon (±10%)
R-12. 34000 ohms, 10 watts, wire wound (±5%)
R-13. 2000 ohms, 5 watts, wire wound (±5%)
R-14. 4000 ohms, 10 watts, wire wound (±5%)
T-4. Thordarson T-22R00 transformer or equivalent (primary 115 volts, secondary 250-0-250 volts at 40 ma., 5 volts at 2 amperes, 6.3 volts at 2 amperes)
5Y3. Rectifier tube (glass envelope)
6SN7. Twin triode (glass envelope)

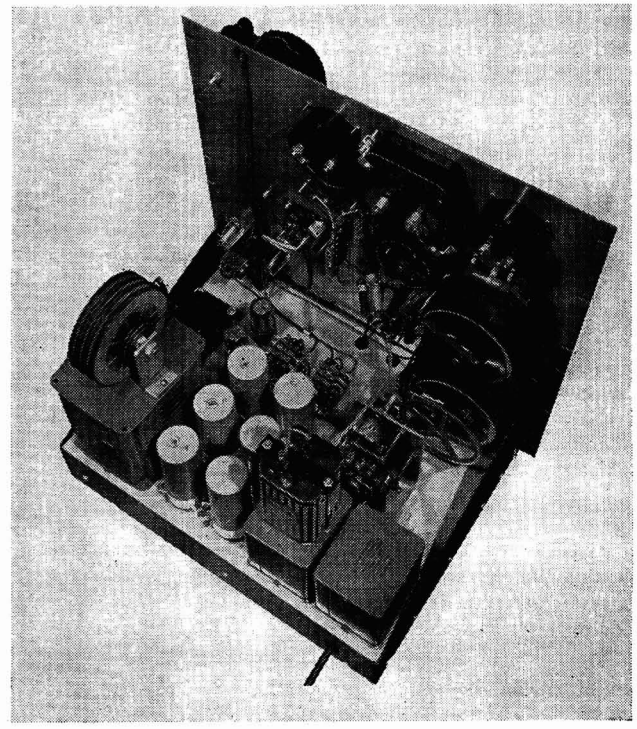

Figure 4. Chassis Assembly

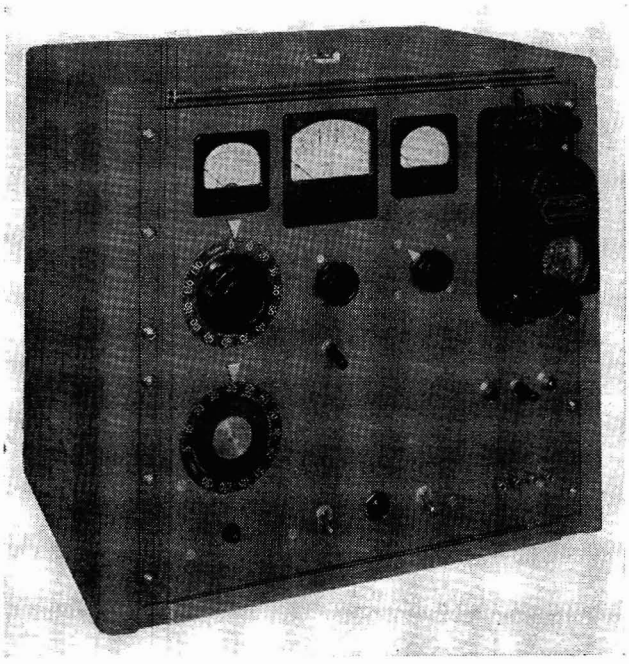

Figure 5. Complete Potentiostat

obtained by selecting a stepdown transformer with a smaller ratio, but more than 25 volts have never been found necessary in any analytical applications of controlled potential electrolysis. The output supply is designed for a continuous maximum current of 5 amperes, but the components are all conservatively rated and currents up to 7 or 8 amperes can be drawn for short periods without damage.

The inductance-capacitance filter comprising *C*-1, *L*-1, and *C*-2 must have an efficiency great enough to reduce the residual ripple voltage to the order of only a few hundredths of a volt at full load, because the residual pulsations produce an oscillation of the potential of the working electrode. The ripple voltage increases with the current output. On the other hand, when the electrolysis current is large the resultant oscillation in the potential of the working electrode actually is much smaller than with small currents because a much greater fraction of the total voltage applied to the cell is dissipated as *iR* drop in the cell. The residual ripple voltage was tested with a calibrated cathode ray oscilloscope. With 25 volts output and a current smaller than 0.1 ampere it was less than ±0.005 volt (less than ±0.02%), with 1.5 amperes at 20 volts it was ±0.02 volt (±0.1%), and with 7.4 amperes at 4.3 volts it was ±0.04 volt (±0.9%).

Because of the large capacity of the filter circuit a very large momentary current surges into the power supply at the instant the main alternating current switch, *S*-1, is closed. The fixed resistors, *R*-1 and *R*-2, were provided to limit this momentary surge to prevent blowout of fuse *F*-1.

Voltmeter *M*-2 (left meter on panel) which indicates the output voltage need not be very accurate, and the same applies to ammeter *M*-1 (right meter on panel) which indicates the electrolysis current. Switch *S*-3 serves as an on-off switch for the direct current output, and also, in combination with shunts *R*-3 and *R*-4, as a selector switch to provide three current ranges of 0.1, 1, and 10 amperes. Switch *S*-3 (below right-hand meter on panel) should be of good quality with low resistance contacts. Shunts *R*-3 and *R*-4 are constructed of copper wire adjusted to the correct resistance values empirically at the time of assembly.

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Machine Computation of Relation between Resistance and Temperature of a Resistance Thermometer

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MODERN science is becoming more and more conscious of the role played by accurate physical data. In the design of process equipment and manufacturing apparatus, factors must be taken into consideration that received scant notice 20 years ago. These factors depend upon exact physical data for their elucidation. Exact physical data in many instances require the accurate measurement of temperature.

Physical methods of analysis and testing depend heavily also on accurate measurement of temperature. There are a number of accepted methods of accurate temperature measurement, but the international temperature scale from −182.97° to 630° C. is defined in terms of a standard platinum resistance thermometer (*6*). Increased emphasis on accurate measurement of temperature means increasing use of platinum resistance thermometers.

This excellent tool has been slow in gaining acceptance because of its high cost, fragility, size, calibration, and the elaborate accompanying electrical measuring equipment. The measuring equipment has recently been automatized and made recording (*7*), and the work of Meyers (*4*) has brought the size down to where it is comparable to an ordinary mercury thermometer. The tedium of the calibration was the motivating force for the present effort.

THE EQUATIONS

In the early days of resistance thermometry, Callendar (*1*) found that the formula relating the resistance to the temperature was

$$t = 100\,\frac{R_t - R_0}{R_{100} - R_0} + \delta\left(\frac{t}{100} - 1\right)\frac{t}{100} \qquad (1)$$

where R_t is the resistance in ohms at t° C., R_0 and R_{100} are the resistances at 0° and 100° C., and δ is a constant determined from measurements of the resistance at the boiling point of sulfur (444.60° C.).

This equation served well from −40° C. upward, but below this temperature becomes increasingly in error as the temperature is lowered. In 1925, Van Dusen (*8*) proposed an additional term which compensates the discrepancy down to the oxygen point (−182.97° C.). The modified equation is:

$$t = 100\,\frac{R_t - R_0}{R_{100} - R_0} + \delta\left(\frac{t}{100} - 1\right)\left(\frac{t}{100}\right) + \beta\left(\frac{t}{100} - 1\right)\left(\frac{t}{100}\right)^3 \qquad (2)$$

Precision platinum resistance thermometry employs a four-constant equation

$$R_t = R_0[1 + At + Bt^2 + C(t - 100)t^3]$$

relating the resistance (in ohms) of the thermometer, R_t, to the temperature, $t°$ C. In use, the problem of finding the temperature for a measured value of the resistance is solved by constructing a table of resistances for even values of temperature. For a precision of 0.001° C., these temperature values should be at about every degree for linear interpolation in between. In this way, repetitive calculation demanding six-place accuracy arises. International Business machines have been employed in this computation. The information is supplied to these machines in the form of punched cards, and the machines report the computation in punched form. The final step is the production of a printed record of the entire computation, including the answer. The time required for manual calculating machine operation is approximately decimated by the automatic machines operating with punched cards, while the fatigue of the operation is small by comparison.

where the symbols have the same meaning as in Equation 1 and β is a constant determined by calibration at the boiling point of oxygen (−182.97° C.).

In 1948, the international temperature scale was defined (*6*) above 0° C. by

$$R_t = R_0 (1 + At + Bt^2) \qquad (3)$$

where R_t and R_0 have the same meaning as before and A and B are the calibration constants. Below 0° C. a higher power term with a calibration constant, C, is added which holds down to −182.97° C. and the equation becomes

$$R_t = R_0 [1 + At + Bt^2 + C (t - 100) t^3] \qquad (4)$$

In a recent publication Schwab and Smith (*5*) have shown that Equations 1 and 2 can be transformed into 3 and 4.

UTILITY OF THESE EQUATIONS

In use, the problem of finding the temperature associated with a measured resistance is a serious one, even after the coefficients of the equations are known. It seems that the simplest solution to the problem is the computation of a table of values of R_t for even values of t. For 0.01° C. accuracy, the resistance must be known to the nearest 0.001 ohm, which necessitates 5-place accuracy in the computation. In such a table (to the nearest 0.001 ohm) ΔR for a 10.00° interval differs from its neighbors by 0.002 to 0.003 ohm, so that linear interpolation over a 10° interval does not introduce an error greater than about ±0.02° C. Computation of the values of R_t at values of t for 10° intervals from −200° to +500° C. requires about a 5- or 6-hour job with a manually operated calculating machine.

When the resistance can be read to 0.0001 ohm, linear interpolation over a 10° interval is not sufficiently accurate, so 6-place accuracy must be used, and the table made for intervals of 1° C. This enlarges the job of computing the table to 50 or 60 hours of work with a manually operated calculating machine. Inasmuch as the only operations involved are multiplication and summation and the work is highly repetitive, automatic calculation is not only indicated but highly desirable.

Having an installation of International Business machines available, it was decided to apply them to this problem. Eckert (*2*, *3*) has outlined the functions and operating principles of the various International Business machines, and reference should be made to his work if further familiarity with these machines is desired.

APPLICATION OF MACHINE COMPUTATION TO THE PRESENT PROBLEM

Figure 1 shows a card specially printed for the calculations on the present problem.

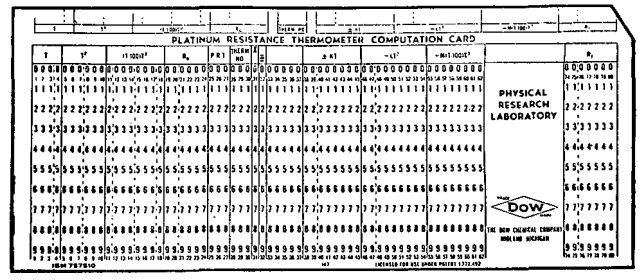

Figure 1. Specially Printed Card with Fields Delineated for This Computation

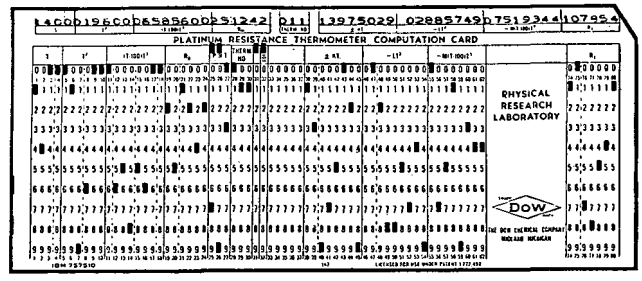

Figure 2. Card Showing Computation Complete for One Temperature

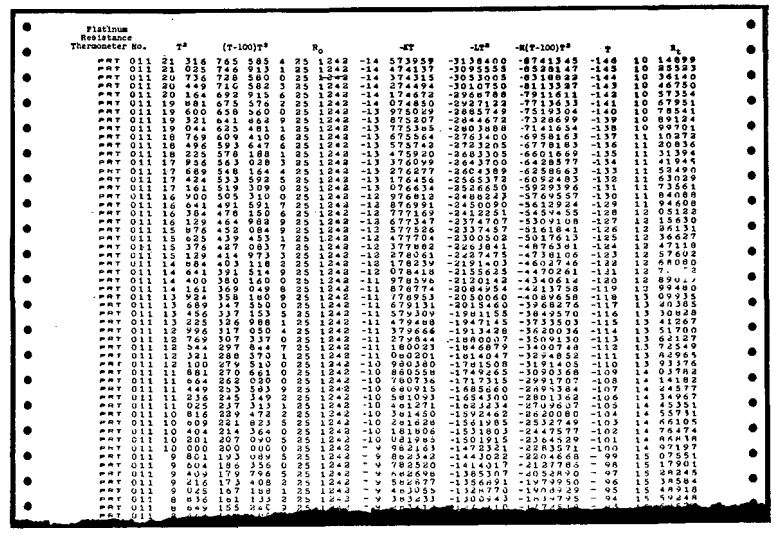

Figure 3. Section of Final Table Giving Values of Resistance for Each Degree and All of the Computation

Various "fields" are delineated and marked specifically. Columns 1 to 4 contain the value of t (the temperature); columns 5 to 10 the values of t^2; columns 11 to 18 the values of $(t - 100)t^3$; columns 19 to 24 the value of R_0, and so on.

For automatic calculation, Formula 4 has been replaced by the equivalent formula:

$$R_t = R_0 + Kt + Lt^2 + M\,(t - 100)\,t^3 \qquad (5)$$

where $K = R_0A$, $L = R_0B$, and $M = R_0C$. In this equation, the four constants R_0, K, L, and M define the resistance of a given thermometer.

Values of t, t^2, and $(t - 100)t^3$ from $-200°$ to $+500°$ C. were computed and punched into 700 cards in the first 18 columns. Each card represents one temperature value. Next, the value of R_0 is "gang punched" (in each card) by running the whole deck through the high speed reproducer. In the same operation the information in columns 25 to 32 (number of the platinum resistance thermometer and the cards with negative temperatures) is also gang punched.

After locking the value of K into a "multiplier," and passing the deck through, the multiplier will punch product Kt in columns 38 to 45, at the rate of about 700 cards per hour. In like manner, products Lt^2 and $M\,(t - 100)t^3$ are multiplied and punched in columns 46 to 62. [The product $M(t - 100)t^3$ is required only on negative values of t.]

The deck is now run again through the multiplier, which is now wired to add these products algebraically to the R_0 term and punch the R_t sum in columns 74 to 80. Figure 2 shows one of the cards with the complete computation. The deck is now run through an "accounting machine," which reads the information in the holes and makes a printed record of all the information. Figure 3 shows a section of the final table giving the values of R_t for every degree, and all the computations in the event that checking is necessary.

Thus in approximately 4 hours' machine time the entire computation of 700 temperatures is complete, and a printed record (triplicate if desired) of all the computations is rendered.

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Sampling Certain Atmospheric Contaminants by a Small Scale Venturi Scrubber

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A portable device for sampling air contaminants permits the scrubbing of large volumes of air by a small volume of liquid. This facilitates chemical analysis of the collected materials by making it possible to obtain relatively high concentrations of contaminants in the scrubbing liquid. The action of this portable field unit is based on the principle used in the Venturi scrubbers employed to remove fumes from industrial stack gases. Its efficiency for the collection of several gaseous and particulate air pollutants is discussed. These pollutants include ammonia, sulfur dioxide, sulfuric acid, and sodium chloride.

RECENT investigations of air pollution in metropolitan areas have emphasized the need for a sampling device which makes it possible to scrub large volumes of air with a small amount of scrubbing liquid. A device is also needed which will sample these large volumes in a relatively short time. Most previous sampling instruments designed for the collection of air pollutants in a scrubbing liquid, such as impingers or bubbler trains (*1*, *8*), have the disadvantage of comparatively slow sampling rates or unduly large liquid volumes. Filtration techniques have been developed which are useful, and many advances have been made in this direction (*10*). However, filtering rates are usually slow and the necessity for removing the filtered material from the filtering agent is often objectionable.

The apparatus here described is a portable laboratory scale model of the Venturi scrubbers used for fume recovery in plant stack gases, and is based in principle on the industrial models described by Anthony (*2*). Essentially, it affords a means of injecting a scrubbing liquid into a rapidly moving stream of air at a Venturi throat; the liquid stream is there reduced to a fine spray with droplet acceleration, and subsequently the spray-gas mixture is decelerated and separated. The efficient collection of particulate impurities probably is due chiefly to collision with water droplets and diffusion into the water (*2*). Johnstone and Roberts (*4*) have advanced a "diffusion theory" in which it is shown that aerosol particles of 0.1-micron diameter or less have sufficient Brownian movement to be considered as acting like large gas molecules; thus the collection of such particles might be considered to be analogous to gas absorption in liquid droplets.

This type of scrubber has proved especially useful to this laboratory in sampling polluted city atmospheres, inasmuch as chemical analysis of trace substances is facilitated by their relatively high concentration in the scrubber liquid, as compared to other methods of sampling. It is valuable also because of its ability to sample large volumes of air quickly during periods of peak contamination, which may be of short duration. In addition to this application, the small Venturi-scrubber should also be useful in industrial plants and other locations where it is desired to know the concentration of toxic substances in working areas. The use of this scrubber has been mentioned in several reports from this laboratory (*6*, *7*, *11*).

APPARATUS

Functional details of the small scale Venturi scrubber used in this laboratory are diagrammed in Figure 1.

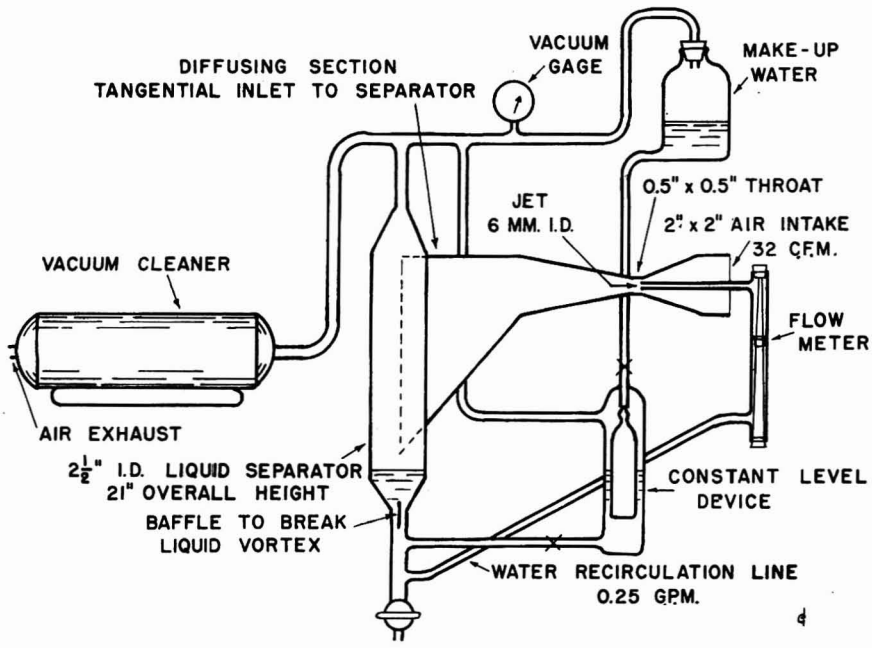

Figure 1. Functional Details of Laboratory Scale Venturi Scrubber

through a constant-level device, is provided to a reservoir in order to make up for evaporation losses. The total liquid in the scrubber is about 150 ml. An area-type flowmeter is located in the water circulation line. It was found that the maximum possible flow gives optimum results. This is achieved by positioning the water jet in the Venturi throat by a trial and error method until the maximum flow rate is attained. This rate was equivalent to 8 to 9 gallons of water per 1000 cubic feet of air scrubbed (about 0.25 gallon per minute) in an instrument of the design and dimensions shown in Figure 1. Connected to the air line leading from the separator is a vacuum gage which may be calibrated to indicate the rate of air flow through the instrument. The width of the diffusing section at the point of attachment to the separator is about 0.75 inch.

It is noteworthy that the velocity of air through the Venturi throat and the liquid-to-air ratio of this apparatus were comparable to those of highly efficient industrial Venturi scrubbers (*4, 5*).

Figure 2 shows the scrubber conveniently housed in a single portable unit for field use. The over-all dimensions of the unit are 26 × 21.5 × 10 inches.

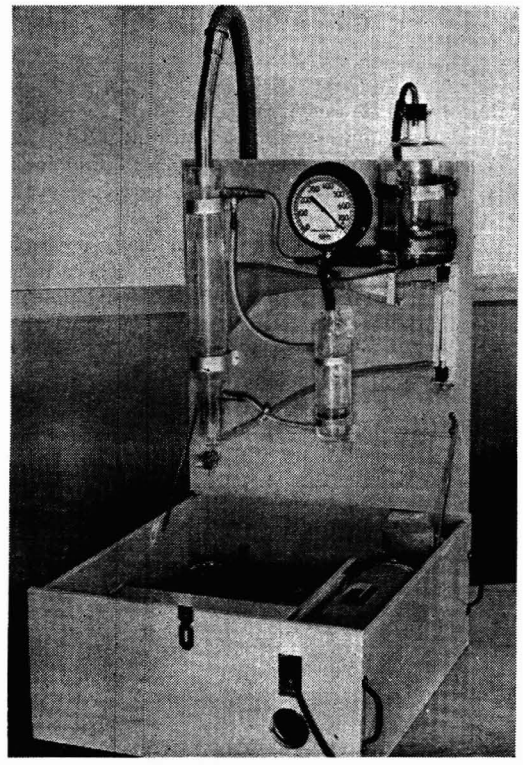

Figure 2. Portable Venturi Scrubber

It is built of Lucite, which permits observation of the spray and separation action. Lucite was preferred to glass because of ease of construction. Air is drawn through the device at the rate of 32 cubic feet per minute by a tank-type vacuum cleaner. At the Venturi throat, the gases have a velocity of 250 to 300 feet per second. After passing this point, the air is separated from the scrubbing liquid in the cyclonic separator section.

The scrubbing liquid is recirculated from the bottom of the separator back through the spray jet in the Venturi throat. The drop in pressure at the Venturi throat produces the "driving force" which causes the recirculation of the scrubbing liquid. A satisfactory water jet tube was found to be a length of glass tubing, 6 mm. in inside diameter, with a straight-cut end; this end is beveled toward the center to produce a sharp edge. A connection,

COLLECTING EFFICIENCY

The efficiency of this small Venturi scrubber was investigated for particulate and gaseous air contaminants.

The particulate contaminants were sodium chloride aerosol, which is relatively easy to collect, and sulfuric acid mist, which is notoriously difficult to collect by wet scrubbing. The gases used were ammonia and sulfur dioxide. These contaminants were introduced at a known rate into a 40-foot mixing tube through which air was passing at 1000 cubic feet per minute. The atmosphere leaving the tube was sampled by means of the Venturi scrubber, and the resulting scrubber solutions were subjected to appropriate chemical analyses. Sampling time in each case was 15 minutes.

The results show that the efficiency of the scrubber may be markedly different for various contaminants or for contaminants in different physical conditions.

Sodium Chloride. Sodium chloride aerosol was produced by dispersing a 15% aqueous solution of the salt into the 40-foot tube. Evaporation of the droplets left behind crystalline particles. Figure 3 shows a gold-shadowed electron micrograph of

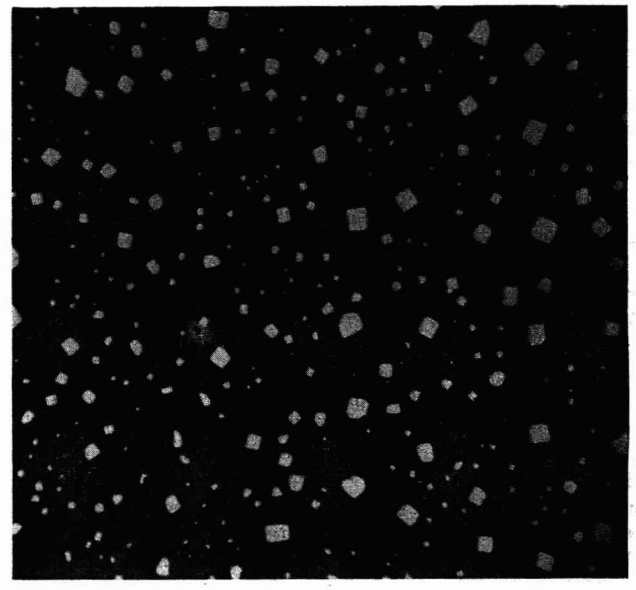

Figure 3. Sodium Chloride Particles from Aerosol

Table I. Scrubber Recovery of Sodium Chloride Aerosol

NaCl Concentration in Air, Mg. per Cu. Meter		
Calcd. from wt. of NaCl lost from generator	Calcd. from amount of NaCl in scrubber solution	Recovery, %
0.355	0.318	89.8
0.389	0.360	92.7
0.400	0.412	102.8
0.355	0.358	100.7
0.424	0.392	92.7
0.392	0.392	100.0
0.426	0.397	93.0

particles collected on a screen which had been in a sedimentation chamber containing the aerosol for 3 hours. Although the picture may overemphasize the percentage of large particles because of the short time allowed for sedimentation, it shows that all the particles were about 5 microns or less in diameter. The concentration of the salt in the air was determined by difference from (*a*) the known weight of sodium chloride originally introduced into the generator, and (*b*) the amount of salt remaining in the generator after the dispersion period. This latter was determined by thoroughly washing the remaining solution from the generator, evaporating it to dryness, and weighing the residue. The scrubber solutions were analyzed for sodium content by the flame spectrophotometer. These recoveries, which averaged 96%, are presented in Table I.

Sulfuric Acid. The sulfuric acid aerosol was formed by bubbling dried nitrogen through a small tube of fuming sulfuric acid. This was weighed immediately before and after the generation period to determine, through the weight of sulfur trioxide lost, the concentration of sulfuric acid produced in the test atmosphere. (Sulfur trioxide reacts rapidly with any moisture in the air to form a sulfuric acid aerosol.) One set of experiments was performed to determine the scrubber's efficiency in picking up the sulfur trioxide (sulfuric acid aerosol) immediately after it was generated from the bubbler. The sulfate content of the scrubber solution was measured turbidimetrically (*9*). Results of these experiments are shown in Table II. The scrubber removed about 44% of this mist from the air.

Table II. Scrubber Recovery of Sulfuric Acid Aerosol, as Sampled Directly from Generator

SO_3 Generated, Mg.	SO_3 Recovered by Scrubber, Mg.	Recovery, %
10.8	3.9	36
9.7	3.8	39
6.8	3.8	56
2.2	0.9	41
1.6	0.8	50

The sulfuric acid particles prepared in the manner described above are probably much smaller, and therefore more difficult to collect, than those found in normal atmospheres, because little time was allowed for them to grow by accumulating water. Another set of experiments was performed in which the sulfur trioxide was generated into the previously described 40-foot mixing tube, where several seconds elapsed between the time the mist was generated and the time of its collection by the scrubber. Again the scrubber solutions were analyzed turbidimetrically. The results of the tests carried out in this manner are presented in Table III. The scrubber averaged 65% efficiency in the ten trials.

A further check of the concentration of sulfuric acid in the aerosols was carried out by drawing the test atmosphere through a train of four Gooch crucibles, each three fourths filled with tightly packed asbestos (*12*). After sampling, the asbestos was washed until free of sulfuric acid, and an aliquot of the filtered wash water was taken for turbidimetric analysis. This has been shown to be a very accurate method for determining the concentration of sulfuric acid in aerosol form. Sulfuric acid concentrations as determined in this manner also appear in Table III.

The efficiency of this device for sulfuric acid in aerosol form was lower than might be desired and was somewhat less than that reported by Jones (*5*) for an industrial scrubber. However, the concentrations of sulfuric acid in the gases at the inlet of the scrubber described by Jones were at least 1300 times the concentration used in the present work, and the concentrations of sulfuric acid in the exhaust gases from the scrubber he described were at least ten times greater than the concentrations in the atmospheres that were sampled by the laboratory scale scrubber. Moreover, the sulfuric acid aerosol Jones sampled may have had a larger mean particle size than those used in the present investigation. Because an extensive study of the design of the Venturi, solution jet, and flow rates was not undertaken, it is possible that the recovery of such aerosols could be improved through some modification of the apparatus.

Table III. Scrubber Recovery of Sulfuric Acid Aerosol from Mixing Tube

SO_3 in Air, Mg. per Cu. Meter			
Detd. from loss in wt. of bubbler	Detd. by crucible train sampling	Detd. by Venturi-scrubber	Recovery, %
0.278	..	0.149	53.7
0.268	..	0.178	66.5
0.156	..	0.104	66.7
0.096	..	0.070	72.9
0.148	..	0.097	65.8
0.073	..	0.055	75.0
0.053	..	0.031	58.6
1.75	1.82	0.118	67.4
1.95	1.98	0.110	56.7
1.90	1.90	0.129	67.8

A few runs were made to determine the effect on the efficiency for the collection of sulfuric acid of substituting a 0.5% aqueous solution of sodium hydroxide for water as the scrubbing agent. There was apparently no advantage in using a basic scrubbing agent.

These results indicate that the apparatus can be used to determine in a semiquantitative manner very low concentrations of sulfuric acid in air. For precise results, it would probably be necessary to apply a predetermined correction factor which would depend on the efficiency of the scrubber for the acid in the particular atmosphere being investigated.

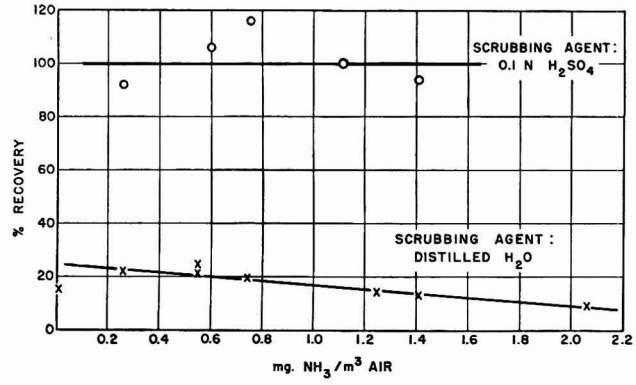

Figure 4. Recovery of Ammonia by Small Scale Venturi Scrubber

Ammonia. The long mixing tube was also used to determine the efficiency of the Venturi scrubber for removing ammonia from air by metering this gas from a cylinder into the intake end. Both distilled water and 0.1 *N* sulfuric acid were used as the solution in the scrubber during 15-minute runs. The ammonia content of the collecting solution was determined colorimetrically using Nessler's reagent (*3*). The efficiency using distilled water as the scrubbing solution increased with decreasing air concentrations of ammonia, approaching a maximum recovery of about

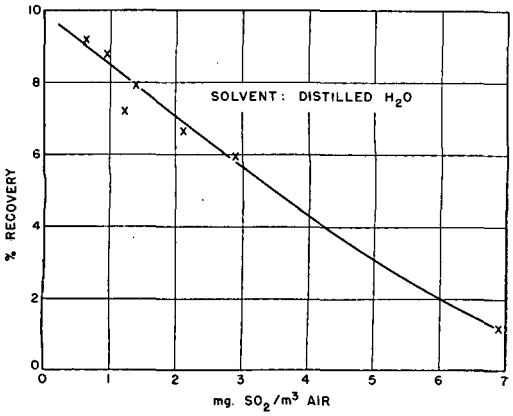

Figure 5. Recovery of Sulfur Dioxide by Small Scale Venturi Scrubber

20% at a concentration of 0.2 mg. per cubic meter of air. However, the recoveries averaged about 100% when 0.1 *N* sulfuric acid was used. The results are plotted in Figure 4.

The Venturi scrubber would be expected to be inefficient for volatile substances, such as ammonia, unless some substance is present in the scrubber solution to convert these materials to a nonvolatile form. For a very small increment of time at the beginning of the sampling period the scrubber is efficient. During this time the scrubber solution consists essentially of pure water. As the scrubber operates, however, the solution rapidly absorbs the gas, until in a short time equilibrium conditions exist and the vapor pressure of the volatile substance in solution equals the partial pressure of this substance in the air. Obviously, gas collection by the scrubber from that time on would be zero. Thus the over-all efficiency of the scrubber will decrease with increasing lengths of runs. The decreasing efficiency with increasing concentration must result from deviations from Henry's law.

Sulfur Dioxide. Finally, because sulfur dioxide is often found along with sulfur trioxide in the atmosphere, the efficiency of the scrubber for collecting this gas was studied. It was expected that when pure water was used in the scrubber the efficiency for sulfur dioxide collection would be low, as was the case in ammonia sampling. This would be an advantage when using the scrubber to collect sulfuric acid, inasmuch as interference from the dioxide, which in dilute solution oxidizes rapidly to sulfuric acid, would be minimized. To determine the possible extent of such interference, sulfur dioxide was metered into the mixing tube, and the scrubber sampled the air–sulfur dioxide mixture for 15-minute periods using distilled water as the solvent. The sulfur dioxide content of the resulting solution was determined by oxidizing the sulfite to sulfate, which was then determined turbidimetrically. The results are presented in Figure 5. The over-all efficiency varied from 1 to 9% as the sulfur dioxide concentration was decreased from about 7 to 0.6 mg. per cubic meter. Probably high efficiencies for sulfur dioxide, if desired, could be obtained by using sodium hydroxide solutions in the scrubber.

ACKNOWLEDGMENT

Acknowledgment is due the Smoke and Fumes Committee of the Western Oil and Gas Association, whose financial support and encouragement for the study of smog in Los Angeles made this work possible.

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Spectrophotometric Determination of Phosphorus in Organic Phosphates

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The molybdivanadophosphoric acid procedure for the determination of orthophosphate is adapted to the analysis of aliphatic phosphates after hydriodic acid conversion and to both aliphatic and aromatic phosphates after conversion by catalytic oxidation.

IN A previous paper (*6*) the authors reported that hydriodic acid conversion of aliphatic phosphates yields colorless solutions, whereas similar treatment of aromatic phosphates gives solutions of varying color from pale green to pale orange; and that conversion of both aliphatic and aromatic phosphates by sulfuric-nitric-perchloric acid oxidation in the presence of a molybdenum catalyst produces slightly greenish colored solutions. Although the solutions obtained by hydriodic acid conversion of aliphatics appear to be colorless, when viewed through a long column they show a faint yellowish cast. Because of the importance of the molybdivanadophosphoric acid colorimetric method of determining orthophosphate, reported by Misson (*5*) and modified by Kitson and Mellon (*4*), it appeared desirable to establish the degree of applicability of the method to organic phosphates decomposed by hydriodic acid or by catalytic oxidation. The present paper describes this study.

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The residual color after hydriodic acid conversion of aromatic phosphates is stable, requiring vigorous oxidation for complete removal. The addition of small amounts of ammonium persulfate, bromine water, perchloric acid, hydrogen peroxide, or potassium permanganate to the boiling solution has little effect in reducing the color. Evaporation to fumes with perchloric acid destroys the color in the case of tri-*p*-cresyl and tri-*o*-cresyl phosphates, but the meta derivative requires evaporation to dryness. From the outset, therefore, it appeared doubtful whether the molybdivanadophosphoric acid method would find convenient adaptation to hydriodic acid conversion in the analysis of aromatic phosphates. The residual color after conversion of organic phosphates by catalytic oxidation is due to the presence of molybdenum which is found not to interfere in the procedure herein described for the determination of orthophosphates.

Gibbs (*3*) reported that molybdiphosphoric acids form readily when soluble phosphates and molybdates are brought into solution together. The green color present after catalytic oxidation is attributed to some heteropoly acid of phosphate and molybdate, the exact nature of which is dependent upon the pH and the relative concentrations of phosphate and molybdate (*2*). Kitson and Mellon (*4*) found that unless too much ammonium ion is present the addition of ammonium vanadate solution readily converts molybdiphosphoric acids to the heteropoly molybdivanadophosphoric acid. This observation was confirmed in the present investigation. The necessity for adding vanadate before molybdate for the complete conversion of phosphate to molybdivanadophosphoric acid exists only under conditions favorable for the formation of ammonium molybdiphosphate which is not readily converted to the desired complex upon the addition of vanadate. Bolin and Stambery (*1*) reported that the presence of a small amount of molybdenum does not interfere in the analysis of various agricultural materials by the molybdivanadophosphoric acid procedure using a Cenco photelometer with a 420 mμ filter. In the present study measurements were made on a Beckman Model DU spectrophotometer at 460 mμ, the wave length used by Kitson and Mellon (*4*). The acidities of the solutions in the present study were approximately 0.6 *N*.

SOLUTIONS

A. Blank for Hydriodic Acid Conversion. Thirty milliliters of reagent grade hydriodic acid, specific gravity 1.7, 55 and 58%, were diluted to 100 ml., decomposed by the cautious addition of 30 ml. of concentrated nitric acid, diluted somewhat, boiled down to 100 ml. to remove all iodine, transferred to a 250-ml. volumetric flask, and diluted to the mark.

B. Blank for Catalytic Oxidation. A mixture of 5 ml. of catalytic oxidation reagent (*6*), 10 ml. of concentrated nitric acid, and 2 ml. of concentrated perchloric acid was evaporated to fumes of perchloric acid, transferred to a 250-ml. volumetric flask, and diluted to the mark.

C. Nitric Acid, 1 to 2.

D. Ammonium Vanadate, 0.25%. Two and one half grams of ammonium vanadate were dissolved in 500 ml. of warm water, the solution was cooled, 20 ml. of concentrated nitric acid were added, and the mixture was diluted to 1 liter.

E. Ammonium Molybdate, 5%. Fifty grams of ammonium molybdate were dissolved in water and diluted to 1 liter.

PROCEDURE

Preparation of Standard Graphs for Hydriodic Acid Conversion. A 0.4393-gram sample of twice-recrystallized potassium dihydrogen phosphate was dissolved in distilled water and diluted to 1 liter in a volumetric flask. This solution contained exactly 0.1 mg. of phosphorus per ml. Ten aliquots ranging from 1 to 10 ml. were measured from a 10-ml. buret into 50-ml. volumetric flasks. To each aliquot 5 ml. of each of solutions A, C, D, and E were successively added, and the mixtures were diluted to the mark, shaken, and allowed to stand for 10 minutes. The photoelectric spectrophotometer was adjusted to read 100% transmittance for a *blank containing* 5 ml. of each of solutions A, C, D, and E, diluted to 50 ml. The results were *plotted* on semilogarithmic paper as per cent transmittance against concentration of phosphorus in milligrams per 50 ml. The straight line provided a standard graph for reference of all analyses made on this instrument with the same reagents at the same dilution. A second series of ten standards was similarly prepared, using twice the volume of all reagents with the exception of solution A, of which only 5 ml. was used, and diluting to the mark in 100-ml. volumetric flasks. These standards were for use in making measurements on those phosphate solutions which were diluted to 100 ml. to keep the concentration of phosphorus less than 1.0 mg. per 50 ml., the highest concentration at which measurements were to be made. For both series of dilutions the slope was found to be −0.4989. The weight of phosphorus in 50 ml. of solution is given by the expression

$$\text{Mg. of P in 50 ml.} = \frac{2 - \log T_{460}}{0.4989} \qquad (1)$$

where T is the per cent transmittance.

Preparation of Standard Graphs for Catalytic Oxidation. Two graphs were prepared as described above, except that solution B was substituted for solution A. The slopes of these graphs were the same and identical with those for hydriodic acid conversion within the limits of experimental error.

Measurement of Hydriodic Acid Conversion Samples. Five-milliliter aliquots of samples of organic phosphates decomposed by hydriodic acid conversion were transferred from their 250-ml. volumetric flasks to those used for color development. Aliquots containing less than 1.0 mg. of phosphorus were transferred to 50-ml. volumetric flasks and aliquots containing from 1.0 to 2.0 mg. of phosphorus were transferred to 100-ml. volumetric flasks. For the 50-ml. dilutions, 5 ml. each of solutions C, D, and E were added in the order named, and the solution was made up to the mark and allowed to stand 10 minutes. For the blank, 5 ml. each of the solutions A, C, D, and E were similarly mixed, diluted, and allowed to stand 10 minutes. The per cent transmittance was measured and the concentration of phosphorus was calculated from Equation 1. For the 100-ml. dilutions, 10 ml. each of solutions C, D, and E were added, and the final solution was made up to the mark and allowed to stand 10 minutes. For the blank, 5 ml. of solution A and 10 ml. each of solutions C, D, and E were similarly diluted. The per cent transmittance was measured and the concentration of phosphorus was calculated. The percentage of phosphorus pentoxide in the unknown samples was calculated as follows:

Dilution volume

50 ml. $$\%\ P_2O_5 = \frac{\text{mg. of P in 50 ml.} \times 11.456}{\text{grams of unknown sample}} \qquad (2)$$

100 ml. $$\%\ P_2O_5 = \frac{\text{mg. of P in 50 ml.} \times 22.912}{\text{grams of unknown sample}} \qquad (3)$$

Table I. Comparison of Methods for Determination of Phosphorus in Organic Phosphates

Compound or Mixture	Theoretical P_2O_5 Equivalent[a], %	Hydriodic Acid Conversion		Catalytic Oxidation	
		Alkalimetric titration	Spectrophotometric procedure	Alkalimetric titration	Spectrophotometric procedure
		P_2O_5 Found, %			
Hexaethyl tetra	56.1	55.7	56.79	55.7	56.51
Hexaethyl tetra	56.1	54.7	55.53	56.8	55.13
Monoethyl	56.3	55.0	55.87	56.4	56.01
Monoethyl	56.3	56.2	57.66	57.2	57.38
Tetraethyl[b] pyrobase	48.9	48.6	49.45	48.4	49.13
Tributoxy ethyl	17.8	17.5	17.74	17.4	17.84
Triethyl	38.9	37.6	38.18	37.9	38.03
Triethyl	38.9	37.8	39.37	37.9	38.69
Mean for aliphatics	46.16	45.39	46.32	45.96	46.11
Vapotone[c]		43.3	44.17	44.8	43.70
Triphenyl[d]	21.8	21.3	35.82	21.8	21.19
Triphenyl	21.8	21.1	37.89	20.7	21.20
Tri-*o*-cresyl	19.2	19.5	19.68	18.8	19.18
Tri-*m*-cresyl	19.2	18.6	21.66	19.9	19.40
Tri-*p*-cresyl	19.2	19.0	19.62	20.0	19.36
Tricresyl[e]	19.2	17.3	17.64	18.7	18.71
Tri-*o*-phenyl phenyl	12.8	11.7	12.98	13.1	12.77
Mean for aromatics	19.03	18.36	23.61	19.00	18.81

[a] Upon basis of 100% purity.
[b] Technical tetraethyl pyrophosphate.
[c] 50% technical tetraethyl pyrophosphate with organic solvent and emulsifier.
[d] Recrystallized twice from methanol and water.
[e] Isomer not specified, but identified through derivatives as chiefly meta isomer.

Measurement of Catalytic Oxidation Samples. Measurement on 5-ml. aliquots of samples decomposed by catalytic oxidation were made as described above, except that solution B was substituted for solution A in the blanks.

DISCUSSION

In Table I each value for the per cent phosphorus pentoxide found represents the mean of measurements on duplicate aliquots from each of two to eight samples of the organic phosphate. The data for alkalimetric titration of aliquots of the same solutions analyzed here and reported previously (*6*) for both aliphatics and aromatics are included for comparison.

Hydriodic Acid Conversion. For aliphatic phosphates, results by the molybdivanadophosphoric acid method are slightly higher than by the alkalimetric titration procedure. For the aromatics they are somewhat high, in general, and in the case of triphenyl phosphate and tri-*m*-cresyl phosphate the results are so high as to make the method completely unreliable when applied to them. These results are attributed to the presence of residual colored organic matter. The time required for the destruction of this color by evaporation with oxidizing agents makes the method of hydriodic acid conversion of aromatics less desirable than catalytic oxidation for the spectrophotometric determination. The standard deviation for the aliphatics was 0.38%, and the results, while higher than by alkalimetric titration, are in agreement with those similarly obtained after conversion by catalytic oxidation. Comparison of results recorded in Table I suggests that the results obtained by alkalimetric titration after hydriodic acid conversion may be slightly low and those by the spectrophotometric procedure after similar conversion slightly high.

Catalytic Oxidation. Values obtained by the molybdivanadophosphoric acid method agree well with those obtained by the alkalimetric titration procedure. The standard deviation for aliphatics was 0.28% and for aromatics 0.09%.

SUMMARY

Analyses of organic phosphates, decomposed by hydriodic acid and by catalytic oxidation, were completed by the molybdivanadophosphoric acid spectrophotometric procedure, and compared with values obtained for aliquots of the same solutions by the molybdiphosphate-alkalimetric titration procedure. Results obtained by the spectrophotometric procedure compare favorably with results obtained by the titration procedure for aliphatic phosphates converted by hydriodic acid and for both aliphatic and aromatic phosphates converted by catalytic oxidation. The results obtained by the spectrophotometric procedure for aromatics after conversion by hydriodic acid are unreliable.

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Determination of Small Amounts of Chromium and Vanadium by Amperometric Titration

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An amperometric method for the rapid determination of chromium and vanadium is described. Chromium and vanadium are oxidized to chromate ion and vanadate ion by heating with perchloric acid and treatment with permanganate. Chromate and vanadate ions are titrated amperometrically with ferrous solution at the rotating platinum electrode. Reduced vanadium is selectively reoxidized to vanadate ion with permanganate ion and again titrated amperometrically. Chromium is obtained by difference. The method has been successfully applied to steel, crude oil, oil residuum, and asphalt samples. As little as 5 micrograms of chromium or vanadium can be determined.

KOLTHOFF and May (*5*) have shown that chromate ion in very dilute acid solution can be accurately determined by amperometric titration with ferrous ion using a rotating platinum indicator electrode. Ducret (*3*) determined chromate and vanadate ions in mixtures by titration with ferrous ion using sulfonated diphenylamine as indicator; he first determined chromate plus vanadate ions by reduction with ferrous ion, and then determined vanadium alone after selective reoxidation with permanganate ion. This method fails when dealing with small amounts of these ions because of the large indicator correction (*5*).

The chemistry and electrode reactions involved in the amperometric titration of chromate ion have been amply discussed by Kolthoff and May (*5*). In these laboratories, their method was found to be applicable also to the determination of small amounts of vanadium by titration of vanadate ion with ferrous ion. The principle is the same, except that the reduction of vanadate ion to vanadyl ion involves the addition of only one electron. Using 0.001 *N* ferrous solution, as little as 5 micrograms of vanadium can be readily titrated.

Oxidation of chromium and vanadium to chromate and vanadate ions may be accomplished by a number of oxidizing agents, such as bromate ion (*6, 12*), persulfate ion (*13*), permanganate ion (*13*), or perchloric acid (*10, 11, 13*). Of these reagents, perchloric acid was selected because of the simple and rapid manner in which the oxidation can be performed. However, some reduction of the chromate ion occurs by virtue of the hydrogen peroxide formed by decomposition of the perchloric acid (*10, 13*). Rapid cooling and dilution were found to be inadequate as a means of avoiding this reduction. Complete oxidation of the mixture was achieved by treating with permanganate ion after cooling and diluting.

In the method described, chromate and vanadate ions are titrated amperometrically with ferrous solution to measure the

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chromium plus vanadium. Vanadium is then selectively oxidized with permanganate ion according to the method of Ducret (*3*) and titrated amperometrically with ferrous solution. Chromium is obtained by difference. The method has been applied successfully to steel, crude oil, asphalt, and oil residuum.

APPARATUS

The apparatus used for amperometric titration was similar to that described by Laitinen, Jennings, and Parks (*7*), and consisted of a Fisher Elecdropode (Fisher Scientific Company, Pittsburgh, Pa.) equipped with a saturated calomel reference electrode, salt bridge, constant-speed stirring motor, and rotating platinum indicator electrode.

REAGENTS

Perchloric acid, approximately 60 to 72%.

Potassium permanganate solution, approximately 0.1 *M*.

Ferrous Ammonium Sulfate Solutions. To prepare 0.1 *N* ferrous solution, dissolve 39 grams of reagent grade ferrous ammonium sulfate hexahydrate in 1 liter of 1 *N* sulfuric acid. Prepare 0.01 *N* and 0.001 *N* solutions by dilution of the 0.1 *N* solution. Standardize these solutions immediately before use by amperometric titration with standard potassium dichromate solution of suitable strength.

Sodium azide, 4% aqueous solution.

PROCEDURE

To the solution of chromium and/or vanadium contained in a 50-ml. borosilicate glass Erlenmeyer flask, add 2 to 3 ml. of concentrated sulfuric acid and 3 ml. of 60 to 72% perchloric acid. Boil until heavy fumes appear or until the mixture becomes orange in color ($Cr_2O_7^{--}$). *Continue* boiling for approximately 3 minutes after the appearance of heavy fumes of perchloric acid or 2 minutes after coloration of the mixture. Cool the flask rapidly by plunging the lower half of the hot flask into ice water for a fraction of a second, withdraw it rapidly, then quickly immerse a second time, swirling for 6 to 7 seconds. This procedure permits rapid cooling of borosilicate glass flasks with a minimum danger of breakage. Immediately dilute with 10 to 15 ml. of water. Heat to boiling and add 0.1 *M* potassium permanganate dropwise until a pink color persists for 2 minutes. Add one drop of concentrated hydrochloric acid, boil 3 minutes, and cool to approximately 25° C.

Transfer the solution to a 100-ml. tall-form beaker, adjust the volume (see Table I), and place in position at the rotating platinum anode. Set the potential of the platinum anode to +1.0 volt. Using the conditions given in Table I, record the galvanometer reading, add 0.2 ml. of freshly standardized ferrous solution, and again record the galvanometer reading. Slowly add titrant until an increase in current is obtained; record the reading and volume of titrant added. Continue adding titrant in increments of 0.05 ml., recording the galvanometer reading after each addition, until 4 or 5 points on a straight line are obtained. Draw a straight line through the points obtained with no titrant and 0.2 ml. of titrant, and through the points obtained well after the "bend" in the curve. The point of intersection of these lines represents the end point of the titration.

After titration of chromium plus vanadium add 0.1 *M* potassium permanganate dropwise to the mixture (without turning off the stirring electrode) until a pink color persists for 2 minutes. Add 2 ml. of 4% sodium azide solution and stir for 5 minutes. If a pink color is still discernible, add 1 ml. of 4% sodium azide solution and stir 5 minutes. Titrate the mixture amperometrically, as before, conforming to the conditions given in Table I, but do not readjust the volume of solution.

From the result of the second titration, calculate the vanadium content of the sample. Subtract the milliequivalents of vanadium (second titration) from the total number of milliequivalents (first titration) to obtain milliequivalents of chromium. From this value calculate the chromium content of the sample.

Table I. Optimum Conditions for Amperometric Titration of Chromium and Vanadium

	Chromium plus Vanadium Content, Me.		
Titrating Conditions	1.0–0.1	0.1–0.01	<0.01
Volume of solution, ml.	40	30	30
Galvanometer sensitivity, microamperes per mm.	0.50	0.20	0.02
Normality of ferrous solution	0.1	0.01	0.001

Table II. Analysis of Solutions Containing Known Amounts of Chromium and Vanadium

Chromium, Mg.		Vanadium, Mg.		Approximate Normality of Titrant (Fe^{++})
Present	Found	Present	Found	
4.96	5.04	...	...	0.01
0.991	0.988	...	...	0.01
0.495	0.497	...	...	0.01
0.050	0.049	...	...	0.001
0.010	0.010	...	...	0.001
0.005	0.004	...	...	0.001
0.000	0.0007	...	...	0.001
0.000	0.0009	...	...	0.001
...	...	4.90	4.93	0.01
...	...	4.90	4.97	0.01
...	...	1.96	1.98	0.01
...	...	0.490	0.494	0.01
...	...	0.098	0.100	0.001
...	...	0.010	0.011	0.001
...	...	0.005	0.007	0.001
...	...	0.000	0.002	0.001
...	...	0.000	0.003	0.001
4.96	4.92	4.90	4.96	0.1, 0.01
4.96	4.97	0.490	0.491	0.1, 0.01
4.96	4.93	0.490	0.460	0.1, 0.01
4.96	4.94	0.000	0.000	0.1
0.000	−0.010	4.90	4.94	0.01

Table III. Chromium and Vanadium in National Bureau of Standards Steel Samples

N.B.S. Sample No.	Approximate Sample Wt., Mg.	Chromium, %		Vanadium, %	
		Present[a]	Found	Present[a]	Found
50a (chrome-tungsten-vanadium steel)[b]	100	3.52	3.50	0.97	0.94
61a (ferrovanadium)[c]	18	0.68	0.75	50.2	50.2
116a (ferrotitanium)[d]	100	0.23	0.23 0.23	0.33	0.35 0.34
161 (nickel-chromium casting alloy)[e]	50	16.9	16.8 16.5	0.03	0.04 0.03

[a] Values taken from N.B.S. certificate of analysis.
[b] Also contained Mn, Si, P, Cu, Ni, As, Sn, Mo.
[c] Also contained Mn, Si, P, Al.
[d] Also contained Si, Al.
[e] Also contained Mn, P, Si, Cu, Mo, Co.

RECOVERY OF CHROMIUM AND VANADIUM FROM KNOWN SOLUTIONS

Two aqueous solutions, one of pure potassium dichromate and the other of ammonium metavanadate (98.0% pure by electrometric titration, *4*), were prepared. The compounds were reduced by boiling the solutions with sulfuric acid and ethyl alcohol; excess ethyl alcohol was removed by subsequent heating to fumes of sulfuric acid. The results obtained for these solutions, separately and mixed, are shown in Table II.

APPLICATION TO STEEL SAMPLES

The reliability of the method for the analysis of complex steel samples is illustrated by the data in Table III for the analysis of four samples obtained from the National Bureau of Standards. These samples were dissolved in sulfuric acid or in a mixture of nitric and sulfuric acids. Nitric acid, when used, was removed by heating to fumes of sulfuric acid. The data show that little or no interference was caused by the various elements present.

APPLICATION TO PETROLEUM PRODUCTS

The data in Table II indicate that the method is sufficiently sensitive to be useful for the determination of small amounts of chromium and vanadium in petroleum products. Therefore, the method was applied to crude oil, asphalt, residuum, and a simulated high-additive oil to which was added known amounts of National Bureau of Standards steel samples.

The samples (60 grams) were burned in air and the residues ignited in a muffle furnace to remove carbon. Three to 4 ml. of concentrated sulfuric acid and 5 drops of concentrated nitric acid were added to the residue and the mixture was heated to fumes of sulfuric acid. Four additional portions of nitric acid were added (heating to fumes of sul-

Table IV. Chromium and Vanadium in Various Petroleum Products

Sample Description	Chromium, P.P.M. Added	Chromium, P.P.M. Found	Vanadium, P.P.M. Added	Vanadium, P.P.M. Found
Simulated high-additive[a] oil + ferrotitanium	0.20	0.33	0.29	0.24
	0.21	0.21	0.30	0.26
	0.09	0.11	0.13	0.15
	0.05	0.05	0.07	0.06
Simulated high-additive[a] oil +chrome-tungsten vanadium steel	11.5	10.6	3.2	3.0
Texas crude oil	...	0.0	..	7.8
		0.0		7.8
Refined mineral oil	...	0.0	..	0.0
		0.0		0.0
California residuum	...	<0.1	..	10.4
		<0.1		9.7
Venezuela crude oil	...	<0.1	..	178
				181
Texas crude oil	...	<0.1	..	5.9
				5.5
California crude oil	...	<0.1	..	6.0
				5.4
Asphalt	...	0.1	..	20.4
				18.2
Venezuela residuum	...	<0.1	..	272
				277

[a] Composition of high-additive oil:

	%		%
Cd	0.06	Si	<0.01
Pb	0.23	Cu	0.01
Mg	0.29	Fe	0.03
Na	0.35	Ca	0.14
Al	0.07	Sn	0.12
Ba	0.41	Cl	1.60
		P	0.67

furic acid after each addition) and the resulting mixture was oxidized and tested as described above.

Results obtained are shown in Table IV. The samples that contained the high-additive oils gave milky solutions and also gave a slightly less rapid increase in diffusion current following the end point. However, the results appear to be satisfactory. The results for various natural petroleums shown in Table IV indicate the presence of appreciable amounts of vanadium. This is in agreement with the findings of Shirey (*9*) and De Golyer (*2*), who report presence of vanadium in many petroleums. The large amount of vanadium found for the Venezuela samples is not surprising; a vanadium content of as high as 45% has been reported in the ash from a Venezuela petroleum (*1*). The data show that 5 micrograms (or less) of chromium or vanadium can be determined in 60 grams of oil.

DISCUSSION AND CONCLUSIONS

Small amounts of chromium and vanadium can be determined by the amperometric method without interference from moderate amounts of almuinum, barium, cadmium, calcium, chlorine, copper, iron, lead, magnesium, manganese, nickel, phosphorus, tin, titanium, tungsten, and zirconium. Approximately 0.5 hour is required to complete the analysis after bringing the sample into solution.

Several of the samples analyzed yielded milky solutions which would have seriously handicapped an indicator titration, but caused little difficulty in the amperometric titration. This method eliminates indicator corrections which are possible sources of error and are particularly undesirable when dilute solutions are employed.

The use of 0.001 *N* ferrous solution in the titration makes the method very sensitive and presents little difficulty due to change in titer. Normally the ferrous solution is freshly standardized against dichromate by titration at the rotating platinum electrode, but Schäfer (*8*) has recently shown it is possible to maintain ferrous solution of constant normality by using a reductor in the dispensing system.

Although the amperometric method for chromium and vanadium is most accurate when the metals occur in about equivalent concentrations, the data indicate that small amounts of either metal can be determined satisfactorily in the presence of the other, although because chromium is determined by difference, small amounts of chromium are determined less accurately in the presence of a large amount of vanadium than vice versa. This is particularly true when a more dilute ferrous solution can be used to titrate the vanadium.

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Determination of Small Amounts of Sulfuric Acid in the Atmosphere

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DURING the study of air contaminants it became necessary to determine the amount of free sulfuric acid in the atmosphere. The purpose was to determine the absolute amounts of this aerosol at various times of the day in connection with the observed variation in visibility, and to establish the rate of oxidation of sulfur dioxide into sulfuric acid mist under the meteorological conditions prevalent in the Los Angeles Basin. Specific measurements were also needed to determine the harmful effect of sulfuric acid on animal tissues as well as deleterious effects on vegetation.

Various methods have been tested for their suitability in the determination of very small concentrations of this aerosol in the air. The method used by Coste and Courtier in England (*2*) consists of the removal of sulfur dioxide from the air sample and the subsequent saturation of the air with water vapor. Following this, the sulfuric acid aerosol is condensed in a flask immersed in ice. The sulfuric acid, if present in the atmosphere sample, forms the nucleus for the water droplets. The particle sizes of these acid droplets grow sufficiently large to be collected as condensates in the cooled flask. Although this method produced

good results with solutions of known concentrations, it had the disadvantage that the atmosphere could be sampled only at the rate of 30 cubic feet in 24 hours. Therefore, because this represents about a minimum sample, only an average result for the entire period could be obtained.

Another method for the collection of the sulfuric acid component in the atmosphere makes use of a train of four to five Gooch crucibles which are inserted into one another (*8*). Each of the crucibles is tightly packed with asbestos, glass wool, or cotton wool. It was found that the filtering material itself showed a considerable alkalinity or acidity to water, even after repeated washings. In addition, the sampling rate had to be maintained at the low rate of 6 cubic feet per hour as a result of the back-pressure caused by the tightly packed filtering material. Results obtained by this method were erratic and the efficiency varied from 40 to 85%.

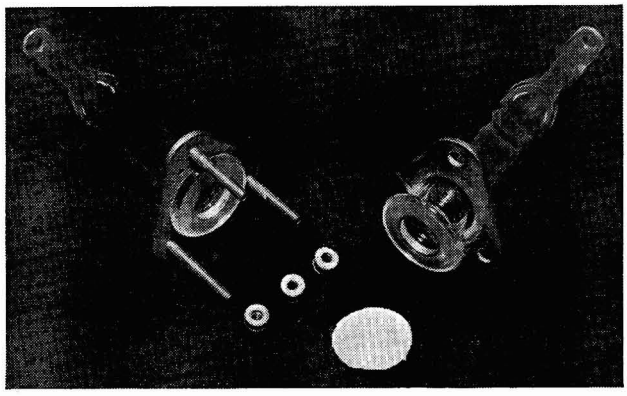

Figure 1. Holder for Filter Paper Disks

The Jena-type glass sintered filter, as recommended by Goodeve (*5*) of University College, London, for the removal of the sulfuric acid aerosol was also tested. Results from this type of filter confirmed the high efficiencies reported by Goodeve. However, because of the high resistance of the filters, the maximum sampling rate of air was limited to 10 cubic feet per hour. This rate was insufficient to determine the sulfuric acid collected over a 1-hour period because of the low concentrations normally involved. Goodeve's method is satisfactory for a 7- to 9-hour sampling period which would represent 70 to 90 cubic feet of air, but it proved unsatisfactory for shorter periods of sampling at the same rate.

Because none of the above procedures was sufficiently rapid for the authors' purpose, a modified sampling method has been developed, which is based upon the use of specially prepared Whatman No. 4 filter papers. The use of these filter papers had been suggested in 1924 (*9*) for the determination of sulfuric acid mist within chemical industries. Because of the fairly high concentrations of this acid encountered within the plant, no special consideration had to be given to the acidity or alkalinity of the filter papers. However, with the presence of very minute quantities of sulfuric acid mist in the atmosphere, these filter papers can be used for atmospheric sampling only after their own acidity or alkalinity has been carefully removed by special treatment. Failure to take this factor into consideration would lead to erroneous results. Owing to the low resistance and the high collecting efficiency of these filter papers, sampling rates as high as 60 cubic feet per hour can be used. Inasmuch as a 60-cubic-foot sample was found sufficient to determine the free sulfuric acid present, this method permits the measurement of hourly variations of the sulfuric acid aerosol in the atmosphere.

The presence of interfering substances in the atmosphere has been investigated. It was found that particulate matter normally present as dust is neutral and that gases such as sulfur dioxide, hydrogen chloride, oxides of nitrogen, and ammonia pass through the filter without affecting its pH. Therefore, for all practical purposes, the residual acidity measured by this method is equal to the total acidity of the sulfuric acid aerosol collected.

Although the pore size of the Whatman No. 4 filter papers is given by the manufacturer as 2 to 5 microns, particle sizes of 0.01 micron can be effectively trapped because of the centrifugal action on the particle being drawn through the filter paper. A gas containing an aerosol, on being drawn through a mechanical filter, is broken up into a large number of small eddy currents (*4*). It is thereby brought into contact with a relatively enormous surface and the aerosol will be deposited in or on the mechanical filter by the centrifugal force.

The efficiency of the Whatman No. 4 filter papers for the collection of the sulfuric acid aerosol was tested by generating known concentrations of sulfuric acid of various particle sizes and measuring quantitatively the amount of acid that was recovered. The recoveries were found to be 91 to 97% of the amount of sulfuric acid passed into the system (see Table I).

In the efficiency studies no Tyndall beam effect was observed in the air after it had passed the filters. According to Goodeve, no Tyndall beam effect can be observed with particles less than 0.01 micron in diameter. It is therefore possible that the small amount of acid unaccounted for was due to particle sizes of less than 0.01 micron. In this case no great significance is attached to the small amount of acid not recovered by the Whatman filters, because the particle size of sulfuric acid mist most frequently encountered in the Los Angeles atmosphere is of the order of 0.75 micron. This has been shown on atmospheric samples which were previously collected by this laboratory, using a modified Sonkin's cascade impacter. However, the possibility also exists that a slight buffering action of the filter paper and/or absorption on the walls of the sampling tubes contributed to this loss of about 5%.

EXPERIMENTAL

A glass holder was built to hold small disks of filter paper, 1 inch (2.5 cm.) in diameter. This holder is shown in Figure 1 and is similar to filter paper holders devised by Clarke and Hermance (*1*) and Gettler and Goldbaum (*3*). It consisted of two pieces of borosilicate glass tubing (15 mm. in inside diameter), each of which had been flared to a cross-sectional diameter of 30 mm. and ground flat on the flared ends. These two ends were coupled by means of two perforated metal collars which were clamped together and pressure-tightened by screws with knurled nuts. The disks of filter paper were cut to a diameter of 25.4 mm., seated between the ground ends of the holder, and clamped. Two disks, superimposed, were used for each test.

Table I. Efficiency Study of Whatman No. 4 Filter

Test Run	Theoretical Wt. of Sulfur Trioxide Generated, Mg.	Total Wt. of Sulfur Trioxide Recovered, Mg.	Efficiency, % Recovery
1	3.73	3.39	90.8
2	7.79	7.32	94.0
3	5.84	5.43	93.0
4	13.60	12.84	94.5
5	14.60	14.10	96.6

The filter paper disks were cut from large Whatman No. 4, 18.5-cm. filter papers. Three of these filter papers were washed and dried together to form a given batch of filter disks. Washing consisted of leaching with copious quantities of distilled water successively over an extended period of time. Satisfactory results were obtained with five 12-hour leachings, using 500 ml. of distilled water for each. A 19-cm. borosilicate glass crystallizing dish was used for the washing. After washing, the filter papers were oven-dried at 100° C.

By means of a sharpened cutting tool, each filter paper was cut into 1-inch disks, which were placed in a dry, clean jar representing a filter batch containing 70 to 75 filter disks.

A method has been developed for determining small concentrations of sulfuric acid mist in the atmosphere. It employs specially prepared filter papers for the removal of this aerosol from the air stream and determines the acidity by titration. With the help of this method it is possible to measure variations of the amount of free sulfuric acid in the air on an hourly basis.

The pH of the filter batch was tested by placing two disks, selected at random, in a given volume (20 ml.) of carbon dioxide–free, distilled water of known pH. The disks were thoroughly macerated and stirred by means of two glass rods to form a slurry of pulp. After standing for 3 minutes, the pH of the water–filter paper mixture was determined with the use of a Beckman pH meter. Similarly, two other pH tests were made on the filter batch to determine the uniformity of pH. If the three pH batch tests indicated a consistency of 0.03 pH unit and a deviation of not more than 0.10 pH unit from that of the distilled water used in the measurement, the filter batch was considered satisfactory for use in determining atmospheric sulfur trioxide.

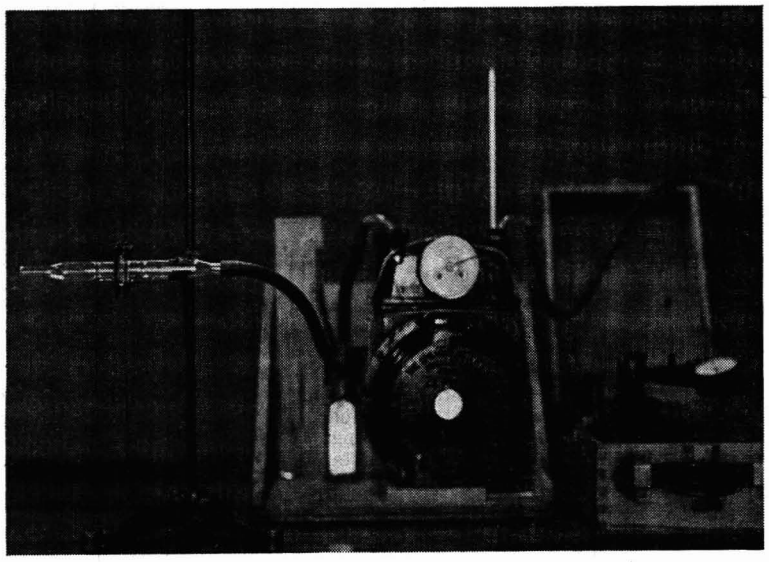

Figure 2. Apparatus for Field Tests

Table II. Results Obtained in Downtown Los Angeles

Date, 1949	Hour Sample Taken	Relative Humidity at 8:30 A.M.	Sample, Cu. Feet	Sulfuric Acid, P.P.M. (Vol.)	Remarks
Dec. 2	9–10 A.M.	94	54	0.036	Dense fog, intense air pollution
Nov. 22	1–2 P.M.	91	60	0.033	Dense fog, intense air pollution
Sept. 1	9–10 A.M.	71	59	0.015	Less fog, moderate air pollution
Aug. 31	9–10 A.M.	61	61	0.0056	Very little fog, very slight haze
Dec. 4	9–10 A.M.	30	62	0.0	Clear day, no pollution

Table III. Results Obtained in London, England

Date, 1934	Volume of Air, Liters	Time, Hours	H_2SO_4, P.P.M. (Vol.)	Remarks
Feb. 12	3440	7.5	0.034	Slight fog
Feb. 14	2360	9.5	0.019	Less fog
Feb. 15	3660	9.75	0.015	Less fog
Feb. 16	3580	9.5	0.009	Fog in morning
Feb. 23	1390	7	0.0	Clear

Field tests for atmospheric sulfur trioxide were made using two filter disks in the holder as described. Air was drawn through the filter at a rate of 50 to 60 cubic feet per hour with the pressure drop through the filter and air temperature being recorded (Figure 2). After sampling for 1 hour, the filter disks were removed and placed in a dry, clean 2 × 2 inch jar.

Following the foregoing procedure, the test filters were macerated in 20 ml. of distilled water and the resulting solution was then measured for pH and titrated with 0.002 *N* sodium hydroxide. The end point for the titration was that of carbon dioxide–free, distilled water corrected for filter batch acidity or alkalinity. It was determined by the use of a Beckman pH meter.

The titrated acidity was expressed as parts per million of sulfuric acid by volume according to the following formula:

$$\text{p.p.m.} = \frac{\text{ml. of base} \times \text{normality} \times 10^3 \times 22.41 \times 10^6}{98 \times 28.32 \times \text{cu. feet of air (S.T.P.)}}$$

RESULTS

The amounts of sulfuric acid found in typical samples from the Los Angeles area are shown in Table II. They are of the same order of magnitude as the data which were taken by the University College, London, employing the sintered filter method. Results shown in Table III (*6*) are the data which were collected in London, England, under clear and foggy weather conditions.

The results obtained by this method indicate that the sulfuric acid content of the atmosphere ranges from 0.0 to 0.036 p.p.m. by volume. The highest readings for sulfuric acid were obtained in days that started with a high relative humidity. A high moisture saturation of the atmosphere, when water droplets are condensed on the surface of dust particles, salt spray, metallic oxides, etc., appears to provide a highly satisfactory medium for the sulfur dioxide of the atmosphere to be dissolved and then rapidly oxidized to sulfuric acid. After the temperature increases during the day and the fog particles dissipate, the sulfuric acid mist remains in the atmosphere because of its low vapor pressure. In dry air the oxidation of sulfur dioxide to sulfuric acid seems to proceed at a very slow rate. Even in the presence of relatively high sulfur dioxide concentrations, the sulfuric acid content remained very low or immeasurable at a low relative humidity.

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Microdetermination of Oxalate in Fermentation Media

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An improved volumetric method is presented for the determination of small quantities of oxalate, capable of analyzing 3.5 mg. of oxalic acid with a recovery of 100.55% (coefficient of variation 0.24) down to 0.4 mg. with a recovery of 99.71% (coefficient of variation 0.40). Comparisons are made with contemporary techniques. The effect of citrate, original sample volume (up to 10 ml.), and medium constituents upon the accuracy is also given.

A METHOD is described for the estimation of small quantities (0.4 to 4.0 mg.) of oxalic acid in the presence of other organic acids.

The method of Pucher, Wakeman, and Vickery (*4*), on which it was based, entailed precipitation of the calcium salt at pH 5.0; separation was effected by means of a microcrucible containing special asbestos, prepared as directed by Kirk and Moberg (*2*). Neither this separation, which was unreliable and involved a transference of the precipitate, nor separation by centrifugation was found suitable. Advantageous though the latter process might be in theory (*3*), it had the disadvantage that floating of the precipitate was troublesome, in spite of the addition of an acid-alcohol mixture.

Table I. Standard Method

(1 ml. of oxalate solution in each case. 8 replicates)

Theoretical $(COOH)_2$	Recovery	Difference		Range of Recoveries	Coefficient of Variation
Mg.	*Mg.*	*Mg.*	%	%	
3.646	3.666	+0.020	+0.55	100.3-100.7	0.24
3.599	3.614	+0.015	+0.42	100.0-100.7	
1.370	1.366	−0.004	−0.29	99.1-100.3	0.37
1.286	1.281	−0.005	−0.39	99.3-99.8	
0.4061	0.4049	−0.0012	−0.29	99.2-100.3	0.40
0.3990	0.3989	−0.0001	−0.03	99.5-100.3	

The present author employed King filter sticks (*1*) in the early part of the work for the separation of the precipitate, but occasional reaction of the filter paper with the permanganate caused their discontinuance, although they were handier to use. Sintered-glass filter sticks were found most suitable for the separation. Titration of the oxalate with 0.02 *N* potassium permanganate at 100° C. gave unsatisfactory end points (variation of ±2.5% for 0.4 mg. and ±5.0% for 7 mg. in 12 ml., *4*). Instead, titration was done with 0.10 *N* or 0.05 *N* permanganate at room temperature (about 25° C.) in the presence of manganese sulfate as a catalyst. This was very satisfactory if the volume of liquid to be titrated was kept below about 3 ml. and if horizontal microburets capable of delivering 0.8 to 0.001 ml. or 0.2 to 0.0002 ml. were used.

Table II. Modifications of Filtration and Titration Procedure

(1 ml. of oxalate solution in each case)

Filtration Method	Titration Method	Theoretical $(COOH)_2$	Replicates	Recovery	Difference		Coefficient of Variation
		Mg.		*Mg.*	*Mg.*	%	
King filter stick	0.02 *N* $KMnO_4$, 10-ml. buret graduated in 0.01 ml., 100° C. (*4*)	3.519	8	3.593	+0.075	+2.10	1.7
		3.501	8	3.589	+0.088	+2.51	
Pucher (*4*)	Pucher (*4*)	3.626	6	3.817	+0.191	+5.3	2.5
		3.588	6	3.773	+0.185	+5.1	
Pucher (*4*)	Excess $KMnO_4$, KI, and titration with $Na_2S_2O_3$ in 10-ml. buret graduated in 0.01 ml. (*3*)	3.390	6	3.501	+0.111	+3.3	1.3
		3.426	6	3.549	+0.124	+3.6	
King filter stick	Powers (*3*)	3.521	5	3.577	+0.056	+1.60	1.5
		3.500	7	3.566	+0.066	+1.90	

REAGENTS

Sodium oxalate, analytical reagent grade, dried at 110° C. for 2 hours. Solutions are made up to contain approximately 5.0, 1.0, and 0.6 mg. per ml.

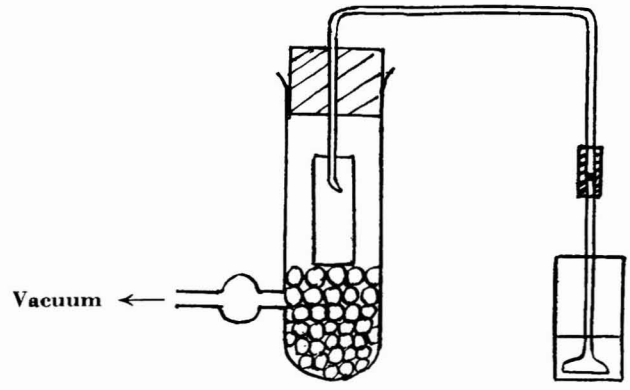

Figure 1. Method of Filtration

Potassium permanganate, analytical reagent grade, 0.10 *N* and 0.05 *N*. The requisite amount is dissolved in slightly more than the theoretical quantity of distilled water, boiled 30 minutes, then allowed to stand overnight. After being filtered through sintered glass the solution is standardized against sodium oxalate. Permanganate so prepared is stable for at least 9 months.

Citric acid, analytical reagent grade. Solutions are prepared to contain approximately 25.0, 6.0, and 2.0 mg. per ml.

Ammonia, 6 *N*, analytical reagent grade.

Acetic acid, 2 *N*, analytical reagent grade.

Sulfuric acid, 3 *N*, analytical reagent grade.

Manganese sulfate tetrahydrate, analytical reagent grade.

Bromocresol purple indicator, 0.04% in distilled water.

Calcium acetate, pure, 5% solution.

PROCEDURE

One milliliter or less of the solution containing oxalic acid (0.4 to 4.0 mg.) was measured into a microbeaker and made just alkaline to the bromocresol purple (already present) with 6 *N* ammonia; 2 *N* acetic acid was then added to a yellow color (pH 4.0 to 5.6), followed by 0.5 ml. of 5% calcium acetate in the cold, and was left to stand a minimum of 2 hours, after the beaker had been gently swirled to mix the contents.

The supernatant was removed under slight vacuum by means of a 7-mm. H4 sintered-glass filter stick and the precipitate was washed with a saturated solution of calcium oxalate prepared according to Stanford and Wheatley (*5*). The washings were removed in the same manner and the precipitate was finally sucked dry by appropriate manipulation with the filter stick.

The beaker containing the precipitate and filter stick was now treated with 1 ml. of 3 *N* sulfuric acid and warmed on the hot plate. The filter stick was used to assist the solution of the calcium oxalate, especially on the sides of the beaker, where there was usually a tendency to upward creep of the precipitate during the washing with saturated calcium oxalate. The resultant solution was then removed under reduced pressure through the filter stick, followed by washing with distilled water.

The product was collected in a second microbeaker as illustrated in Figure 1. About 4 mg. of manganese sulfate were now added and shaken to dissolve, and the solution was titrated at room temperature (about 25° C.), with 0.1 *N* or 0.05 *N* permanganate using a horizontal microburet. The titration was carried out for two minutes with the usual additional care near the end point. This time was required to obtain the accuracy of determination in Table I.

Table III. Effect of Titration Temperature

(King filter stick, 0.8- or 0.2-ml. microburet, and 0.10 or 0.05 *N* $KMnO_4$. 1 ml. of oxalate solution in each case)

Temp., ° C.	Theoretical $(COOH)_2$	Replicates	Recovery	Difference		Coefficient of Variation
	Mg.		*Mg.*	*Mg.*	%	
100	3.525	9	3.553	+0.028	+0.79	0.87
	3.499	6	3.551	+0.052	+1.48	
60	3.525	11	3.529	+0.004	+0.11	0.71
	3.499	6	3.527	+0.028	+0.80	
25	3.596	4	3.593	−0.003	−0.08	0.23
	3.608	9	3.618	+0.010	+0.28	

DISCUSSION

The tables show that the standard method of titration at about room temperature with manganese sulfate as catalyst was by far the most accurate. All other titration techniques gave greater variability and a slightly high result (Table II). Raising the temperature had a similar effect on the results of the standard method (Table III). The volume of solution at the time of precipitation had a small but definite effect in raising the titer slightly and introducing a little greater variation (Table IV). Although the presence of citrate produced statistically significant differences from the expected titers, these effects were relatively small under the conditions described in Table V. Possibly the additive effect of the larger citrate concentration was due to a slight insolubility of calcium citrate in the small volume of precipitated solution (2 ml.) used (but see Vickery and Pucher, *6*). It has never proved serious in the author's experience in the range indicated. These values of citrate (0 to 26 mg.) and oxalate (3.6 mg.) were chosen to cover the expected values in a research program on the metabolism of *Aspergillus niger*.

Table IV. Effect of Volume of Precipitating Solution

(Present standard procedure. 6 replicates)

Volume	Theoretical $(COOH)_2$	Recovery	Difference		Coefficient of Variation
Ml.	*Mg.*	*Mg.*	*Mg.*	%	
10	3.476	3.490	+0.014	+0.40	0.21
	3.575	3.593	+0.018	+0.50	
0.5	3.476	3.488	+0.012	+0 34	0.15
	3.575	3.586	+0.011	+0.31	

Table V. Effect of Citric Acid on Oxalate Precipitation

(Present standard procedure. 1 ml. of oxalate solution and 1 ml. of citrate solution in each case, save with oxalate alone which contained 1 ml. of oxalate solution and 1 ml. of distilled water. 7 replicates. Theoretical $(COOH)_2$, av., 3.627 mg.)

Citric Acid (Av.)	Recovery (Av.)	Difference		Coefficient of Variation
Mg.	*Mg.*	*Mg.*	%	
25.94	3.658	+0.031	+0.86	0.79
10.67	3.641	+0.014	+0.39	0.33
5.55	3.644	+0.017	+0.47	0.39
3.24	3.654	+0.027	+0.75	0.25
1.34	3.633	+0.006	+0.17	0.42
0	3.634	+0.007	+0.19	0.23

Table VI. Recovery of Oxalic Acid in Presence of Large Excess of Citric Acid

(Present standard procedure. 4 ml. of oxalate solution and 1 ml. of citrate. 74.5 mg. of citric acid per ml. 4 replicates)

Theoretical $(COOH)_2$	Recovery	Difference		Range of Recoveries	Coefficient of Variation
Mg.	*Mg.*	*Mg.*	%	%	
0.4176	0.4155	−0.0021	−0.50	99.2-99.7	
0.4211	0.4195	−0.0016	−0.38	99.3-99.9	0.22

Table VI provides data on the recovery, in the presence of a rather greater excess of citrate. This concentration of oxalate (0.4 mg.) was used because it provided the practical minimum for the present improved method of oxalate determination. This weight, however, may be contained in up to 10 ml. of solution with only slight changes in efficiency (Table IV). Titration of less than 0.4 mg. of oxalic acid with permanganate provides difficulties in a poor end point appreciation and the recoveries presented here no longer hold.

Table VII. Recoveries from Media Containing Citrate

(18.3 mg. of citric acid per ml. of media. Present standard procedure. 1 ml. of oxalate solution and 1 ml. of media in each case. 4 replicates)

Medium $(COOH)_2$	$(COOH)_2$ Added	Recovery of Added $(COOH)_2$	Difference		Range of Recovery	Coefficient of Variation
Mg.	*Mg.*	*Mg.*	*Mg.*	%	%	
0.1589	1.6811	1.6778	−0.0033	−0.20	99.5-100.3	0.34
0.1963	1.8516	1.8516	0.0	0.0	99.5-100.4	0.43
0.1826	1.8477	1.8436	−0.0041	−0.22	99.7-100.6	0.44

Table VII indicates that recoveries from fermented media (*Aspergillus niger* grown on original media containing ammonium nitrate 0.22%, potassium dihydrogen phosphate 0.10%, magnesium sulfate heptahydrate 0.025%, and glucose 10% as carbon source) were in good agreement with theory.

To apply the method to very dark colored material, such as molasses or plant tissues, the material is treated as for "preparation of organic acid fraction" (Pucher *et al., 4*). An aliquot of the neutralized extract is then used for the determination of oxalate as described above.

The method has been used for routine analysis in these laboratories over the past year and has proved fully satisfactory. When twenty samples were run together the method required about 5 hours, excluding the time involved in the precipitation and subsequent standing. If a less accurate determination will suffice—e.g., coefficient of variation not more than 0.9% (Table III)—then to speed up the titration, the acid solution of calcium oxalate may be heated to boiling on the hot plate with a crystal of manganese sulfate and titrated without further heating, the catalyst compensating for the falling temperature.

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Amperometric Titration of Sulfhydryl Groups

Microgram Analysis

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A modification of the amperometric titration method for sulfhydryl groups has been developed for use with microgram quantities. A vibrating platinum electrode is used as a combination electrode-stirrer. With this technique it is possible to determine with reasonable accuracy amounts of sulfhydryl as small as about 1 microgram. The method was tested with denatured protein solution and yielded results which were in agreement with previous studies of this type.

KOLTHOFF and Harris (*6*) described an argentometric titration for compounds containing the sulfhydryl group, using an amperometric end point and a rotating platinum cathode. In an effort to adapt the method to determination of microgram quantities, Perrone and Kirk in 1946 were able to show that a vibrating platinum electrode was equally effective. Using the standard vibrating stirrer (*5*) commonly employed in microgram analysis, but substituting a platinum wire for the glass thread and attaching the wire to the electrical system, the end points obtained with the Kolthoff and Harris arrangement were as good as those obtained with the rotating electrode, inasmuch as the rapidity of movement of the platinum wire in the solution could be made as great. Since that time, the vibrating electrode has been used for polarography but not amperometry with larger volumes of solution by Harris and Lindsey (*4*). Benesch and Benesch (*1*) have recently demonstrated the applicability of the rotating electrode to determination of the sulfhydryl group in relatively large amounts of biological materials. In this article is described the modification of standard microgram analysis to the similar determination of microgram quantities of this group in biological and other materials, using the vibrating platinum stirrer-electrode.

APPARATUS

The vibrating electrode consisted of a standard stirrer, shown in Figure 1, in which the glass thread was replaced by a platinum wire. Because the vibrator is constructed of steel, electrical connection was made to the screw holding it at the attached end. The electrode also served as stirrer, agitation being produced in the small volume of solution by the 60-cycle vibration. Titrations were performed in porcelain titration dishes, carried on the standard titration table (*5*). Titrating liquid was added either from the capillary buret described by Sisco, Cunningham, and Kirk (*8*) or from a modified horizontal buret (*5*) whose total capacity was only about 20 microliters. This buret of very small capacity made possible greater precision in adding small increments when the amounts titrated were in the lower range of the method.

As reference cell, a vessel similar to that previously described (*8*) was filled. In the bottom of the cell was placed about 0.6 cm. of mercury, on top of which was added an electrolyte solution made by dissolving 4.2 grams of potassium iodide and 1.3 grams of mercuric iodide in 100 ml. of saturated potassium chloride solution. The salt bridge was filled with a gel of 3% agar containing 30% potassium chloride. After a few weeks' use the agar gel tended to crack and required replacement. This could be retarded by keeping the gel immersed in potassium chloride solution when not in use. Precautions against the inclusion of air bubbles in the agar were necessary at all times; this was readily accomplished by gently sucking the melted agar solution into the bottom of the salt bridge tube.

The current was measured by a galvanometer of the swinging light-beam type having a sensitivity of 0.21 μa. per division. A resistance wire was provided across the leads in case the deflection of the instrument became too great. This could be a high resistance radio potentiometer allowing the adjustment of the resistance as needed. The substitution of a fine Nichrome wire about 45 cm. (15 inches) long, having a resistance of about 25 ohms per inch, was satisfactory.

REAGENTS

Silver nitrate solution, about 0.001 *M*, was made by dilution of a stronger stock solution which was usually 0.1 *M*. The stock and diluted solutions were carefully standardized against potassium chloride on a larger scale, making use of dichlorofluorescein indicator.

Supporting electrolyte solution was made to contain 0.25 *M* ammonium hydroxide and 0.1 *M* ammonium nitrate. This solution prevented precipitation of chloride and functioned in the electron transport system.

PROCEDURE

A convenient aliquot of the solution being analyzed was measured with a micropipet into the porcelain dish, using two or three rinsings. The half-cell and vibrating platinum electrode were immersed in the solution and the current was allowed to equilibrate while the buret was being filled with silver nitrate solution.

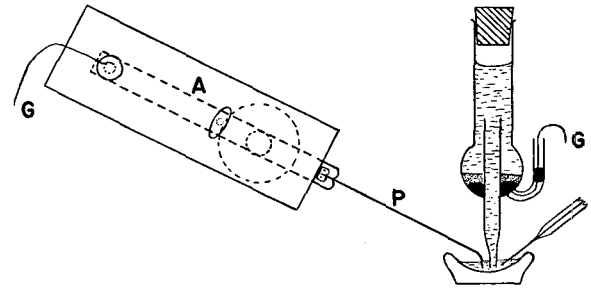

Figure 1. Vibrating Cathode Assembly

A. **Magnetic vibrator**
G. **Leads to galvanometer**
P. **Vibrating electrode**

Table I. Cysteine Solutions of Known Content

Cysteine HCl Taken, γ	Sulfhydryl Group, γ	Time of Standing, Min.	No. of Detns.	Cysteine HCl Found[a], γ	Average Recovery, %	Standard Error[b], %
30.6	6.23	10–30	6	30.4 ± 1.03	99.6	1.6
15.3	3.11	2–3 hours	6	11.98 ± 0.6	77.7	2.0
5.06	1.029	10–30	5	4.904 ± 0.22	97.8	2.9
3.03	0.616	10–30	8	2.765 ± 0.08	91.2	1.3
1.01	0.205	10–30	3	0.943 ± 0.03	93.0	2.4
1.01	0.205	4–5 hours	3	0.83 ± 0.05	82.2	4.5

[a] Mean ± mean deviation.

[b] Calculated from formula S.E. = $\sqrt{\frac{\epsilon d^2}{N(N-1)}}$.

Table II. Glutathione, Solutions of Known Content

Glutathione Taken, γ	Sulfhydryl Group, γ	Time of Standing, Min.	No. of Detns.	Glutathione Found[a], γ	Average Recovery, %	Standard Error[b], %
50	5.39	10–30	5	50.46 ± 0.24	100.1	0.27
21.1	2.27	10–30	4	21.55 ± 0.55	102.0	1.7
21.1	2.27	6–7 hours	5	19.3 ± 0.4	91.5	1.5
5.40	0.58	10–45	5	4.84 ± 0.29	88.50	3.4
5.40	0.58	2–3 hours	6	4.64 ± 0.49	86.0	4.5

[a] Mean ± mean deviation.

[b] Calculated from formula S.E. = $\sqrt{\frac{\epsilon d^2}{N(N-1)}}$.

Titration was not started until the current remained steady. At times this required as long as 10 minutes when a new reference cell was first employed. The vibrating electrode required a shorter time for equilibration than the rotating electrode, usually operating at the very beginning of its movement. Titration was carried out by adding increments of silver nitrate of convenient size and noting the galvanometer deflections after each addition. When the first large deflection was observed, the current was recorded for at least three more additions of reagent. Blank determinations were made under each set of experimental conditions. Blanks were uniformly low, corresponding to 0.2 to 0.8 microliter of titrating solution.

A graph of the results showing galvanometer deflections as ordinate versus increment as abscissa was constructed. The shape of the curves was the same as those of Kolthoff and Harris and others.

RESULTS

Using the technique described, analyses were made of cysteine hydrochloride, glutathione, and eventually protein solutions, some of which had been denatured. The range of the method was tested by analysis of cysteine solutions of known content (Table I). The recoveries were satisfactory when the experimental conditions were controlled with reasonable accuracy. When long times of standing were employed, the recovery fell uniformly, as shown by the small standard error combined with considerable lack of recovery. The smallest amounts of cysteine when run rapidly still yielded a reasonably high recovery, though not uniformly theoretical. As the amounts became larger the theoretical recovery was approached more closely.

Similar results were obtained with known quantities of glutathione (Table II). Here, also, the time of standing was significant, any time over a half hour causing low recovery. The smaller quantities here also were not completely recovered. Considering that the amount of sulfhydryl group present in glutathione is only 10.77%, the lower limit of accurate analysis is at least as low as 1 to 2 micrograms of sulfhydryl group.

After the procedure had been tested with known concentrations of simple compounds, it was thought desirable to test its utility with denatured proteins. For this purpose, bovine serum albumin was titrated to determine the amount of sulfhydryl normally present. It was also denatured by various denaturing agents, as shown in Table III. The amounts of sulfhydryl obtained with denatured proteins by this procedure were found to be reasonably constant. The values were in good agreement with the value of 0.34% found by Greenstein (*3*). There is no method by which the exact amount of sulfhydryl which should be present may be demonstrated. The agreement between denaturation with various concentrations of alcohol and that with guanidine hydrochloride was striking, whereas the value with urea was somewhat lower.

Table III. Sulfhydryl Group in Bovine Serum Albumin

Denaturing Agent and Solvent	No. of Detns.	Sulfhydryl as Cysteine γ/100 γ Protein
Alcohol[a], %		
31.6	4	0.32 ± 0.02
42.5	3	0.29 ± 0.01
63.3	4	0.30 ± 0.02
None	4	No titration
Guanidine hydrochloride[b]		
Distilled water	6	0.33 ± 0.03
63.3% alcohol	4	0.31 ± 0.02
Urea[c]		
Distilled water	5	No titration
43.5% alcohol	5	0.21 ± 0.01

[a] Protein placed in alcohol solution and allowed to stand at room temperature 0.5 hour before titration.

[b] Protein solution added to guanidine hydrochloride according to method of Greenstein (*2*).

[c] Denatured according to method of Anson (*7*).

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Microbiological Determination of Sulfur in Yeast

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THE recent investigation of the ability of various yeasts to use the sulfur of amino acids and other sulfur compounds (*11*) suggested the possible development of a microbiological assay method for sulfur. A search of the literature revealed that most of the values given for the sulfur content of yeast were obtained by direct ashing of the samples. Fink and Just (*9*) used the Eschka mixture as a fixative during the ashing of yeast samples and obtained much higher values than those obtainable by direct ashing. Highest total sulfur values found were obtained by Block and Bolling (*4*) using the Pregl method. These authors reported that sulfur analyses on dried yeasts are difficult to carry out and may at times be low. In view of the widely divergent values for sulfur in yeast reported by different investigators, an attempt has been made to clarify this problem by the development of a microbiological method.

The success of such a microbiological assay method is partially dependent upon the utilization of a test organism capable of responding equally well to all forms of available sulfur in the sample. In previous work (*11*) *Torula utilis* gave indications of responding to both organic and inorganic sulfur sources in this manner. Further investigation has revealed that although its growth responses to small increments of various sulfur sources are not identical, they are more alike than those of any other or-

A microbiological assay method for the determination of total sulfur in yeast is described in which *Torula utilis* is the test organism and methionine is the reference standard for sulfur. The microbiological assay results on yeast are compared with results from four known chemical methods. The method may be applicable to other substances. Results on whole soybean flour, defatted soybean flour, and vitamin-free casein indicate that the selection of the reference standard is important.

ganism tested. This yeast also possesses other desirable attributes, such as fast growth and ease of handling.

Another requisite of a microbiological method is the treatment of the sample in such a manner that all the sulfur will be in assimilable form. The test organism selected is capable of utilizing sulfur from amino acids and inorganic compounds but not directly from proteins. This problem has been studied and the results are presented in later paragraphs.

The procedure described has been developed on these bases and the results are compared with those obtained with several known chemical methods for the determination of total sulfur.

APPARATUS

Colorimeter. A Lumetron 400 colorimeter, fitted for use with matched 18 × 150 mm. borosilicate glass test tubes without lips, was employed with a filter made of a gray glass and wire screen combination. The glass transmitted 20% of the incident light and the screen 33%. This combination is supplied as part of the regular complement of filters if requested. Other photoelectric colorimeters which may be adapted to turbidimetric measurement are also suitable for the test.

Shaker. During the growth period test tubes were shaken on a reciprocating platform shaker driven at 160 cycles per minute through a $3^3/_{16}$ inch stroke by a 0.25-hp. motor. The platform was equipped with 3 wooden blocks $11^3/_4 \times 7^3/_4 \times 2^3/_8$ inches drilled with holes $^7/_8$ inch in diameter and 2 inches (5 cm.) deep. The holes were purposely made larger than the test tubes to permit vigorous shaking, which prevents the yeast from settling out. Properly spaced, 40 holes can be made in each block.

Temperature Control. Growth tests were conducted at 30° C. by placing the entire shaking apparatus in an incubator.

SOLUTIONS

In the preparation of solutions and in carrying out the assay it was found necessary to wash all glassware thoroughly and rinse it in distilled water just prior to use. All pipets were rinsed twice in the solution to be pipetted just before use.

Sugar and Salts Solution (SAS No. 1). One liter contained 40 grams of C.P. dextrose (anhydrous), 2.2 grams of potassium dihydrogen phosphate, 1.7 grams of potassium chloride, 0.5 gram of magnesium chloride hexahydrate, 0.5 gram of calcium chloride dihydrate, and 0.01 gram of ferric chloride.

Potassium Citrate Buffer. One liter contained 100 grams of potassium citrate ($K_3C_6H_5O_7.H_2O$) and 20 grams of citric acid ($H_3C_6H_5O_7.H_2O$).

Ammonium Chloride Solution. One liter contained 30 grams of ammonium chloride.

These solutions were distributed in Erlenmeyer flasks, plugged with cotton, and sterilized by heating in flowing steam at 100° C. for 30 minutes on three successive days. They were then stored at room temperature until opened.

Growth Factor Concentrate. Two hundred milliliters contained 2 ml. of 50 mg. per ml. inositol solution, 4 ml. of 10 mg. per ml. calcium pantothenate solution, 0.2 ml. of 100 micrograms per ml. biotin solution, 2 ml. of 1 mg. per ml. thiamine solution, 2 ml. of 1 mg. per ml. pyridoxine solution, and 2 ml. of 1 mg. per ml. nicotinic acid solution. This mixture should not be sterilized; it may be stored in the refrigerator for about a week.

DL-Methionine Standard Solution. One hundred milliliters contained 4.65 mg. of DL-methionine, equivalent to 10 micrograms of sulfur per ml. This solution was stored in the refrigerator in a tightly stoppered flask.

Basal Medium. A basal medium sufficient for 60 test tubes was prepared by mixing in the following proportions:

SAS No. 1	150 ml.
Potassium citrate buffer	30 ml.
Ammonium chloride solution	30 ml.
Growth factor concentrate	30 ml.

This basal medium was prepared fresh on the day that the assay was to be run.

PREPARATION OF SAMPLES FOR ASSAY

One gram of dried yeast or 3 grams of pressed yeast were weighed out accurately and suspended in 20 ml. of 10 *N* sodium hydroxide. The sample was autoclaved for 2 hours at 20 pounds' pressure. Then the hydrolyzate was cooled and the volume was made to about 700 ml. with distilled water. The pH was adjusted to 8.6 electrometrically with approximately 3 *N* hydrochloric acid and the volume was made to 1 liter with distilled water. A convenient amount of this solution (at least 15 ml.) was centrifuged for 10 minutes at 1500 r.p.m. and the supernatant liquid to be used for the assay was decanted.

ASSAY PROCEDURE

To a series of five test tubes set up in quadruplicate, amounts of DL-methionine standard solution to supply 0, 1, 2, 4, and 6 micrograms of sulfur were added. It is recommended that each sample solution to be assayed be run in quadruplicate at two different levels. The proper aliquots of each sample solution were pipetted into test tubes. Four milliliters of basal medium were added to each tube and the volume of the tubes was made to 9.5 ml. with distilled water. They were then plugged tightly with cotton, steamed for 15 minutes at 100° C., and cooled rapidly.

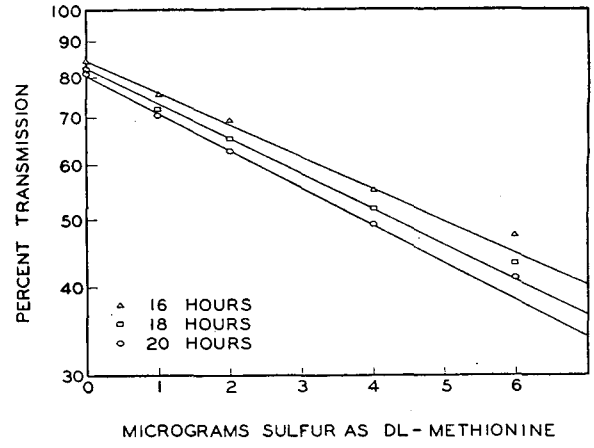

Figure 1. Reference Curve

A fresh 24-hour agar slant of *Torula utilis* was used for the test organism. Sufficient yeast to give a colorimeter reading of 20% absorption of light was transferred with a sterile wire loop to a tube containing 10 ml. of 0.9% sterile saline. This amounts to approximately 0.7 mg. per ml. of moist yeast. The contents of the tube were added to an Erlenmeyer flask containing 40 ml. of sterile saline, giving a total volume of 50 ml.

Using a sterile 2-ml. graduated pipet, 0.5 ml. of this suspension was transferred to each test tube. The cotton plugs were pushed down so there were no loose cotton ends. Then the sets of DL-methionine standards were distributed among the sets of sample tubes on the shaker blocks to correct for slight temperature differences from one end of the shaker to the other. The tubes were shaken for 20 hours in an incubator maintained at 30° C.

The amount of growth was measured after 16, 18, and 20 hours by measuring percentage absorption of light on the colorimeter.

Part of a typical assay is given in Table I. For a full run the standards should be run in quadruplicate rather than in dupli-

Table I. Typical Protocol

[To each tube are added 4 ml. of basal medium plus the ingredients noted below. After sterilization 0.5 ml. of yeast suspension (0.07 mg. of moist yeast) is added to each tube. The tubes are shaken at 30° C. for 20 hours. Absorption, %, of light is measured at 16, 18, and 20 hours.]

Tube No.	H_2O, Ml.	Added, Ml.	% Absorption at 16 hours	18 hours	20 hours
		46.5 γ/ml. DL-methionine[a]			
1	5.5	0	15	16	17
2	5.4	0.1	25	28	29
3	5.3	0.2	30	33	36
4	5.1	0.4	45	47	50
5	4.9	0.6	52	56	58
		Hydrolyzed yeast A solution[b]			
6	5.0	0.5	27	31	33
7	5.0	0.5	27	31	34
8	5.0	0.5	28	31	34
9	5.0	0.5	28	32	34
10	4.5	1.0	40	43	45
11	4.5	1.0	39	42	45
12	4.5	1.0	38	43	45
13	4.5	1.0	38	43	44
		46.5 γ/ml. DL-methionine[a]			
14	5.5	0	16	19	21
15	5.4	0.1	24	28	30
16	5.3	0.2	31	36	38
17	5.1	0.4	45	49	51
18	4.9	0.6	53	57	59
		Hydrolyzed yeast B solution[b]			
19	5.0	0.5	30	32	35
20	5.0	0.5	30	33	34
21	5.0	0.5	29	31	34
22	5.0	0.5	31	33	35
23	4.5	1.0	43	46	47
24	4.5	1.0	44	46	50
25	4.5	1.0	44	46	48
26	4.5	1.0	43	46	47

[a] Solution contains 10 γ of sulfur per ml.
[b] Solution contains 3 grams of moist yeast per liter treated as described.

cate. The averaged values of the sulfur standards were plotted as per cent transmission (100 − % absorption) on semilogarithmic graph paper, making a curve for each time of measurement (Figure 1). Replicate transmission values of the samples were averaged and the corresponding amount of sulfur was determined from the graph.

RESULTS AND DISCUSSION

In the preparation of the yeast samples for analysis, acid hydrolysis was attempted and abandoned because of sulfur loss as hydrogen sulfide. Alkaline hydrolysis was tried with encouraging results. A series of tests was run hydrolyzing samples with 1, 5, 10, and 15 *N* sodium hydroxide. The averaged results of two such test series are presented in Table II. Hydrolysis with 10 *N* sodium hydroxide consistently yielded maximum results and consequently has been used throughout this method. Enzymatic hydrolysis was not attempted, but may also give satisfactory results if possible sulfur losses can be avoided.

Table II. Hydrolysis of Yeast C with Varying Normalities of Sodium Hydroxide

	Microbiological Assay				Chemical Assay
NaOH normality	1	5	10	15	...
% sulfur (dry basis)	0.241	0.408	0.436	0.393	0.430

The growth curve obtained from the DL-methionine standard series can be plotted as a straight line on semilogarithmic graph paper only between 0 and 4 micrograms of sulfur. It is apparent upon examining the curves in Figure 1 that linearity is not obtained above the level of 4 micrograms of sulfur. Evidence presented in a recent paper by Fels and Cheldelin (*8*) shows the nonlinear growth response of *Saccharomyces cerevisiae* with respect to level of added sulfate. For this reason it is important that aliquots of samples to be assayed contain between 1 and 4 micrograms of sulfur.

Values obtained for bakers' yeasts using the microbiological assay method were for the most part between 0.35 and 0.45% sulfur, agreeing with values reported by Block and Bolling (*4*) but much higher than those obtained by Fink and Just (*9*), who found from 0.17 to 0.25% sulfur in various yeasts. In view of the wide range of results reported in the literature it seemed desirable to investigate various chemical methods in an attempt to find a procedure that would yield reliable data on the sulfur content of yeast, thus providing a means of evaluating the accuracy of the microbiological assay.

Four commercial pressed bakers' yeast samples were obtained and dried in the laboratory to preserve them for use in comparison tests. The following different methods of ashing the samples for sulfur analysis were carried out:

Eschka Mixture Method. This is the method used by Fink and Just (*9*) in their work on the sulfur content of yeasts. Results by this method on the dried yeast samples ranged from 0.256 to 0.306% sulfur (Table III).

Acidic Magnesium Nitrate Method (*2*). This is a tentative Association of Official Agricultural Chemists method for sulfur in fruit and fruit products. Sulfur values on the same four yeast samples varied from 0.389 to 0.473%.

Alkaline Magnesium Nitrate Method (*1*). This is an official A.O.A.C. method for the determination of total sulfur in plants. The collaborative work carried out on this method by the A.O.A.C. (*10*) prior to its adoption as official demonstrated that results thus obtained agreed very closely with the values obtained using the bomb method or the sodium fusion technique. The samples used in the collaborative work were cottonseed meal, soybean meal, and mustard seed meal, all plant seed products of high protein content. The results obtained with this method on the four dried yeast samples ranged from 0.406 to 0.465% sulfur.

The alkaline magnesium nitrate method, the acidic magnesium nitrate method, and the microbiolgocial method yield total sulfur values which are in close agreement with one another, as shown in Table III. These results also confirm those of Block and Bolling (*4*) for total sulfur in yeast.

Table III. Microbiological and Chemical Sulfur Assay of Yeast

(All results reported as % sulfur on dry basis)

Yeast	% Protein ($N \times 6.25$)	Eschka Mixture	Acidic Magnesium Nitrate	Alkaline Magnesium Nitrate	Microbiological
C	56.9	0.306	0.430	0.430	0.405
D	46.8	0.256	0.406	0.406	0.401
E	54.6	0.290	0.473	0.465	0.447
F	46.5	0.256	0.389	0.407	0.377

When *T. utilis* was originally grown on sodium sulfate and DL-methionine at a sulfur level of 10 micrograms, the amount of growth was approximately the same in each case. However, when sodium sulfate was used as a standard in the present method, results were found to be about 20% higher than when DL-methionine standard was used. In comparing these compounds in increments of from 1 to 6 micrograms of sulfur it became apparent that sodium sulfate produces somewhat slower growth than does DL-methionine. In view of the work of Block and Bolling (*4*), which shows that yeast contains about 70% methionine sulfur, the authors' sulfur values using DL-methionine as a standard should be essentially correct.

An attempt was also made to apply this microbiological method to other high-protein natural products. Defatted soybean flour, whole soybean flour, and vitamin-free casein were analyzed by the alkaline magnesium nitrate method and by the microbiological method using both DL-methionine and sodium sulfate as standards. Results of this test are recorded in Table IV. The total sulfur values for the soybean products using DL-methionine as a standard are low compared to the chemical results, whereas values obtained with the sodium sulfate standard agree well with the results of the chemical method. Experience has shown

Table IV. Microbiological and Chemical Sulfur Assays of Various Substances

(All results reported as % sulfur on dry basis)

Sample	% Protein	Alkaline Magnesium Nitrate	Microbiological DL-Methionine standard	Microbiological Na_2SO_4 standard
Soybean flour (defatted)	($N \times 6.25$) 54.10	0.519	0.417	0.522
Soybean flour (whole)	($N \times 6.25$) 53.06	0.443	0.338	0.436
Vitamin-free casein	($N \times 6.38$) 98.18	0.711	0.679	...

that cystine and sodium sulfate produce very similar yeast growth response. This observation coupled with data given by Evans (*7*) and Chang and Murray (*5*), showing that the sulfur in soybean is predominantly from cystine, adequately explains this apparently anomalous behavior. Admittedly these authors' data are at a variance with a considerable portion of the literature, which shows soybeans to contain a greater relative amount of methionine. However, it is the authors' belief that Evans (*7*) presents sufficient justification for his results.

Microbiological results on the total sulfur content of casein using DL-methionine as the standard are in good agreement with those obtained with the chemical method. This is in accord with data cited by Cohn and Edsall (*6*), Evans (*7*), and Block and Bolling (*3*, *4*), who report casein to contain 85 to 90% methionine sulfur and 10 to 15% cystine sulfur.

CONCLUSIONS

It is believed that this microbiological assay method, using DL-methionine as a standard, will yield reliable total sulfur values when used on yeast. However, care must be taken in the application of the method to substances other than yeast. An understanding of the composition of the sample to be assayed is essential for the selection of a standard which will produce a growth response corresponding to that of the sulfur in the sample.

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Indicators for Titration of Fluoride with Thorium

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In a study of colorimetric and fluorometric indicators for the titration of fluoride with thorium, the best colorimetric indicators found were, in order of decreasing effectiveness, the two-color indicators purpurin sulfonate, Alizarin Red S, Eriochromcyanin R, dicyanoquinizarin, and Chrome Azurol S; and the best fluorescent indicators were pure sublimed morin and quercetin. Optimum conditions for the use of the better indicators are presented.

THE need for a better indicator for the titration of fluoride with thorium nitrate has been a matter of study for some time. Yoe, Salsbury, and Cole (*8*, *9*) studied the color change of some 54 dyes in admixture with Alizarin Red S in the hope of enhancing the color change of the latter. Shevdov (*7*) studied several zirconium-hydroxyanthraquinone lakes as indicators for the same purpose.

Others have compared the effectiveness of only two or three indicators. Komaroviskiĭ (*4*) and Frers and Lauchner (*3*) employed a zirconium-quinalizarin lake as indicator; Zakhar'evskiĭ (*10*), methyl red; Elsworth and Barritt (*2*), a mixture of Alizarin Red S and 1,2,5,8-tetrahydroxyanthraquinone; Clifford (*1*), a zirconium-purpurin lake; Milton (*5*), Sollochrome Brilliant Blue BS and Chrome Azurol S; and Shevdov (*7*), an Alizarin Cyanin R–zirconium lake.

A large number of compounds for use in the titration of fluoride with thorium nitrate were studied to determine the most sensitive and effective indicator for this method. Compounds that might form soluble colored or fluorescent complexes or lakes with the thorium ion as well as the compounds tested by Yoe and co-workers (*8*, *9*) were tested.

The testing procedure consisted of a selection of the most promising compounds by preliminary Procedures I and II, followed by a more complete examination of the more promising materials.

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PRELIMINARY PROCEDURE I

Solutions of the indicator compounds containing 0.5 mg. per ml. were prepared using water or ethyl alcohol as the solvent wherever possible. Each compound was examined in the vicinity of the titration equivalence point for its color change at three or more pH ranges (pH 2, 3, and 4.7), and at pH 3.0 in ultraviolet light for fluorescence changes. Sufficient buffer from 0.5 *M* to 2 *M* stock solutions was added so that each test solution contained in about 50 ml. 1 millimole of dichloroacetate buffer for pH 2.0, monochloroacetate for pH 3.0 to 3.5, and acetate for pH 4.1 to 4.7. Sodium fluoride samples containing either 45 or 300 micrograms of fluoride were titrated in a volume of about 50 ml. using tall-form Nessler tubes. In cases where alcohol concentration might have an effect, titrations were carried out at several concentrations of ethyl alcohol.

The colors in visible light were compared in a Nessler rack, equipped with an opal-glass reflecting mirror with illumination from a Mazda 20-watt Daylight tubular fluorescent light mounted 45 cm. (18 inches) from the mirror. For fluorescence studies a Hanovia AH-4 lamp covered by a Wood's filter (Corning 5850) was mounted at the side of the Nessler tubes and the fluorescence was observed from the top in a darkened room. Each sample was titrated well beyond the calculated end point with standard thorium nitrate, and the color or fluorescent change, if any, was

Table I. Compounds Showing No Visual or Fluorescent Change of Indicators for Titration of Fluoride with Thorium

Alkaloids

Atropine sulfate
Quinine bisulfate
Brucine
Strychnine

Amines, Hydroxyamines, and Related Compounds

1 - Acetylamino - 4 - aminonaphthalene-7-sulfonic acid
2-Amino-2-ethyl-1,3-propanediol
2 - Amino - 8 - naphthol - 6 - sulfonic acid
1,3-(Bisdiethyl)-diaminoisopropyl alcohol
1,3-Diaminoisopropyl alcohol
1,3-Diaminopropionic acid.HBr
1,1-(Dimethyol)-1-aminoethane
Ethylenediamine
Ethylenediamine tetraacetic acid
m-Phenylenediamine
1,5,7-Trisulfonamido-2-naphthol
m-Toluenediamine.HCl
2-Amino-1-butanol
2-Ethylaminoethanol
2-Amino-2-methyl-1-propanol
2-Amino-2(hydroxymethyl)1,3-propanediol
β-Bromoethylamine.HBr
2,5-Diamino-1-naphthol.HCl
2-Diethylaminoethyl chloride
N-Ethylaniline
2-Aminoethanol
1-Amino-2-propanol
p-Phenylenediamine sulfonate
N,N,N',N'-Tetrakis(2-hydroxyethyl)-ethylenediamine

Anthraquinones and Related Compounds

Alizarin blue
Anthraflavin
Chrysazin
Diaminoanthrarufin
1,5 - Dihydroxy - 4,8 - diaminoanthraquinone-2,6-disulfonic acid
Frangula extracts
1-Hydroxy-4-chloroanthraquinone
1 - Hydroxy - 2,4 - dianilinoanthraquinone
1-Hydroxy-4-toluidoanthraquinone
Rufigallic acid
1,4,5,8 - Tetrahydroxyanthraquinone-2-sulfonic acid
Alizarin orange
Anthrarufin
Cochineal
Dinitroanthrarufin
1,4 - Dihydroxy - 2 - phenoxyanthraquinone
1 - Hydroxy - 2 - carboxy - 4 - bromoanthraquinone
1 - Hydroxy - 2,4 - dichloroanthraquinone
Leucoquinizarin
1,4,5,8-Tetrahydroxyanthraquinone

Azo, Disazo, and Related Compounds

Acid Alizarin Black RA
Alizarol Orange R
Benzoazodiphenylamine
Benzoazurin 3R
1,3 - Dihydroxybenzo - 4 - azo - 3'-(4'-hydroxybenzene)-sulfonic acid
Erie Fast Yellow WB
Flavazin
Methyl red
Pontacyl Sulfon Violet R
Pontamine Sky Blue 6BX
Pontamine Green BX
Purpurine 4B
Superchrome Blue B extra
Tartrazine
Acid Alizarin black SEA
Alizarol Verdon S
Benzoazophenol
Chrysoidine
1,3 - Dihydroxybenzo - 4 - azo - 2'-benzoic acid
Evans blue
Methyl orange
Pontacyl Carmine 6B
Pontamine Blue BBF
Pontamine Violet N
Pontamine Brown CG
Rainbow Orange G
Superchrome Black PV
Superchrome Garnet Y
Superchrome Violet B

Fluorescein-Type Compounds

Dichlorofluorescein
Fluorescein
Tetrabromosulfonphthalein
Diiodofluorescein
Phenolphthalein
Thymolphthalein

Quinoline Derivatives

5,6-Benzoquinoline oxalate
8-Hydroxyquinaldine
5-Nitroso-8-hydroxyquinoline
7 - Nitroso - 8 - hydroxyquinoline-5-sulfonic acid
x-(*p*-Sulfophenylazo)-8-hydroxyquinoline-5-sulfonic acid
Bromoxine
8 - Hydroxyquinoline - 5 - azo - 4'-nitrobenzene-2-sulfonic acid
5-(*p*-Sulfophenylazo)-8-hydroxyquinoline (sulfenazoxine)
1-(*o*-Hydroxyphenyl)-2-benzazine
2-(*o*-Hydroxyphenyl)-quinoline

Triphenylmethane-Type Compounds

Alkali blue 4GP
Aniline blue
Crystal violet
p-Fuchsin
Malachite green
Methyl Violet 5B
Triphenylchloromethane
Wool Violet 4BN
Alphazurine A
Aurine
Fast Acid Green B
Guinea Green B
Methyl violet
Rosaniline
Victoria Blue B

Flavonoid-Type Compounds

D-Catechol
Naringin
Phloretin
l-Epicatechin
3,5,7,3',4'-Pentahydroxyflavanone
Rutin

Miscellaneous Dyes and Compounds

Acridine
Acridone
Brasilin
Chlorogenic acid
o-Dianisidine
2,4-Dihydroxybenzene sulfinic acid
Diphenyl carbazide
Fluorene
Hematein
Indigo disulfonate
Indophenol B.G.
Litmus
Luminol
β-Methylumbelliferone
Methylene blue
Nordihydroguaiaretic acid
Phenosafranine hydrochloride
Primuline NAC
Quinaldine
Resazurin
Riboflavin
Salicylaldoxime
Thiamine chloride
Vitamin C
Acridine orange
Auramine
Cacotheline
Cupferron
Anthraquinonyl sulfide
α-Dinitrodiphenylamine sulfoxide
Dithio-oxamide
Gallic acid
Hemoglobin
Indole
Leuco Indophenol R
Logwood extracts
Methane trisulfonic acid, NH_4 salt
Methyl-β-phenylethylmalonic acid dihydrazide
o-Phenanthroline
Phosphine
Printing Violet R
Resorufin
Rhodamine B
Safranine A
Sodium diethyl dithiocarbamate
Violamine RR
Xanthone

compared to the change given by Alizarin Red S at pH 3.0. The amounts of the indicator solutions (0.2 to 2.0 ml.) used were selected by trial to permit the maximum change at the end point.

PRELIMINARY PROCEDURE II

The compounds available in small amounts only were tested by this procedure, primarily for fluorescent effects.

Solutions containing 0.5 mg. per ml., or in a few cases 0.2 mg. per ml., were prepared in 10% ethyl alcohol if possible; 95% ethyl alcohol, propylene glycol, or pyridine if necessary. For each compound a series of eight Klett No. 802 test tubes was set up, using 10 ml. of water in four tubes and 10 ml. of 50% ethyl alcohol in the second four tubes.

The proper pH in each series of four tubes was maintained by the addition of 1 millimole of pH 2.0, 3.0, or 4.15 buffer to the first three, respectively, and water of pH 7 to the last tube. To each tube was added 0.15 mg. of indicator, usually in solution, and the total volume was brought to 11 ml.

The initial color under visible light and the fluorescence of each of these solutions were noted. Fluorescence intensities were also measured with a Klett fluorometer equipped with a Corning 5850 primary and a Wratten No. 4 secondary filter with the balance diaphragm set at 14 in most cases. To each of the 8 tubes were added 0.3, 0.3, 0.4, and 0.6 ml. of 0.0157 *M* thorium nitrate, and the fluorescent reading was noted for each addition. The color under ultraviolet light was also finally observed after introduction of an additional Corning 5790 filter in the primary ultraviolet beam to cut out blue light passing the Corning 5850 filter. If a turbidity or precipitate formed, its color was noted.

RESULTS OF PRELIMINARY PROCEDURES

The compounds tested which showed no color or fluorescent change by Procedure I or II are listed in Table I. In addition, 30 compounds of unknown structure previously investigated by Yoe and co-workers (*8, 9*) were tested. The compounds which showed color or fluorescent change by Procedure I are listed in Table II, and the indicating compounds tested by Procedure II are listed in Table III.

DISCUSSION OF PRELIMINARY PROCEDURE

Samples of the same indicator compound from different suppliers generally gave comparable results by either procedure. The agreement was especially notable for samples of Alizarin Red S. However, two samples of 2',6'-dichlorohydroxydimethylfuchsondicarboxylic acid, Alizurol Azurine ECA, and Basic Color MD-1293, differed in sensitivity as did two samples of *o'*-sulfohydroxydimethylfuchsondicarboxylic acid, Eriochromcyanin R and Pontachrome Blue ERC (see Table II). Differences in sensitivity were especially pronounced for various samples of quercetin and morin. Quercetin obtained by the hydrolysis of rutin supplied by S. B. Penick & Company and sublimed Schuchardt's morin recrystallized from acetic acid proved the most sensitive fluorescent indicators.

SELECTION OF OPTIMUM INDICATOR AND OPTIMA STUDIES

The indicators which showed a color or fluorescent change in this titration were studied further to determine the best indicator

Table II. Compounds Showing Some Change in Visible or Ultraviolet Light as Indicators for Titration of Fluoride (Procedure I)

O no change; VS very slight change; S slight change; M moderate change; G good change (comparable to Alizarin Red S at pH 3.0 in water); X very good change; XX excellent change in the color or fluorescence; ? doubtful result

Compound	Best pH	Class	% Alcohol
Changes in Visible Light			
Anthraquinone Types			
Alizarin	3.0, 3.5	G	0
Alizarin Red S	3.0	G	0
	3.2	X	0
Alizarin sapphire	2.0	VS	0
Alizarin Y	4.7	G	10
Anthragallol	4.2	S-M	20
Anthrapurpurin	4.7	G	0–20
Cascara sagrada (50% alcohol extract)	4.7	M	0
2-Chloroquinizarin	3.0	VS	0–20
Dicyanoquinizarin	4.2	G	0
Leucotetra[a]	3.0, 3.5	S	0–20
Purpurin	4.7	M-G	0–25
Purpurin sulfonate	3.5, 4.2	G	0
Quinalizarin	3.5	S-M	0–20
Quinizarin	3.0	G	10–25
Synthracene Blue WRS[b]	3.5	S	0
Azo-Type Compounds			
Diamine Pink BD (C.I. 128)	4.2, 4.7	VS	0
p-Nitrophenylazoresorcinol	3.0, 4.7	VS	0
Pontachrome Blue R	3.0	S	0
Pontamine Light Yellow 5GX (C.I. 346)	3.0, 4.7	VS	0
Pontamine Yellow 5X (C.I. 620)	3.0, 4.7	VS	0
Azo Quinoline Compound			
5 (or 7)-(4-nitro-1-phenylazo-2-sulfonic acid)-8-hydroxyquinoline	4.7	M-G	0
Triphenylmethane-Type Compounds			
Alizurol Azurine ECA[c] (C.I. 720)	2.0–4.7	M	0
Aluminon	4.2, 4.7	M-G	0
Basic Color MD 1293[d] (C.I. 720)	4.7	G-X	0
Chrome Azurol S (C.I. 723)	3.0	G	0
Eriochromcyanin R (C.I. 722)[e]	4.2, 4.7	G	0
Pontachrome Blue ERC[f]	4.7	M	0
Miscellaneous Compounds			
Alkali Fast Green 2G (C.I. 735)	2.0	S	0
Alkannin (C.I. 1240)	4.7	VS	0
	6.0	S	10
Chrome Blue GD (C.I. 878)	4.2, 4.7	S-M	0
p-Dimethylaminobenzilidine rhodanine	4.7	M	0
2-Nitroso-1-naphthol	4.7	S	0–20
Pontacyl Fast Violet 10B (C.I. 696)	4.7	S	0
Rose petal powder, 50% alc. extract	4.2	M-G ppt.	0
Polyporic acid	4.2	S	0–50

[a] 1,4,5,8-tetrahydroxyanthraquinol.
[b] 1,2,4,5,6,8-hexahydroxyanthraquinone-3(or 7)-sulfonic acid.
[c] From National Aniline Co.
[d] Obtained through Chemical Corp.
[e] From German source.

Compound	Best pH	Class	% Alcohol
Compounds Also Showing Fluorescent Change			
Changes in Visible Light			
Congo Corinth (C.I. 375)	3.0	S	0
Pontachrome Blue-Black RM (C.I. 202)	3.5	S	0
Superchrome Blue-Black 6BP (C.I. 201)	3.0, 4.2	S	0
Ferron	4.7	S-M	0
Isoquinoline	2.0–4.2	S ppt.	35
Oxine	4.7	M ppt.	30
Oxine sulfonate	4.7	M	0
Flavonoid-Type Compounds			
Cyanidin chloride (crude, impure)	4.7	M	0
Quercetin (S. B. Penick & Co.)	3.5	X	50
Quercetin (S. B. Wender)	3.2–3.5	X	50
Quercetin (Am. Dyewood Corp.)	3.5	G	50
Quercetin, quercetin mixture[g]	3.0–4.2	M	50
Quercetin-6′-sulfonic acid, Na salt[h]	3.0–4.2	X	20
Quercetin-6′-sulfonic acid, Na salt[i]	3.0, 3.5	VS	0
Morin, tech. (Eastman Kodak Co.)	3.5	M	50
Morin (Schuchardt, Germany)	3.5	G-X	50
Morin (sublimed and recryst. from above)	3.0, 3.5	M-X	50
Morindin chloride (crude, impure)	4.2	S	0
Morin-*x*-sulfonic acid	2.0–4.7	O-VS	0–50
Kaempferol (S. B. Wender)	3.0–4.2	G	50
Gossypetin (W. Clark)	4.2	G	50
Changes in Ultraviolet Light			
Congo Corinth	3.5	VS	0
Pontachrome Blue-Black RM	4.7	VS	0
Superchrome Blue-Black 6BP	4.7	VS	0
Ferron	3.0–4.7	M-G	0
Isoquinoline	2.0	VS	40
Oxine	4.7	M	30
Oxine sulfonate	4.7	G	0–30
Flavonoid-Type Compounds			
Cyanidin chloride (crude, impure)	2.0–4.2	S-M	0
Gossypetin (W. Clark)	3.0	S	50
Kaempferol (S. B. Wender)	3.0	X	50
Quercetin (S. B. Penick & Co.)	3.0, 3.5	X-XX	50
Quercetin (Am. Dyewood Corp.)	3.0, 4.7	M-G	50
Quercetin (S. B. Wender)	3.0, 3.5	X-XX	50
Quercetin, quercetin mixture[g]	3.0	G	50
Quercetin-6′-sulfonic acid, Na salt[h]	3.0	X	50
Quercetin-6′-sulfonic acid, Na salt[i]	3.0	O	50
Morin, tech. (Eastman)	3.5	M	50
Moron (Schuchardt)	3.5	X	50

[f] From Jackson Lab., Du Pont.
[g] Sample from Schuchardt, Germany, ratio unknown.
[h] From W. Clark, freshly prepared solution.
[i] From W. Clark, solution after standing 12 days, oxidation apparent.

in each class. The best indicators are listed in Table IV with a rank of effectiveness assigned to each compound and the optimum conditions for titration as determined in the studies outlined below.

Both 35- and 300-microgram aliquots of standard fluoride were titrated visually at the optimum pH and alcohol content previously approximately determined with varying indicator concentrations to select the best indicator concentration for a volume of 50 ml. The illumination conditions specified in Procedure I were used for both visible and ultraviolet studies. In addition, fluorescence changes were followed using a modified Klett fluorometer equipped with a 140-ml. rectangular cell and the filters mentioned previously. It was generally observed that the smallest amount of indicator which would give a light color with the buffer in the presence of fluoride in the case of two-color indicators, or a light color and moderate fluorescence in the presence of a slight excess of thorium for the fluorescent indicators, was more sensitive in change at the equivalence point than larger amounts of indicator.

Using the optimum indicator concentration, variations in the color or fluorescence change were studied at several pH values. The proper proportion of alcohol was added where necessary. The optimum pH for the best color change of the better indicators is given in Table IV. The studies confirmed previous conclusions (*6*) that pH 3.0 to 3.5 is the optimum for the titration of fluoride ion with thorium nitrate. Because the optimum color change of Alizurol Azurine ECA, Basic Color MD-1293, and 5(or 7)-(4-nitro-1-phenylazo-2-sulfonic acid)-8-hydroxyquinoline does not occur in this range, they were eliminated from further consideration.

The most suitable alcohol content was redetermined for indicators which required alcohol. Alizarin Red S, Alizurol Azurine, Chrome Azurol S, Eriochromcyanin R, and purpurin sulfonate do not require alcohol. A high alcohol content with the flavonoid fluorescent indicators, quercetin and morin, is accompanied by greater intensity of fluorescence and sensitivity in the titration. For these a limiting alcoholic concentration of 50% was selected to avoid interference of foreign ions at greater concentrations, even though greater fluorescence intensities might be obtained with higher alcohol concentrations.

Using the best conditions determined above, the responses in the titration of 5 to 50 micrograms, 250 to 500 micrograms, and 1.0 to 5.0 mg. of fluoride in a total volume of 50 ml. were determined. The color changes observed for the better indicators are given in Table V. From the results of these studies the best two-color indicators in visible light in order of decreasing effectiveness are:

1. Purpurin sulfonate
2. Alizarin Red S
3. Eriochromcyanin R

Table III. Compounds Showing Change in Visible or Ultraviolet Light When Used for Titration of Fluoride (Procedure II)

Class Symbols. Symbols used in Table II: max maximum change for intermediate amount of thorium; min minimum change for intermediate amount of thorium; neg decrease in color or fluorescence of class as indicated; ? doubtful fluorescence; vis. visual; fluor. fluorescent.

Color Symbols. bk black; bkh blackish; bl blue; blh bluish; br brown; bri bright; buf buff or buffish; d dark or dull; g green; gh greenish; l light; lav lavender; mag magenta; no none; or orange; pk pink; ppt precipitate; pu purple; r red; rh reddish; sl slight; tur turbidity; T Tyndall blue scattering by a turbidity; v very; vio violet; y yellow; yh yellowish; ... not determined. For color changes, if original solution is practically colorless or nonfluorescent, only color formed after addition of thorium is given. In other cases initial color is given, followed by final color.

Data Presented. To conserve space, visual and fluorescent changes are given only for pH and alcohol content, if any, at which best change occurred or where some unusual effect was noted. Additional details are given in thesis from which this paper is abstracted. Tests in neutral solution are not considered.

Compound	Type Observed	Best pH	% Alcohol	Class	Color or Fluorescence
Flavonoid Types					
3′-Carbethoxyhesperidin	vis.	2.0, 3.0	50	VS	l y-g
	fluor.	3.0	0, 50	S-M	bl-g
D-Catechol	vis.	2.0–7.0	0, 50	O	no
	fluor.	2.0	50	VS max	bl
L-Epicatechol	vis.	2.0–7.0	0, 50	O	no
	fluor.	4.1	50	VS	bl
Cyanin chloride	vis.	3.0, 4.1	50	VS	l lav to rh-lav
	fluor.	3.0, 4.1	0	S max	pu-bl
	fluor.	3.0, 4.1	50	S neg	bl
Dianiline gossypol oxime	vis.	2.0–4.1	0, 50	M	y
	fluor.	3.0	0, 50	S max	bl, bl-g
3,4′-Dihydroxy-4′-methoxychalcone-4-phosphate, disodium salt	vis.	2.0–4.1	0, 50	M	y-g
	fluor.	2.0–4.1	50	VS	bl-g
Eriodictyol	vis.	2.0, 3.0	0, 50	ppt	w
	vis.	4.1	0, 50	O	no
	fluor.	2.0	50	M-G	bl
Gossypetin	vis.	3.0, 4.1	0	G	gh-bk
	fluor.	3.0	50	S	br ?
Gossypin	vis.	3.0, 4.1	0, 50	M	y-g
	fluor.	4.1	0	S	y-g
	fluor.	4.1	50	S neg	bl-g
Gossypitrin	vis.	2.0–4.1	0, 50	S	y
	fluor.	3.0, 4.1	50	S-M	g
Hesperidin	vis.	4.1	50	S-M	y-g
	fluor.	4.1	50	M	bl-g
Homoeriodictyol	vis.	2.0	0, 50	M	w ppt, w tur
	fluor.	2.0	0, 50	M-G	T bl
Isoquercitrin	vis.	2.0–4.1	50	S-M	l y-g
	fluor.	2.0	50	S-M	w
	fluor.	2.0	0	VS neg	w
Kaempferol	vis.	3.0, 4.1	50	X	y-g
	fluor.	2.0, 3.0	50	X	bri g
Morin (Schuchardt)	vis.	3.0	50	X-XX	bri y-g
	fluor.	3.0	50	X-XX	bri yh-g
Morin (sublimed and recryst. from Schuchardt)	vis.	3.0	50	G	bri y-g
	fluor.	3.0	50	XX	bri g
Naringin	vis.	2.0–4.1	0, 50	O	no
	fluor.	3.0	0	VS	bl-g
Neohesperidin	vis.	2.0, 3.0	0, 50	...	w ppt, w tur
	fluor.	2.0	50	M-G	T bl
	fluor.	4.1	0	S max	bl
3,5,7,3′,4′-Pentahydroxyflavanone	vis.	2.0–4.1	0, 50	O	no
	fluor.	3.0	50	M-max	bl
Phloretin	vis.	2.0–4.1	0, 50	O	no
	fluor.	7	0	VS neg	...
Flavonoid Types (Contd.)					
Pomiferin	vis.	2.0–4.1	0, 50	O	no
	fluor.	4.1	0	S neg	T bl
Quercetagetin	vis.	4.1	50	S-M	or
	fluor.	2.0	0	S max	l d g
Quercetin (S. B. Penick)	vis.	3.0, 4.1	50	X-XX	bri y-g
	fluor.	3.0, 4.1	50	XX	bri yh-g
6′-Sulfoquercetin, Na salt (fresh solution)	vis.	2.0–4.1	0, 50	G	v y
	fluor.	2.0, 3.0	50	X	bri g
6′-Sulfoquercetin, Na salt (old solution)	vis.	4.1	0, 50	S	gh-y, y
	fluor.	3.0	0	VS	l g
Quercitrin	vis.	3.0	50	S-M	l y-g
	fluor.	4.1	50	S max	l y-g
Rhamnetin	vis.	2.0, 3.0	0	G ppt	y ppt
	fluor.	2.0–4.1	50	X	bri g
Robinin	vis.	2.0, 3.0	50	S	y-g
	fluor.	2.0	50	S-M	yh bl-g
Rutin	vis.	2.0, 3.0	0, 50	O	no
	fluor.	4.1	0	VS	gh-bl
Rutin methylglucamine	vis.	2.0–4.1	50	VS	v l g
	fluor.	3.0	50	VS	v l bl
2′,3,4-Trihydroxychalcone	vis.	3.0, 4.1	50	S-M	y-g
	fluor.	2.0–4.1	0, 50	O	no
Xanthorhamnetin	vis.	2.0	50	S	y-g
	fluor.	2.0, 3.0	50	S	l yh-g
Other Types of Compounds					
4-Carboxyesculetin, Na salt	vis.	2.0–4.1	50	S	l y-g
	fluor.	2.0, 4.1	50	VS	gh-bl
Chlorogenic acid	vis.	2.0–4.1	0, 50	O	no
	fluor.	2.0, 4.1	50	VS	bl-g
Curcumin	vis.	2.0–4.1	0, 50	O	no change
	fluor.	2.0, 3.0	50	B neg	bri g,g
Chloroxine	vis.	3.0	0	S	l g
	fluor.	2.0–4.1	50	S-M	g
N,N′-Disalicylidene-*o*-phenylenediamine	vis.	3.0, 4.1	0, 50	VS	l y-g
	fluor.	3.0	0	S neg min	bl-g
Esculetin	vis.	2.0–4.1	50	S	l g
	fluor.	3.0	50	S-M	bri l bl
Nordihydroguaiaretic acid	vis.	2.0–4.1	0, 50	O	none
	fluor.	3.0	0	VS	bl
	fluor.	3.0, 4.1	50	VS neg	bl
Polyporic acid	vis.	3.0	50	M	lav to g tur
	fluor.	3.0	50	S-M max	blh-g

Table IV. Optimum Conditions for Best Indicators by Procedure I

Rank[a]	Compound	Opt. Indicator Concn. for 50 Ml., Mg.	Best pH	For Range of F^-, Mg.	Opt. Alc. Content, %
2A	Alizarin Red S	0.20	3.4	0.6 up	None
			3.0	Under 0.2	None
4A	2,3-Dicyanoquinizarin	0.60	3.8	All ranges	10–20
1A	Purpurin sulfonate	0.15	3.7	0.6 up	None
			3.3	Under 0.2	None
9A	5(or 7)-(4-nitro-1-phenylazo-2-sulfonic acid)-8-hydroxyquinoline	0.20	4.2	All ranges	30–40
6A	Alizurol Azurine ECA	0.15	3.5–3.9	All ranges	0–5
8A	Aluminon	0.4–0.9	4.2–4.5	Best all ranges	None
			3.6	Satisfactory	
7A	Basic Color MD1293	0.10	3.75	All ranges	0–10
5A	Chrome Azurol S	0.15–0.25	3.0–3.2	All ranges	None
3A	Eriochromcyanin R	0.6–0.8	4.2	Best all ranges	None
			3.6	Satisfactory	
1B	Quercetin (Penick)	0.50	3.5	All ranges	50
1B	Morin (Schuchardt)	0.50	3.5–3.8	All ranges	50
2B	Morin (sublimed)	1.0	3.5–3.8	All ranges	50
3B	Quercetin-6′-sulfonate, Na salt (fresh solution)	0.3–0.7	3.2	All ranges	50

[a] A, two-color indicators; B, one-color visual indicators and also fluorescent indicators.

Table V. Color Change of Best Indicators in Visible Light by Procedure I Using Optimum Conditions Listed in Table IV

Compound	Color With excess fluoride	At end point	With slight excess of thorium	With large excess of thorium
Alizarin Red S	Yellow-green	Pinkish buff	Light pink	Rose
Dicyanoquinizarin	Dull lavender	Dull purple	Bluish violet	Lavender blue
Purpurin sulfonate	Orangish yellow	Light pink	Light lavender	Purple
5(or 7)-(4-nitro-1-phenylazo-2-sulfonic acid)-8-hydroxyquinoline (at pH 4.7)	Yellow	Buffish orange	Orange	Deep orange
Alizurol Azurine ECA	Pink	Lavender pink	Lavender	Lavender blue
Aluminon	Pink	Pinkish rose	Pink-rose	Dull dark rose
Basic color MD1293	Light pink	Light pink-lavender	Lavender blue	Medium blue
Eriochromcyanin R	Orangish pink	Reddish orange	Orangish red	Rose red
Quercetin (Penick)	Almost colorless	Light green	Yellow-green	Bright greenish yellow
Morin (Schuchardt)		Same as quercetin		
Morin (sublimed)	Colorless	Light green	Yellow-green	Bright yellowish green
Quercetin-6′-sulfonic acid, Na salt (fresh solution)		Same as quercetin		

4. 2,3-Dicyanoquinizarin
5. Chrome Azurol S

The best one-color visual indicators similarly are:

1. Quercetin (S. B. Penick & Co.)
2. Morin (T. Schuchardt)

The best fluorescent indicators similarly are:

1. Morin (sublimed and recrystallized from Schuchardt's morin)
2. Quercetin (S. B. Penick & Co.)
2. Morin (Schuchardt)
3. Ferron
4. 8-Hydroxyquinoline-5-sulfonic acid
5. Kaempferol

Foreign ions such as sulfate, chromate, calcium, and iron were found to affect *all two*-color indicators to a similar extent. Halide ions and sodium acetate or chloroacetate affect all the two-color indicators slightly, when present in high concentrations. The chloride, bromide, iodide, chlorate, perchlorate, acetate, and chloroacetate ions did not affect the results with the flavonoid fluorescent indicators. Thus, the latter would be especially suitable for determination of the fluoride obtained by alkaline decomposition of halogenated organic compounds. Of the best two-color indicators, purpurin sulfonate has some advantage over Alizarin Red S, as it lacks the grayish buff color range just before the end point. The last three two-color indicators are inferior to both purpurin sulfonate and Alizarin Red S in the sharpness of their color change at the equivalence point.

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Photofluorometric Titration of Fluoride

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A procedure is presented for the photofluorometric titration of fluoride in the presence of considerable sodium chloride, using thorium nitrate as the titrant and quercetin as the fluorescent indicator. The method may be used following alkaline decomposition of fluorocarbons or after the Willard-Winter separation.

ALIZARIN Red S and Chrome Azurol S are now commonly used as visual colorimetric indicators for the titration of fluoride with thorium nitrate. These indicators have the disadvantage of a subtle color change at the end point and interference by semicolloidal thorium nitrate when large amounts of fluoride are titrated.

Fluorescent indicators are readily adaptable for the instrumental titration of fluoride with thorium nitrate. The best fluorescent indicators, in order of decreasing effectiveness, have been reported to be (*1*): pure sublimed and recrystallized morin (2′,3,4′,5,7-pentahydroxyflavone), pure quercetin (3,3′,4′,5,7-pentahydroxyflavone), ferron (8-hydroxy-7-iodo-5-quinolinesulfonic acid), kaempferol (3,4′,5,7-tetrahydroxyflavone), and 8-hydroxyquinoline-5-sulfonic acid. Because pure morin is at present generally unavailable, the work reported here is restricted to the use of quercetin as an indicator.

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APPARATUS

Modified Klett Fluorometer. The regular cell compartment lid, filter support, cell support, and mirror assembly of a Klett fluorometer were removed. A cardboard support was used to hold the secondary filter in front of the photocell. A special high cell house cover was constructed, with a hole for the buret tip located to the rear out of the path of the ultraviolet and fluorescent light paths. The arrangement is shown in Figure 1.

Filters. The fluorescence of the thorium-quercetin complex using 50% ethyl alcohol and monochloroacetate buffer of pH 3.0 showed a wide band from 530 to 580 mμ with a maximum at about 575 mμ. Thus Corning No. 3385 was chosen as the secondary filter; however, Corning No. 3384 or Wratten No. 4 or No. 8 is also satisfactory. Optimum sensitivity in the titration of 0.1 mg. of fluoride was obtained using a standard thickness Corning No. 5850 primary filter.

Stirrer. A magnetic stirring motor was located in the wooden instrument box, centered under the cell, with its speed control projecting beyond the case of the fluorometer. A small rod magnet encased in plastic was used in the cell. If a magnetic stirrer is unavailable, an ordinary small two-bladed glass stirrer may be suspended midway down in the solution. When this is used, the upper portion should be painted dull black and a guard ring should be used at the point where the stirrer enters the cell house. The rate of stirring should be moderate, neither too slow nor too fast.

Cells. Four types of cells were used: a Klett "100-ml." colorimeter cell with cemented sides, a similar cell with two aluminum foil reflectors located on the sides opposite the light paths, a 150-ml. quartz cell with similar reflectors, and a blown borosilicate glass cell. The special cells were about 37 × 42 mm. and approximately 120 mm. high. The quartz cell with reflectors gave somewhat better results for the titration of less than 0.2 mg. of fluoride, the other cell with reflectors gave better results under 1 mg. of fluoride, and all cells worked equally well for the titration of more than 1 mg. of fluoride.

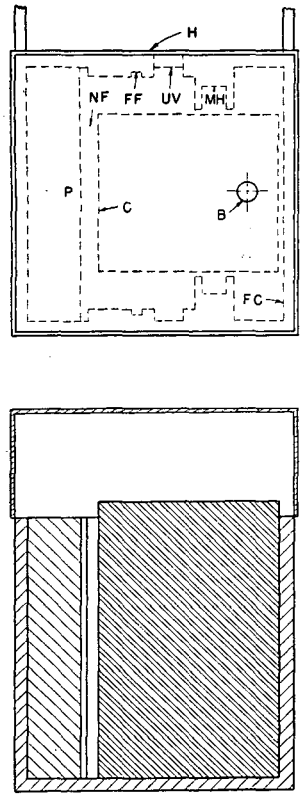

Figure 1. Modified Klett Fluorometer

Cooling. It was found necessary to cool the fluorometer lamp house with the full pressure of an air stream introduced at the bottom of the housing.

Buret. A long tip was fused to the bottom of a side-filling 10-ml. buret graduated in 0.05-ml. divisions or less. The buret was introduced so that the tip just dipped into the solution to be titrated and its position was not changed during the titration.

REAGENTS

Ethyl Alcohol, 95%. Ethyl alcohol denatured with methanol is satisfactory.

pH indicator, 0.05% solution of *p*-nitrophenol in 50% ethyl alcohol.

Titration Indicator. Dissolve 100 mg. of pure quercetin from S. B. Penick & Company in 500 ml. of 95% ethyl alcohol. Store in borosilicate glass.

Buffer. Dissolve 94.5 grams of C.P. monochloroacetic acid and 30.0 grams of reagent grade sodium hydroxide separately in cool water, slowly mix, and then dilute to 1 liter with water. Store in borosilicate glass in a cool place. The pH of 4 ml. of buffer diluted to 50 ml. with water should be 3.00 ± 0.15.

Monochloroacetic acid, C.P., 0.5 *M*, or any convenient strength.

Thorium Nitrate Solutions, 0.05 *N* and 0.01 *N*. Prepare from C.P. thorium nitrate tetrahydrate dried in a desiccator at room temperature. Standardize against sodium fluoride or sodium silicofluoride using the procedure below.

Standard Sodium Fluoride Solution. Dissolve 1.6800 grams of reagent grade sodium fluoride in 2 liters of water. One milliliter equals 0.38 mg. or 20 micromoles of fluoride.

FACTORS INFLUENCING TITRATION

The variables involved were studied using the apparatus described above. Preliminary experiments (*1*) showed that in approximately 50% alcohol containing 1 mg. of quercetin per 100 ml., buffered at pH 3, fluorescence increased with increasing thorium concentration and decreased again on addition of fluoride. In the titration of a given amount of fluoride with thorium nitrate, there was a sharp increase in fluorescence at the end-point region. The appearance or disappearance of fluorescence was rather difficult to detect visually. The intensity of fluorescence, the sharpness of the end point, and the sensitivity of the titration were influenced by several variables regardless of the fluorescent indicator used.

Type of Alcohol. Using 50 ml. of various alcohols, about 2 mg. of fluoride were titrated with 0.0125 *M* thorium nitrate using 1 mg. of pure quercetin and 2 ml. of 1 *M* monochloroacetate buffer at pH 3.0 in a total volume of 100 ml. Ethyl alcohol gave the most satisfactory results, methanol almost as satisfactory, isopropyl alcohol not quite so satisfactory, and other alcohols such as *n*-propyl and *tert*-butyl alcohol unsatisfactory results. Absolute ethyl alcohol (benzene-free) or 95% denatured alcohol are equally good and need not be redistilled except when it is desired to extend the method below 0.1 mg. of fluoride.

Variation in Alcohol Concentration. The fluorescence of a given amount of thorium in the presence of quercetin increases linearly with increasing ethyl alcohol concentration up to at least 80% ethyl alcohol. It was shown that a minimum of 30% ethyl alcohol is necessary for the satisfactory titration of 2 to 40 mg. of fluoride. The ethyl alcohol content must be increased to 50% to extend the method down to 0.1 mg. of fluoride or less. The effect of alcohol in increasing fluorescence of a given amount of thorium was similar for titrations at pH 2.0, 3.0, 3.2, 3.5, and 4.15. Increasing the alcohol content, however, increases the initial fluorescence of the system and increases the deleterious effect of foreign ions by decreasing the solubility of their salts and also makes control of pH more difficult. Thus the alcohol content was fixed at 50% for the titration of less than 1 mg. of fluoride, and 40% for over 1 mg. of fluoride.

For 50% alcohol a variation of ±4%, and for 40% alcohol a variation of ±5% did not affect the titration of 0.5 and 2.0 mg. of fluoride, respectively. For titration of large amounts of fluoride, the change in alcohol concentration caused by addition of the aqueous titrant did not adversely affect the sensitivity of the method.

Concentration of Quercetin. Using either 40 or 50% ethyl alcohol, buffered at pH 3.0 or 3.5 with monochloroacetate, it was found by a series of titrations of 1 mg. of fluoride that a concentration of 1.0 mg. of quercetin per 100 ml. of solution titrated gave optimum results in the titration. If the concentration is less than 0.8 mg., the maximum fluorescence will be reached too soon after the end point; if over 1.5 mg., the strong color of the thorium-quercetin complex may cause a deviation from Beer's law of the fluorescence with increase in thorium concentration after the end point.

Variation in pH. Previous work has shown that pH 3.0 to 3.2 is most effective for the titration of fluoride using thorium nitrate. Although the fluorescence of the thorium-quercetin complex shifts with pH, it was found that a variation of ±0.15 pH unit, using a pH 3.0 monochloroacetate buffer, had no effect on the end point in fluoride titrations in the 0.5- to 10-mg. range.

Effect of Temperature. The initial fluorescence reading before titration does not vary appreciably between 10° and 50° C. For the titration of less than 0.5 mg. of fluoride the sharpness of the inflection at the end point is improved by starting the titration at 10° to 15° C. Less than 0.15 mg. of fluoride cannot be titrated at 30° C. using 0.0025 *M* thorium nitrate. Above 2 mg. of fluoride, variation of the initial temperature from 10° to 50° C. did not affect the end point. To prevent excessive heating in the fluorometer during titration it is necessary to cool the lamp housing with a stream of air.

Variations Due to Stirring. A few typical titrations with stirring are shown in Figure 2. Near the region of the extrapolated end point the observed fluorescent readings fall on a curve, which is more gradual in the titration of large amounts of fluoride or silicofluoride. This curve is due to maximum solubility and maximum dispersion of colloidal thorium fluoride in the region of stoichiometric equivalence for the two ions. On either side of this region solubility and colloidal tendencies are

reduced by an excess of one of these ions. Thus, linear fluorescence readings are obtained at the beginning of the titration and somewhat after the end point. The distinctness of the end point decreases at very low fluoride concentrations. Some of the fluorescence registered by the photocell is really due to Tyndall-type scattering of light by the dispersed thorium fluoride and this accounts in part for the curved portion of titration curves. The effect is even more noticeable when ferron rather than quercetin is used as the indicator. This effect cannot be reduced by use of narrower band filters in the Klett fluorometer without sacrificing sensitivity in the titration of very low concentrations of fluoride. Figure 3 shows the fluorescence with stirring compared to the fluorescence after settling without stirring when ferron is used as the indicator. The difference in the end-point values is negligible. Thus, continual stirring gives correct end-point values, and with steady slow addition of titrant, reproducible readings of the intensity of fluorescence are usually obtained even in the end-point region.

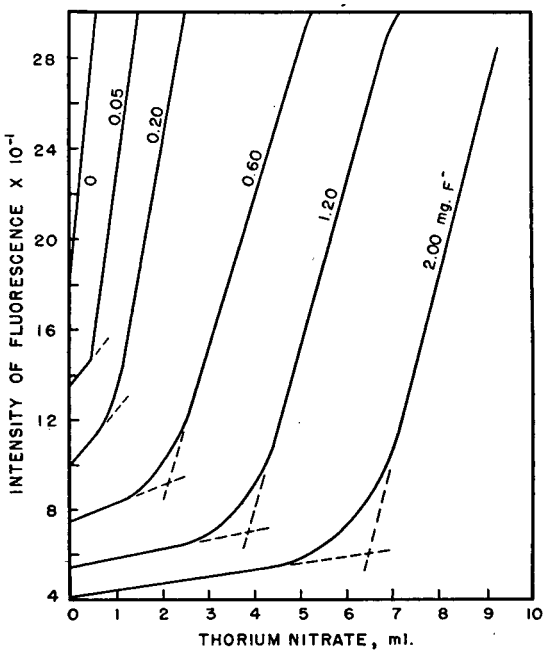

Figure 2. Typical Titrations with Stirring

Interferences. In general, the same types of ions interfere in this photofluorometric method using quercetin indicator as in the visual Alizarin Red S method. These interferences include, naturally, all ions which precipitate or form very stable complexes with thorium in acidic solutions, such as sulfate, phosphate, and iodate; those which also give a color or fluorescent change with the indicators, such as aluminum, iron, or zirconium; highly colored ions which mask the color or fluorescence such as chromate, permanganate, cobalt, or nickel; and ions which form insoluble fluorides in acidic solution such as calcium, lead, and the rare earths. Silver, nitrite, and borate ions also interfere in this method. Phosphate ion is titrated sharply, just like fluoride, and a titration of a mixture of fluoride and phosphate will give the total of these two ions. Sulfate interferes seriously in the fluorescence of the complex and cannot be titrated.

The quercetin photofluorometric procedure is not affected, however, by several ions which interfere to some extent in the visual method using Alizarin Red S. This is especially true for large amounts of thorium fluoride, which mask the color change in the visual titrations. High concentrations of the alkali metals, ammonium, manganous, chlorate, perchlorate, cyanide, acetate, chloroacetate, and other halide ions do not affect this

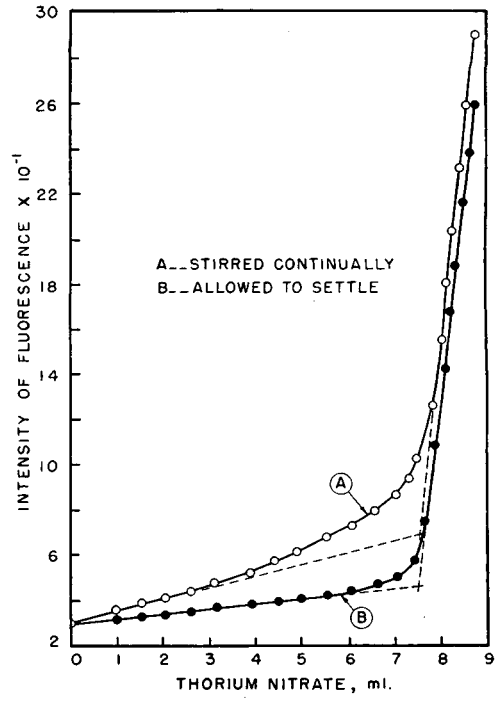

Figure 3. Fluorescence with Stirring and after Settling without Stirring

procedure. Thus the method is especially suited for titration of samples of fluorocarbons after decomposition with sodium or sodium peroxide.

Silicate ion interferes to some extent in the procedure, giving somewhat higher values in the titration of fluoride. However, because in hydrofluosilic acid there is a constant ratio of the silicate to the fluoride, a linear calibration curve is obtained when milliliters of titrant are plotted against milligrams of fluoride

Table I. Precision and Range of Photofluorometric Method

F^- Taken *Mg.*	F^- Found by A *Mg.*	Deviation *Mg.*	F^- Found by B *Mg.*	Deviation *Mg.*
		0.00317 *M* $Th(NO_3)_4$		
0.090	0.085	−0.005	0.078	−0.012
0.090	0.094	+0.004	0.102	+0.012
0.105	0.108	+0.003	0.167	+0.062
0.140	0.151	+0.011	0.144	+0.004
0.2105	0.210[a]	...	0.211[a]	...
0.2105	...	...	0.242	+0.032
0.281	...	...	0.286	+0.005
0.421	0.430	+0.009	0.421	0.000
0.461	...	...	0.474	+0.013
0.491	0.485	−0.006	0.472	−0.019
0.561	0.570	+0.009	0.541	−0.020
0.702	0.697	−0.005	0.703	+0.001
0.772	0.763	−0.009	0.777	+0.005
0.900	0.900[a]	...	0.903[a]	...
0.922	...	...	0.920	−0.002
1.382	...	...	1.387	+0.005
2.000	...	...	2.000[a]	...
2.304	...	...	2.30	−0.004
		0.0157 *M* $Th(NO_3)_4$		
1.382	...	...	1.43	+0.05
1.843	...	...	1.81	−0.03
2.765	...	...	2.82	−0.10
3.005	3.00[a]	...	3.02[a]	...
3.69	...	...	3.46	−0.23
3.69	3.71	+0.02	3.69	0.00
5.53	5.50	−0.03	5.53	0.00
8.75	9.05	+0.30	8.94	+0.19
8.75	...	...	8.82	+0.07
9.22	9.16	−0.06	9.21	−0.01
10.50	10.48[a]	...	10.51[a]	...
11.98	11.93	−0.05	11.97	−0.01
12.90	12.85	−0.05	13.05	+0.15
13.82	13.70	−0.12	14.10	+0.28
14.94	14.90	−0.04	15.00	+0.06

[a] Values used for standardization purposes.

present as hydrofluosilic acid or sodium silicofluoride. Thus, the procedure may be applied to distillates from the Willard-Winter method (*2*).

RANGE, PRECISION, AND REPRODUCIBILITY OF METHOD

To determine the precision and range of this method the procedure given below was employed.

Two thorium nitrate solutions were prepared, 0.0157 *M* and 0.0032 *M*, respectively, based on precipitation of thorium as the hydroxide and ignition to the oxide. The weaker titrant was standardized at 0.3, 0.9, and 2.0 mg. of fluoride, the stronger at 3.0 and 10.5 mg., using sodium fluoride that had been especially purified by Winter (*2*). The experimental results obtained by two analysts using these standardization levels are presented in Table I. The results all fit one of the following equations closely: For the 0.0157 *M* solution, theoretical factor 1.19 mg. per ml., mg. F = 1.188 mg. per ml. (ml. − 0.15); for the 0.0032 *M* solution, theoretical factor 0.2415 mg. per ml. in the 0.3- to 0.9-mg. range, mg. F = 0.2406 mg. per ml. (ml. − 0.15), in the 0.9- to 2.0-mg. range, mg. F = 0.266 mg. per ml. (ml. − 0.3). The precision was determined using 0.0123 *M* titrant. Ten samples of sodium fluoride were titrated at three levels. The deviation, on the 95% confidence level for a single determination, was ±0.013 mg. at 1.00 mg., ±0.09 mg. at 15.00 mg., and ±0.154 at 30.0 mg. of fluoride.

The lower limit of the method using the apparatus and procedure given below is about 0.04 mg. of fluoride using a quartz cell, 50% alcohol, and 0.01 *N* titrant. The upper limit is above 40 mg. of fluoride using a blown borosilicate glass cell, 40% alcohol, and 0.0125 *M* titrant.

Procedure I (0.1 to 2.0 mg. of fluoride). Place an aliquot of not more than 40 ml. of the sample in the special cell, add 1 to 2 drops of the pH indicator, and adjust the acidity by adding monochloroacetic acid slowly until the yellow indicator color just disappears. Add 5 ml. of buffer solution, 5 ml. of quercetin indicator solution, and sufficient 95% ethyl alcohol to form a 50% solution, taking into account the alcohol added with the indicator. Make all additions with pipets to keep the alcohol content constant.

Place the cell in the modified Klett fluorometer after cooling to 10° to 15° C., and adjust the stirrer to obtain a moderate stirring rate. The fluorometer should have warmed up for 15 minutes. Close the cover of the cell chamber, insert the tip of the buret filled with 0.0025 *M* titrant through the hole, and allow it to dip just into the solution. Adjust the balance diaphragm slit of the Klett to about 15 and balance the intensity potentiometer in the usual manner. The galvanometer should be steady on zero; if not, decrease the stirring rate slightly.

Add several small increments (usually 0.05 or 0.1 ml.) of thorium nitrate, balancing the galvanometer to zero after each addition and plotting the observed intensity readings against volume of titrant added. Do this until sufficient points have been obtained definitely to establish a straight line. Allow the titrant to follow slowly into the solution, watching for a sharp increase in galvanometer reading, indicating that the end point is near. At this point make several more small additions of titrant (usually 0.05 ml.) with galvanometer adjustment as before. Continue this until sufficient points have been obtained to establish a straight line. Extrapolate the straight lines graphically to the point of intersection which indicates the equivalence point, and read the end-point volume from the graph.

Procedure II (1.0 to 40 mg. of fluoride). Use Procedure I, but omit cooling of the cell and adjust the alcohol content to 40% instead of 50%. Use 0.0125 *M* titrant. The first increments may be larger (0.1 to 0.6 ml.), but the increments after the end point should be 0.1 or 0.05 ml.

RESULTS AND DISCUSSION

This procedure has given very good results on certain fluoro-organic compounds after treatment by alkaline decomposition methods. Results agreed much more closely than those obtained visually using Alizarin Red S. After one series of distillations for standardization purposes, results by this method applied to distillates from the Willard-Winter separation checked more closely for amounts of fluoride greater than 2 mg. than by the Alizarin Red S method. The method avoids the personal factor of judging subtle visual color changes and takes no longer than the visual procedure.

ACKNOWLEDGMENT

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Spectrographic Microdetermination of Beryllium in Air Dust Samples

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ONE of the important tools in the prevention of beryllium poisoning is the monitoring of the concentration of beryllium in the air. The critical concentration for outside air may be as low as 0.01 microgram per cubic meter (*4*), and it is necessary to measure at the level of 0.001 microgram per cubic meter. Even with a sensitive spectrographic method, the necessity for taking large volume air samples leads to many difficulties in the analytical process.

The most widely adopted method for spectrographic analysis for microquantities of beryllium is that of Cholak and Hubbard (*2*, *3*). Barnes, Piros, Bryson, and Wiener (*1*) have described a method for urine, tissue, and electrostatic separator dust samples. The general methods of Steadman (*9*) and Feldman (*5*) have also been applied to the determination of traces of beryllium. All these methods had certain disadvantages for the particular type of samples run in this laboratory, and it was necessary to develop a suitable procedure.

The method is not sufficiently sensitive to be useful in the analysis of urine and tissue samples. Further work is being done on the development of a more sensitive, yet reproducible method.

Air dust samples run in this laboratory are collected by drawing known volumes of air through paper filters. Depending on the atmosphere sampled, varying types and amounts of impurities are collected with the beryllium. This makes it necessary to use chemical separations to obtain the beryllium in relatively pure solution, not because of spectral interference, but in order to obtain a more uniform matrix for excitation. Any of the usual dilution methods reduces the sensitivity below the required minimum.

Although the techniques of air sampling are outside the scope of

this paper, certain features of the sampling influence the analytical method and must be described.

Three types of filters are in common use, the Whatman No. 41 ashless filter paper, the Mine Safety Appliances Company's all dust paper No. 2133 and Type S unimpregnated filter. The first filter, in a 10-cm. (4-inch) diameter, allows a sampling rate about 0.7 cu. meter per minute, but clogs rapidly in dusty atmospheres. The second paper allows a sampling rate of 1.0 to 1.3 cu. meters per minute and does not clog so rapidly. The Type S filter, because of the large area of the pleated structure, allows a sampling rate of about 2 cu. meters per minute and is very resistant to clogging. However, it has an ash content of over 1% (125 mg. per filter), which adds to the impurities in the sample. Blanks run on these papers show up to 0.3 microgram of beryllium per filter.

Table I. Precision of Spectrographic Procedure

γ Be per Ml.	γ Be Found	% Relative Error
0.10	0.13	30
0.15	0.18	20
0.20	0.22	10
0.50	0.72	44
	0.4	20
1.0	1.0	0
1.5	1.6	7
	1.4	7
2.0	1.8	10
	2.6	30
5.0	4.8	4
	5.8	16
7.5	6.9	8
10	9.0	10
	8.6	14
20	22	10
	19	5
50	64	28
	51	2
	44	12
	47	6
100	116	16
	107	7
	100	0
150	190	27
200	220	10
	Av.	13

The total volume of air sampled may range from 100 to 20,000 cu. meters. This means that at the 0.001 microgram per cubic meter level from 0.1 to 20 micrograms of beryllium would be present.

REAGENTS

All chemicals used are reagent grade.

Standard Beryllium Solution. Spectroscopically pure beryllium sulfate (0.982 gram) is dissolved in 100 ml. of 20% hydrochloric acid and diluted to 1 liter with water. The nominal composition is 50 micrograms of beryllium per ml. Further dilutions are made with water just before use.

Standard Aluminum Solution. A solution containing approximately 2.5 mg. of aluminum per ml. is prepared by dissolving aluminum nitrate nonahydrate in water. This solution is prepared in large amounts and standardized gravimetrically.

Oxine Solution. Twelve grams of oxine (8-quinolinol,8-hydroxyquinoline) are dissolved in glacial acetic acid and made up to 100 ml. The solution should be prepared fresh weekly and kept out of the light when not in use.

CHEMICAL PROCEDURE

The procedure given covers the analysis of dust on the Type S filter only, as the treatment of the other papers differs only in that smaller quantities of reagents are used in the wet-ashing and extraction steps.

The paper is placed in a 400-ml. beaker and 100 ml. of nitric acid are added. It is stirred to a pulp, 5 ml. of sulfuric acid are added, and the beaker is covered and taken to sulfur trioxide fumes on a hot plate. It is cooled slightly, 25 ml. of nitric acid and 0.5 ml. of 60% perchloric acid are added, and the samples refumed. The addition of nitric acid is repeated until all organic matter is destroyed. The solution is transferred to a 50-ml. platinum dish, 2 ml. of hydrofluoric acid are added and taken to dryness on a sand bath.

To the dry samples, 4 ml. of hydrochloric acid are added, the sample is transferred to a 50-ml. centrifuge tube, and the volume is adjusted to about 20 ml. It is neutralized to the first precipitate of iron or aluminum hydroxide with ammonium hydroxide, and 2 ml. of glacial acetic acid and then hydrochloric acid are added dropwise, until the precipitate dissolves. Then 5 ml. of 12% oxine in glacial acetic acid and some paper pulp are added. The pH is adjusted to 6 with ammonium hydroxide, using test paper as indicator, and the sample is centrifuged at about 3000 r.p.m. for 5 minutes. The liquid is decanted through a loose-textured paper into a 125-ml. separatory funnel, the precipitate is washed with water, and the washings are added to the original filtrate.

The filtrate and washings are extracted with 10 ml. of chloroform to remove excess oxine, repeating if the organic layer is not colorless. The aqueous phase is transferred to a clean 50-ml. centrifuge tube and the pH adjusted to 7.

If a visible precipitate forms at this point, the removal of iron and aluminum was incomplete and the oxine separation must be repeated. If the solution is clear, 1.0 ml. of an aluminum solution (2.5 mg. of aluminum per ml.) is added, centrifuged, and the supernatant liquid is discarded. The precipitate is dissolved in 0.5 ml. of 1 to 3 sulfuric acid and rinsed into a graduated 15-ml. centrifuge tube with water. It is evaporated to a volume of 1.0 ml. under an infrared lamp.

SPECTROGRAPHIC PROCEDURE

Spectrograph. Baird 3-meter grating, 50-micron slit.

Source Unit. Baird with Sola constant voltage transformer.

Lower Electrode. National Carbon Company 0.25-inch (0.6-cm.) nominal diameter graphite, regular grade, 6.3 by 50 mm., with a 4.5-mm. cup, 5 mm. deep.

Upper Electrode. Same type, sharpened to a 45° point.

Excitation. Direct current arc, 10 amperes, 2 minutes.

Densitometer. Baird nonrecording.

The cupped electrodes are waterproofed with a solution of

Table II. Recovery of Beryllium from Synthetic Samples

Type of Filter	γ Be Added	γ Be Found	% Recovery[a]
None	0	<0.2	...
		<0.2	...
	1.0	0.7	70
	5.0	4.5	90
No. 2133	0	0.3	...
	0.5	0.9	120
	1.5	1.2	60
	5.0	4.0	75
		5.2	100
	25	19.4	75
Type S	0	0.3	...
		0.3	...
		0.2	...
		0.3	...
		0.4	...
		<0.15	...
	0.05	0.5	400[b]
	0.10	0.6	300[b]
	0.50	0.3	0
	1.0	1.2	90
		0.8	50
	1.25	2.0	140
	1.75	1.5	70
	2.0	2.3	100
		3.0	135
		1.9	80
		1.7	70
	3.5	3.3	85
	5.0	5.6	105
		4.0	75
		4.2	80
		3.8	70
		4.4	80
	10	12	120
	25	20	80
		22	90
		21	85
		28	110
		26	105
	50	45	90
		60	120
		44	90
		Average recovery	90%
		Average error	20%
		(All samples over 1 microgram)	

[a] Corrected for blank.
[b] Omitted from average.

A spectrographic method for determining beryllium in air dust samples collected on filter paper covers from 0.05 to 200 micrograms of beryllium per sample. The filter paper is wet- or dry-ashed, treated with hydrofluoric and sulfuric acids, and taken to dryness. After solution in hydrochloric acid, the iron, aluminum, and other metals are removed by precipitation with oxine in acetate-buffered solution. The excess oxine is removed with chloroform, and the beryllium is precipitated as hydroxide, using 2.5 mg. of aluminum as a carrier. The hydroxide is dissolved in sulfuric acid, and the volume brought to 1 ml. of 1 to 7 acid. Aliquots of this solution are used for the spectrographic analysis. A 0.05-ml. portion is transferred to each of three cupped, waterproofed carbon electrodes containing about 45 mg. of sodium chloride as spectrographic buffer and carrier. Excitation is with the direct current arc at 10 amperes for 2 minutes. The average of triplicate density ratios of Be 2348/Al 2367 or Be 2650/Al 2367 is used as the analytical curve function. Check analyses show a relative error of ±20%, except at the extreme lower range, and over-all recovery of the chemical procedure is 90% of the beryllium content.

Duco cement in acetone and filled with sodium chloride to within 1 mm. of the top by tamping in a dish of the salt. The weight of sodium chloride has been found to be 45 ± 5 mg. in all cases measured. Three electrodes for each sample are treated with 0.05 ml. of the solution prepared above and dried in an oven at 110° C.

The lower electrode is made the anode in the arc. The electrode spacing of 4.5 mm. and the length of anode extending from the water-cooled electrode holders are set manually, using an auxiliary lens and screen. The arc image is focused on the grating with a long-focal-length quartz lens.

The spectrum is recorded on Eastman Type 33 plates, and standard development and fixing procedures are used. The densities of Be 2348.6, Be 2650.8, and Al 2367.1 are read and the ratio of the suitable beryllium line to the aluminum line is used as the analytical function.

No plate calibration procedure is used; the ratio Be 2348/Al 2367 gives a straight line from 0.005 to above 0.2 microgram of beryllium on the electrode, as does the ratio Be 2650/Al 2367 from 0.1 to 10 micrograms of beryllium. Standards are run periodically and the analytical curve is remade if excessive shifts appear.

The sample burns smoothly during the excitation period, giving a negligible background on the plate. Although reburning the electrodes shows that some beryllium is left, complete burning does not increase sensitivity or accuracy because an increased continuous background is produced.

RESULTS OF ANALYSES

The values in Table I were obtained by adding appropriate amounts of the standard beryllium solution to 0.5 ml. of 1 to 3 sulfuric acid, adjusting the volume to 1 ml., and running through the standard spectrographic procedure.

The values in Table II were obtained by adding appropriate amounts of the standard beryllium solution to the paper filter in a beaker and running through the complete procedure. It was not found possible to prepare synthetic samples with beryl, but the procedure used has been shown to give complete solution of beryl and other beryllium ores.

The accuracy in the lower range is limited by the high blank (0.3 microgram) and no results showing under 1 microgram of beryllium per sample are considered reliable.

EXPERIMENTAL

Ashing Procedure. Three ashing procedures were tried: dry ashing in silica and in platinum dishes and wet ashing in borosilicate glass beakers (as in the procedure). The results shown in Table III indicate that the use of silica leads to the loss of considerable beryllium, and that dry ashing in platinum is inferior to wet ashing, although it is used if speed is essential.

Known amounts of beryllium solution were dried on paper filters in the dishes used. Before ashing, the filters were moistened with 1 to 1 sulfuric acid to aid in conversion to beryllium oxide. Organic matter was burned off at a low temperature and the sample ignited for a few minutes in a muffle at 900° C. Beryllium was recovered from the ash by heating with 3 ml. of sulfuric acid, transferring to platinum if necessary, and completing the procedure from the hydrofluoric acid treatment on.

Oxine Separation. Kolthoff and Sandell (*7*) and Knowles (*6*) have used oxine for separating beryllium from iron, aluminum, and other heavy metals. However, no data on the recovery of microgram quantities of beryllium from large amounts of precipitable elements were available.

Table III. Recovery of Beryllium from Dry Ashing Procedures

Type of Filter	Be Salt Used	Type of Dish	γ Be Added	γ Be Found
Type S	Sulfate	Silica	2	1.2
				1.2
			5	2.0
				2.2
			10	2.4
				0.3
			50	52
				13
Type S	Fluoride	Silica	50	24
				5
Type S	Chloride	Silica	5	1.4
				2.7
				1.9
No. 2133	Fluoride	Silica	50	4.4
				6.2
Type S	Sulfate	Platinum	5	9.3
				5.2
				3.3
				3.5
				2.2
				2.9
				4.1
Type S	Fluoride	Platinum	5	2.5
				5.5
				3.5
				4.6
				3.1
				3.5
Type S	Chloride	Platinum	5	3.0
				2.9
				3.5

So that all samples received could be handled by the same method, the use of a large quantity of oxine was indicated. Five milliliters of a 12% solution were found to remove iron and aluminum completely from all but a very few samples. Theoretically, 0.6 gram of oxine should precipitate 77 mg. of iron or 37 mg. of aluminum.

The pH is not critical, and indicator paper is suitable for the adjustment. If the excess oxine is incompletely removed, the aluminum is not quantitatively precipitated. Both the extraction and the phase separation after extraction must be done carefully.

The oxine separation cannot be checked separately, but the results given for the entire process in Table II show that the beryllium recovery is good.

Precipitation with Aluminum as Carrier. Table IV shows the results obtained when various known amounts of beryllium were carried through the precipitation step.

Table IV. Recovery of Beryllium with Aluminum Hydroxide Carrier

γ Be/Ml. Added	γ Be/Ml. Found	% Relative Error
0.05	0.042	15
0.10	0.095	5
0.20	0.19	5
0.30	0.35	17
	0.32	7
1.0	0.6	40
5.0	5.0	0
	4.4	12
	7.0	40
10	9.4	6
	13.4	34
40	45	13
50	56	12
	58	16
	56	12
	41	18
		Av. 16

The chief interference seems to be the incomplete precipitation of aluminum (and possibly beryllium) caused by carry-over of organic matter from the oxine separation. However, this is readily detectable by the resultant decrease in the density of the aluminum 2367 line, and such results are discarded. Care in the operations reduces this error to negligible proportions, and, if necessary, the solution and precipitate may be digested briefly in a water bath to aid coagulation.

Selection of Spectroscopic Buffer Carrier. Several salts were tested as buffer carriers for the beryllium, using a crater 5 mm. deep packed with the salt to within 1 mm. of the lip. All samples were arced for 2 minutes at 10 amperes. Sodium chloride was selected on the basis of sensitivity and freedom from background. The cup depth was selected to hold a sufficient quantity of salt to last for the full 2-minute excitation period.

Other Spectrographic Variables. The National Carbon *spectroscopically pure graphite electrodes* showed a slight increase in sensitivity over the regular grade, but not enough to warrant the difference in cost.

The burning time was selected as a reasonable compromise between speed and complete volatilization of the beryllium.

The concentration of the sulfuric acid used has a considerable effect on the spectrographic results. This was first pointed out to the authors by Landis (*8*) and confirmed in their laboratories. The exact concentration is less critical in the range of 3 to 5 *N* and the 1 to 7 acid concentration was selected for this reason. It should be noted that 0.5 mg. of phosphate ion on the electrode lowered the sensitivity to 0.05 microgram of beryllium.

The use of aluminum as internal standard was recommended by Barnes, Piros, Bryson, and Wiener (*1*), who used Al 2321.6. The present authors have selected Al 2367 for its suitable density and ability to correct slight variations in operating conditions.

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Micromethod for Estimation of Potassium by Paper Chromatography

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A VARIETY of methods exists for the determination of small quantities of potassium. Relatively few laboratories are equipped with spectrographic equipment or flame photometers, however, and the latter instrument is frequently troublesome and inaccurate when applied to the analysis of complex samples (*8*). Polarographic analysis does not distinguish between potassium and sodium (*10*). Chemical tests involving gravimetric, volumetric, turbidimetric, and colorimetric analyses of the perchlorate, cobaltinitrite, chloroplatinate, iodoplatinate, phosphotungstate, and dipicrylamine complex are frequently laborious (*4*) and occasionally dangerous (perchlorate, *6*), and require considerable skill (*9*). In most of these cases, quantities of potassium exceeding 0.100 mg. are required, and the results obtained from complex mixtures vary from ±2 to ±10% (*1, 3, 7*).

In the course of studies on potassium metabolism there has been occasion to develop a technique for potassium determination which is somewhat simpler than many of the usual methods and requires the use of no equipment beyond that generally found in smaller laboratories. A total sample of less than 0.1 mg. of potassium is required, which for most biological fluids represents less than 0.1 ml. For this reason it is felt that the technique has broad applicability in general, clinical, research, and classroom work. The method involves a rapid chromatographic separation on paper, development with a sensitive and specific reagent, and quantitation to eliminate errors caused by extraneous materials. By employing a modification of the technique described by Berry and Cain (*2*), each sample serves both as a standard and as an unknown at the same time.

PROCEDURE

The sample solution to be analyzed is diluted or concentrated so as to contain approximately 0.1% potassium. Portions of this sample are placed on a 30 × 12 cm. sheet of Whatman No. 1 filter paper as follows: Ten points are marked with a pencil 2 cm. from one edge of the paper and 2.5 cm. apart from each other. To each of the first five of these spots are added from a suitable pipet 5.0 μl. of the sample, and all the spots are allowed to dry. A standard solution of potassium chloride containing 1.91 grams per liter (1.00 gram of potassium) is then prepared and distributed upon the spots in amounts as follows: 0, 5, 10, 15, 20, 0, 5, 10, 15, and 20 μl. On the sheet as finally prepared, the first five spots contain 5 μl. of unknown plus 0, 5, 10, 15, and 20 micrograms of

A paper chromatographic method for the quantitative estimation of potassium involves a rapid chromatographic separation on paper, development of the chromatogram with sodium lead cobaltous hexanitrite solution, and quantitation of the potassium spots by planimetry. A technique of chromatogram preparation is described which eliminates errors due to foreign materials in the sample. Using the method described, potassium in concentrations of 0.1% may be determined with an error of less than 10% on a 0.1-ml. sample.

potassium, respectively, and the second five spots contain 10 μl. of unknown with the same amounts of added potassium as the first five.

The filter paper is fixed in the form of a cylinder with staples, and set in a 1- or 2-liter beaker with the spots along the bottom of the cylinder and in such a manner that the paper cylinder does not touch the sides of the beaker. Into the bottom of the beaker is introduced, to a depth of about 1 cm., a solution consisting of 80 parts of ethyl alcohol and 20 parts of 0.1 N hydrochloric acid. The beaker is covered with a watch glass and the solvent is allowed to ascend to the top of the filter paper cylinder. This ascent requires about an hour. The cylinder of filter paper is then removed from the beaker and dried in air.

Sodium lead cobaltous hexanitrite (*5*) is used to develop the chromatogram. The reagent is conveniently prepared by dissolving 11.5 grams of lead nitrate and 15.0 grams of sodium nitrite in 50 ml. of distilled water. When solution has occurred, 10.0 grams of cobaltous nitrate are added and the volume is made up to 100 ml. with distilled water. This solution is allowed to stand for 1 hour, diluted to 200 ml. with distilled water, and filtered just prior to use. The reagent is sprayed lightly over the chromatogram with a hand atomizer or pressure spray, after which a green spot at R_f 0.4 rapidly develops, owing to the presence of potassium. Under the conditions of the test, no interference occurs as a result of the presence of ammonia, calcium, or magnesium, which give positive reactions in crystallographic work employing this reagent (*10*). The chromatogram is then allowed to dry, and the spots are carefully circumscribed with a pencil. The areas of the spots may then be measured with a grid system or with a polar planimeter, or quantitated by excision and weighing on an analytical balance. The results reported here were obtained by planimetry.

When the areas of the first five spots are plotted against the amounts of added potassium, a straight line is obtained, as shown in Figure 1. A second straight line, parallel to the first, is obtained by plotting the corresponding areas against the potassium added to the 10-μl. samples of unknown. It is apparent from Figure 1 that the difference between the intercepts of these two parallel lines with the x-axis is equivalent to the potassium content of 5 μl. of unknown solution (K in Figure 1). This follows because K corresponds to the amount of potassium that must be added to the 5-μl. aliquot of unknown to produce a spot of the same size as the 10-μl. aliquot. It is generally best to analyze the data by this graphic method. By employing simple mathematical formulas, however, it is possible to calculate the potassium content of the sample from the equation:

$$K_{(\text{mg./ml.})} = \frac{\bar{A}_{10} - \bar{A}_5}{0.01\ \Sigma(K_s A) - \Sigma 0.1\ A}$$

where $\bar{A}_{10}$ = the mean area of the spots containing 10 μl. of the unknown, $\bar{A}_5$ = the mean area of the spots containing 5 μl. of the unknown, $\Sigma(K_s A)$ = the sum for the ten spots of the products of the area of each spot and the microliters of potassium standard added to that spot, and A = the total area of all ten spots. Minor deviations in technique, however, may greatly increase the errors obtained by the use of this formula, so that a graphic solution is preferable except when experienced workers are conducting routine analyses. The accuracy of the procedure may be further increased by increasing the number of increments of known potassium concentrations on the chromatogram, or by determination in replicate.

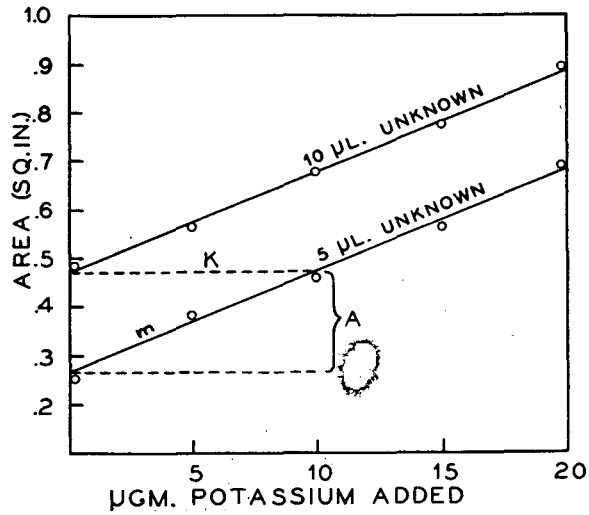

Figure 1. Illustration of Graphic Method of Analysis of Potassium Determination Data

Table I. Typical Analytical Data Based on Determination of Potassium in Urine and Unknowns

Sample		Potassium Found, Mg./Ml.	% Error[a]
Urine sample 1		1.75	+5
		1.60	−3
		1.65	−1
	Mean	1.67	
Urine sample 2		1.30	+6
		1.10	−10
		1.25	+2
	Mean	1.22	
Urine sample 2 + 0.2 mg. K/ml.[b]		1.47	+4
+ 0.6 mg. K/ml.[b]		1.70	−7
+ 1.0 mg. K/ml.[b]		2.35	+6
Unknown A[c] (1.70 mg. K/ml.)		1.75	+3
Unknown B (1.16 mg. K/ml.)		1.10	−5
Unknown C (0.67 mg. K/ml.)		0.75	+12
Unknown D (2.07 mg. K/ml.)		2.25	+9
Unknown E (1.35 mg. K/ml.)		1.25	−7

[a] Values on urine samples based on deviation from mean; other urine recovery data based on mean value for sample 2.
[b] Average of duplicate determinations.
[c] Quantitative unknown solutions of potassium salts prepared by other workers and analyzed by author in duplicate.

The technique described was originally designed for the determination of potassium in urine, and the analytical data in Table I were obtained from this material and from a series of unknowns such as are used in quantitative analysis courses. Tests on other materials have shown that the method works well in general analytical work, although slight changes in technique may be desirable in some cases. Thus, when large amounts of foreign materials interfere with the potassium spot, the test should be run on a larger sheet of filter paper, so that greater resolution of the components of the sample is obtained.

DISCUSSION

The technique employed largely embodies the usual methods of paper chromatography. The modifications concerned with the

analysis of the data, however, involve several unique factors. When the same increments of potassium are added to the two series containing different amounts of unknown, and the areas of the resulting spots are measured, the rate of increase of the areas within the two series should be equal, and the plotted results should produce two parallel lines. It is therefore easy to devise a series of simultaneous equations which could be solved for the potassium content of the unknown. The equation presented is based on these considerations and provides a simplified method for calculating the result. The quantitation of results obtained by paper chromatography is extremely subject to errors due to the effects of foreign materials in the sample, and a method based on the principle of sample distribution employed here is essential for a suitable degree of accuracy, as pointed out by Berry and Cain (*2*). The concentration of the extraneous materials in a sample spot on paper may markedly influence the degree of spreading of any particular constituent. Consequently, comparison cannot validly be made of the areas of urinary potassium spots with standard spots in which large concentrations of other materials are absent. It is essential that the potassium standard be influenced in its "spreading" and migrating characteristics in the same manner as is the potassium in the unknown sample. This may be achieved by preparing the standard solution so that it contains representative amounts of the major constituents of urine. Such a procedure is analogous to that employed in the flame photometry of complex mixtures, where the reference standard is made up with foreign materials present so as to resemble the type of unknown under study. The use of the unknown itself as a base for the standard, however, presents a more accurate means of achieving this effect.

Sodium lead cobaltous hexanitrite has been previously used for the detection of potassium by microcrystallographic means (*5*), under which circumstances ammonium, cesium, lithium, rubidium, thallium, arsenic, antimony, calcium, chromium, iron, magnesium, silver, and tin are reported to interfere with the results. Under the conditions of the test described here, none of these materials interferes, although some difficulty may result when these elements are present in very high amounts. Using the method described, potassium in concentrations of about 0.1% may be determined with an error of less than 10% on a sample of 0.075 ml. of solution. Although the accuracy of the present method is thus not so great as that of some other methods, it seems adequate for many analyses in which greater precision is not required, and where limitations exist on equipment, sample size, and time.

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Round-Table Discussion

FLAME PHOTOMETRY

Digest of stenographic report of round-table discussion held by Division of Analytical Chemistry, 117th Meeting, A.C.S., Houston, Tex., March 1950

Moderator: W. G. SCHRENK, *Department of Chemistry, Agricultural Experiment Station, Manhattan, Kan.*

FLAME photometry offers excellent possibilities for the development of analytical procedures for those metallic ions which can be excited by the relatively low excitation levels available in the flame. The procedure is not new and has been used for years as a qualitative test for ions such as potassium and sodium.

Interest in quantitative procedures, however, has increased rapidly in the past few years. This has been due, first, to the development and availability of instruments, designed for this purpose (*1, 2, 4, 6*), and secondly, to the need for simpler and more accurate methods for the determination of the ions of the alkali metals. Particular attention has been given to the determination of sodium and potassium because of their importance in biological systems. Other elements, however, can be determined by this procedure. Of particular interest at the discussion were calcium and magnesium.

Although flame methods appear promising with regard to the determinations of these elements, any new technique involves certain difficulties not found in established methods. As a result discussion centered around two general topics concerning this method of analysis. First, were factors influencing instrument stability and precision and, second, were the preparation of samples and calibration techniques.

FACTORS INFLUENCING INSTRUMENT REPRODUCIBILITY

Included among the factors which govern the reproducibility of any given instrument are such items as the atomizer system, air supply, gas supply, and the stability of the electronic amplifier circuit, including the photocell.

Discussion brought out the point that atomizer construction is critical. Atomizers of apparently identical construction require separate calibration and therefore are not entirely interchangeable. The atomizer described by Weichselbaum and Varney (*6*) was mentioned, but no performance data were available other than those described by them in their paper.

Several people mentioned the need for extreme cleanliness in handling the atomizer. It was pointed out that if the atomizer does not drain properly erratic results will occur. Some workers clean the atomizer with distilled water and alcohol after each determination, followed by periodic cleanings with other materials. Others routinely clean after a definite number of determinations.

It was also pointed out that the type of sample plays a role in determining the number of analyses which can be made before cleaning is required. Biological samples, in general, tend to reduce the number of times the atomizer can be used before thorough

cleaning is required. All agreed that cleanliness with respect to the atomizer is a critical point in flame photometry.

Air supply also needs to be controlled. If the air supply in the laboratory is not clean it should be filtered. Dust particles may cause two effects: first, they may clog the orifice of the atomizer, and secondly, they may cause luminous spots to appear in the flame. Both effects are undesirable. Fumes from a chemical laboratory may cause difficulty also. One report was made of erratic behavior caused by mercury vapor in the air. It follows, therefore, that care is necessary with the air supply with regard to elements which are not usually considered in flame photometry.

Adequate control of air, gas, and oxygen pressures is necessary also. It has been shown (*1*, *3*) that these factors influence the sensitivity of the analytical procedure and therefore not only need adequate control, but also must be easily reset to predetermined values. Diaphragm-type pressure regulators do not give as steady a flow of gas as is required. If used as pressure reducers, they should be followed by some additional control such as a needle valve.

Discussion also indicated that electronic amplifier tubes can cause errors in analyses. These errors are due in part to the lack of stability and uniformity of the tubes. Errors may also be due to variations in line voltage when instruments are alternating current operated. Tube errors can be minimized by buying on the open market and selecting by trial and error those that function best in the instrument. Line voltage variation can be stabilized in part by the use of "constant voltage" transformers. One reference was made to literature (*3*) which reported on a modification of an exsiting electronic circuit which gave improved performance.

Several other factors which were discussed briefly included atomizer chambers, the use of alcohol in the test solution, and surface tension effects, all of which appear to be related in instrument stability. The use of isopropyl alcohol in the test solution increases the sensitivity. The heated jacket on the atomizer chamber also increases sensitivity and gives more reproducible results. The effect is apparently one of getting more of the sample into the flame. The alcohol apparently increases the volatility of the sample and the heated atomizer chamber tends to prevent condensation of the atomized sample before it reaches the flame.

Surface tension apparently affects the rate of atomization of the sample and consequently the sensitivity. This effect can be minimized by using dilute test solutions, adding alcohol, and eliminating as far as possible the larger organic molecules which may be present in some cases.

CALIBRATION AND SAMPLE PREPARATION

Special care is required in the preparation of standard solutions for the calibration of flame photometers. It was suggested that consideration be given spectroscopically pure samples for such calibrations. Some laboratories reported that they run a standard for every four or five unknowns. Others calibrate their instruments at the beginning and end of each series of determinations. Another technique reported was that in which two standards are run for each unknown, one of higher and the other of lower concentration than the unknown. This technique permits interpolation of results on the unknown.

The accuracy of the determination is influenced by the composition of the test solution. Thus, it has been shown (*2*, *3*, *5*) that extraneous elements may influence the intensity of emission of the test element. In cases where the extraneous elements are present in small amounts standards containing only the test element may be used. It was pointed out, however, that such standard solutions have limited application. In most cases it was felt desirable to make standard solutions of approximately the composition of the unknown.

Included among the types of samples which were being analyzed for sodium and potassium were such substances as soil, food products, blood serum, urine, plant tissue, and water. Discussion emphasized the need for careful sample preparation. Most organic substances need to be eliminated by ashing before analysis. In some cases a simple extraction process can be used—for example, potassium can be quantitatively extracted from many dried plant materials with hot water. Care in checking such a method is required, however, because oil-bearing substances such as cottonseed meal cannot be handled by this procedure.

Samples of blood serum also require special care. If serum is not removed from the blood soon after the blood is drawn, erroneous results on potassium will be obtained. Anticoagulants cause errors on sodium analysis. Hemolysis also may cause errors in the determination of potassium. If liquid biological materials can be diluted sufficiently, ashing may not be required. Other laboratories routinely eliminate all organic substances by ashing. If the surface tension of the fluid is close to that of water, it is possible that some organic material is permissible.

No mention was made during the discussion regarding the internal standard technique which may be used with some equipment. This method of improving accuracy should not be overlooked, however, by those who may be starting work in this field.

Most laboratories were using flame methods primarily for the determination of sodium and potassium, although some were considering calcium and magnesium also and indicated considerable interest in their determination. In answer to questions concerning flame methods for calcium and magnesium it was pointed out that errors were greater and sensitivity less than for sodium and potassium. Suggestions regarding changes in instruments to accommodate such determinations included (1) a better lens system between the flame and the photocell, (2) an improved amplifier circuit, and (3) an increase in flame temperature.

In answer to a question concerning the use of flame photometric techniques for the continuous determination of calcium and magnesium and water it was suggested that if total calcium and magnesium was desired a technique using a resin exchange might possibly be developed. Readings could then be made on sodium and translated to combined calcium and magnesium content.

The lack of sensitivity in the determination of magnesium was also discussed. At low magnesium levels the errors are considerably larger than in the determination of sodium and potassium. It was suggested that a hotter flame might increase sensitivity. This could possibly be accomplished by the substitution of an acetylene-oxygen mixture to replace a gas-oxygen mixture in the burner. It was also pointed out that such a change would increase the hazard of backfiring in a burner and that a new burner design might be of help.

Among those taking extensive part in the discussions were V. W. Meloche of the University of Wisconsin, H. A. Frediani of Merck and Co., Inc., W. R. Lowstuter of Hercules Powder Company, J. H. Gast of Baylor University, and C. E. Bills. Thanks are due them for the success of the discussion period as well as to all others who attended and took part in the round table discussion.

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RECEIVED August 4, 1950.

NOTES ON ANALYTICAL PROCEDURES . . .

Quantitative Hydrogenation of Unsaturated Fatty Acid Derivatives

Hydrogenation of Less Than Milligram Amounts in the Warburg Apparatus

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RECENT publication (*7*) of a method for the determination of unsaturation by microhydrogenation prompts a report of a procedure developed some time ago (*5*) to serve a similar purpose.

Having in hand a promising method (*6*) of isolating very small, pure samples of unsaturated fatty acid esters derived from natural sources, the authors were faced with the problem of determining the extent to which these samples were unsaturated. As it did to Ogg and Cooper (*7*), microhydrogenation appeared to be much more attractive than microhalogenation because of its less capricious dependence upon structural factors. Some years ago, Kuhn and Möller (*3*) published details of a method based on similar reasoning.

Although a number of microhydrogenation apparatus have been described (*2*), these were considered unsuitable because they involved more or less complicated construction or were incapable of measuring accurately the small hydrogen uptake anticipated in the experiments.

The commercially available Barcroft-Warburg apparatus, with no more complicated modification than is customary for studies involving gases other than air, serves admirably for the microhydrogenation of less-than-milligram amounts of material containing as little unsaturation per molecule as that found in methyl oleate. Coupled with a colorimetric determination (*1*) of carbomethoxy content, this procedure suffices to distinguish very small amounts of such esters as methyl oleate, linoleate, and linolenate from one another.

PROCEDURE

Standard single-side-armed 15-ml. Warburg flasks are charged as follows: Approximately 5 mg. of catalyst (10% palladium black on charcoal) and 2.5 ml. of redistilled 96% ethyl alcohol are placed in the main compartment of the flasks, while 0.5 ml. of an ethyl alcohol solution of the unsaturated compound (1 to 10μ moles per ml.) is added to the side arm (which should be sufficiently large to prevent spilling into the body of the flask during the equilibration-period shaking).

The charged flasks are connected to the Warburg manometers and the air in them is replaced by hydrogen, by means the apparatus described by Burris (*8*), except that screw clamps are used to prevent rising of the manometer fluid from its reservoir bulb during evacuation.

Table I. Hydrogen Uptake of Unsaturated Acid Derivatives

Substrate	Amount, Mg.	Amount, μ moles	Hydrogen Uptake, μ Moles, Calcd.	Observed	Error, %	Double Bonds Found
Maleic anhydride	0.23	2.37	2.37	2.50	+5.5	1.06
				2.47	+4.2	1.04
				2.42	+2.1	1.02
Methyl oleate	0.54	1.81	1.81	1.85	+2.2	1.02
				1.67	−7.7	0.92
	1.08	3.62	3.62	3.48	−3.9	0.96
Methyl linoleate	0.35	1.19	2.38	2.29	−3.8	1.93
				2.47	+3.8	2.08
Methyl linolenate	0.19	0.64	1.92	1.86	−3.1	2.91
	0.47	1.60	4.80	5.02	+4.6	3.14

Without disconnecting the assembly from the hydrogen reservoir, the manometer stopcocks are closed by a quarter turn, and the shaking is started. When the absorption of hydrogen by the catalyst and the solutions has stopped (this requires about 30 minutes and is followed manometrically), the manometer stopcocks are reopened to the system, and the manometer fluid is readjusted to the zero point (usually 250 mm.). The stopcocks are again closed, the manometers are disconnected from each other, and the system is equilibrated for 5 minutes. The contents of the side arms are then tipped into the main compartments, and the shaking is continued for 5 minutes. At this time a preliminary reading is taken and, by appropriate tipping of the manometers, the side arms are rinsed with some of the reduced solution; the shaking is then continued for an additional 5-minute period. The final readings are corrected against a blank manometer, identically charged and manipulated, but containing no unsaturated material, and the corrected hydrogen uptake is converted to micromoles by the usual procedures.

Using this technique, the hydrogen uptake of less-than-milligram amounts of several unsaturated acid derivatives has been measured and is reported in Table I.

The maleic anhydride was freshly sublimed; the methyl esters were purchased from the Hormel Foundation and were reported to have the following iodine numbers (Wijs): oleate, 85.43 (theory 85.6); linoleate, 172.4 (theory 172.4); linolenate, 258.6 (theory 260.4).

ERRORS AND PRECAUTIONS

A survey of the literature failed to reveal data on the solubility of hydrogen in 96% ethyl alcohol, which are required in the evaluation of the Warburg flask constant. However, because this factor affects only about 1% of the flask constant, using data on the solubility of hydrogen in absolute ethyl alcohol at 30° C. (*4*) probably introduces no great error. Absolute ethyl alcohol is to be avoided as a solvent in these microhydrogenations because of its usual benzene content.

The reading of the manometers, especially where small differences of level are involved, is probably the greatest single source of low precision. To minimize the change in readings which occurs when the shaking of the flasks in the bath is interrupted, the method of reading is standardized in any convenient way and accomplished as quickly as possible. The temperature of the bath should also be standardized, and should be as close to room temperature as proper functioning of the bath thermostat will permit.

The lubricant used on the ground surfaces of the apparatus should be insoluble in 96% ethyl alcohol and must not itself take up hydrogen under the conditions of the determinations. The silicone-type high vacuum grease manufactured by the Dow Corning Company was found to be satisfactory from these standpoints; use of another common lubricant of a different type led to continued erratic hydrogen absorption.

Following preliminary examination of various hydrogenation catalysts, Adams' platinum oxide was discarded because the small amounts required were not adequately dispersed in the solution by the rather moderate shaking typical of the Warburg apparatus. A catalyst which proved to be eminently satisfactory both from the point of view of easy suspension in the solution and for its

activity is the 10% palladium on charcoal manufactured by the American Platinum Works.

The volume of the solution of substrate is important in that it must be large enough for adequate transfer from the side arm to the main compartment and small enough for adequate equilibration with hydrogen before the substrate and catalyst solutions are mixed. In this connection, it is wise not to fill the side arm too full, lest premature mixing occur during evacuation of the system or during the equilibration shaking period. For the type of flask used in this laboratory (American Instrument Company, No. 5-201 without center well), 0.5 ml. of substrate solution was entirely satisfactory.

A cursory examination of Table I discloses the fact that the factors discussed above may lead to very appreciable errors in terms of per cent; however, the precision is adequate for the determination of the number of carbon-to-carbon double bonds in less-than-milligram amounts of fatty acid derivatives.

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Elastomeric Test Films from Acidic Latices

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THE necessity for obtaining homogeneous "flaw-free" samples for testing the stress-strain properties of elastomeric films is well recognized. Tensile strength, elongation, modulus, and tear resistance are all affected by the presence of flaws such as air bubbles, surface cracks, ridges, edge nicks, and thickness heterogeneities in the films tested. Previous authors (*3*) considering the problem have recommended carefully pouring the latex on flat glass plates and using controlled humidity and precipitating atmospheres to prevent the formation of an impervious surface film which would prevent further escape of water from the underlayers within the latex film. Use of this method in this laboratory has in many cases yielded flawed and irregular films of acidic copolymer latices of ethyl acrylate and acrylonitrile, even after the alkalinity had been adjusted to pH 10.5 with sodium hydroxide. It became necessary therefore to devise a method for casting, from such acidic type latices, films which would be essentially flaw-free.

Thick plaster of Paris molds are generally used to cast commercial articles from latex. The mechanism of film formation against such a plaster mold is usually considered as being due to absorption of water through the minute plaster of Paris pores, the size of which prevents passage of the solid polymer particles. The polymer film is deposited as a more or less porous mass through which diffusion of water may occur, and drying may take place simultaneously from both surfaces—e.g., through the plaster mold and from the side exposed to the atmosphere. This has a distinct advantage over casting against glass, where drying may take place through one surface only. For these reasons, it was decided to attempt to apply this technique to casting flat films for test purposes.

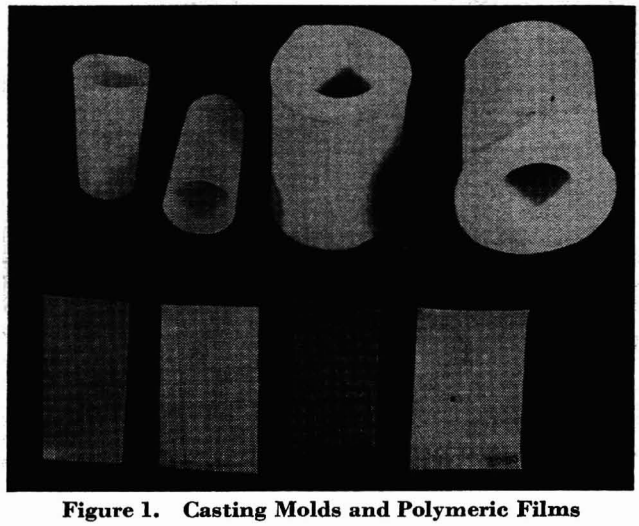

Figure 1. Casting Molds and Polymeric Films

In the method used, thick plaster molds with an interior cavity the size and shape of a 250-ml. high-form beaker were prepared. This shape of mold was used for several reasons. Its cylindrical shape allows the film to distribute evenly any stresses that develop in drying. In addition, under these conditions of casting, the film, during formation, is maintained in contact with an aqueous medium that prevents the formation of an impervious surface skin, which would cause entrapment of water in the subsurface layers. Experience has shown that such a skin is likely to develop wrinkles and other flaws as the film dries. Films deposited on plaster molds were built up rapidly and wet films 0.060 to 0.090 inch thick could be deposited in 1 to 2 hours.

MOLDS

The molds were prepared from Coe Dental Laboratories Hydrocal dental stone and distilled water. To 2100 grams of stone, 700 ml. of distilled water were slowly added and the resulting slurry was kneaded between the fingers until all lumps had been dispersed, evacuated in a vibrating vacuum chamber for 2 minutes, and then poured into a 1-liter beaker. A 250-ml. high-form beaker, which had previously been dipped into an alcoholic solution of green soap and allowed to dry, was now moistened and a thin coat of the plaster slurry was rubbed onto its outer surface. The high-form beaker was filled with water to counteract its buoyancy, inserted into the slurry with a gentle rotary motion, and centered by eye. The plaster was then allowed to harden. When the plaster set it expanded slightly, breaking the outer beaker. At this point, a gentle, rotary pull freed the inner beaker, which was readily removable, owing to the soap and its tapered shape. The mold was then dried for 24 hours in a circulating air oven at 60° C. An occasional mold prepared in this fashion yielded consistently poor films. No explanation of this effect has been discovered as yet.

CASTING

To prepare test sheets from latex, the dry plaster mold was filled with latex, previously filtered through cotton gauze to remove any coagulum. The latex was allowed to remain in the mold from 30 minutes to 2 hours, depending upon the solids content of the latex. Latices containing 50% solids yielded films

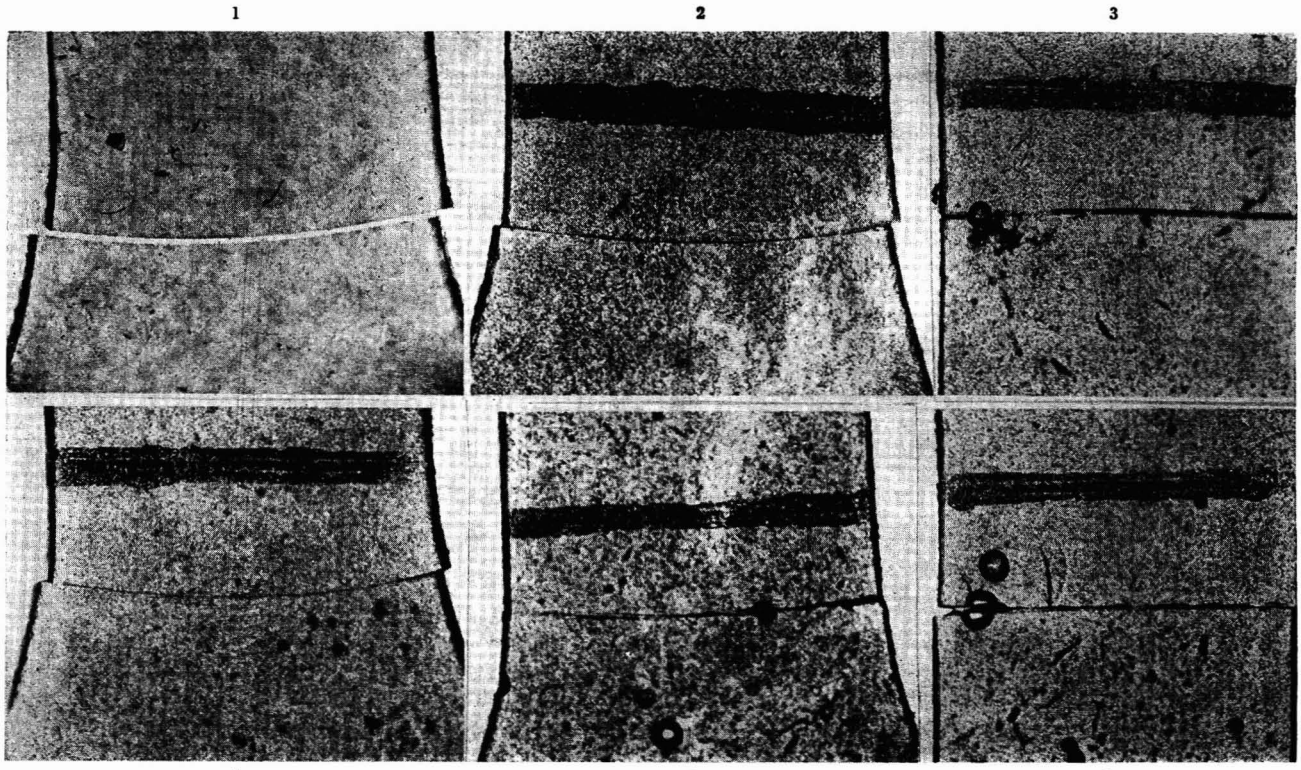

Figure 2. Photomicrographs of Tensile Test Specimens (× 20)

from 0.060 to 0.090 inch thick in 2 hours. After the time required for deposition had elapsed, the balance of the latex was poured out, and the mold, which then had a wet film of polymer deposited upon the surface of its inner cavity, was placed in a circulating air oven at 60° C. until dry. The drying time varied from 3 to 12 hours, depending upon the type of latex used. The film could then be stripped from the mold, cut along one side to form a sheet, and given any further vulcanizing or curing treatment desired. Certain high-solids latices deposited films that adhered tenaciously to the molds. The removal of these films was greatly expedited by pouring water under the edge of the film as it was loosened from the plaster.

PREPARATION OF LATEX

Ethyl acrylate (90 grams) and acrylonitrile (10 grams) were copolymerized in emulsion using 100 grams of a 1% Santomerse D solution as emulsifying agent in the presence of 0.5 gram of potassium persulfate and 0.5 gram of sodium thiosulfate. The reaction was allowed to proceed for 4 hours at 20° C. and the latex steam stripped to remove unreacted monomer. The yield was 94 to 96% of latex at pH 2.2.

TESTING

The tensile strength tests were conducted according to A.S.T.M. D 412–41 using die C. The testing machine used was a Scott L-6 rubber tester. All samples were conditioned for 7 days at 50% relative humidity and 75° F. prior to testing.

RESULTS

The casting technique described gave excellent results when applied to the acidic copolymer latices of ethyl acrylate and acrylonitrile. The films were visually bubble-free, ridge-free, and homogeneous in thickness. Similar results were obtained with

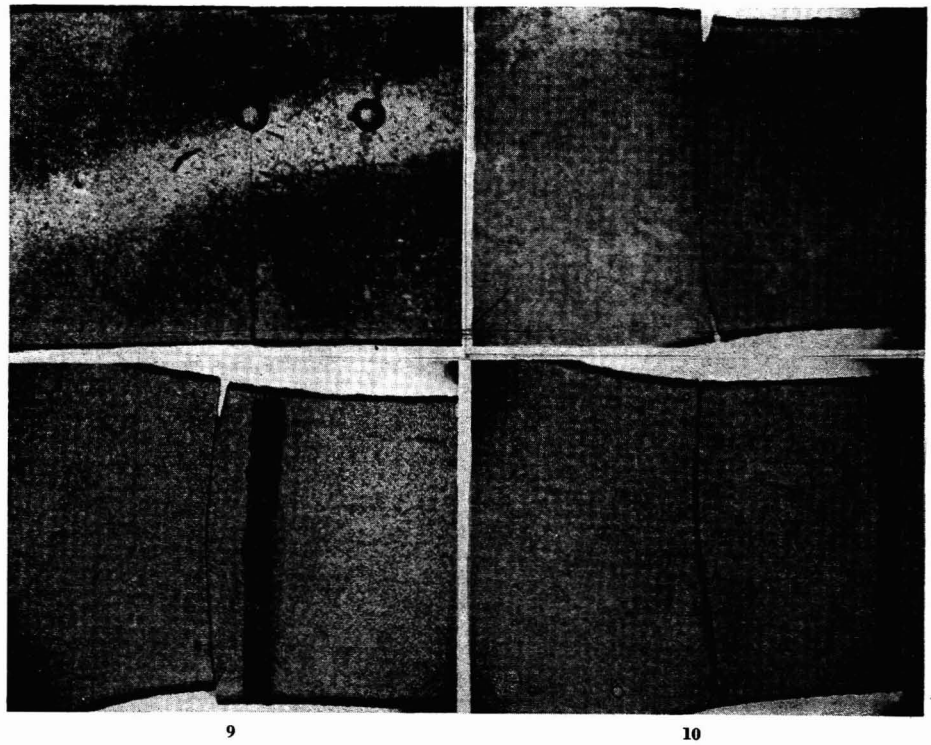

Figure 3. Photomicrographs of Tensile Test Specimens (× 20)

commercial latices of neoprene, natural rubber, and Geon polyblend.

The method is rapid, reproducible, and easily applied, and produces films of excellent appearance. Figure 1 shows the molds and films of various polymeric materials prepared by this technique.

Despite the fact that the films appeared to be flaw-free, determination of the tensile values of a very large number of specimens gave inordinately low values in approximately 5% of the cases. The data tabulated in Table I show five specimens with low values of tensile strength and five reference specimens having higher values. All the samples tested were examined under a microscope using 20 to 1350 power magnification, and it was established that those samples which had low tensile values had broken through a bubble. These bubbles were visible only under the microscope. Figures 2 and 3 show a series of photomicrographs of these ten specimens taken at 20×, which demonstrate this effect.

Table I. Tensile Strength of Polyethyl Acrylate–Acrylonitrile 90–10 Copolymers

Specimen No.	Tensile Strength, Lb./Sq. Inch	Ultimate Elongation, %
1	747	730
2	800	730
3	330, bubble present	740
4	800	750
5	227, bubble present	600
6	267, bubble present	680
7	302, bubble present	710
8	817	750
9	744	750
10	392, bubble present	670

Despite the fact that the specimens with low tensile strength broke through a bubble, it is not axiomatic that a specimen containing a flaw will fail at the flaw. It has been shown that the stress concentration in the shoulder of the specimen is often sufficient to cause rupture at that point in preference to another point of lower stress concentration that contains a flaw (*1*, *2*, *4*).

Examination of Figures 2 and 3 and Table I also shows that the presence of bubbles does not appear to affect the ultimate elongation materially.

DISCUSSION AND CONCLUSIONS

The knowledge of the tensile strength of a polymer is of importance to a polymer research laboratory in order to serve as a guide for further modification of polymer structure. The results obtained from flawed samples will frequently lead to unfruitful research. A product fabrication laboratory, however, is primarily interested in the strength of the polymer and the ability of the fabricator to make a flaw-free product. This situation suggests the adoption of two techniques by the testing laboratory. In testing for the polymer laboratory, each specimen is examined after testing under a microscope. Any specimen that breaks through a bubble or flaw visible when examined in this fashion is rejected. The results of five specimens that show no flaws are averaged and reported as the polymer tensile strength. For a product fabrication laboratory, any specimens that show visible flaws that would cause rejection of the finished product should be discarded and the balance tested. The average of five specimens that show no flaws under unaided visual inspection is reported as the average tensile strength of the article.

SUMMARY

A method for casting and testing essentially flaw-free films from acidic type latices consists of casting against the interior cavity of a cylindrical gypsum mold. The method permits drying of the film from both surfaces and prevents the formation of an impervious surface film, which would later cause imperfections. Films 0.060 to 0.090 inch thick can be deposited in 1 to 2 hours using a latex containing 50% solids. Measurements of tensile strength and microscopic examination of samples cut from the films cast according to this technique showed that those samples which gave inordinately low values of tensile strength broke through a bubble visible only under the microscope. Suggestions are given for evaluating tensile test results.

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Effect of Temperature on Tributyl Phosphate as Extracting Agent for Organic Acids

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THE use of *n*-tributyl phosphate for extracting organic acids from aqueous solution at 25° ± 2° C. was reported earlier (*1*). Work recently completed shows the effect of temperature from 0° to 35° C. for acetic, citric, tartaric, and glycolic acids.

PROCEDURE

Fifty milliliters of ester (previously saturated with water to minimize volume change on mixing) and 50 ml. of the aqueous acid were pipetted into a 150-ml. glass-stoppered separatory funnel. The mixture was allowed to reach the bath temperature and then shaken vigorously for 30 seconds at about 5-minute intervals for at least 0.5 hour without being removed from the bath. Agitation for longer periods gave identical results. After phase separation had taken place, two 20-ml. pipetted portions from each phase were titrated with 0.1 or 0.5 *N* carbonate-free standard sodium hydroxide, using thymol blue indicator.

Table I. Effect of Temperature on Distribution of Organic Acids in the Two-Phase System Water–*n*-Tributyl Phosphate

Acid	Concn., *N*	Temp., ±0.1° C.	Extracted[a], % (±0.1)	C (Ester) / C (Water)
Acetic	1.125	0	66.3	1.97
		15	64.4	1.81
		25	63.3	1.73
		35	62.3	1.65
Acetic	0.0988	0	71.5	2.51
		15	69.5	2.28
		25	68.1	2.14
		35	66.4	1.98
Citric	1.000	0	71.3	2.48
		15	62.4	1.66
		25	56.5	1.30
		35	50.5	1.02
Citric	0.0992	0	80.1	4.03
		15	70.9	2.44
		25	63.9	1.77
		35	56.6	1.30

(*Continued on next page*)

Table I (*Continued*)

Acid	Concn., *N*	Temp., ±0.1° C.	Extracted[a], % (±0.1)	C (Ester) / C (Water)
Tartaric	1.001	0	41.8	0.72
		15	34.6	0.53
		25	30.5	0.44
		35	26.7	0.36
Tartaric	0.0988	0	49.5	0.98
		15	39.3	0.65
		25	33.5	0.50
		35	28.7	0.40
Glycolic	0.992	0	34.9	0.54
		15	31.8	0.47
		25	29.9	0.43
		35	28.3	0.40
Glycolic	0.0983	0	39.1	0.64
		15	35.0	0.54
		25	32.6	0.48
		35	30.5	0.44

[a] Based on analyses of equal volumes of aqueous and ester phases.

In titrating the ester phase sample with aqueous base a two-phase system is formed, wherein the indicator is almost completely extracted into the ester. Sharp end points were obtained, however, by injecting a small amount of indicator into the aqueous layer (lower) after each addition of base.

In several instances the densities of the two phases were nearly identical, in which cases the rate of phase separation was very slow. A study of the effect of adding inorganic salts to increase the density of the aqueous phase to hasten phase separation, and the effect of such salts on the distribution constant, is in progress.

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Colorimetric Determination of Sodium Pentachlorophenate

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METHODS have been developed to meet the need for a simple procedure for determining small amounts of sodium pentachlorophenate in solutions for mildewproofing, algae control, etc. The methylene blue procedure was developed first and at a time when the author was not aware that any other colorimetric procedure existed (at Cannon Mills, Kannapolis, N. C.). The copper method was developed much later. A good colorimetric procedure consists of oxidizing the compound to a quinone (*4*). The oxygen bomb method and the lime ignition method are also available (*4*).

COPPER METHOD

It was found that the copper salt formed by treating sodium pentachlorophenate with copper sulfate is soluble in 70% isopropyl alcohol with the production of an opalescent colloidal solution which clears with acidification. The desired acidity for the reaction of potassium ferrocyanide with copper is also obtained,

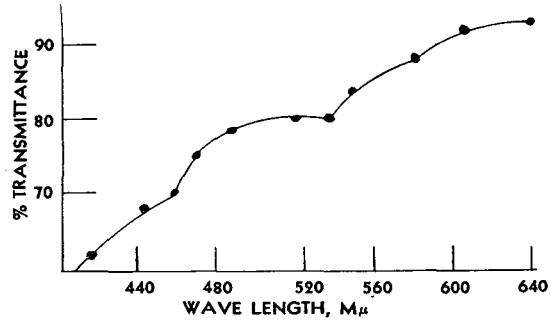

Figure 1. Absorption of Colored Solution

as outlined in the procedure. The amount of sodium pentachlorophenate can be determined by treating the foregoing colorless solution with potassium ferrocyanide, which is a very sensitive reagent for copper. The method has a range of 15 mg. and adheres to Beer's law over this range. The abridged spectrophotometric curve shown in Figure 1 was obtained with a Leitz-Rouy photometer. Maximum absorption occurs at 415 mμ, and a filter covering this range should be used.

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Table I. Recoveries

Amount in Solution, Mg.	Amount Recovered, Mg.	Recovery, %
5	4.8	96.0
5	5.0	100.0
5	5.0	100.0
10	10.0	100.0
10	10.0	100.0
10	9.8	98.0
15	15.0	100.0
15	14.7	98.0
15	15.0	100.0

PROCEDURE

Reagents used are 1% copper sulfate, 70% isopropyl alcohol, 0.67 *N* sulfuric acid, and 1% potassium ferrocyanide. The sample should contain approximately 10.0 mg. of active material. The sample is placed in a 125-ml. separatory funnel and 20 ml. of 1% copper sulfate are added. The solution is filtered, the separatory funnel is rinsed with several portions of water, and the residue on filter paper is washed thoroughly with distilled water. Then 50 ml. of 70% isopropyl alcohol, acidified to the optimum point with 3 drops of 0.67 *N* sulfuric acid, are added to remove any residue present, and this alcohol is used to dissolve residue on filter paper into 100-ml. flask. The paper is washed with a little distilled water. Then 5 ml. of 1% potassium ferrocyanide are placed in the flask and the solution is mixed, diluted to 100 ml., and allowed to stand for 5 minutes. The colorimeter is set to its zero position with distilled water, and is read at the end of a 5-minute period with a filter transmitting at 415 mμ.

Sodium pentachlorophenate may be precipitated from water by copper sulfate, forming a purple salt which dissolves with a loss of color in acid alcohol. By treating the solution with potassium ferrocyanide the amount of copper involved in the reaction is determined. One mole of copper combines with 2 moles of sodium pentachlorophenate. Absolute or 70% ethyl alchol may be used; however, 70% isopropyl alcohol is cheaper and more available.

This is an accurate method and a rapid one. The greatest error in recovery was 4%, which is within the range tolerated for colorimetric procedures. A determination may be completed in about 15 minutes. It is necessary to adhere to the time factors pointed out in the procedure, for the colored copper ferrocyanide is unstable, and its optical density changes rapidly with time. A large source of error will develop unless these points are kept in mind.

Other phenols react in the same manner and will introduce a source of error. Commercial sodium pentachlorophenate contains some tetrachlorophenate plus a few related impurities commonly

classified as tars (*2*). This procedure was calibrated with technical sodium pentachlorophenate, which cancels out the effect of these substances.

METHYLENE BLUE METHOD

Methylene blue hydrochloride combines with sodium pentachlorophenate quantitatively at a pH of 10.9, producing a blue colored complex which is soluble in chloroform. The reaction obeys Beer's law up to 1 mg. and the maximum absorption band is at 600 to 640 mμ as determined with a photoelectric colorimeter The blank, which is prepared with sodium hydroxide, chloroform, methylene blue, and water, is a magenta colored solution prior to being filtered through cotton. Upon being filtered, this solution turns a very pale blue, much lighter than that found with sodium pentachlorophenate. A certain part of the color of the unknown is due to this reaction, but is taken care of by setting the instrument to zero with the blank.

The reaction producing the magenta colored blank seems to be physical and is probably a hydration phenomenon. Water is essential for the production of the red color, and removal of the water results in a blue solution. It is believed that the alkali reacts with some impurity in methylene blue, such as methylene violet or azure blue (*1*). Azure blue will react with an alkali, producing the same red color and the same type of absorption curve. All the azure blue may be used up in this fashion, which is contrary to what is observed with methylene blue.

SUMMARY

Two methods have been developed for the determination of sodium pentachlorophenate. The copper method is superior because of its wider range and greater simplicity. The methylene blue procedure is interesting from the standpoint of the reactions described with regard to this and similar compounds with alkalies, etc., which may be of some value in determining these substances.

These methods were worked out for solutions containing sodium pentachlorophenate, but they can also be applied to textiles, wood, etc., if one takes advantage of the fact that this compound is soluble in water, alcohol, and benzene (water at 25° 26.1%, acetone 32 to 33%, alcohol 32 to 33%, and benzene 0.1%) (*3*).

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Determination of Tryptophan with *p*-Dimethylaminobenzaldehyde Using Photochemical Development of Color

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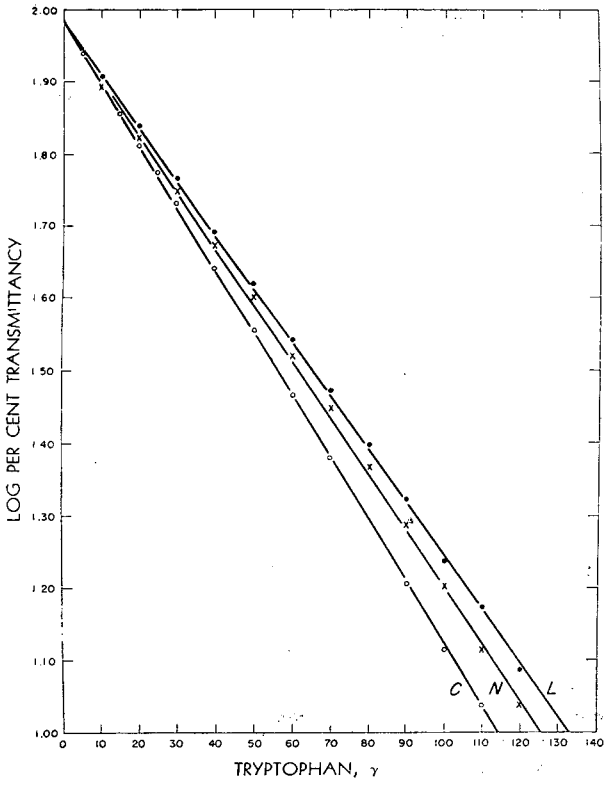

Figure 1. Standard Curves for Tryptophan

L. **Color developed with light**
N. **Color obtained by addition of sodium nitrite to previously illuminated test solutions**
C. **Color developed with sodium nitrite**

TWO reactions are involved in previous methods for the determination of tryptophan (*2*). Reaction I is the condensation of tryptophan and *p*-dimethylaminobenzaldehyde in 19 *N* sulfuric acid to form a colorless product, and reaction II is the development of a blue color by oxidation of this compound with sodium nitrite. The photochemistry of these reactions has been reported (*4*). This note describes a method for the estimation of tryptophan, using light as the agent for inducing oxidation. This method may have special applications in some of the many complex problems related to tryptophan analysis, but it is not intended to replace previously described procedures using sodium nitrite as oxidant.

The rate of reaction II, caused photochemically, was rapid at first but became slow as the reaction approached completion as 70, 88, 91, 93% of the potentially available color (as compared with that obtainable with sodium nitrite) developed on illumination of test solutions for 5, 20, 60, and 120 minutes, respectively. Illumination caused slight destruction or alteration of the chromogen, because after 5 minutes' illumination 93 to 94% of the total color obtainable by sodium nitrite alone could be obtained by subsequent oxidation with sodium nitrite. This effect was not progressive, because whether illumination was for 5 or 120 minutes 93 to 94% of the total color was subsequently obtainable with sodium nitrite.

The transmittancy-concentration relationship obtained when reaction II was carried out photochemically or with sodium nitrite is shown in Figure 1, where the log of the per cent transmittancy is plotted against weight of tryptophan. *C* was obtained using sodium nitrite, *L* was obtained by illumination for 20 minutes, and *N* was obtained by sodium nitrite oxidation of test solutions previously illuminated for 20 minutes. That *C* and *N* do not coincide shows that light destroys or modifies some of the chromogen. The conformity of these results to Beer's law, for transmittancies ranging from 12 to 82%, is shown because *C*, *L*, and *N* are straight lines.

Table I. Tryptophan Content of Proteins Using Light and Sodium Nitrite to Develop Color

Protein	Tryptophan Content[a] Light	Sodium nitrite	Difference
	%	%	%
Casein	1.75 ± 0.01(3)[b]	1.70	2.9
β-Lactoglobulin	2.50 ± 0.01(3)	2.52	0.8
Ovalbumin, denatured	1.55 ± 0.02(3)	1.46	6.2
Conalbumin, denatured	3.38 ± 0.04(3)	3.01	12.3
CS-54R (*1*)	0.907 ± 0.013(3)	0.991	8.5
CS-56R (*1*)	0.524 ± 0.005(2)	0.561	6.6

[a] Same protein samples used for analyses using light and sodium nitrite. Results are on ash- and water-free basis and values were corrected as described in (*3*). Values obtained using sodium nitrite were determined by Procedure O and were taken from Table XIII (*3*).
[b] Numbers in parentheses show number of determinations made on each protein.

The relative color intensities obtained on 20 minutes' illumination of tryptophan, casein, ovalbumin, and a cottonseed allergenic polysaccharidic protein CS-54R (*1*) were 97, 99, 100, and 99%, respectively, of the color intensity obtainable by a 60-minute period of illumination of each substance. These data indicate that the photochemical development of color, reaction II, is slightly more rapid for proteins than for free tryptophan and for greatest accuracy in an analytical method the exact time of maximum color development for tryptophan and each protein should be determined as was done previously for reaction II with sodium nitrite (*2*, *3*). In the present work, however, a 20-minute period of illumination was adopted as sufficiently accurate to illustrate the method.

Results of tryptophan analysis of several representative proteins using light to develop the color are compared in Table I with values obtained using sodium nitrite. The difference between values varied from a minimum of 0.8% with β-lactoglobulin to a maximum of 12.3% with conalbumin, and the average difference for six proteins was 6.2%. The precision using photochemical oxidation was ±1%, while that using sodium nitrite was ±0.88%. These results indicate that photochemical oxidation could be used in an analytical procedure with a reasonable degree of accuracy if use of sodium nitrite were not feasible.

APPARATUS, MATERIALS, AND METHODS

Apparatus, materials, and general procedures have been described in detail (*2–4*). Reaction I for free tryptophan and for proteins can be carried out by any appropriate procedure. The apparatus and procedure for illumination of test solutions have been described (*4*). *C*, Figure 1, is the same as described in Figure 4 of (*2*). *L* and *N* were obtained as described below using Procedure E (*2*) for reaction I.

Procedure S. To 1.003 mg. of tryptophan and 250.8 mg. of *p*-dimethylaminobenzaldehyde (30 mg. per 10 ml.) in a 125-ml. glass-stoppered Erlenmeyer flask were added 83.6 ml. of 19 *N* sulfuric acid at 25°, solution A. In another flask 100 ml. of 19 *N* acid were added to 300 mg. of *p*-dimethylaminobenzaldehyde, solution B. Required volumes of solutions A and B were mixed immediately in 25-ml. glass-stoppered flasks to give test solutions containing 10 to 120 micrograms of tryptophan and 30 mg. of *p*-dimethylaminobenzaldehyde in a volume of 10 ml. The solutions were reserved in the dark at 25° for 19 hours. Each solution was then illuminated for 20 minutes at 25° and transmittancies were read using a blank solution similar to the test solution, except that tryptophan was omitted. The lowest transmittancy obtained over the wave length ranging from 580 to 620 mμ was used for calculation of results. Results are plotted as *L*. To each solution was then added 0.1 ml. of 0.04% sodium nitrite solution and after standing for 30 minutes transmittancies were read. Results are plotted as *N*.

For the proteins, Procedure O (*3*) was used with approximately individual optimum times for reaction I (*3*). Solutions were illuminated for 20 minutes. Transmittancies were converted to weight of tryptophan from *L*, Figure 1.

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RECEIVED November 2, 1949. Paper V in series entitled "Chemical Determination of Tryptophan." For previous paper see (*3*).

Determination of Cholesterol

Adaptation of Schoenheimer-Sperry Method to Photoelectric Instruments

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THIS paper describes a modification of the Schoenheimer-Sperry method (*1*), simple enough for clinical use and accurate enough for research purposes. A similar adaptation, published by Sperry (*5*), has limited distribution and is at present out of print. The authors have tried to eliminate some of the difficulties of the method which in the past have been made its introduction into the routine of any laboratory a tedious and time-consuming procedure.

REAGENTS

With three exceptions, the reagents used are the same as described by Sperry (*4*). Instead of redistilled alcohol and acetone, C.P. acetone and absolute alcohol are entirely satisfactory. The 0.4% aqueous digitonin solution has been replaced by an 0.5% digitonin solution in 50% alcohol, as recommended by Sobel and Mayer (*2*). The use of alcoholic digitonin solution results in an evenly dispersed, fine precipitate. The precipitate is not sticky, and the washed precipitate easily dissolves in glacial acetic acid without the development of the sediment referred to by Sperry (*4*). The stock standard used contained 2 mg. of cholesterol per ml., and three different working standards containing 0.250, 0.500, and 0.750 mg. were prepared with each series of determinations.

APPARATUS

The apparatus was that described by Sperry (*4*), except that 15-ml. glass-stoppered, heavy-duty borosilicate glass centrifuge tubes (obtained from MacAllaster-Bicknell, Cambridge, Mass.) were substituted for the stirring rods and preserving jars.

EXPERIMENTAL

In attempting to adapt the method to the spectrophotometer and photoelectric colorimeter it soon became evident that the development of the color and the determination of its intensity are the most sensitive points of the reaction. The time relationship of the color development and the relationship between the color intensity and the Beer-Lambert law seemed to be the most important in this respect.

In his earlier publications Sperry recommended that if the color is developed at 25° C. it should be read at 30 minutes (*3*, *4*). In his

Table I. Optical Density ($-\log T$) of Samples Containing 0.5 Mg. of Cholesterol

Sample	30 Min.	30.5 Min.	31 Min.	31.5 Min.	32 Min.	33 Min.
Determined with Spectrophotometer						
1	48.9	48.9	48.9	48.9	48.9	..
2	49.0	49.1	49.1	49.1	49.1	..
3	49.0	49.1	49.1	49.0	49.0	..
4	..	..	49.3	..	49.3	..
5	..	..	..	..	49.8	..
6	..	..	..	..	..	49.9
Determined with Photoelectric Colorimeter						
1	48.8	48.8	48.8	49.1	48.9	..
2	48.5	48.5	48.5	48.5	48.6	..
3	48.4	48.4	48.5	48.7	48.5	..
4	..	..	48.7	..	48.7	..
5	..	..	..	..	48.5	..
6	..	..	..	..	..	48.5
	40 Min.	40.5 Min.	41 Min.	41.5 Min.	42 Min.	43 Min.
Determined with Spectrophotometer						
7	48.7	48.5	48.3	48.3	48.1	..
8	49.0	48.9	48.5	48.4	48.2	..
9	49.0	49.0	48.8	48.7	48.5	..
10	..	..	48.7	..	48.2	..
11	..	..	..	..	48.2	..
12	..	..	..	..	..	47.5
Determined with Photoelectric Colorimeter						
7	49.8	49.8	49.7	49.7	49.7	..
8	49.7	49.6	49.7	49.6	49.6	..
9	49.7	49.7	49.7	49.7	49.7	..
10	..	..	49.7	..	49.3	..
11	..	..	..	..	49.4	..
12	..	..	..	..	..	49.3

latest publication (*5*) he specified 40 minutes as the optimal time for the determination of the color intensity.

To clarify this point, color was developed at 25° C. in a series of standards containing 0.5 mg. of cholesterol dissolved in glacial acetic acid, by the addition of the acetic anhydride–sulfuric acid reagent. When the color intensity was to be determined in the photoelectric colorimeter (Fisher Scientific Company, Pittsburgh, Pa.) 0.5 mg. of cholesterol was dissolved in 1 ml. of glacial acetic acid to which 2 ml. of acetic anhydride–sulfuric acid reagent were added, and the color was read in a microcuvette of approximately 10-mm. diameter, using a 650 mμ filter. When a spectrophotometer (Coleman Junior Model 6a, Maywood, Ill.) was used, 0.5 mg. of cholesterol was dissolved in 2 ml. of glacial acetic acid, 4 ml. of acetic anhydride–reagent were used, and the reading was made in a cuvette of 19-mm. diameter at a wave length of 625 mμ.

Each series consisted of six samples, and the acetic anhydride reagent was added at 3-minute intervals. The color intensity in the first and second series was determined in the spectrophotometer and in the third and fourth series in the photoelectric colorimeter. In three samples of the first and third series the color was read at 30 minutes, then the samples were left in the cuvette and the color was read again at 30.5, 31, 31.5, and 32 minutes. Another sample was left in the water bath a minute longer and the color was read at 31 and 32 minutes, and in two other samples the color was read at 32 and 33 minutes, respectively. A similar procedure was carried out with the second and fourth series; the color was read at 40, 41, 42, and 43 minutes. Findings are presented in Table I.

It can be seen from the table that there is little or no difference in the color intensity read at or around 30 minutes, or at or around 40 minutes. At 30 minutes there is a slight tendency to a further increase of the color intensity with time, and at 40 minutes a similar tendency to a decrease of the color intensity, but for practical purposes the color can be considered stable, and its intensity can be determined at any time between 30 and 40 minutes.

To study the relationship between the color intensity and the Beer-Lambert law, color was developed in samples containing 0.125, 0.250, 0.500, 0.750, and 1.000 mg. of cholesterol. When the determinations were made on the spectrophotometer, the above quantities of cholesterol were dissolved in 2 ml. of glacial acetic acid, to which 4 ml. of acetic acid anhydride reagent were added. When photoelectric colorimeter was used the cholesterol was dissolved in 1 ml. of glacial acetic acid and 2 ml. of acetic acid anhydride were used. The results are presented in Table II.

The color intensity as determined with either instrument follows the Beer-Lambert law fairly closely. The parallelism is closer when the color intensity (or optical density) is determined with the spectrophotometer than when it is determined by the photoelectric colorimeter. The spectrophotometer in higher concentrations tends to give slightly higher values. The results obtained with the photoelectric colorimeter are somewhat higher in low concentrations, and considerably lower in higher concentrations than would follow from the Beer-Lambert law. The error caused by this discrepancy can be largely eliminated by the use of several standards of different concentrations and comparison of the unknown with the standard nearest to it. This point is brought out more clearly in connection with the results of the recovery tests.

PROCEDURE FOR ANALYSIS OF CHOLESTEROL IN BLOOD OR PLASMA

Extraction. Add 5 ml. of heparinized plasma (or whole blood) rapidly, in a fine stream, to 75 to 80 ml. of 1 to 1 alcohol-acetone mixture in a 100-ml. volumetric flask. Bring to boil, and keep at boiling point for 30 seconds in a water bath, rotating to avoid bumping. After cooling to room temperature, make up to volume with 1 to 1 alcohol-acetone, mix, and filter through fat-free filter paper into a glass-stoppered bottle.

Table II. Optical Density of Cholesterol Solutions Read 40 Minutes after Development of Color

Cholesterol Content of Sample, Mg.	Photoelectric Colorimeter	Spectrophotometer
0.125	14.3	11.3
	14.5	11.7
0.250	28.4	23.3
	28.5	23.4
0.500	49.6	45.9
	50.0	46.1
0.750	67.8	69.0
	67.8	69.6
1.000	80.0	96.0
	80.0	95.0

Determination of Total Cholesterol.

1. Place 2 drops of 50% potassium hydroxide in a 15-ml. glass-stoppered centrifuge tube, add 5 ml. of extract, and shake vigorously.
2. Place the centrifuge tube in a water bath at 40° C. for 30 minutes. Do not allow the temperature of the bath to fall below 37° C.
3. Remove centrifuge tube from water bath, cool to room temperature, add 1 drop of phenolphthalein solution, and titrate with 10% acetic acid solution. Add 1 drop in excess.
4. Add 3 ml. of 0.5% alcoholic digitonin solution to the slightly acid extract in centrifuge tube, stopper, mix thoroughly, and keep at room temperature overnight.
5. Remove stopper, centrifuge at 2500 to 2800 r.p.m. for 15 minutes, and decant supernatant fluid. Blow in 2 ml. of 1 to 2 acetone-ether mixture in a rapid stream to break up sediment and wash down the wall of centrifuge tube with another 2 ml.
6. Centrifuge again for 10 minutes, decant, and wash with 4 ml. of ether.
7. Centrifuge again for 10 minutes, decant, and evaporate ether with a stream of filtered air for 1 minute.
8. Heat a 3- to 4-cm. layer of sand in a pan to 110° to 115° C. in an oven. Replace the stopper loosely in the centrifuge tube (preferably by placing a fine string about halfway down the stopper), place the centrifuge tube in hot sand, and keep in oven for 30 minutes.
9. Take the centrifuge tube from the sand bath, remove the stopper, and allow 2 ml. of glacial acetic acid to run down its wall. Replace stopper, shake vigorously, and put back into sand bath for 2 to 3 minutes.

Determination of Free Cholesterol. Put 10 ml. of extract into a glass-stoppered centrifuge tube. Omit steps 2 and 3 and carry out steps 4 to 9, inclusive, as described for total cholesterol, but do step 6 twice.

Development of Color. Remove centrifuge tubes from the oven and cool in a 25° C. water bath.

Prepare standards containing 0.25, 0.50, and 0.75 mg. of cholesterol in 2 ml. of glacial acetic acid and a blank containing 2 ml. of glacial acetic acid.

Prepare acetic anhydride–sulfuric acid (20 to 1) reagent and keep in a glass-stoppered bottle in an ice-cold water bath.

Add at regular intervals (1 minute is suitable after some practice) 4 ml. of ice-cold anhydride reagent to blank, each unknown, and standards, mix well, and place in 25° C. water bath kept in a dark cabinet.

Adjust the instrument to zero with blank in place.

At an exact time (any time between 30 and 40 minutes may be chosen) from the addition of the anhydride reagent, and in the same order the reagent was added, read the color.

If the photoelectric colorimeter is used, dissolve both unknowns and standards in 1 ml. of acetic acid and use only 2 ml. of anhydride reagent. This change is made necessary by the fact that with this type of instrument the color is read in a 10-mm. cuvette and the decreased thickness of the solution must be compensated for by increased concentration. It was also necessary to use a cushion in the cuvette holder to raise the cuvette and thereby bring the small quantity of solution into the path of the light of the instrument.

CALCULATION

For accurate results calculations should be made with the standard that gave the nearest reading to the unknown in question. The readings are made on the optical density ($-\log T$) scale of the instruments.

$$\text{Mg. of cholesterol in sample} = \text{mg. of cholesterol in standard} \times \frac{\text{reading of unknown}}{\text{reading of standard}}$$

To obtain results in milligrams per cent, the cholesterol content of the sample must be multiplied by the dilution factor of the sample. If 5 ml. of plasma (whole blood) are extracted in 100 ml. (dilution 1 to 20) and 5 ml. of the extract are used in a sample, the factor converting to milligrams per cent will be $20 \times 20 = 400$. If 10 ml. of extract are used (as in the determination of free cholesterol), the factor will be $10 \times 20 = 200$. Simpler formulas can be obtained if the milligrams of cholesterol in the sample are multiplied by the dilution factor used. For convenience the factors to be used are presented in Table III.

With the use of the above combined factors

$$\text{Mg. \% of cholesterol in sample} = \frac{\text{reading of unknown}}{\text{reading of standard}} \times F$$

Table III. Dilution Factors

Cholesterol in Standard, Mg.	Factor F	
	5 ml. of extract	10 ml. of extract
0.25	100	50
0.50	200	100
0.75	300	150

CONTROL OF METHOD BY RECOVERY OF KNOWN QUANTITIES OF CHOLESTEROL

The cholesterol concentration was determined as total cholesterol in 50, 100, 200, and 300 mg. % acetone-alcohol solutions. The total and free plasma cholesterol content was determined in two pregnant women, with and without the addition of 0.125 mg. of cholesterol to the samples. It follows from the above calculations that the addition of 0.125 mg. of cholesterol to a sample should produce a 50 mg. % increase in the total cholesterol concentration and a 25 mg. % increase in the free cholesterol concentration. The results are presented in Tables IV and V. Table IV shows good agreement between the expected and determined cholesterol concentrations, when the calculations were made with the nearest standard. The maximum difference was 3%. When the results were calculated with a single standard, the maximum difference between the calculated and determined cholesterol concentration was as high as 12%.

Table IV. Recovery of Cholesterol from Acetone-Alcohol Solutions of Known Concentration

Cholesterol Concentration, Mg. %	Photoelectric Colorimeter, Mg. % Calculated		Spectrophotometer, Mg. % Calculated	
	Nearest standard	Single standard[a]	Nearest standard	Single standard[a]
50	49.0	55.0	49.0	45.0
	50.0	56.0	50.0	46.0
100	99.0	111.0	98.0	97.0
	99.0	111.0	97.0	96.0
200	197.0	197.0	199.0	199.0
	198.0	198.0	200.0	200.0
300	300.0	265.0	299.0	298.0
	300.0	265.0	300.0	300.0

[a] Standard used contained 0.5 mg. of cholesterol ≃ 200 mg. %.

Table V. Recovery of Cholesterol Added to Plasma Extracts

Sample	Total Cholesterol, Mg. %		Free Cholesterol, Mg. %	
	Detd.	Calcd.	Detd.	Calcd.
Plasma 58				
Without added cholesterol	222.0	..	56.5	..
	219.0	..	57.0	..
With added cholesterol	271.5	271.5	82.0	81.8
	272.0	..	81.0	..
Plasma 59				
Without added cholesterol	255.0	...	77.5	..
	257.0	..	77.0	..
With added cholesterol	308.0	306.0	103.5	102.8
	306.0	..	103.0	..

Table V shows that good recovery was obtained when 0.125 mg. of cholesterol was added to samples of plasma extracts in which total and free cholesterol was determined.

DISCUSSION

If a given color reaction is to be considered adaptable to analysis with photoelectric instruments, the optical density—i.e., the concentration of the sample—must be within the sensitive range of the instruments. Preliminary experiments indicated that accurate results could be expected if the cholesterol content of the samples was between 0.125 and 0.750 mg. Thus, when a 5-ml. blood or plasma aliquot is extracted with 100 ml. of 1 to 1 alcohol acetone and 5 and 10 ml. of the extract are used for the determination of total and free cholesterol, respectively, the cholesterol content of the samples will be between 0.125 and 0.750 mg., provided that the total cholesterol concentration is between 50 and 300 mg. %, and the free cholesterol concentration is between 25 and 150 mg. %. If lower or higher concentrations are encountered the determinations should be repeated using more, or less, extract.

The use of glass-stoppered test tubes offers several advantages. The extract can be mixed with the potassium hydroxide used in the total cholesterol determination more satisfactorily. Frequently, in the last washing of cholesterol digitonide a small quantity of ether is trapped under the centrifuged precipitate. When the tubes are placed in the oven for drying, the trapped ether evaporates rapidly. This may scatter the precipitate and lead to a loss of material. This can be prevented by loosely stoppering the centrifuge tube. Furthermore, in the course of the repeated washing of the precipitate some of it unavoidably sticks to the wall of the centrifuge tube. This precipitate can be dissolved more completely with the relatively small quantity (1 to 2 ml.) of acetic acid used if the centrifuge tube can be stoppered and its contents well shaken. Another advantage of the stoppered test tube is that it will prevent absorption of atmospheric moisture by the hygroscopic glacial acetic acid and acetic acid anhydride, thereby eliminating the interference with the color development caused by the presence of water.

Close agreement was found (especially with the use of the spectrophotometer) with the Beer-Lambert law when the color was developed in glacial acetic acid solutions of cholesterol. Re-

covery tests on acetone-alcohol solutions of cholesterol, however (Table IV), gave better results when the samples were compared to standards containing approximately the same quantity of cholesterol. The discrepancy resulting from the use of a single standard was greater when the photoelectric colorimeter was used. It is therefore suggested that three different standards containing 0.250, 0.500, and 0.750 mg. be prepared for each series of photoelectric determination of plasma (or blood) cholesterol.

It was necessary for the authors' purposes to extract 5 ml. of plasma (or whole blood), but total cholesterol can be determined in 0.25 ml. and free cholesterol in 0.50 ml. of plasma. In this case the extraction should be done as recommended by Sobel and Mayer (*2*), and from then on the procedure continued as described here.

SUMMARY

The Schoenheimer-Sperry method of cholesterol determination has been adapted to photoelectric instruments. The introduction of glass-stoppered centrifuge tubes obviates the use of stirring rods and preserving jars recommended in the original method. Cholesterol may be determined accurately by photoelectric methods in samples containing 0.125 to 0.750 mg. of cholesterol. The accuracy of the Schoenheimer-Sperry method may be enhanced by the use of serial standards; calculations are based on the comparison of the unknown to the standard of approximating concentration.

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Dropping Mercury Electrode Apparatus

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VARIOUS dropping mercury electrode assemblies have been suggested (*1–7*). In the majority of these, rubber tubing with its inherent disadvantages is used to some extent. For polarographic analysis based on standardized diffusion current constants, the apparatus of Lingane (*4*) with the stop-clock circuit for the rapid, automatic determination of the rate of flow of mercury is probably the most advantageous. The apparatus described in this paper is particularly well suited for routine polarographic analysis by comparative methods. Designed entirely of glass and having ground-glass connections, there is no contact between mercury and rubber and hence no possibility of the mercury being fouled from this source. The three-piece assembly is compact, is more readily disassembled and cleaned than the glass-rubber or all-glass apparatus currently in use, and is not considered by the authors as extremely complicated or fragile. These advantages offset the initial expense.

Figure 1 illustrates the entire apparatus, an enlarged view of one section of the stand tube, and a hand-drawn capillary.

The apparatus is made up of three pieces: *A*, the capillary; *B*, the mercury reservoir vessel; and *C*, the stand tube with adjoining stopcock and blood pressure bulb and valve. In *A* a 5.5-cm. length of Corning marine barometer tubing is joined to 6-mm. soft-glass tubing have a 7/25 ⅋ female joint. Electrical contact to the mercury in *B* is made by means of the tungsten contact and mercury well, *D*. The details of the construction of the stand tube, *C*, in the region of the ground-glass joint, *E*, 12/30 ⅋, are shown in Figure 1, center.

The operation of the apparatus is simple. By means of the blood pressure bulb and valve, *F*, mercury can be raised to any desired height in the stand tube. The air forced in when the bulb is squeezed (valve is closed and stopcock is open during this operation) enters the reservoir vessel through a hole, *G*, close to the lower inner seal. As a result of the pressure which is built up in the reservoir vessel, the mercury proceeds up tube *H*. The stopcock is closed when the mercury has reached the desired level. When the polarographic analysis has been completed, the capillary is washed carefully with a stream of distilled water and immersed in either distilled water or pure mercury. Then the mercury column is lowered by opening the stopcock and the valve, whereupon the pressure in the vessel is returned to atmospheric.

In polarographic work hand-drawn capillaries can be used as well as marine barometer tubing. Various features of such capillaries have been discussed by Novak (*8*). The major disadvantage in their use is the fact that they are easily breakable. Figure 1, right, shows a hand-drawn capillary for which this disadvantage has been overcome. Such a capillary has been used

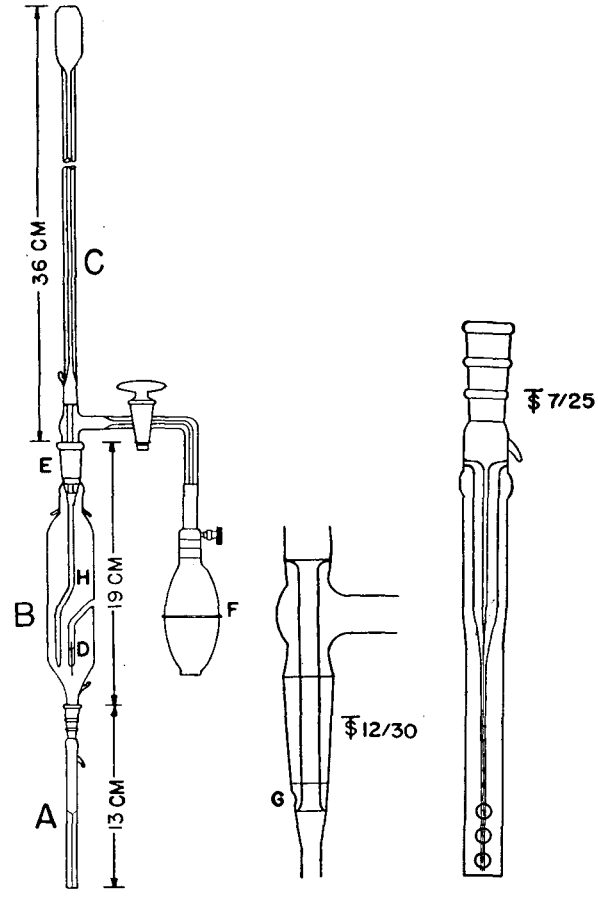

Figure 1. Dropping Mercury Electrode Apparatus

Left. **Entire apparatus**
Center. **Enlarged view of section of stand tube**
Right. **Hand-drawn capillary with protective shield**

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successfully for many months and the operator has been relieved of any fear of losing his capillary through breakage.

The capillary is protected against mechanical shock by a shield of 7-mm. tubing. Six holes, three on opposite sides, in the shield near the tip of the capillary permit circulation of the solution. A hole in the shield near its upper end permits air to escape when the capillary is immersed. During routine analysis a stream of distilled water from a wash bottle directed through one of the ports in the shield serves to wash the capillary free of any solution. Adhering water is removed readily by wiping the capillary and shield with a small piece of filter paper.

The following is a very efficient way to clean the capillary, either the hand-drawn model or one of marine barometer tubing.

Having removed all mercury from the capillary (by carefully jarring the capillary or by suction supplied by an aspirator), one first draws hot concentrated nitric acid through the capillary, using an aspirator. Whether or not the acid is passing through the capillary can be determined by lifting the capillary out of the acid and draining any excess off the tip. If the passage is free, a column of liquid will be seen moving up the capillary. Next one draws distilled water through the capillary, then a little acetone, and finally a little air.

The hand-drawn capillary with the protective shield can be cleaned as easily as the same capillary without the shield or a capillary of marine barometer tubing, but it may be a little more difficult to determine whether or not liquid is passing up the capillary.

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Rate-Indicating Mariotte Bottle

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A MARIOTTE bottle is frequently employed in the laboratory to obtain low but constant rates of liquid flow. If the liquid is used to displace a gas, this bottle serves also to meter the gas at rates far below the threshold of the ordinary wet-test gas meter. Its utility suffers, however, from the fact that it is not an indicating instrument and must be set at the required flow rate by a method of trial and error. The present note deals with some simple additions to the Mariotte bottle that transform it into a rate-indicating device. These additions are based on the approximate theory of the operation of the bottle.

THEORY OF MARIOTTE BOTTLE

A typical Mariotte bottle, shown in Figure 1, consists essentially of a jar provided with a liquid-exhaust line, A, and an air-intake line, B. As the liquid flows out of the jar at C it is replaced by air which enters at B and bubbles up through the liquid level, D.

The rate of discharge of the liquid, considered as ideal and nonviscous, may be calculated from Bernoulli's equation, which requires that the energy per unit volume of liquid at any two points along a streamline must be constant. Its familiar form for an incompressible liquid is

$$\frac{d}{2}v^2 + P + dgz = \text{constant} \qquad (1)$$

where d is the density of the liquid, v its velocity at any point, P the hydrostatic pressure at the same point, and z the vertical distance of the point above some arbitrary datum line. If this datum line is taken at level C, it is necessary that

$$\frac{d}{2}v_e^2 + P_o = K \qquad (2)$$

where v_e is the velocity of discharge, P_o is atmospheric pressure, and K is a constant. Similarly, because the velocity at liquid surface D is negligibly small,

$$P_i + dgh_a = K \qquad (3)$$

where P_i is the air pressure above the liquid surface. Hence, on combining Equations 2 and 3,

$$\frac{d}{2}v_e^2 + P_o = P_i + dgh_a$$

or

$$v_e = \sqrt{(2/d)\,(P_i - P_o + dgh_a)} \qquad (4)$$

It is also necessary that

$$P_o = P_i + dgh_b \qquad (5)$$

Putting this value in Equation 4 gives

$$v_e = \sqrt{2g(h_a - h_b)}$$

or

$$v_e = \sqrt{2gh_1} \qquad (6)$$

The velocity of discharge is therefore proportional to the square root of the hydrostatic head between the points of liquid discharge and air entry. The rate of discharge may accordingly be regulated by changing the distance between these points.

An alternative way of controlling the rate is to insert a resistance in the air-intake line.

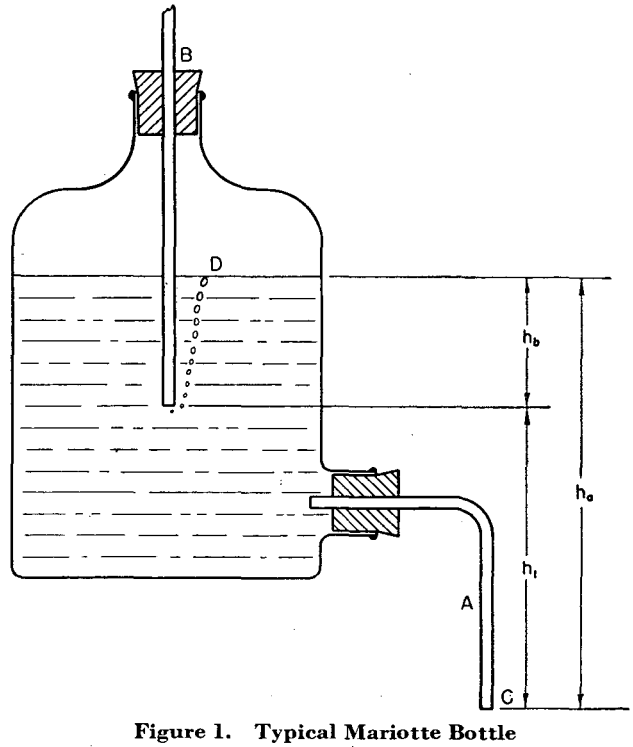

Figure 1. Typical Mariotte Bottle

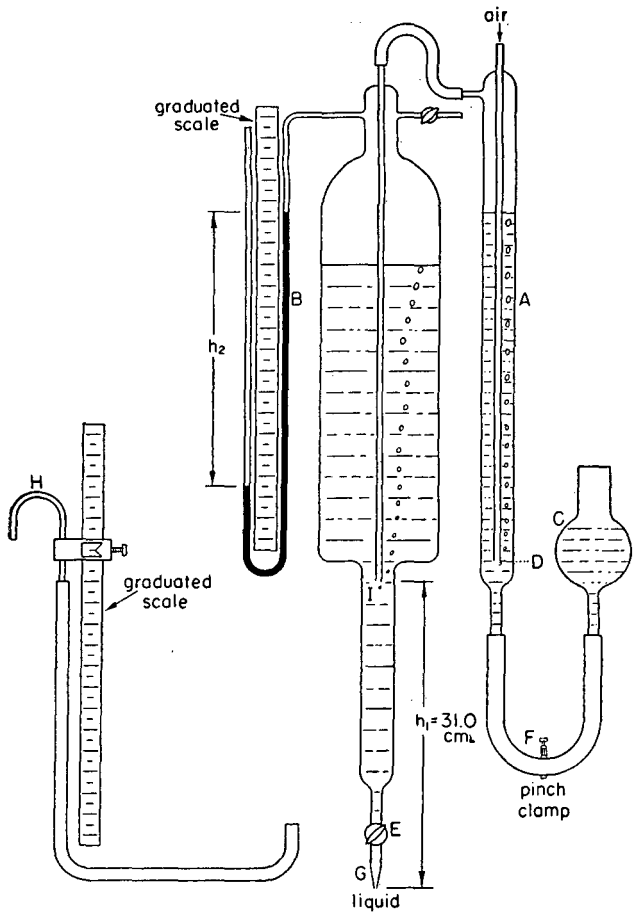

Figure 2. Development of Modified Mariotte Bottle

Without inquiring into the nature of this resistance, assume that it lowers the pressure of the intake air an amount equal to a hydrostatic head of height h_2. Then, in place of Equation 5, one has

$$P_o - dgh_2 = P_i + dgh_b \tag{7}$$

Combining this equation with Equation 4 yields

$$v_t = \sqrt{2g(h_1 - h_2)} \tag{8}$$

For an actual, viscous fluid the velocity of discharge will be somewhat smaller than that given by Equation 8. The rate of liquid discharge, R, should then follow the equation

$$R = k\sqrt{(h_1 - h_2)} \tag{9}$$

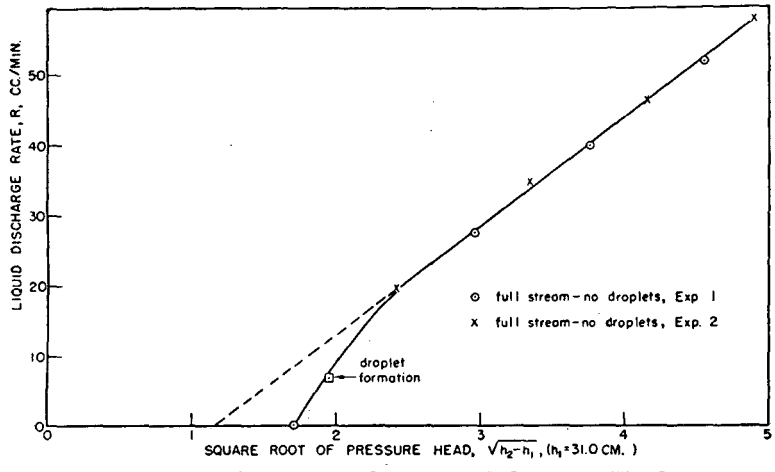

Figure 3. Calibration Curve for Hydrostatic Head

where k is a constant, provided the liquid is flowing in a steady stream. If, however, the rate of flow is so small that droplets are formed at the exit point, C, surface tension will play a role and Equation 9 will no longer be valid. Nevertheless this equation serves as a guide for transforming the Mariotte bottle into a rate-indicating device.

MODIFIED MARIOTTE BOTTLE

From Equation 9 it is apparent that the liquid rate, R, will be a function of h_1 alone if h_2 is held constant, or, conversely, of h_2 if h_1 is held constant. The problem of designing a satisfactory rate-indicating Mariotte bottle therefore consists of varying and measuring h_1 or h_2 in a simple manner. Two methods of effecting this result are illustrated in Figure 2.

In one of them, h_1 is held constant and h_2 is varied; in the other, the reverse is true. To vary h_2, the air is allowed to leak into the Mariotte bottle through the liquid trap, A. This operation reduces the pressure in the air-intake line below atmospheric pressure by an amount indicated on the liquid manometer, B. In operating this device, the leveling bulb, C, is used to bring the liquid level in the trap above point D. Stopcock E is then opened and liquid is allowed to discharge from the Mariotte bottle. This action draws the liquid from the leveling bulb up into the liquid trap until the discharge ceases. The pinchcock, F, is then closed and the leveling bulb dropped below level D. Immediately thereafter the pinchcock is reopened, air flows into the Mariotte bottle, and h_2 is adjusted to any desired value.

In this manner the calibration curve in Figure 3 was obtained. Here the rate of discharge of water, R, was plotted against $\sqrt{h_1 - h_2}$ in order to check the validity of Equation 9. When the liquid was discharged in a full stream, the rate of discharge was indeed proportional to $\sqrt{h_1 - h_2}$. When the discharge was dropwise, surface tension played a role, and the rate of discharge was considerably less than one would expect from the back-extrapolation of the straight-line calibration curve.

Two additional features of the calibration curve are attributable to surface tension—the discharge rate does not fall to zero for $(h_1 - h_2) = 0$ as would be expected from Equation 9, and the back-extrapolated straight line (dotted portion) does not pass through the origin of coordinates. The first feature can be quantitatively explained as follows:

The bubble formed at the discharge tip, G, is capable of supporting a column of liquid, h, whose magnitude is defined by the equation

$$hdg = 2\gamma/r \tag{10}$$

where γ is the surface tension of the liquid and r is the radius of the discharge orifice. Because water was used, $\gamma = 72$ dynes per cm. while $r = 0.045$ cm. Consequently $h = 3.3$ cm. At zero flow rate the calibration curve gives $\sqrt{h_1 - h_2} = 1.7$, so that $(h_1 - h_2) = 3$ cm., a figure in rough agreement with the preceding one. (A more careful consideration of this point may show that the Mariotte bottle is adaptable to surface-tension measurements.)

That the back-extrapolation of the straight-line portion of the calibration curve does not pass through the origin is probably related to the fact that the flowing liquid loses energy in forming fresh liquid surfaces around the intake air bubbles and at the exhaust orifice, G.

The method of calibrating the Mariotte bottle by varying h_1 and keeping h_2 constant is also illustrated in Figure 2.

One need merely connect the discharge tip, G, to the gooseneck, H, by means of a rubber tube. If the gooseneck is lifted above level I, the flow will be entirely shut off. As it is lowered, the rate of discharge will increase in a manner similar to that depicted by the calibration curve in Figure 3. If there is negligible flow resistance in the air-intake line, $h_2 = 0$, and consequently the rate of discharge will be simply proportional to $\sqrt{h_1}$.

Very accurately reproducible microflow rates may be obtained by inserting one or more pieces of glass capillary tubing in series with the air-intake line, using rubber tubing connections. Glass thermometer tubing serves well for this purpose.

APPLICATION OF MARIOTTE BOTTLE

The Mariotte bottle has been utilized to calibrate a fine-capillary flow-rate indicator in the flow-rate range below 100 cc. of gas per minute. In this range the usual laboratory instruments, such as a wet-test meter, will not serve, and the Mariotte bottle affords a simple but accurate means of doing the job.

The technique of calibration may easily be understood with reference to Figure 4.

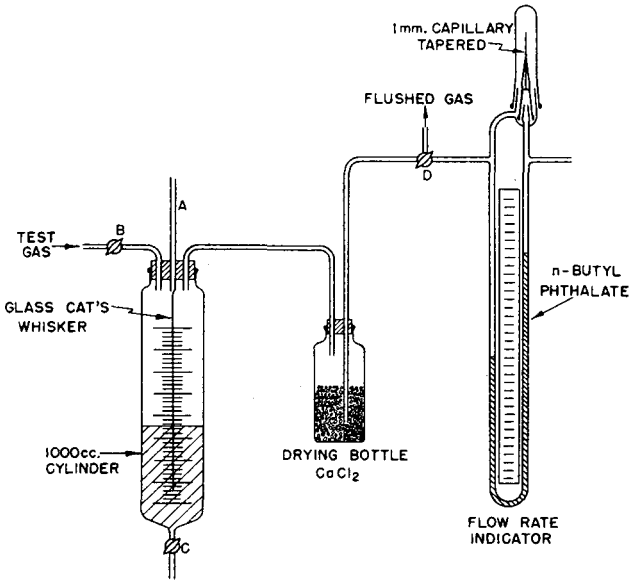

Figure 4. Setup for Calibration

The discharge tip of the Mariotte bottle is connected to tube *A*. Water is then run into the graduated cylinder at a fixed, invariable rate. The gas in the cylinder is thereby displaced and caused to pass through the capillary. To make the setup easy to handle, various refinements are incorporated. Stopcock *B* is used to fill in the test gas at the same time the foreign gas is being flushed out through stopcocks *C* and *D*. To prevent splashing and to smooth out the flow of water, the lip of the discharge tube is provided with a very fine glass cat's-whisker which extends into the graduated cylinder. The drying bottle is used to present a moisture-free gas to the capillary.

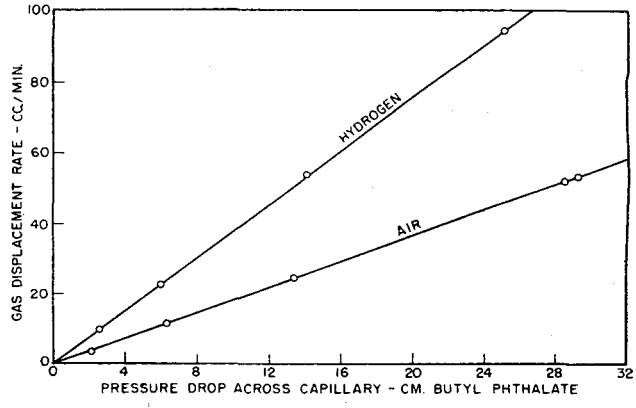

Figure 5. Two Calibration Curves

In Figure 5 are two calibration curves obtained with the arrangement shown in Figure 4. The upper curve refers to hydrogen, the lower to air. The straight-line calibration holds good down to flow rates in the neighborhood of 5 cc. per minute and presumably lower. To test the accuracy of the work, the inverse ratio of the slopes of these two curves was compared to the ratio of the viscosities of the respective gases at the experimental temperature of 25° C. The ratio of the viscosity of air to that of hydrogen at this temperature is 2.05, whereas the inverse ratio of the slopes is 2.06.

There are several fine points to be considered if very high accuracy is required. In the first place, the solubility of the test gas in the water is of no importance, because the volume of the displaced gas alone is measured. But this displaced gas is water-saturated, so that the gas displacement rate of Figure 2 should strictly be corrected downward for the water vapor held up in the drying bottle. On the other hand, the gas is displaced at a pressure higher than atmospheric by the pressure drop across the capillary. The gas displacement rate of Figure 5 should accordingly be corrected upward if all gas volumes are to be referred to atmospheric pressure. These two effects therefore tend to cancel out and for most work calibration curves similar to those depicted in Figure 5 will be adequately accurate.

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Determination of Phosphorus in Alloys

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THE analytical usefulness of the double-strength citromolybdate solution which was used in the determination of phosphorus in iron ore (*5*) has been extended to include the determination of phosphorus in alloys. The analysis of several Bureau of Standards samples indicates that the accuracy and precision of this method are good, and average deviations in results are well within the range recommended for iron and steel analysis (*3*).

Standard procedures other than those given may be used in dissolving the samples, provided that the volume of the solution before the addition of the citromolybdate solution is not over 80 to 100 ml., and that the solution contains not more than 10 grams of ammonium nitrate or its equivalent in addition to not more than 6 ml. of concentrated nitric acid (specific gravity 1.42) or not more than 6 ml. of 60% perchloric acid. The mixed indicator (*4*) used in the determination of phosphorus pentoxide in phosphate rock is used in determining the end point.

REAGENTS AND STANDARD SOLUTIONS

The reagents for this method are essentially those used in the determination of phosphorus in iron ore (*5*).

DETERMINATION OF PHOSPHORUS IN STEEL

Steels Soluble in Nitric Acid. The procedure for dissolving the steel samples is essentially that adopted by the American Society for Testing Materials (*1*) and others (*7*, *9*). The volume of acid should be determined by the size of the sample, so that it will not be necessary to use more than 8 ml. of concentrated ammonium hydroxide (specific gravity 0.90) in adjusting the acidity. From this point, proceed as in the determination of phosphorus in iron ore (*5*) (Table I).

Steels Insoluble in Nitric Acid. Weigh out a 0.5- to 2-gram sample of the steel into a 400-ml. beaker, add 30 ml. of hydrochloric-nitric acid (*10*) mixture and 0.5 to 1.0 ml. of hydrofluoric acid, and heat on a hot plate until the sample is in solution and the silica is volatilized (*1*). Add 30 ml. of 60 to 70% perchloric acid, evaporate to fumes of perchloric acid, and fume for 5 to 10

Table I. Analyses of Standard Steels, Soluble in Nitric Acid

Bur. Stds. Sample No.	Type	Phosphorus Value, % Certified	Exptl.[a]	Deviations, %
8g	Bessemer steel	0.093	0.093	0.000
10e	Bessemer steel	0.083	0.083	0.000
			0.081	−0.002
129A	Bessemer steel	0.094	0.091	−0.003
			0.098	+0.004
			0.091	−0.003
11e	B.O.H. steel	0.015	0.015	0.000
			0.012	−0.003
15D	B.O.H. steel	0.018	0.017	−0.001
21C	A.O.H. steel	0.062	0.060	−0.002
			0.063	+0.001

[a] Time of boiling for complete precipitation about 5 minutes.

Table II. Analyses of Standard Steels, Insoluble in Nitric Acid

Bur. Stds. Sample No.	Type	Phosphorus Value, % Certified	Exptl.[a]	Deviations, %
30c	Cr-V steel, V 0.235%	0.019	0.020[b]	+0.001
			0.021[b]	+0.002
			0.014[c]	−0.005
32b	Cr-Ni steel	0.016	0.015	−0.001
			0.014	−0.002
100	Mn rail steel	0.023	0.020	−0.003
			0.021	−0.002
106A	Cr-Mo-Al steel	0.016	0.018	+0.002
			0.014	−0.002
111A	Ni-Mo steel	0.017	0.020	+0.003
			0.016	−0.001
121A	Stainless steel, 18% Cr, 10% Ni, titanium bearing	0.023	0.020	−0.003
			0.022	−0.001

[a] Time of boiling for complete precipitation 10 to 15 minutes.
[b] Reduced with ferrous sulfate, allowed to stand overnight before filtering, 150 ml. of double-strength citromolybdate solution used.
[c] Reduced with ferrous sulfate, filtered immediately, 150 ml. of double-strength citromolybdate solution used.

minutes. Cool, add 50 ml. of water and a saturated solution of sulfur dioxide or sodium sulfite until chromium and vanadium are reduced, and boil until all the sulfur dioxide has been expelled.

From this point, the procedure for the determination of phosphorus in steels insoluble in nitric acid is the same as that for steels soluble in nitric acid, except that the boiling time for complete precipitation is 10 to 15 minutes (Table II).

DETERMINATION OF PHOSPHORUS IN CAST IRON

The samples were dissolved according to the standard procedure (*2*, *11*), precipitated, and titrated as when determining phosphorus in steel (Table III).

The nitric and perchloric acid procedure (*11*), which is similar to the procedure for steels insoluble in nitric acid, can be used for cast iron.

DETERMINATION OF PHOSPHORUS IN FERROPHOSPHORUS

Prepare a 1-gram sample for analysis as described by Lundell, Hoffman, and Bright (*8*), and dilute to 500 ml. in a volumetric flask. Pipet a 25-ml. aliquot into a 400-ml. beaker; add 50 ml. of water, 3 to 5 ml. of concentrated nitric acid (specific gravity 1.42), and 3 to 5 ml. of 2.5% solution of potassium permanganate; and boil carefully until manganese dioxide is precipitated. Add a saturated solution of sodium nitrite, dropwise, until all the manganese dioxide disappears. Boil to expel oxides of nitrogen, add 100 ml. of double-strength citromolybdate solution, heat to boiling, and boil 5 to 10 minutes. From this point, proceed as in the determination of phosphorus pentoxide in phosphate rock (*4*) (Table IV).

PROCEDURE FOR PHOSPHORUS IN PHOSPHOR BRONZE

Weigh out a 2-gram sample of phosphor-bronze bearing metal into a 400-ml. beaker, add 15 ml. of concentrated nitric acid (specific gravity 1.42) and 5 ml. of concentrated hydrochloric acid (specific gravity 1.19), and heat on a hot plate until the sample is in solution except for a white residue. Add 15 ml. of hot water and digest at 80° to 90° C. until the residue goes in solution. Dilute to about 80 ml. with water, add 100 ml. of double-strength citromolybdate solution, heat to boiling, and boil for 5 to 10 minutes. From this point, proceed as in the determination of phosphorus pentoxide in phosphate rock (*4*) (Table IV).

DISCUSSION AND NOTES ON PROCEDURE

In using the perchloric acid procedure as outlined for steel, it was found unnecessary to adjust the acidity by adding ammonium hydroxide when the volume of perchloric acid used did not exceed 30 ml. of 60 to 70% acid.

When the sample contained a large amount of vanadium, it was found advisable to add 150 ml. of the citromolybdate solution and allow it to stand overnight before filtering.

If arsenic is present in large amount, it should be removed as recommended by Lundell, Hoffman, and Bright (*6*).

ACKNOWLEDGMENT

This research was sponsored by a grant from the Research Fund of the University of Alabama.

Table III. Analyses of Standard Cast Irons

Bur. Stds. Sample No.	Type	Phosphorus Value, % Certified	Exptl.[a]	Deviations, %
4g	Cast iron	0.121	0.126	+0.005
			0.123	+0.002
6d	Cast iron, As 0.26%	0.486	0.502	+0.016[b]
			0.502	+0.016[b]
			0.474	−0.012[c]
7c	Cast iron, As 0.071%	0.778	0.787	+0.009[c]
			0.791	+0.013[c]
			0.796	+0.018[b]
			0.806	+0.028[b]
82	Ni-Cr cast iron	0.102	0.104	+0.002
			0.103	+0.001
107	Ni-Cr-Mo cast iron	0.197	0.195	−0.002
			0.195	−0.002
115	Cu-Ni-Cr cast iron	0.113	0.107	−0.006
			0.107	−0.006

[a] Time of boiling for complete precipitation about 5 minutes.
[b] Arsenic not removed; 100 ml. of double-strength citromolybdate used.
[c] Arsenic not removed; 40 ml. of double-strength citromolybdate used.

Table IV. Analyses of Other Bureau of Standards Samples

Sample No.	Type	Certified Phosphorus Value, %	Exptl. Phosphorus Value, %	Deviations, %
90	Ferrophosphorus	26.2	26.2	0.0
			26.2	0.0
63	Phosphor-bronze bearing metal	0.62	0.61	−0.01
			0.61	−0.01

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Preparing Extruded Specimens for X-Ray Diffraction Analysis

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X-RAY powder diffraction analysis has many features that qualify it as a method for microanalysis of organic compounds in the solid state. The extruded binderless (*2, 5, 6*) specimen probably represents the best type of specimen for microanalytical work because of its minuteness (2 to 3 mg.) and absence of foreign materials.

The disadvantages of other types of specimen are: (*a*) excessive size [wedge specimen (*1*), McLachlan specimen (*7*), the flat specimens used in Geiger counter spectrometry, etc.]; (*b*) contamination of the specimen so that it must be repurified for further use [specimens mounted on fibers with adhesive (*3*), specimens extruded with binder (*3*), specimens mounted on a stage with adhesive (*8*), etc.];(*c*) and the presence of foreign materials which contribute to the background scattering [specimens in little tubes (*3*) of cellophane, nylon, or glass, or coated on fibers]. This last difficulty is particularly important for organic specimens whose scattering power is not much greater than the containing tubes and supporting fibers.

There are several objections to the use of extruded binderless specimens in microanalysis. Although the specimen is very small, a relatively large amount of sample is required to fill the extrusion tube by the usual "scoop and tamp" technique. It is hard to prepare a sample that is mechanically strong. Extrusion of samples at high pressures is said to lead to preferred orientation of crystallites.

Morse (*8*) has designed a loader which apparently was intended to cope with some of these difficulties. Unfortunately, specimens produced in this way are mechanically weak and insecurely anchored. Moreover, the use of adhesive for anchoring the specimen tends to contaminate the specimen so that it cannot be used subsequently without repurification. Morse also mentions that high extrusion pressures tend to cause preferred orientation in the specimen. The present authors have been unable to substantiate this claim, although they have used pressures up to ~40,000 pounds per square inch. In all cases (naphthalene was especially troublesome), preferred orientation could be completely avoided by grinding the sample to a small enough particle size. Specimens of 59 aromatic hydrocarbons (*4*) whose diffraction patterns are soon to be published, could be prepared so that no preferred orientation was evident.

PROCEDURE

The details of the equiqment are shown in Figure 1.

Basically it consists of a tiny funnel just large enough to accommodate the amount of sample necessary to produce a specimen, and a holding device which keeps the specimen tube properly positioned with respect to the funnel. By means of a plunger wire fastened in a small pin vise, the sample material is pushed into the specimen tube with constant tamping to consolidate the specimen uniformly throughout its length. The plunger should be a stiff No. 22 steel wire (Piano wire has the requisite stiffness). The specimen tubes are 19-gage stainless steel tubing cut to the proper length (available from the Superior Tube Co., Norristown, Pa.). When the specimen tube is filled to the proper depth (about 4 mm.) the loading device is disassembled and the specimen is extruded from the specimen tube with the plunger wire. The plunger wire should protrude from the pin vise a distance just 1 mm. short of the specimen tube length, so that the specimen cannot be completely extruded out of the tube, which thus serves as a handle for it. Specimens produced in this way contain no binder and are sufficiently rugged to permit mounting in typical Debye-Scherrer cameras.

The neck prevents the specimen tube from being pulled out of the loading device during tamping. The anvil rod acts as a spacer and serves to keep the specimen tube against the neck. By using anvil rods of different lengths, specimen tubes of different lengths can be accommodated. The relation between the thickness of the funnel block and the length of the specimen tube should be such that the specimen tube projects out of the funnel block even when it has made contact with the neck. The apparatus, with the exception of the alignment pins and the studs, is made of brass. It is now used routinely in the authors' laboratory.

This apparatus has been used only with organic compounds for which there are noncorrosive organic solvents. Inorganic samples present a difficult cleaning problem. Although the apparatus shown is very convenient, a much simpler form, consisting of funnel block and anvil block clamped together with external clamps, can be used.

ACKNOWLEDGMENT

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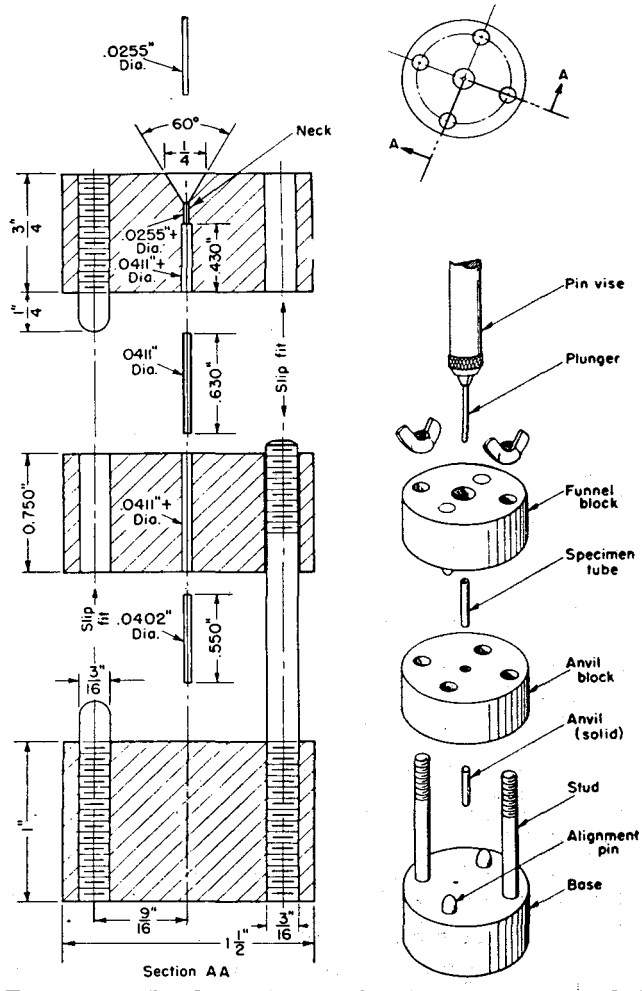

Figure 1. Loading Device for Preparing Extruded Samples for X-Ray Diffraction Analysis

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Interference of Methyl Anthranilate in Estimations of Parathion

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WITH the initial application of the insecticidal material *O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate (parathion) to agricultural products has coincided the introduction of a method for the quantitative estimation of its residues upon these products (*1*). The basis of this very sensitive method is the quantitative reduction of the nitro to the amino group, which is then diazotized and coupled with *N*-1-naphthylethylenediamine to give an intense magenta color ("dyed" parathion). However, Gunther and Blinn (*3*), Norris (*4*), and Edwards (*2*) have reported that aniline and some of its analogs afford essentially the same magenta color by this procedure. This suggested that methyl anthranilate, reported by Power and Chestnut (*5*) to be found in grapes and by Small (*6*) to be found in citrus fruits, might interfere with the determination of parathion residues on and in these products, particularly as the methyl anthranilate content may vary with both the age and the variety of the fruit. The present report deals with the spectral study of "dyed" methyl anthranilate.

DISCUSSION

After being carefully purified, methyl anthranilate hydrochloride was subjected to the exact dyeing procedure used for parathion estimations (*1*); the resulting intense magenta color was visually very similar to that produced by parathion. The absorption characteristics, determined on a Beckman spectrophotometer Model DU, in the visible range are compared with those given by dyed technical parathion in Figure 1. The molar extinction coefficients were based upon the concentrations of the undyed materials.

Although the spectral characteristics of the two dyed compounds differ slightly, it can be seen that methyl anthranilate would interfere seriously in a determination for parathion by the above-mentioned procedure. The maxima are separated only by 8 mμ, but they differ in molecular extinction coefficient by about 800 units (2%). Maximum color development is achieved by methyl anthranilate well within the 10-minute period specified for parathion.

The presence of methyl anthranilate in a "strip" solution can be demonstrated, even though intermixed with parathion, by the use of the dyeing procedure (*1*) without the preceding reduction step. However, any interference caused by methyl anthranilate can largely be removed from the strip solution by modifying a procedure originally reported by Gunther and Blinn (*3*).

To illustrate, 100-ml. portions of benzene were each fortified with 81 micrograms of methyl anthranilate, then washed one, two, or three times with 25 ml. of 10% hydrochloric acid solution. After evaporation of the benzene in the usual manner, the dyeing procedure was applied. It was found that one hydrochloric acid wash removed 60.5% of the methyl anthranilate present. With two and three washes with the dilute acid, there was left no detectable amount of methyl anthranilate in the benzene.

It is therefore suggested that strip solutions, in which methyl anthranilate is suspected, be washed at least twice with dilute hydrochloric acid.

It was incidentally noticed that the color characteristics of dyed methyl anthranilate behaved according to Beer's law within the range of 18 to 360 micrograms of parent material. This suggests a possible method for the quantitative estimation of methyl anthranilate.

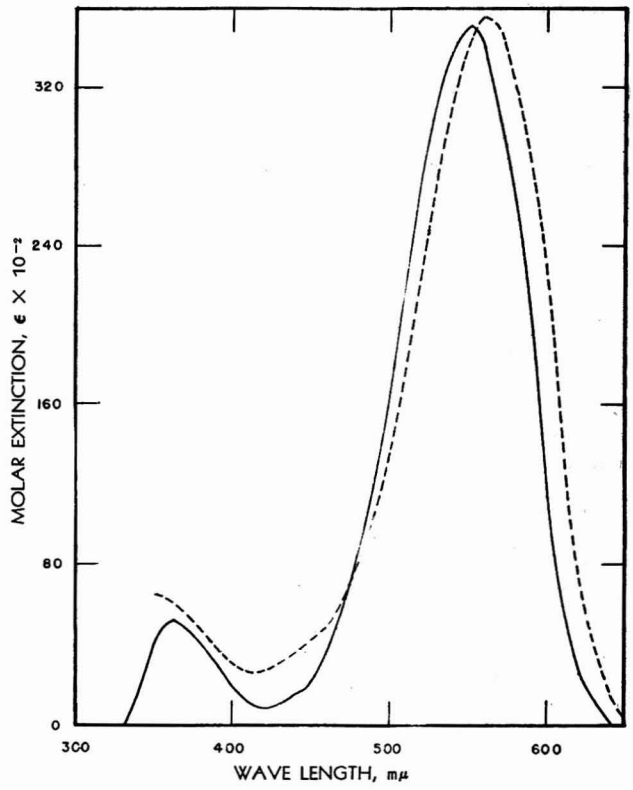

Figure 1. Absorption Characteristics of Dyed Methyl Anthranilate Compared to Dyed Parathion

——— Dyed methyl anthranilate
- - - - Dyed parathion

ACKNOWLEDGMENT

The authors wish to thank W. E. Baier, California Fruit Growers Exchange, Ontario, Calif., for suggesting the problem and for a supply of methyl anthranilate.

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Electromechanical Integrator for Coulometric Analysis

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COULOMETRIC analysis based on controlled potential electrolysis requires the accurate measurement of the quantity of electricity passed through the electrolysis cell (*2*). Any of the classical types of chemical coulometers can be used for this purpose. However, they require considerable manipulation and it is very desirable to have a more convenient, direct-reading device to perform this function of integrating the current-time curve.

In controlled potential coulometric analysis the current usually decreases more or less exponentially with time from a relatively large value (which may be anywhere between a few tenths and several amperes) to a final value which is practically zero. The elapsed time may vary from about 10 minutes to an hour or more. These characteristics require that the integrating instrument function accurately over the entire range of current values from zero up. The design of an instrument which will fulfill this requirement, will have the necessary precision and accuracy of the order of 1% or better, and will not be so complicated and temperamental that its use is more involved than the chemical coulometer it is intended to replace, is not so simple as it might seem at first thought. The instrument described in this paper is one solution to this problem.

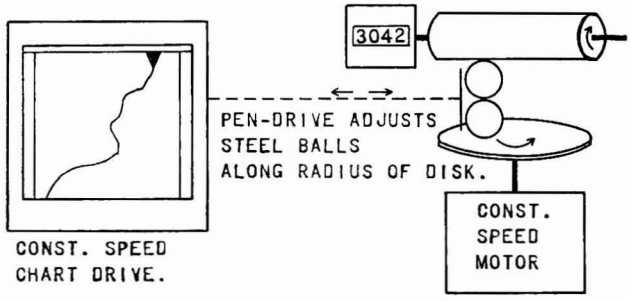

Figure 1. Ball and Disk Transmission

As indicated schematically in Figure 1, the heart of the device is a precision ball and disk transmission. The disk is rotated at precisely constant speed and this motion is transmitted to a cylinder by two steel balls. The speed of the cylinder, and hence the counting rate of the revolution counter driven by it, are directly proportional to the position of the balls along the radius of the disk. Because the balls are positioned by the same mechanism that drives the pen of the recording potentiometer, the counting rate at any instant is directly proportional to the reading of the recording potentiometer, and the total counts which accumulate are directly proportional to the area under the recorded curve. By appropriate selection of the mechanical characteristics and the range of the recording potentiometer, the counter can be caused to read directly in coulombs or other desired units.

The ball and disk transmission was taken from a Type K-4 Automatic Computing Sight for Turret (Lower Ball), purchased as war surplus and manufactured originally by the Sperry Gyroscope Company. This particular unit employs a screw to position the balls; one revolution of the screw shaft shifts the balls from the center to the edge of the disk. The unit was small enough to be mounted on the rear of the carriage of a standard Brown Electronik potentiometer recorder, together with the small synchronous motor used to drive the disk and the revolution counter, as shown in Figure 2. An aluminum spur gear is fitted over the hub of the large gear which drives the recording pen, and is held in place by compression exerted by a threaded ring screwed on the hub, which permits easy adjustment of its angular position relative to the potentiometer reading. This gear engages an aluminum spur gear attached to the screw shaft which positions the balls. The gear ratio is approximately 1 to 1, and because the "pen drive gear" rotates through about 320°, a complete excursion of the recording pen causes the balls to shift over nearly the entire radius of the disk.

The disk is rotated at 45 r.p.m. by a Type RWC 2505 Holtzer-Cabot synchronous motor. The integrating unit includes a spring-loaded screw adjustment which enables the compression between the disk, balls, and cylinder to be adjusted.

The maximum speed of the cylinder is twice the disk speed. The Model S13, 5-digit Veeder-Root revolution counter is connected by two spur gears to the cylinder, the gear ratio being 1 to 1. Because this counter counts each tenth of a revolution, the maximal counting rate is 900 counts per minute for full scale recorder deflection.

To minimize error and dead zone caused by lost motion in the gears and screw which positions the balls, a strip of spring bronze is mounted to maintain constant tension in one direction against the sliding carriage in which the balls are mounted.

To measure and integrate current a precision resistor (1 to 10 ohms depending on the current range) is placed in series with the electrolysis cell and the recording potentiometer is connected across this resistor to record the iR drop. The integrator is calibrated with a known constant current which is evaluated by measuring the iR drop across the precision resistor with a potentiometer. The counter reading over a measured time interval with the known current is observed. These data are combined to compute the calibration factor expressed as coulombs per count. A voltage-divider arrangement (*1*) can be used conveniently to change the range of the integrator over very wide limits to measure relatively large or very small quantities of electricity.

The gear that drives the ball-positioning screw is adjustable with respect to the recorder deflection, which allows the zero of the integrator to be set to correspond to any desired position of the recorder pen. The zero of the recorder and integrator can thus be set to an intermediate scale position and the instrument used to integrate curves in cases where the measured quantity changes sign during the course of an experiment.

The precision (reproducibility) of the instrument is demonstrated by typical calibration data obtained when twelve different constant voltages, accurately measured with a potentiometer, were applied to the instrument to obtain deflections of the recording potentiometer from one tenth to full scale. The calibration factor was computed from the observed counting rate at each constant applied voltage. The average was 0.0530 coulomb per count with an average deviation from the mean of ± 0.0003 or $\pm 0.6\%$.

The accuracy—i.e., reliability of the calibration factor obtained with known constant voltages—was then tested under actual use conditions by operating the instrument in series with a silver coulometer during the deposition of copper from a 0.5 M sodium

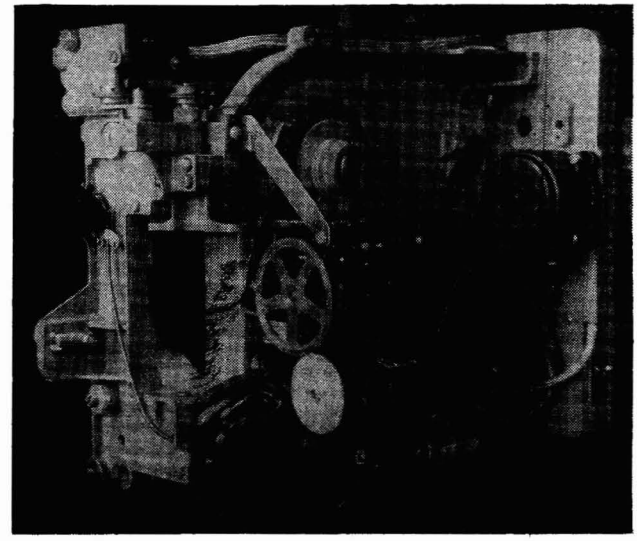

Figure 2. Electromechanical Integrator

tartrate electrolyte of pH 5 to 6 with the potential of the platinum cathode held constant by an automatic potentiostat (*2*) at −0.32 volt versus the saturated calomel electrode. When 50- to 60-mg. quantities of copper were used, the current decreased exponentially from about 500 to 2 ma. over a period of 20 to 30 minutes. In three trials the average calibration factor of the integrator, computed from the weight of silver deposited in the silver coulometer, was 0.0529 coulomb per count with an average deviation from the mean of ±0.0003 or ±0.6%. This value agrees very well with the calibration factor (0.0530) determined with known constant voltages.

The particular ball and disk unit used was small, the disk having a radius of only 1.6 cm., and a higher degree of precision probably could be obtained by employing a larger integrating unit. Precision ball and disk units with disk radii of 3.2 and 6.3 cm. (1.25 and 2.50 inches) are available from the Ford Instrument Company, Long Island City, N. Y. It is evident that the inherent precision of the recording potentiometer itself, rather than mechanical limitations, is the factor that determines the ultimate precision and accuracy attainable with this type of integrator.

Lag in the recording potentiometer is a possible inherent source of error in cases where the current varies rapidly with time. This can be minimized or eliminated altogether by employing a recording or indicating potentiometer whose speed of response is great compared to the time rate of change of the current. The time required for full scale deflection of the particular Brown Elektronik recorder used in this study was 10 seconds. This is amply fast for purposes of coulometric analysis.

Although designed specifically as a coulometer, the integrator has other possible uses. It will integrate any curve which the recording potentiometer is capable of drawing. One interesting possibility is the integration of polarographic current-voltage curves recorded over a precisely regulated time interval corresponding to an applied voltage range which includes the polarographic wave.

SUMMARY

A mechanical ball and disk integrator controlled by the pen-drive mechanism of an ordinary recording potentiometer serves as a convenient electromechanical integrator. The instrument integrates any curve which the recording potentiometer can draw, the integral (area under the recorded curve) appearing as a reading on a revolution counter. The precision and accuracy are better than ±1%. The instrument was designed to replace the more cumbersome classical types of chemical coulometer in coulometric analysis.

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Automatically and Continuously Recording Flow Refractometer

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IN 1946 Claesson (*1*) described an instrument for the continuous analysis of the eluate from an adsorption column, using the change in refractive index as an indication of change in composition. The essential features were as follows:

Light is conducted through a lens system and the emerging beam is passed through two solutions (A and B) separated from each other in a refractometer cell by a thin glass plate set at an angle of 45° to the beam of light. Solution B is a liquid of known, constant refractive index, and solution A is the eluate from an adsorption column. As the beam of light passes from A through B, any change in refractive index in A will deflect the beam. This emergent beam is then divided into two beams by passing through a hexagonal glass prism, one edge of which is pointed toward the refractometer cell. At the start the two beams may be equal, but any change in A will make them unequal. Each beam is received by a photovoltaic cell. The change in the amount of light received by each cell is measured by means of a bridge circuit and a recording galvanometer. This change is directly related to the change in refractive index occurring in A.

In connection with some studies to be reported separately, it became necessary to use methods of adsorption analysis. Claesson's procedure seemed attractive, but it soon became apparent that his apparatus was needlessly elaborate for the authors' purpose and prohibitively expensive. The present paper describes a much simpler and cheaper instrument which, however, is comparable in sensitivity and utility to Claesson's. Attention is called to another continuous recording refractometer (*3*) designed and built for industrial control use, the action of which depends on intensity of internal reflection, near the critical angle, in a prism in contact with the stream of sample. The design of another recording refractometer was also recently reported by Zaukelies and Frost (*4*) of this laboratory. Their design, an important feature of which was the use of a twin-cathode photocell, was conceived and developed independently of the authors' but both came as a result of a talk on frontal analysis given by Claesson at Northwestern University in 1947. The present paper is submitted in view of the importance and potentialities of this analytical approach, and because of the modifications in the optical systems and electrical circuits.

The major change (suggested by C. T. O., July 1947) was the use of sensitive phototubes in a balanced circuit to provide high voltage sensitivity and a single vacuum tube in a stable circuit which provides sufficient current to operate a rugged milliammeter, in place of Claesson's relatively insensitive circuit using photovoltaic cells. This permitted a much more compact instrument because the optical lever arm was reduced from 100 to 10 cm. Another important change was the elimination of the weight-recording device. Claesson's apparatus recorded the weight of the eluate as a function of the refractive index by means of a spring scale attached to the galvanometer mirror. This has been eliminated by recording the refractive index on a motor-driven chart which moves at a constant rate. A General Electric photoelectric recording milliammeter was used. A more insensitive Esterline-Angus instrument was tested also in the preliminary work, but only the General Electric instrument was used in the final setup. Inasmuch as constant flow rate is maintained through the column, the chart can be calibrated to read the volume of eluate.

Claesson found it necessary to incorporate a complex constant pressure device in his apparatus because with the adsorbent selected—namely, carbon—the flow of solution was too slow. The adsorbents used in the present work, silica gel and alumina, allowed a rapid passage of the solution and at the same time maintained the necessary selective adsorption characteristics.

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A minor difference was the use of a stainless steel, right-angle reflecting prism instead of the hexagonal glass prism.

DESCRIPTION OF INSTRUMENT

The optical parts essential to the instrument are shown in Figure 1. These include the light source, the refractometer cell, the prism, and the phototubes. Figure 2 portrays the electronic circuit.

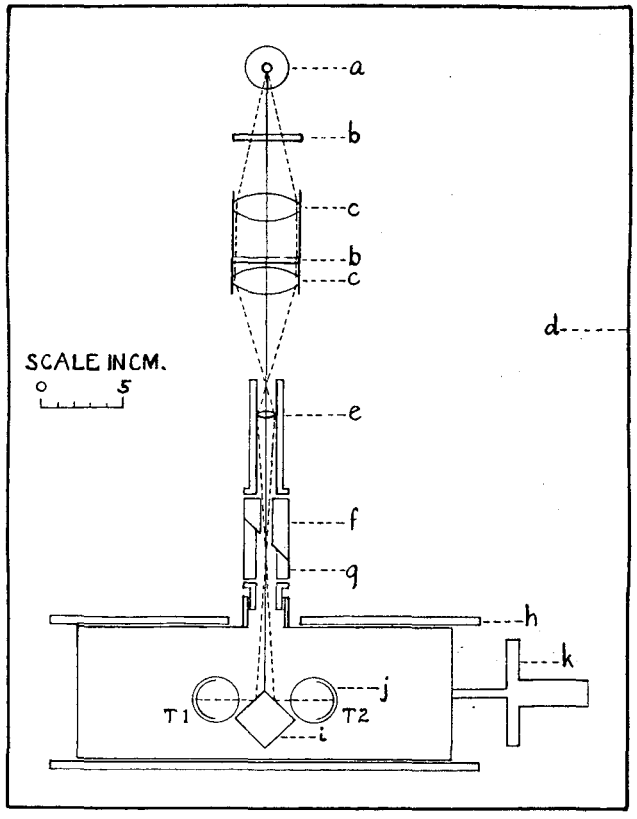

Figure 1. Refractometer Assembly

a. Tungsten lamp
b. Heat-absorbing glass plates
c. Condensing lenses
d. Brass plate for base
e. Focusing lens
f. Cell 1
g. Cell 2
h. Track
i. Reflecting prism
j. Phototubes T_1, T_2
k. Micrometer

The light source was a 50-candlepower, 6- to 8-volt, automobile headlight bulb used in conjunction with a set of achromatic condensing lenses (obtained from the Edmund Salvage Co., Audubon, N. J., Stock No. 6179; focal length, 78 mm.; diameter, 46 mm.) and a focusing lens (focal length, 25.4 mm.; diameter, 12.5 mm.). The light source was adjusted in such a way that it focused in the middle of the refractometer cell. The bulb was operated at 6.3 volts from a transformer fed by a Sola constant voltage regulator operating from a 115-volt alternating current line. Although higher operating voltages increased the sensitivity of the instrument, the bulbs burned out more rapidly.

The refractometer cell was constructed from a brass block as shown in Figure 3. The volume of cell 1 was 0.7 ml. The angle between cell 1 and cell 2 was set arbitrarily at 45°, but could be changed if greater sensitivity were desired. The cells were separated from each other by microscope cover glasses, which were used also as windows at the ends of the cells. Seepage of liquid around the cover glasses was prevented by use of lead-foil washers. Cell 1 was connected to the vertical adsorption column by means of a brass-to-glass ground joint. The eluate entered through *G* and made its exit through *H*. Cell 2 contained the solvent used in the adsorption column.

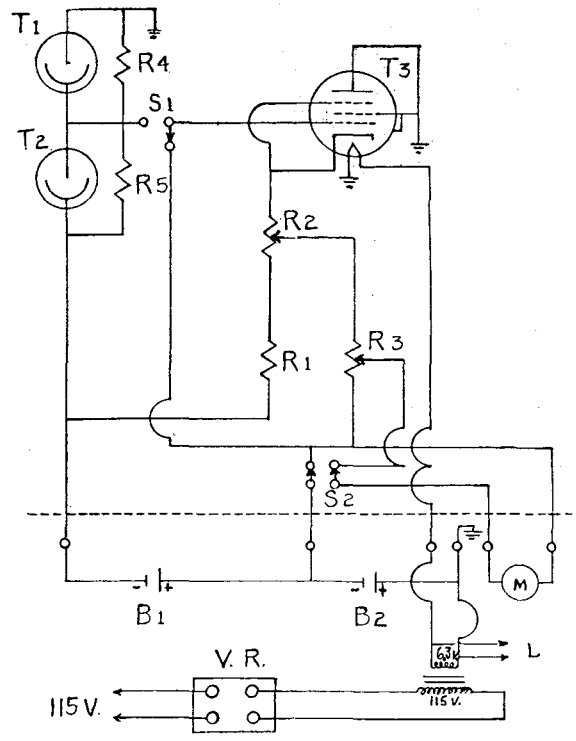

Figure 2. Electronic Circuit

V. R. Voltage regulator
L. To source of light

The highly polished stainless-steel, right-angle prism was 2 cm. on each edge. This prism divided the beam into two parts, each of which was received by a phototube. The prism and the two phototubes were housed in a light-tight box, which could be moved in a track perpendicular to the beam by means of a depth micrometer.

The circuit, Figure 2, was essentially a cathode-follower circuit in which the grid potential was controlled by the potential existing between the two phototubes connected in series.

Two RCA929 phototubes, T_1 and T_2, shown in Figure 2, were chosen for the balanced circuit because of their high sensitivity to light from a tungsten lamp. In this type of circuit a large voltage change is produced by a small unbalance of phototube current because of the high dynamic impedance of the vacuum-type phototube. In fact, for the authors' purposes the sensitivity was decreased by inserting two matched 10-megohm resistors, R_4 and R_5, across the phototubes because stability was the limit on useful sensitivity. By increasing the stability of the optical system and selecting the phototubes and amplifier tube, a ten- or twentyfold increase in sensitivity can be obtained.

The single 6SJ7 vacuum tube, T_3, connected as a triode, served as a current amplifier or an impedance match between the phototubes and the recording meter. The circuit was a cathode-follower type which proved to be very stable and relatively free from drift. To balance this circuit, switch S_1 was turned to the position at which the control grid was connected directly to the junction between B_1 and B_2, which were 45-volt B batteries. Then the balancing control, R_2, was adjusted until no potential existed across the sensitivity control, R_3, as indicated by a null on the meter, M.

Optical balance was then obtained with liquids in both sections of the refractometer cell. Starting with the sensitivity control near zero, S_1 was turned to connect the control grid to the phototube circuit and the micrometer screw was adjusted until a null was observed on the meter. Then the sensitivity was increased to any desired value and subsequent changes in the refractive index of the solution were clearly presented on the recorder tape by suitably adjusting the micrometer control.

Suitable values for R_1, R_2, and R_3 were found to be 50,000, 5000, and 5000 ohms, respectively.

No thermostat was used, because temperature was not found to be of prime consideration in the present investigation. The concentration of the solutions used was of the order of 1%, so that the change of refractive index of the solution with tem-

perature approximated the corresponding change for the solvent. Variations in room temperature were transmitted to both cells concurrently through the brass block. Heating by the light source was reduced by inserting two heat-absorbing glass plates in the optical system.

Stability of the optical path was of utmost importance, for a distortion of 0.06 mm. would cause full-scale deflection on the milliammeter. In order to acquire this optical stability, the parts of the instrument were secured to a $^3/_8$-inch brass plate which in turn rested on a three-point mount.

SENSITIVITY AND CALIBRATION OF INSTRUMENT

In Figure 4 is plotted the meter reading when the reflecting prism was moved across the beam by means of the depth micrometer mentioned above.

Curve *A* demonstrated that a 0.1-mm. shift in the prism gave full-scale deflection (about 0.2 ma.) on the G.E. recorder at full sensitivity setting (R_3 at 5000 ohms) and that the meter reading was a linear function of the prism position. At the lowest sensitivity used (R_3 at about 500 ohms), a 0.23-mm. shift gave full-scale deflection. Curve *D* indicates that a 1.2-mm. shift in prism position corresponded to full-scale deflection (1 ma.) on an Esterline-Angus recording meter. The relationship was not completely linear but could be made so by minor changes in the circuit. With optimum adjustment of the optical path, a shift of 0.06 mm. gave full-scale deflection on the G.E. meter.

By placing solutions of known refractive index in the cell, it was determined that a change of 2×10^{-3} unit in refractive index corresponded to a 0.2-mm. shift of the prism. Thus, with the instrument as shown, the full-scale sensitivity was conveniently adjustable in the range from 6×10^{-4} to 1.2×10^{-2} refractive index unit. In the work to be reported (*2*) the G.E. meter was used at a full-scale sensitivity of 2×10^{-3} unit, which was ample. In a room free from drafts, over-all stability during the course of a typical experiment was better than 10^{-5} unit. Accordingly, a change in refractive index of 2×10^{-5} unit was easily detectable.

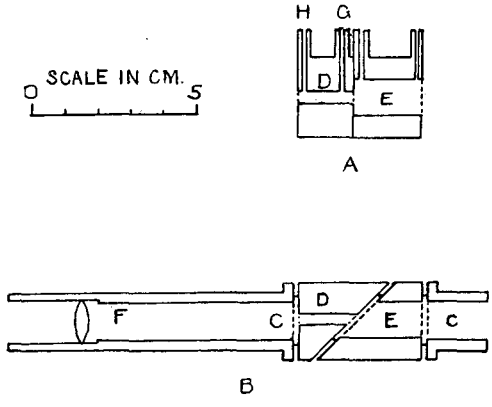

Figure 3. Refractometer Cells

A. Vertical cross section
B. Horizontal cross section
C. Microscope cover glasses
D. Cell 1
E. Cell 2
F. Focusing lens
G. Eluate entering cell
H. Eluate leaving cell

Experience showed that thermostating the cell alone decreased stability, probably because of temperature differences between the column and cell. Careful jacketing of the entire apparatus would increase the optical stability. With other refinements an ultimate usable sensitivity between 10^{-6} and 10^{-7} refractive index unit is considered feasible for this type of instrument. Minor changes in the circuit also extend the lower range of sensitivity.

Utility of this instrument in separating various mixtures of ethers and sulfides by adsorption methods is described in an article (*2*) to appear elsewhere. Specifically, these mixtures were studied using alumina and silica gel as adsorbents with cyclohexane as solvent; butyl ether and 1,2-diethoxyethane; propyl sulfide and 1,2-bis(ethylmercapto)ethane; 2-chloroethyl sulfide and 1,8-dichloro-3,6-dithiaoctane. Straight-line curves were obtained for each mixture by plotting percentage of the more strongly adsorbed component (the diethers or the dithia compounds) against L_1/L_2. L_1 and L_2 are the lengths of the lines on the chart of the recording G.E. milliammeter from zero volume to the volumes (V_1 and V_2) at which points the first and second components, respectively, are just ready to appear in the effluent liquid. For analysis, therefore, one prepares such a curve from known mixtures, then obtains the L_1/L_2 value for an unknown, and reads off the percentage of *A* from the curve.

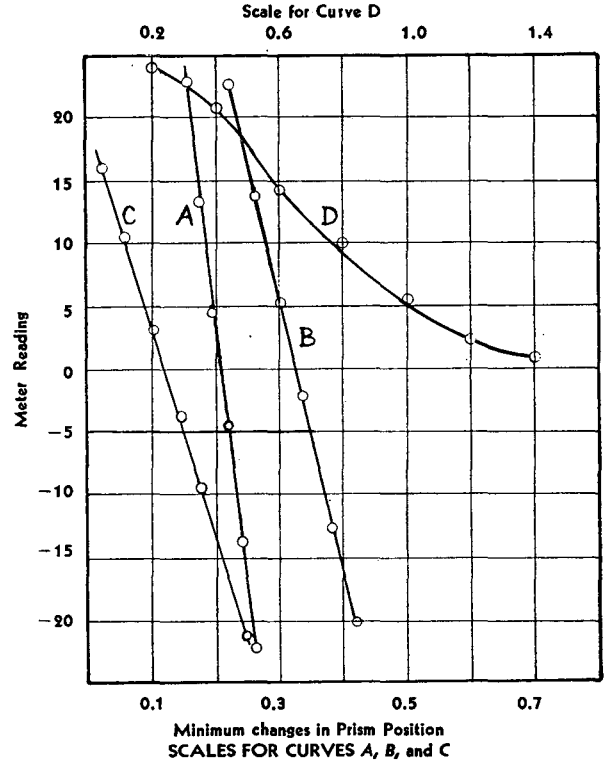

Figure 4. Meter Readings at Various Sensitivities

General Electric meter used for curves *A*, *B*, *C*. Full-scale deflection equals 50 units on *Y*-axis.
A. Maximum sensitivity (setting at 90), R_3 = 5000 ohms
B. Intermediate sensitivity (setting at 50)
C. Lowest sensitivity used (setting at 10), R_3 = about 500 ohms
Esterline-Angus meter used for curve *D*, with setting at 90. Full-scale deflection equals 25 units (not 50) on *Y*-axis.

SUMMARY

The design and construction of an automatically recording flow refractometer useful in adsorption analyses are described. Full-scale sensitivity of this unit was conveniently variable between 1.2×10^{-2} and 6×10^{-4} refractive index unit. Over-all stability corresponded to less than 10^{-5} unit, which was the lower limit of sensitivity for the apparatus in the described form. An ultimate sensitivity between 10^{-6} and 10^{-7} refractive index unit is regarded as feasible for this type of instrument.

ACKNOWLEDGMENT

The authors are grateful to John Kamper for help in part of the machine work.

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(2) Hurd, Thomas, and Frost, *J. Am. Chem. Soc.*, **72**, 3733 (1950).
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RECEIVED November 1, 1949.

Mass Spectrometric Analysis of Low Concentrations of Vapors in Air

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Kodak Research Laboratories, Rochester, N. Y.

ALTHOUGH it is well known that the mass spectrometer can determine low concentrations of one gas in the presence of large amounts of another, as in the analysis of atmospheres in annealing furnaces for traces of oxygen, or of soil gases for hydrocarbons, no description has been found of techniques suitable for concentrating and determining contaminants in air in the range below 100 p.p.m. The following procedure has given useful results when substances that can be trapped by liquid nitrogen are to be determined. This would appear to include most of the ordinary atmospheric contaminants with the exception of carbon monoxide. Typical results are given for several mixtures of solvent vapors in air, sampled in a small test room.

The inherent advantages of the mass spectrometric method of gas analysis are rapidity and freedom from interference by chemically related substances. In addition, the spectrum provides a complete qualitative record, which should be particularly important when the source of atmospheric contamination is not completely known. Two recent discussions of mass spectrometry outline the principles and techniques of this instrumental method of analysis (*4*, *6*).

APPARATUS AND PROCEDURE

The mass spectrometer was a 60° sector-type instrument constructed in this laboratory and used successfully for general analytical work. The all-glass inlet manifold, including several stopcocks and a 1-liter expansion volume, operated without auxiliary heating, as did the ion source. A glass capillary leak was located at the end of the inlet tube inside the ion source. For calibration, pure liquids in volumes up to 0.0005 ml. were admitted, through a sintered-glass valve, from a graduated micropipet.

Ion currents were amplified and then recorded on a Leeds & Northrup Speedomax recorder, with manual selection of shunts. All scanning was done by varying the magnetic field. The maximum over-all sensitivity of the apparatus as used in this work was approximately 30 chart divisions for the principal peak of acetone, mass 43, with an expanded sample pressure of 1 micron. This corresponds to an acetone content of 30 p.p.m. in the 50-ml. sample bulb. The observed peak heights may be measured to 0.2 chart division, or 0.2 p.p.m. for acetone.

Air samples were taken from a static atmosphere in a small, tightly closed laboratory room with painted walls and a volume of 290 cubic feet. Mixed solvents were evaporated on a hot plate, and the air was circulated with an electric fan. Sampling started 20 minutes after completion of the evaporation. Instantaneous samples for the mass spectrometer were taken in previously evacuated 50-ml. bulbs with stopcocks.

For comparative chemical analysis, continuous samples were drawn from the same point in the room into suitable absorbers. The volume of air was measured with rotameters. Activated silica gel was used to absorb acetone, chloride-free activated carbon to absorb chlorinated hydrocarbons, and a sulfuric acid–potassium nitrate nitrating mixture for aromatics. The chemical determination of acetone was by the iodometric method (*3*), of chlorinated solvents by decomposition with liquid ammonia and sodium (*5*), followed by precipitation with silver nitrate, and of aromatics by extraction and weighing of the nitrated products (*2*).

The bulb samples for the mass spectrometer were attached to the inlet manifold through an 8-mm. glass U-trap, 4 inches (10 cm.) deep, which was cooled in liquid nitrogen. The contents of the bulb were exhausted through the U-trap by means of a mechanical pump preceded by a second liquid nitrogen trap. Careful manipulation of the stopcock on the bulb prevented any large initial surge. Evacuation was continued for a total of 5 minutes. The U-trap and manifold were then isolated (eliminating the volume of the sample bulb), the liquid nitrogen was removed, and the collected solvents were vaporized into the fixed volume of the fore-part of the sample system. After 2 minutes, this volume was opened to the main expansion volume and to the leak into the spectrometer. With this procedure, there was no indication of fractionation in the condensation-evaporation process.

Despite the use of stopcocks and lubricated ground joints, there was negligible carry-over from one sample to the next, if care was taken to use the sample bulbs only at low concentrations, and if back-diffusion from the oil of the mechanical pump was prevented by the second liquid nitrogen trap. Pump-out times between samples varied from 5 to 15 minutes, depending upon the compounds present and their concentrations. In the most recent work, 200-ml. sample bulbs, with break-seals instead of stopcocks, have been used with good results.

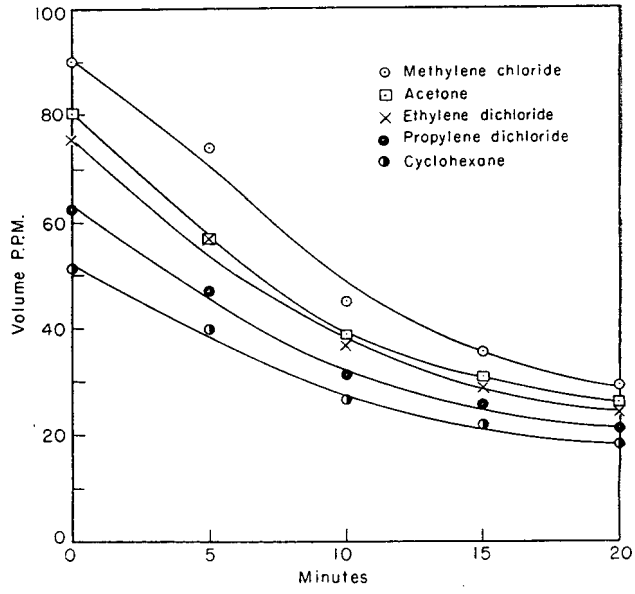

Figure 1. Variation of Solvent Concentrations in Air with Time

Five-component mixture

Typical curves of concentration *vs.* time of sampling for a five-component mixture, shown in Figure 1, indicate a die-away effect due to absorption by the walls and contents of the room. Average concentrations obtained graphically from these and other similar results are shown in Table I, together with the

Table I. Analysis of Test-Room Atmospheres

Sample No.	Component	Volume Found, P.P.M. Mass spectrometer	Chemical
1	Acetone (see Figure 1)	44.4	48.2
	Cyclohexane	30.6	[a]
	Methylene chloride	53.6	[a]
	Ethylene dichloride	43.4	[a]
	Propylene dichloride	36.4	[a]
	Total chlorinated solvents	133.4	145.5
2	Acetone	19.8	19.3
	Methylene chloride	36.1	39.4
3	Acetone	41.8	46.4
	Methylene chloride	11.6	13.3
4.	Toluene	229.1	[a]
	Benzene	161.3	[a]
	Total aromatics	390.4	360

[a] Not determined separately.

comparable chemical analyses. Direct comparison with the amount evaporated was not possible because of the absorption during the necessary mixing period in the closed room.

The results deviate, on the average, by 8.3% from the amount found chemically, with the spectrometer low in five out of the seven cases. Although this accuracy is sufficient for many studies in industrial hygiene, particularly when conventional methods are not suitable because of the complexity of the mixture or the need to take instantaneous samples, further work is expected to result in considerable improvement. A further description of this technique and its application will be published (*1*).

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(2) Manning, A. B., *J. Chem. Soc.*, **1929**, 1014.
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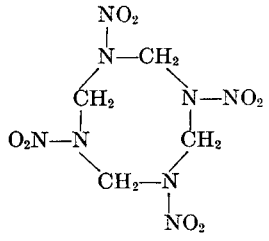
RECEIVED April 14, 1950.

CRYSTALLOGRAPHIC DATA

36. Cyclotetramethylene Tetranitramine (HMX)

Contributed by WALTER C. McCRONE, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula for HMX

HMX is a high melting by-product in the manufacture of RDX. The cystallography of HMX is therefore important in order to recognize this component in RDX products. It is also particularly interesting because it exists in four polymorphic forms, each of which can be obtained at will from a variety of solvents by varying the rate of cooling during crystallization.

HMX I

The room-temperature-stable form of HMX can be prepared by very slow cooling of solutions of HMX in acetic acid, acetone, nitric acid, or nitromethane. Agitation favors formation of I.

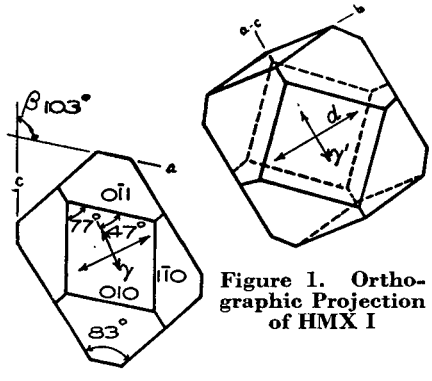


Figure 1. Orthographic Projection of HMX I

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Massive crystals showing the forms: prism, {110}; clinopinacoid, {010}; clinodome, {011}; and both the positive and negative hemiorthodomes, {101} and {$\bar{1}$01}.

Interfacial Angles (Polar). 101 ∧ 10$\bar{1}$ = 83°

Beta Angle. 103°.

Density. 1.96.

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.589 \pm 0.002$; $\beta = 1.594 \pm 0.002$; $\gamma = 1.73 \pm 0.01$.

Optic Axial Angles (5893 A.; 25° C.). $2V = 20°$; $2E = 33°$.

Dispersion. Slight horizontal, $r > v$.

Optic Axial Plane. ⊥010; $\gamma \wedge c = 30°$ in acute β.

Sign of Double Refraction. Positive.

Acute Bisectrix. γ.

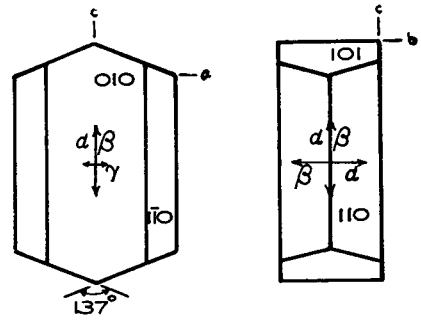


Figure 2. Orthographic Projection of HMX II

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.64$. R (calcd.) = 58.0. R (obsd.) = 56.1.

FUSION DATA

The three low temperature modifications of HMX transform through the solid phase to HMX IV at about 156° C.; hence, the sublimate and the crystals formed on cooling are of the latter modification. Some decomposition occurs during melting at 279° C. and the preparation should be quickly cooled.

The sublimate shows slightly rounded hexagonal pinacoid and bipyramid combinations. All possible orientations are usually shown with a few giving a centered uniaxial negative interference figure. The crystals from the melt show the same random orientations, but again some six-sided outlines can be recognized showing low birefringence and a centered figure.

HMX II

HMX II can be prepared from the same solvents as I but with more rapid cooling. It is the stable form from about 115° to 156° C.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Needles from all solvents with no well developed forms except perhaps prism, {110}; brachypinacoid {010}. Occasional well formed ends are macrodomes, {101}.

Interfacial Angles (Polar). 110 Λ $\bar{1}10$ = 116°; 101 Λ $\bar{1}01$ = 43°.

Density. 1.87.

Optical Properties

The optical properties vary from crystal to crystal and even from one end of the same crystal to the opposite end over a narrow range as follows: α = 1.561 to 1.565; β = 1.562 to 1.566; γ = 1.72 to 1.74.

Optic Axial Angles (5893 A.; 25° C.). $2V$ = 10 to 30° (red); −8° to 30° (blue).

Dispersion. Crossed axial plane dispersion (see table below).

Optic Axial Plane. 010 (red); 001 (blue).

Sign of Double Refraction. Positive.

Acute Bisectrix. γ.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.61. R (calcd.) = 58.0. R (obsd.) = 55.7.

Dispersion of Optic Axial Angle ($2E$) for Two Different Crystals of HMX I

Wave Length, A.	$2E$ Crystal 1	$2E$ Crystal 2
7000	17.5°	23.0°
6400	17.0°	20.0°
6000	15.5°	16.5°
5800	10.0°	10.0°
5600	0.0°	1.5°
5400	−9.2°	−9.0°
5100	−17.5°	−16.5°
4500	−25.5°	..

HMX III

HMX III is also obtained from the same solvent as I and II, but with much more rapid cooling rates. HMX III has only a very narrow temperature stability range around 156° C., if at all, at atmospheric pressure.

Crystal Morphology

Crystal System. Monoclinic.

Form and Habit. Massive or plates lying on 001; the usual forms are prism, {110}; orthopinacoid, {100}; and basal pinacoid, {001}.

Interfacial Angles (not a polar angle). 110 Λ $\bar{1}10$ (projection on 001) = 74°.

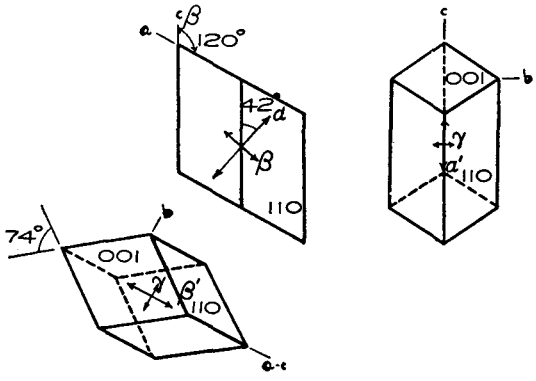


Figure 3. Orthographic Projection of HMX III

Beta Angle. 120°.

Twinning Plane. 001.

Density. 1.82.

Optical Properties

Refractive Indexes (5893 A.; 25° C.). α = 1.537 ± 0.002; β = 1.585 ± 0.002; γ = 1.666 ± 0.002; β' = 1.583 ± 0.004 (in the 001 plane).

Optic Axial Angles. (5893 A.; 25° C.). $2V$ = 75°.

Dispersion. Strong crossed, $v > r$.

Optic Axial Plane. ⊥010; $\alpha \Lambda c$ = 42° in obtuse β.

Sign of Double Refraction. Positive.

Acute Bisectrix. γ.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.597. R (calcd.) = 58.0. R (obsd.) = 55.4.

HMX IV

HMX IV can be crystallized most readily from solvents in which it is only slightly soluble and by very rapid chilling, preferably in small amounts poured over ice. This modification is stable from about 156° C. to the melting point, 279° C.

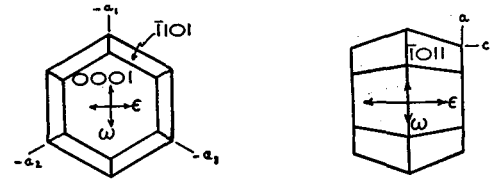


Figure 4. Orthographic Projection of HMX IV

Crystal Morphology

Crystal System. Hexagonal.

Form and Habit. Rods and needles elongated parallel to c with the forms: first-order bipyramid, {10$\bar{1}$1}; first-order prism, {10$\bar{1}$0}; and basal pinacoid, {0001}.

Profile Angle. 37° (lying on 10$\bar{1}$0).

Density. 1.78.

Optical Properties

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma}$ = 1.593. R (calcd.) = 58.0. R (obsd.) = 55.9.

Most of the work described above was carried out on a contract between Cornell University and the Office of Scientific Research and Development during June 1943 to July 1944. It was described in OSRD Report No. 3014 recently declassified by the Ordnance Department of the Army. Acknowledgment is due to Alfred T. Bloomquist who was technical representative of OSRD Section B-2-A during the progress of this work and to Colonel C. H. M. Roberts of the Ordnance Department for his efforts in regrading this report.

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.

The Analyst's Calendar

Analytical and Microchemical Group of Philadelphia Section

The Analytical and Microchemical Group of the Philadelphia Section has planned meetings for October, November, and December, to be held at the Philadelphia Museum School of Art, Broad and Pine Sts. Topics and speakers are:

Rapid Microspectrophotometric Procedures Used in Studies of Iron-Containing Proteins. Britton Chance, University of Pennsylvania. October 3

Coulometric Titrations. John W. Sease, Wesleyan University. November 7

Methods for Molecular Weight Determinations. Robert E. Kitson, Polychemicals Department, E. I. du Pont de Nemours & Co., Inc. December 5

Optical Society of America. Cleveland, Ohio, October 26 to 28

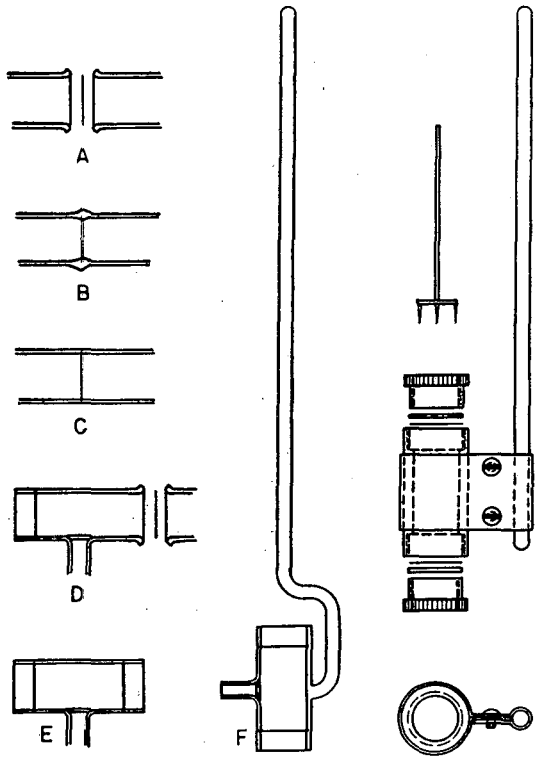
Fourth Symposium on Analytical Chemistry. Louisiana State University, Baton Rouge, La., January 29 to February 1, 1951

Fourth Annual Summer Symposium. Washington, D. C., June 14 to 16, 1951

AIDS FOR THE ANALYST.....

A Calorimetric Pipet for Liquids or Solids. John E. Wertz, University of Chicago, Chicago, Ill. (Present address, University of Minnesota, Minneapolis, Minn.)

For some accurate measurements of the integral heat of solution of solid sodium chloride it was necessary to devise a container that would hold the salt in a dry state below the surface of water in a calorimeter until thermal equilibrium had been established. It was desirable that the container have a negligibly small heat of opening or that this heat be reproducible in magnitude. This requirement excluded the possibility of using thin-walled glass bulbs, because their heat of breakage is irregular and not negligible in comparison with the magnitude of the heat quantity that was being measured. Furthermore, the fragments may alter the heat of stirring.



A pipet which performed better than was anticipated is shown in two forms in the diagram.

The pipet on the left is constructed of soft glass with platinum foil windows 0.0005 (0.0125 mm.) or 0.001 inch (0.025 mm.) in thickness. The platinum disks, 10 to 15 mm. in diameter, are punched from foil by a very sharp die. Two pieces of soft glass of internal diameter 2 mm. less than that of the disk are flared, care being taken that the end lies in one plane. A disk is carefully centered on one of these while it is hot enough to cause the disk to be held. Both ends are then heated and joined as in *B*. By heating, pulling, and blowing, the thick glass at the seal can be worked down to uniform wall thickness as in *C*. A side tube of small diameter is connected to one of the tubes at a point near the middle of the finished pipet and the other is cut or broken off 5 mm. from the disk. The second disk is incorporated in a similar manner and the end is again shortened (*E*). A solid rod is affixed opposite the side tube and is then bent (*F*) so as to be coaxial with the pipet. After filling, the side tube is pulled off.

A properly made pipet will be water-tight. Tightness can be tested by filling the pipet with phenolphthalein solution and immersing it in sodium carbonate solution. (The author is indebted to T. F. Young for suggesting this procedure.) Leaks are indicated by fine red lines. To ensure vapor tightness, the ends may be given a thin coat of glyptal lacquer. Although a foil window is occasionally broken on evacuation, one may usually both heat and evacuate the pipet for outgassing a contained powder.

The pipet was coaxial with a sharply pointed tungsten wire, having four smaller pointed wires sealed into a borosilicate glass bead on the central one. The platinum disks were easily torn by impalement, leaving a hole almost the full inside diameter of the glass tube through which the water can pass.

The platinum is completely recoverable, for it rolls toward the walls and remains as one piece, so that the net cost per pipet is only the difference between the cost of the new platinum and the exchange value of scrap.

The requirement of small heat of breakage was met satisfactorily, for in experiments with disks 0.0005 inch thick and 13 mm. in diameter, no detectable heat of breaking could be observed. This indicated that heat of breaking was less than 0.02 calorie. This was confirmed by G. Jura, who found similar results for platinum 0.001 inch thick. This pipet has been successfully used also for heat of immersion measurements.

For preliminary determinations and for work in which evacuation of the container was unnecessary, an all-silver pipet of similar construction, shown at right, was machined from a solid silver rod 0.75 inch in diameter, and threaded at each end to receive hollow nuts which were screwed firmly against a silver washer pressing on the disk. After the piece of platinum was seated, it was lightly coated around the edges with a mixture of vaseline and mineral oil to ensure water-tightness.

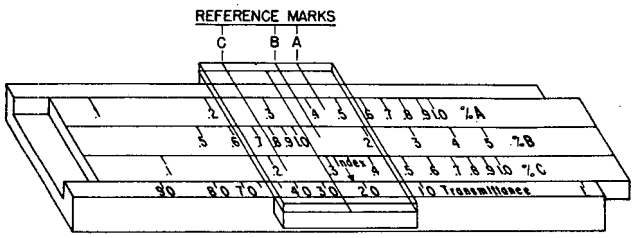
The knurled nuts could easily be tightened with the fingers sufficiently to prevent leakage, which never occurred in more than thirty experiments that were run with the pipet. The convenience of this device, which requires only cleaning and replacement of the disks, commends it for use where possible.

Slide Rule for Quantitative Spectrochemical Analysis. Duane D. Harmon, Gulf Research & Development Company, Pittsburgh, Pa.

To permit rapid interpretation of quantitative spectrographic plates, a slide rule has been developed in this laboratory. It carries the calibration curve as a transmittance scale on the base of the slide rule and the working curves as concentration scales on the slide. No relative intensity scale is necessary, so long as it is remembered that the transmittance scale and the concentration scales are plotted against the same relative intensity scale. The concentration scales are so constructed as to have their indexes at one point on the slide. The index is defined as the concentration of the element where $\frac{I \text{ analysis line}}{I \text{ internal standard line}} = 1$. This index is marked on the slide. The indicator is a broad sheet of Lucite carrying a hairline and possessing a roughened upper surface capable of taking a pencil mark. Merely rubbing the surface with an eraser roughens it sufficiently and yet preserves the transparency of the Lucite.

Over lengthy periods the index has been found to move with respect to the concentration. This is known as curve shift and must be corrected on the slide rule. One method would be to change the position of the index on the slide. This is impractical in cases where the same internal standard line is used for several elements which have different curve shifts and consequently give several indexes.

Another method of curve shift correction permits the use of the original index. A standard containing the elements to be determined in known concentrations must be photographed on each plate. The plate is processed and the internal standard and analytical lines are read on the densitometer.



The index is placed at the transmittance of the internal standard line by moving the slide. The hairline of the indicator is then referred to the transmittance of each element line in turn and the percentages of the elements are read from the proper concentration scale under the hairline. If a curve shift has occurred, the values obtained for the elements in the standard will not agree with the known concentrations. To correct for the curve shift, the hairline is placed at the values first obtained and reference marks are drawn on the surface of the indicator at the known concentrations. Subsequent analyses are made in the same manner, except that the concentrations of the elements are read from the proper concentration scales under the penciled reference mark instead of under the hairline.

This technique could be adapted to a calculating board to permit frequent plate calibration. For this the indicator would act as the vertical scale on the board and the slide carrying the concentration scales would act as the horizontal scale.

New Design of Rubber Stoppers for Microchemistry. Committee for the Standardization of Microchemical Apparatus, Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY, Al Steyermark, Chairman, Hoffmann-LaRoche, Inc., Nutley, N. J.

THE micro rubber stoppers used in the field of microchemistry are usually supplied in a solid and in a one-hole type. Because of variations in the internal diameters of the wide variety of apparatus with which they are used—combustion tubes, filter tubes, centrifuge cones, and test tubes—they do not always fit the individual object properly. Therefore, universal extra-long rubber stoppers have been designed; these can be trimmed to the desired length in order to obtain a diameter which will best fit the apparatus in question. Figure 1 gives the dimensions of the solid stopper and Figure 2 gives those for the one-hole type. It is impracticable to make the one-hole rubber stopper of the same length as the solid stopper, because the wall thickness on the smaller end would be too thin. In order to permit proper fit of the stoppers in the various necks and turbulatures of microchemical apparatus, the rubber composition should be comparatively soft and pliable. Both new rubber stoppers are commercially available from the Arthur H. Thomas Company, Philadelphia, Pa.

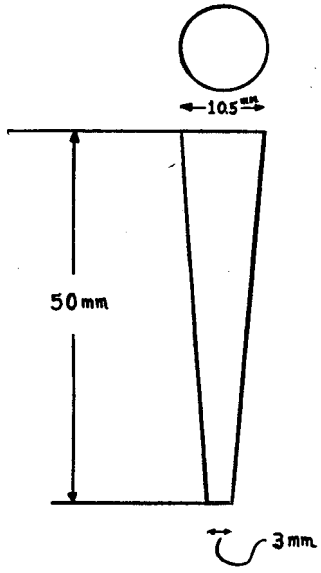


Figure 1. Dimensions of Solid Stopper

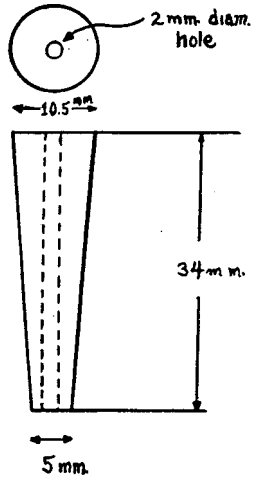


Figure 2. Dimensions of One-Hole Stopper

Large-Size Sodium Press. Raymond P. Mariella, Northwestern University, Evanston, Ill.

PROBABLY there is a sodium press in every chemical research laboratory. The capacity is usually small, the plunger frequently gets stuck in the sleeve, and much time is spent getting the apparatus in a state to receive the next charge.

In the author's laboratory, in recent years, frequent condensations have been performed using appreciable quantities of sodium metal, averaging 2 gram atoms or more. Because to produce 50 grams of ribbon from the conventionally sized press would be difficult, a large scale press (Figure 1) was designed. The press is made from cold-rolled steel, and when used, is attached to an arbor press.

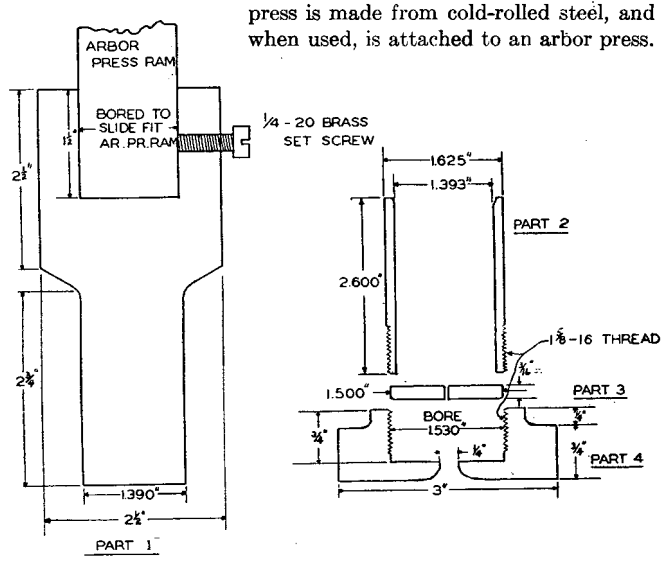


Figure 1. Sodium Press

1. Plunger (fastened to arbor press). 2. Sleeve (sliding fit on 1 and screwed into 4). 3. Die (hole in center of convenient size and shape). 4. Base (aligned accurately with arbor press ram and clamped to arbor press table)

The capacity of the sleeve is approximately 50 grams per downward sweep of the plunger. If the plunger does get stuck, it is easily removed. By inverting the press, removing the base and die, and securing the sleeve, the plunger can be instantly rammed out, using the arbor press. The apparatus is easily assembled, easily cleaned (dip parts in alcohol), and kept free from rust by being covered with vaseline or a heavy oil when not in use.

The sodium should be clean, for, if too much scale is present, the hole in the die will be plugged up. The amount of sodium left in the press, after the plunger has descended as far as possible, averages 0.5 gram. The sodium shoots out of the die and into the desired solution at a rapid rate, so that there is little chance for the clean sodium surface to react with oxygen or atmospheric moisture.

The author is indebted to Nick Dallas, who helped design the press, and John Kamper, of the shop staff.